A STUDY OF THE BEHAVIORAL AND NEUROGENIC EFFECT AND MECHANISM OF ACTION OF LAVENDER ESSENTIAL OIL AND BIS-7-COGNITIN: POTENTIAL TREATMENT OPTIONS FOR DEPRESSION

DALINDA ISABEL SÁNCHEZ VIDAÑA

PhD

The Hong Kong Polytechnic University

2019

The Hong Kong Polytechnic University

Department of Rehabilitation Sciences

A Study of the Behavioral and Neurogenic Effect and Mechanism of Action of Lavender Essential Oil and Bis-7-Cognitin: Potential Treatment Options for Depression

Dalinda Isabel SÁNCHEZ VIDAÑA

A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

July 2018

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

Singed: _____

Name: Dalinda Isabel SÁNCHEZ VIDAÑA

DEDICATION

I dedicate this research to my dad**, José Victor Sánchez Peña**, who taught me to observe and love nature, to have discipline and to dedicate myself to my studies. Above all, he taught me to enjoy life fully. I could not have had a better dad. Everything I have achieved I owe to you and the great example you were for me, the many hours I saw you studying, preparing your materials your books and teaching your students. I dedicate this thesis to you, who always took advantage of any situation to teach us. For the hours on the road you helped me and my sister to remember the type of vegetation, the weather, the classification of the clouds, the types of forests and the characteristics of the soil, I remain eternally grateful. You taught me to be the passionate researcher that I am today. Thank you very much for everything pa.

Le dedico esta investigación a mi papi, **José Victor Sánchez Peña**, quién me enseñó a observar y amar a la naturaleza, a tener disciplina, a dedicarme a mis estudios. Pero, sobre todo, me enseñó a disfrutar la vida plenamente. No pude haber tenido un mejor papá. Todo lo que he logrado te lo debo a ti, al gran ejemplo que fuiste, las muchas horas que te vi estudiando, cargando tus libros y enseñando a tus estudiantes. Te dedico esta tesis a ti pa, que siempre aprovechaste cualquier situación para enseñarnos. Por las horas en la carretera en donde mis hermanas y yo repasábamos contigo el tipo de vegetación, el clima, la clasificación de las nubes, los tipos de bosque, las características del suelo. Tu me enseñaste a ser la investigadora apasionada que soy hoy. Muchas gracias por todo pa.

ABSTRACT

Background: Depression is a mood disorder projected to be the highest cause of years of life lived with disability by 2030. Although a wide variety of antidepressants is currently available on the market, several concerns arise that affect the effectiveness of the treatment. Therefore, there is an urgent need to develop novel and more efficient treatment options for depression. The first step for the strategic drug design involves thinking outside the box to explore alternatives that may assist in the development of more efficient and novel pharmacological treatments. Therefore, the strategy should involve the most recent understanding of the pathophysiology of depression focusing on physiological processes not previously considered in the development of the currently available antidepressants. Understanding the pathophysiological elements involved in depression may provide a scenario to design target/network-based approaches towards drug development.

Rationale of the present study: Neurogenesis has been highlighted as a promising target process to develop novel therapeutic interventions for depression. A set of considerations were established to discover novel potential treatment options for depression: to take neurogenesis as the target physiological mechanism for the evaluation of candidates with antidepressant effect, the selection of promising candidates to be included in the present study was based on the following criteria: (1) promising beneficial effect on depression based on the literature, (2) the neurogenic effect had not been previously evaluated, (3) no previous study on the molecular pathways involved in its beneficial effects on depressive symptoms and (4) at least one of the candidate(s) should act via the olfactory system in order to study the olfactory system-emotion-neurogenesis relationship in detail. The criteria enabled the identification of interesting candidates whose neurogenic effect was not previously

evaluated, thereby adding to the novelty and originality of the present study. One of the candidates evaluated in the present study was lavender essential oil (LEO) that is frequently used in aromatherapy. Previous studies have evaluated LEO showing promising effects on mood in both animal studies and clinical trials. The nature of LEO and its action on the olfactory system is an interesting feature, making LEO relevant to the present study. Finally, the effect of LEO on neurogenesis has not been explored. The other candidate evaluated was Bis-(7) cognition (B7C), a dimer formed by two tacrine molecules linked by a spacer containing 2 methylene groups considered as a treatment option for Alzheimer's disease (AD). The literature review on B7C pointed out that (1) the physicochemical characteristics of B7C makes it a good candidate for the treatment of central nervous system (CNS) disorders, (2) the beneficial effects of B7C on AD animal models and the high comorbidity of AD and depression highlight a research gap that invites an evaluation of the effect of B7C on depression, (3) no previous studies have evaluated the potential antidepressant effect of B7C, and (4) the neurogenic effect of B7C has not been explored.

Aim: To evaluate the behavioral and neurogenic effect of lavender essential oil and bis-(7)-cognitin in rats and to explore the molecular mechanism of action *in vivo* and *in vitro*.

Methods: An extensive literature review was carried out on LEO and B7C to explore the effectiveness on depressive symptoms and the molecular targets relevant in neurological disorders respectively. As a second stage of the project, both candidates were evaluated *in vivo* to assess their effect on behavior, and neurogenesis in a high corticosterone (CORT) animal model for depression. An *in vitro* evaluation was carried out to assess the effect of LEO and B7C on cell proliferation and explore the molecular pathways underlying their cell proliferation effect.

Results: The analysis of RCT studies focused on the effect of aromatherapy on depressive symptoms demonstrated the promising effect of aromatherapy in alleviating depressive symptoms. The animal studies selected in the systematic review to assess the effectiveness of LEO on depressive symptoms clearly demonstrated the positive effect of LEO and linalool to decrease depression-like behavior compared with the effect of commercial antidepressants. The effect of LEO on behavior and neurogenesis was evaluated in a high corticosterone model in rats. The results demonstrated that treatment with LEO decreased the CORT-induced depression like behavior. Also, neurogenesis was found to be the physiological progress involved in the antidepressant effect of LEO, as treatment with LEO increased the number of positive BrdU cells in the hippocampus and subventricular zone (SVZ). Additionally, LEO reverted the CORT-induced dendritic branching atrophy in doublecortin (DCX) positive cells in the hippocampus. The overall results of the evaluation of LEO in a high-CORT animal model for depression demonstrated that LEO is a promising treatment option for the treatment of depression as it has positive effects on mood and neurogenesis. The results from the *in vitro* experiments demonstrated that 0.05 µl/ml LEO reverted the CORT-induced decreased cell proliferation. Also, it was demonstrated that the mechanism underlying the cell proliferation effects of LEO involved the regulation of the PI3K/Akt and MAPK/ERK signaling pathways. B7C was found to have a positive effect on behavior and neurogenesis. Also, the optimal concentration of B7C was 0.3mg/kg which was further evaluated in a high-CORT animal model for depression. Treatment with B7C showed improvement in the CORT-induced anxiety-like behavior and decreased neurogenesis. The evidence of the present study supports further investigation of B7C on mood and the molecular mechanism underlying its effect. The findings of the *in vitro* experiments clearly

demonstrated the effect of B7C in increasing cell proliferation. Also, B7C showed a restorative effect on cell proliferation in a CORT-induced decreased cell proliferation *in vitro* experiment. Finally, the findings of the present study indicate that the mechanism underlying the cell proliferation effects of B7C involves the regulation of the PI3K/Akt and MAPK/ERK signaling pathways.

Conclusions: LEO and B7C demonstrated to have a positive effect on mood and to increase neurogenesis. The present study is the first to address the effect of both candidates on behavior, neurogenesis and to examine the underlying mechanism of action *in vitro*.

PUBLICATIONS ARISING FROM THE THESIS

PUBLICATIONS

Sánchez-Vidaña Dalinda Isabel; Po, Kevin Kai-Ting; Fung, Timothy Kai-Hang; Chow, Jason Ka-Wing; Lau, Way Kwok-Wai; So, Pui-Kin; Lau, Benson Wui-Man; Tsang, Hector Wing-Hong. Lavender essential oil abolishes high dose corticosterone-induced depression-like behavior and reduction in neurogenesis in rats**. Under review**

Sánchez-Vidaña Dalinda Isabel; Chow, Jason Ka-Wing; Fung, Timothy Kai-Hang; Ho, Jeffrey; Wu, William Ka Kei; Ngai, Shirley Pui-Ching; Lau, Benson Wui-Man; Tsang, Hector Wing-Hong. Effectiveness of lavender essential oil depression: A systematic review on the preclinical and clinical evidence. **Under review**

Sánchez-Vidaña Dalinda Isabel, Ngai SP-C, He W, Chow JK-W, Lau BW-M, Tsang HW-H **(2017)** The Effectiveness of Aromatherapy for Depressive Symptoms: A Systematic Review. *Evidence-Based Complement and Alternative Medicine* 2017:21.

Sánchez-Vidaña Dalinda Isabel, Chan JN-M, Chan AHL, Hui KKY, Lee S, Chan H-Y, Law YS, Sze MY, Tsui W-CS, Fung TKH, Lau BW-M, Lai CYY **(2016)** Repeated treatment with oxytocin promotes hippocampal cell proliferation, dendritic maturation and affects socio-emotional behavior. *Neuroscience* 333:65–77.

Sánchez-Vidaña, Dalinda Isabel, Rahim Rajwani, Man-Sau Wong, Rahim Rajwani, and Man-Sau Wong (2017) The Use of Omic Technologies Applied to Traditional Chinese Medicine Research. *Evidence-Based Complementary and Alternative Medicine* 2017:1–19.

Po KK-T, Leung JW-H, Chan JN-M, Fung TK-H, **Sánchez-Vidaña DI**, Sin EL-L, So K-F, Lau BW-M, Siu AM-H (2017) Protective effect of *Lycium Barbarum* polysaccharides on dextromethorphan-induced mood impairment and neurogenesis suppression. *Brain Research Bulletin* 134.

Chan, Jackie N-M, Jada C-D Lee, Sylvia S P Lee, Katy K Y Hui, Alan H L Chan, Timothy K-H Fung, **Dalinda I Sánchez-Vidaña**, Benson W-M Lau, and Shirley P-C Ngai (2017) Interaction Effect of Social Isolation and High Dose Corticosteroid on Neurogenesis and Emotional Behavior. *Frontiers in Behavioral Neuroscience* 11. Frontiers Media SA: 18.

MANUSCRIPTS IN PREPARATION

Sánchez-Vidaña Dalinda Isabel, Hu Sheng Quan, Lee Jada Chia-Di, Liu Cinthia, Cho Macey, Lam Ivy, Fung Timothy Kai Hang, Chan Jackie Ngai Man, Chow Jason Ka Wing, Lau Benson Wui Man, and Han Yi-Fan. Behavioral and neurogenic effect of Bis (7)-cognitin on rats with corticosterone-induced depression-like behavior and study of the mechanism of action.

Sánchez-Vidaña Dalinda Isabel, Hu Sheng Quan, Lau Benson Wui Man, and Han Yi-Fan. Molecular targets of Bis (7)-cognitin and its relevance in neurological disorders: A review.

Sánchez-Vidaña Dalinda Isabel, Lau Benson Wui Man, Tsang, and Hector Wing-Hong**.** A study of the molecular mechanism of lavender essential oil *in vitro* and its relevance for depression.

CONFERENCE PRESENTATIONS

Sánchez-Vidaña Dalinda Isabel, Po KK-T, Tsang HW-H, Lau BW-M **(2017)** Effect of lavender essential oil on behavior and neurogenesis in an animal model for depression. *My Neuro 2017: Reconnecting the Synapses. Joint meeting of 27th Malaysian Society of Neurosciences (MSN) and 17th Neurosurgical Association of Malaysia (NAM) and in conjunction with Paedriatric Neurology update 2017 and 4th Federation of Asian-Oceaninan Neuroscience Societies (FAONS) Symposium*. 11th to 13th August, Kuala Lumpur, Malaysia. **Best Poster Award, 2nd place in the category of "Basic Neuroscience".**

ACKNOWLEDGEMENTS

I would like to immensely thank my Chief Supervisor, Dr. Benson Wui-Man Lau, for his wonderful support, trust and guidance. He was not only my mentor but a role model as a researcher and as human being. This Ph.D. project would not have been possible without his kind patience, trust, encouragement and direction. This journey was specially meaningful thanks to your mentorship, kindness and teachings. You show me with a clear example how to enjoy doing research and build golden collaboration relationships. Thank you Benson.

I would also like to thank my Co-Supervisor Professor Hector Tsang, for giving me the opportunity to join the Department of Rehabilitation Sciences, for his guidance and support during my Ph.D. studies.

Colleagues and collaborators

Special thanks to Dr. Shirley Pui Ching Ngai and Veronika Schoeb for their teachings and guidance in multidisciplinary collaboration. I would like to thank the amazing lab team members Jackie Ngai Man Chan, Kevin Kai-Thing Po, Timothy Kai Hang Fung, Jason Ka Wing Chow, for the warm welcome to Benson's lab team, for the training, patience, team work and fun moments in the lab. You guys are awesome!!! Also, special thanks to my friend and colleague Aaron Wanjia He for sharing his knowledge and guiding me. I learned a lot from you Aaron! I would like to acknowledge Professor Yi-Fan Han, Dr. Eagle Sheng Quan Hu, Dr. Cynthia Lai, Dr. Way Kwok-Wai, Dr. Jada Chia-Di LEE, Dr. Joseph Wai Hin Leung, Dr Pui-Kin So, Dr. Jeffrey Ho, Professor William Ka Kei Wu, Alex Cheung and the FYP and MOT students Alan H.L Chan, Katy K.Y. Hui, Sylvia Lee, Hoi-Yit Chan, Yuen Shan Law, Mei Yi Sze, Sarah, Wai-Ching Tsui, Cinthia Liu, Macey Cho, Ivy Lam for your advice and contribution to publish the findings of this project.

Family

I would like to thank my father, **Professor José Víctor Sánchez Peña,** and my sisters **Diana Lorena Sánchez Vidaña, Mariel del Rosario Sánchez Vidaña, Adriana Elizabeth Sánchez Vidaña,** you inspired me to think big and go higher. Thanks for supporting me, for the laughter, amazing moments and delicious Mexican food. Let´s keep showing to the world the Sánchez heritage. Sigan siendo ñoñas, ya saben que su hermana, la ñoña mayor, las apoyara siempre. I would also like to thank my grandma (mi hermosa ague) **Balbina Villegas,** for believing in me and supporting all my crazy ideas and ambition to go out to explore the world. Ague, you hold me in your arms when I first arrived to this world, and keep doing it. Thank you! Special thanks to the **Family Trejo Vidaña (Tía Carmen, Tío Trejo, prima Are** and **Jair)** for all your support during my journey.

Game of tones team

Thank you **Pablo Cruz González, Daniel Kwasi Ahorsu, Donald Lipardo, Harun Venkatesan, Parth Shah, Talha Qasim, Shilpa Gurung, Anchalee Peung, and Sachin Shende** for your kindness and selfless love, commitment and hard work in the Suicide Prevention project "Game of Tones: You matter, we care". Working with you have been an unforgettable learning experience.

Colleagues at the Department of Rehabilitation Sciences

Thank you so much my dear friend **Pablo Cruz González,** for being a crucial element to start my Ph.D. journey in RS. Without you, I would not be in PolyU. Thanks for the long talks in Spanish, for listening, and the friendship. Thank you **Ivan Gómez, Tiev Miller, Kartik Krishnamurthy, Chloe Chung, Wendy So, Bella Ye, Abiot, Michael,** and **Figo** for the wonderful time in RS.

PolyU friends

I would like to thank my dear PolyU friends **Brenda Guanjin Wang (my wonderful roommate, princess Brenda), Isaac Mwinlaaru (Grammar Lord), Aquil Mirza, David Ciccoria, Anna Laszlo, Nikolina Dragičević, Babak Beygi, Antwi-Afari Maxwell Fordjour, Ainur Kubasheva, Karen Wu, Ivy, Denny Juan, Hanxi Wu, Malin Victor Angell, Chi Zhang, Elvis Wu, Suhas Mutalik Desai, Derry Law, Ismael, Sebastian K Valenzuela, Micky Wong,** and many many other friends I met in PolyU. You all were a strong support during my Ph.D. journey. Thank you for all the memorable moments!!! I would like to specially thank **Mark Nartey** for the wonderful moments in ChengDe hall, the Hall Editorial Committee, his friendship and his kind contribution to editing this dissertation. Thank you Mark!!!

PolyU Hung Hom Hall Staff

I would like to thank **Dr. Eric Tam**, the SRRL team **Erica Yi, Christopher Ho, Grace Tang** and **Ivan Chung** for the opportunity to be part of the Tutor team, for all the training, and support. I would like to specially thank you, **Ivan Chung,** for the life teachings, for being such a wonderful role model, for being a listening ear and for all the wonderful collaboration we have built to contribute to the people's wellbeing. Also, I would like to thank the wonderful PMO team in Hung Hom Hall who greeted me with a big smile every morning for the past 3 years and 9 months.

Hong Kong Friends

My dear Hong Kong friends, **Fiona Cheung, Gary Wei Ho Chan (my amigo loco Hong Konger Mexicano), Portia Chan, Freddy,** thank you for all the unforgettable moments, for the long meaningful talks (and the crazy ones), for the parties, for the great meals!!! **Hakgwai Lau, Messie Yip, Mr. MC Yan, Edmund Leung (The Interzone Collective), Keith Wong, Jeffrey So (Dusty Bottle), Tong Wun Fung (Tie Shu Lan),** thank you for the music and for sharing your talent. My Hong Kong experience was multiplied exponentially thanks to you.

International Friends

What would have been of my stay in Hong Kong without all of you? Saurabh Jejurikar (my dear K friend), Faiza, Ayush Thakkar, and Maria Kintzel thank you for all the happy moments, for the laughter, for the parties, the holidays, for your support and encouragement. I love you all!!! I also want to thank Nathan Muyinda, despite the distance, you supported and encouraged me in my Ph.D. Journey. Thank you Nathan. I would also like to thank Greg Fairley and Madhi, for the wonderful talks and advice.

Friends in The Netherlands

I would like to thank my dear friends and former colleagues Ana Izquierdo, Isabel de los Rios, Hendrina Wattimury, Libette, Diana Edo Matas, Nigel Stapleton, Rinske Hulsker, and Liselotte Roosen for the encouragement and support.

My Mexican Friends

To my dear Mexican friends , Antonieta Goytia (la quiero mucho profa, gracias por todo), Noelia Villegas, Nadia Jiménez Peña, Guillermo Castillo Rivera, Xiukiauitzincheko Escandon Mosqueira (mi estimado hermano chaman), Felipe Mendoza thank you for your friendship, all the support and for being part of my journey.

My soulmate

I would like to thank my dear soulmate, Marvin Misael Vásquez (MAO), for the friendship, the laughter, the unconditional support. Despite the distance, the connection has just become stronger. Gracias Marrrrrvin, Mente Corazón y Fuerza.

My extended family

My wonderful partner, **Kristofer Söderqvist**, thank you for all the support, guidance, and selfless love. You helped me in the moment I most needed it and thanks to your kindness and guidance, I could find the right direction and balance to complete this chapter in my life. I love you immensely!!! I also would like to thank my extended family **Inger Söderqvist** and **Bill Söderqvist** for the warm welcome and the support to start a new stage in my life in Sweden. Thank you so much!!!

My Dhamma brothers

I have no words to thank my dear dhamma brothers **Sachin Shende** and **Sushil Kumar** for all the group meditation sessions in Hung Hom Hall and campus. Thanks for the strong determination and for sharing metta!!! I would also like to thank **Venerable Lamma Tashi** for his invaluable spiritual guidance that started in Bhutan, the Land of the Thunder Dragon. Thanks for all the blessings!!!

Thank you **Universe**!!!

May all beings be happy

TABLE OF CONTENTS

CHAPTER 9: GENERAL CONCLUSIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACh	Acetylcholine	ADHD	Attention deficit hyperactivity disorder
AchE	Acetylcholinesterase	Akt	Serine threonine kinase
ACTH	Adrenocoticotropic hormone	AMED	Allied and Complementary Medicine Database
AD	Alzheimer's disease		

Aβ	Amyloid β
B7C	Bis-7-Cognitin
BACE-1	Beta secretase
BDI	Beck Depression Inventory
BDNF	Brain derived neurotrophic factor
BrdU	Bromodeoxyuridine
BuChE	butyryl cholinesterase
CAF	Centralized animal facility
CAM	Complementary and alternative medicine
CAS	Catalytic active site
CCRCT	Cochrane Central Register of Controlled Trials
CES-D	Center of Epidemiological Studies Depression
CGN	Cerebellar granule neurons
ChAT	Choline acetyl transferase
CINAHL	Cumulative Index to nursing and Allied health
CNS	Central nervous system
CORT	Corticosterone
CRF	Corticotropin releasing factor
Ctr	Control
DAG	Diacylglycerol
DAG	Diacylglycerol
DCX	Doublecortin
DG	Dentate gyrus
DRG	Dorsal rood ganglion
DSM-5	Diagnostic and Statistical Manual of Mental Disorders fifth edition
DSM-IV	Modified Diagnostic and Statistical Manual of Mental Disorder criteria
EPDS	Edinburgh Postnatal Depression Scale
ERK1/2	Extracellular signal-related kinase
Fluox	Fluoxetine
FST	Forced swimming test
Grb2	Growth factor receptor-bound protein 2
HADS	Hospital anxiety and depression scale
HPA	Hypothalamic-pituitary-adrenal axis
i.p.	Intraperitoneal
IA	Inhalation aromatherapy

ICD-10	International Classification of Diseases and Related Health Problems
IGF1	Insulin-like growth factor 1
JNK 1/2/3	Jun amino terminal kinases
LEO	Lavender essential oil
MA	Massage aromatherapy
MADRS	Montgomery-Asberg Depression Rating Scale
MAO	Monoamine-oxidase
MAPK	Ras-microtubule-associated protein kinase
MRS	Menopause Rating Scale
NA	Not available or not applicable
NCCAM	National Center for Complementary and Alternative Medicine
NDRI	Norepinephrine and dopamine inhibitor
NGFs	Neurotrophic/Growth factors
NIP	No information provided
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
NR	Not reported
NT3	Neurotrophin 3
NT4	Neurotrophin 4
OB	Olfactory bulb
OFT	Open field test
OT	Oxytocin
PAS	Peripheral active site
PI3K	Phosphoinositide 3 kinase
PKC	Protein kinase C
PLCγ	Phospholipase C γ
POMS	Profile of mood states rating scale
RCT	Randomized controlled trials
RGC	Retinal ganglion cells
s.c.	Sub-cutaneous
SCOP	Scopolamine
SD	Sprague-Dawley
SGZ	Subgranular zone
Shc	Src homology 3 domain containing adaptor protein
SIT	Social interaction test

SNRI	Serotonin and norepinephrine reuptake inhibitor
SOS	Son of sevenless
SSRI	Serotonin selective reuptake inhibitor
SVZ	Subventricular zone
TG	rat trigeminal ganglion
TMD	Total Mood Disturbance
TMS	Total Mood Score
TrkB	Tropomyosin-related kinase B
TST	Tail suspension test
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

CHAPTER 1

GENERAL INTRODUCTION

Painting by Wei Ho Chan, 2018 (permission to use of the painting in this thesis was granted by the artist)

1.1. Depression: A global health concern

Depression is a life-threatening mood disorder characterized by the manifestation of affective, physical and cognitive symptoms including sadness, anhedonia, lack of interest in daily activities, feeling of worthlessness, fatigue, changes in appetite and sleep patterns, suicidal thoughts, poor concentration and memory impairment that last more than 2 weeks (Baquero and Martín, 2015; Ren et al., 2015; Tizabi, 2015; Qaseem et al., 2016). The quality of life of people suffering from depression is greatly affected. The presence of depressive symptoms has a negative impact on a person's ability to perform daily tasks, resulting in disability and a high risk of perpetrating self-harm that may lead to suicide (Ernst et al., 1998; Yim et al., 2009).

The global prevalence of depression has increased dramatically and it is reported by the WHO as the largest health concern of the 21st century (WHO, 2013). More than 350 million people are suffering from depression (WHO, 2016) and this mood disorder is projected to be as the highest cause of years of life lived with disability by 2030 (Ferrari et al., 2013; Bhattacharya et al., 2016). Depression is also linked with a high mortality rate accounting for about one million suicide cases every year (Caraci et al., 2010; Bhattacharya et al., 2016). Depression causes a significantly high economic burden accounting for an annual economic loss of USD 210 billion only in the USA (Greenberg et al., 2015; Wittenborn et al., 2015).

The multifactorial nature of depression creates a complex scenario for its diagnosis and treatment management. This mood disorder involves a complex interplay of genetic and a number of environmental factors such as childhood maltreatment, lack

of parental care, loss of a parent during childhood, poor social support, low physical activity, and drug abuse (Köhler et al., 2018). Furthermore, the development of the gene-environment interactions, which cannot be easily predicted, and epigenetic mechanisms may play a role to the pathophysiology of depression (Köhler et al., 2018) bringing additional factors to bear on the complexity of this affective disorder.

Although no reliable classification tools have been developed to associate the etiology of depression with the response to the treatment, several attempts have been made to define and classify the severity of this mood disorder. The diagnosis, treatment and evaluation of the treatment's effectiveness rely on the assessment of the presence and severity of symptoms (Schmidt et al., 2011). Examples of validated assessment tools include the Hamilton Depression Rating Scale that comprises a clinical-rated and a self-reported assessment (Hamilton, 1960; Anderson et al., 2002) and the Beck Depression Inventory that includes a self-reporting tool (Anderson et al., 2002). Two major systems have been used to describe and classify the gravity of depression: the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5) and the International Classification of Diseases and Related Health Problems (ICD-10) (WHO, 1993; National Collaborating Centre for Mental Health, 2010). These systems have classified depression in terms of severity, duration, course of the disorder and subtypes according to the symptom profile (National Collaborating Centre for Mental Health, 2010). The complexity of depression can be observed in the classification systems which describe more than 60 different types of major depressive disorders comprised of different combination of symptoms (Culpepper et al., 2015). Because the neurobiological abnormalities that contribute to the heterogeneity of depression are

complex and poorly understood, psychiatrists still rely on subjective tools for the assessment of the depressive symptoms (Schmidt et al., 2011).

1.2. Pharmacotherapy: The first line of treatment for depression

Pharmacotherapy for a long time has been the first line of treatment for depression (Richelson, 2013). The first antidepressants introduced back in the 50s were the so-called tricyclic antidepressants (e.g. imipramine and desipramine) and monoamine oxidase inhibitors (e.g. tranylcypromine and phenelzine). In the 80s, selective serotonin reuptake inhibitors (SSRI) such as sertraline, paroxetine and citalopramin were introduced for the treatment of depression (Berton and Nestler, 2006; Richelson, 2013; Saltiel and Silvershein, 2015; Hiemke, 2016). In the 90s, serotonin and norepinephrine reuptake inhibitors (SNRI) such as venlafaxine, duloxetine and desvenlafaxine and norepinephrine and dopamine reuptake inhibitors entered the market (Richelson, 2013; Saltiel and Silvershein, 2015; Hiemke, 2016).

The criteria to choose the most appropriate antidepressant out of 30 antidepressants available in the market largely depend on the symptom profile of the patient (Saltiel and Silvershein, 2015; Hiemke, 2016). Some of the symptoms observed in depression include cognitive impairment and anxiety. For example, anxiety has been shown to have a high comorbidity with major depressive disorder (Richelson, 2013). In a study carried out in the Netherlands, about 67% of the patients diagnosed with depression showed a comorbid anxiety disorder and 63% of the patients with an anxiety disorder showed comorbid depressive symptoms (Lamers et al., 2011). Because of the high comorbidity of depression and anxiety and the overlapping neurobiochemical

disturbances in both conditions, effective antidepressants with anxiolytic effect, such as SSRIs and SNRIs, have been found to be effective for both, anxiety and depression (Richelson, 2013; Saltiel and Silvershein, 2015). Therefore, patients exhibiting anxiety in the repertoire of depressive symptoms can be prescribed SSRIs or SNRIs (Saltiel and Silvershein, 2015).

1.3. The challenges of using pharmacotherapy for the treatment of depression

Although a wide variety of antidepressants is currently available on the market, several concerns arise that affect the effectiveness of the treatment. First of all, full remission is seldom achieved in patients with depression (Wilkinson, 1995; Arroll et al., 2005; Chan et al., 2015). The degree of responsiveness to the pharmacological treatment considers a reduction of less than 50% of the symptomatology related to depression (Saltiel and Silvershein, 2015). Therefore, remission is only considered when the scores in the rating scales are low but this cannot be translated as absence of symptoms (Saltiel and Silvershein, 2015).

Another issue related to the use of antidepressants is the degree of responsiveness. About 30 to 40 % of the patients with depression do not respond to treatment (Caraci et al., 2010; Qureshi and Al-Bedah, 2013; Saltiel and Silvershein, 2015) and up to 60% may experience some level of treatment resistance that may lead to prolonged depression episodes and increased severity of the symptoms (Strawbridge et al., 2017).

Low compliance to antidepressant treatment is another concern closely linked to the side effects. The most frequently reported side effects include nausea, insomnia,

agitation, weight gain, somnolence, sexual dysfunction and cardiovascular disturbances (Nezafati et al., 2015; Tizabi, 2015; Yeung et al., 2015). The presence of side effects plays a crucial role in the patient's compliance to the treatment. About 52% of patients taking antidepressants quit the treatment due to their inability to tolerate to the side effects (Qureshi and Al-Bedah, 2013; Saltiel and Silvershein, 2015). Another limitation of antidepressants is the long time required for the therapeutic effect (Tizabi, 2015). Averagely, it takes about 6 to 7 weeks to experience alleviation of depressive symptoms (Richelson, 2013).

When poor response to the pharmacological treatment is observed or the intolerance to the side effects greatly affects the compliance to the treatment, clinicians put in practice several strategies. For example, clinicians may consider the adjustment of the dose and the possibility to substitute the antidepressant with another available pharmacological option (Richelson, 2013; Culpepper et al., 2015). Also, revision of the diagnosis takes place accompanied by a full exploration of the presence of other conditions that might be contributing to the symptomatology including substance abuse, other psychiatric disorders or any other medical condition (Richelson, 2013; Culpepper et al., 2015). However, the above-mentioned strategies take a long time to determine whether the new approach implemented benefits the patient; subsequently this may negatively affect compliance to the treatment (Richelson, 2013).

The drawbacks of the use of antidepressants have a negative impact on the patient's treatment and necessitates the developing of novel and more effective options for the treatment of depression. In fact, an increasing number of patients with depression have turned to non-pharmacological alternatives given all the issues surrounding treatment

with the current antidepressants (Hiemke, 2016). Some of the non-pharmacological approaches explored by patients include psychoeducation, physical exercise, problem solving therapy, guided self-help and behavioral activation treatments (Dirmaier et al., 2012) and complementary and alternative medicine (CAM) (Ernst et al., 1998; Yeung et al., 2015).

As discussed above, depression is a critical health concern that continues to affect an increasing number of people all over the world. The complex nature of the neurobiology of depression that confers on it a high degree of heterogeneity and the issues related to pharmacotherapy of depression make it urgent to develop new therapeutic options. The first step for the strategic drug design involves thinking outside the box to explore alternatives that may assist in the development of more efficient and novel pharmacological treatments. Therefore, the strategy should involve the most recent understanding of the pathophysiology of depression focusing on physiological processes not previously considered in the development of the currently available antidepressants. This approach is the main focus of the present research project.

1.4. Thinking outside the box: An exploration of the hypotheses on the pathophysiology of depression

Target screening, clinical practice, pharmacotherapy, epidemiology, and pathology-centered approaches are drug discovery strategies used to develop novel therapeutic options (Pangalos et al., 2007). Particularly, basic research focused on the pathology of the disease has been the most commonly implemented discovery strategy in

neurological disorders (Pangalos et al., 2007). Knowing the pathophysiology of a given disease provides an insightful picture of the disease process (Pangalos et al., 2007). Consequently, key signaling pathways and potential drug targets can be identified (Pangalos et al., 2007). Despite the complex, sometimes poorly understood, pathophysiology in neurological disorders, the pathology-centered approach not only increases knowledge on the disease but also offers a platform for therapeutic innovation (Pangalos et al., 2007). Therefore, understanding the pathophysiological elements involved in depression may provide a scenario to designed target/network-based approaches towards drug development.

The neurobiology of depression comprises a complex interaction of genetic, epigenetic, biochemical and psychosocial factors. The details of how those elements interact with each other in the pathology of depression are still not well understood (Saltiel and Silvershein, 2015). However, knowledge based on extensive studies has resulted in numerous hypotheses that encapsulate different factors, molecular networks and key players. This has been useful in shedding light on the pathophysiology of depression (Lee et al., 2010).

Rather that evaluating individual hypothesis on depression, researches should have in mind the interaction and overlapping key elements involved in each hypothesis (Lee et al., 2010; Petrik et al., 2012). Taking that direction, it would be possible to get a bigger picture of the pathophysiology of depression (Lee et al., 2010) and develop more effective and novel therapeutic options (Richelson, 2013).

1.4.1. Classical monoamine hypothesis

Most antidepressants available on the market are based on serendipitous discoveries made more than half a century ago (Berton and Nestler, 2006; Wainwright et al., 2013). In 1928, an enzyme causing oxidative disamination of biogenic amines was fist described (López-Muñoz and Alamo, 2009). It was not until 1937 that this enzyme, which was also found in the brain, was characterized and named monoamine-oxidase (MAO) (López-Muñoz and Álamo, 2016). After World War II, large amounts of hydrazine, a compound used by the German army as rocket fuel, was distributed to chemical and pharmaceutical companies at a very low price (López-Muñoz and Alamo, 2009). Hydrazine derivatives were found to have antitubercular effects (López-Muñoz and Alamo, 2009). Back in 1952, iproniazid, a MAO inhibitor, was already being used to treat patients with tuberculosis. The side effects observed in the patients treated with iproniazid included psychological changes such as greater vitality, improved sleep and social activity and positive mood (López-Muñoz and Alamo, 2009). Later on, the psychostimulant side effect of iproniazid was considered as a potential primary effect for the treatment of psychiatric disorders and the term antidepressant was first used to refer to the effects of this drug on patients with depression (López-Muñoz and Álamo, 2016).

The mechanisms by which most antidepressants act follow the hypothesis of monoamine depletion (Tizabi, 2015). Monoaminergic transmission is the basis of important neural processes such as mood, reward, pleasure, motivation, cognition and executive functions (Saltiel and Silvershein, 2015). The monoamine hypothesis was postulated based on the inhibitory action of iproniazid on MAO and further studies on

the blockade of synaptic reuptake of monoamines (López-Muñoz and Álamo, 2016). The monoamine hypothesis was first postulated in 1965 by Joseph J. Schildkraut of the Massachusset Mental Health Center in Boston and it stated that depression was the result of low neurotransmitter levels (López-Muñoz and Álamo, 2016).

The mechanism involved in the monoamine hypothesis considers the restoration of the disturbances in monoaminergic neurotransmission by enhancing the availability of neurotransmitters in the synaptic cleft (Wainwright et al., 2013; Tizabi, 2015). Therefore, antidepressants in this category target the reuptake or degradation mechanisms of neurotransmitters (Saltiel and Silvershein, 2015; Tizabi, 2015). For instance, SSRI, SNRI and norepinephrine and dopamine reuptake inhibitor (NDRI) inhibit the serotonin transporter, serotonin and norepinephrine transporters and norepinephrine and dopamine transporters respectively, to increase the concentration of their respective monoamine in the synaptic cleft (Saltiel and Silvershein, 2015). Later research in the field of depression has strengthened the arguments behind the actions of monoamines and depression. Saltiel and Silvershein has provided a hypothetical map that establish the relationship between brain structures, monoamine function and depressive symptoms – this map represents a comprehensive summary of the monoamine hypothesis in depression (Figure 1) (Saltiel and Silvershein, 2015).

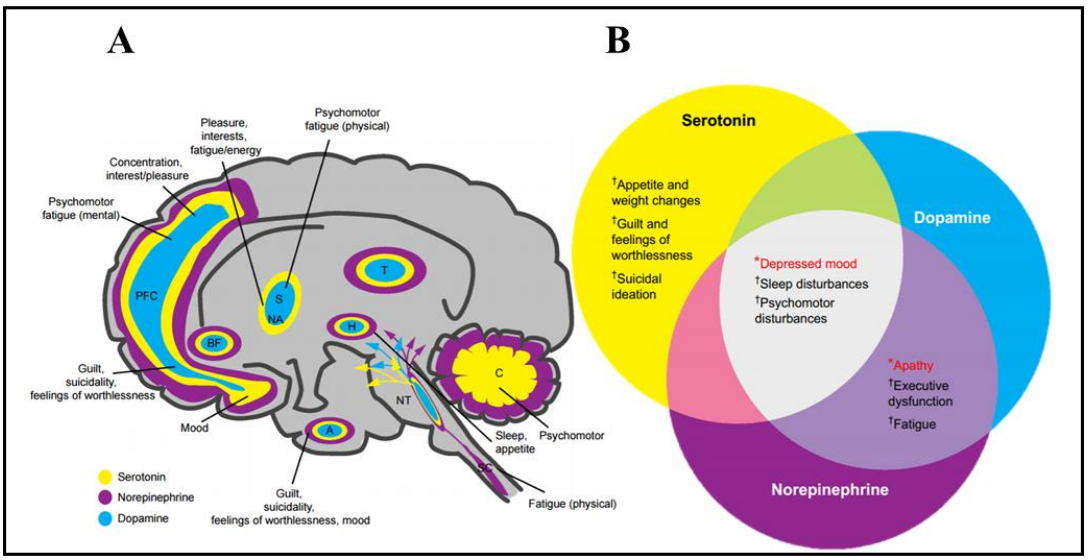


Figure 1. Hypothetical map establishing the relationship between brain structures, monoamine function and depressive symptoms. **A:** Brain map of the hypothetical malfunctioning brain circuits of neurotransmitters and their relationship with depressive symptoms. **B:** At least one of the symptoms shown in * and four or more of the symptoms shown as † should be present to diagnose depression. Abbreviations: A, amygdala; BF, basal forebrain; C, cerebellum; H. hypothalamus; NA, nucleus accumbens; NT, neurotransmitter centers; PFC, prefrontal cortex; S, striatum; SC, spinal cord; T, thalamus. Figures modified from Saltiel and Silvershein, 2015.

The advances in antidepressant development that followed the iproniazid serendipitous discovery led to the generation of other categories of antidepressants design under the principles of the monoamine hypothesis of depression (Wainwright et al., 2013). Nowadays, antidepressants available on the market target the monoaminergic system and they are used as first-line treatment for depression (Pilar-Cuéllar et al., 2013; Saltiel and Silvershein, 2015). However, due to the undesired side effects that lead to low compliance, in addition to relapses, low recovery rates and

drug resistance observed in clinical practice, there is an urgent need to develop novel therapeutic options that explore alternative research approaches (Berton and Nestler, 2006; Lang and Borgwardt, 2013).

Traditionally, the monoamine hypothesis was the classical approach in depression research until the late 90s (Pilar-Cuéllar et al., 2013). New evidence has led to the conclusion that the physiological aberrations observed in depression go beyond the monoamine hypothesis (Pilar-Cuéllar et al., 2013). As novel hypotheses develop, knowledge on the pathophysiology of depression is constructed to contribute to a better understanding of depression (Petrik et al., 2012). Therefore, future research directions should consider the integration of knowledge from the different hypotheses of depression.

1.4.2. Hypothalamic-pituitary-adrenal axis hypothesis

As illustrated in Figure 1, several brain structures have been associated with the behavioral abnormalities observed in mood disorders (Saltiel and Silvershein, 2015). Normal mood functioning depends on a complex network involving different brain regions (Campbell and Macqueen, 2004). Understanding how the abnormal neural circuitry functioning leads to mood disorders remains a challenging task in neuroscience. However, evidence from brain imaging studies, based on blood flow or related measurements in different brain areas has made it possible to identify brain regions such as the prefrontal cortex, cingulate cortex, hippocampus, striatum, amygdala and thalamus that showed dysregulation and implication in the affective-emotional network in depression (Berton and Nestler, 2006; Bartsch, 2012).

Post mortem analysis of the brain has shown correlation between abnormalities observed in the above-mentioned brain regions and depressive symptoms (Berton and Nestler, 2006; Duman et al., 2016). A clear example of altered brain structure and function is the reduced volume of the prefrontal cortex and hippocampus which has shown strong association with the severity of depression (Duman et al., 2016). Additionally, depressive symptoms such as impaired memory, feelings of hopelessness, guilt and suicidal thoughts have been linked to functional and structural alterations in the prefrontal cortex and hippocampal regions (Berton and Nestler, 2006). Cell atrophy, cell loss and circuitry malfunctioning found in the above-mentioned brain structures, although not fully understood, are important contributing factors in the pathophysiology of depression and more research efforts are required to enable a full understanding of the brain structure-connectivity-functioning cross-talk (Campbell and Macqueen, 2004; Duman and Li, 2012).

Different lines of research have pointed out that the abnormalities in the hippocampal structure and functioning are closely implicated in the symptomatology observed in depression (Campbell and Macqueen, 2004; MacQueen and Frodl, 2011). The hippocampus is a relatively small structure and part of the limbic system that has been extensively studied (Bartsch, 2012) and plays a pivotal role in cognition, consolidation of short and long term memory (Campbell and Macqueen, 2004) and regulation of emotional responses (Taupin, 2006). Structurally speaking, the hippocampus consists of the cornu Ammonis subregions CA1 to CA4 and the dentate gyrus (Campbell and Macqueen, 2004). In terms of hippocampal connectivity and functioning, the dorsal hippocampal region has been found to play a preferential role in cognition and

memory while the ventral hippocampal region plays a primordial role in affective behavior (Zhao et al., 2008a). Regarding the cellular composition, about 90% of the neurons found in the hippocampus are glutaminergic neurons and granule cells while the remaining 10% are mainly GABA producing interneurons (Campbell and Macqueen, 2004).

The hippocampus is rich in mineralocorticoid and glucocorticoid receptors (Wainwright et al., 2013). These receptors participate in the maintenance of the normal hypothalamic-pituitary-adrenal (HPA) axis function and the negative-feedback regulatory processes involved in the flight-or-flight stress response (Wainwright et al., 2013). As a result, the hippocampus is particularly sensitive to behavioral stress and high levels of glucocorticoids (MacQueen and Frodl, 2011). While mild stress has enhancing effects on cognition and memory, sustained stress has been one of the most significant factors linked to susceptibility for depression and closely associated with structural and functional alterations in the hippocampus (Calabrese et al., 2009; MacQueen and Frodl, 2011; Duman et al., 2016).

The HPA axis is an essential coping mechanism to stress controlled by the regulation of glucocorticoid release (Figure 2) (Berton and Nestler, 2006; Krugers et al., 2010). In depression, hyperactivity of the HPA axis, elevated glucocorticoid levels and disruption of the negative feedback regulatory mechanisms have been established as one of the most consistent findings in mood disorder research (Berton and Nestler, 2006; Pariante, 2006; Duman et al., 2016). The hypothalamus releases corticotrophin-releasing factor (CRF) and vasopressin that stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland which enhances the

secretion of glucocorticoids from the adrenal cortex (Berton and Nestler, 2006; Pariante, 2006; Pariante and Lightman, 2008; Lee et al., 2010). The feedback loop of the HPA axis involves brain structures such as the hippocampus and amygdala (Lee et al., 2010). Glucocorticoids activate feedback inhibition of the release of CRF from the hypothalamus and ACTH via interaction with their glucocorticoid and mineralocorticoid receptors (Pariante and Lightman, 2008).

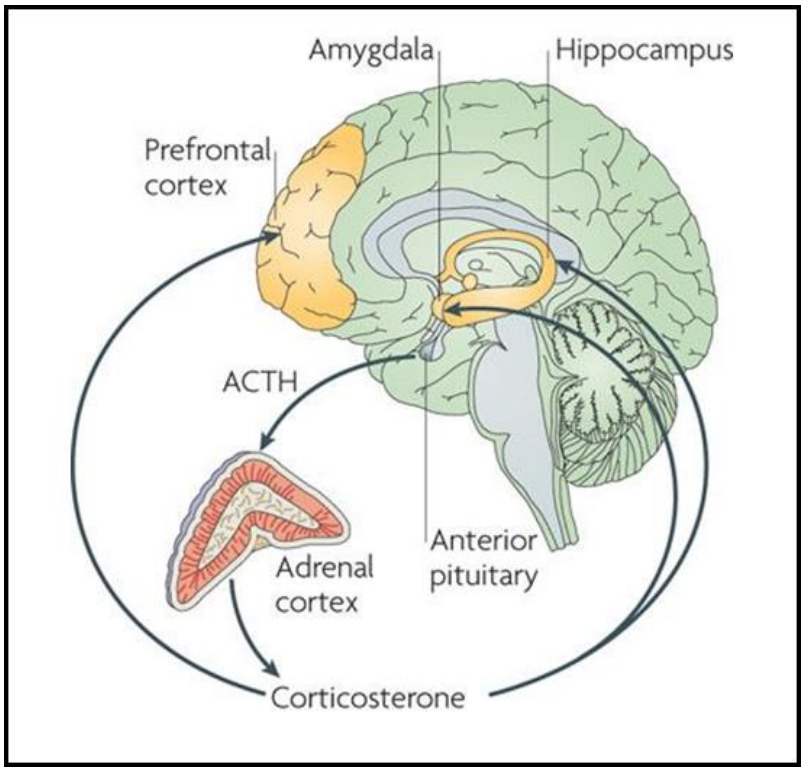


Figure 2. Representation of the HPA axis. The intrinsic feedback connectivity of the HPA axis including the anterior pituitary and the adrenal cortex with structures from the limbic system (amygdala, hippocampus, prefrontal cortex) is depicted. Figure modified from Krugers et al., 2010.

Increased levels of circulating glucocorticoids resulting from excessive activation of the HPA axis lead to higher expression of CRF in the hypothalamus which contributes

to the stimulation of ACTH release and therefore higher glucocorticoid levels (Berton and Nestler, 2006). In parallel, high glucocorticoid levels increase the excitotoxicity of CA3 neurons in the hippocampus and this results in dendritic atrophy, spine loss, apoptosis of neurons, and inhibition of the generation of new granule cells neurons (Berton and Nestler, 2006; Lee et al., 2010). The negative alterations in the hippocampus caused by HPA axis hyperactivity disrupt the feedback regulatory mechanisms and lead to hippocampal dysfunction (MacQueen and Frodl, 2011). Finally, aberrant hippocampal functioning has been associated with some of the symptoms observed in depression such as cognitive impairment, memory deficits and anhedonia (MacQueen and Frodl, 2011).

Treatment with antidepressants has been associated with the restoration of the negative feedback in the HPA axis (Pariante and Lightman, 2008), leading to improvement of depressive symptoms (Wainwright et al., 2013). The restored negative feedback inhibition of the HPA axis by antidepressants has been linked to the functional restoration of the hippocampus (Wainwright et al., 2013) via upregulation of glucocorticoid receptor levels in the hippocampus (Calabrese et al., 2009).

1.4.3. Neuroplasticity, neurogenesis and neurotrophic hypotheses

The neuroplasticity hypothesis emerged in the late 90s based on evidence gotten from the effect of stress on the hippocampus and decreased synaptic plasticity in the prefrontal cortex (Pilar-Cuéllar et al., 2013). Neuroplasticity is a dynamic brain mechanism in which information is received and processed via the neuronal circuitry

to adapt and respond to internal and external environmental stimuli (Calabrese et al., 2009; Wainwright et al., 2013). Through neuronal plasticity, changes in structural and functional mechanisms in the brain involving neuronal remodeling, the formation of new synaptic connections (synaptic plasticity) and birth of new neurons (neurogenesis) take place (Calabrese et al., 2009). Because patients with depressive symptoms show difficulties in adaptation to environments and vulnerability to challenging situations, neuroplasticity has been associated with those behavioral abnormalities (Calabrese et al., 2009). Therefore, disturbances in neuroplasticity mechanisms have been linked to the aberrant emotional processes observed in depression (Calabrese et al., 2009; Lau et al., 2013).

The involvement of several signaling pathways in neuroplasticity processes such as neural maintenance and remodeling, synaptogenesis and neurogenesis have been studied in depression. Due to the close interrelation of neuroplasticity mechanisms, key molecular elements and signaling pathways have been found to be involved in more than one neuroplasticity process. The information, sub-classified as neurotrophic, neurogenesis and synaptogenesis hypotheses, comprises crucial molecular elements and signaling pathways involved in different neuroplasticity mechanisms.

There is a strong body of evidence that suggests a pathophysiological relationship of depression regarding neurogenesis (Gage et al., 2008; Tizabi, 2015). A healthy adult brain is equipped with about 100 billion neurons characterized by long and complex dendritic arboritization patterns that connect neurons in a network comprising about 100 trillion synapses (Alzheimer's Association, 2015). The brain synaptic network

allows rapid signal transmission and is the key foundation for the creation of memories, thoughts, sensations, emotions, locomotion and skills (Alzheimer's Association, 2015).

Neurodegenerative events have been established in different regions of the brain in depressed patients including the hippocampus. The hippocampus plays a pivotal role in cognitive and emotional processes and it is the primary memory structure in the brain (Sierksma et al., 2010). Reduction of the hippocampal volume in patients with major depression has been previously established (Sierksma et al., 2010; Alzheimer's Association, 2015). Furthermore, depressive symptoms have been correlated with decreased hippocampal neurogenesis, defined as the process of producing new neurons from neural stem cells (Apple et al., 2016). In fact, the behavioral benefits of antidepressants have been linked with improved neurogenesis in the hippocampus (Apple et al., 2016). Consequently, neurogenesis has been highlighted as a promising target process to develop novel therapeutic interventions for depression (Apple et al., 2016).

For a long time, the birth of new neurons was limited to occur during embryonic and early postnatal developmental stages (Jessberger and Gage, 2014). Besides, loss of neuron was thought to be an irreversible process in the adult brain because of the idea of disruption of the stability in the brain if replacement of the dying neurons could take place (Eriksson et al., 1998; Gage et al., 2008; Chesnokova et al., 2016). The integration of newborn cells into the complex brain circuitry was assumed to be impossible as it could destabilized the circuit connections governing preexisting information and acquired skills (Jessberger and Gage, 2014). However, back in the

90s, Erikson and his colleagues were the first in demonstrating neurogenesis processes in the hippocampal sub granular zone of the adult brain. The analysis of postmortem brain tissue of cancer patients, that underwent incorporation of the nucleotide analogue bromodeoxyuridine (BrdU) used as a diagnostic tool in cancer patients, revealed the existence of neurogenesis in the adult brain (Eriksson et al., 1998; Apple et al., 2016; Chesnokova et al., 2016).

Hippocampal neurogenic processes are of particular importance for the formation and consolidation of memories (Apple et al., 2016). In mammals, neurogenesis is limited to occur in specific brain regions including the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus and the subventricular zone (SVZ) which is located along the lateral wall of the lateral ventricles (Figure 3) (Aimone et al., 2014; Apple et al., 2016). Neural stem cells are generated in both regions which give rise to neural progenitor cells with the ability to differentiate into neurons or glia cells. Migration of neural progenitor cells generated in the SVZ occurs along the rostral migratory stream and serves as a supply of neurons for the olfactory bulb (OB). On the other hand, neural progenitor cells generated in the subgranular zone translocate to the granular cell layer of the DG where cells integrate into the existing neuronal network in the hippocampus (Aimone et al., 2014; Vadodaria and Gage, 2014). Every month, approximately 6% of the total population of granule cell neurons in the DG are newly generated neurons in rats (Hanson et al., 2011).

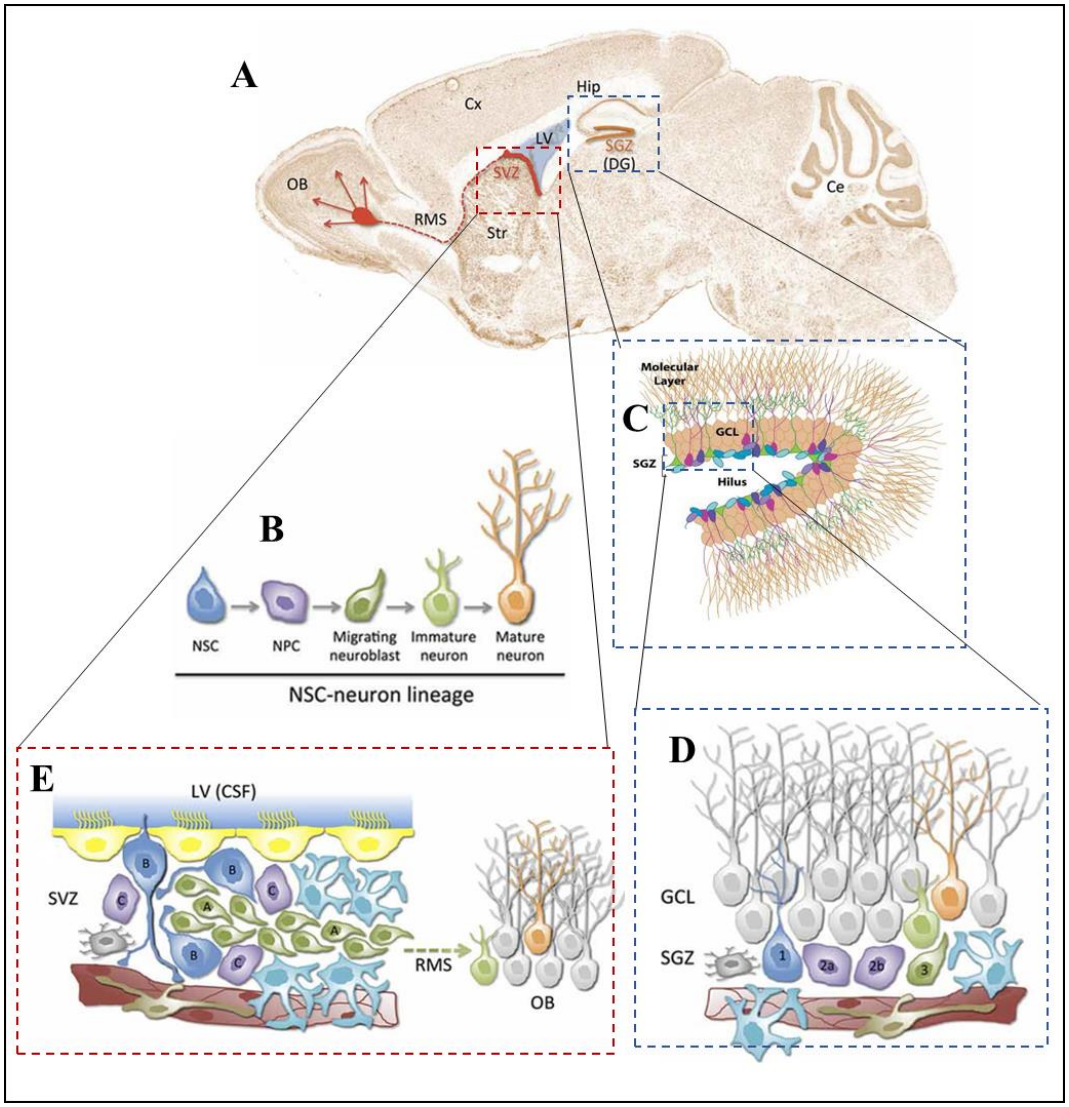


Figure 3. Neurogenesis in the adult mammalian brain. **A**: Sagittal section of the adult mouse brain depicting the neurogenic regions in the DG of the hippocampus (Hip) and the SVZ along the lateral ventricle (LV). New-born neurons originated in the SVZ migrate via the rostral migratory stream (RMS) towards the olfactory bulb (OB). **B:** Neuron lineage derived from neural stem cells (NSCs) in the neurogenic regions. **C:** SGZ situated on the border of the granule cell layer (GCL) of the DG in the Hip and hilus. **D:** Cell type (as depicted in **B**) and cytoarchitecture in the SGZ neurogenic niche. The radial type 1 cells (NSCs) give raise to type 2a/b neural progenitor cells (NPCs) that differentiate into neuroblasts (3). After neuroblasts migration and maturation into neurons, neurons integrate into the GCL **E:** Cell type

(as depicted in **B**) and cytoarchitecture in the SVZ neurogenic niche and in the OB. The active type B NSCs, in close contact with the cerebrospinal fluid (CSF), generate type C NPCs that proliferate and give rise to type A neuroblasts. Neuroblasts migrate long distances via the RMS towards the OB where they mature into interneurons. Ce, cerebelum; Cx, cortex; Str, striatum (Eisch et al., 2008; Bátiz et al., 2016). Figure modified from Eisch et al., 2008 and Bátiz et al., 2016.

Neurogenesis is a multistep process driven by cell proliferation that involves several developmental stages in the neurogenic regions (Nowakowski and Hayes, 2008). The neurogenesis developmental process includes cell proliferation, cell cycle exit and faith choice, cell migration, cell differentiation, cell maturation and integration into the existing circuitry (Figure 4) (Nowakowski and Hayes, 2008; Vadodaria and Jessberger, 2013).

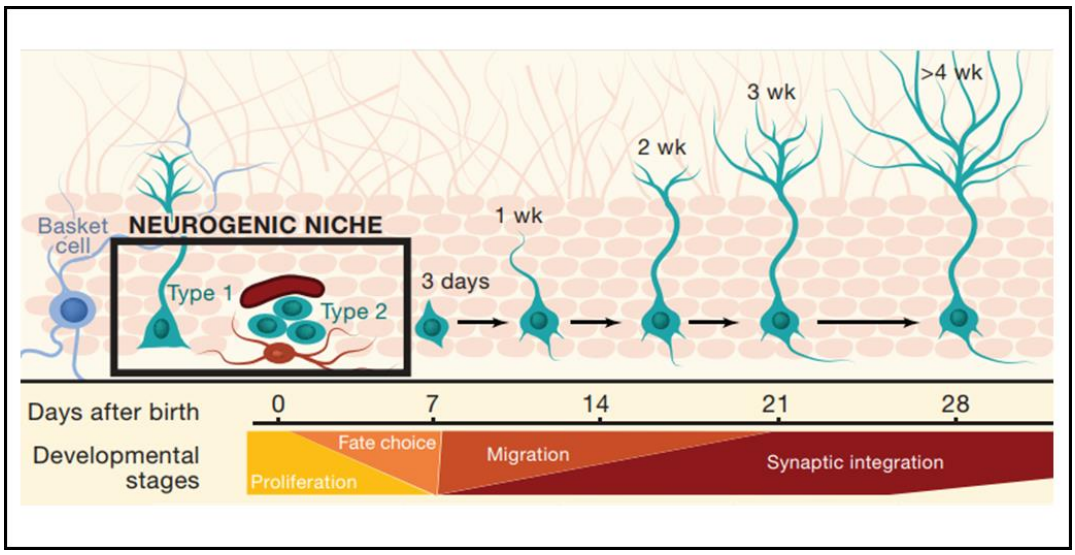


Figure 4. Developmental stages in the neurogenesis process. Figure modified from Vadodaria and Gage, 2014.

Neurogenesis in the adult brain is regulated by intrinsic (e.g. neurotrophic factors and epigenetic mechanisms) and extrinsic factors (e.g. exercise and a rich environment) while other factors such as stress and aging have been found to have a negative impact on the regulatory mechanisms (Figure 5) (Vadodaria & Jessberger, 2013). Neurotrophic/Growth Factors (NGFs) are members of a family of molecules essential in cell maintenance and survival, function and regulation of neuroplasticity mechanisms of neurons in the adult brain (Nunciato et al., 2013; Vilar and Mira, 2016). The most extensively studied NGFs in depression include the Brain Derived Neurotrophic Factor (BDNF), nerve growth factor, Neurotrophin 3 and Neurotrophin 4 (NT3 and NT4) (Duman and Voleti, 2012; Nunciato et al., 2013; Duman et al., 2016). Additional NGFs involved in depression include the Vascular Endothelial Growth Factor (VEGF), Insulin-Like Growth Factor 1 (IGF1) and Fibroblast Growth Factor 2 (FGF2) (Duman and Voleti, 2012). VEGF and ILGF1 are mainly expressed in peripheral tissues while the others, although also expressed in peripheral tissues, are largely expressed in the brain, suggesting that peripheral and central system cross-talk takes place (Duman and Voleti, 2012).

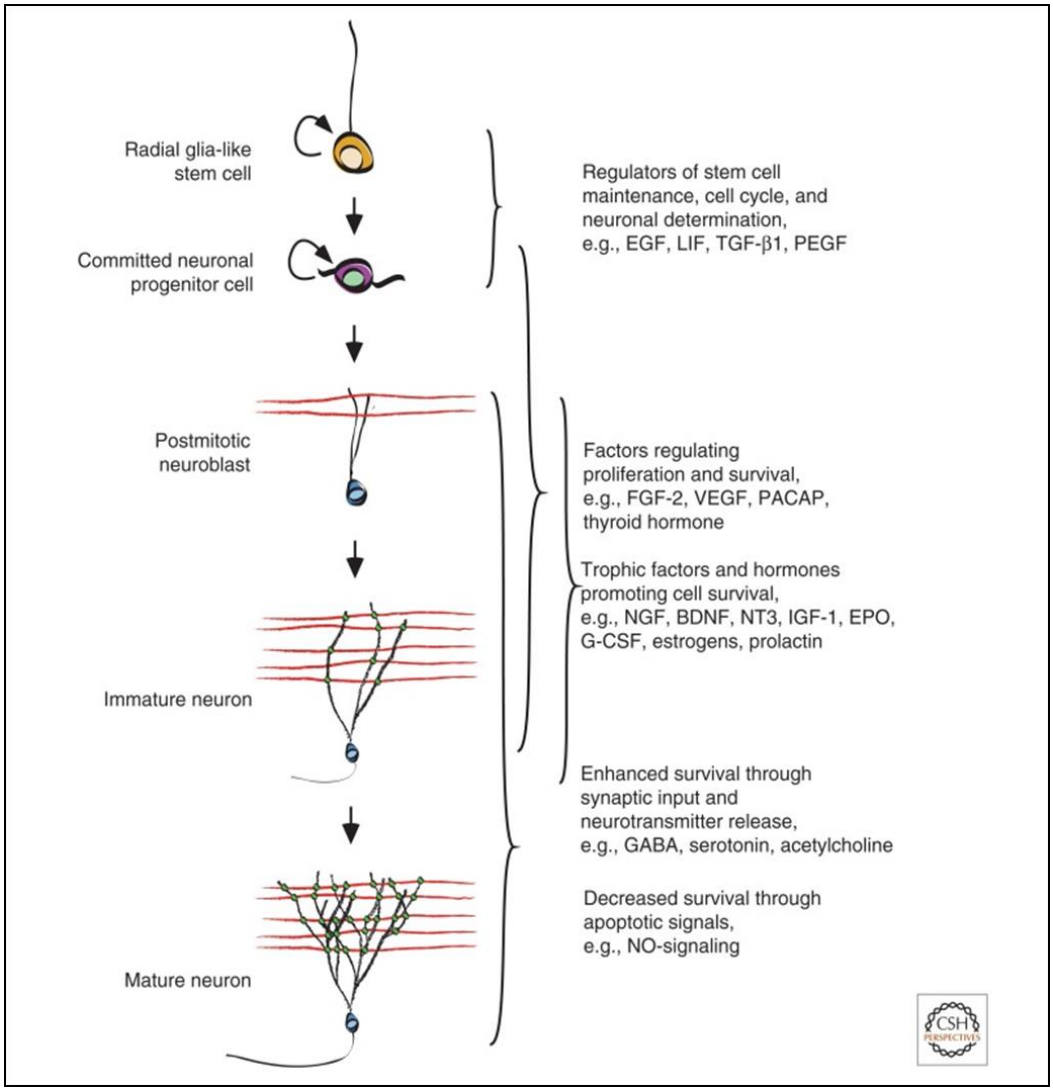


Figure 5. Factors involved in the regulation of adult neurogenesis. EGF, Epidermal growth factor; FGF-2, fibroblast growth factor 2; GABA, γ-aminobutyric acid; G-CSF, granulocyte colony-stimulating factor; LIF, leukemia inhibitory factor; TGF-β1, transforming growth factor β1; NO, nitric oxide; PACAP, pituitary adenylate cyclase-activating peptide; PEGF, platelet-derived endothelial cell growth factor (Kuhn, 2015). Modified from Kuhn, 2015.

BDNF is an essential molecule involved in the regulation of plasticity in the hippocampus being an active player in cell survival, differentiation and migration, axonal and dendritic growth and synaptogenesis (Berton and Nestler, 2006;

MacQueen and Frodl, 2011; Bekinschtein et al., 2014). A pre-BDNF isoform of 35 kDa is synthetized and later cleaved to either a 28kDa pro-BDNF form or a14kDa mature BDNF form (Hill et al., 2014). BDNF is also a crucial element for optimal functioning and integrity of the hippocampus (Sierksma et al., 2010). BDNF is required in the DG for the encoding and consolidation of pattern separated memories and this factor stimulates neurogenesis and survival of newborn neurons (Bekinschtein et al., 2014).

Under stress conditions, BDNF mRNA expression is decreased in the dentate gyrus and CA3 region of the hippocampus (Sierksma et al., 2010). In patients with depression, low mRNA levels of BDNF in the hippocampus have been found. This suggests that changes in hippocampal neurogenesis regulated by BDNF is a key component of the pathophysiological processes in depression (Sierksma et al., 2010). Early studies on stress showed decreased levels of BDNF in the plasma of patients with depression which were restored after antidepressant treatment (Duman and Voleti, 2012; Duman et al., 2016). The BDNF upregulation mediated by antidepressant action in the hippocampus and prefrontal cortex suggests potential neuroprotective effects (Duman and Voleti, 2012; Tizabi, 2015). However, BDNF depletion itself is not enough to induce depressive behavior (Duman and Voleti, 2012) but downregulation of BDNF increases the susceptibility to other factors such as stress that may ultimately lead to depressive phenotype (Duman and Li, 2012). Restoration to normal BDNF levels has been observed after electroconvulsive shock therapy or antidepressant treatment (Hanson et al., 2011). BDNF mediated antidepressant effects have been reported after administration by infusion in the hippocampus and peripheral administration (Pilar-Cuéllar et al., 2013). Rather than promoting cell proliferation,

BDNF plays a key role in neurogenesis by stimulating cell survival and activation of the BDNF-TrkB pathway (Hanson et al., 2011; Duman and Voleti, 2012).

The effect of BDNF starts with the interaction with the extracellular domain of its receptor, Tropomyosin-Related Kinase B (TrkB) triggering a series of downstream signaling pathways clearly illustrated and explained in Duman and Voleti, 2012 (Figure 6). BDNF induces dimerization of TrkB that triggers a downstream cascade of post-receptor pathways (Berton and Nestler, 2006; Duman and Voleti, 2012). The intracellular tyrosine kinase domain of TrkB causes autophosphorylation of the tyrosine residues which interact with adaptor proteins and activate signaling pathways such as the Ras-microtubule-associated protein kinase (MAPK), phosphoinositide 3 kinase (PI3K)/serine threonine kinase (Akt) and phospholipase C γ (PLCγ) (Duman and Voleti, 2012; Duman et al., 2016). Two intracellular tyrosine residues play an important role in the formation of protein signaling complexes and the activation of phosphorylation pathways (Duman and Voleti, 2012). Phosphorylation of the tyrosine 515 residue of the intracellular domain of TrkB causes the recruitment of proteins including the Src homology 2 domain containing (Shc) adaptor protein, growth factor receptor-bound protein 2 (Grb2), son of sevenless (SOS) which ultimately activates the Ras-MAPK pathway (Duman and Voleti, 2012). Activation of the Ras-MAPK pathway mediates cell survival, cell growth and neuroplasticity (Duman and Voleti, 2012). The formation of the Shc-Grb2 complex can also recruit Grb2-associated binder 1 (GAB1) resulting in the activation of the PI3K/Akt pathway which is involved in cell survival and neuroplasticity (Duman and Voleti, 2012). Phosphorylation of the tyrosine 816 residue recruits PLCγ that stimulates the formation of PI3, regulation of intracellular Ca^{2+}, and diacylglycerol (DAG), leading

to the activation of CAMK and protein kinase C (PKC) pathways involved in neuroplasticity (Duman and Voleti, 2012).

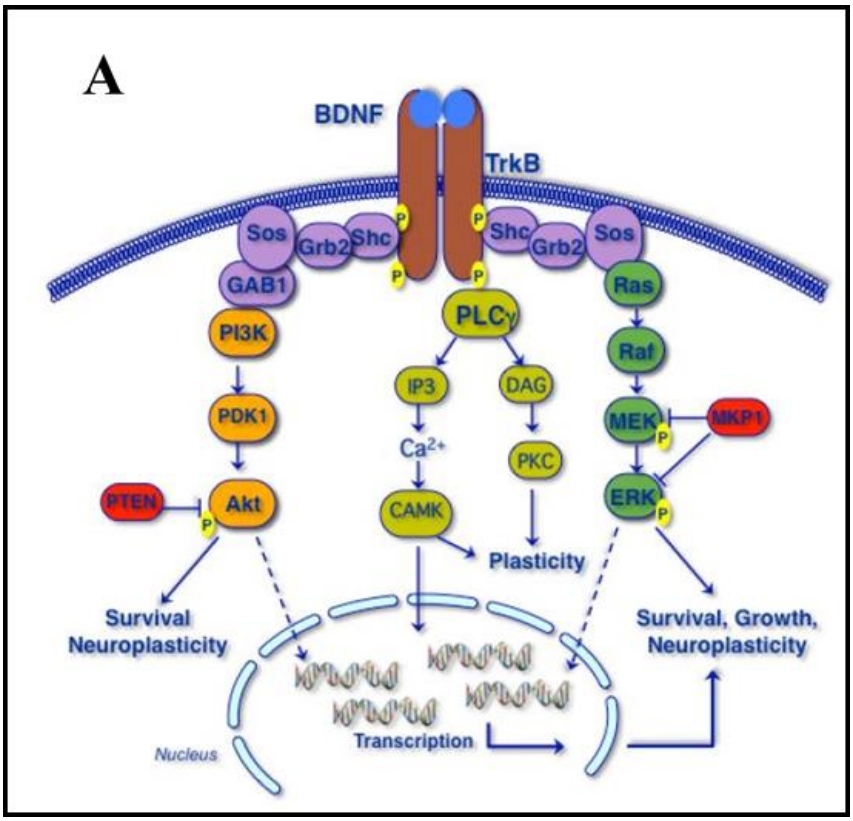


Figure 6. BDNF-TrkB mediated signaling pathways. Abbreviations: ERK, extracellular signal regulated kinase; MEK, MAP/ERK kinase; MKP1, MAP kinase phosphatase 1; PDK1, 3-phosphoinositide-dependent protein kinase 1. Figure modified from Duman and Volety, 2012.

Based on preclinical and clinical studies, a correlation has been found between stress and depression and decreased levels of BDNF and components of the Ras-MAPK and PI3K-Akt pathways which are found to be restored after antidepressant treatment (Duman and Voleti, 2012). Therefore, BDNF and its receptor TrkB have been considered potential targets for developing antidepressant agents (Lee et al., 2010).

1.5. The olfactory system-emotion-neurogenesis relationship and its implications in depression

Olfaction, the most ancient sense, is not only associated with primitive needs such as reward, threat, reproduction and homeostasis but also with mood, memory and cognition (Brennan, 2010; Soudry et al., 2011; Krusemark et al., 2013; Woolley et al., 2015). As a vitally important sense, olfaction can have significant attributes considering its capacity to regulate behaviors (Sarafoleanu et al., 2009).

In humans, the olfactory area contains about 50 million sensory cells with 8-20 cilia down in a mucous of the olfactory epithelium (Sarafoleanu et al., 2009). Volatile chemicals can interact with the receptors of the sensory cells and trigger a cascade of signal transport, transduction and response (Ache and Young, 2005; Sarafoleanu et al., 2009). The OB, comprising about 8000 glomeruli, is the outer structure of the olfactory system that receives the stimulus which is transported through the primary olfactory neuron axons (Figure 7) (Soudry et al., 2011). The extensive network of axonal terminals has projections onto the primary olfactory cortex where the chemosensory signals are processed and then projected to structures of the limbic system including the amygdala, thalamus, hypothalamus and hippocampus (Bartocci et al., 2000; Soudry et al., 2011; Krusemark et al., 2013). After the initial olfactory stimulus reaches the limbic system, the signal travels to the olfactory associative cortex in the orbitofrontal region (Bartocci et al., 2000; Mouly and Sullivan, 2010). The olfactory signal is processed in different parts of the brain where it is identified and triggers behavioral responses such as feeding, social interaction, reproduction, consolidation of memories and emotional responses (Arisi et al., 2012). The intimate

connection of the olfactory system with brain regions responsible of the regulation of emotions makes the emotion-olfactory system relationship relevant for the study of mood disorders such as anxiety and depression (Krusemark et al., 2013).

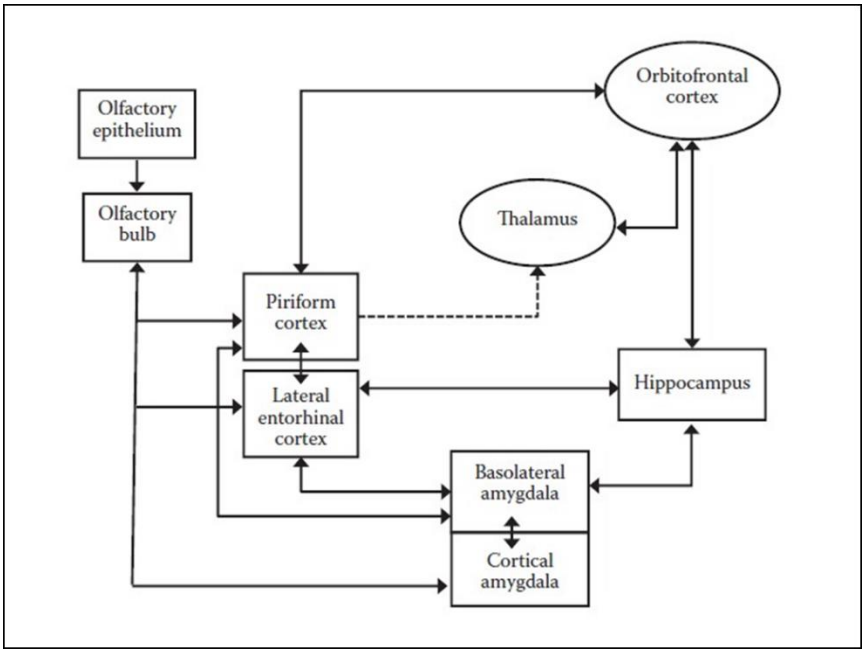


Figure 7. Representation of OB pathways connecting the OB and the limbic system structures. Figure modified from Mouly and Sullivan, 2010.

Positive (appetitive), negative (aversive or avoidance) or neutral responses can be triggered by pleasant or unpleasant odors (Bartocci et al., 2000; Horii et al., 2013). For example, predator odors can induce stress leading to behavioral and homeostatic changes by the activation of the HPA axis and the stimulation of the frontocortical system and the dopaminergic system in the amygdala (Horii et al., 2013). Conversely, pleasant odors activate regions of the orbitofrontal cortex (Rolls et al., 2003; Shen et al., 2005) and the positive effects of pleasant odors on emotion include stress relief, relaxation and improvement of mood (Han et al., 2017).

The link between olfaction and depressive symptoms have been previously proposed. A negative correlation between the olfactory sensitivity and depressive symptoms in both healthy subjects and patients with depression has been previously reported (Negoias et al., 2010; Kohli et al., 2016). Further evidence shows a significant negative correlation between the OB volume and depressive-symptoms scores (Negoias et al., 2010). In animal studies, changes in the OB volume occurred as a result of changes in olfactory sensory stimulation and removal of the OB affected the hippocampal function and showed to induce depressive symptoms (Taalman et al., 2017). Findings from animal studies suggest that the behavioral responses observed after olfactory bulbectomy are caused by dysfunction in the cortico-hippocampal-amygdala circuit (Taalman et al., 2017). Furthermore, it has been suggested that reduced neurogenesis in depression could also have an impact at the level of the OB (Negoias et al., 2010). Improvement of depressive symptoms have been associated to increased neurogenesis in the hippocampus as previously discussed. The theory of neurogenesis in the hippocampus and the positive effects on depression as an approach can also be explored in the OB (Taalman et al., 2017). As it occurs in the hippocampus, a similar reduction in volume and neurogenesis has been observed in the OB (Taalman et al., 2017). Several studies have suggested a relationship between reduced neurogenesis in the olfactory system and neurodegenerative and psychiatric disorders (Höglinger et al., 2004; Negoias et al., 2010; Bergmann et al., 2012).

The genetic and molecular regulatory mechanisms governing neurogenesis in the OB are largely conserved between the embryonic and adult stages (Brann and Firestein, 2014). New interneurons are continually added to the OB in the mammalian brain as a result of the neurogenesis process occurring in the SVZ, the germinative zone

situated along the lateral ventricle facing the stratum (Pignatelli and Belluzzi, 2010; Lim and Alvarez-Buylla, 2016). The stem cells in the SVZ give rise to neuroblasts that migrate through the RMS to the OB where they differentiate into interneurons (Altman, 1969; Pignatelli and Belluzzi, 2010). The SVZ-OB system is an interesting physiological mechanism useful in highlighting the role of neurogenesis and plasticity linked to psychiatric disorders such as depression (Apple et al., 2016; Lim and Alvarez-Buylla, 2016).

1.6. Oxytocin: Its role in affective behavior and neurogenesis

Oxytocin is a neuropeptide produced in the paraventricular and supraoptic nuclei of the hypothalamus involved in social behavior (Gimpl and Fahrenholz, 2001; Guastella et al., 2010; Lin et al., 2017). Oxytocin plays a role at peripheral (reproduction) and central (social and bonding behavior) processes (Gimpl and Fahrenholz, 2001; Guastella et al., 2010; Lin et al., 2017) regulating social behavior such as mother-child bonding, trust and olfactory-based social processing (Woolley et al., 2015). Oxytocin receptors are present in the OB where oxytocin acts in the creation of olfactory-social memories (Woolley et al., 2015) facilitating social recognition and social memories which are essential for the formation of social bonds (Burke et al., 2015).

The beneficial effects of oxytocin on behavior have been reported in several studies and are worth considering for the treatment of mood disorders. Improvement of social behavior has been observed in both animals and human subjects after nasal administration of oxytocin (Leuner et al., 2012; Wacker and Ludwig, 2012). Also, positive behavioral and neuroendocrine effects under stress conditions have been

demonstrated in the dorsal hippocampus after oxytocin treatment (Lin et al., 2017). Protective effect against the stress-induced damage to the hippocampus suggests that oxytocin may facilitate hippocampal plasticity by stimulating neurogenesis within the hippocampus (Leuner et al., 2012).

The role of oxytocin on positive social behavior via the olfactory system have been extensively studied. Furthermore, oxytocin has shown a positive effect on hippocampal neurogenesis. Therefore, oxytocin represents an important key player to be considered in the study of novel treatment options for depression.

1.7. The rationale of the dissertation

As previously discussed, neurogenesis is an important physiological process worthy of exploration in the development of novel pharmacological alternatives for the treatment of depression. Furthermore, the olfactory system-emotion-neurogenesis relationship represents an interesting process worthy of further in-depth investigation. Additionally, key role players such as the BDNF and oxytocin are important elements to be included in the evaluation of potential treatment options for depression due to the implications they hold in the mechanism of neurogenesis.

In order to continue with the "thinking outside the box" approach to discover novel potential treatment options for depression, a set of considerations were established. The first consideration was to take neurogenesis as the target physiological mechanism for the evaluation of candidates with antidepressant effect. The second consideration was the selection of promising candidates to be included in the present

study based on the following criteria: (1) promising beneficial effect on depression based on the literature, (2) the neurogenic effect had not been previously evaluated, (3) no previous study on the molecular pathways involved in its beneficial effects on depressive symptoms and (4) at least one of the candidate(s) should act via the olfactory system in order to study the olfactory system-emotion-neurogenesis relationship in detail. The criteria established served as a guide to narrow the options to be included in the present study. The criteria enabled the identification of interesting candidates whose neurogenic effect was not previously evaluated, thereby adding to the novelty and originality of the present study.

1.7. 1. Lavender Essential Oil and Bis-(7)-Cognitin: The selected candidates in the research project

The rationale of the present study has clearly stated that (1) neurogenesis can be used as a target physiological process for the screening of potential candidates for the treatment of depression, (2) exploration of the effect of the selected candidate(s) on the olfactory system-emotion-neurogenesis relationship could open a new opportunity not only to build knowledge on the pathogenesis of depression but also to discover novel pharmacological treatments and (3) the BDNF and oxytocin stand out as important players that are worth to be monitored when screening the effect of candidates that have not been evaluated under the scenarios presented in (1) and (2). The next step to complete the strategy of the present research study was the selection of the candidates to be included.

Lavender essential oil

Aromatherapy is a practice reported as early as 6000 BC which was used by ancient civilizations in China, India, Greece, Egypt and the Romans for the healing of the mind, body and soul (Ali et al., 2015; Koo, 2017; Manion and Widder, 2017). Contemporary aromatherapy emerged in the 20th century in France thanks to the work of the chemist Rene Maurice Gattefosse, who used the term aromatherapy for the first time in 1920 (Koo, 2017). In 1940, Jean Valnet, inspired in Gattefosse' work, carried out research on the antiseptic properties of essential oils during World War II and used aromatherapy with psychiatric patients (Shirsat et al., 2013). The effect of essential oils on the central nervous system, massage and cosmetics was investigated by the Austrian biochemist, Margaret Maury, in 1964 (Shirsat et al., 2013). The increased popularity of aromatherapy popularity and its potential for clinical application was observed in the United States in the 80s (Koo, 2017).

The health effects of aromatherapy have been evaluated on a wide variety of conditions such as pain management (Lakhan et al., 2016), insomnia (Lillehei et al., 2015) and mood disorders including stress (Liu et al., 2013), anxiety (Barati et al., 2016), and depression (Conrad and Adams, 2012). The volatile compounds found in the essential oils are chemosignals detected in olfactory epithelium that travel through the OB and reach structures of the limbic system triggering effects on mood (Andrade et al., 1999; Cavanagh and Wilkinson, 2002; Perry and Perry, 2006). Since essential oils have shown positive effects on mood, e.g. alleviation of depressive symptoms (Louis and Kowalski, 2002; Burnett et al., 2004), they represent a valuable potential

treatment option that needs further evaluation (Ernst et al., 1998; Andreescu et al., 2008; Setzer, 2009).

From the rich repertoire of essential oils used in aromatherapy, LEO is of particular interest for the purpose of the present study. Firstly, LEO is frequently used in aromatherapy (Andrade et al., 1999; Cavanagh and Wilkinson, 2002; Greenberg and Slyer, 2017). Secondly, previous studies have evaluated LEO showing promising effects on mood in both animal studies and clinical trials (Perry and Perry, 2006; Wu and James, 2011; Perry et al., 2012). Thirdly, the nature of LEO and its action on the olfactory system is an interesting feature, making LEO relevant to the present study. Despite the beneficial effect on mood including the relief of depressive symptoms, no further investigation has been done to elucidate the mechanism of action of LEO. Finally, the effect of LEO on neurogenesis has not been explored. Going by the afore stated reasons, it was clear that LEO fulfilled, to a great extent, the criteria established in the present study for the selection of candidates to be evaluated.

Bis-(7)-Cognitin (B7C)

Bis(heptyl)-cognitin (B7C) is a novel acetylcholinesterase inhibitor synthesized at the Department of Applied Biology and Chemical Technology of The Hong Kong Polytechnic University by Professor Yi-Fan Han and his research team (Wang et al., 1999b). B7C is a dimer formed by two tacrine molecules linked by a spacer containing 2 methylene groups (Hu et al., 2015a, 2015c). This particular molecule has caught the attention of researchers, especially as a treatment option for Alzheimer's disease (AD).

Because of the high comorbidity of AD and depression, and the positive effects of B7C on AD animal models, B7C was included in the present study.

Epidemiological and clinical evidence has shown a strong relationship between depression and dementia, specifically with AD. Co-morbidity of depression and AD has been suggested since depression is found to be a risk factor for cognitive dysfunction that leads to the development of dementia and ultimately AD (Caraci et al., 2010; Baquero and Martín, 2015). Indeed, it has been demonstrated that higher levels of depressive symptoms result in rapid decline in cognition (Sierksma et al., 2010; Baquero and Martín, 2015). Therefore, the close relationship between these two conditions presents a potential area of research to develop novel therapeutic options with a dual effect as mood stabilizers and neuroprotective agents that may have cross-benefit for depression and AD (Tizabi, 2015).

Neurodegenerative events have been established in different regions of the brain, including the hippocampus, in depressed patients. In AD, hippocampus is one of the first brain areas to show alterations in its structure (Caraci et al., 2010). Reduction of the hippocampal volume in patients with major depression significantly correlates with cognitive and memory impairment which are the major symptoms commonly observed in AD (Sierksma et al., 2010). Depression and AD symptoms have been correlated with decreased hippocampal neurogenesis, defined as the process to produce new neurons from neural stem cells.

B7C has neuroprotective effects by inhibition of the enzymes acetylcholinesterase, neuronal nitric oxygen synthase and the N-methyl-D-aspartate receptor (Hu et al.,

2015a). An advantageous feature of the B7C molecule is that it easily crosses the brain blood barrier due to its highly lipophilic profile making it a promising drug candidate for central nervous system disturbances (Hu et al., 2015a). Furthermore, evidence shows that B7C reverses cognitive impairments in a scopolamine-induced spatial and recognition memory deficit animal model (Han et al., 2012). Depression has been linked with AD because it is found to be a risk factor for cognitive dysfunction (Caraci et al., 2010; Baquero and Martín, 2015). The promising results revealed by B7C in neuroprotection presents an opportunity to explore its effects on depression.

The literature review on B7C pointed out that (1) the physicochemical characteristics of B7C makes it a good candidate for the treatment of CNS disorders, (2) the beneficial effects of B7C on AD animal models and the high comorbidity of AD and depression highlight a research gap that invites an evaluation of the effect of B7C on depression, (3) no previous studies have evaluated the potential antidepressant effect of B7C, and (4) the neurogenic effect of B7C has not been explored.

As described above, the strategy followed to design the present study drives from a thorough analysis of a global health concern, a critical evaluation of the treatment options available that supported the urgent need to explore new therapeutic alternatives, an analysis of the pathophysiology of depression, the selection of an important physiological process namely neurogenesis to screen candidates with potential antidepressant effect, and a critical selection of the candidates included in the present study, LEO and B7C.

CHAPTER 2

AIMS, RESEARCH APPROACH AND THESIS OUTLINE

Painting by Wei Ho Chan, 2018 (permission to use the painting in this thesis was granted by the artist)

2.1. Aims

General aim

To evaluate the behavioral and neurogenic effects of LEO and B7C in rats and to explore the molecular mechanism of action *in vivo* and *in vitro*.

Specific aims

Lavender Essential Oil (LEO)

- To explore the effectiveness of the aromatherapy intervention in reducing depressive symptoms in clinical studies.
- To evaluate the evidence on the effect of LEO in reducing depressive symptoms in preclinical and clinical studies.
- To evaluate the effect of LEO on behavior and neurogenesis in a high corticosterone animal model.
- To identify the molecular key players and mechanisms involved in the effect of LEO *in vitro*.

Bis-(7)-Cognitin (B7C)

- To investigate the molecular targets and mechanism of action of B7C on neurological disorders.
- To evaluate the dose dependent effect of B7C on behavior and neurogenesis.
- To evaluate the effect of B7C on behavior and neurogenesis in a high corticosterone animal model.
- To identify the molecular key players and mechanisms involved in the effect of B7C *in vitro*.

2.2. Research approach

The present study is divided into 3 main approaches: (1) literature review, (2) *in vivo* effect studies and (3) *in vitro* effect studies. The scope of the present study, research questions and the research approach are stated in the table below.

Table 1. Scope, research questions and research approach of the PhD project

Lavender Essential Oil (LEO)		
Aims	**Research question**	**Research approach**
To explore the effectiveness of the aromatherapy intervention to reduce depressive symptoms in clinical studies.	Is aromatherapy effective to reduce depressive symptoms?	Literature review
To evaluate the evidence on the effect of LEO to reduce depressive symptoms in preclinical and clinical studies.	Is LEO efficacious in reducing depressive symptoms in preclinical and clinical studies? What is the mechanism of action of LEO involved in the reduction of depressive symptoms?	
To evaluate the effect of LEO on behavior and neurogenesis in a high corticosterone animal model.	Does LEO decrease depression-like behavior in an animal model for depression? Is neurogenesis involved in the antidepressant effect of LEO?	Evaluation by *in vivo* study
To identify the molecular key players and mechanisms involved in the effect of LEO *in vivo* and *in vitro*.	What is the molecular mechanism involved in the cell proliferation effect of LEO?	Evaluation by *in vivo* and *in vitro* study
Bis-(7)-Cognitin (B7C)		
Aims	**Research question**	**Research approach**
To investigate the molecular targets and mechanism of action of B7C on neurological disorders.	What are the molecular targets and mechanism of action of B7C on neurological disorders?	Literature review
To evaluate the dose dependent effect of B7C on behavior and neurogenesis. To evaluate the effect of B7C on behavior and neurogenesis in a high corticosterone animal model.	Does B7C improve depression-like and anxiety like behavior? Does B7C affect neurogenesis? What is the optimal concentration of B7C that increases neurogenesis? Does B7C decrease depression-like behavior and affect neurogenesis in an animal model for depression?	Evaluation *in vivo*
To identify the molecular key players and mechanisms involved in the effect of B7C *in vivo* and *in vitro*.	What is the molecular mechanism involved in the cell proliferation effect of B7C?	Evaluation by *in vivo* and *in vitro* study

2.3. Thesis outline

The present study aimed to investigate the effect of the acetylcholinesterase inhibitor B7C and LEO, a commonly used essential oil in aromatherapy, on depression and to explore whether neurogenesis is the physiological process behind the therapeutic effect of both candidates. In addition to an extensive literature review on B7C and LEO, a series of animal and *in vitro* experiments were performed. The research objectives and a brief outline of the studies included in the thesis are organized in different chapters as presented below:

CHAPTER 3 PART A: A systematic review of the effectiveness of aromatherapy for depressive symptoms was carried out in this chapter. The aim was to gather all the clinical and up to date evidence available to analyze the effect of aromatherapy and to identify the essential oils most commonly used in clinical trials evaluating depressive symptoms. The review in this chapter allowed an in-depth analysis of the clinical evidence and supported further investigation of LEO.

CHAPTER 3 PART B: This section reviews the preclinical and clinical evidence on the effectiveness of LEO on depression. Suggested by evidence presented in CHAPTER 3 part A, LEO was the most commonly used essential oil in clinical trials evaluating its effect on depressive symptoms. Therefore, this chapter aimed to gain more in-depth knowledge on the effect of LEO by analyzing the findings from preclinical and clinical studies. The results of a systematic review to evaluate the preclinical and clinical studies of LEO on depression are presented and discussed.

CHAPTER 4: CHAPTER 3 part B showed that LEO has been evaluated in animal studies showing promising results of being able to relieve depression-like behavior. However, no studies have explored the effect of LEO in animal models for depression. In addition, there were no animal studies that had reported on the mechanism of action involved in the effect of LEO. Therefore, the aim of this chapter was to evaluate the effect of LEO on behavior and neurogenesis in a high dose corticosterone model in rats. The animal model employed is frequently used to evaluate antidepressant activity and neurogenesis as administration of high corticosterone induces depression-like behavior and reduces neurogenesis.

CHAPTER 5: As no previous study had evaluated the effect of B7C on depression, a literature review on B7C was carried out. The aim of this chapter was to explore the effect of B7C on the CNS to identify its molecular targets and evaluate the relevance of B7C as potential therapeutic agent for neurological disorders. Also, the literature search in this chapter was used to have an updated overview of the research done on B7C and support the study on B7C on depression.

CHAPTER 6: The aim of this chapter was to evaluate the behavioral and neurogenic effect of B7C on rats. The study was divided into two phases. The first phase included a dose-dependent experiment to (1) evaluate whether B7C affected neurogenesis and (2) to identify the dose of B7C that showed a positive effect on behavior and stimulated neurogenesis. The second phase of this study was to evaluate the effect of B7C on rats with corticosterone-induced depression-like behavior and reduced neurogenesis. The experimental design used in the second phase included a

comparison with the antidepressant fluoxetine to evaluate whether the effect of B7C was equal or better than the effect of a widely used antidepressant.

CHAPTER 7: A positive effect of LEO and B7C on behavior and neurogenesis was demonstrated in CHAPTER 4 and 6. Therefore, this chapter aimed to study the molecular mechanism of LEO and B7C *in vitro*. The *in vitro* model was designed to study two well-known molecular pathways involved in cell proliferation, that is the Phosphoinositide 3-kinase signaling pathway (PI3K/Akt/mTOR) and the Mitogen-Activated Protein Kinases/Extracellular Signal-Regulated Kinases signaling pathway (MAPK/ERK).

CHAPTER 8: In the animal studies carried out in CHAPTERS 4 and 6, the levels of oxytocin were measured in serum to investigate whether oxytocin played a role in the mechanism of action of LEO and B7C. Since oxytocin was found to be upregulated, an animal experiment was designed to evaluate the effect of oxytocin alone on neurogenesis.

CHAPTER 3 PART A

The content of his chapter was published in Evidence-Based Complementary and Alternative Medicine

THE EFFECTIVENESS OF AROMATHERAPY FOR DEPRESSIVE SYMPTOMS: A SYSTEMATIC REVIEW

Sánchez-Vidaña DI, Ngai SP-C, He W, Chow JK-WJ, Lau BW-M, Tsang HW-H (2017). *Evidence-Based Complement Alternative Medicine*. 2017:21.

Model: Fiona Chung

Abstract

Background: Depression is one of the main global health concerns of our time. Although many antidepressants are currently available, the long start of therapeutic effect, intolerance to the side effects and low efficacy in a significant number of patients has resulted in low compliance to treatment among patients. People with depressions frequently search for new therapeutic options such as complementary and alternative therapies including aromatherapy. There is, therefore, a growing and urgent need for effective alternatives that do not compromise compliance in the treatment of depression. Aromatherapy represents a promising therapeutic option that needs further investigation.

Aim: The systematic review aimed to analyze aromatherapy in terms of efficacy for the alleviation of depressive symptoms in clinical trials.

Methods: Relevant articles were retrieved using predefined search terms in 5 databases (AMED, CINHAL, CCRCT, MEDLINE, and PsycINFO). Articles were selected based on pre-established inclusion and exclusion criteria. The outcome measure was the level of depressive symptoms.

Results: Twelve clinical studies were included. Two main administration methods were identified including inhalation aromatherapy (5 studies) and massage aromatherapy (7 studies). Alleviation of the depressive symptoms according to the scores measured using validated tools was reported in 7 studies.

Conclusions: The analysis of the clinical evidence showed positive effect of aromatherapy in decreasing depressive symptoms in a wide variety of subjects. Further investigation is needed as half of the studies obtained low scores in the quality assessment.

3.1. Introduction

Depression is a life-threatening mood disorder with a significant negative impact on people's quality of life and professional performance due to disability, suffering and high risk of perpetrating self-harm (Ernst et al., 1998; Yim et al., 2009). According to the definition stated in the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5), depression is manifested as a combination of symptoms accompanied by cognitive and physic phenomenology that leads to decreased interest in daily life activities (Baquero and Martín, 2015). Depressive symptoms include feelings of guilt, sadness, worthlessness and desperation, inability to experience pleasure, changes in appetite and sleep patterns, lack of energy, poor concentration and memory, motor retardation, fatigue and recurrent suicidal and death ideation which are experienced for more than 2 weeks (Baquero and Martín, 2015; Chan et al., 2015; Tizabi, 2015; Qaseem et al., 2016). The WHO reports depression as the largest health concern of the 21st century and the major cause of years of life lived with disability worldwide (Ren et al., 2015; Bhattacharya et al., 2016). Major depressive disorder, for example, is associated with significant impairment to carry out daily household tasks and reduced engagement in personal and social activities due to decreased mental and physical performance which translates into lower quality of life (Smith et al., 2010).

Despite the wide variety of antidepressants available on the market, concerns regarding side effects and compliance have a significant impact on the efficacy of pharmacotherapy for depression. For instance, it has been reported that about 60% of patients taking SSRI respond favorably to the treatment while nearly 30% of patients

do not respond (Caraci et al., 2010; Chan et al., 2015). Some of the side effects associated with the use of antidepressants include nauseas, insomnia, agitation, weight gain, somnolence and sexual dysfunction (Tizabi, 2015; Yeung et al., 2015). Treatment with benzodiazepines increases the risk of excessive sedation, dependence, cognitive impairment and decreased physical activity. All this negatively affects the daily performance of the patients exacerbating the already unpleasant depressive symptoms (Fißler and Quante, 2014). In addition to the side effects, it usually takes a long time for one to start experiencing the beneficial effect of antidepressants –for example, the taking of SSRI which prolongs the depressive symptoms in the patients. Due to the ineffectiveness of the treatment in some patients or their inability to endure the side effects, a large number of patients do not comply with the treatment and search for other therapeutic options (Qureshi and Al-Bedah, 2013; Chan et al., 2015; Yeung et al., 2015).

Due to the dissatisfaction experienced and the limitations of conventional therapy, patients have turned to CAM as an option for the treatment of depression (Ernst et al., 1998; Yeung et al., 2015). Positive findings in clinical studies have led to increased use of CAM in patients with depression (Qureshi and Al-Bedah, 2013). About 56% of patients were reported to have used CAM as an adjuvant and a supportive practice to their regular depression treatment (van der Watt et al., 2008; Yeung et al., 2015). Surveys carried out in Australia have shown that self-help and use of CAM are the preferred options for depression (Smith et al., 2010). Also, a large number of people with depressed symptoms make use of CAM according to reports in the US population (Smith et al., 2010). Therefore, special attention has been paid to the effectiveness of these therapies on depression.

The Cochrane Collaboration defines CAM as a health system that comprises a wide variety of healing resources for the prevention, diagnosis and treatment of diseases. It functions as a complement to the mainstream medicine system (Ernst et al., 1998). The complementary input and the conventional therapy combine to form a common whole in order to satisfy the unmet demands by the first line treatment (i.e. pharmacotherapy) or by enriching the conceptual contexts of medicine (Ernst et al., 1998; Wieland et al., 2011).

A wide variety of healing resources including medical products and practices forms part of the CAM (Ernst et al., 1995). According to the National Center for Complementary and Alternative Medicine (NCCAM), CAM therapies can be classified into five. Examples of CAM therapies include herbal medicine, aromatherapy, massage, traditional Chinese Medicine, meditation, yoga and acupuncture (Figure 8) (Ventola, 2010; Wieland et al., 2011; Singh and Chaturvedi, 2015). The CAM therapies shown in Figure 8 can also be categorized as invasive and non-invasive therapies from which acupuncture is the only invasive procedure and the rest of the therapies such as meditation, aromatherapy, herbal medicine and so on are categorized as non-invasive (Singh and Chaturvedi, 2015).

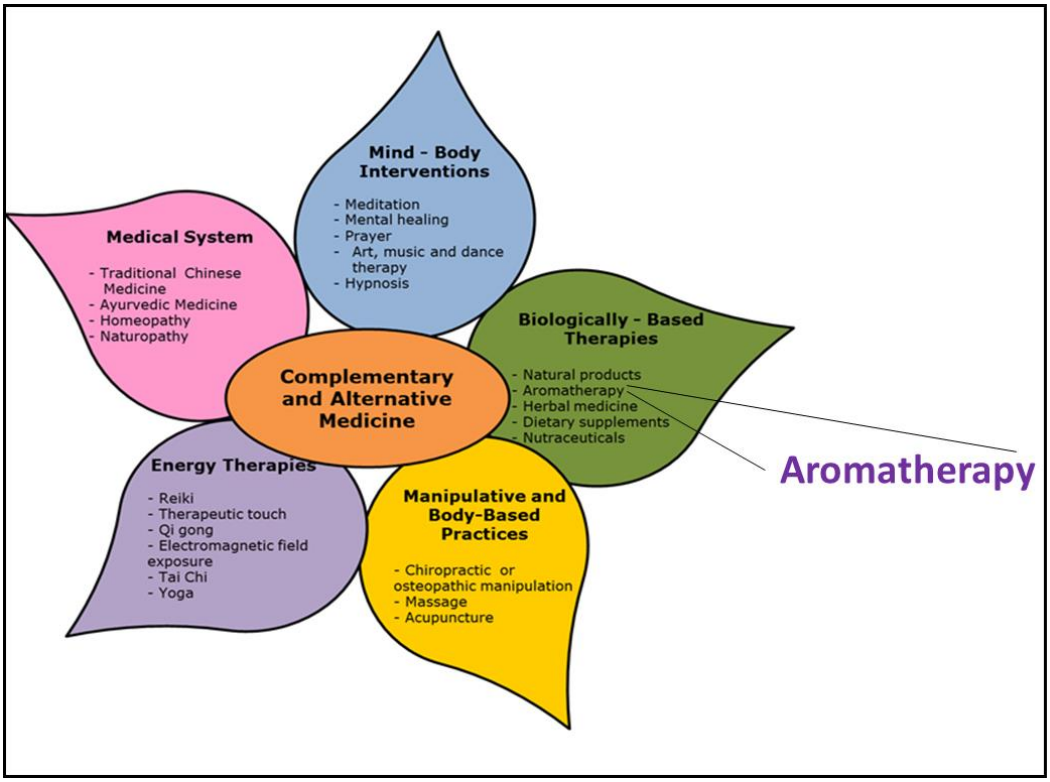


Figure 8. CAM categories and practices

In the UK, an increasing popularity of aromatherapy, one of the most preferred CAM therapy used, has been observed (Hur et al., 2014; Joswiak et al., 2016). Aromatherapy is an inexpensive and non-invasive modality of CAM that makes use of plant-derived essential oils for the treatment of a wide variety of diseases in order to improve psychological health and wellbeing (Cooke and Ernst, 2000; Setzer, 2009; Ndao et al., 2012). There is a body of evidence supporting the beneficial effect of aromas on mood since pleasant fragrances trigger a state of relaxation, promote comfort, pleasant memories, invigoration and management of stress and are associated with positive mood (Louis and Kowalski, 2002; Burnett et al., 2004; Ali et al., 2015). Also, several essential oils have been used to improve depressive symptoms representing a valuable therapeutic option that is worthy of future exploration clinical studies (Ernst et al., 1998; Andreescu et al., 2008; Setzer, 2009). Essential oils employed in aromatherapy

are absorbed into the body via the olfactory and respiratory system through the nasal mucosa by inhalation, transdermally through the external skin or orally by mouth (Perry and Perry, 2006; Fung et al., 2012; Ndao et al., 2012).

The classification of essential oils is based on the botanical classification of the plant from which the essential oils are extracted (Carke, 2008). The use of chemotypes is another classification of essential oils based on the subspecies of a plant with the same morphological characteristics that produce essential oils with different chemical profile e.g. type and quantity of chemical components (Buckle, 2003). The chemotype describes the main compound within certain essential oil (Buckle, 2003). Frequently, essential oils are used at different concentrations depending on the route of administration: (1) for aromatherapy massage, 1-5% essential oil, (2) for oral administration, 8-50% essential oil, and (3) concentrated essential oil is used in inhalation aromatherapy (Tisserand and Balacs, 1995). However, the dosage and dilution of essential oil chosen are not standardized in practice (Tisserand and Balacs, 1995). The most potent and effective administration method is oral administration in which the components of the essential oil reach the bloodstream (Carke, 2008). Since essential oils are lipophilic, they can easily be carried to all organs in the body (Carke, 2008). In inhalation aromatherapy, the inhaled air containing essential oils do not only reach the circulation system via the blood capillary network in the nose and the bronchi in the lungs but also stimulate brain areas directly via the olfactory epithelium (Tisserand and Balacs, 1995; Carke, 2008).

Essential oils trigger mechanisms in the brain via the olfactory system. The mechanism of action of essential oils administered by inhalation involves stimulation

of the olfactory receptors cells in the nasal epithelium, about 25 million cells, connected to the OB (Burnett et al., 2004; Ali et al., 2015). After stimulation, the signal is transmitted to the limbic system and hypothalamus in the brain through the OB and olfactory tract (Burnett et al., 2004). Once the signals reach the olfactory cortex, release of neurotransmitters e.g. serotonin takes place which results in the expected effect on emotions related to essential oil use (Burnett et al., 2004; Price and Price, 2011; Ali et al., 2015).

A previous systematic review evaluated the effects of aromatherapy on depressive symptoms. The scope of the systematic review included studies from 2000 to 2008 (Yim et al., 2009). New clinical studies focusing on the effectiveness of aromatherapy on depressive symptoms have been carried out since the publication of Yim et al's work. Consequently, the need to update the analysis of the effectiveness of aromatherapy for the alleviation of depressive symptoms arises. Therefore, the aim of the present systematic review was to provide an updated critical assessment of the evidence from randomized controlled trials on the effectiveness of aromatherapy in the treatment of depression.

3.2. Methods

Search strategy

A set of predefined key terms were used in the following databases according to the search strategy (Table 1): Allied and Complementary Medicine Database (AMED), Cochrane Central Register of Controlled Trials (CCRCT), Cumulative Index to Nursing and Allied health (CINAHL), MEDLINE and PsycINFO. The search was

restricted to studies in English. The search procedure was carried out by 2 independent authors and discrepancies were resolved by consulting a third author.

Table 2. Search terms and database search strategy

Table modified from Sánchez-Vidaña et al., 2017.

ID	Disease search terms
1.	Depress*
2.	Major depress*
3.	Mood disorder
4.	Depressive disorder
5.	1 OR 2 OR 3 OR 4
ID	**RCT search terms**
6.	Controlled clinical trial*
7.	Random* controlled trial*
8.	6 OR 7
ID	**Aromatherapy search terms**
9.	Aroma
10.	Aromatherapy
11.	Aromatic therapy
12.	Essential oil*
13.	Fragrance
14.	Fragrant oil*
15.	Scent
16.	Massage therapy
17.	Medical massage
18.	Massage
19.	9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
20.	5 AND 8 AND 19

*truncation symbol used in database search

Inclusion and exclusion criteria and outcome measure for the selection of studies

Randomized controlled trials (RCT) using any type of study design were included. No restriction in the year of publication was considered. Studies using validated

standardized tools to measure depressive symptoms were included disregarding the health condition evaluated in the study. Examples of assessment tools for depressive symptoms are Profile of Mood States rating scale (POMS) or Hospital Anxiety and Depression scale (HADS). Eligible studies evaluated the effect of essential oils using any administration method. Studies combining aromatherapy with any type of massage were included. Reviews and meta-analyses on aromatherapy on mood disorders were not included.

Strategy for the selection of studies

After pooling the articles retrieved from the databases, the duplicates were eliminated. The titles were screened and articles that did not meet the inclusion and exclusion criteria were eliminated. Afterwards, the full text of the pre-selected articles was checked to refine the selection. The database search and selection of the studies was carried out by 2 independent authors. Any disagreements were discussed and resolved and where necessary, a third author was consulted.

Extraction of the data

The data extracted included the study design, number of subjects, description of the subjects, and inclusion criteria of the studies. Further data extracted included the description of the comparison group, the method of administration, duration of the study, frequency of the treatment, assessment tools, outcome measures and conclusion.

Quality assessment

The Jadad scale was used to evaluate the quality of the studies included. The criteria of the Jadad scale take the following into consideration: the randomization, double

blinding, withdrawals and dropouts (Jadad et al., 1996). In a scale from 0 to 5, higher scores correspond to higher quality of the study.

3.3. Results

Database search and study selection

The database search was done from in May 2016. A total of 875 studies were retrieved from the 5 databases (Figure 9). After removing the duplicates, 668 remained and when they were further screened, 522 studies were excluded because they did not evaluate depressive symptoms. Additionally, 84 studies were not RCTs, not in English and/or no depressive symptoms were measured; therefore, those studies were excluded. The remaining studies (n = 32) were checked in detail. Four studies could not be accessed as the full texts were not available. The authors of the studies that could not be accessed were contacted. However, the articles could not be retrieved because the authors could not be reached or the study was still unpublished. Therefore, the 4 studies were excluded. Twelve RCT studies met the pre-established criteria and, therefore, were included in the present systematic review (Graham et al., 2003; Lemon, 2004; Soden et al., 2004; Wilkinson et al., 2007; Araki et al., 2012; Conrad and Adams, 2012; Serfaty et al., 2012; Igarashi, 2013; Taavoni et al., 2013; Wu et al., 2014; Matsumoto et al., 2014; Sehhatie et al., 2015).

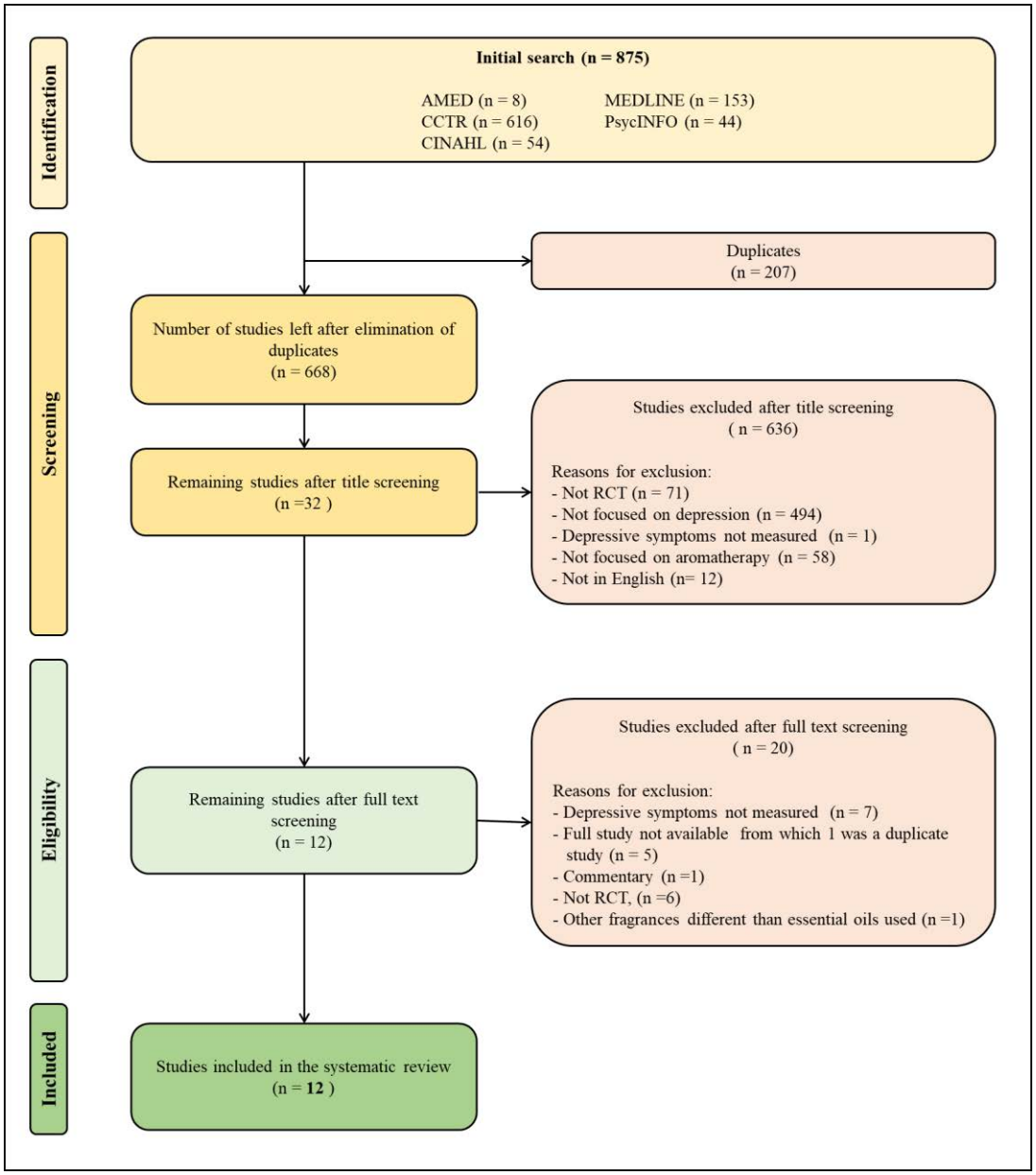


Figure 9. Study selection flowchart

Figure modified from Sánchez-Vidaña et al., 2017.

Description of the subjects

The data extracted from the subject description is shown in Table 3. A total of 1226 subjects were included out of which 80% were female and 18% were male participants. In one study (Lemon, 2004), the number of female and male participants were not stated representing 1.4% of the total population. The mean age of the subjects was 47

and the age range was 21 to 73 years. The majority of the subjects included were pregnant women (n = 333), followed by women in the menopausal phase (n = 90), patients with cancer (n = 82), patients with depression and or/anxiety disorder (n = 32), post-partum women (n =28), women with children diagnosed with attention deficit/hyperactivity disorder (n = 25), healthy female volunteers (n = 20) and subjects diagnosed with idiopathic environmental intolerance (n = 16).

Table 3. Characteristics of the participants included in the selected studies

Table modified from Sánchez-Vidaña et al., 2017.

Reference	Study design	Subjects					
		Number of subjects	Mean subject age (range)	Gender (n)	Subject description	Diagnostic systems/Inclusion criteria	Baseline score for depressive symptoms
Inhalation aromatherapy							
(Graham et al., 2003)	Placebo-controlled randomized double-blind RCT	313	65 (33-90)	Female (150) Male (163)	Individuals with cancer receiving radiotherapy treatment	Patients prescribed with 8 or more fractions of radiotherapy	Baseline depression status: Odds ratio of 29 using HADS.
(Conrad and Adams, 2012)*	Randomized observational pilot study with repeated measures	28	32 (25-43)	Female (28)	Postpartum women	0-18 months postpartum women with scores of 10 or higher on either the Edinburgh Postnatal Depression Scale or the Generalized Anxiety Disorder Scale	The baseline score using the Edinburgh postnatal Depression Scale for the control group was 15.9 and 16.1 for the intervention group.
(Igarashi, 2013)	Prospective RCT	13	27.3 for the control group (NA) 29.3 for the treatment group (NA)	Female (13)	Pregnant women	28 week-pregnant women, singleton pregnancy	Depression-dejection scale baseline score using POMS was 2.7 in the control group and 1.6 in the treatment group.
(Matsumoto et al., 2014)	Randomized controlled crossover study	20	20.5	Female (20)	College students	Healthy volunteers	The depression-dejection scale baseline score using POMS was not provided, but the change difference between pre and post treatment was reported. The change in depression-dejection score was lower than -1 in the treatment group and statistically significant when compared to the change in the control group.
(Sehhatie et al., 2015)	Controlled double-blinded RCT	320	20-30, average age NA	Female (320)	Pregnant women	Women between 18-35 years, with a pregnancy age between 38 to 42 weeks, a score of 12 or less in the Edinburgh test	Depression grade baseline in the Edinburgh test was 6.3 in the control group and 6.1 in the intervention group.

Reference	Study design	Subjects					
		Number of subjects	Mean subject age (range)	Gender (n)	Subject description	Diagnostic systems/Inclusion criteria	Baseline score for depressive symptoms
Aromatherapy massage							
(Lemon, 2004)	RCT	32	32.9 (23-53) in the treatment group	Female (10 in the treatment group) Male (4 in the treatment group) No information provided on the number of female and male subjects in the control group	Patients with depression and/or anxiety	Patients scoring more than 7 in the Montgomery-Asberg Depression Rating scale (and/or the Tyrer brief Anxiety scale	The baseline using the Montgomery-Asberg Depression rating scale was 19.8 in the control group and 30 in the treatment group. The baseline using the HADS was 14.6 and 15.3 in the control and treatment group, respectively.
(Soden et al., 2004)	Double-blind RCT	42	73, (44-85)	Female (32), male (10)	Individuals with cancer	Individuals with cancer with a wide variety of levels of physical and psychological symptoms	Baseline score using HADS was not stated. Only the median change in HADS was provided being 0 for the aromatherapy group, -1.5 for the massage group, -0.5 for the aromatherapy massage group and 0.5 for the control.
(Wilkinson et al., 2007)	RCT	288	52.1; 52.8 for the usual care group and 51.5 for the usual care plus aromatherapy group	Female (250), male (38)	Individuals with cancer	Patients diagnosed with cancer, a prognosis of more than 3 months, with clinical anxiety or depression	The baseline score using the Center for Epidemiological Studies Depression Scale was 26.1 for the aromatherapy group and 26 for the group receiving usual care (control).
(Araki et al., 2012)	Non-blinded randomized crossover trial	16	46.1 (37.9-54.3)	Female (15), Male (1)	Patients diagnosed with idiopathic environmental intolerance	Clinical examination by a physician and scoring above 26 for men and 30 for women in the Chemical Odor Sensitivity scale	Depression subscale baseline score using POMS was around 2.8 in the control period.
(Conrad and Adams, 2012)*	Randomized observational pilot study with repeated measures	28	NA	Female (28)	Postpartum women	0-18 months postpartum women with scores of 10 or higher on either the Edinburgh Postnatal Depression Scale or the Generalized Anxiety Disorder Scale	The baseline score using the Edinburgh postnatal Depression Scale for the control group was 15.9 and 16.1 for the intervention group.

Reference	Study design	Subjects					
		Number of subjects	Mean subject age (range)	Gender (n)	Subject description	Diagnostic systems/Inclusion criteria	Baseline score for depressive symptoms
(Serfaty et al., 2012)	Single-blind RCT	39	52.5; 51.1 for the aromatherapy group and 54 for the cognitive behavior therapy group	Female (31), male (8)	Individuals with cancer	Patients diagnosed for at least one month, who also had at least a predicted survival of 6 months and score 11 or more in the HADS for anxiety or depression	The baseline score in the depression-dejection subscale of POMS was 11.2 for the aromatherapy massage group and 13.4 for the control group.
(Taavoni et al., 2013)	RCT	90	53.70 for the control group (49.42-57.98), 52 (47.12-56.88) for the massage therapy group, 53.35 (49.01-57.69)	Female (90)	Women who entered their menopausal period naturally	Woman, age between 45 and 60 years, with amenorrhea for at least 1 year	At baseline, according to the Menopause Rating Scale, the frequency of the severity of the depressive mood was reported as mild (14.9%), moderate (36.8%), severe (20.7%) and very severe (2.3%). No difference was found among the groups at baseline.
(Wu et al., 2014)	RCT	25	34-48, average age NA	Female (25)	Woman with children	Women whose children were diagnosed with attention deficit hyperactivity disorder	Baseline using the Beck Depression Inventory was 8.6 in the control group and 10.8 in the treatment group.

*: In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. Abbreviations: NA, Not Available; HADS, Hospital Anxiety and Depression scale; POMS, Profile of Mood States.

Intervention

Control group

Several comparison groups were used in the RCTs included. In 6 studies (Soden et al., 2004; Araki et al., 2012; Igarashi, 2013; Taavoni et al., 2013; Wu et al., 2014; Sehhatie et al., 2015) no intervention was used as the comparison group. Four studies used a vehicle group (carrier oil or water) (Graham et al., 2003; Lemon, 2004; Conrad and Adams, 2012; Matsumoto et al., 2014) and an active control group consisting of usual supportive care while cognitive behavior therapy was used as comparison group in 2 studies (Wilkinson et al., 2007; Serfaty et al., 2012).

Description of the intervention groups

Aromatherapy was administered as inhalation aromatherapy in 5 studies (Graham et al., 2003; Conrad and Adams, 2012; Igarashi, 2013; Matsumoto et al., 2014; Sehhatie et al., 2015), followed by with massage in 8 studies wherein 1 also used inhalation aromatherapy (Lemon, 2004; Soden et al., 2004; Wilkinson et al., 2007; Araki et al., 2012; Conrad and Adams, 2012; Serfaty et al., 2012; Taavoni et al., 2013; Wu et al., 2014). The detailed description of the intervention protocol is shown in Table 4. Caution should be taken when analyzing the evidence from the study carried out by Sehhatie et al. (Sehhatie et al., 2015) since two interventions (aromatherapy being part) for pain relief in labor. The concern arising from the combined intervention is that no discrimination between the contribution from each intervention in the final outcome is possible to carry out.

Table 4. Description of the interventions and protocols

Table modified from Sánchez-Vidaña et al., 2017.

Reference	Intervention and protocol							
	Comparison group (n)	Treatment group(s) (n)	Type of essential oil used	Duration of the study	Administration method	Treatment frequency	Duration per session	Total no. of sessions
Inhalation aromatherapy								
(Graham et al., 2003)	Control with sweet almond cold-pressed pure vegetable oil with no fragrance (NA)	- Carrier oil with fractionated low (NA) grade essential oil - Pure essential oil (NA)	- Fractionated oils of unknown purity diluted 1:3 in carrier oil - Mixture of lavender, bergamot and cedarwood (2:1:1)	8 weeks	3 drops of oil applied to a bib worn during the administration of the treatment	Daily	15-20 min	56
(Conrad and Adams, 2012)*	Control, jojoba oil (14)	- 2% dilution of a mixture of essential oils (6)	- 0.25 rose otto essential oil and 0.75 lavender,2% dilution of the essential oil mixture	4 weeks	8 drops of oil applied to a cotton pad. Subjects were instructed to smell the cotton pad for 15 min	Twice a week	15 min	8
(Igarashi, 2013)	Control, no intervention (6)	- Pure essential oil (7)	- Lavender - Petitgrain - Bergamot	1 day	5 drops of oil applied on a filter placed in a diffuser.	Once	5 min	1
(Matsumoto et al., 2014)	Control, water (20)	- Pure essential oil (20)	- Yuzu	2 days	10µl oil in a cotton pad used in a diffuser set in the subject's nostrils	Twice	10 min (sessions separated in intervals around 2.6 days)	2
(Sehhatie et al., 2015)	Control group which did not receive any non-pharmacological method for pain relief of labor (160)	- Non-pharmacological methods for pain relief of labor including: Showering, being in upright position, aromatherapy and soft music without word (160)	-20% lavender essential oil	During labor	10x10 cm cloth impregnated with 1 ml 20% lavender essential oil which was attached to the mother's breast at the beginning of the active phase. The aromatherapy intervention was combined with other non-pharmacological interventions	Once	Duration of the active phase of labor	1
Aromatherapy massage								
(Lemon, 2004)	Control, grape seed oil (16)	- Diluted essential oil (16)	- 9 essential oils (bergamot, lemon Clary sage, lavender, roman chamomile, geranium, rose otto,	12 weeks	15 ml grape seed carrier oil with (4 drops) or without essential oils applied in a full body massage	Once a fortnight	40 min	6

Reference	Intervention and protocol							
	Comparison group (n)	Treatment group(s) (n)	Type of essential oil used	Duration of the study	Administration method	Treatment frequency	Duration per session	Total no. of sessions
			sandalwood, jasmine). A combination of essential oils chosen by the aromatherapist on each treatment session (16)		using gentle effleurage and petrissage			
(Soden et al., 2004)	Control, no intervention (13)	- Aromatherapy massage (16) - Massage with inert carrier oil (13)	- 1% Lavender essential oil diluted in sweet almond oil	2 years	Back massage	Weekly	30 min	4
(Wilkinson et al., 2007)	Usual supportive care	- Usual supportive care and aromatherapy massage	- 20 essential oils	10 weeks	Standardized massage agreed by the therapists	Weekly	1 h	4
(Araki et al., 2012)	Control, no intervention	-Aromatherapy massage	- 1% massage oil containing melissa, juniper and rosemary essential oils mixed into jojoba oil (1:2:2 ratio)	8 weeks	Standardized massage on the back, shoulders, arms, hands, lower legs and feet using 20-30 ml massage oil	Every two weeks	1 h	4
(Conrad and Adams, 2012)*	Control, essential oil blend unscented white lotion (14)	- 2% dilution of a mixture of essential oils (8)	- 0.25 rose otto essential oil and 0.75 lavender,2% dilution of the essential oil mixture	4 weeks	Topic application of the oil or lotion on both hands with gentle strokes of homogeneous pressure and speed	Twice a week	15 min	8
(Serfaty et al., 2012)	Cognitive Behavior Therapy (19)	- Aromatherapy massage (20)	- 20 essential oils	2 years	Standardized massage combined with treatment as usual (routine support)	Weekly	1 h	Up to 8 sessions in 10 weeks
(Taavoni et al., 2013)	Control, no intervention (30)	- Aromatherapy massage (30) - Massage (30)	- 3% oil mixture containing lavender, geranium, rose and rosemary (4:2:1:1 ratio) in almond and evening primrose oil	4 weeks	Massage in the abdomen, tights and arms using massage oil containing essential oils or odorless liquid petrolatum. Massage was applied with clockwise circular movements and light pressure.	Twice a week	30 min	8
(Wu et al., 2014)	Control, no intervention (12)	- Aromatherapy massage (13	- Jojoba oil containing 2% lavender and 2% geranium essential oils	4 weeks	Massage on the neck, shoulders, arms, back and legs including effleurage, friction, petrissage and vibration at a moderate pressure using 20 ml of massage oil	Twice per week	40 min	8

*: In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. Abbreviations: NA, Not Available; min, minutes; h, hour.

Essential oils used in the selected studies

The essential oils were used in 3 forms: pure, diluted or in combination with other essential oils. The procedure used to select the essential oils was based on (1) recommendation by an aromatherapist, (2) the effect of essential oils on physical and mood states, (3) the subject's preference, (4) and the safety profile to be used during pregnancy. However, some studies did not mention the criteria used in the selection of the essential oils chosen neither did they state the type of essential oils used. Lavender essential oil was the most commonly used essential oil in 8 of 12 studies (Graham et al., 2003; Lemon, 2004; Soden et al., 2004; Conrad and Adams, 2012; Igarashi, 2013; Taavoni et al., 2013; Wu et al., 2014; Sehhatie et al., 2015).

Inhalation aromatherapy

In the inhalation aromatherapy intervention, the essential oils most frequently used were lavender and bergamot either in their pure form or in combination with other oils (Graham et al., 2003; Lemon, 2004; Soden et al., 2004; Conrad and Adams, 2012; Igarashi, 2013; Taavoni et al., 2013; Wu et al., 2014; Sehhatie et al., 2015). Graham et al. (2003) reported the use of a mixture of fractionated essential oils. However, the type of essential oils used was not stated (Graham et al., 2003). Other essential oils used included petitgrain (Igarashi, 2013) and yuzu (Matsumoto et al., 2014) both in their pure form while cedarwood (Graham et al., 2003) and rose otto (Conrad and Adams, 2012) were used mixed with other essential oils.

Aromatherapy massage

In 2 studies, the therapist chose the essential oils from a set of 20 different options, but the type of essential oil chosen was not reported (Wilkinson et al., 2007; Serfaty

et al., 2012). In the study by Lemon (2004), the essential oils were selected from a list of 9 options and the author reported the type of essential oil chosen. The rest of the studies reported the use of lavender essential oil and a mixture of 2 to 4 essential oils, or lavender essential oil in combination with rose otto.

Administration protocol

Inhalation aromatherapy

Several administration modalities were used in the inhalation aromatherapy intervention. For example, Matsumoto et al. (2014) used a diffuser placed directly in the nostrils of the subjects and a cotton soaked with the essential oil which was placed in the diffuser (Matsumoto et al., 2014). The source of the aroma was placed 30 cm from the nose of the subject in two studies (Graham et al., 2003; Igarashi, 2013). The essential oil was applied on a bib worn by the subjects in two other studies (Graham et al., 2003; Sehhatie et al., 2015). Different amounts of essential oils were used from 10 µl to 1 ml or 3, 5 or 8 drops. Another parameter that varied in the studies included was the time of exposure, ranging from 5 to 20 minutes (Graham et al., 2003; Conrad and Adams, 2012; Igarashi, 2013; Matsumoto et al., 2014) (Table 4). The frequency of the treatment varied significantly from one unique time to once or twice a week, or daily. The acute and chronic effect of the aromatherapy intervention was evaluated implementing the duration of the treatment from 1 to 2 days and from 4 to 8 weeks.

Aromatherapy massage

Standardized protocols were used to perform the aromatherapy massage intervention. However, the areas of the body where the massage was administered were not mentioned in 3 studies (Wilkinson et al., 2007; Araki et al., 2012; Serfaty et al., 2012).

In one study, the massage target area was the back (Soden et al., 2004). The remaining studies focused the massage intervention on the abdomen, thighs and arms (Taavoni et al., 2013), and the neck, shoulders, arms, back and legs (Wu et al., 2014). In the study of Conrad and Adams (Conrad and Adams, 2012), the m'technique, a hand massage therapy, was applied in both hands. The administration protocol in most of the studies was carried out weekly (Soden et al., 2004; Wilkinson et al., 2007; Conrad and Adams, 2012; Serfaty et al., 2012; Taavoni et al., 2013; Wu et al., 2014). In the study by Araky et al. (2012) the administration of the intervention was done every 2 weeks (Araki et al., 2012). The duration of the sessions varied significantly among the studies from 15, 30, 40 min to 1 h.

Outcome measures

The scales used for the assessment of the depressive symptoms and a summary of the results is shown in Table 5. The assessment tools that were used in most of the experiments were HADS and POMS (Graham et al., 2003; Soden et al., 2004; Araki et al., 2012; Serfaty et al., 2012; Igarashi, 2013; Matsumoto et al., 2014) followed by EPDS in 2 studies (Conrad and Adams, 2012; Sehhatie et al., 2015) and other tools such as MADRS (Lemon, 2004), MRD (Taavoni et al., 2013), BDI (Wu et al., 2014) and CES-D (Wilkinson et al., 2007).

Table 5. Description of the measurement tools, outcomes and conclusions

Table modified from Sánchez-Vidaña et al., 2017.

Reference	Outcome measures				
	Scale	Comparison group	Intervention group(s)	Outcome	Improvement of depressive symptoms
Inhalation aromatherapy					
(Graham et al., 2003)	- HADS	Control group	- Carrier oil with fractionated low grade essential oil - Pure essential oil	Increased outcome measurement when compared to the baseline (18%-22% in HADS) using multivariate analysis. No statistically significant difference observed.	No
(Conrad and Adams, 2012)*	-EPDS	Control group	- 2% dilution of a mixture of essential oils	The mean difference in EPDS scores between the control group and the intervention group at end point was -3.981 but no statistically significant difference observed. Combined analysis of inhalation aromatherapy and massage aromatherapy showed statistically significant difference with a mean difference of -4.8.	Yes
(Igarashi, 2013)	- POMS	No intervention	- Pure essential oil	Depression-dejection sub scale scores pre and posttest were 2.7 and 1.2 in the comparison group and 1.6 and 0.6 in the intervention group respectively. No statistically significant difference observed.	No
(Matsumoto et al., 2014)	- POMS in TMD	Control group	- Pure essential oil	Change in TMD score was 0.5±2.2 in the control group and -1.28±2.6 in the intervention group (statistically significant difference).	Yes
(Sehhatie et al., 2015)	- EPDS	No intervention	- Non-pharmacological methods for pain relief of labor including: Showering, being in upright position, aromatherapy and soft music without words	Depression grade (0-30) pre and post-delivery were 6.3 and 8.8 in the no intervention group and 6.1 and 7.8 in the intervention group respectively. No statistically significant difference observed.	No
Aromatherapy massage					
(Lemon, 2004)	- MADRS	Control group	- Diluted essential oil	The scores in the MADRS at baseline and end point were 19.8 and 21.1 in the control group and 30 and 18.1 in the treatment group. Statistically significant difference was observed between the test and control group.	Yes
(Soden et al., 2004)	- HADS	No intervention	- Aromatherapy massage - Massage with inert carrier oil	The median change in HADS at baseline and end point was 0.5 in the no intervention group, 0 in the aromatherapy massage group and -1.5 in the massage group. No statistically significant difference among the groups.	No
(Wilkinson et al., 2007)	- CES-D	Active control (usual supportive care)	- Usual supportive care and aromatherapy massage	The scores using CES-D at baseline and end point were 26.0 and 4.6 in the active control group and 25.9 and 6.2 in the intervention group respectively. No statistically significant difference observed between the 2 groups.	No
(Araki et al., 2012)	- POMS	No intervention	- Aromatherapy massage	Statistically significant difference between the 2 groups when comparing the pre and post sessions in all the POMS sub-scales including depression-dejection.	Yes
(Conrad and Adams, 2012)*	- EPDS	Control group	- m'technique (hand massage)	The mean difference between the baseline and end point using EPDS was -6.031	Yes

Reference	Outcome measures				
	Scale	Comparison group	Intervention group(s)	Outcome	Improvement of depressive symptoms
(Serfaty et al., 2012)	- POMS-TMS	Active control (cognitive behavior therapy)	- Aromatherapy massage	The POMS-TMS decreased in both groups after intervention from 46.3 to 26.5 in the active control group and 44.5 to 29 in the intervention group.	Yes
(Taavoni et al., 2013)	- MRS	No intervention	- Aromatherapy massage - Massage	The mean difference in psychological symptoms (including depressive mood) was -0.379 in the no intervention group (no statistically significant difference), -3.49 in the aromatherapy massage group (statistically significant difference) and -1.20 in the massage group (statistically significant difference).	Yes
(Wu et al., 2014)	- BDI	No intervention	- Aromatherapy massage	The BDI score pre and posttest was 8.6 and 8.5 in the control group and 10.8 and 6.5 in the intervention group (statistically significant difference).	Yes

*: In this study, both aromatherapy modalities were tested: inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. Abbreviations: CES-D, Center of Epidemiological Studies Depression (self-reported depression); BDI, Beck Depression Inventory; DSM-IV, modified Diagnostic and Statistical Manual of Mental Disorder criteria; EPDS, Edinburgh Postnatal Depression Scale; HADS, Hospital Anxiety and Depression scale; MADRS, Montgomery-Asberg Depression Rating Scale; MRS, Menopause Rating Scale; POMS, Profile of Mood States;; TMD, Total Mood Disturbance; TMS, Total Mood Score (shortened version of the profile of mood states).

Efficacy of aromatherapy

Inhalation aromatherapy

Reduction of the depressive symptoms was reported in 2 studies using inhalation aromatherapy (Conrad and Adams, 2012; Matsumoto et al., 2014). In the study of Conrad and Adams (Conrad and Adams, 2012), the subjects were postpartum women that received two different aromatherapy interventions, inhalation aromatherapy and massage aromatherapy. The control and the intervention groups showed similar scores in the EPDS scale ($p = 0.8$). At the end of the intervention, a significant reduction of depressive symptoms was observed in the intervention group when compared to the control group (EPDS: $p = 0.01$). The study of Matsumoto et al. (Matsumoto et al., 2014) showed a positive effect on the negative emotional stress after 2 sessions of 10 min in healthy subjects. The following scores significantly decreased after inhalation of yuzu: TMD ($p < 0.001$), depression-dejection ($p = 0.041$), tension-anxiety ($p < 0.018$), anger-hostility ($p =0.002$) and confusion ($p =0.019$).

The 3 other studies did not show any significant benefit of the inhalation aromatherapy intervention (Graham et al., 2003; Igarashi, 2013; Sehhatie et al., 2015). No reduction of depressive symptoms was observed in the study by Graham et al. (Graham et al., 2003) in cancer patients undergoing radiotherapy. Furthermore, no effect was observed in the scores of the POMS scale on the pregnant women exposed to inhalation aromatherapy in the studies by Igarashi and Sehhattie et al. (Igarashi, 2013; Sehhatie et al., 2015).

Aromatherapy massage

Positive effects of aromatherapy massage were observed in 5 studies (Lemon, 2004; Araki et al., 2012; Serfaty et al., 2012; Taavoni et al., 2013; Wu et al., 2014). Improvement of the depressive symptoms were demonstrated in the study of Lemon (Lemon, 2004). The author concluded that aromatherapy massage had beneficial effect on subjects with a mild or higher degree of depressive symptoms. Furthermore, a statistically significant difference ($p < 0.001$) was observed in the study by Araki et al. (2012) when comparing the POMS scores at baseline and end point. Conrad and Adams (2012) evaluated the effect of both inhalation aromatherapy and aromatherapy massage. A clear improvement of depressive symptoms was observed in the aromatherapy massage group (EPDS, $p = 0.025$). In this study, the effect of inhalation aromatherapy showed a beneficial effect on the depressive symptoms but only when the data was pooled with the results of the aromatherapy massage group in an attempt by the authors wanted to show the effect of the aromatherapy intervention (using both administration methods). Therefore, no conclusive remark can be drawn in the case of inhalation aromatherapy alone. The study of Serfaty et al. also demonstrated alleviation of the depressive symptoms with the use of aromatherapy massage (Serfaty

et al., 2012). Positive effects in the depression-dejection and tension anxiety sub scores were observed in both interventions using the POMS-TMS assessment. The aromatherapy massage group showed positive effects in the last week of treatment while the comparison group, the cognitive behavior therapy, showed a more effective and sustained effect. The study carried out by Taavoni et al. (2013) measured depressive mood using the MRS assessment. A significant difference ($p < 0.001$) was observed among the three groups evaluated, with the massage and aromatherapy massage groups showing improvement in the outcome measure. The study demonstrated that massage aromatherapy was more effective in reducing the psychological scores evaluated when compared to massage alone. The last study that showed a positive effect on depressive symptoms was the study conducted by Wu et al. (2014). An improvement in the depressive symptoms scored using the BDI scale was observed in the aromatherapy massage group ($p = 0.04$).

Quality assessment

The quality of the studies included was evaluated using the Jadad scale – in this scale, high scores reflect higher quality. The results of the quality assessment are shown in Table 6. One out of 12 studies scored 5 (Soden et al., 2004), 2 studies scored 4 (Graham et al., 2003; Sehhatie et al., 2015), 3 studies scored 3 (Wilkinson et al., 2007; Serfaty et al., 2012; Igarashi, 2013), 3 studies scored 2, and 3 studies scored 1 (Lemon, 2004; Matsumoto et al., 2014; Wu et al., 2014).

Table 6. Quality assessment of studies included according to the Jadad scale

Table modified from Sánchez-Vidaña et al., 2017.

Reference	Quality assessment of methodology based on Jadad scale							
	Randomization	Appropriate method of randomization and description	Blinding				Description of dropouts / withdrawals	Jadad score (score out of 5)
			No blinding	Single blind	Double blind	Appropriate method of double blinding and description		
Inhalation aromatherapy								
(Graham et al., 2003)	Yes	Yes	No	No	Yes	Yes	No	4
(Conrad and Adams, 2012)*	Yes	No	Yes	No	No	NA	Yes	2
(Igarashi, 2013)	Yes	Yes	Yes	No	No	NA	Yes	3
(Matsumoto et al., 2014)	Yes	No	No	Yes	No	No	No	1
(Sehhatie et al., 2015)	Yes	Yes	No	No	Yes	No	Yes	4
Aromatherapy massage								
(Lemon, 2004)	Yes	No	Yes	No	No	NA	No	1
(Soden et al., 2004)	Yes	Yes	No	No	Yes	Yes	Yes	5
(Wilkinson et al., 2007)	Yes	Yes	No	No	Yes	No	Yes	3
(Araki et al., 2012)	Yes	Yes	Yes	No	No	NA	NA	2
(Conrad and Adams, 2012)*	Yes	No	Yes	No	No	NA	Yes	2
(Serfaty et al., 2012)	Yes	Yes	No	Yes	No	No	Yes	3
(Taavoni et al., 2013)	Yes	Yes	Yes	No	No	No	No	2
(Wu et al., 2014)	Yes	No	Yes	No	No	NA	No	1

*: In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. Abbreviations: NA, Not applicable

3.4. Discussion

The aim of the present systematic review was to analyze the clinical evidence on the effect of aromatherapy in the alleviation of depressive symptoms. The study also served as an update of a systematic review previously published by Yim et al. (2009). The systematic review by Yim et al. highlighted a couple of limitations that were addressed in the present study. For instance, the authors pointed out that the small

sample size of the studies included and the low number of RCT studies included could not support a conclusive analysis of the findings. In the present study, the focus was to analyze the evidence based on RCT studies. Also, from 2009 till date, more clinical trials on aromatherapy have been carried out, making it possible for higher sample size to be used in the present study.

Effectiveness of aromatherapy to relieve depressive symptoms

A very heterogeneous group of subjects was included in the systematic review. They included pregnant women, postpartum women, women at their menopause, women with children diagnosed with attention deficit hyperactivity disorder (ADHD), healthy female volunteers, patients diagnosed with cancer, depression/anxiety and idiopathic environmental intolerance. Different conclusions were reached when either inhalation aromatherapy or massage aromatherapy was administered. The reasons behind the discrepancy of the results observed can be attributed to the diversity in the administration protocol and the type of subjects. Another important consideration is that proper olfaction function should be evaluated when studying the effect of inhalation aromatherapy since poor function can affect the effectiveness of the treatment. Furthermore, when carrying out a study on aromatherapy massage, researchers should consider the degree of the effect of aromatherapy alone when combined with massage and evaluate both cases separately and in combination to make a more appropriate evaluation of the effect of aromatherapy alone.

The chemical profile of the essentials oil used is another important factor to be considered in analyzing the effectiveness of aromatherapy. The essential oils included in the studies have shown positive effects on mood such as anxiolytic, antidepressant

and sedative effect (Setzer, 2009; Bagetta et al., 2010; Kageyama et al., 2012; Matsumoto et al., 2016). For example, lavender (Kageyama et al., 2012), bergamot (Bagetta et al., 2010) and sandalwood (Setzer, 2009) improved the depressive symptoms while yuzu relieved the negative emotional stress (Matsumoto et al., 2016). The remaining essential oils included in the present study contained volatile compounds such as limonene, linalool and linalyl acetate. These oils are well known to have a positive effect on anxiety and to induce sedation, thereby possibly contributing to the effect observed in the clinical trials.

Inhalation aromatherapy

In the present systematic review, 2 studies showed positive effects in inhalation aromatherapy using the essential oil yuzu (Matsumoto et al., 2014) and in combination with rose otto and lavender (Conrad and Adams, 2012).

The lack of efficacy observed in the study by Graham et al. can be attributed to the quality of the essential oil whose purity was unknown as well as the combining of 3 pure oils used in the intervention group. Thus, using low quality essential oils or a mixture could affect the efficacy of the treatment. It is, therefore, recommended that pure essential oils be used and then be evaluated individually.

The acute beneficial effect of inhalation aromatherapy was observed in the study carried out by Igarashi (Igarashi, 2013) in pregnant women. However, it is important to highlight that the data analysis in the study showed positive results within group comparison and not between group comparisons. In addition, the short duration of the treatment and frequency of the intervention was too short to make a more detailed

evaluation. The study by Sehhatie et al. was also carried out in pregnant women but no significant effect was observed. The lack of effect observed in both studies can be attributed to the short time of exposure.

Another population in the present systematic review was postpartum women including a group receiving inhalation aromatherapy and another group receiving aromatherapy massage applied to both hands (Conrad and Adams, 2012). This study showed improvement in depressive symptoms of both interventions. However, the authors combined the data of both interventions for the statistical analysis and concluded that both aromatherapy interventions were effective. The data presented only supported the effectiveness of aromatherapy massage but not that of inhalation aromatherapy. However, no group receiving only hand massage without aromatherapy was included to assess the contribution of the hand massage alone on the effectiveness of the intervention. Hence, it is not possible to conclude that the effect should be to aromatherapy massage.

Another study showed positive results with inhalation aromatherapy using healthy volunteers. The discrepancy between the studies showing positive findings and the studies that did not find any beneficial effect may stem from the administration procedure with regard to the distance between the nostrils and the aroma. For example, Matsumoto et al. (Matsumoto et al., 2014), which found a positive effect of aromatherapy, placed the diffuser in direct contact with the subject nostrils. The close proximity of the volatile compounds of the essential oil and the nasal mucous may have enhanced the interaction with the olfactory receptors. Additionally, Matsumoto et al. performed a small olfactory function trial involving 2 sessions of 10 min prior

the RCT study to make sure that the olfactory function of the subjects included was normal. Factors such as the distance between the nostrils and the aroma and a prior olfactory function test might be the reason for the difference in findings among the inhalation aromatherapy studies. The administration procedures of inhalation aromatherapy that showed alleviation of the depressive symptoms included cotton impregnation and the use of a diffuser.

Aromatherapy massage

Aromatherapy massage is the administration method found to be more efficacious for decreasing depressive symptoms, as 5 of the 12 studies included showed positive results. Aromatherapy massage offers a combination of the health benefits of both aromatherapy and massage and is commonly used by health individuals especially to reduce stress (Wu et al., 2014). Massage is a popular alternative option used for about 2.1% of the patients diagnosed with severe depression (Coelho et al., 2008) and it is frequently used in the palliative care of patients with cancer (Wu et al., 2014).

The difficulty to evaluate the effectiveness of aromatherapy administered by aromatherapy massage is due to the lack of discrimination to separate the effect induced by aromatherapy and massage alone. Since both aromatherapy and massage show health benefits when applied alone, it should be expected that both therapies would have a combined beneficial effect. Therefore, caution should be taken when designing an RCT study and inclusion of a control group that receives massage alone should be considered. Lack of important information such as the area of the body where the massage was applied was not reported in some studies (Wilkinson et al., 2007). Also, the criteria used to decide the type of massage to be administered were

not stated in all the aromatherapy massage studies. Examples of massage techniques include effleurage, kneading, petrissage, friction, tapotement, and vibrations and shaking (Goats, 1994). The type of massage and area of the body where the massage is administered is essential to support the use of a certain type of massage above the other and to evaluate the health benefits related to the technique used. Different massage techniques are associated with different physiological effects for instance increase blood flow or production of short-induced analgesia (Goats, 1994). Therefore, a detailed description of the massage therapy used is essential in analyzing the combined effect of aromatherapy massage.

There were some differences in the frequency of the intervention. This can be used to explain the positive findings in the study by Serfaty et al. (2012) and the lack of effect observed in the studies by Soden et al. and Wilkinson et al. intervention (Soden et al., 2004; Wilkinson et al., 2007). For example, Serfaty et al. (2012) used twice the number of sessions than the other 2 studies. Aromatherapy massage applied weekly for 8 weeks was found to be effective in cancer patients when compared to the active control. The absence of therapeutic effect observed in some of the aromatherapy massage studies included may be as a result of the effect of the massage itself whish was so strong that it concealed the added effect of aromatherapy. Therefore, no significant difference was observed when aromatherapy and massage were combined (Corner et al., 1994; Wilkinson, 2007; Russell et al., 2008; Hou et al., 2010). Also, caution should be taken when interpreting the massage therapies since the effects observed in people with cancer are not compelling (Ernst, 2009). In the study of Fellowes et al. (Fellowes et al., 2004) a positive effect on the alleviation of depressive symptoms was observed in subjects with cancer. Therefore, more investigation is

needed to clarify the mixed findings. Furthermore, it is important to highlight that the aromatherapy massage intervention in the study by Serfaty et al. (2012) was administered in combination with cognitive behavioral therapy. Therefore, the most appropriate conclusion from the study by Serfaty et al. (2012) is that aromatherapy massage assisted in the alleviation of depressive symptoms when combined with cognitive behavioral therapy.

Two other studies found positive effects of aromatherapy massage on depressive symptoms involved women at menopause (Taavoni et al., 2013) and women with children diagnosed with ADHD (Wu et al., 2014). The intervention protocol in both studies was similar involving 8 sessions in which the intervention was administered twice a week for 30-40 minutes. Soden et al. and Wilkinson et al. did not report any beneficial effect of aromatherapy massage. In both studies, the frequency (once a week) and number of sessions were lower than the studies in which positive effects were observed. Thus, the duration and number of sessions might be one of the reasons for the lack of efficacy.

Regarding the administration protocol in the studies of Lemon and Serfaty et al., alleviation of the depressive symptoms was observed. Interestingly, these studies used a higher number of sessions (6-8) with a duration of 40 minutes to 1h per session. The study by Araki et al. also demonstrated positive effects of aromatherapy massages applied with a lower number of sessions (4) and a lower frequency of treatment (every two weeks, 1h per session) (Araki et al., 2012). To sum up, the findings in the studies using aromatherapy massage suggest that at least more than 4 sessions that take place

once or twice a week for at least 30 min per sessions are needed to observe beneficial effects on depressive symptoms.

Limitations

The main concern of the analysis carried out in the present systematic review is the quality of the studies included. Low scores were observed in half of the studies studied (score of 1 or 2). As a result, the poor blinding procedure could have contributed to the effectiveness of the treatment reported. Also, the administration protocol differed considerably among the studies – for instance the massage technique was not described in detail in the aromatherapy massage studies. Furthermore, the contribution of massage alone could not be established in the aromatherapy massage intervention. Finally, a comparison of the findings presents a complex-cum-challenging situation, as different assessment tools were used in various studies.

Clinical recommendations

In future RCT studies evaluating inhalation aromatherapy, a pre-test that will conform normal olfactory function should be included. Additionally, longer exposure time and a higher number of sessions should be considered. When evaluating aromatherapy massage, at least 8 sessions once or twice per week should be included.

3.5. Conclusion

In the systematic review carried out by Yim et al. (2009) sufficient evidence was not available to conclude that aromatherapy had a beneficial effect on depressive symptoms. However, the analysis of the evidence from the present systematic review

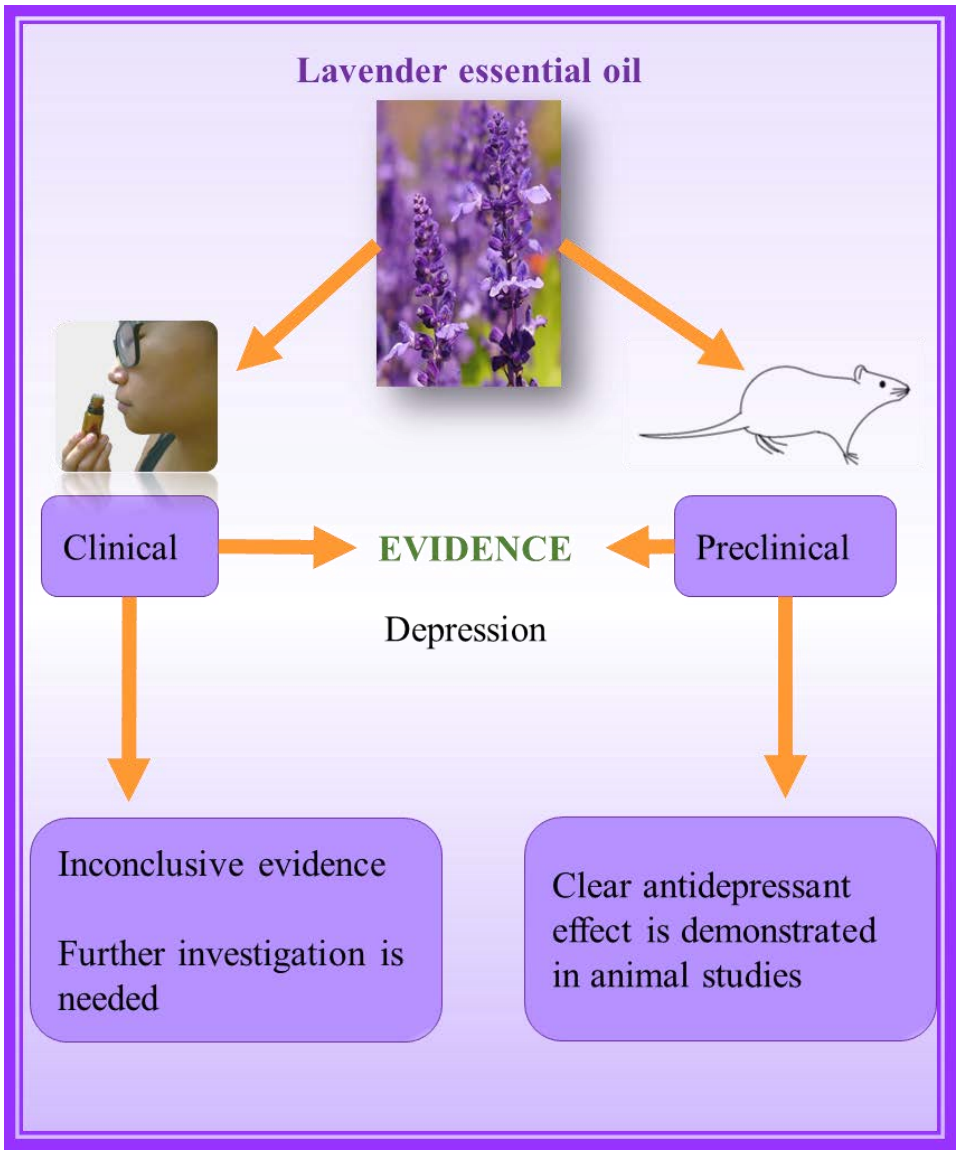
points to a different finding. A critical analysis of the most updated findings of the effect of aromatherapy on depressive symptoms was carried out in the present study and it was found that aromatherapy massage was more efficacious than inhalation aromatherapy. Although promising results were observed in inhalation aromatherapy, more investigation is needed to corroborate this position. In conclusion, this systematic review has demonstrated that aromatherapy represents a potential alternative for pharmacotherapy as far as the alleviation of depressive symptoms is concerned.

CHAPTER 3 PART B

The content of his chapter was sent for publication and is under review.

EFFECTIVENESS OF LAVENDER ESSENTIAL OIL ON DEPRESSION: A SYSTEMATIC REVIEW OF THE PRECLINICAL AND CLINICAL EVIDENCE

Sánchez-Vidaña DI, Chow JKW, Fung TKH, Ho J, Wu WKK, Ngai SPC, Lau BWM, Tsang HWH. **(Under review)**



Model: Fiona Chung

Abstract

Background: As suggested by the literature reviewed in the previous chapter, aromatherapy, a form of complementary and alternative medicine (CAM), is widely used and has potential for the treatment of mood disorders. Certain essential oils are used to alleviate depressive symptoms and they represent a promising therapeutic alternative. Aromatherapy is a form of CAM that has widespread use and potential for the treatment of psychiatric disorders. Essential oils such as lavender essential oil (LEO) have been used to ameliorate depressive symptoms and they represent a valuable therapeutic alternative. In this chapter, the antidepressant effect of LEO has been evaluated in clinical trials and has revealed promising results.

Aims: The present systematic review aimed to analyze the evidence of the effect of LEO in preclinical and clinical studies on depressive symptoms. Also, the mechanism of action of LEO and its major chemical constituents were explored.

Methods: A set of predefined search terms were input in the following databases: AMED, CINHAL, CCRCT, MEDLINE and PsycINFO. *In vitro*, *in vivo* and RCT studies focusing on the evaluation of LEO, lavender-based extract or LEO's major chemical constituents were included.

Results: A total of 42 studies were retrieved from all the databases. Thirteen studies that fulfilled the inclusion and exclusion criteria were selected. Four RCT studies and 4 animal studies demonstrated alleviation of depressive symptoms and depression-like behavior respectively. One *in vitro* study explored the mechanism of action of LEO, linalool and linalyl acetate.

Conclusions: LEO and linalool improved the depression-like behavior in animal studies. The clinical evidence was not conclusive. Therefore, further clinical investigation is needed.

3.6. Introduction

CAM practices are often used by patients with depression (van der Watt et al., 2008; Yeung et al., 2015). Aromatherapy belongs to the wide repertoire of CAM therapies and has a valuable potential for the treatment of mood disorders including depression (Perry and Perry, 2006). In aromatherapy, essential oils are used and this helps in improving the physical and psychological wellbeing of patients (Cooke and Ernst, 2000; Perry and Perry, 2006; Herz, 2009; Setzer, 2009).

Aromatherapy can be applied by inhalation, topically or orally (Perry and Perry, 2006; Fung et al., 2012; Ndao et al., 2012). Essential oils comprise many lipophilic compounds that can easily reach circulation after topic or oral administration (Buckle, 2003; Carke, 2008). After inhalation of essential oils, the volatile compounds are absorbed in the mucous of the olfactory epithelium and reach the blood stream but they can also induce different mechanisms in the brain. (Tisserand and Balacs, 1995; Carke, 2008). Chemosignals are detected in the nasal epithelium formed by about 25 million cells connected to the OB. The signal triggered by inhaled essential oils is sent to different structures of the limbic system including the hippocampus. The signal reaches the olfactory cortex where neurotransmitters are released resulting in effects on mood (Burnett et al., 2004; Price and Price, 2011; Ali et al., 2015). Pleasant aromas are associated with relaxation, comfort, pleasant memories and reduction of stress (Louis and Kowalski, 2002; Burnett et al., 2004; Ali et al., 2015). Because of the action of essential oils on mood, they represent a promising alternative for the treatment of mood disorders, thereby warranting further investigation (Ernst et al., 1998; Andreescu et al., 2008; Setzer, 2009).

Lavender essential oil is derived from flowering heads of *Lavandula aungustifolia* Mill. (syn. *Lavandula officinalis* Chaix) and it is commonly used in aromatherapy (Andrade et al., 1999; Cavanagh and Wilkinson, 2002; Greenberg and Slyer, 2017). The biological activity attributed to LEO includes smooth muscle relaxation, antimicrobial activity, sedative, analgesic, anxiolytic, and antidepressant properties (Wu and James, 2011; Perry et al., 2012). In clinical studies, LEO is the most frequently used essential oil to evaluate its effect on depressive symptoms. Since LEO is extensively used and there is clinical evidence available that supports its promising antidepressant effects, more in-depth investigation on LEO and its molecular mechanism is needed. Therefore, this systematic review aimed to analyze the findings pertaining to the effectiveness of LEO on the alleviation of depressive symptoms and its mechanism of action in preclinical and clinical studies. To my best understanding, there is no systematic review on these issues previously done.

3.7. Methods

Search terms and strategy

A list of relevant search terms was established considering depression, type of study, aromatherapy, LEO and its major compounds (Table 7). The search strategy was implemented using the following databases: AMED, CCTR, CINAHL, Pubmed and PsycINFO. The database search was carried out independently by two authors and a third author was consulted in instances of discrepancy.

Table 7. Search terms and database strategy

ID	Disease search terms
1.	Depress*
2.	Major depress*
3.	Mood disorder
4.	Depressive disorder
5.	1 OR 2 OR 3 OR 4
ID	**Type of study search terms**
6.	Controlled clinical trial*
7.	Randomi* controlled trial*
8.	In vitro
9.	In vivo
10.	Cell culture*
11.	Tissue culture
12.	Preclinical stud*
13.	Animal stud*
14.	Animal model
15.	6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14
ID	**Aromatherapy search terms**
16.	Aroma
17.	Aromatherapy
18.	Aromatic therapy
19.	Essential oil*
20.	Fragrance
21.	Fragrant oil*
22.	Scent
23.	Massage therapy
24.	Medical massage
25.	Massage
26.	Inhalation
27.	16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26
ID	**Lavender essential oil and its main components**
28.	Lavender
29.	Lavandula
30.	Lavender oil
31.	Linalool
32.	Linalyl acetate
33.	1-8-cineole
34.	Lavandulyl
35.	Terpene-4-ol
36.	Lavandulol
37.	Cis-ocimene
38.	Trans-ocimene
39.	28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38
40.	5 AND 15 AND 27 AND 39

*truncation symbol used in database search

Inclusion and exclusion criteria

Studies that evaluated the effect of any form of LEO including LEO, lavender derived extract or LEO's major compounds (mentioned in the search terms) were selected. RCT studies using validated scales for the assessment of depressive symptoms were also included. Further preclinical studies using i*n vivo* and/or *in vitro* models relevant for depression that evaluated the mechanism of action of LEO or any of its major compounds were included. Additionally, only full text studies in English were included. Finally, no restriction in the year of publication was considered, and no systematic reviews or meta-analyses were included.

Study selection strategy

After retrieving relevant articles from the databases, all the articles were pooled and the duplicates were excluded. The pre-selection of the studies was carried out by screening the title. The whole text of the remaining articles was checked in detail to select the articles to be included in the present systematic review. Two independent authors carried out the database search and selection of articles. Any disagreement was resolved by discussion in consultation with a third author and agreement on the studies to be included in the systematic review was achieved.

Data extraction

The data extracted from all the studies included the type of study, form of LEO evaluated, and focus of the study. The type of study, study design, subjects, intervention, administration protocol, validated scale for the measurement of the depressive symptoms, outcomes, and conclusions were extracted from the clinical studies. The animal description, cell line, treatment, biological test, outcome and conclusions were extracted from the preclinical studies.

Quality assessment

The Jadad scale was used to assess the quality of the clinical studies (Jadad et al., 1996). The scale evaluation is based on parameters such as randomization, double blinding, withdrawals and dropouts. Higher scores correspond to higher quality studies (Jadad et al., 1996).

3.8. Results

Study selection

The combined database search from inception to August 2017 resulted in 42 studies (AMED: 2, CCTR: 0, CINAHL: 11, Pubmed: 20, and PsycINFO: 9) (Figure 10). After removal of duplicates, the title of the remaining articles (n = 34) was screened. The full text of the pre-selected studies was reviewed and 13 studies were selected for the systematic review including 7 clinical trials and 6 preclinical studies (5 *in vivo* and 1 *in vitro* study).

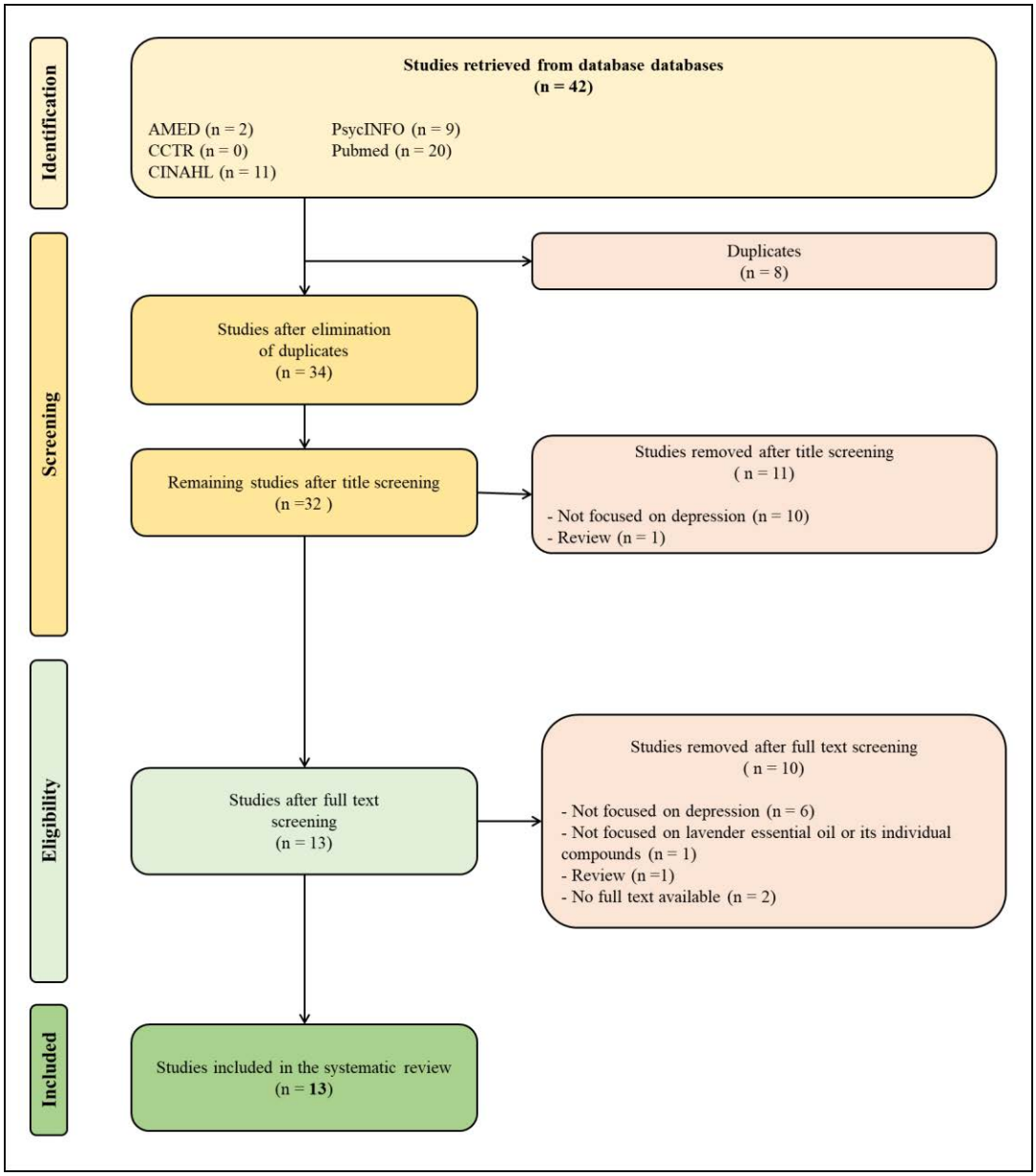


Figure 10. Study selection flow diagram

Description of the studies included

The RCT studies included (n = 7) evaluated the effect of LEO on depressive symptoms (Table 8) (Diego et al., 1998; Graham et al., 2003; Soden et al., 2004; Conrad and Adams, 2012; Igarashi, 2013; Chen and Chen, 2015; Blackburn et al., 2017). The *in vivo* studies selected (n = 5) evaluated the effect of LEO or its major components on depression-like behavior (Komiya et al., 2006; Seol et al., 2010; Guzmán-Gutiérrez et

al., 2012; Hritcu et al., 2012; Coelho et al., 2013). The effect of LEO was evaluated in 3 out of 5 *in vivo* studies (Komiya et al., 2006; Seol et al., 2010; Hritcu et al., 2012) and the effect of linalool, one of the most common components in LEO, was studied in the remaining 2 *in vivo* studies (Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013). The study carried out by Guzmán-Gutiérrez et al. (2012) focused on the evaluation of an essential oil derived from *Litsea glaucescens*. However, the authors also evaluated the effect of linalool, a major compound present in LEO (Guzmán-Gutiérrez et al., 2012). Finally, the only *in vitro* study selected focused on the mechanism of action of LEO and two of its major compounds (López et al., 2017).

Table 8. Overview of the selected studies

Reference	Type of study	Form of LEO evaluated		Focus of the study	
		Essential oil or oral infusion (dried lavender flowers)	Single compound	Effectiveness	Mechanism of action
(Diego et al., 1998)	Clinical	✓		✓	
(Graham et al., 2003)	Clinical	✓		✓	
(Soden et al., 2004)	Clinical	✓		✓	
(Conrad and Adams, 2012)	Clinical	✓		✓	
(Igarashi, 2013)	Clinical	✓		✓	
(Chen and Chen, 2015)	Clinical	✓		✓	
(Blackburn et al., 2017)	Clinical	✓		✓	
(Komiya et al., 2006)	Preclinical (*in vivo*)	✓		✓	
(Seol et al., 2010)	Preclinical (*in vivo*)	✓		✓	
(Guzmán-Gutiérrez et al., 2012)	Preclinical (*in vivo*)		✓	✓	
(Hritcu et al., 2012)	Preclinical (*in vivo*)	✓		✓	
(Coelho et al., 2013)	Preclinical (*in vivo*)		✓	✓	
(López et al., 2017)	Preclinical (*in vitro*)	✓	✓		✓

Clinical studies

Study design and description of subjects

A detailed description of the study design and description of the subjects is shown in Table 9. A total of 562 subjects were considered in the present systematic review out of which 315 (62.5%) were female and 211 (37.5%) were male. The mean age of the subject population was 40.3 years. Only one study (Blackburn et al., 2017) did not report the age of the subjects. The type of subjects comprises 405 patients with cancer (72.1%) (Graham et al., 2003; Soden et al., 2004; Blackburn et al., 2017), 104 post-partum women (18.5%) (Conrad and Adams, 2012; Chen and Chen, 2015), 40 healthy volunteers (7.1%) (Diego et al., 1998), and 13 pregnant women (2.3%) (Igarashi, 2013).

Table 9. Study design and description of subjects

Reference	Study design	Subject description		
		No. of subjects and gender	Mean age	Type of subjects
(Diego et al., 1998)	Randomized controlled trial	40 (30 female, 10 male)	30.9	Faculty and staff members of a university
(Graham et al., 2003)	Placebo-controlled double-blind randomized trial	313 (150 female, 163 male)	65	Patients with cancer under radiotherapy treatment
(Soden et al., 2004)	Double-blind randomized controlled trial	42 (32 female, 10 male)	73	Patients with cancer
(Conrad and Adams, 2012)	Randomized observational pilot study with repeated measures	28 (female)	32	Post-partum women with scores of ≥ 10 in the Edinburg Postnatal Depression Scale or the Generalized Anxiety disorder
(Igarashi, 2013)	Prospective Randomized controlled trial	13 (female)	27.3 (control group) 29.3 (treatment group)	28-weeks pregnant women (singleton pregnancy)
(Chen and Chen, 2015)	Pretest-posttest randomized controlled trial	76 (female)	32.68 (control group) 32.05 (treatment group)	Post-partum women with a score of ≥16 in the Postpartum Sleep Quality Scale
(Blackburn et al., 2017)	Randomized crossover washout trial	50 (22 female, 28 male)	NA (age range from 19-73 years)	Patients newly diagnosed with acute myelogenous leukemia initiating 4 weeks of intensive induction chemotherapy

Intervention protocol

The description of the intervention protocol is show in Table 10. Inhalation aromatherapy was used in 5 out of the 7 RCT studies (Diego et al., 1998; Graham et al., 2003; Conrad and Adams, 2012; Igarashi, 2013; Blackburn et al., 2017). Massage aromatherapy was evaluated in 2 studies (Soden et al., 2004; Conrad and Adams, 2012) and oral administration was used in 1 study (Chen and Chen, 2015). Both inhalation aromatherapy and massage aromatherapy were evaluated in the study of Conrad and Adams (2012) (Conrad and Adams, 2012).

The essential oil used in 2 RCT studies was extracted from *Lavandula angustifolia* while the remaining 5 studies did not state the type of lavender used. The subjects could choose the essential oil of their preference (1 out of 3 options) in 2 studies (Igarashi, 2013; Blackburn et al., 2017). However, Igarashi (2013) did not present a separate analysis with respect to the essential oil chosen. Therefore, caution should be taken when interpreting the results of this study as the final effect observed cannot be attributed to LEO alone. On the other hand, the study by Blackburn et al. (2017) mentioned that the most selected essential oil was LEO. All the studies selected used different comparison groups. For instance, the studies on inhalation aromatherapy included no intervention (Igarashi, 2013), fragrance free oil (Graham et al., 2003), and rose water (Blackburn et al., 2017) as comparison groups. Other comparison groups included treatment groups that made use of different essential oils other than LEO (Diego et al., 1998) and traditional medical care without aromatherapy (Conrad and Adams, 2012). In the case of the aromatherapy massage intervention, the comparison group considered was no intervention (Soden et al., 2004) and traditional medical treatment without aromatherapy (Conrad and Adams, 2012). Finally, the study by

Chen and Chen (2015) took the regular post-partum care as comparison group for the orally administered lavender intervention (Chen and Chen, 2015).

The nature of the essential oils varied significantly and they entailed using undiluted single essential oils (Igarashi, 2013; Blackburn et al., 2017), undiluted essential oils mixed with other essential oils (Graham et al., 2003), diluted single essential oils (Diego et al., 1998; Soden et al., 2004), and diluted essential oils mixed with other essential oils (Conrad and Adams, 2012). Also, dried lavender flowers were used to prepare the orally administered tea in the study by Chen ad Chen.

In the studies using inhalation aromatherapy as administration method, a cotton soaked with the essential oils was used in the studies by Diego et al. (1998 and Conrad and Adams (2012). In two other studies a diffuser was the technique used to administer the essential oils (Igarashi, 2013; Blackburn et al., 2017) and a bib full of essential oil was used by Graham et al. (2003) in 1 study. With regard to the aromatherapy massage method, standardized back massage (Soden et al., 2004) and standardized hand massage (Conrad and Adams, 2012) were the techniques employed. Regarding the frequency of the treatment, the inhalation aromatherapy intervention was administered once (Diego et al., 1998; Igarashi, 2013), daily (Graham et al., 2003; Blackburn et al., 2017) and twice a week (Conrad and Adams, 2012). The frequency of the treatment in the aromatherapy massage studies was once a week (Soden et al., 2004) and twice a week (Conrad and Adams, 2012). Finally, the lavender tea was consumed daily for 2 weeks (Chen and Chen, 2015). The time of exposure to the essential oils in the inhalation aromatherapy intervention varied from 3, 5, 15 min, 15-

20 minutes to 9 hours whereas the massage intervention was applied in sessions of 15 to 30 minutes.

Table 10. Description of the intervention and administration protocol

Reference	Groups		LEO				Intervention				
	Comparison group (n)	Treatment group(s) (n)	Species	Treatment description	Alone	Mixed		Administration protocol	Treatment frequency	Duration per session	Total no. of sessions
Inhalation Aromatherapy											
(Diego et al., 1998)	Two treatment groups (40)	1. LEO (20) 2. Rosemary essential oil (20)	NR	1. 10% LEO in grapeseed oil 2. 10% rosemary essential oil in grapeseed oil	✓		IA	Essential oil was applied to a cotton dental swab in a 100ml plastic vial held 3 inches from the nose of the subjects. Subjects were sitting quietly in a massage chair, breathing normally with their eyes closed.	1 day	3 min	1
(Graham et al., 2003)	Fragrance-free sweet almonds cold pressed pure vegetable oil (NA)	1. Fractionated low-grade essential oils (NA) 2. Pure essential oils (NA)	NR	1. Fractionated essential oils of unknown purity (1:3 dilution in carrier oil) 2. Mixture of pure LEO, bergamot and cedarwood essential oils (2:1:1)		✓	IA	Three drops of oil applied to a bib worn during the treatment period.	Daily	15-20 min	NR
(Igarashi, 2013)	No intervention (6)	Pure essential oil (7)	*Lavandula angustifolia*	Pure essential oil. Each Participants chose 1 out of 3 essential oil options (LEO, petitgrain and bergamot essential oils)	✓		IA	5 drops of oil applied to a filter placed in a diffuser. The diffuser was placed 30cm away from the participant.	Once	5 min	1
(Conrad and Adams, 2012)*	1. Traditional medical treatment without any aromatherapy (14)	1. Diluted essential oil mixture (IA) (6) 2. Diluted essential oil mixture (AM) (8)	*Lavandula angustifolia*	1. 2% essential oil mixture (0.25 rose otto , 0.75 lavender essential oils) in jojoba oil 2. 2% essential oil mixture (as above) in in carrier lotion		✓	IA and AM	Cotton pad infused with 8 drops of essential oil mixture for inhalation aromatherapy and m'technique (standardized hand massage) with the essential oil mixture for aromatherapy massage	Twice a week for 4 weeks	15 min	8

Reference	Groups		LEO				Intervention				
	Comparison group (n)	Treatment group(s) (n)	Species	Treatment description	Alone	Mixed		Administration protocol	Treatment frequency	Duration per session	Total no. of sessions
(Blackburn et z al., 2017)	Rosewater (50)	Pure essential oil (50)	NR	Patients chose their preferred essential oil (lavender, chamomile or peppermint). Lavender was the most selected essential oil.	✓		IA	A diffuser containing 8 drops of either essential oil or rose water (placebo) was placed in each patient's room and was turned on for about 8 hours from 9:00 pm onwards.	Daily for 2 weeks with 1-week washout period between week 1 and 2	Approx. 9 h	14
Aromatherapy Massage											
(Soden et al., 2004)	No intervention (13)	1. Massage with LEO (16) 2. Massage with inert carrier oil (13)	NR	1. 1% LEO in sweet almond oil	✓		AM	Standardized back massage	Weekly for 4 weeks	30 min	4
(Conrad and Adams, 2012)*	1. Traditional medical treatment without any aromatherapy (14)	1. Diluted essential oil mixture (IA) (6) 2. Diluted essential oil mixture (AM) (8)	*Lavandula angustifolia*	1. 2% essential oil mixture (0.25 rose otto and 0.75 lavender essential oils) in jojoba oil 2. 2% essential oil mixture (0.25 rose otto and 0.75 lavender essential oils) in in carrier lotion		✓	IA and AM	Cotton pad infused with 8 drops of essential oil mixture for inhalation aromatherapy and m'technique (standardized massage) with the essential oil mixture for aromatherapy massage	Twice a week for 4 weeks	15 min	8

Reference	Groups		LEO				Intervention			
	Comparison group (n)	Treatment group(s) (n)	Species	Treatment description	Alone	Mixed	Administration protocol	Treatment frequency	Duration per session	Total no. of sessions
Oral Administration										
(Chen and Chen, 2015)	Regular post-partum care (38)	Infusion of lavender flowers (38)	NR	Teabags with 2 g of dried lavender flowers	✓		O	One cup of lavender tea (1 teabag infused for 10-15 min in 300 ml hot water) ingested 1h before bedtime. Before drinking, participants smelled/appreciated the tea aroma for some time.	Daily for 2 weeks	NR	14

Abbreviations: IA, inhalation aromatherapy; AM, aromatherapy massage; O, oral administration; NR, not reported
*This study included both administration methods: IA and AM.

Assessment tools, outcome, and conclusions

The assessment tools, outcomes, and conclusions are summarized in Table 11. The RCT studies used validated tools for the assessment of the severity of the depressive symptoms and for the assessment of mood in general (Blackburn et al., 2017). The tools employed included the Edinburgh Postnatal Depression Scale (EPDS) (Conrad and Adams, 2012; Chen and Chen, 2015); the Hospital Anxiety and Depression Scale (HADS) (Graham et al., 2003; Soden et al., 2004), the Profile of Mood States (POMS) (Diego et al., 1998; Igarashi, 2013) and the Edmonton Symptoms Assessment Scale-Revised Scores (ESASr).

Four out of the 7 RCT studies reported a significant improvement of the depressive symptoms. Three studies using inhalation aromatherapy demonstrated that treatment with LEO alone (Diego et al., 1998; Blackburn et al., 2017) or in combination with other essential oils (Conrad and Adams, 2012) significantly decreased the depressive symptoms when compared to the control groups. However, it is important to highlight that the findings from the study by Conrad and Adams (2012) considered a combined analysis of the effect using both inhalation aromatherapy and aromatherapy massage. Additionally, the positive effect observed in the studies from Conrad and Adams (2012) and Blackburn (2017) cannot be fully translated to LEO since LEO was given in a mixture with other essential oils and the effect of other essential oils alone (from which lavender was the most commonly chosen) was also considered to analyze the combined effect of the aromatherapy intervention. In the aromatherapy massage intervention, only one study using a mixture of essential oils including LEO, significantly decreased the depressive symptoms (Conrad and Adams, 2012). A significant reduction of the depressive symptoms was demonstrated after daily administration of lavender tea (Chen and Chen, 2015). Finally, no side effects

associated with the use of LEO applied by inhalation, massage or orally as lavender tea were reported in any of the RCT studies.

Table 11. Description of the assessment tools for depressive symptoms, outcomes, side effects and conclusions

Reference	Scale to assess depressive symptoms	Baseline	Outcome	Side effects	Improvement of depressive symptoms
Inhalation Aromatherapy					
(Diego et al., 1998)	POMS	The baseline for depressed mood was 2.66 in the POMS in the lavender group. Lower POMS score indicates better mood.	After the intervention, statistically significant difference was observed. The lavender group showed lower POMS score (1.16 after intervention).	No side effects were reported.	Yes
(Graham et al., 2003)	HADS	The baseline depression status was odds ratio of 29 in the HADS.	No statistically significant difference among the groups was observed.	No side effects were reported.	No
(Igarashi, 2013)	POMS	The depression-dejection scale baseline using the POMS was 2.7 and 1.6 in the control and intervention group, respectively. Lower POMS score indicates better mood.	No statistically significant difference between the groups was observed.	No side effects were reported.	No
(Conrad and Adams, 2012)*	EPDS	The baseline using the EPDS was 15.9 and 16.1 in the control and intervention groups, respectively. A score greater than 10 indicates possible depression. Lower scores indicate an improvement in depressive symptoms.	No statistically significant difference between the control and intervention group was observed, However, a significant difference was observed in a combined analysis of inhalation and massage aromatherapy when compared to the control group (mean difference of -4.8).	No side effects were reported.	Yes
(Blackburn et al., 2017)	ESASr	No baseline calculated. Reduced ESASr. High scores indicate increased the severity of the symptoms.	The weekly average ESARr score after 2 weeks treatment was calculated. The weekly average burden of the depression subscale of the ESASr was 2.08 and 1.24 in the control and intervention group, respectively. Statistically significant difference was observed between the control and the aromatherapy group in which aromatherapy group showed reduced score in the depression subscale of the ESARs.	NA	Yes
Aromatherapy Massage					
(Soden et al., 2004)	HADS	The baseline score was not reported. The median change in HADS was 5, -1.5 and -0.5 for the control, aromatherapy and massage group, respectively.	No statistically significant difference among the groups was observed.	No side effects were reported.	No
(Conrad and Adams, 2012)*	EPDS	The baseline using the EPDS was 15.9 and 16.1 in the control and intervention groups, respectively. A score greater than 10 indicates possible depression. Lower scores indicate an improvement in the depressive symptoms.	Statistically significant difference observed between the control and massage aromatherapy group (endpoint scores of 12.1 and 7.1, respectively).	No side effects were reported.	Yes

Reference	Scale to assess depressive symptoms	Baseline	Outcome	Side effects	Improvement of depressive symptoms
			Statistically significant difference was observed in a combined analysis of inhalation and massage aromatherapy together compared with the control group (mean difference of -4.8).		
Oral Administration					
(Chen and Chen, 2015)	EPDS	The baseline scores using the EPDS were 9.71 and 7.50 in the control and intervention group, respectively. Statistically significant difference between the control and intervention group was observed at baseline.	Statistical significant difference between the control and intervention group was observed in the EPDS scores after 2 weeks test (10.47 and 7.37 in the control and intervention group, respectively; the education level and pretest score were the ANCOVA covariates).	No side effects were reported.	Yes

Abbreviations: EPDS, Edinburgh Postnatal Depression Scale; ESASr, Edmonton Symptoms Assessment Scale-Revised Scores; HADS, Hospital Anxiety and Depression Scale; NA, Not applicable; POMS, Profile of Mood States.
*This study included both administration methods: IA and AM.

Quality assessment

The quality of the RCT studies was evaluated using the Jadad scale, a 0-5 points scale that considers randomization, description of the randomization method and description of dropouts/withdrawals (Jadad et al., 1996). A high score indicates a high quality of the study. The majority of the studies selected (4 out of 7 studies) received high scores. The studies of Soden et al. (2004) and Blackburn et al. (2017) obtained the highest score, followed by the study of Graham et al. (2003) that scored 4. The study by Igarashi (2013) scored 3 while 2 studies – Conrad and Adams (2012) and Chen and Chen (2012) – scored 2. One RCT study (Diego et al., 1998) obtained scored 1 in the Jadad scale.

An important consideration regarding the quality of the RCT studies is that the studies that demonstrated reduced depressive symptoms after the aromatherapy intervention obtained low scores in the Jadad scale. The main concerns in the low quality of the studies included the lack of information of the blinding procedure used, no appropriate method of double blinding and the lack of description of the double blinding method.

Table 12. Quality assessment based on the Jadad scale

Reference	Quality assessment (Jadad scale)							
	Random.	Appropriate method of random. and description	Blinding				Description of dropouts	Score (0-5)
			No blinding	Single blind	Double blind	Appropriate method of double blinding and description		
(Diego et al., 1998)	Yes	No	No	No	No	No	No	1
(Graham et al., 2003)	Yes	Yes	No	No	Yes	Yes	No	4
(Soden et al., 2004)	Yes	Yes	No	No	Yes	Yes	Yes	5
(Conrad and Adams, 2012)	Yes	No	Yes	No	No	NA	Yes	2
(Igarashi, 2013)	Yes	Yes	Yes	No	No	NA	Yes	3
(Chen and Chen, 2015)	Yes	No	Yes	No	No	No	Yes	2
(Blackburn et al., 2017)	Yes	Yes	No	No	Yes	Yes	Yes	5

Abbreviations: NA, Not Applicable; Random, Randomization.

Preclinical studies

A total of 6 preclinical studies, 5 animal studies and 1 *in vitro* study that evaluated the antidepressant effect of LEO and some of the most abundant single compounds found in LEO were selected. Relevant information extracted from the preclinical studies is summarized in Table 13. The antidepressant effect of LEO and linalool was evaluated in mice in 3 studies (Komiya et al., 2006; Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013). Furthermore, the effect of LEO and two of its major components namely linalool and linalyl acetate, were evaluated in rats in 3 studies (Seol et al., 2010; Hritcu et al., 2012; López et al., 2017). Two different species of lavender were used to extract LEO, *Lavandula angustifolia* (3 studies) and *Lavandula hybrida* (1 study). The study of Komiya et al. (2006) did not report the species of lavender used to extract the LEO evaluated. Furthermore, single compounds such as linalool (Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013; López et al., 2017) and linalyl acetate (López et al., 2017) were also investigated.

The chemical profile of LEO and confirmation of the chemical nature of the isolated compounds tested were determined in 3 studies (Guzmán-Gutiérrez et al., 2012; Hritcu et al., 2012; López et al., 2017). Intraperitoneal administration of LEO and its isolated compounds was the method most frequently used in the selected studies (3 studies) (Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013) while inhalation aromatherapy was used in 2 studies (Komiya et al., 2006; Hritcu et al., 2012).

In addition to the investigation of the effect of LEO and its most common components, several biological analyses were performed. In the evaluation done by López et al. (2017), cortex homogenate from rat brains were used to study the molecular targets involved in the antidepressant effect *in vitro*.

The control groups considered in the LEO-intraperitoneally-administered studies consisted of different concentration of tween-80 (0.5 and 10%) (Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013) and almond oil (Seol et al., 2010). In the inhalation aromatherapy studies, ethanol (Komiya et al., 2006) and saline (Hritcu et al., 2012) were used as control groups. Two controls were used in the study carried out by Komiya et al. (2006), however, the nature of one of the control groups was not reported.

The treatment groups consisted of LEO alone, other essential oils such as rose, lemon, chamomile, clary sage and rosemary (Komiya et al., 2006; Seol et al., 2010; López et al., 2017). Also, the single compounds linalool and linaly acetate (López et al., 2017) when used alone and when combined with scopolamine were evaluated (Hritcu et al.,

2012). Guzmán-Gutiérrez et al. (2012) and Coelho et al. (2013) included single isolated compounds as the treatment groups.

Five out of the 6 preclinical studies evaluated the acute effect of LEO and its major compounds while 1 study focused on the chronic effect of LEO on depression-like behavior (Hritcu et al., 2012). The acute antidepressant effect of LEO administered by inhalation aromatherapy was investigated by exposure of the animals to 1 ml LEO mixed with ethanol in the study by Komiya et al. (2006). Nineteen minutes after exposure, the behavioral tests were carried out. In the study by Seol et al. (2010), LEO was intraperitoneally administered using 5, 10 and 20% LEO and the behavioral tests were carried out 60 min after the treatment. Coelho et al. (2013) and Guzmán-Gutiérrez et al. (2012) treated the animals with a single injection of linalool at different concentrations (10, 50, 54.8, 100, 173.2, and 200 mg/kg) followed by the behavioral test. The administration protocol used by Guzmán-Gutiérrez et al. (2012) included 3 sessions. The first administration of linalool was carried out 15 min before the pre-test, followed by a second and third administration 18h and 1h prior the behavioral test. Finally, the chronic effect of LEO was evaluated by Hritcut et al. (2012) by 1h daily exposure to 200 µl LEO for 7 consecutive days given in co-treatment with scopolamine (Hritcu et al., 2012).

Table 13. Description of the preclinical studies

Ref.	Animal description		Cell line	LEO		Treatment						
	Species (total no. of animals used)	Strain		Species or isolated compound	Chemical profile of LEO or isolated compound	Admin. method	Control	Treatment group(s)	Concentration of LEO or isolated compound	Admin. protocol	Acute effect	Chronic effect
(Komiya et al., 2006)	Male mice (25*)	ICR	NA	NIP	No	IA	- Control - Ethanol	1. LEO 2. Rose essential oil 3. Lemon essential oil	1 ml essential oil mixed with 1 ml ethanol	90 minutes before the behavioral test	✓	
(Seol et al., 2010)	Rats (10-12 per treatment group)	Sprague-Dawley	NA	*Lavandula angustifolia*	No	i.p.	Almond oil	1. LEO 2 Chamomile essential oil 3. Clary sage essential oil 4. Rosemary essential oil 5. Imipramine 6. Fluoxetine	5, 10 and 20% essential oil dissolved in almond oil.	1 injection 60 min before behavioral test	✓	
(Guzmán-Gutiérrez et al., 2012)	Male mice (70 and 60 *)	ICR	NA	Linalool	Yes	i.p.	0.5% tween 80 in saline solution	1. Linalool 2. Eucalyptol 3. Limonene 4. α-pinene 5. β-pinene 6. Imipramine	100 mg/kg linalool	3 times: 15 min after pre-test, 18 and 1 h before the FST	✓	
							0.5% tween 80 in saline solution	1. Linalool 2. Imipramine	54.8, 100, and 173.2 mg/kg linalool			
(Hritcu et al., 2012)	Male rats (50)	Wistar	NA	*Lavandula angustifolia* (LEO1) and *Lavandula hybrida* (LEO 2)	Yes	IA	Saline	1. Scopolamine (SCOP) 2. Silexan (capsules containing 80mg standardized LEO) + SCOP	200 µl LEO	1 h daily exposure for 7 consecutive days. Behavioral tests were done on the		✓

Ref.	Animal description		Cell line	LEO		Treatment						
	Species (total no. of animals used)	Strain		Species or isolated compound	Chemical profile of LEO or isolated compound	Admin. method	Control	Treatment group(s)	Concentration of LEO or isolated compound	Admin. protocol	Acute effect	Chronic effect
								3. LEO1 + SCOP 4. LEO2 + SCOP		last day 60 min after exposure.		
(Coelho et al., 2013)	Male mice (113)	Swiss	NA	Linalool	No	i.p.	10% tween 80	1. Linalool 2. Imipramine	10, 50, 100 and 200 mg/kg linalool (dissolved in 10% tween 80)	1 injection 30 min before the behavioral test	✓	
(López et al., 2017)	Male rat **	Sprague-Dawley	SH-SY5Y neuroblastoma cells	*Lavandula angustifolia* Linalool Linalyl acetate	Yes	*In vitro* assays	Different controls were used in the bioassay	1. LEO 2. Linalool 3. Linalyl acetate	Different concentrations were used in the bioassays.	Different incubation times were used in the bioassays	✓	

Abbreviations: IA, inhalation aromatherapy; i.p., intraperitoneal; NA; Not applicable; NIP: No information provided.
*: Only the number of animals used in the behavioral test in which LEO or its major components were evaluated was considered.
** Animals were used to obtain rat cortex suspensions for the target activity evaluation. The exact number of rat used was not stated.
# In addition to the ethanol control, another control was used. However, authors did not provide information on the conditions used for the control group.

A summary of the behavioral tests, bioassays, outcomes, and conclusion of the preclinical studies is provided in Table 14. Depression-like behavior was measured using two behavioral tests, namely the forced swimming tests in 4 studies (Komiya et al., 2006; Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Hritcu et al., 2012) and the tail suspension test in one study (Coelho et al., 2013). The majority of the animal studies (4 out of 5) demonstrated a significant reduction in depression-like behavior induced by LEO or its single compounds tested (Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Hritcu et al., 2012; Coelho et al., 2013). Interestingly, the study by Seol et al. (2010) showed a comparable effect between LEO and fluoxetine and imipramine which are commonly used antidepressants in clinics. Similar positive results were observed in the study by Gutiérrez et al. (2012) and Coelho et al. (2013). Both studies clearly demonstrated a significant reduction of depression like behavior induced by linalool at doses of 100 and 200 mg/kg to a level comparable to that of imipramine. The evidence from the animal studies strongly supports the positive effect of LEO and linalool in reducing depression-like behavior.

López et al. (2017) studied the molecular mechanism involved in the antidepressant effect of LEO, linalool and linalyl acetate. The authors found that LEO (4 and 8 µl/ml) and linalool (0.8 and 8 µl/ml) prevented the binding to the serotonin transporter and demonstrated a dose-dependent affinity of LEO (IC_{50} of 0.4 µl/ml), linalool (IC_{50} of 2.97 µl/ml) and linalyl acetate (IC_{50} of 0.74 µl/ml) to the NMDA receptor. Both findings strongly suggested that the mechanism of action behind the antidepressant effect of LEO and its most common compounds involved the inhibition of the serotonin transporter and the regulation of the NMDA receptor. Additionally, the

authors identified linalool and linalyl acetate as key players in the mechanism of action by which LEO exerts its antidepressant effects. Finally, the neuroprotective effect of LEO was demonstrated in a hydrogen peroxide toxicity test. Finally, no severe side effects were reported in any of the preclinical studies. However, Guzmán-Gutiérrez et al. (2012) showed that linalool induced sedation that reduced the exploratory activity of mice.

Table 14. Behavioral tests, bioassays, outcomes and conclusions from the *in vivo* and *in vitro* studies included

Reference	Behavioral tests	Bioassays	Outcome	Side effects or toxicity	Antidepressant effect
(Komiya et al., 2006)	FST	NA	LEO showed no effect on the immobility time in the FST.	NR	No
(Seol et al., 2010)	FST	NA	LEO decreased depression-like behavior in rats as observed in the shorter immobility time in the FST at all LEO concentrations tested. The reduced depression-like behavior was comparable to the effect of imipramine and fluoxetine (the conclusion was drawn from observation of the graph provided since no statistical comparison available was available).	NR	Yes
(Guzmán-Gutiérrez et al., 2012)	FST	NA	Linalool at a concentration of 100 mg/kg decreased depression-like behavior in the FST. The antidepressant effect of linalool was comparable to the effect of imipramine (no statistically significant difference between the imipramine and linalool groups).	Linalool showed sedative effect as observed in the reduced exploratory activity of mice in the exploratory cylinder test.	Yes
(Hritcu et al., 2012)	FST	NA	Silexan, LEO1 and LEO2 decreased depression-like behavior as observed in decreased immobility time in the FST	NR	Yes
(Coelho et al., 2013)	Tail suspension test (TST)		Linalool reduced the immobility time in the TST at doses of 100 and 200 mg/kg showing antidepressant-like activity. The antidepressant effect observed in the 100 and 200 mg/kg linalool groups is comparable to the effect of imipramine (no statistical comparison done between the linalool and imipramine groups, but the graph shows similar effect between the imipramine and high concentration of linalool groups).	Linalool showed no genotoxic effects.	Yes

Reference	Behavioral tests	Bioassays	Outcome	Side effects or toxicity	Antidepressant effect
(López et al., 2017)	NA	Serotoninergic targets 1. Monoamine Oxidase-A (MAO-A) inhibition assay 2. Serotonin transporter assay Ionotropic receptors 1. Affinity for $GABA_A$ receptor 2. Affinity for NMDA receptor Neuroprotection on SK-SY5Y cells 1. MTT cell viability assay	LEO did not show any inhibitory effect on MAO-A. In the serotonin transporter assay, the dose-dependent effect of LEO and linalool on the displacement of ^{3}H-citalopram from binding to the serotonin transporter was observed. The findings suggest that LEO and linalool exert antidepressant properties by preventing binding to the serotonin transporter. Linalyl acetate did now show affinity to the serotonin transporter. LEO, linalool and linalyl acetate didn't show affinity to the $GABA_A$ receptor. LEO, linalool and linalyl acetate showed dose-dependent affinity to the NDMA receptor. LEO showed neuroprotective properties against hydrogen peroxide toxicity in SK-SY5Y cells.	NR	Yes

Abbreviations: FST, forced swimming test; NA; Not applicable; NR, not reported; TST, tail suspension test.

3.9. Discussion

LEO has been reported as the most frequently used essential oil in clinical trials evaluating the effect of aromatherapy on depressive symptoms (Sánchez-Vidaña et al., 2017). LEO is highly preferred because of its relaxing, sedative and antidepressant properties (Perry and Perry, 2006). The present systematic review aimed to analyze the findings of preclinical and clinical studies on the effect of LEO and its most common compounds on depressive symptoms. Additionally, the molecular mechanism behind the effects of LEO and its major components were examined.

Analysis of the clinical evidence

Positive effects of LEO on the alleviation of depressive symptoms were observed in the clinical studies included in the present systematic review. However, there were limitations that need to be considered. First of all, only two RCT studies evaluated the

effect of either LEO alone (Diego et al., 1998) or lavender-derived tea (Chen and Chen, 2015). Although positive effects were observed in other studies, the effect cannot be fully attributed to LEO alone. A study included LEO as one of the options to choose from (Blackburn et al., 2017). However, the positive effect observed stemmed from a combined effect of all the essential oil options provided and, thus, cannot be attributed to the contribution of LEO alone. Another study that showed positive effects evaluated a mixture of essential oils including LEO which was administered by inhalation aromatherapy and massage aromatherapy (Conrad and Adams, 2012). Another concern in the study of Conrad and Adams' (2012) study is that the overall conclusion considered the combined effect of both the inhalation aromatherapy and the massage aromatherapy interventions. The main issue with the combined analysis of the modalities of aromatherapy is the impossibility of discriminating the added effect of the massage alone since a no massage group was not included as a control group. A recommendation for future clinical trials evaluating the effect of LEO would be to include LEO alone in a treatment group and a massage group without aromatherapy when investigating massage aromatherapy. Another concern arising from the analysis of the clinical evidence is the low quality of the studies that demonstrated a reduction in the depressive symptoms. Three out of the 4 RCT studies that showed positive effects obtained low scores, 1 and 2, in the Jadad scale and only 1 study scored 5. Therefore, the need for higher quality clinical trials to be used in evaluating the effectiveness of LEO on depressive symptoms is apparent. The overall analysis of the clinical evidence suggested that LEO administered by inhalation aromatherapy, aromatherapy massage or lavender drank in the form of tea showed promising effects. However, the inclusion of other essential oils, the use of a mixture of essential oils together with LEO as well as the low quality of the studies indicate that a solid

conclusion cannot be made on the effectiveness of LEO in relieving depressive symptoms in clinics.

The findings of previous studies on the effect of LEO on mood have contributed to an understanding of the effect of LEO and support the analysis done in the current systematic review. The antidepressant, anxiolytic, and sedative effects of LEO have been previously reported (Perry and Perry, 2006; Kageyama et al., 2012; Taavoni et al., 2013; Wu et al., 2014). The complex mixture of volatile compounds found in LEO contains around 59 chemicals from which linalool and linalyl acetate are the most abundant (Buchbauer et al., 1991; Chatzopoulou and Goliaris, 2003; Perry et al., 2012). Linalool has sedative effects while linalyl acetate has shown narcotic properties and the evidence from the present systematic review suggests that both compounds contribute to the biological activity of LEO (Ali et al., 2015).

Analysis of the preclinical evidence

The findings of the preclinical studies showed strong evidence of the effect of LEO and its major compounds in reducing depressive-like behavior in contrast with the promising but inconclusive evidence from the RCT studies.

Four of the 5 animal studies selected showed positive effects of LEO and linalool on the alleviation of depression-like behavior (Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Hritcu et al., 2012; Coelho et al., 2013). The forced swimming test and the tail suspension test are behavioral tests commonly used in academia and industry to screen compounds with antidepressant activity (Can et al., 2012a, 2012b; Slattery and Cryan, 2012). Interestingly, three of the 4 animal studies that demonstrated the antidepressant

effect of LEO included treatment groups that received the antidepressants imipramine and fluoxetine and the comparison between the aromatherapy treatment and the antidepressant treatment was very promising. The tricyclic antidepressant imipramine introduced in the 1950s, and the serotonin reuptake inhibitor fluoxetine first described in 1974, are widely used in clinical practice (Preskorn, 1995; Dean, 2012; Magni et al., 2013). Therefore, including imipramine or fluoxetine as positive controls in preclinical trials is a useful practice.

The evidence from the preclinical studies does not only support the positive effect of LEO and linalool, but also demonstrate a promising antidepressant effect compared with gold standard antidepressants. Furthermore, the molecular mechanism of LEO and its major components have been previously investigated and one study was included in the present systematic review. In the study by López et al. (2017), the effect of LEO, linalool and linalyl acetate on MAO-A, the serotonin transporter and the NMDA receptor were analyzed (López et al., 2017). The importance of screening the potential antidepressant effect of novel alternatives should include the above-mentioned targets as they are mostly involved in the molecular mechanism of most of the antidepressants currently available. The results reported by the authors provide strong evidence that supports the antidepressant effect of LEO, linalool and linalyl acetate. Firstly, the inhibitory effect of LEO and linalool on the serotonin receptor was clearly demonstrated. The relevance of the inhibition of the serotonin transported for depression has been previously established. (Owens and Nemeroff, 1994) and the findings from the *in vitro* study showed that the serotonin receptor is targeted by LEO and linalool. Secondly, LEO, linalool and linalyl acetate exhibited a regulatory effect on the NMDA receptor. Stimulation of the NMDA receptor is, particularly, important

in the treatment of depression as it causes rapid antidepressant effect (Ates-Alagoz and Adejare, 2013).

The findings of Lopez et al.'s (2017) study on the molecular mechanism, the evidence from the animal studies and the promising, although not conclusive, evidence from the clinical studies support the antidepressant effect of LEO and its major most common compounds.

3.10. Conclusions

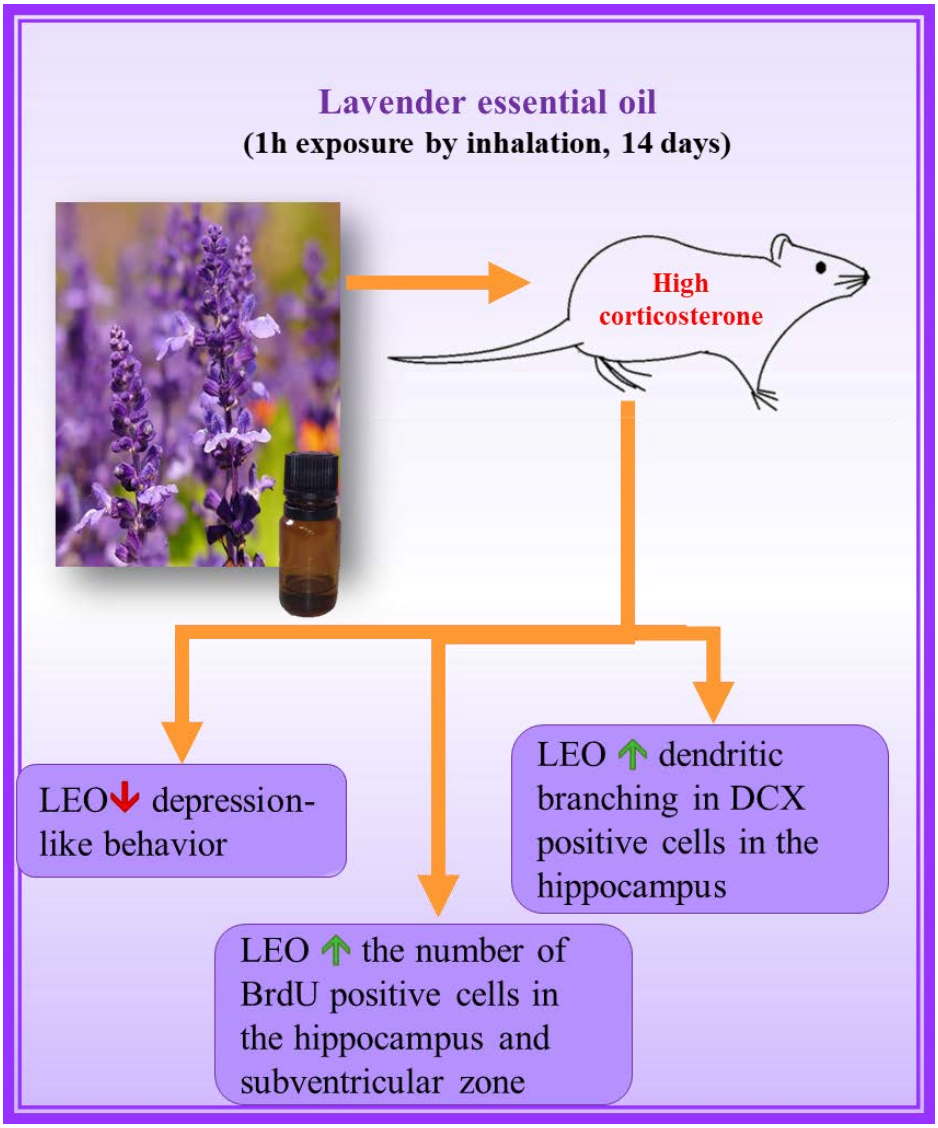
The present systematic review is one of the first studies to analyze the preclinical and clinical evidence of the effect of LEO and some of its most common compounds in reducing depressive symptoms. One of the highlights of the present systematic review is the analysis of the preclinical studies which clearly demonstrated the positive effect of LEO and linalool in mitigating depression-like behavior compared with the effect of commercial antidepressants. Also, the relevant targets for depression were analyzed in one study providing further evidence that supports the antidepressant effect of LEO and its single compounds linalool and linalyl acetate. Finally, despite the inconclusive evidence from the RCT studies, the evidence presented suggest a promising antidepressant effect of LEO than needs further investigation.

CHAPTER 4

The content of his chapter was sent for publication and is under review.

LAVENDER ESSENTIAL OIL ABOLISHES HIGH DOSE CORTICOSTERONE-INDUCED DEPRESSION-LIKE BEHAVIOR AND REDUCTION OF NEUROGENESIS IN RATS

Sánchez-Vidaña DI, Po KKT, Fung TKH, Chow JKW, Lau WKW, So PK, Lau BWM, Tsang HWH. **(Under review)**



Abstract

Background: Depression is a global health issue with negative societal and economic health burden. Aromatherapy, a practice that uses essential oils for preventive and therapeutic purposes, represents a promising therapeutic alternative for the alleviation of depressive symptoms. Lavender essential oil (LEO) has been the focus of clinical studies due to its positive effect on mood.

Aims: To evaluate the effect of LEO on depression and anxiety-like behaviors and neurogenesis in a high dose corticosterone animal model.

Materials and methods: Adult male Sprague Dawley rats (n = 24) were assigned into 4 groups (n = 6 animals per group): Control, corticosterone (CORT), LEO administered by inhalation, and LEO+CORT. Exposure to vehicle or LEO was for 1h for 14 consecutive days. At the end of the treatment period, behavioral tests were carried out. After perfusion, brains and serum samples were collected for immunohistochemical analysis to detect BrdU and DCX positive cells in the hippocampus and subventricular zone and biochemical analyses to measure BDNF, corticosterone and oxytocin in serum.

Results: Treatment with LEO reverted the depression-like behavior and the CORT-induced decreased number of BrdU positive cells in the hippocampus and the SVZ as observed in the LEO+CORT group. Increased dendritic complexity of immature neurons was observed in the LEO+CORT group. The levels of BDNF were upregulated in LEO and LEO+CORT groups. LEO alone upregulated the levels of oxytocin in serum.

Conclusions: The present study has provided evidence of the biological effect of LEO on neuroplasticity and neurogenesis. Also, this study contributes to the understanding of the mechanism of action of LEO in an animal model for depression.

4.1. Introduction

Depression is an affective disorder characterized by symptoms such as sadness, anhedonia, difficulties to concentrate and changes in appetite that last for more than 2 weeks (Baquero and Martín, 2015; Ren et al., 2015; Tizabi, 2015; Qaseem et al., 2016). Antidepressants are the first line of treatment for patients with depression (Richelson, 2013). Even though numerous pharmacological options have been developed since the mid-1950s, there are still concerns about the effectiveness and efficiency of antidepressants (Wilkinson, 1995; Arroll et al., 2005; Chan et al., 2015). Due to the drawbacks of antidepressants, novel and more effective treatment options are urgently needed.

Aromatherapy is a form of CAM that uses essential oils for the prevention and treatment of a wide variety of health conditions (Ali et al., 2015; Koo, 2017). Administration of aromatherapy can be done orally, topically or inhaled (Tisserand and Balacs, 1995; Louis and Kowalski, 2002; de Groot and Schmidt, 2016). The evidence from clinical trials shows the promising therapeutic effect of aromatherapy in reducing depressive symptoms (Sánchez-Vidaña et al., 2017). Lavender essential oil (LEO) is an essential oil commonly used in aromatherapy and has been shown to have anxiolytic and antidepressant effects in preclinical and clinical studies (Andrade et al., 1999; Cavanagh and Wilkinson, 2002; Perry et al., 2012; Greenberg and Slyer, 2017). However, the mechanism behind the antidepressant effect of LEO remains unexplored.

Neurogenesis, a neuroplasticity process, is an important physiological target for the screening of drugs with potential antidepressant effect (Wainwright et al., 2013). In adult mammals, neurogenesis occurs in the dentate gyrus of the hippocampus and the subventricular zone along the lateral ventricle (Aimone et al., 2014; Apple et al., 2016). Improved neurogenesis in the hippocampus has been correlated with the use of antidepressants (Hanson et al., 2011). A key player in the regulation of neurogenesis is the brain-derived neurotrophic factor (BDNF) (Calabrese et al., 2009; Lee and Kim, 2010; Hanson et al., 2011). In animal studies, downregulation of BDNF induced by stress has been identified as a causative factor in depression (Calabrese et al., 2009; Lee and Kim, 2010). On the other hand, the beneficial effect of antidepressants has been closely linked to upregulation of BDNF (Calabrese et al., 2009). Another key player to consider in depression is oxytocin, a neuropeptide that modulates social interaction and reproductive behavior (Wacker and Ludwig, 2012). Improvement in social behavior has been observed after nasal administration of oxytocin in animals and human subjects (Leuner et al., 2012; Wacker and Ludwig, 2012). Furthermore, positive effect on behavior has been observed as a result of oxytocin protective effect against stress (Kirsch et al., 2005) and oxytocin-induced stimulation of hippocampal neurogenesis (Leuner et al., 2012; Sánchez-Vidaña et al., 2016). Because essential oils have shown positive effects on mood (Louis and Kowalski, 2002; Burnett et al., 2004) and oxytocin is an important regulator of social behavior via the olfactory system (Wacker and Ludwig, 2012), oxytocin represents an interesting target worthy of investigation.

At present, no previous study has evaluated whether neurogenesis could be the physiological process involved in the antidepressant effect of LEO. Also, the molecular key players associated with LEO had not been previously explored. The present study, therefore, aimed to evaluate the behavioral and neurogenic effect of LEO in a high corticosterone animal model of depression. Furthermore, the mechanism of action behind the effect of LEO was investigated.

4.2. Materials and methods

Animals

Adult male Sprague Dawley rats (240-260 g body weight) were purchased from the Centralized Animal Facility (CAF) at the Hong Kong Polytechnic University. The animals were grouped in 3 rats per cage and kept under 22±2°C and 12h dark/light cycle (lights on at 7 am). Food and tap water was provided *ad libitum*.

Drugs

The LEO was purchased from DK aromatherapy and extracted from flower tops of *Lavandula aungustifolia* Mill. (syn. *Lavandula officinalis* Chaix). The essential oil was stored at room temperature protected from the light. A 1% solution of Tween 20 was used to prepare a 2.5% LEO that was used in the treatments. Corticosterone was obtained from Sigma Aldrich. For the chemical analysis, LEO was diluted in hexane (Duksan Reagents).

Chemical characterization of LEO

GC-MS was used to qualitatively characterize the LEO used in the present study. A 1:200 dilution in hexane was prepared. The diluted LEO was analyzed in an Agilent

7890A GC coupled to a Waters GCT Premier electron ionization-time-of-flight mass spectrometer. Chromatographic separation was performed using an Agilent HP-5ms GC column (30 m length x 0.25 mm ID x 0.25 μm film thickness) and helium as carrier gas. The temperature program for chromatographic separation was initially held at 30° C for 1 minute, raised to 270°C with a rate of 10°C/min, and finally held at 270°C for 10 minutes (totally run time 35 minutes). The electron energy of the electron ionization source of the mass spectrometer was set at 70 eV. The mass spectrometer was first externally calibrated with heptacosa. During data acquisition, internal calibration with the heptacosa was enabled. Identification of LEO was carried out by performing a database search within the National Institution of Standards and Technology (NIST) library (version 2008) (Shellie et al., 2000; Chatzopoulou and Goliaris, 2003).

Experimental design

The effect of LEO on depression and anxiety-like behaviors was studied using a high corticosterone animal model (Gregus et al., 2005; Brummelte et al., 2006; Lee et al., 2013). A dose of 40 mg/kg corticosterone was used to induce depression- and anxiety-like phenotype in rats (Sánchez-Vidaña et al., 2016). The animals were randomly allocated into 4 groups (n = 6 rats per group): (1) the control group received a subcutaneous (s.c.) injection of propylene glycol (vehicle) and 1 hour exposure to a cotton saturated with 1% tween-20; (2) the corticosterone group (CORT) received a daily s.c. injection of 40 mg/kg corticosterone and 1-hour exposure to a cotton saturated with 1% tween-20; (3) the LEO group was exposed to a cotton saturated with 2.5% LEO and received a s.c. injection of propylene glycol; (4) the CORT+LEO was administered a s.c. injection of 40 mg/kg CORT and exposed to a cotton impregnated with 2.5% LEO. CORT was administered 20 min after exposure to either

vehicle or LEO (Hancianu et al., 2013). The administration of the different treatments was done daily for 14 consecutive days. An intraperitoneal injection of 50 mg/kg/day BrdU was administered to all the animals at days 12, 13 and 14 in order to label the proliferating cells (Taupin, 2007). The behavioral tests were performed on days 15 and 16. After assessment of behavior, intracardial perfusion on day 16 and 17 was carried out and the brains, adrenal glands and plasma were collected.

Inhalation equipment and treatment administration

Individually, rats were introduced into the inhalation equipment made of an acrylic fiber box (42 x 30 x 29 cm). The front and back walls of the chamber had four holes (2 cm diameter/hole) with a perforated acrylic fiber wall placed 3 cm apart from the front and back walls. The cotton soaked with the respective treatment was placed between the outer and inner walls and out of the animals' reach (Figure 11). In order to allow proper ventilation in the chamber, the top wall contained 25 small holes (Tsang et al., 2013). All animals were exposed to the 1 ml of vehicle or 1 ml of 2.5% LEO for 60 min. The dose and the exposure time were established based on previous studies (Linck et al., 2010; Hritcu et al., 2012; Hancianu et al., 2013) and based on the analysis of the *in vivo* studies included in CHAPTER 3b. Two different chambers were used: one exclusively assigned for the treatment with vehicle and the other one for the treatment with LEO. After each animal was exposed to the treatment, the equipment was cleaned with 70% ethanol.

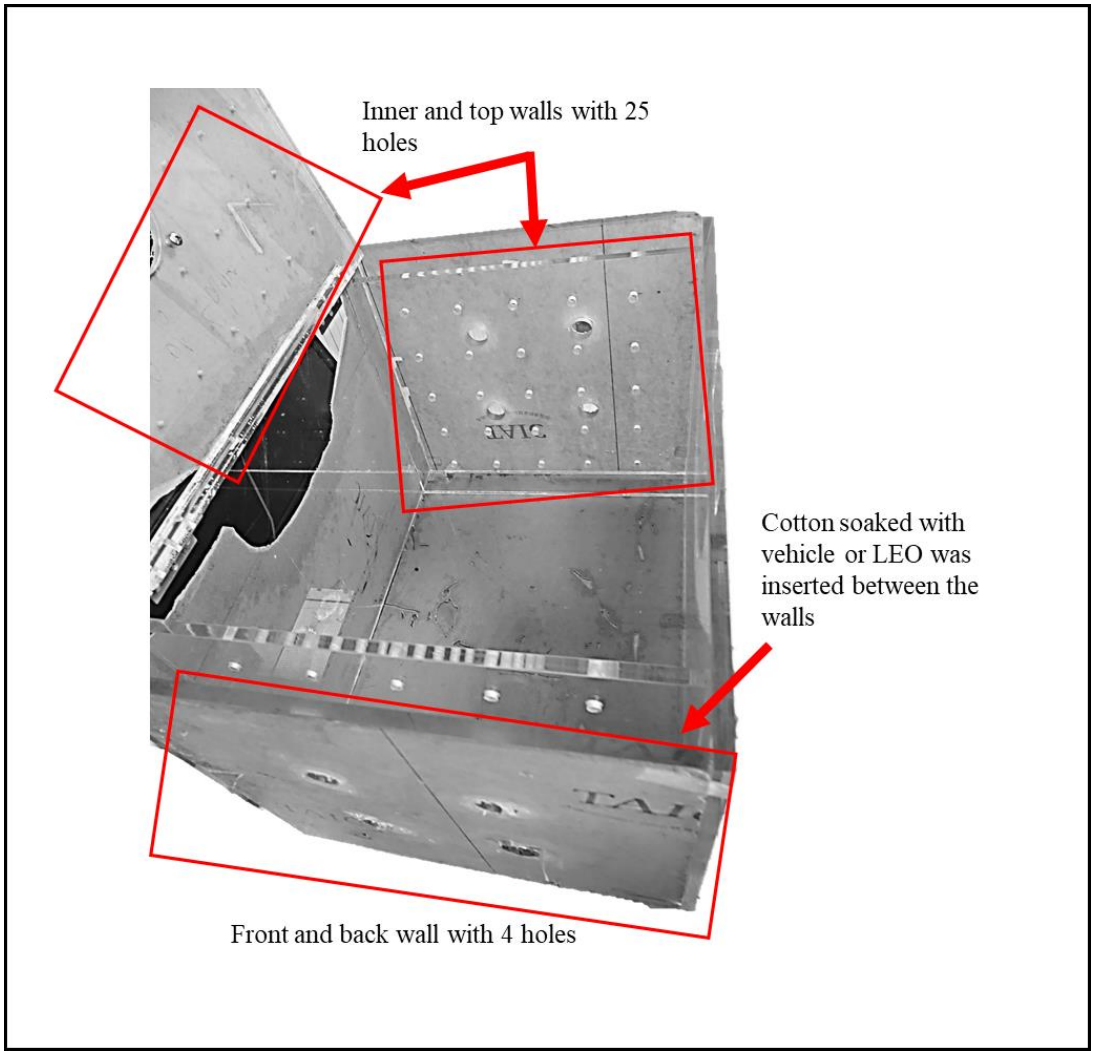


Figure 11. Inhalation equipment

Behavioral tests

Forced Swimming Test (FST): The assessment of depressive-like behavior was performed by using the FST as previously described (Hansen et al., 1997; Gregus et al., 2005; Brummelte et al., 2006; Slattery and Cryan, 2012). The equipment for the FST consisted of a vertical transparent (40 cm height x 30 cm diameter) filled with tap water (room temperature) at a depth of 30 cm. Two sessions were involved in the FST: (1) a 15 min long pre-test to induce a state of helplessness followed by (2) a 10 min test carried out 24h after the pre-test. The second session was video recorded for later analysis by an experimenter blinded to the treatment groups. The following

behaviors were scored: (1) time spent immobility or floating characterized by the minimal to absence of movement to keep from drowning; (2) time spent swimming characterized by active movement of both the fore and hind limbs in a paddling manner, the movement is more than necessary to keep the head above the water, but less motion as the one shown in climbing or struggling behavior; (3) time spent climbing or struggling which is characterized by fast and vigorous movements of the limbs breaking the surface of the water in an attempt to get out of the container. Depression-like behavior is observed when the animals have a longer time of immobility (Gregus et al., 2005; Lau et al., 2011b).

Social Interaction Test (SIT): Anxiety-like behavior was assessed as previously described using the SIT (Becker et al., 1999; Gregus et al., 2005). An open-field arena of 72 cm length x 72 cm width x 40 cm depth with a camera mounted on top of the field was used to record the behavior to be scored by an experimenter blinded to the treatment groups. Two unfamiliar rats were placed in the test arena for 10 min and the initiation of the following behavior was scored: (1) positive social behaviors including sniffing, following, crawling, social play, and grooming; (2) aggressive behavior comprising kicking, boxing, wrestling, biting; (3) no interaction identified when the rats were apart from each other. Increased anxiety-like behavior is shown with a low number of positive social behaviors (Becker et al., 1999; Gregus et al., 2005; Bailey and Crawley, 2009).

Animal perfusion and tissue processing

A high dose of sodium pentobarbital (200 mg/kg, intraperitoneal) was injected intraperitoneally. Samples from truncal blood were taken and centrifuged at 1,000 x g for 15 minutes to collect the serum and later stored at -80°C. The serum was used

to carry out biochemical analyses to determine the concentration of BDNF, corticosterone, and oxytocin. Transcardial perfusion was performed using 4% paraformaldehyde as previously described (Leuner et al., 2009; Gage et al., 2012). The dissected brains were post-fixed overnight at 2-8°C in 4% paraformaldehyde. After post-fixation, the brain tissues were stored in cryoprotectant solution (30% sucrose solution in 0.1M phosphate-buffered saline) and stored at 2-8°C (Hillerer et al., 2014). The adrenal glands were collected and stored at -80°C. Coronal brain sections, 40 μm thick, were prepared using a Cryostat in a 1-in-12 series of consecutive sections using a Cryotome E (Thermo Electron Corporation) and stored in the cryoprotectant solution at -20°C. Sections of the hippocampus and sub ventricular zone were fixed on glass slides coated with gelatin for immunostaining.

Immunoperoxidase staining of BrdU positive cells

Detection of BrdU (neuronal cell proliferation marker) positive cells was performed as previously described (Lau et al., 2012). Hippocampal and subventricular zone sections mounted on glass slides were rinsed (3 times for 10 min) with 0.01M PBS. The slides were incubated in preheated citrate buffer at 90°C for antigen retrieval. DNA denaturation was carried out by incubating the slides in 2N HCl at 40°C for 30 min. Neutralization of the acid was done by incubating the slides in 0.1M borate buffer for 15 min at room temperature. After neutralization, the slides were washed (3 times for 10 min) with 0.01M PBS before incubation with 1:1000 mouse anti-BrdU antibody (Roche) overnight. After incubation, slides were washed (3 times for 10 min) with 0.01M PBS and the slides were incubated with 1:200 biotinylated goat anti-mouse antibody (Dako) for 2 h at room temperature. After incubation with the secondary antibody, the slides were washed (3 times for 10 min) with 0.01M PBS. Signal amplification was performed using an avidin-biotin complex system (Vector) to

visualize the BrdU labeled cells with diaminobenzidine hydrochloride as the chromogen (Lau et al., 2011a; Leuchtweis et al., 2014). Immunostained sections were air dried, counter-stained with 10% eosin in 70% ethanol for 3 min, dehydrated as follows (3 min incubation in every solvent): 2 times in 90% ethanol, 3 times in 100% ethanol and 3 times in xylene at room temperature. DPX mounting media was used to coverslip the slides (Thermo Scientific) (Hattiangady and Shetty, 2010).

Immunoperoxidase staining of DCX positive cells

Detection of immature neurons was carried out by immunostaining of doublecortin (DCX) which is a protein expressed by immature neurons (Lau et al., 2011a). The DCX immunostaining protocol is similar to the BrdU protocol except that the incubations with HCl and borate buffer were skipped and the primary and secondary antibody used were rabbit anti-DCX antibody (1:300, Cell signaling) and goat anti-rabbit biotinylated antibody (1:200, Dako) respectively (Lau et al., 2011a).

Quantification of BrdU and DCX positive cells

Quantification of BrdU and DCX positive cells was done using the optical fractionator probe in an Stereo Investigator system (version 11, MBF Bioscience) as previously described (Sánchez-Vidaña et al., 2016). Brain sections from six animals per group were analyzed including 6 hippocampus sections and 4 SVZ sections per animal. The BrdU positive cells were identified as dark-brown round spots along the dentate gyrus of the hippocampus and the SVZ. In the hippocampus, the total number of BrdU cells, automatically calculated by the software, was estimated by counting the BrdU positive cells in 6 sections, and then calculating the average number of cells, and multiplying the average number of cells by 12 since the systematic sampling was done on every 12^{th} section. In the SVZ, the average number of BrdU positive cells per section was

calculated by taking the total number of cells counted per section (4 SVZ sections in total) and calculating the average number of cells. The DCX positive cells were identified as the whole cell, including the cell body and dendrites, and they were stained. The counting procedure used to quantify the BrdU positive cells was used to quantify the DCX positive cells in both regions of the brain. The following settings were used in the optical fractionator probe for counting both BrdU and DCX positive cells: dissector height of 25 μm and 60X60μm sampling site size. In the hippocampus, the total number of positive BrdU and DCX cells was expressed as mean ± SEM. In the SVZ, the average number of BrdU or DCX positive cells per section was expressed as mean ± SEM.

Dendritic complexity of immature neurons

Sholl analysis, a tool used to determine changes in the neuronal dendritic arborization, involves a systematic procedure to count the number of intersections of the dendrites to concentric circles drawn at fixed distances from the soma (Sholl, 1953; O'Neill et al., 2015). Images of immature neurons exhibiting tertiary of higher dendrite arborization with untruncated dendrites were captured at 40X magnification. A total of 10 neurons per animal was selected (60 neurons per treatment group) (Ramirez-Rodriguez et al., 2011). Images of the selected immature neurons were processed using the NeurphologyJ plugin for the ImageJ software (Gutierrez and Davies, 2007; Meijering, 2010). The assessment of the spatial distribution and dendritic complexity were carried out by tracing the cell body and dendrites in the software (Meijering, 2010; Lau et al., 2011a). The fixed settings for the Sholl analysis included a starting point of 10 μm, a step size of 10 μm and an ending radius of 200 μm. Additional measurements comprise the distance from the soma to each intersection and the total number of intersection that corresponds to the number of times the dendrites

intersected the fixed concentric circles. The Sholl analysis was performed by an experimenter blinded to the treatments. A high dendritic complexity is reflected in a high number of intersections.

Biochemical analyses in serum

ELISA kits were used to determine the concentration in serum of BDNF (Millipore), corticosterone (Enzo Life Science) and oxytocin (Enzo Life Science) following the instructions from the manufacturers of the kits.

Statistical analysis

The statistical analysis was carried out using the SPSS software (version 13.0). One-way ANOVA with Tukey posthoc test was used when the data to be analyzed met the criteria of homogeneity and equal variance. In the event that either the assumptions of normality, checked by Shapiro-Wilks test, or homogeneity of variance, checked by Levene's test, were not met, the non-parametric tests Kruskal-Wallis and Mann-Whitney U were used. Significant difference was determined using a p-value < 0.05.

4.3. Results

Chemical characterization of LEO

The chemical profile of the LEO used in the present study was determined by qualitative GS-MS analysis. Linalool, β-pinene, o-cymene, α-ocimene, β-ocimene, allo-ocimene, α-terpineol, camphene, δ-3-Carene, γ-terpinene, terpinen-4-ol, Caryophyllene, β-humulene were identified in LEO. The chemical characterization of LEO is in line with the chemical profile of LEO reported in previous studies (Shellie

et al., 2000, 2002; Chatzopoulou and Goliaris, 2003; Zagorcheva et al., 2013; Khairul et al., 2015).

Body weight and adrenal gland weight

Before the treatment started, the body weight of the animals was measured and no significant difference in the body weight was found among the groups (Figure 12 A). At the end of the treatment, no difference in the weight of the animals was observed. However, a significant difference in the weight gain was found (Figure 12 B, p = 0.001). Significant difference in the weight gain was observed between the control (mean ± SEM; 59.3 g ± 7.11) and CORT (34.6 g ± 3.4) groups in which the CORT group showed lower weight gain. Similarly, a significant difference (p = 0.024) was found between the CORT+LEO (38 g ± 4.57) and the control group (59.3 g ± 7.11). On the contrary, treatment with LEO alone (59.66 g ± 2.92) did not show any difference when compared to the control group (59.3 g ± 7.11). A significant difference was observed among the groups in the adrenal gland weight (Figure.12 C and D; p = 0.001). The lowest adrenal gland weight was found in the CORT group (5.53 g ± 0.61) and was statistically different when compared with the control group (p = 0.04). No significant difference in the adrenal gland weight was found when comparing the control (9.56 g ± 0.79) and LEO group (10.22 g ± 0.71). Interestingly, the comparison between the control (9.56 g ± 0.79) and CORT+LEO (6.86 g ± 0.78) showed no significant difference.

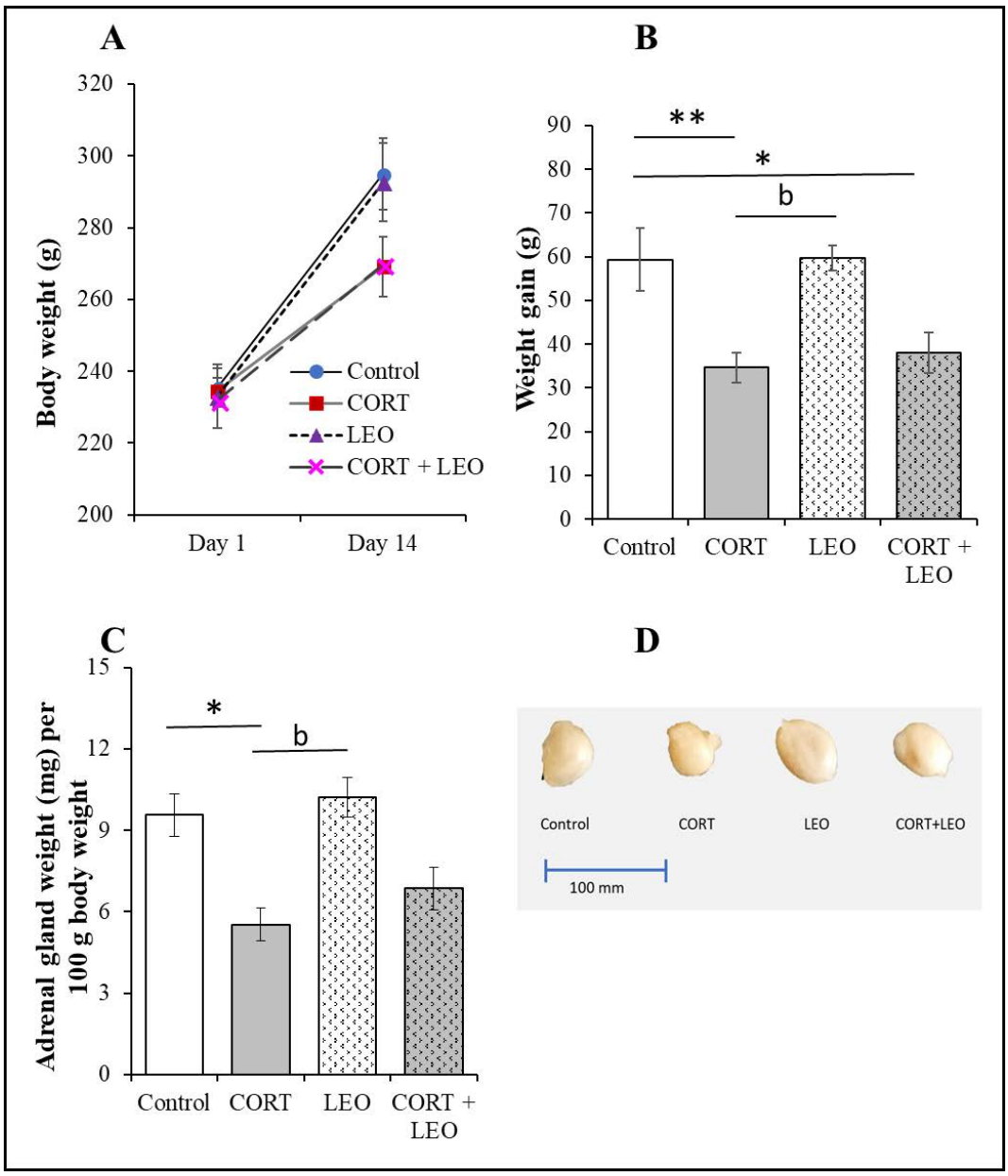


Figure 12. Body weight and adrenal gland weight. Treatment with LEO protected against the negative effects on behavior and neurogenesis induced by high CORT treatment, but LEO did not protect against the decreased weight gain induced by CORT. **(A)** Body weight at day 1 and 14. **(B)** Weight gain. **(C)** Adrenal gland weight. **(D)** Representative images of the adrenal glands per treatment group. Results are expressed as Mean ± SEM; **p* < 0.05 and ***p* < 0.01 when compared to the control group; **b** *p* < 0.05 when compared to the CORT group.

Behavioral tests

The time spent floating in the FST showed a significant difference among the groups in the FST (Figure 13 A, $p = 0.004$). The longest time spent floating was observed in the CORT group (201.33 sec ± 19.19) indicating depression-like behavior and it was statistically different ($p = 0.004$) when compared with the control group (99.60 sec ± 11.52). The findings showed more severe depression-like behavior in the CORT group. Co-treatment with LEO reverted the CORT-induced depression-like behavior and this was shown as decreased time spent floating (129 sec ± 10.05) which was comparable to the floating time in the control group (99.60 sec ± 11.52). Also, the time spent floating in the LEO group (119.25 sec ± 8.04) was comparable to that of the control group (99.60 sec ± 11.52). In the SIT, no aggressive behavior was displayed by all the animals. The SIT assesses anxiety-like behavior and a significant difference among the groups was observed with regard to the number of positive social interactions (Figure 13 B, $p = 0.013$). The total number of positive social interactions was significantly reduced in the CORT treatment (9.67 ± 2.30) when compared with the control group ($p = 0.035$, 17 ± 2.30) indicating increased anxiety-like behavior in the CORT group. Conversely, co-treatment with LEO reverted the reduced number of positive social interactions, therefore it reduced the anxiety-like behavior induced by CORT in the CORT+LEO group (16.40 ± 2.15). LEO treatment did not affect the number of positive social interactions (18 ± 1.50) but showed significant difference when compared with the CORT group ($p = 0.013$). No significant difference was found between the CORT and the CORT+LEO group ($p = 0.074$), but the increased number of positive interactions in the CORT+LEO group was comparable to the control group showing no difference.

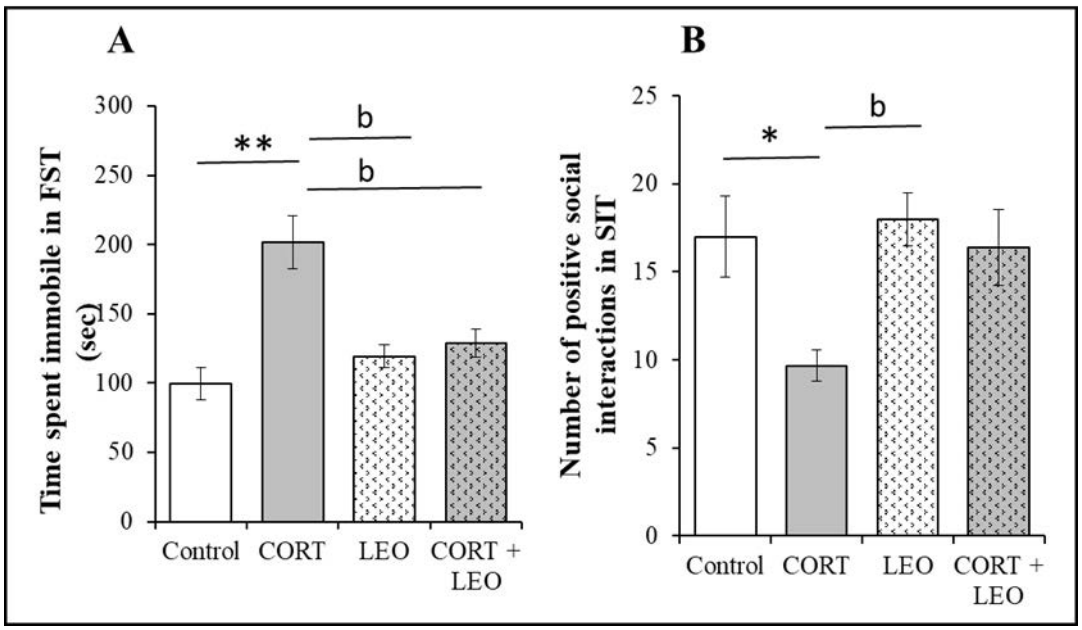


Figure 13. Results from the forced swimming test and social interaction test. **(A)** Results from the FST showing the time spent floating. **(B)** Total number of positive social interactions in the SIT.

BrdU and DCX positive cells

A significant difference in the total number of BrdU positive cells in the hippocampus was observed (Figure 14 A, $p = 0.021$, Figure 15). Treatment with CORT significantly reduced the cell proliferation in the hippocampus as reflected in the reduced number of BrdU positive cells (1354.8 ± 130.38) when compared with the control group (2556 ± 389.46) ($p = 0.016$). Treatment with LEO alone did not affect the number of BrdU positive cells (1917.75 ± 252.14). Interestingly, LEO treatment reverted the CORT-induced reduction in the number of BrdU positive cells in the LEO+CORT group (3069.33 ± 557.85; $p = 0.016$). The findings demonstrate that treatment with LEO protected the hippocampus against the negative effects induced by CORT by stimulating cell proliferation.

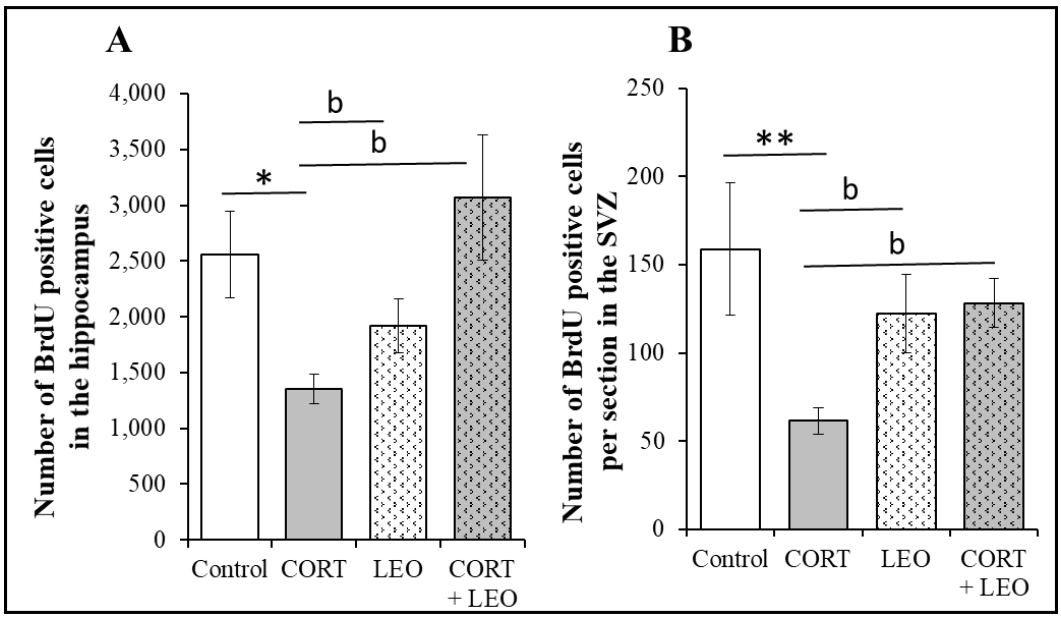


Figure 14. Number of BrdU positive cells in the hippocampus and SVZ

In the SVZ, the number of BrdU positive cells showed a significant difference (Figure 14 B, p = 0.006, Figure 15). The number of BrdU positive cells was significantly reduced in the CORT group (61.5 ± 7.62) when compared with the control group (158.80 ± 37.66, p = 0.004). Treatment with LEO alone did not affect the number of BrdU positive cells in the SVZ (122 ± 22.26). However, LEO showed a positive effect on cell proliferation in the SVZ by reverting the CORT-induced reduction in the number of BrdU positive cells as shown in the CORT+LEO group (128.20 ± 13.73) when compared with the control group (158.80 ± 37.66).

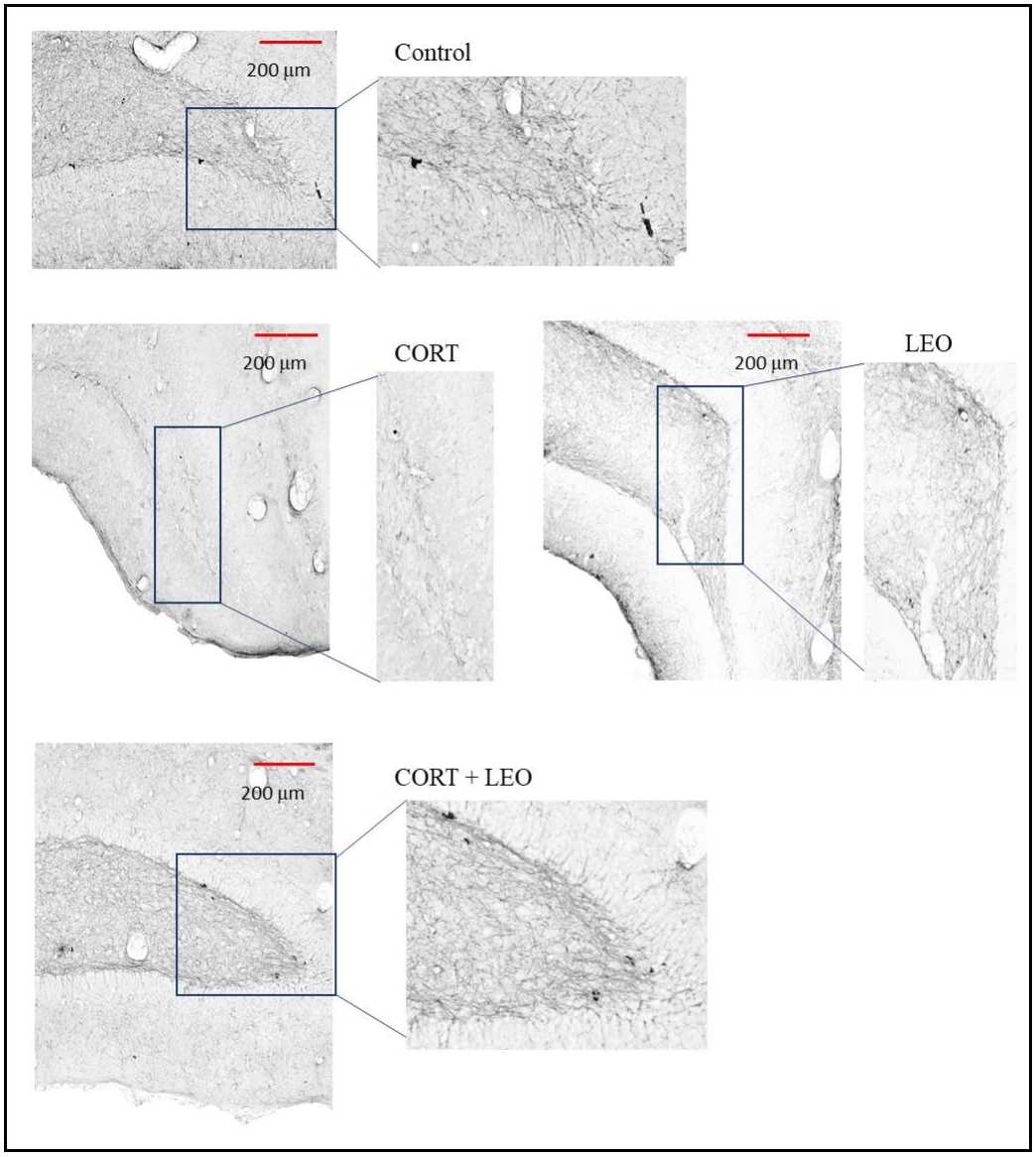


Figure 15. Representative images of the BrdU positive cells in the dentate gyrus of the hippocampus

DCX is a marker of immature neurons. In the hippocampus, a significant difference was found in the total number of DCX positive cells (Figure 16 B, $p = 0.011$, Figure 17). Treatment with CORT had a negative impact on the number of immature neurons in the hippocampus (3720 ± 948.68; $p = 0.042$) and it showed significant difference when compared with the control group (8335.33 ± 372.45). Treatment with LEO alone did not affect the number of DCX positive cells (8131.75 ± 2247.03) when compared

with the control group. Treatment with LEO alone did not have any effect on the number of immature neurons (8131.75 ± 2247.03). However, significant difference was observed in the number of DCX positive cells in the CORT+LEO group (3716 ± 552.21) when compared with the control group (8335.33 ± 372.45; p = 0.041), demonstrating that treatment with LEO did not revert the CORT-induced negative effect on the number of immature neurons.

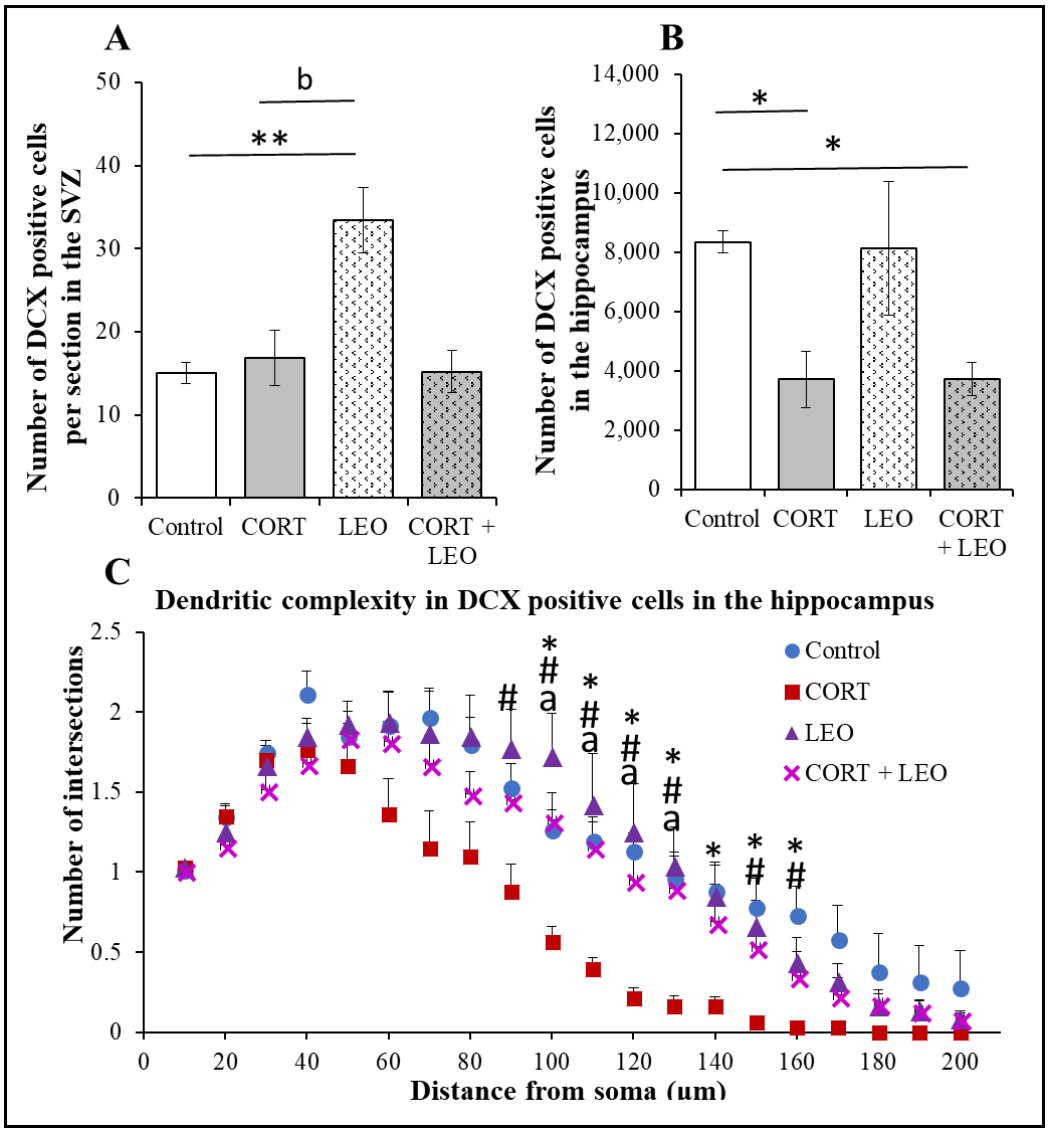


Figure 16. Number of DCX positive cells in the SVZ and hippocampus, and analysis of the dendritic complexity

In the SVZ, a statistically significant difference in the number of DCX positive cells was observed (Figure 16 A, $p = 0.001$, Figure 17). Treatment with LEO alone (33.40 ± 3.95) caused a significant increase in the number of immature neurons in comparison with the control group (15 ± 1.29; p = 0.001). The CORT group (16.83 ± 3.28) and CORT+LEO group (15.17 ± 2.54) did not show any statistically significant difference when compared with the control group (15 ± 1.29). However, a significant difference between the CORT and LEO group was observed.

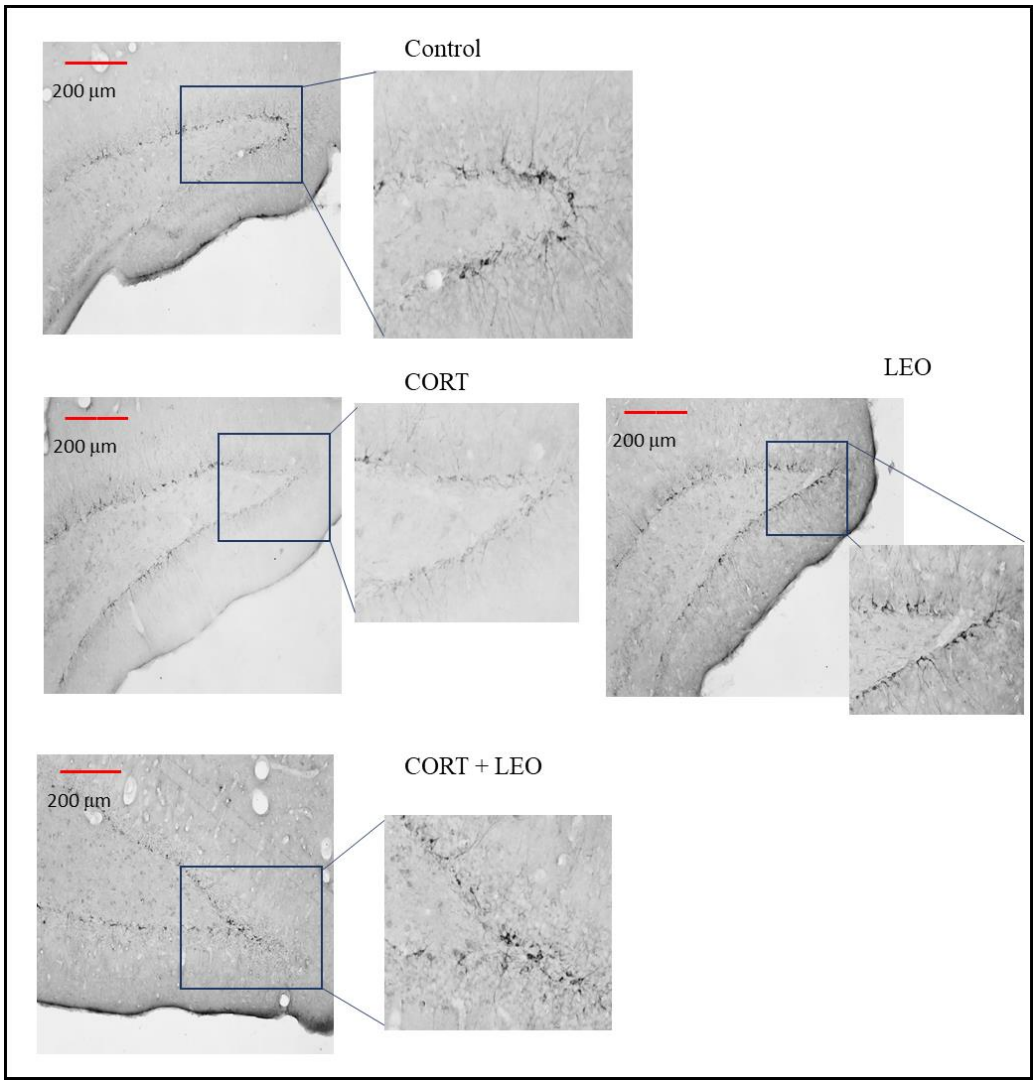


Figure 17. Representative images of the DCX positive cells in the dentate gyrus of the hippocampus

Dendritic complexity of immature neurons

The dendritic complexity of DCX positive cells showed interesting results in the Sholl analysis (Figure 16 C). A significant difference in the dendritic complexity was observed between the control and CORT group in which the CORT group showed a lower number of intersections. No significant difference between the control, LEO and CORT+LEO groups was found (Figure 16 C) indicating a positive effect of LEO to revert the decreased dendritic complexity induced by CORT.

Concentration of corticosterone, BDNF and oxytocin in serum

A significant difference was observed in the corticosterone levels in serum (Figure 18 A, $p = 0.009$). The CORT group showed a lower level of corticosterone (142.57 ng/ml ± 27.99) when compared with the control group (340.99 ng/ml ± 51.89, $p = 0.004$). The corticosterone levels in serum was similar in the control (340.99 ng/ml ± 51.89), LEO (294.68 ng/ml ± 19.86) and CORT+LEO groups (215.55 ng/ml ± 39.31). However, a significant difference was observed between LEO (294.68 ng/ml ± 19.86) and CORT group (142.57 ng/ml ± 27.99, $p = 0.004$).

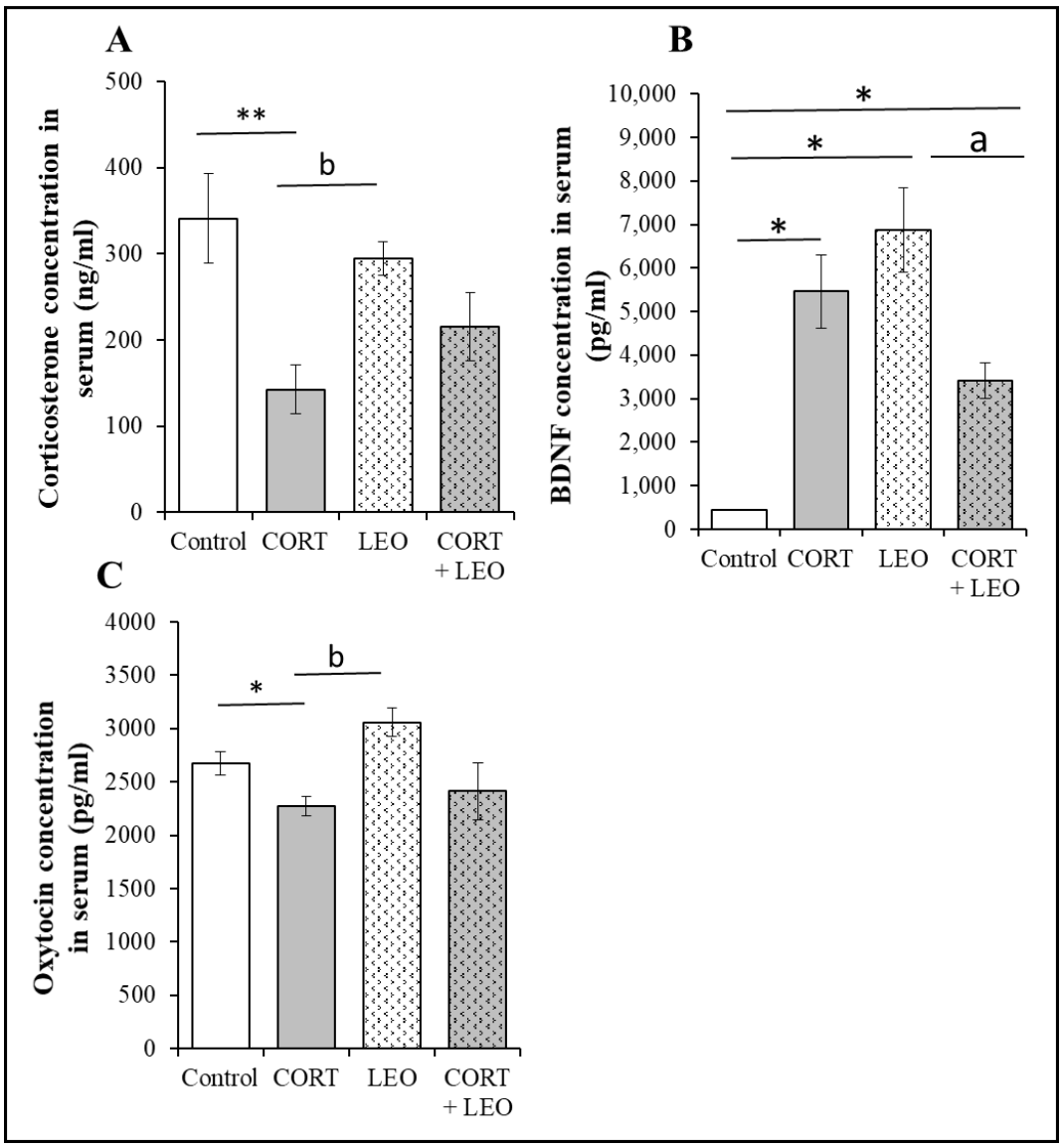


Figure 18. Concentration of corticosterone, BDNF and oxytocin in serum

The BDNF levels in serum were significantly different among the treatment groups (Figure 18 B, $p = 0.005$). High BDNF levels were observed in the CORT (5464.36 pg/ml ± 843.98, $p = 0.016$), LEO (6871.46 pg/ml ± 974.01, $p = 0.016$) and CORT+LEO groups (3414.95 pg/ml ± 453, $p = 0.029$) when compared to the control group (442.03 pg/ml ± 22.38). Treatment with LEO alone (6871.46 pg/ml ± 974.01) caused a significant increase in BDNF when compared with the CORT+LEO group (3414.95 pg/ml ± 453, $p = 0.016$). However, no significant difference was found

between the CORT (5464.36 pg/ml ± 843.98) and LEO groups (6871.46 pg/ml ± 974.01).

The level of oxytocin in serum showed a statistically significant difference among the treatment groups (Figure. 18 C, $p = 0.022$). The concentration of oxytocin in the CORT group (2273.04 pg/ml ± 93.44) was lower than the concentration in the control group (2677.35 pg/ml ± 110.55; $p = 0.038$). Treatment with LEO (3057.69 pg/ml ± 132.23) increased the levels of oxytocin when compared with the CORT group (2273.04 pg/ml ± 93.44, $p = 0.004$). No significant difference was found when comparing the control, LEO and CORT+LEO groups.

4.4. Discussion

The present study demonstrated a positive effect of LEO in alleviating depression-like behavior in a high-dose corticosterone animal model. Treatment with LEO increased the number of BrdU-positive cells in the hippocampus and SVZ and reverted the CORT-induced suppression of neurogenesis. In the hippocampus, LEO improved the dendritic complexity in the CORT+LEO group, but the number of immature neurons was not increased when compared to the CORT group. In the SVZ, treatment with LEO alone increased the number of DCX positive cells. The concentration of BDNF and oxytocin was increased after treatment with LEO alone. Both BDNF and oxytocin are relevant in the regulation of the neurogenesis process. Although the concentration of BDNF and oxytocin did not show any significant difference between the CORT+LEO and CORT group, the increase induced by the treatment with LEO alone suggests a promising effect of LEO that should be investigated in detail. For instance,

a longer treatment period, 3 to 4 weeks instead of 2 weeks, could be implemented. However, the findings suggest a novel perspective with respect to the treatment effect of LEO highlighting that neurogenesis may be an underlying mechanism. The findings enhance understanding of the pharmacological effects of LEO and support its potential clinical application as an antidepressant. However, further investigation is needed.

Chemical characterization of LEO and its effect on body weight and adrenal gland weight

The chemical characterization of LEO used in the present study is in line with the findings of previous reports (Shellie et al., 2002; Chatzopoulou and Goliaris, 2003; Khairul et al., 2015). LEO contains a complex mixture of volatile compounds. Linalool and linalyl acetate are the most commonly found components in LEO (Chatzopoulou and Goliaris, 2003; Perry et al., 2012). Of these, linalool has been proposed as the most biological active compound in LEO (Ali et al., 2015).

CORT treatment significantly reduced the body weight gain in the CORT and CORT+LEO groups. The results agree with previous studies that attributed the weight loss to the chronic administration of a high dose of CORT in rats (Brummelte et al., 2010; Lebedeva et al., 2017). Treatment with LEO did not affect the body weight gain. However, a study conducted by Shen et al. (2005) showed the opposite findings (Shen et al., 2005). The contrast in findings may be attributed to the shorter duration of Shuen et al.'s study when compared with the present study. The adrenal gland weight was significantly lower in the CORT group which is consistent with the findings of previous studies where decreased adrenal gland weight was observed after corticosterone treatment (Qiu et al., 2007). The reduced adrenal gland weight is

associated with hypoactivity of the hypothalamic-pituitary-adrenal axis (HPA axis). CORT is the end product of the activation of the HPA axis which mimics the physiological stress response (Kott et al., 2016). The adrenal gland is an important component of the HPA axis, a physiological regulator system of the stress response that uses glucocorticoids such as corticosterone, as an effector molecule (O'Connor et al., 2000). Upon activation, the HPA axis triggers the release of corticotropin-releasing factor (CRF) which in turn stimulates the synthesis and release of glucocorticoids from the adrenal gland (Scherer et al., 2011). In rodents, high concentrations of circulating corticosterone cause a negative feedback in the HPA axis that inhibits the release of CRF (Scherer et al., 2011). At high corticosterone conditions, the activation of the HPA axis is downregulated (Scherer et al., 2011) and lower adrenal gland weight is observed as a result of adrenal gland atrophy caused by chronic administration of corticosterone (Donner et al., 2012; Staní et al., 2017). Our study demonstrated that co-treatment with LEO showed an increase in adrenal weight, which reverts the effect of the CORT-induced low adrenal gland weight.

Effect of LEO on depression-like and anxiety-like behavior

Treatment with LEO attenuated the depression-like behavior under high dose corticosterone conditions. Treatment with CORT significantly increased the time that animals spent floating in the FST, thereby indicating increased depression-like behavior (Gregus et al., 2005; Marks et al., 2009). Exposure with LEO and linalool reduced the depression-like behavior in previous animal studies (Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013). The findings of the present study showed the reduction of the depression-like behavior caused by LEO exposure, thereby reverting the negative effects induced by CORT treatment.

In the SIT, treatment with CORT decreased the number of positive social interactions as previously reported (Sánchez-Vidaña et al., 2016). In clinical studies, high levels of stress or chronic exposure to stress hinders individual engagement in social interactions due to social anxiety (Sandi and Haller, 2015). An increased number of positive social interactions was found after treatment with LEO in the CORT+LEO-treated group to levels comparable to those of the control group. The results in the SIT found in the present study suggest that treatment with LEO under stress conditions improves positive social interactions. The increased number of positive social interactions in the CORT+LEO group was not enough to show significant difference when compared with the CORT group. However, the trend to increase the number of positive social interactions in the CORT+LEO group is clearly suggesting that further investigation should be carried out using a longer treatment period.

In previous studies, an increased engagement in social interaction was observed when animals were treated with linalool by inhalation (Linck et al., 2010). Again, an increased social interaction was observed by Kumar (2013) in a dose-dependent manner when administering the LEO formulation Silexan. Therefore, improved social interaction has been previously reported and this is corroborated by the results observed in the present study. We demonstrated that treatment with LEO increased the number of positive social interactions in the CORT+LEO group to a level comparable to the one of the control group. The results suggest a potential protective action of LEO against the anxiogenic effect of CORT, but further investigation is needed using a longer duration of the treatment in order to confirm the effect of LEO on anxiety-like behavior.

Effect of LEO on the number of BrdU and DCX positive cells

Hippocampal neurogenesis is subjected to regulation by glucocorticoids (Snyder et al., 2011) as a high density of mineralocorticoid and glucocorticoid receptors are present in the DG of the hippocampus (Wong and Herbert, 2005). Although it has been hypothesized that aromatherapy may promote neurogenesis in the hippocampus (Perry and Perry, 2006; Scuteri et al., 2017), no evidence concerning the effect of LEO on neurogenesis has been identified. To the best of our knowledge, the present study is the first to report the effect of LEO on neurogenesis. Suppression of newborn cells, labeled by BrdU, was observed in the CORT group which is accordance with the stress-mediated decreased cell proliferation phenomenon described above (Qiu et al., 2007; Murray et al., 2008). Remarkably, treatment with LEO reverted the suppression of cell proliferation induced by CORT treatment indicating that LEO protected hippocampal neurogenesis from the effect of CORT. Antidepressants increase cell proliferation in the hippocampus restoring the suppressed neurogenesis observed in stress and depression (Duman et al., 2016). The restoration of the number of BrdU positive cells in the hippocampus observed in the CORT+LEO treatment showed that LEO exhibited an effect on the hippocampus similar to the effect caused by antidepressants. The findings of the present study suggest that LEO may be a promising treatment alternative for depression.

CORT treatment suppressed cell proliferation in the SVZ. Similar results have been reported in previous studies as well (Lau et al., 2012; So et al., 2012). We observed that LEO treatment protected the SVZ against the suppressed cell proliferation induced by CORT treatment as it was observed in the CORT+LEO group. LEO treatment alone did not increase the number of BrdU positive cells which suggests that LEO treatment only increased cell proliferation under stress conditions. The SVZ

is another neurogenic region where new neurons are continually added to the OB within the mammalian brain (Lim and Alvarez-Buylla, 2016). The SVZ is a distinctive neurogenic region apart from the hippocampus. For this reason, the SVZ has been suggested to play a significant role in neural plasticity within the adult brain (Lim and Alvarez-Buylla, 2016). The results from the present study demonstrated that LEO stimulates cell proliferation not only in the hippocampus but also in the SVZ under high corticosterone conditions. This seems to indicate an interesting effect of LEO on SVZ neurogenesis.

Treatment with LEO did not improve the suppression of the number of DCX cells induced by CORT treatment in the hippocampus as observed in the CORT+LEO group. However, LEO significantly increased the number of DCX positive cells in the SVZ when administered alone, but no change was observed under high CORT conditions. The effect of LEO on the number of immature neurons in the SVZ suggests that LEO stimulates cell differentiation, but the effect is not present under stress conditions. The dendritic complexity of DCX positive cells determined by the Sholl analysis showed a decrease in the frequency of intersections in the CORT group. This is consistent with previously reported findings (Lang and Borgwardt, 2013; Sánchez-Vidaña et al., 2016; Conrad et al., 2017; Olescowicz et al., 2017). Although the number of DCX positive cells in the hippocampus and the SVZ did not increase in the CORT+LEO group, the higher dendritic complexity in the CORT+LEO group may partially explain the positive behavioral effect observed. In the early post-mitotic maturation phase in hippocampal neurogenesis, immature neurons increase the dendrite and axon extensions, thereby facilitating an increase in synaptic plasticity (Kempermann et al., 2015). Antidepressants have been shown to reverse the harmful

effects of stress on synaptic and dendritic structures (Bessa et al., 2008). Therefore, the improved dendritic complexity within the hippocampus in the LEO+CORT group suggests that LEO treatment stimulates dendritic branching. As previously reported (Lussier et al., 2013), increased dendritic branching is correlated with reduction in depression-like behavior. In the present study, LEO reverted the negative effect of corticosterone on dendritic complexity as shown in the CORT+LEO group.

Effect of LEO on peripheral corticosterone, BDNF and oxytocin levels

BDNF is a growth factor present in most tissues, highly expressed in the hippocampus and cortical region of the brain and found in blood (Polacchini et al., 2016; Numakawa et al., 2017). It is an important regulator of survival, differentiation, and growth functions in the brain (Hansson et al., 2006). BDNF can cross the blood-brain barrier and it is found in serum, plasma and stored in high amounts in platelets (Lee and Kim, 2010; Piepmeier and Etnier, 2015). Changes in BDNF expression in the brain have been correlated with BDNF levels in serum (Lee and Kim, 2010; Pilar-Cuéllar et al., 2013). Clinical studies focused on the measurement of circulating BDNF have reported lower levels of BDNF in patients with major depression (Polacchini et al., 2016). Due to its presence in serum and its correlation with the disease states, BDNF has been considered a promising biomarker for brain disorders (Polacchini et al., 2016). Furthermore, not only has the treatment with antidepressants increased the levels of BDNF in serum, but also other neuro-rehabilitation approaches have shown the same effect (Polacchini et al., 2016). The peripheral administration of BDNF has shown antidepressant effect similar to the increased BDNF in serum observed after antidepressant treatment (Pilar-Cuéllar et al., 2013). Several studies have demonstrated that upregulation of BDNF increases neurogenesis (Hanson et al., 2011; MacQueen and Frodl, 2011; Numakawa et al., 2017) since BDNF increases the

survival of newborn cells in the dentate gyrus of the hippocampus (Numakawa et al., 2017). Reduced BDNF expression and hippocampal neurogenesis have been correlated with depressive behavior in animal models of depression and the presence of newborn neurons contributed to the recovery of depression-like behavior (Numakawa et al., 2017). Therefore, depressive symptoms have been associated with changes in the expression of BDNF and reduced neurogenesis (Numakawa et al., 2017). Although several molecular pathways regulated by BDNF have been studied, it is not known which one is the most crucial for the antidepressant effects observed in animal models (Berton and Nestler, 2006). Yet, it is still important to explore the involvement of BDNF in the mechanism of action of novel treatment options for depression. Not only is the direct infusion into the hippocampus vital but also the peripheral administration of BDNF has been shown to regulate neurogenesis by increasing the survival of cells in the dentate gyrus in the hippocampus (Hanson et al., 2011). Because of its regulatory function on neurogenesis, BDNF appears to be a potential candidate to explore in order to ascertain whether it plays a role in the mechanism of action of LEO to decrease depressive symptoms.

The mechanism of action by which LEO acts to relieve depressive symptoms has not been previously documented. In the present study, we showed that LEO significantly increased the BDNF levels. Previous studies have reported that increased BDNF stimulates neurogenesis in the adult hippocampus and SVZ (Liu and Song, 2016). BDNF plays a key role in the regulation of adult neurogenesis (Zhao et al., 2008a; Calabrese et al., 2009) and the link between BDNF and depression has been previously established (Taupin, 2006; Marais et al., 2009; Lang and Borgwardt, 2013; Gersner et al., 2014; Chen et al., 2015a). The results of our study showed that LEO

increased the concentration of BDNF in serum in the CORT+LEO group. This may positively affect depression-like behavior and neurogenesis. Therefore, BDNF may be involved in the mechanism of action by which LEO exerts an antidepressant effect. Interestingly, the corticosterone group also showed an increase in the BDNF level in serum, a situation which might have occurred as a result of the time the serum sample was taken. After 2-3 days of stopping the administration of the treatments, the rats were sacrificed and serum samples were taken. The expression of BDNF in the brain is a regulated mechanism. In a previous study, down regulation of BDNF mRNA levels was observed after administration of corticosterone (Hansson et al., 2006). However, 24h after administration of corticosterone, the expression of BDNF mRNA as well as the BDNF in serum returned to levels comparable to the levels of the control (Hansson et al., 2006). High levels of corticosterone decreased the excitability of neurons in the hippocampus which led to decreased secretion of BDNF (Hansson et al., 2006). The increased BDNF level observed after corticosterone treatment might be due to potential build-up of BDNF in the neurons as a result of the decreased secretion induced by corticosterone (Hansson et al., 2006). The BDNF build-up phenomenon after corticosterone treatment may explain the raise in the concentration of BDNF in the CORT group at the time the serum sample was taken.

The CORT concentration in serum in the CORT group was low. This was expected after chronic administration of CORT leading to a negative feedback in the HPA axis (Scherer et al., 2011). Activation of the HPA axis takes place after exposure to stressors, leading to increased levels of circulating glucocorticoids (Koutmani and Karalis, 2015). The HPA axis is subjected to negative feedback which functions as a regulatory mechanism to prevent hormone over secretion and to maintain the

hormonal level within a homeostatic range (Keller-Wood, 2015). Exogenous administration of glucocorticoid leads to suppression of the HPA axis activity which can last several hours and it is reversible at low doses of glucocorticoid (Andrews et al., 2012). Chronic exogenous administration of CORT results in reduced adrenal gland weight which leads to reduction in the normal endogenous compensatory CORT release (Coburn-Litvak et al., 2004). In the present study, the exogenous administration of CORT was stopped 2-3 days before taking the serum sample. Due to the suppression of the secretion of endogenous CORT as a result of the chronic CORT administration, the CORT level was low in the CORT group at the time the serum sample was taken. However, the adrenal gland atrophy, negative behavioral changes, and decreased cell proliferation in the hippocampus showed the effect of the chronic administration of CORT which have been previously reported (Coburn-Litvak et al., 2004; MacQueen and Frodl, 2011; Wolkowitz et al., 2011; Koutmani and Karalis, 2015). Treatment with LEO alone did not affect the CORT levels in serum which are comparable to those of the control group. Interestingly, in the co-treatment group, LEO ameliorated the negative feedback in the HPA axis observed when exogenous CORT was administered.

Oxytocin is a hypothalamic neuropeptide produced in the paraventricular and supraoptic nuclei of the hypothalamus. It plays a role in both peripheral (reproduction) and central (social and bonding behavior) processes (Gimpl and Fahrenholz, 2001; Guastella et al., 2010; Lin et al., 2017). Attenuation of the behavioral and neuroendocrine effects induced by stress have been observed after oxytocin administration in the dorsal hippocampus (Lin et al., 2017). Oxytocin has shown a protective effect against the stress-induced damage to the hippocampus. It is thought

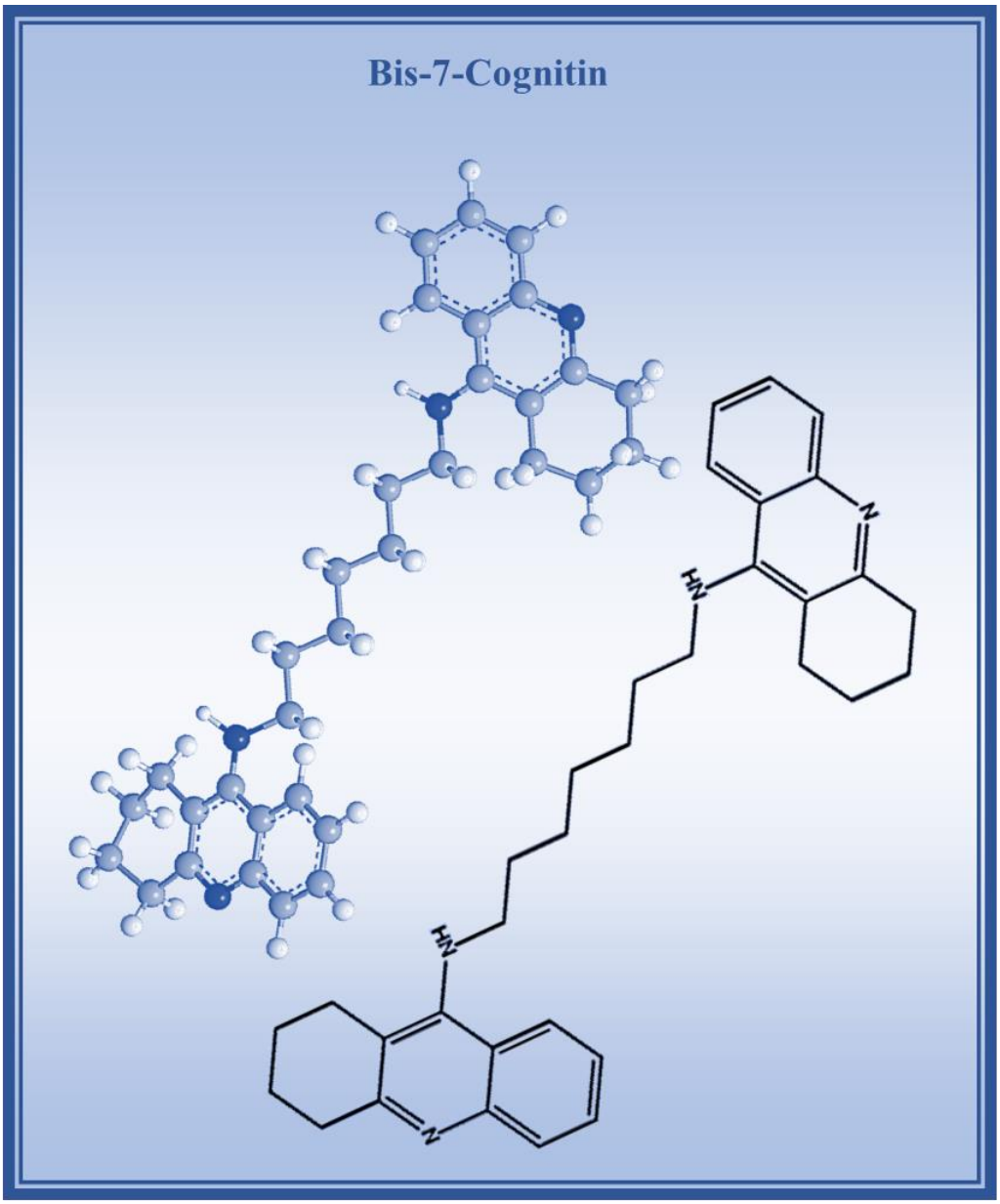
to facilitate hippocampal plasticity by increasing cell proliferation and neurogenesis within the hippocampus (Leuner et al., 2012). However, in the present study treatment with LEO did not affect the peripheral levels of oxytocin. We have further studied the effect of oxytocin on neurogenesis and the findings are presented and discussed in chapter 8. Briefly, we found that oxytocin has a positive effect on depression and anxiety-like behavior, stimulates neurogenesis, and increases dendritic complexity in immature neurons. Therefore, it is important to explore whether new treatment options for depression, such as LEO, act using oxytocin as mediator.

4.5. Conclusions

The present study has demonstrated the potential therapeutic effect of the application of LEO. The observed *in vivo* effects at both behavioral and cellular level support the use of LEO as a promising treatment option for depression. Not only did LEO improve depression-like behavior but it also promoted neurogenesis and improved dendritic branching. The findings indicate that neurogenesis may play a significant role in the mechanism of action involved when using LEO to treat depression. The effect of LEO on serum BDNF and oxytocin suggests that those molecules may also play a role in the beneficial behavioral and cellular outcomes. However, the evidence from the present study on the upregulation of BDNF and oxytocin is not conclusive. Finally, the results from the present study provide a better understanding of the potential use of LEO for the treatment of depression.

CHAPTER 5

MOLECULAR TARGETS OF B7C AND ITS RELEVANCE ON NEUROLOGICAL DISORDERS: A REVIEW



Structures from the ChemACX database in the ChemOffice software (version 17.1) with the Chem 3D PlugIn

Abstract

The exact mechanisms involved in the pathogenesis of neurodegenerative conditions are not fully known. However, it is clear that the multifactorial pathogenesis nature of neurodegenerative disorders requires the use of several drugs to tackle the multiple symptoms present in these disorders.

B7C is s synthetic drug that has been studied for over 20 years and it represents a promising multi-target drug for the treatment of neurodegenerative disorders such as Alzheimer's Disease (AD). Therefore, the present chapter aimed to review the studies on B7C to summarize the molecular targets of B7C and to discuss the relevance of B7C on neurological disorders, and its potential application on treatment of depression.

5.1. Introduction

Neurodegenerative disorders are disabling conditions characterized by the loss of neurons that leads to chronic degeneration and deterioration of the brain (Pérez-Hernández et al., 2016). Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis, Huntington's disease and amyotrophic lateral sclerosis are some of the major causes of death in the elderly population and they have been projected to be the second leading cause of death by 2040 (Valera and Masliah, 2016). AD is the most common cause of dementia and the most frequent neurodegenerative disorder characterized by decline in cognition, memory ability, language and problem-solving skills due to failure in synaptic signal transfer, decreased number of synapses and neuronal death (Baquero and Martín, 2015; Teipel et al., 2016).

The exact mechanisms involved in the pathogenesis of neurodegenerative conditions are not fully understood (Li et al., 2007b). However, it is clear that the multifactorial pathogenesis nature of neurodegenerative disorders requires the use of several drugs to tackle the multiple symptoms present in these disorders (Li et al., 2007b, 2009). The one-drug-one-target approach offers limited action and do not postpone the progression of neurodegeneration (Li et al., 2007b). Drugs with multitarget properties might provide a more significant effect by acting on different brain regions relevant to the symptomatology of the disorder.(Li et al., 2007b). Thus, the one-drug-multiple-targets approach represents a valuable tool for developing single drugs with multitarget activity for the treatment of neurodegenerative disorders (Li et al., 2007b).

Depressive symptoms are highly frequent in neurodegenerative diseases (Baquero and Martín, 2015). Epidemiological and clinical evidence has shown a strong relationship between depression and dementia, specifically with AD. Co-morbidity of depression and AD has been suggested since depression is found to be a risk factor for cognitive dysfunction that leads to the development of dementia and ultimately AD (Caraci et al., 2010; Baquero and Martín, 2015). Also, psychiatric disturbances such as depression and apathy are highly manifested in AD (Wuwongse et al., 2010). In fact, it has been demonstrated that higher levels of depressive symptoms result in a rapid decline in cognition (Sierksma et al., 2010; Baquero and Martín, 2015). Also, a history of depression has been suggested as a strong risk factor for developing AD (Wuwongse et al., 2010). Therefore, the close relationship between these two conditions presents a potential area of research aimed at developing novel therapeutic options with a dual effect as mood stabilizers and neuroprotective agents that may have cross-benefits for depression and AD (Tizabi, 2015).

B7C is s synthetic drug that has been studied for over 20 years and it represents a promising multi-target drug for the treatment of neurodegenerative disorders such as AD (Ros et al., 2001). Therefore, the present review analyzed studies on B7C in order to summarize the molecular targets of B7C and to discuss the relevance of B7C on neurological disorders.

5.2. B7C: A product of the structure-activity-relationship drug design

Although the pathological processes in AD are not well understood, it is clear that disturbances in the cholinergic system and other neurotransmitters play a pivotal role

in the pathogenesis of this neurodegenerative disorder (Han et al., 2012). Strategies to develop drugs for AD have focused on acetylcholinesterase (AChE) as a target for drug design based on the cholinergic hypothesis for AD (Ros et al., 2001; Lopes et al., 2017). The cholinergic hypothesis states that increased levels of acetylcholine in the brain alleviate the cognitive deficiencies observed in AD (Ros et al., 2001). Although a series of AChE inhibitors have been extensively studied, none of them represent a real cure for AD (Ros et al., 2001; Lopes et al., 2017).

Tacrine (9-amino-1,2,3,4-tetrahydroacridine, Figure 19) under the trade name Cogniotex®, was the first drug approved for the treatment of AD in 1993 (Han et al., 2012). Other AChE inhibitors such as donepezil (Aricept ®), rivastigmine (Exelon ®) and galantamine (Reminyl ®) introduced in 1996, 2000 and 2001 respectively, in addition to the N-methyl-D-aspartate (NMDA) receptor antagonist memantine (Namenda ®) followed the release of tacrine (Han et al., 2012; Lopes et al., 2017). Tacrine binds in a reversible mode to AChE and it is considered a classical AChE pharmacophore (Lopes et al., 2017). Due to side effects such as hepatotoxicity and myopathy as well as with poor pharmacokinetic properties, including low bioavailability and narrow therapeutic index, a series of new tacrine-based compounds have been developed (Luo et al., 2004; Han et al., 2012).

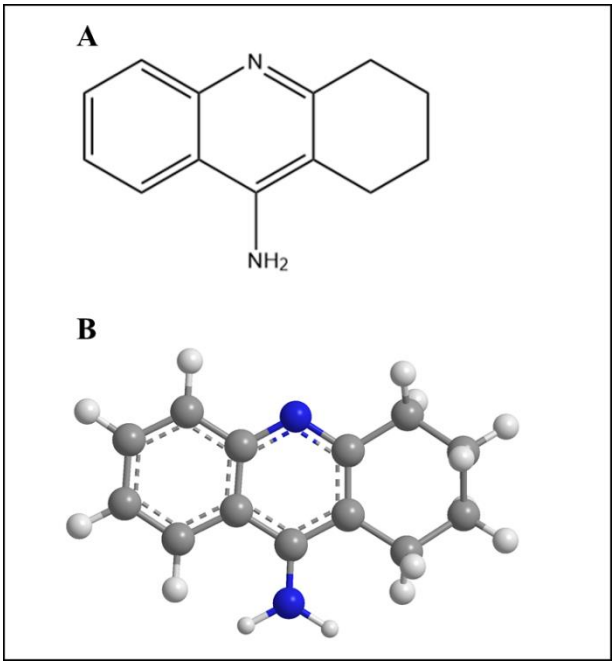


Figure 19. Tacrine chemical structure. **(A)** Tacrine structure shown in stick mode, **(B)** 3D tacrine structure shown in ball and cylindrical bonds mode. Structures from the ChemACX database in the ChemOffice software (version 17.1) with the Chem 3D PlugIn.

The enzyme AChE has 2 sub-active sites within the binding pocket, namely, the esteratic site or catalytic active site (CAS) and the anionic site or peripheral active site (PAS) (Li et al., 2009) (Figure 20). Using computational tools, molecular docking simulations were carried out to design and optimize the synthesis of tacrine analogues (Li et al., 2009). New compounds have been developed using the dual active sites in AChE as a basic hypothesis to increase the therapeutic action of tacrine analogues (Li et al., 2009). Alkaline-linked tacrine dimers that interact with the CAS and PAS that potently inhibit AChE making B7C one of the promising analogues (Lopes et al., 2017).

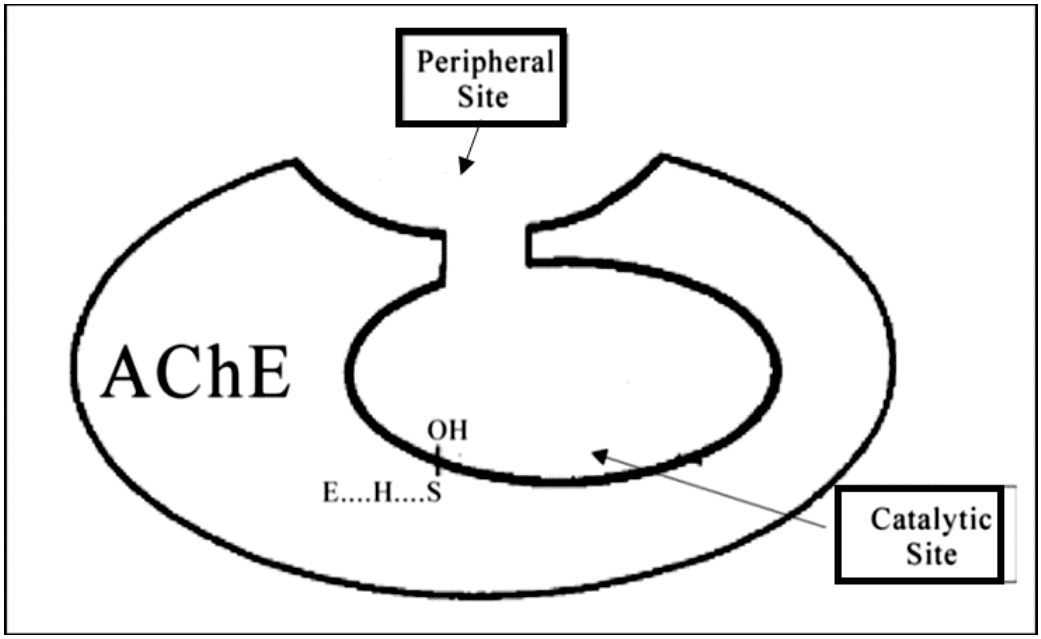


Figure 20. Dual binding site in AChE. Figure modified from Li et al. 2009.

The heptylene-linked bis-tacrine also known as B7C (1,7-N-heptylene-bis 9-9'-amino-1,2,3,4-tetrahydroacridine), have been found to be 150 times more potent and 250 times more selective to inhibit AChE when compared to tacrine (Ros et al., 2001; Li et al., 2009) (Figure 21).

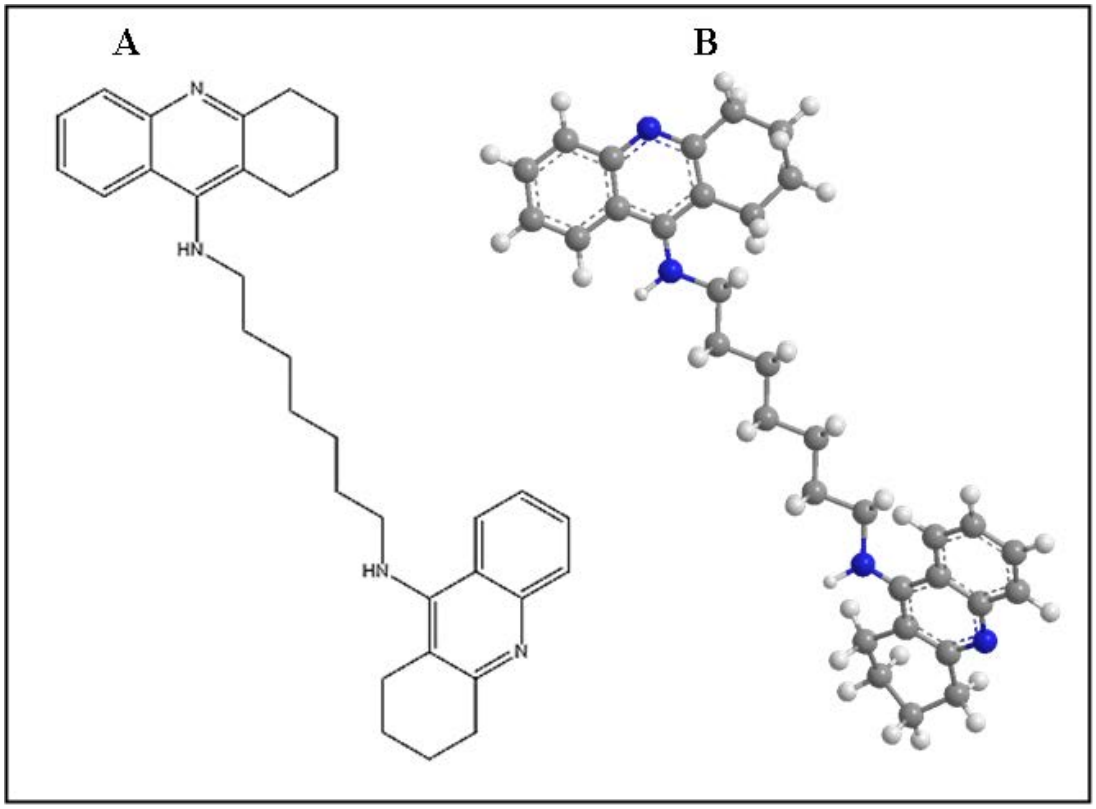


Figure 21. B7C chemical structure. **(A)** B7C structure shown in stick mode, **(B)** 3D B7C structure shown in ball and cylindrical bonds mode. Structures from the ChemACX database in the ChemOffice software (version 17.1) with the Chem 3D PlugIn.

The biological properties of B7C are superior to those of tacrine because B7C interacts simultaneously with the CAS and PAS of the enzyme (Figure 22) (Li et al., 2009). The addition of the heptylene chain to the two tacrine molecules allows the dual interaction with the AChE binding sites which explains its superior activity compared to tacrine (Bolognesi et al., 2010). B7C is a multitarget compound that shows promising biological activity, including inhibition of AChE, prevention of the aggregation of the β-amyloid (Aβ) protein, regulation of the downstream signaling mediated by the NMDA receptor and inhibition of the nitric oxide synthase (NOS)

signaling pathway (Zhang et al., 2011). Recent studies have expanded the understanding of the multitarget activity of B7C.

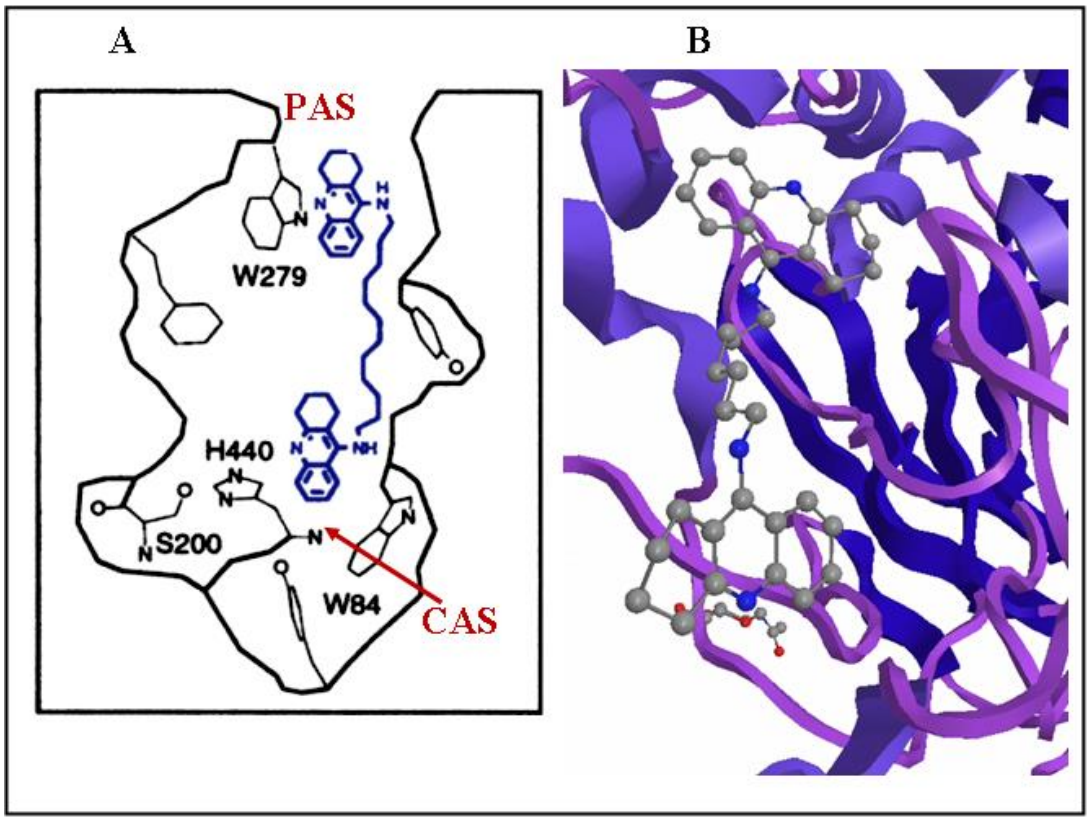


Figure 22. Dual binding site in AChE. **(A)** Schematic representation of the interaction between B7C and the AChE active sites (Figure modified from Li et al. 2009). **(B)** 3D structure of B7C and AChE (structures from the ChemACX database in the ChemOffice software, version 17.1 with the Chem 3D PlugIn; PDB ID: 2CKM).

5.3. B7C molecular targets relevance in neurological disorders

In an attempt to develop more efficient AChE inhibitors, several tacrine-based compounds have been synthetized and evaluated (Hu et al., 2002; Rizzo et al., 2011; Lopes et al., 2017). A summary of studies on B7C is shown in Table 15.

Table 15. Summary of *in vitro* and *in vivo* studies on B7C

Reference	*In vitro* study		*In vivo* study		Molecular/physiological target or behavioral test	Conclusion
	Cell line	B7C concentration	Animal species/strain	B7C dose or concentration		
(Ros et al., 2001)	NA	NA	*Torpedo marmorata* (fish) electric organ Ooscytes from mature females of *Xenopus laevis* (frog)	100 nM	ACh release by recording spontaneous synaptic activity	- B7C increased spontaneous quantal release from cholinergic terminals and blocked the AChE induced currents in lower concentration than tacrine.
(Lopes et al., 2017)	NA	NA	Wistar male rats	Different concentrations	AChE using both computational-based AChE binding affinity predictions and the AChE and BuChE inhibitory activity by the Ellman's method	- B7C showed potent and selective inhibition of AChE at low nanomolar concentration (IC_{50}= 5.33nM).
(Bolognesi et al., 2010)	NA	NA	NA	Different concentrations	AChE and BChE (human recombinant enzymes) BACE-1, Aβ	- B7C inhibited both AChE (IC_{50}= 0.81 nM) and BChE (IC_{50}= 5.66 nM). - B7C inhibited BACE-1 (27%), AChE-induced Aβ aggregation (68%), and Aβ self-aggregation (51 %).
(Rizzo et al., 2011)	NA	NA	NA	Different concentrations	AChE and BChE (human recombinant enzymes), BACE-1	- B7C inhibited both AChE (IC_{50}= 0.81 nM) and BChE (IC_{50}= 5.66 nM) (results were taken as reference from (Bolognesi et al., 2010)). - B7C inhibited BACE-1 (IC_{50}= 7.5µM), and inhibited AChE-induced Aβ aggregation (results were taken as reference from (Fu et al., 2008)) (68%) (results were taken as reference from (Bolognesi et al., 2010)).

Reference	*In vitro* study		*In vivo* study		Molecular/physiological target or behavioral test	Conclusion
	Cell line	B7C concentration	Animal species/strain	B7C dose or concentration		
(Fu et al., 2006)	Hippocampal pyramidal neurons (isolated from Wistar rats)	0.1, 1, 5, 10, 100, and 500 nM	NA	NA	AChE, L-type voltage-dependent Ca^{2+} channels	- B7C reduced the inward calcium current induced by Aβ induced Ca^{2+} which attenuated neuronal apoptosis.
(Hu et al., 2015b)	SH-SY5S cells	0.1, 0.3, 1, 3, 10 µM	Male Sprague-Dawley rats	0.1 and 0.2 mg/kg	AChE, ChAT, Morris water maze test	- B7C inhibited Aβ fibrlis formation and disaggregated pre-formed Aβ fibrils. - B7C reduced Aβ induced neurotoxicity. - B7C inhibited the memory impairment induced by infusion of Aβ in rats. - B7C reversed the dysfunction ChAT and AChE activity induced by Aβ in rats.
(Mutunga et al., 2013)	NA	NA	Drosophila melanogaster and Blatella germanica (insects)	Different concentrations	AChE from Drosophila melanogaster and AChE from Blatella germanica	- Dimeric tacrines, including B7C, were not toxic and did not cross the insect blood brain barrier. - B7C inhibited AChE from both insect species.
(Hu et al., 2002)	NA		NA	Different concentrations	AChE and BChE	- B7C inhibited both AChE (IC_{50}= 2.7 nM) and BChE (IC_{50}= 2.6 nM).
(Wang et al., 1999a)	NA	NA	Female and male Sprague-Dawley rats	1, 3, 5, 9.5, 19, 38 nM	AChE and BChE	- B7C inhibited both AChE (IC_{50}= 5.1 nM) and BChE (IC_{50}= 159 nM).
(Li et al., 1999)	Hippocampal neurons (isolated from mice)	5, 25, 100 µM	NA	NA	AChE, GABAA	- B7C inhibited both AChE (IC_{50}= 1.5 nM). - B7C antagonizes the $GABA_A$ receptor in a competitive mode
(Yu et al., 2008)	NA	NA	Male ICR mice	3.54 µM/kg	AChE	- B7C inhibited AChE (46.3%).
(Zhang et al., 2011)	RGC (primary cell culture from male Sprague-Dawley rats)	1µM/L	Sprague-Dawley male rats	0.05, 0.1 and 0.2 mg/kg	NMDA receptor	- B7C prevented NMDA-induced apoptosis in GCL. -B7C inhibitory effect of NMDA receptors confers neuroprotection. - B7C reduced the NMDA activated current in RGC which indicates that B7C is antagonist of the NMDA receptor.
(Li et al., 2005)	CGN (primary cell culture from Sprague-Dawley rats)	0.1, 1µM	NA	NA	NMDA receptor	- B7C prevents glutamate induced neuronal apoptosis through blockade of the NMDA receptor. - B7C inhibits AChE and acts as NMDA antagonist.

Reference	*In vitro* study		*In vivo* study		Molecular/physiological target or behavioral test	Conclusion
	Cell line	B7C concentration	Animal species/strain	B7C dose or concentration		
						- B7C inhibits the MAPK and ERK pathway.
(Bai-fang et al., 2001)	Cortical cells (isolated from Sprague-Dawley rats)	0.3-1 µM/L	NA	NA	NMDA receptor	- B7C reduced the NMDA-mediated activity and exhibited protective effect against glutamate-induced excitotoxicity.
(Liu et al., 2008a)	Hippocampal neurons (isolated from Sprague-Dawley rats)	0.001-1 µM	NA	NA	NMDA receptor	- B7C inhibited the NMDA receptor (IC50= 0.68 µM). - B7C prevented the glutamate induced excitotoxicity by inhibition of the NMDA receptor.
(Liu et al., 2008b)	Hippocampal neurons (isolated from Sprague-Dawley rats)	0.5 µM	NA	NA	NMDA receptor	- B7C inhibited the NMDA receptors in a non-competitive manner and showed protective effect against glutamate-induced neurotoxicity.
(Luo et al., 2007)	Hippocampal neurons (isolated from Sprague-Dawley rats)		NA	NA	NMDA receptor	- B7C inhibited the NMDA receptor in a pH dependent manner by desensitizing the receptors to proton inhibition.
(Liu and Li, 2012)	HEK-293	1 µM	NA	NA	NR1, NR2A and NR2B receptors	- B7C inhibited the NR1/NR2B receptor expressed in the cells in a slow onset, non-competitive and voltage dependent manner which is similar to what is observed in rat hippocampal neurons expressing the NMDA receptors.
(Li et al., 2007b)	CGN (isolated from Sprague-Dawley rats)	Different concentrations	NA	NA	NMDA receptor, NOS	- B7C showed to be a moderated NMDA receptor antagonist and a selective nNOS inhibitor.
(Li et al., 2006)	Cortical neurons (isolated from Sprague-Dawley rats)	0.001, 1 µM	NA	NA	NOS	- B7C reduced cell death induced by glutamate, Aβ, and L-arginine. - B7C suppressed the activation of the NOS induced by glutamate. - B7C inhibited the activity of NOS.
(Liu et al., 2000)	NA	NA	Male Sprague-Dawley rats	0.22, 0.44, and 0.89 µM/kg	ChAT, and spatial memory measured by the Morris water maze	- The induced learning and memory deficits were reversed by B7C in a dose dependent manner.
(Zhao et al., 2008b)	NA	NA	Male Sprague-Dawley rats	0.05, 0.1, 0.2 mM/kg	Apoptosis	- B7C showed anti apoptotic effect (0.2mg/kg)

Reference	*In vitro* study		*In vivo* study		Molecular/physiological target or behavioral test	Conclusion
	Cell line	B7C concentration	Animal species/strain	B7C dose or concentration		
(Fang et al., 2010)	RGC (isolated from male Sprague-Dawley rats)	1 µM	Male Sprague-Dawley rats	0.05, 0.1, and 0.2 mg/kg	Apoptosis	- B7C inhibited glutamate-induced cell death (IC_{50}= 0.028 µM). - B7C reduced glutamate-induced apoptosis *in vivo* (0.02 mg/kg).
(Fu et al., 2007)	CGN (isolated from Sprague-Dawley rats)	0.001, 0.01, 0.1, 1 µM	NA	NA	Apoptosis	- B7C protected against the glutamate induced excitotoxicity.
(Han et al., 2000)	Cortical astrocytes (isolated from ICR mice)	0.3, 1, 10, 100 nM	NA	NA	Apoptosis	- B7C (1-10 nM) inhibited the ischemia-induced apoptosis.
(Xiao et al., 2000)	PC12 cells	0.01, 0.1, 1, 10 µM	NA	NA	Cell toxicity	- B7C protected the cells against H2O2-induced cell toxicity improving the redox disequilibrium.
(Li et al., 2007a)	DRG neurons (isolated from Sprague-Dawley rats)	Different concentrations	NA	NA	GABA receptor	- B7C binds to GABA receptor in a potent but reversible manner (IC_{50}= 6.28µM).
(Zhou et al., 2009)	Hippocampal neurons (primary cell culture from Sprague-Dawley rats)			1, 3, 5, 10, 30, 100 µM	$GABA_A$ receptor	- B7C is a competitive $GABA_A$ receptor antagonist.
(Shu et al., 2012)	NA	NA	Male Sprague-Dawley rats	0.2 mg/kg	Morris water maze test	- B7C decreased hippocampal neural apoptosis (it increased neurogenesis) in rats with chronic ischemia - B7C reversed the chronic-ischemia-induced decreased spatial learning and memory.
(Han et al., 2012)	NA	NA	Male Kunming strain mice	0.4, 0.5, and 0.6 µM/kg	Spatial memory measured by the Morris water maze and the NOR task to evaluate recognition memory formation	- B7C mitigated the learning and memory deficits induced by scopolamine.
(Pan et al., 2007)	NA	NA	Male ICR mice	0.06, 1.25, 2.5, 5, 5, 10, 20 µM/kg	Passive avoidance response, spontaneous motor activity, hepatotoxicity	- B7C enhanced cognitive function at a high dose (20 µM/kg) but produced motor disfunction and hepatotoxicity.

Reference	*In vitro* study		*In vivo* study		Molecular/physiological target or behavioral test	Conclusion
	Cell line	B7C concentration	Animal species/strain	B7C dose or concentration		
(Pan et al., 2011)	NA	NA	Male ICR mice	0.25, 1, 5, and 20 μM/kg	OFT	- B7C did not affect locomotion in the OFT - B7C (1 μM/kg) improved the cycloheximide induced amnesia in mice.
(Fu et al., 2009)	Mouse Neuro2 neuroblastoma cells	0.1, 0.3, 1 μM	NA	NA	BACE-1 and α-secretase	- B7C inhibits BACE-1 and activates α-secretase
(Fu et al., 2008)	Mouse Neuro2a neuroblastoma cells	0.1, 0.3, 1, 2, 3 μM	NA	NA	BACE-1	- B7C inhibits BACE-1, therefore decreases the generation of Aβ - B7C activates α-secretase
(Li et al., 2010)	DRG (isolated from Sprague-Dawley rats)	1 μM	NA	NA	Kv4.2 potassium channels	- B7C suppressed the Kv4.2 potassium channels in a concentration dependent manner (IC_{50}= 0.53 μM).
(Nie et al., 2007)	DRG neurons (isolated from Sprague-Dawley rats)	10^{-9}M to 10^{-4} M	NA	NA	Kv4.2 potassium channels	- B7C inhibited the delayed rectifier potassium channel and inhibited the Kv4.2 potassium channels. (IC_{50}= 0.72 μM).
(Luo et al., 2004)	TG neurons		NA	NA	5-HT_3 receptor	- B7C inhibited the 5-HT_3 receptor current in a competitive manner.

Abbreviations: BACE-1, beta secretase; BuChE, butyryl cholinesterase; ChAT, choline acetyl transferase; DRG, dorsal root ganglion; NA, not applicable; NOS, nitric oxide synthase; OFT, open field test; RGC, retinal ganglion cells; TG, rat trigeminal ganglion; CGN, cerebellar granule neurons.

As shown in Table 15, the multitarget activity of B7C has been evaluated in cell-based platforms and *in vivo*. The molecular targets studied included the AChE, BChE, the NMDA receptor, ChAT, GABA receptor, BACE-1, $K_v4.2$ potassium channels, NOS and the 5-HT_3 receptor. The design of the tacrine dimers was driven based on the binding properties of the dimers to AChE using computational docking tools (Lopes et al., 2017). Therefore, AChE was the main targets of B7C studies.

AChE is an important element of the cholinergic system that acts in the synaptic cleft by hydrolyzing the neurotransmitter acetylcholine (ACh) at central and peripheral levels (Figure 23) (Colović et al., 2013). AChE is an important target in neurodegenerative disorders as its inhibition leads to accumulation of ACh by decreasing the ACh breakdown rate (Colović et al., 2013). Also, AChE stimulates Aβ fibrilogenesis through the formation of AChE-Aβ complexes, which is a characteristic feature in AD patients (Pilar Muñoz-Ruiz et al., 2005). Due to the nature of the AChE receptor, dual binding is a highly desirable property for the design of AChE inhibitors such as B7C (Li et al., 2009).

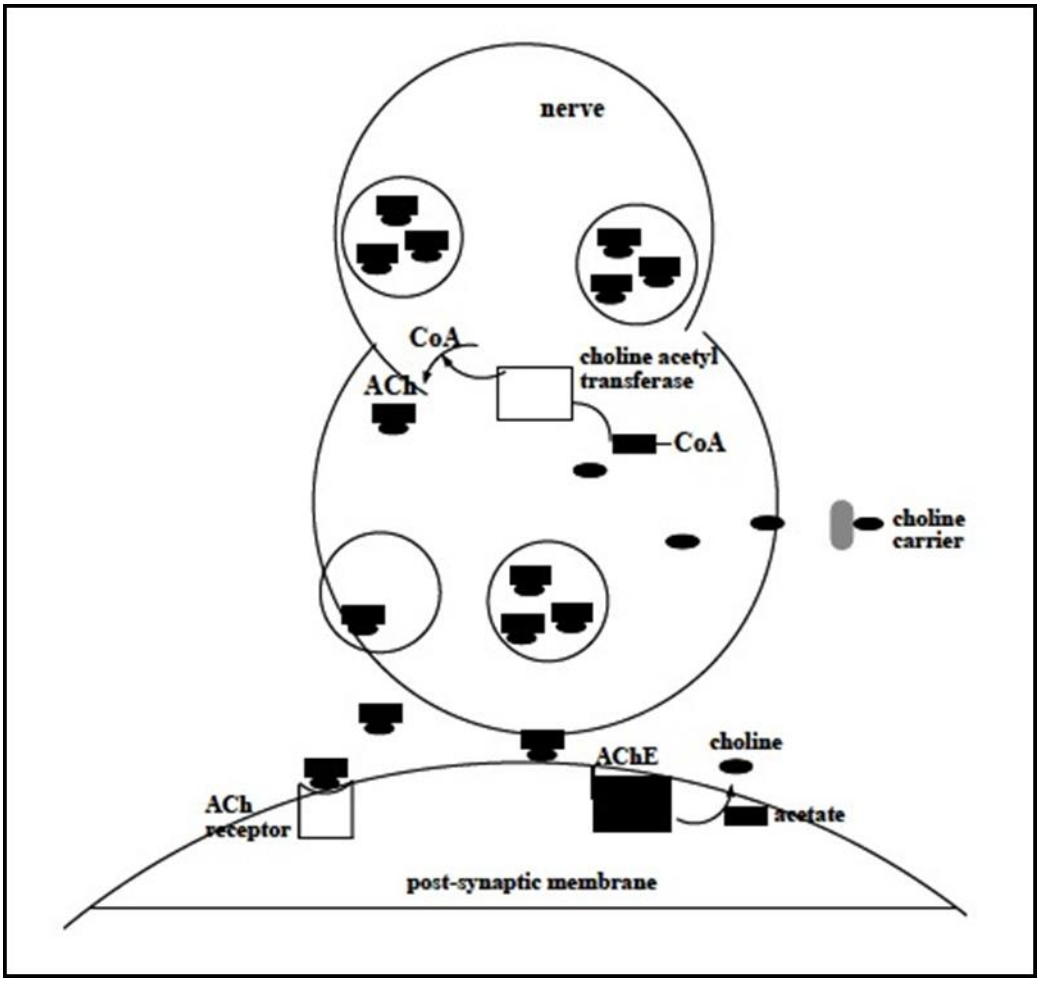


Figure 23. AChE mechanism of action in neurotransmission. Schematic representation of the mechanism of action of AChE in the synaptic cleft. Abbreviations: ACh, Acetylcholine; CoA, coenzyme A. (Figure modified from Čolović et al., 2013).

Several studies (Table 15) have already demonstrated the effect of B7C to inhibit AChE in a selective manner and at lower concentration than tacrine (Li et al., 1999; Wang et al., 1999a; Ros et al., 2001; Hu et al., 2002, 2015b; Fu et al., 2006; Yu et al., 2008; Bolognesi et al., 2010; Rizzo et al., 2011; Mutunga et al., 2013; Lopes et al., 2017). Drugs that inhibit AChE keep the ACh levels high in the synaptic cleft, thereby stimulating cholinergic transmission in regions of the forebrain that compensate for the loss of cells (Pilar Muñoz-Ruiz et al., 2005; Colović et al., 2013).

The findings of the effect of B7C on AChE have also demonstrated inhibition of the Aβ fibrils formation and stimulated the disaggregation of pre-formed Aβ fibrils and improved memory impairment induced by Aβ (Fu et al., 2006; Bolognesi et al., 2010; Rizzo et al., 2011; Hu et al., 2015b).

B7C has also been evaluated on the NMDA receptor (Table 15) and has been found to show an inhibitory effect (Bai-fang et al., 2001; Li et al., 2005, 2007b; Luo et al., 2007; Liu et al., 2008b; Zhang et al., 2011; Liu and Li, 2012). Excitotoxicity significantly contributes to neuronal cell damage and death in neurodegenerative disorders (Lipton, 2004). Excitotoxicity is the result of overactivation of the NMDA glutamate receptor that leads to excessive Ca^{2+} influx in the cell (Figure 24) (Newcomer et al., 2000). Glutamate, the major excitatory neurotransmitter in the brain, is a crucial mediator involved in the normal functioning of the nervous system (Lipton, 2004). It is hypothesized that chronic exposure to elevated levels of glutamate or glutamate receptor hyperactivity triggers apoptotic pathways, a phenomenon of clinical relevance in disorders such as Huntington's disease, Parkinson's disease, multiple sclerosis, HIV-associated dementia, amyotrophic lateral sclerosis, glaucoma and Alzheimer's disease (Lipton, 2004).

Another important mediator of cell death is the NOS which has also been evaluated in B7C studies. In these studies, B7C was shown as having an inhibitory effect on NOS (Li et al., 2006, 2007a). Hyperactivity of NOS leads to excitotoxicity-mediated cell death. The enzyme is tethered to the NMDA receptor and gets activated by the influx of Ca^{2+} which increased the levels of NO associated to stroke and neurodegenerative diseases (Lipton, 2004). As B7C has demonstrated NMDA and

NOS inhibitory activity, it represents a valuable candidate for the treatment of degenerative disorders.

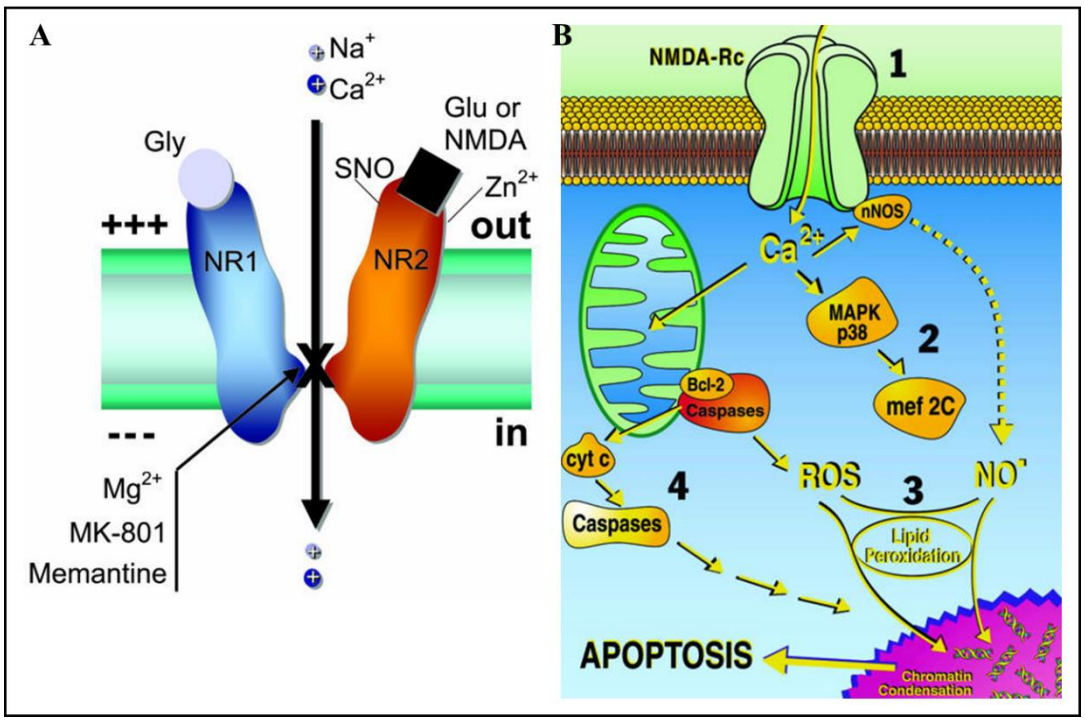


Figure 24. NMDA receptor and cellular mechanism. **(A)** Schematic representation of the NMDA receptor showing the binding sites, the NR1 and NR2 receptor subunits. **(B)** Schematic representation of apoptotic signaling pathways triggered by overactivity of the NMDA receptor. The pathway 1 is the NMDA receptor overactivation (NMDA-Rc). The pathway 2 is the activation of the MAPK-MEF2C pathway that contributes to neuronal cell death. The pathway 3 involves the toxic effect of free radicals and the pathway 4 involves activation of apoptosis-inducing enzymes. Abbreviations: SNO, cysteine sulfhydryl group; cyt c, cytochrome c (Figure modified from Lipton, 2004).

Several studies on B7C focused on apoptosis as a target physiological mechanism (Han et al., 2000; Xiao et al., 2000; Fu et al., 2007; Zhao et al., 2008b; Fang et al., 2010). Abnormal regulation of neuronal cell death has been associated with many neurological disorders (Mattson, 2000). Excessive death of one or more populations of neurons occurs as a result of disease or injury (Mattson, 2000). In AD, loss of hippocampal and cortical neurons is responsible for the symptomatology observed in this neurodegenerative disease (Mattson, 2000). The regulation of apoptosis involves several mediators such as neurotrophic factors (inhibition) or glutamate (activation) (Mattson, 2000). Excessive glutamate mediated activity increases the influx of Ca^{2+} leading to excitotoxicity and ultimately cell death (Mattson, 2000). As shown in Table 15, B7C provided protection against glutamate-induced excitotoxicity and free radical-induced damage (Han et al., 2000; Xiao et al., 2000; Fu et al., 2007; Zhao et al., 2008b; Fang et al., 2010).

Another target for B7C is the GABA receptor (Li et al., 1999, 2007a; Zhou et al., 2009). GABA is the major inhibitory neurotransmitter in the central nervous system and it primarily functions as a metabolite and a neurotransmitter. (Zhou et al., 2009; Best et al., 2014). Together with the excitatory neurotransmitter glutamate, GABA is an important modulator of the inhibitory-excitatory balance that is essential for the proper functioning of the brain (Wu and Sun, 2015). Dysfunctions in the GABA system have been closely linked with neurological disorders such as Huntington's chorea, epilepsy, AD, anxiety, and depression (Krogsgaard-Larsen, 1992; Kim and Yoon, 2017). The $GABA_A$ receptor is a ligand-gated ion channel that regulates the influx of chloride ions, causing hyperpolarization in the postsynaptic neuron (Kim and Yoon, 2017) and mediating the fast inhibitory neurotransmission in the brain (Best et

al., 2014). Changes in the concentration of endogenous modulator or in the composition of the $GABA_A$ receptor lead to downregulation of the neuronal inhibition seen in pathological states (Nuss, 2015). Therefore, drugs that regulate the GABA system such as B7C are highly relevant in the treatment of neurological disorders.

The BACE-1 is the enzyme responsible for the onset of the generation of Aβ. Therefore, it represents a very promising target for AD (Vassar et al., 2009). Aβ is the major components that occur in neuritic plates found in AD (Chen et al., 2012). BACE-1 overexpression or hyperactivity is associated with the pathogenesis of AD while the opposite scenario has been found to have a neuroprotective effect (Chen et al., 2012). The findings of 2 studies shown in Table 15 demonstrated an inhibitory effect of B7C on BACE-1 leading to a decreased generation of Aβ (Fu et al., 2008, 2009).Therefore, B7C showed a protective effect for the treatment of AD. Finally, at a behavioral level, B7C improved cognition and special learning and memory observed in several animal models (Pan et al., 2007, 2011; Han et al., 2012; Shu et al., 2012).

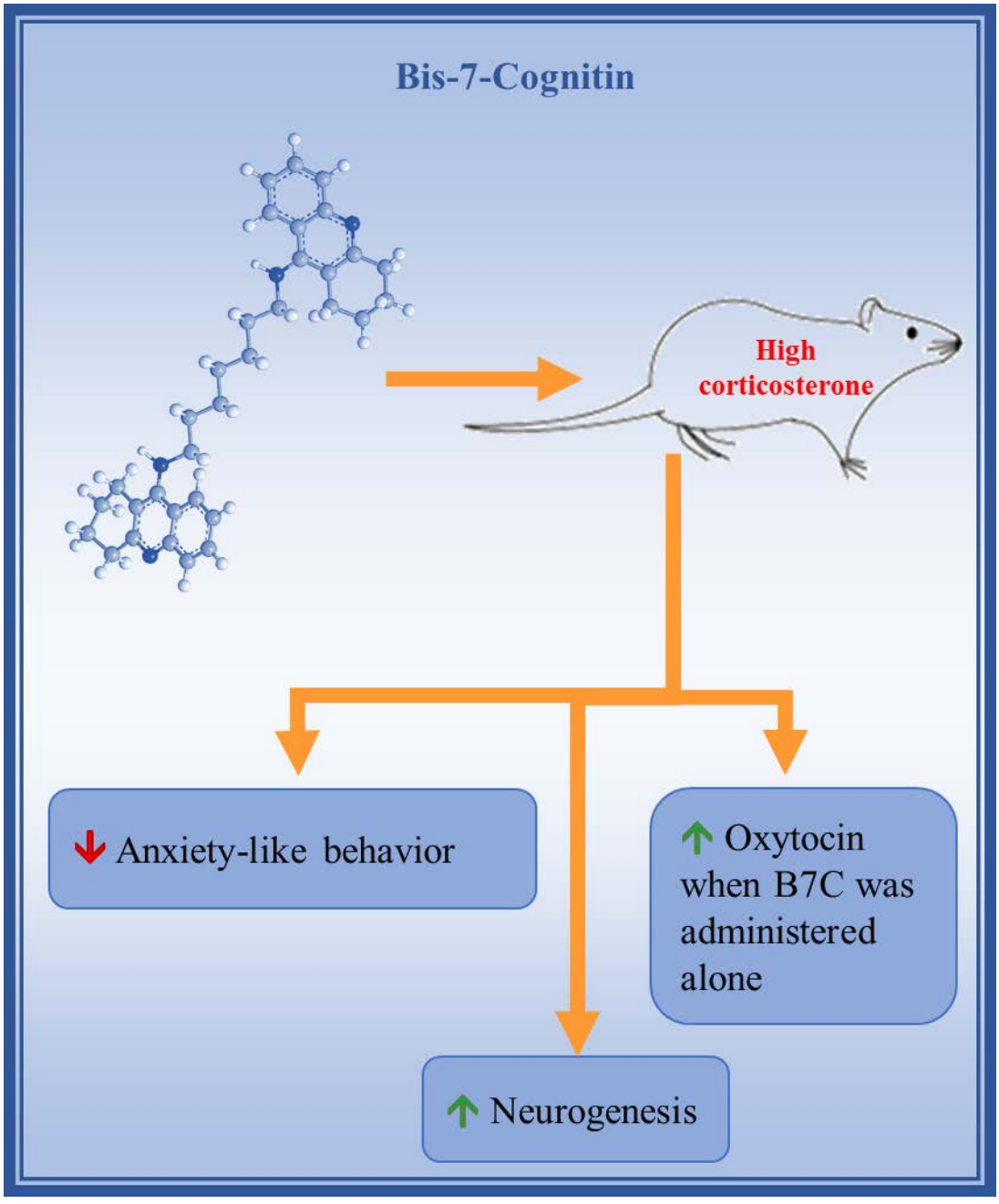
5.4. Conclusions

B7C is a computationally designed drug that has shown promising effects on many *in vitro* and *in vivo* platforms for AD and other neurodegenerative disorders. In the last two decades, several studies have focused on the evaluation of B7C on different targets. From the analysis presented, it is clear that B7C shows a superior activity when compared to its basic structure tacrine. This review presented an overview of the findings of the effect of B7C on several targets and its relevance in neurological

disorders. A very clear finding from the present review is that no studies have been conducted to evaluate the potential antidepressant effect of B7C – such studies will add a significant value to this dimeric molecule. The present review also supports the aim of this dissertation to explore the effect of B7C on neurogenesis. As neurogenesis is under the regulation of different molecular targets stated in this chapter, B7C is likely to regulate neurogenesis via the targets or related pathways.

CHAPTER 6

BEHAVIORAL AND NEUROGENIC EFFECT OF B7C ON RATS WITH CORTICOSTERONE-INDUCED DEPRESSION-LIKE BEHAVIOR



Abstract

Background: Depression is a global health issue with negative societal and economic health implications. B7C is a synthetic multitarget drug with promising effect on neurodegenerative disorders. However, the effect of B7C on depression has not been previously explored.

Aims: To evaluate the effect of B7C on depression and anxiety-like behaviors and neurogenesis in a dose-dependent experiment and a high corticosterone animal model.

Materials and methods: A dose-dependent experiment was carried out using adult male SD rats receiving different doses of B7C (0.2, 0.3 and 0.45 mg/kg). A second experiment involved the evaluation of B7C on behavior and neurogenesis using a high corticosterone (CORT) animal model.

Results: A positive effect on behavior and neurogenesis was observed when B7C was administered at a dose of 0.3 mg/kg B7C. Also, B7C reverted the behavioral and neurogenesis deficits induced by high-CORT treatment.

Conclusions: The positive effects of B7C on behavior and neurogenesis of the present study support further investigation of B7C on mood and the molecular mechanism underlying its effect.

6.1. Introduction

Because of the multifactorial pathogenesis of neurodegenerative disorders, multitarget drugs are highly valuable to tackle the array of symptoms present in this kind of disorders (Li et al., 2007b, 2009). B7C is a synthetic multitarget drug with promising effects including inhibition of AChE (Lopes et al., 2017), reduction of Aβ aggregation (Hu et al., 2015b), regulation of the NMDA receptor (Zhang et al., 2011), neuroprotection (Fang et al., 2010), regulation of GABA receptor-mediated activity (Zhou et al., 2009), inhibition of BACE-1 (Fu et al., 2008) and inhibition of NOS (Li et al., 2006). Most of the studies on B7C have focused on neurodegenerative disorders such as AD, but no study has evaluated the effect of B7C on depression.

Neurodegenerative events have been established in different regions of the brain in depressed patients including the hippocampus. In AD, hippocampus is one of the first brain areas to show alterations in its structure (Caraci et al., 2010). The hippocampus plays a pivotal role in cognitive processes and it is the primary memory structure in the brain (Sierksma et al., 2010). Reduction of the hippocampal volume in patients with major depression significantly correlates with cognitive and memory impairment which are the hallmark symptoms commonly observed in AD (Sierksma et al., 2010). Depression and AD symptoms have been correlated with decreased hippocampal neurogenesis, defined as the process to produce new neurons from neural stem cells. In fact, the behavioral benefits of antidepressants have been linked to improved neurogenesis in the hippocampus. Consequently, neurogenesis has been highlighted as a promising target process in developing novel therapeutic drugs for depression and AD (Apple et al., 2016).

For a long time, loss of neuron was thought to be an irreversible process in the adult brain because of the idea of disruption of the stability in the brain if replacement of the dying neurons could take place (Eriksson et al., 1998). However, in the 1990s, Erikson and his colleagues were the first in demonstrating neurogenesis processes in the sub granular zone in the adult brain (Eriksson et al., 1998; Apple et al., 2016). Hippocampal neurogenic processes are particularly important in the formation and consolidation of memories (Apple et al., 2016). In mammals, neurogenesis is limited to occur in specific brain regions including the subgranular zone of the dentate gyrus in the hippocampus and the SVZ localized next to the lateral ventricles (Aimone et al., 2014; Apple et al., 2016). Neural stem cells are generated in both regions which give rise to neural progenitor cells with the ability to differentiate into neurons or glia, migrate and integrate into the existing neuronal network in the hippocampus (Aimone et al., 2014).

A strong body of evidence suggests a pathophysiological relationship between depression and AD regarding neurogenesis. Previous studies have shown the neurotrophic effects of antidepressants due to upregulation of brain-derived neurotrophic factor (BDNF) in the hippocampus, which suggests potential neuroprotective effects (Tizabi, 2015). BDNF is an essential molecule that plays a role in the regulation of plasticity in the hippocampus (Sierksma et al., 2010). In addition, BDNF is required in the DG for the encoding and consolidation of pattern separated memories and it promotes neurogenesis and survival of newborn neurons (Bekinschtein et al., 2014). In fact, patients with major depression or AD exhibit low mRNA levels of BDNF in the hippocampus, suggesting that BDNF mediates changes in the hippocampus representing a key component of the pathophysiological processes

in depression and AD (Sierksma et al., 2010). Taking all the evidence together, neurogenesis represents a promising target for developing novel therapeutic options for depression and AD. In addition, evidence suggests that neuroprotection and upregulation of neurogenesis are desirable features in new drug candidates for cross-benefit for depression and AD. Therefore, the study of novel therapeutic agents for the treatment of depression and AD should include the evaluation of the neurogenic and neuroprotective effects. Therefore, the aim of this chapter was to evaluate the effect of B7C on depression-like behavior and neurogenesis.

6.2. Materials and methods

Animals

A total of 60 male Sprague-Dawley (SD) rats of 7-8 weeks of age (290 to 300 g body weight) were obtained from the Centralized Animal Facilities of The Hong Kong Polytechnic University and housed in polycarbonate cages (3 rats per cage). The animals had access to food and water *ad libitum*. The room was maintained at 23-25°C with 12-hour light-dark cycle. Approval of the protocol performed was granted by the Animal Subject Ethics Sub-Committee of The Hong Kong Polytechnic University.

Drugs

B7C was a gift from the Department of Applied Biology and Chemical Technology at The Hong Kong Polytechnic University by Professor Yi-Fan Han's research team (Wang et al., 1999). Corticosterone was obtained from Sigma Aldrich and fluoxetine from USP reference standard.

Experimental design

Two independent experiments were carried out. Experiment 1 was a pilot experiment that assessed the dose-dependent effect of B7C on behavior and neurogenesis. The findings from the experiment were used to choose to the optimal dose of B7C to be used in a disease animal model. Experiment 2 was carried out to evaluate the effect of B7C (optimal dose chosen from Experiment 1) on behavior and neurogenesis in a high corticosterone animal model.

Experiment 1: The experiment was designed to evaluate the dose dependent effect of B7C on behavior neurogenesis. The animals were randomly assigned into 4 groups and received the correspondent treatment via intraperitoneal (i.p.) injection of an equal volume of vehicle or B7C. The treatment was administered every day around 13:00-15:00 for 21 consecutive days. Treatment groups: (1) control group (n = 6) which received 0.9% saline; (2) 0.2 mg/kg B7C (n = 6); (3) 0.3 mg/kg B7C (n = 6); and (4) 0.45 mg/kg B7C (n = 6). On days 21-22, behavioral tests were performed. On day 23, all the animals were administered with 100 mg/kg BrdU via i.p. twice a day and transcardial perfusion was carried out on day 24.

Experiment 2: The effect of B7C on depression and anxiety-like behaviors was studied using a high corticosterone animal model (Gregus et al., 2005; Brummelte et al., 2006; Lee et al., 2013). A dose of 40 mg/kg corticosterone was used to induce depression- and anxiety-like phenotype in rats (Sánchez-Vidaña et al., 2016). The animals were randomly allocated into 6 groups (n = 6 rats per group): (1) the control group received a s.c. injection of propylene glycol (vehicle) and an i.p. injection of saline (vehicle); (2) the CORT group received a daily s.c. injection of 40 mg/kg corticosterone and an

i.p. injection of saline (vehicle); (3) the fluoxetine (Fluox) group received an i.p dose of 10mg/kg/day. The fluoxetine dose was based on previous experiments in which fluoxetine was chronically administered showing increased neurogenesis and restoration of depression-like behavior and neurogenesis in an animal model for depression (Malberg and Duman, 2003; Wang et al., 2011). Group (4) was the B7C group and the animals in this group received an i.p. injection of 0.3mg/kg B7C and a s.c. injection of propylene glycol (vehicle); group (5) received both CORT + Fluox, and group (6) was administered with CORT + B7C. The administration of the different treatments was done daily for 21 consecutive days. An intraperitoneal injection of 50 mg/kg/day BrdU was administered to all the animals on days 19, 20, and 21 in order to label the proliferating cells (Taupin, 2007). The weight of the rats was measured on days 1 and 22. On days 21-22, behavioral tests were performed and perfusion was carried out on day 23.

Behavioral tests

Forced Swimming Test (FST): The FST was carried out as described in CHAPTER 4. The scoring of the FST was done manually in Experiment 1 and the Noldus EthnoVision XT software version 11 was used to score the FST of Experiment 2 using the following settings: Activity states, 3 states were selected, the thresholds were above 6% for the highly active state, 3-6% for the moderately active state and below 3 % for the inactive state. The inactive state corresponds to the time spent immobile.

Open Field Test (OFT): An arena (72 cm length x 72 cm wide x 40 cm deep; lighting of 550 lux) was used for the OFT in order to evaluate anxiety in rats when exposed to an unfamiliar environment. A video camera was mounted above the open field adjusted to cover the whole test area. The test was video recorded for scoring later by

an observer blinded to the treatment. In the test, each of the animals was put into the arena for 10 minutes and was allowed to explore the arena freely. For the analysis, the arena was divided into 16 equal squares on screen and the numbers of squares crossed by the rat's neck were counted to measure the locomotor activity. The measurements recorded included: (1) peripheral locomotor – the time spent walking close to the walls of the test field (thigmotaxis); (2) central locomotor activity – time spent in the central 36 cm x 36 cm area of the field. Increased time spent in the central area or decreased time of latency to enter the central area are indications of anxiety-like behavior (Prut and Belzung, 2003; Gregus et al., 2005; Airan et al., 2007; Gamberini et al., 2015). The scoring of the OFT videos was carried out using the Noldus EthnoVision XT software version 11.

Social Interaction Test (SIT): Anxiety-like behavior was assessed using the SIT as described in CHAPTER 4.

Animal Perfusion and tissue processing

Animals were sacrificed as described in CHAPTER 4. Brains and serum samples were collected and processed as described in CHAPTER 4.

Immunoperoxidase staining and quantification of BrdU and DCX positive cells

Immunoperoxidase staining of BrdU and DCX positive cells, quantification of BrdU and DCX positive cells were carried out as described in CHAPTER 4.

Dendritic complexity of immature neurons

Dendritic complexity of immature neurons was carried out as described in CHAPTER 4.

Biochemical analyses in serum

ELISA kits were used to determine the concentration in serum of BDNF (Millipore), corticosterone (Enzo Life Science) and oxytocin (Enzo Life Science) following the instructions from the manufacturer's kits.

Statistical analysis

The statistical analysis was carried out using the SPSS software (version 13.0). One-way ANOVA with Tukey posthoc test was used when the data to be analyzed met the criteria of homogeneity and equal variance. In the event that either the assumptions of normality, checked by Shapiro-Wilks test, or homogeneity of variance, checked by Levene's test, were not met, the non-parametric tests Kruskal-Wallis and Mann-Whitney U were used. Significant difference was determined using a p-value < 0.05.

6.3. Results

Experiment 1

This experiment was a pilot study that evaluated whether B7C had a positive effect on depression and anxiety-like behavior and whether it affected neurogenesis. This pilot experiment was also carried out to select the optimal dose of B7C to be used in a high corticosterone animal model (Experiment 2). Therefore, 3 different doses of B7C were tested.

Body weight

On day 1 and day 22 the weight of the animals was measured (Figure 25 A). The 0.2 mg/kg B7C (291.33 g ± 3.61, $p = 0.002$ vs the control) and 0.45 mg/kgB7C (302.33 g

± 2.13, $p = 0.009$ vs the control group) groups started the treatment with lower weight than the control (319.83 g ± 5.83) and 0.3 mg/kg B7C (322 g ± 8.85, p = 0.015 vs the 0.2 mg/kg B7C group; $p = 0.041$ vs the 0.45 mg/kg B7C group) which showed statistically significant difference. At the end of the treatment, the 0.2 mg/kg B7C group (364.83 g ± 8.30, $p = 0.004$ vs the control group) showed the lowest weight among the control (438.66 g ± 19.91), 0.3 mg/kg B7C (403.33 g ± 11.00) and the 0.45 mg/kg B7C (458.66 g ± 8.73) groups. No statistically significant difference was observed in the weight gain among the group (Figure 25 B).

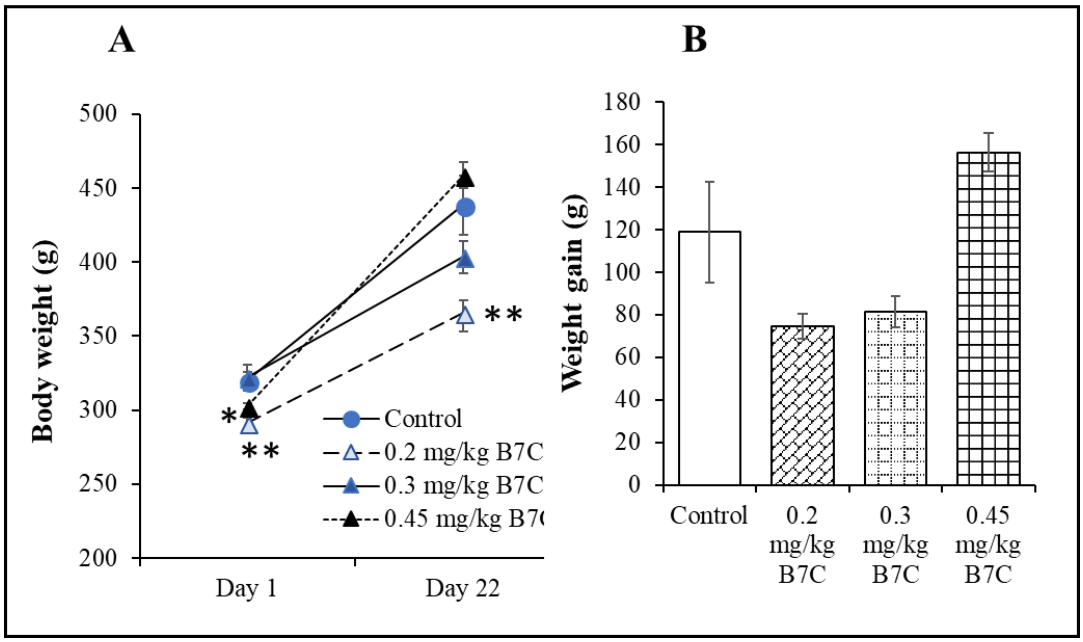


Figure 25. Body weight. (A) Body weight at day 1 and 14. (B) Weight gain. Results are expressed as Mean ± SEM; *$p < 0.05$ and **$p < 0.01$ when compared to the control group.

Behavioral tests

The time spent floating in the FST showed no statistically significant difference when compared with control group to the B7C groups (Figure 26 A; $p = 0.004$). However,

a significant difference was observed between the 0.2 mg/kg B7C (159.80 sec ± 6.58) and the 0.45 mg/kg B7C (77.60 sec ± 13.69, $p = 0.003$) and between the 0.3 mg/kg B7C (131.83 sec ± 16.54) and the 0.45 mg/kg B7C (77.60 sec ± 13.69, p = 0.041). In the SIT, all the B7C groups showed a higher number of positive social interactions when compared with the control group (control 8.66 ± 0.88; 0.2 mg/kg B7C 15.16 ± 1.53; 0.3 mg/kg B7C 21.33 ± 1.45 and 045 mg/kg B7C 18.66 g ± 1.28; $p = 0000$) (Figure 26 B). A significant difference was observed between the 0.2 mg/kg B7C and 0.3 mg/kg B7C ($p = 0.017$).

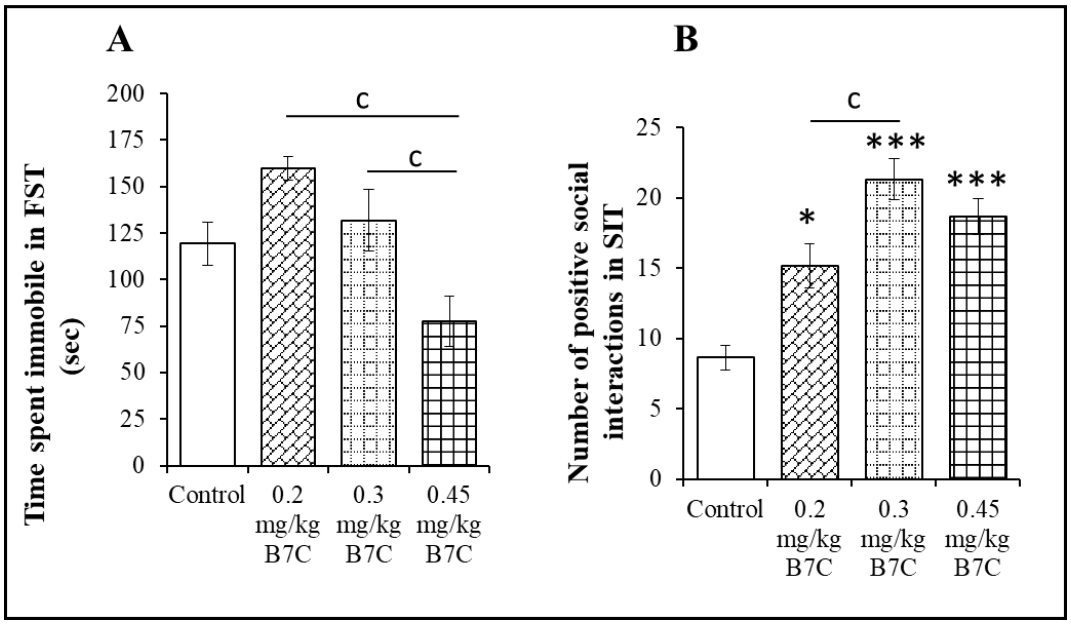


Figure 26. Results from the forced swimming test and social interaction test. **(A)** Results from the FST showing the time spent floating. **(B)** Total number of positive social interactions in the SIT. Results are expressed as Mean ± SEM; *$p < 0.05$ and **$p < 0.01$, ***$p < 0.01$ when compared to the control group, **c** $p < 0.05$ when compared to any B7C group.

In the OFT, no statistically significant difference was found in the time spent in the center of the arena among the groups (Figure 27 A). Also, no significant difference was observed in the distance traveled (Figure 27 B, C).

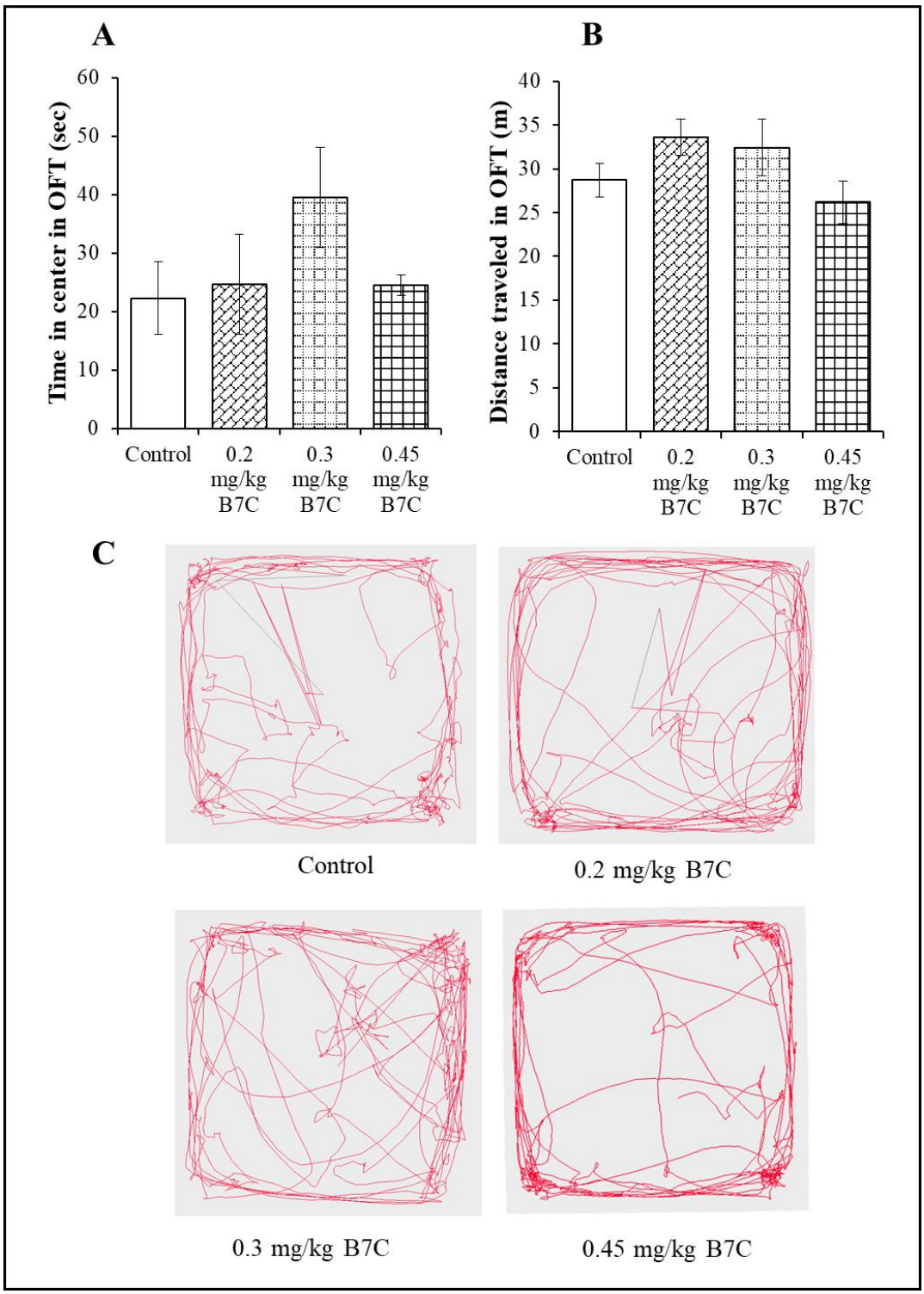


Figure 27. Results from the open field test. **(A)** Time spent in the center in the OFT. **(B)** Total distance traveled. **(C)** Representative moving trace. Results are expressed as Mean ± SEM.

BrdU and DCX positive cells

A significant difference in the number of positive BrdU cells was found in the hippocampus (Figure 28; $p = 0.002$). The 0.2 mg/kg B7C (2283.75 ± 396.79) and 0.3 mg/kg B7C showed higher number of BrdU cells when compared with the control group (913.17 ± 108.84; $p = 0.010$, $p = 0.002$, respectively). Treatment with 0.45 mg/ kg B7C (821.00 ± 133.80) did not increase the number of BrdU positive cells in the hippocampus (Figure 29).

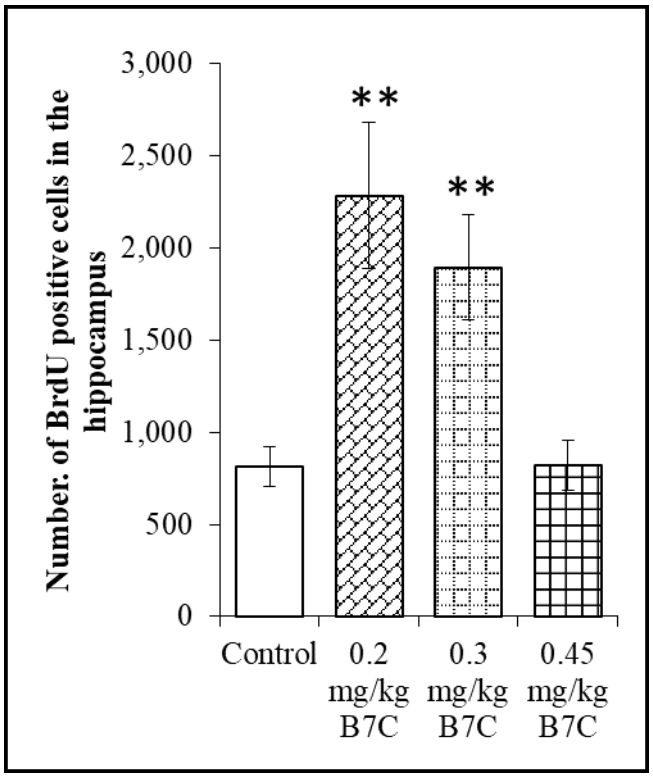


Figure 28. Number of BrdU positive cells in the hippocampus

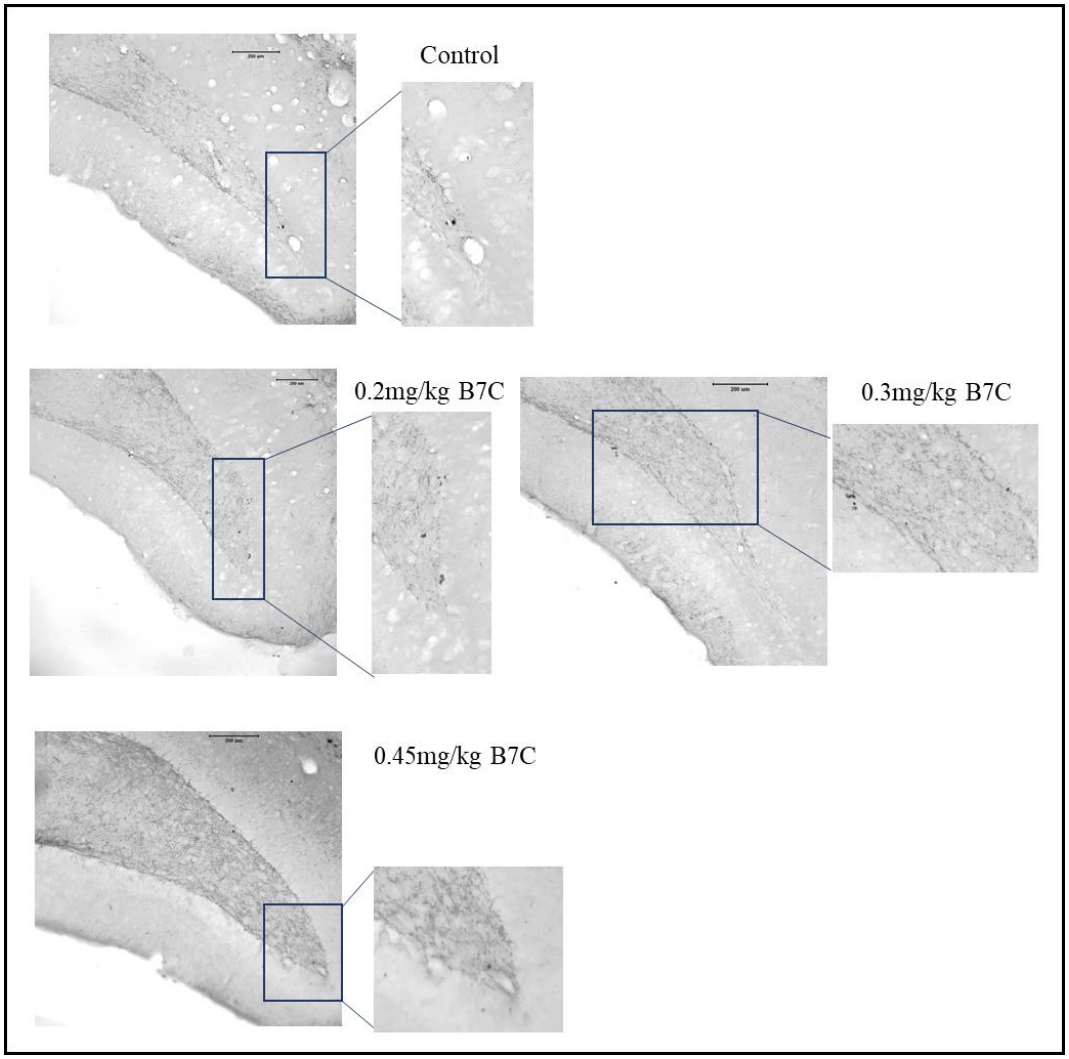


Figure 29. Representative images of the BrdU positive cells in the dentate gyrus of the hippocampus

Treatment with 0.3 mg/kg B7C (1609.20 ± 186.22) increased the number of DCX positive cells in the hippocampus when compared to the control group (769.83 ± 54.36, $p = 0.004$) (Figure 30 and 31). No significant difference was observed in the 0.2 mg/kg B7C group (769.83 ± 54.36).

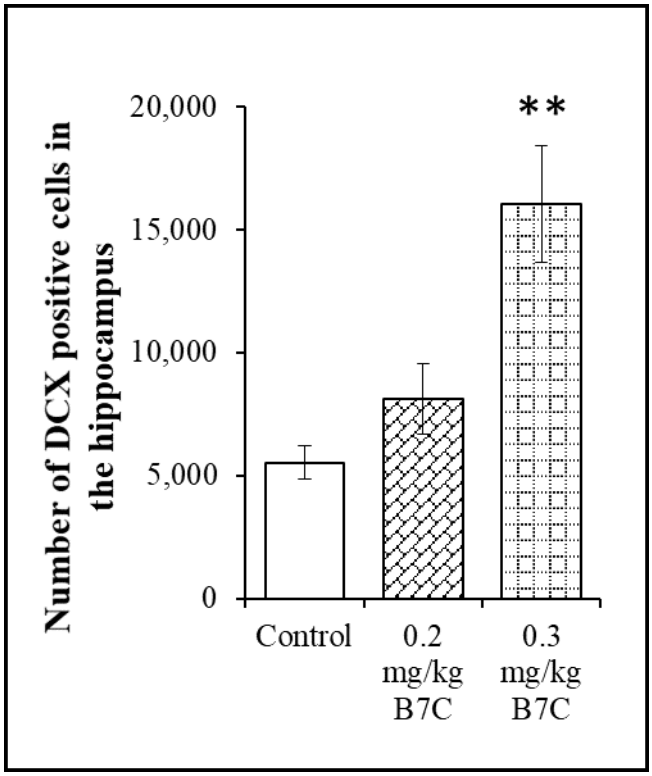


Figure 30. Number of DCX positive cells in the hippocampus

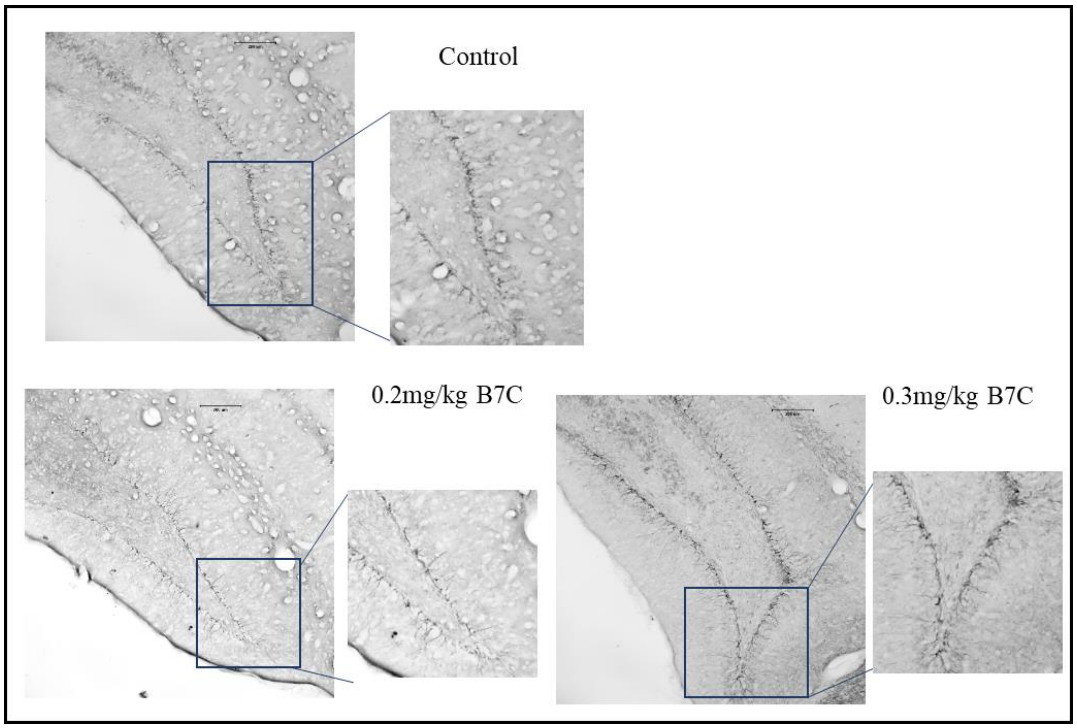


Figure 31. Representative images of the DCX positive cells in the dentate gyrus of the hippocampus

The dose of B7C that showed a positive effect on behavior and neurogenesis was 0.3 mg/kg B7C. Therefore, this dose was selected to evaluate the effect of B7C in a high corticosterone animal model.

Experiment 2

Experiment 2 was carried out to evaluate the effect of B7C in a CORT-induced depression and anxiety-like behavior and in a reduced neurogenesis animal model. Additionally, an antidepressant group was included in the experiment to compare the effect of B7C with the effect of a commonly used antidepressant.

Body weight and adrenal gland weight

On day 1 and day 22 the weight of the animals was measured. No significant difference at the beginning and at the end of the treatment was observed among all the treatment groups (Figure 32 A). However, a statistically significant difference in the weight gain was observed (Figure 32 B, $p = 0.006$). The weight gain in the CORT group (80.83 g ± 11.57) showed significant difference when compared with the control group (142.60 ± 13.55). Also, a significant difference was observed between the 0.3 mg/kg B7C (145.33 g ± 16.42) and the CORT group ($p = 0.016$). No statistically significant difference in weight gain was observed with the other treatment groups. The adrenal gland weight in the CORT (4.64 g ± 0.30), CORT + Fluox (5.64 g ± 0.39), and CORT + B7C (5.02 g ± 0.58) showed lower weight when compared with the control group ($p = 0.000$, $p = 0.002$, $p = 0.000$, respectively) (Figure 32 C).

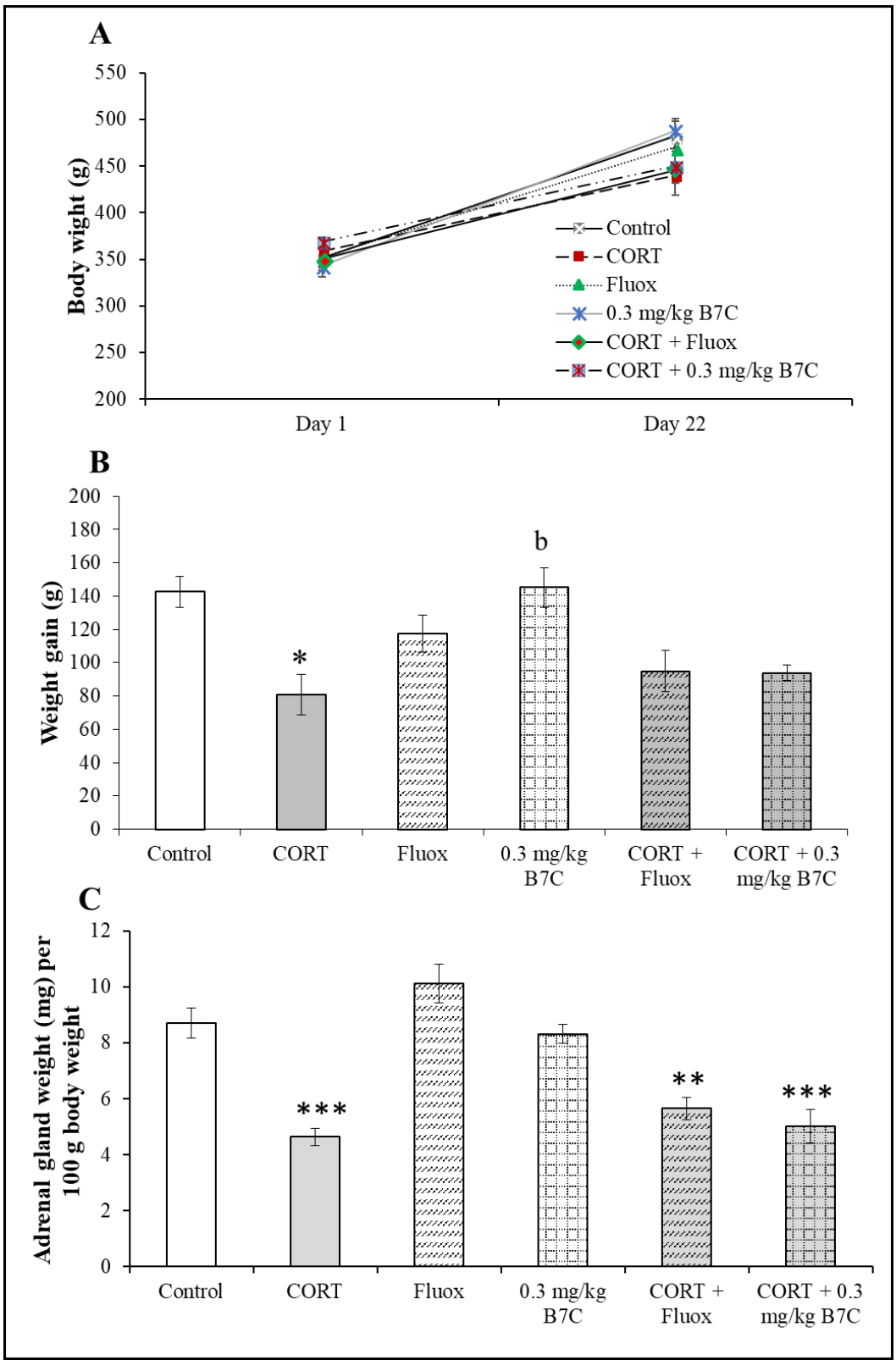


Figure 32. Body weight and adrenal gland weight. **(A)** Body weight at day 1 and 22. **(B)** Weight gain. **(C)**Adrenal gland weight. Results are expressed as Mean ± SEM;

*$p < 0.05$ and **$p < 0.01$; *** $p < 0.001$ when compared with the control group; **b** $p < 0.05$ when compared with the CORT group.

Behavioral tests

In the FST, the time spent immobile showed a statistically significant difference among the treatment groups (Figure 33 A, $p = 0.010$). The longest time spent immobile was observed in the CORT group (220.25 sec ± 51.90) and this was significant different when compared with the control group (67.16 sec ± 12.89, $p = 0.010$). The CORT + B7C (123.00 sec ± 18.08, $p = 0.041$) also showed longer immobile time when compared with the control group. Furthermore, the Fluox (60.00 sec ± 22.73, $p = 0.016$), 0.3 mg/kg B7C (88.83 sec ± 15.81, $p = 0.010$), and CORT + Fluox (105.83 sec ± 18.71, $p = 0.038$) showed a statistically significant difference when compared with the CORT group. In the SIT, a significant difference was observed among the groups (Figure 33 B, $p = 0.002$). The CORT (13.83 ± 1.77, $p = 0.029$) and the Fluox group (9.83 ± 1.75, $p = 0.000$) showed the lowest number of positive social interactions when compared with the control group (21.83 ± 1.70).

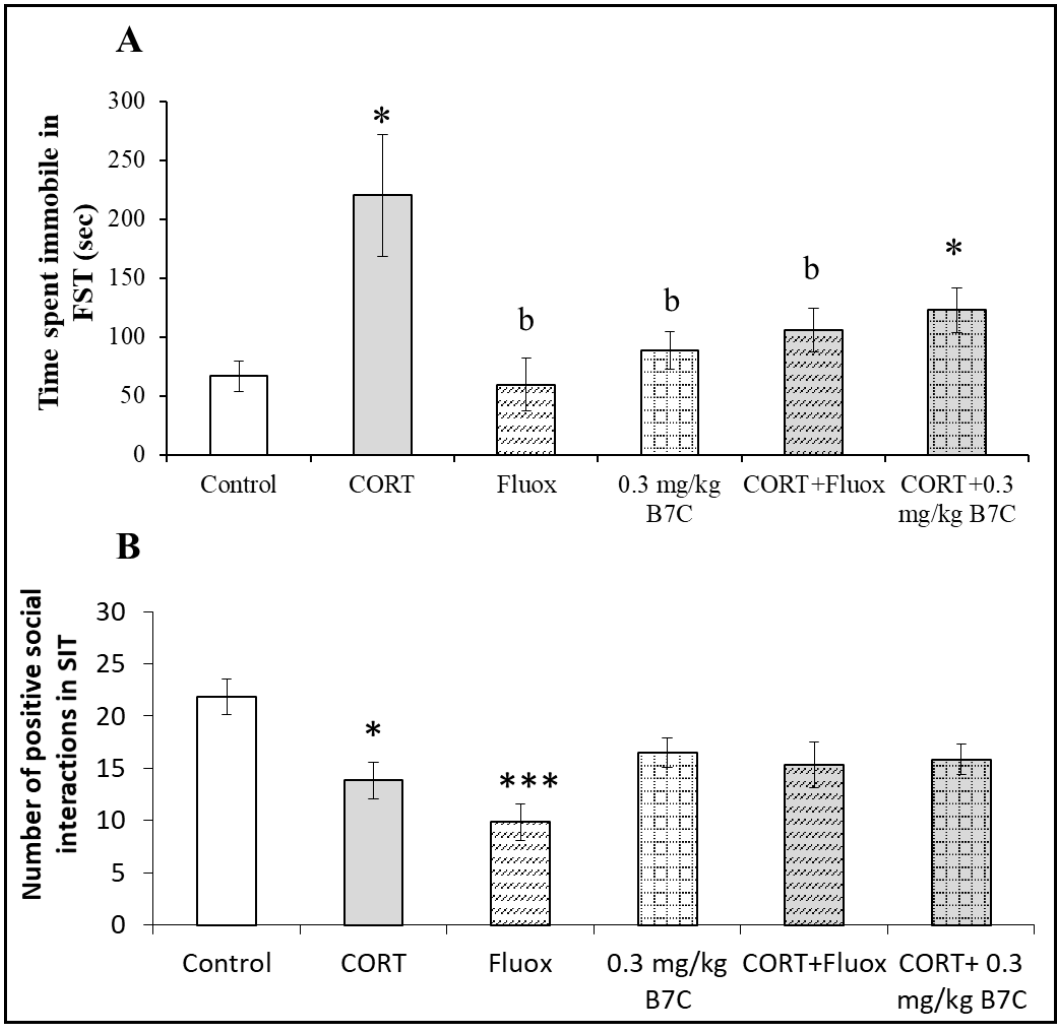


Figure 33. Results from the forced swimming test and social interaction test. **(A)** Results from the FST showing the time spent floating. **(B)** Total number of positive social interactions in the SIT. Results are expressed as Mean ± SEM; *****$p < 0.05$ and ******$p < 0.01$; ******* $p < 0.001$ when compared with the control group; **b** $p < 0.05$ when compared with the CORT group.

In the OFT, no significant difference was observed in the time spent at the center (Figure 34 A). However, a significant difference was observed in the distance traveled (Figure 34 B and C, $p = 0.001$). The 0.3 mg/kg B7C group (346.8 m ± 32.46, $p = 0.016$) and the CORT +Fluox group (578.00 ± 52.06, $p = 0.029$) showed a higher

distance traveled when compared to the control group (30.00 ± 9.96). No difference in the distance traveled was observed in the other treatment groups.

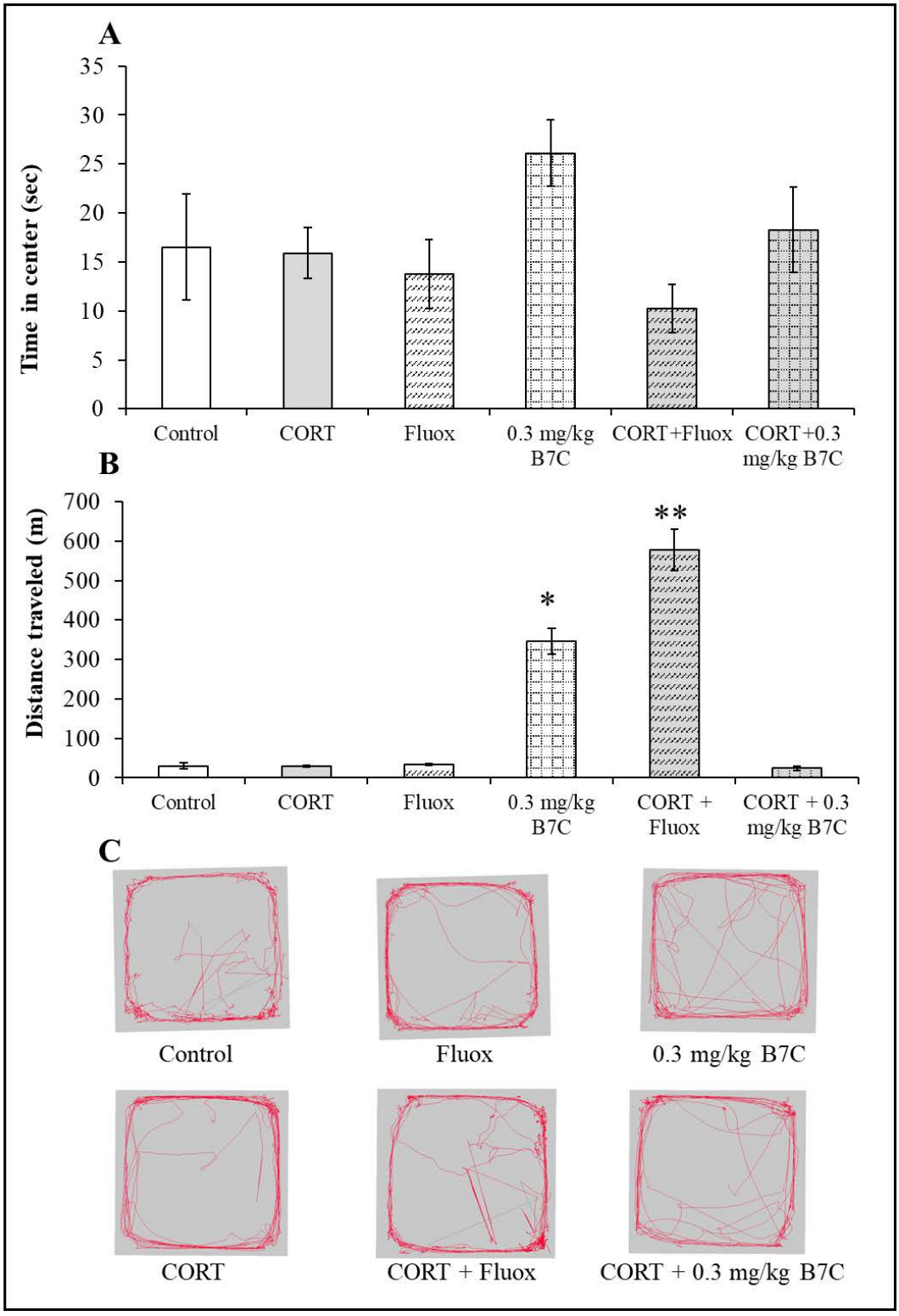


Figure 34. Results from the open field test. **(A)** Time spent in the center in the OFT. **(B)** Total distance traveled. **(C)** Representative moving trace. Results are expressed as Mean ± SEM. $*p < 0.05$ and $**p < 0.01$ when compared with the control group.

BrdU positive cells

The number of BrdU positive cells in the hippocampus showed statistically significant difference among the treatment groups ($p = 0.002$; Figure 35). The CORT group showed the lowest number of BrdU positive cells indicating suppressed neurogenesis and it was statistically significant different when compared with the control group ($p = 0.009$, 1968.2 ± 193.73 and 1122 ± 167.69, control and CORT group respectively). Also, the Fluox group (3518.25 ± 482.87) increased the number of positive BrdU cells when compared with the control group. Conversely, no statistically significant difference was observed in the B7C (1628.83 ± 132.20), CORT + Fluox (2409.33 ± 378.43), and CORT + B7C (2583.33 ± 560.96) groups when compared with the control group (1968.2 ± 193.73). Interestingly, statistically significant difference was observed when comparing the Fluox, B7C, CORT + Fluox, and CORT + B7C group with the CORT group. The results demonstrate that treatment with Fluox alone increased the number of BrdU positive cells and reverted the suppressed neurogenesis induced by CORT treatment as observed in the CORT + Fluox group showing a higher number of BrdU positive cells when compared with the control group. Also, treatment with B7C also reverted the CORT-induced suppressed neurogenesis as observed in the higher number of BrdU positive cells in the CORT + B7C when compared with the CORT group.

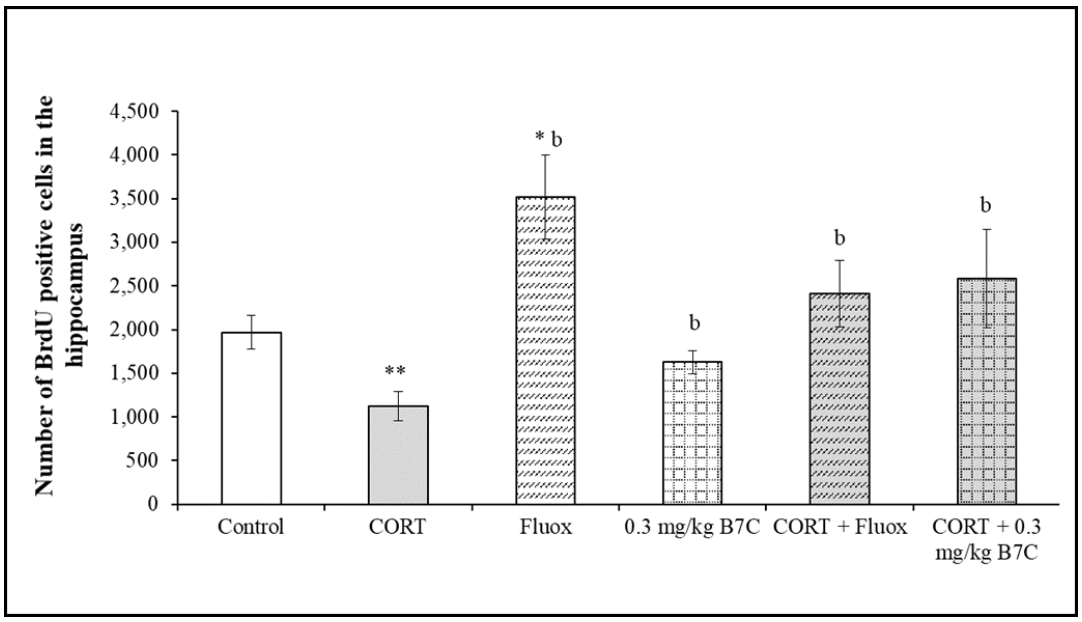


Figure 35. Number of BrdU positive cells in the hippocampus. **p* < 0.05 and ***p* < 0.01; *** *p* < 0.001 when compared with the control group; **b** *p* < 0.05 when compared with the CORT group.

Concentration of corticosterone, BDNF and oxytocin in serum

A significant difference in the concentration of CORT in serum was found among the treatment groups ($p = 0.012$, Figure 36 A). The CORT (180.55 ng/ml ± 37.02, $p = 0.004$) and CORT + B7C group (132.91 ng/ml ± 25.47, $p = 0.004$) showed lower CORT levels in serum when compared with the control group (397.71 ng/ml ± 44.53). No significant different in the levels of BDNF was found (Figure 36 B). Finally, treatment with 0.3 mg/kg B7C group (1367.92 pg/ml ± 85.23, $p = 0.042$, Figure 36 C) increased the level of oxytocin in serum, but the CORT + B7C group (719.19 pg/ml ± 83.46, $p = 0.013$) showed decreased levels of oxytocin in serum when compared to the control group (1059.91 pg/ml ± 72.49).

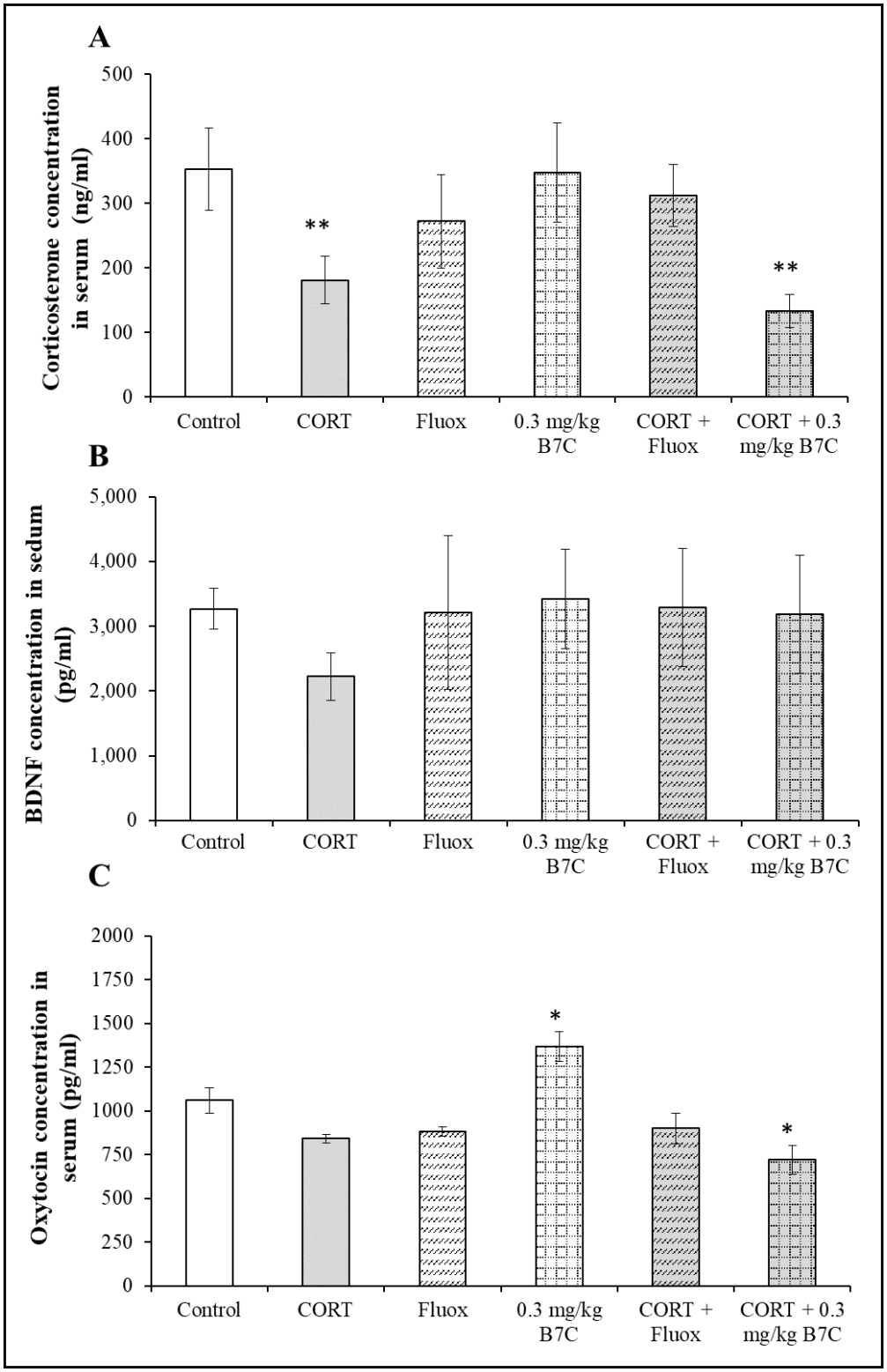


Figure 36. Concentration of corticosterone, BDNF and oxytocin in serum. Results are expressed as Mean ± SEM. *$p < 0.05$ and **$p < 0.01$ when compared with the control group.

6.4. Discussion

The present study is the first to evaluate the effect of B7C on depression and anxiety-like behavior and the first to explore the neurogenic effect of B7C *in vivo*.

Dose-dependent effect of B7C on behavior and neurogenesis

B7C was evaluated at the doses of 0.2, 0.3 and 0.45mg/kg in SD rats administered for 21 days. No effect on weight gain was observed among the B7C treatments when compared with the control group. Also, no difference in the time spent floating was observed. Conversely, B7C at all the doses tested stimulated social interaction as shown in the SIT. B7C increased the number of positive social interactions, demonstrating a positive effect of B7C on anxiety-like behavior. Furthermore, B7C increased the number of BrdU cells in the hippocampus at 0.2 and 0.3 mg/kg B7C. Finally, B7C increased the number of immature neurons (DCX positive cells) at a dose of 0.3 mg/kg. The dose-dependent effect of B7C on depression and anxiety-like behavior and neurogenesis was analyzed in order to identify the optimal dose of B7C that could be used in a high CORT animal model. The results of the dose-dependent experiment showed that B7C at 0.3 mg/kg had a positive effect on anxiety-like behavior and increased the number of BrdU and DCX positive cells in the hippocampus. Therefore, the effect of B7C at a dose of 0.3 mg/kg was evaluated in a high CORT animal model.

Effect of B7C in a high CORT animal model

The present study demonstrated a positive effect of B7C on behavior and neurogenesis. Treatment with B7C and Fluox reverted the reduced weight gain induced by CORT treatment as observed in the CORT + B7C and CORT + Fluox groups. Conversely, treatment with B7C and Fluox did not revert the reduced adrenal gland weight induced by the exogenous administration of CORT. Previous studies have demonstrated weight loss induced by chronic administration of CORT in rats (Brummelte et al., 2010; Lebedeva et al., 2017). Additionally, decreased adrenal gland weight has been reported as a result of CORT treatment (Qiu et al., 2007). The results observed in the present study agree with the reported effect of chronic administration of high CORT. High levels of endogenous glucocorticoids cause reduction in the adrenal gland weight as a result of negative feedback of the HPA axis, which is a physiological mechanism that regulates the stress response (Kott et al., 2016). The present study demonstrated that co-treatment with either B7C or Fluox increased the adrenal gland weight reverting the CORT-induced low adrenal gland weight.

The FST is a behavioral tool used to evaluate depression-like behavior. Previous evidence suggests that CORT treatment significantly increased the immobility time in the FST, thereby indicating increased depression-like behavior (Gregus et al., 2005; Marks et al., 2009). The evidence of the present study showed that treatment with Fluox reverted the CORT-induced depression like behavior as observed in the CORT + Fluox group. Previous studies have demonstrated the alleviation of depression-like behavior by Fluox treatment (David et al., 2009; Sah et al., 2012). Although the CORT + B7C treated group showed decreased time immobile in the FST, it was not

significantly lower than the CORT group. However, the immobility time was much lower than the immobile time in the CORT group.

Chronic administration of CORT led to decreased number of positive social interactions in the SIT as previously reported (Sánchez-Vidaña et al., 2016). The findings of the present study agree with the CORT decreased number of positive social interactions. Interestingly, a significantly higher number of positive social interactions was observed in the CORT + B7C and CORT + Fluox groups demonstrating a restorative effect on anxiety-like behavior by B7C and Fluox. Previous studies have demonstrated the positive effect of Fluox on anxiety-like behavior in the SIT. For instance, treatment with fluoxetine has been found to increase the positive behavior to approach other animals, number of grooming (Young et al., 2014) and a higher number of positive social interactions (Iñiguez et al., 2014). The restorative effect of fluoxetine and B7C against the anxiogenic effect induced by CORT in the SIT demonstrates the positive effect of B7C on mood which is comparable to the effect of the antidepressant fluoxetine.

Treatment with fluoxetine reverted the suppressed number of BrdU positive cells induced by CORT treatment as observed in the CORT + Fluox group. In previous studies, it has been demonstrated that fluoxetine increases the number of BrdU positive cells in the hippocampus (Malberg et al., 2000; Imoto et al., 2015). Also, previous studies showed that fluoxetine reverted the decreased number of BrdU positive cells in high dose corticosterone models (David et al., 2009) and in induced stress models (McEwen et al., 2001). Similarly, as the positive effect of fluoxetine observed in the CORT + Fluox group, B7C reverted the number of BrdU positive cells

in the CORT + B7C group. The results suggest that the positive behavioral effects observed in the CORT + B7C group might be mediated by increased neurogenesis induced by B7C. A previous study evaluated the effect of B7C on the number of BrdU cells in chronic cerebral ischemia model (Shu et al., 2012). The authors concluded that treatment with 0.2 mg/kg B7C for 14 days increased the number of BrdU positive cells (Shu et al., 2012) which agrees with the results observed in the dose-dependent experiment of the present study. Therefore, the present study provides further evidence on the neurogenic effect of B7C. Also, B7C has clearly demonstrated a restorative effect on the suppressed number of BrdU positive cells induced by CORT treatment.

BDNF is a key player in the regulation of adult neurogenesis (Zhao et al., 2008a; Calabrese et al., 2009) which has a clear role in depression (Taupin, 2006; Marais et al., 2009; Lang and Borgwardt, 2013; Gersner et al., 2014; Chen et al., 2015a). BDNF expression in the brain has been found to have a correlation with the BDNF levels in serum. However, no significant difference was observed in the BDNF levels in serum among all the treatment groups in the present study. However, treatment with B7C alone showed increased levels of oxytocin in serum, making the possible involvement of oxytocin in the positive effect of B7C on behavior and neurogenesis worthy of future exploration. However, a significantly lower concentration of oxytocin in serum was observed in the CORT + B7C group. The results observed indicate the possible regulation of oxytocin mediated by B7C. However, the evidence observed in the present study does not allow a clear interpretation of how the B7C regulatory mechanism on circulating oxytocin level occurs. Therefore, further investigation to establish the relationship between oxytocin and B7C is required.

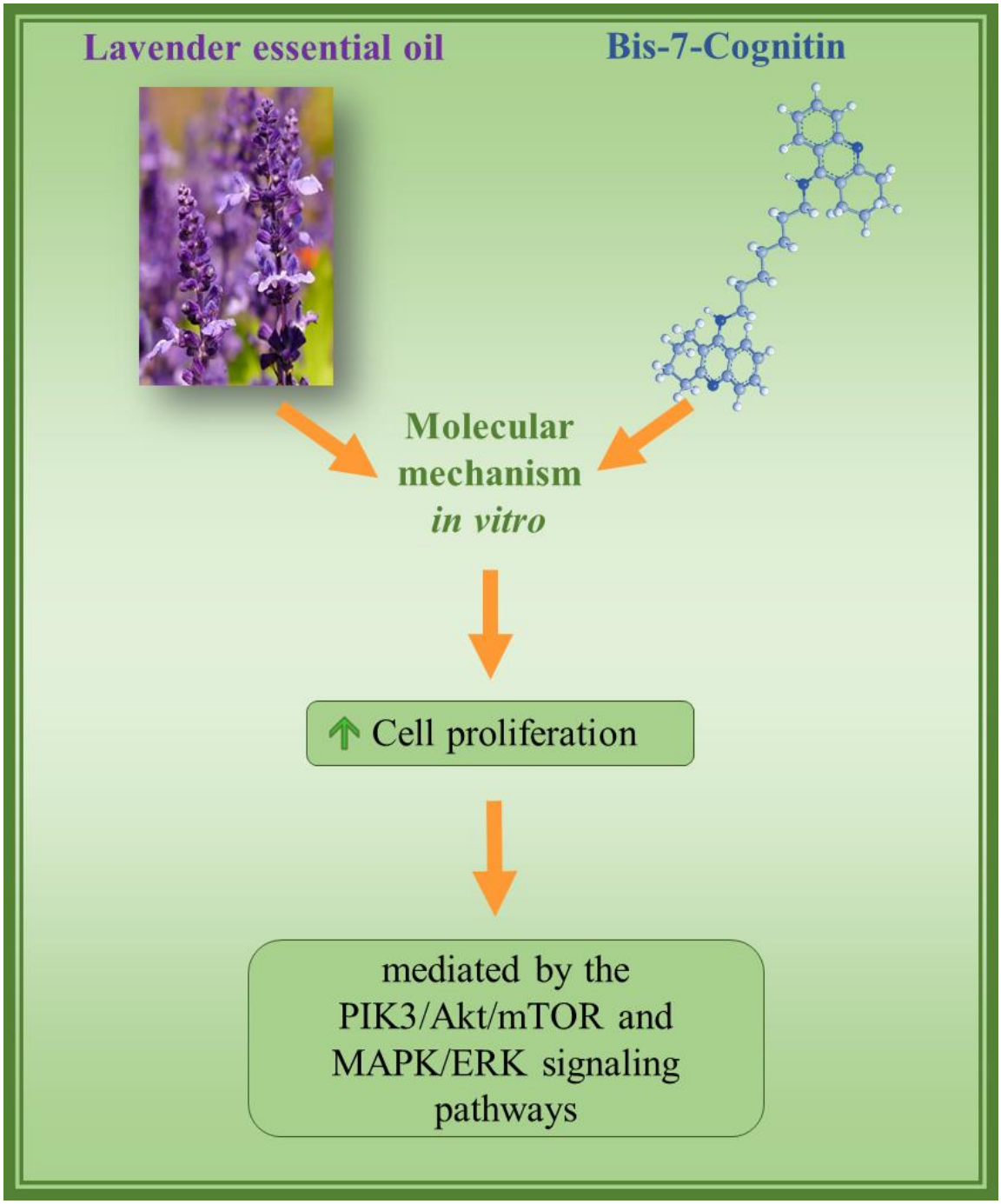
The CORT concentration in the serum of the CORT group was low and this was expected due to the CORT-induced negative feedback in the HPA axis (Scherer et al., 2011). Exogenous administration of glucocorticoid leads to suppression of the HPA axis activity which can last several hours and it is reversible at low doses of glucocorticoid (Andrews et al., 2012). In the present study, the exogenous administration of CORT was stopped 2-3 days before taking the serum sample. Due to the suppression of the secretion of endogenous CORT as a result of the chronic CORT administration, the CORT level was low in the CORT group at the time the serum sample was taken which explains the results observed. Interestingly, the CORT + Fluox group showed a less pronounced negative feedback effect on the CORT levels as shown when comparing the CORT level of the co-treated group with the control group.

6.5. Conclusions

The present study is the first study to evaluate the effect of B7C on depression and anxiety-like behavior and neurogenesis in a high CORT animal model. Treatment with B7C showed improvement in the CORT-induced anxiety-like behavior and decreased neurogenesis. The evidence of the present study supports further investigation of B7C on mood and the molecular mechanism underlying its effect.

CHAPTER 7

A STUDY OF THE MOLECULAR MECHANISM OF LEO AND B7C *IN VITRO*



Abstract

Background: Protection against glucocorticoid-mediated effects on mood and brain is a promising approach in the development of treatment options for depression. The synthetic drug B7C has been demonstrated to be a valuable multi-target drug in the treatment of neurodegenerative disorders. Lavender essential oil (LEO) is a commonly used essential oil in aromatherapy and has been found to have a positive antidepressant effect in animal and clinical studies.

Aims: To evaluate the protective effect of LEO and B7C in a corticosterone-induced cytotoxicity model in PC12 cells and to investigate the molecular mechanism underlying their effect.

Materials and methods: Two sets of experiments were carried out (1) to determine the dose-dependent effect of LEO and B7C on cell proliferation and (2) to evaluate the effect of LEO and B7C on the PI3K/Akt/mTOR and MAPK/ERK signaling pathways. The MTS assay was used to measure cell proliferation in PC12 cells. Corticosterone (CORT) was used to induce cytotoxicity and fluoxetine was used as cell proliferation positive control. The inhibitors LY294002 and U0126 were used to block the PI3K/Akt/mTOR and MAPK/ERK signaling pathways respectively.

Results: LEO and B7C at a concentration increased cell proliferation. LEO and B7C reverted the inhibited CORT-induced reduction in cell proliferation. The protective effect of LEO and B7C was blocked by inhibiting the PI3K/Akt/mTOR and MAPK/ERK signaling pathways.

Conclusions: LEO and B7C showed a protective effect against CORT-induced cell toxicity. Also, regulation of the PI3K/Akt/mTOR and MAPK/ERK signaling pathways was identified as the mechanism involved in the cell proliferation effect of LEO and B7C.

7.1. Introduction

Increased levels of endogenous glucocorticoids are observed as a result of stress (Anacker et al., 2013). Cortisol in humans and corticosterone (CORT) in rodents are glucocorticoids that act as end-point mediators of the HPA axis activation (Waters and McCormick, 2011). Chronic stress leads to hyperactivity of the HPA axis that causes abnormal high levels of glucocorticoids (Numakawa et al., 2017). Hyperactivity of the HPA axis has been linked to disorders such as anorexia nervosa, post-traumatic stress disorder and depression (Gao et al., 2009). Increased levels of circulating glucocorticoids have been shown to decrease neurogenesis in the adult brain (Anacker et al., 2013). Alterations in neurogenesis in the hippocampus caused by high glucocorticoid levels have been suggested to the pathogenesis of depression (Anacker et al., 2013). Therefore, exploring the effect and molecular mechanisms of potential therapeutic alternatives that could offer protection against the glucocorticoid-mediated effects on mood and brain is a promising approach.

Lavender essential oil (LEO) has shown anxiolytic and antidepressant properties and is a promising option for the treatment of depression (Wu and James, 2011; Perry et al., 2012; Sánchez-Vidaña et al., 2017). Although the effect of LEO to decrease depressive symptoms has been explored in animal and clinical studies, the molecular mechanism underlying its effect is unknown. Bis-7 cognitin (B7C) is a synthetic tacrine-based dimer extensively studied for over 20 years for its promising effect in the treatment of Alzheimer's disease (Ros et al., 2001). B7C exerts a potent inhibitory effect on AChE (Li et al., 1999; Wang et al., 1999a; Ros et al., 2001; Hu et al., 2002, 2015b; Fu et al., 2006; Yu et al., 2008; Bolognesi et al., 2010; Rizzo et al., 2011;

Mutunga et al., 2013; Lopes et al., 2017) and it has been demonstrated to act on several targets of great importance for the treatment of neurodegenerative disorders including the GABA receptor (Li et al., 1999, 2007a; Zhou et al., 2009), the NMDA receptor (Bai-fang et al., 2001; Li et al., 2005, 2007b; Luo et al., 2007; Liu et al., 2008b; Zhang et al., 2011; Liu and Li, 2012) and the BACE-1 enzyme. Despite the extensive study of B7C on targets relevant in neurodegenerative disorders, its potential effect on depression and molecular mechanism has not been explored.

The family of mitogen-activated protein kinases (MAPKs) is an important mediator in the transduction of signals form the membrane to the cell nucleus (Gao et al., 2009). Also, the MAPK family is important for the maintenance of homeostasis under stress conditions (Gao et al., 2009). The downstream signaling cascades in MAPK include the extracellular signal-related kinases (ERK1/2), Jun amino terminal kinases (JNK1/2/3), P38-MAPK and the ERK5 (Sun et al., 2015). Particularly, the MAPK/ERK pathway is critical for cell proliferation and differentiation and activation of this signaling pathway by phosphorylation of ERK1/2 leads to modulation of gene expression, mitosis and apoptosis (Gao et al., 2009).

The phosphoinositide kinase (PI3K)/ protein kinase B (Akt)/ mTOR pathway is an important signaling mechanism involved in cell fate by modulation of cell proliferation, cell differentiation and apoptosis (Kotasová et al., 2012; Devesa et al., 2014). The direction of the effect mediated by this pathway is regulated by the interaction with downstream substrates of Akt linked to other signaling pathways (Kotasová et al., 2012). Phosphorylation of Akt activates the phosphoinositide dependent kinase-1 that targets intracellular mediators such as mTOR that regulates

cell proliferation and survival (Devesa et al., 2014). The PI3K/Akt signaling pathway has been shown to be relevant in the pathology of depression and its association with neuronal inflammation where the PI3K/Akt pathway seems to regulate key inflammatory cytokines (Kitagishi et al., 2012). The relevance of the PI3K/Akt/mTOR pathway in depression is also due to the relationship with serotonin. Serotonin activates G-protein-coupled receptors that in turn activate the PI3K/Akt/mTOR signaling cascade which is similar to the activation mediated by VEGF signaling (Kitagishi et al., 2012). Previous findings have also shown that inhibition of Akt phosphorylation increased the number of apoptotic cells (Devesa et al., 2014), thereby adding value to the relevance of studying this signaling pathway when developing new treatment options for depression. Also, upregulation of expression of phosphorylated Akt by the antidepressant fluoxetine that promotes neurogenesis and improves cell survival demonstrates the important of the regulation of the PI3K/Akt signaling pathway in the pathophysiology of depression (Kitagishi et al., 2012).

Therefore, the aim of this study is to evaluate the neuroprotective effect of LEO and B7C on PC12 cells exposed to CORT and to evaluate whether the PI3K/Akt/mTOR and MAPK/ERK signaling pathway might be implicated.

7.2. Methods

Cell culture

PC12 rat pheochromocytoma cells show typical characteristics of neurons and express high levels of glucocorticoid receptors (Gao et al., 2009). Therefore, this cell line was chosen to study the effect of LEO and B7C on cell proliferation in an *in vitro* model involving treatment with corticosterone. PC12 cells were provided by the research group of Professor Yi-Fan Han based at the Department of Applied Biology and Chemical Technology of The Hong Kong Polytechnic University. Cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco) and 100U/ml streptomycin/penicillin (Gibco). Cells were incubated at 37°C with 5% CO_2 humidified atmosphere. Trypsin (Gibco) was used to detach the cells from the bottom of the culture vessel and they were sub-cultured twice a week using a 1:10 split ratio. Cells were seeded in 96 well plates at a cell density of $1x10^5$ c/ml (100µl cell suspension per well) in phenol red free DMEM (Gibco) supplemented with 10% FBS and 100 U/ml streptomycin/penicillin (Gibco) and incubated for 24h before treatment.

Drugs

The LEO was purchased from DK aromatherapy and extracted from flower tops of *Lavandula aungustifolia* Mill. (syn. *Lavandula officinalis* Chaix). B7C was synthesized at the Department of Applied Biology and Chemical Technology of The Hong Kong Polytechnic University by Professor Yi-Fan Han's research team as previously described (Wang et al., 1999). Corticosterone was obtained from Sigma Aldrich and fluoxetine was obtained from USP reference standard. The PI3K inhibitor LY294002 (10 mM) and the MAPK inhibitor U0126 (100 mM) were purchased from

Calbiochem. Stock solutions were prepared as follows: 3 mM B7C stock solution in water, 30 mM CORT stock solution in ethanol, and 0.1 mM fluoxetine in water.

Treatment and cell proliferation assay

Experiment 1: Dose-dependent effect on cell proliferation

In order to identify the concentration of LEO and B7C that increased cell proliferation, cells were treated with 0.05, 0.1, 0.5, 1 and 5 µl/ml LEO and 0.1, 0.3, 0.5, 1 and 5 µM B7C. Several studies have reported a decrease in cell proliferation induced by CORT treatment *in vitro* (Mirescu and Gould, 2006; Lau et al., 2012). Therefore, different concentrations of corticosterone (CORT) were evaluated (1, 2 and 10 µM) to identify the optimal concentration of CORT that decreased cell proliferation. Finally, cells were treated with 1 µM fluoxetine (Fluox) as cell proliferation positive control (Pariante et al., 2003; Sousa-Ferreira et al., 2014). Cells were rinsed with 0.01M PBS before adding the treatments. Cells proliferation was measured by MTS assay (MTS Cell Proliferation Colorimetric Assay Kit, BioVision) 48h after incubation with the treatments. All the treatments (n = 6 per treatment) were prepared in phenol red free DMEM supplemented with 10% FBS and 100 U/ml streptomycin/penicillin. Medium was used as negative control (NC).

Experiment 2: Signaling pathway experiment

In order to identity the potential signaling pathways involved in the cell proliferation effect of LEO and B7C, cell proliferation was measured after 48h treatment with LY294002 (a potent PI3K/Akt/mTOR signaling pathway inhibitor) and U0126 (a potent MAPK/ERK signaling pathway inhibitor). The inhibitors LY294002 and U0126 were used at a concentration of 50 µM and 100 µM respectively based on previous studies (Fujiwara et al., 2008; Gu et al., 2013; Hossain et al., 2017). Briefly, the treatments involving LEO were prepared as follows: 0.05µl/ml LEO, 1µM Fluox,

1 µM CORT, 1µCORT + 0.05 µl/ml LEO, 1 µM CORT + 1 µM Fluox, 50 µM LY294002, 100 µM U0126, 50 µM LY294002 + 0.05 µl/ml LEO, 50µM LY294002 + 1µM Fluox, 100µM U0126 + 0.05 µl/ml LEO and 100 µM U0126 + 1 µM Fluox. The treatments involving B7C were prepared as follows: 0.3 µM B7C, 1 µM Fluox, 1 µM CORT, 1µ CORT + 0.3 µM B7C, 1 µM CORT + 1 µM Fluox, 50 µM LY294002, 100 µM U0126, 50 µM LY294002 + 0.3 µM B7C, 50 µM LY294002 + 1 µM Fluox, 100 µM U0126 + 0.3 µM B7C and 100 µM U0126 + 1 µM Fluox. Cells were rinsed with 0.01 M PBS before adding the treatments. Cells proliferation was measured by MTS assay (MTS Cell Proliferation Colorimetric Assay Kit, BioVision) 48h after incubation with the treatments. All the treatments (n = 6 per treatment) were prepared in phenol red free DMEM supplemented with 10% FBS and 100 U/ml streptomycin/penicillin. Medium was used as NC.

Statistical analysis

Cell proliferation results were expressed as Meam+SEM of three independent experiments. The statistical analysis was carried out using the SPSS software (version 13.0). One-way ANOVA with Tukey posthoc test was used when the data to be analyzed met the criteria of homogeneity and equal variance. In the event that either the assumptions of normality, checked by Shapiro-Wilks test, or homogeneity of variance, checked by Levene's test, were not met, the non-parametric tests Kruskal-Wallis and Mann-Whitney U were used. Significant difference was determined using a p-value < 0.05.

7.3. Results

Effect of LEO on cell proliferation and identification of the signaling pathways

The dose-dependent effect of LEO on cell proliferation is shown in Figure 37. Treatment with CORT at all the concentration tested decreased cell proliferation. LEO at a concentration of 0.05 µl/ml showed increased cell proliferation comparable to the

one induced by treatment with 1 µM Fluox. LEO at 0.05 and 0.1 µl/ml increased cell proliferation. LEO at a concentration of 0.5 µl/ml decreased cell proliferation at a similar degree as CORT while higher concentrations of LEO clearly showed a cytotoxic effect. Based on the results from the dose-dependent experiment, LEO at 0.05 µl/ml was used in the signaling pathway experiment.

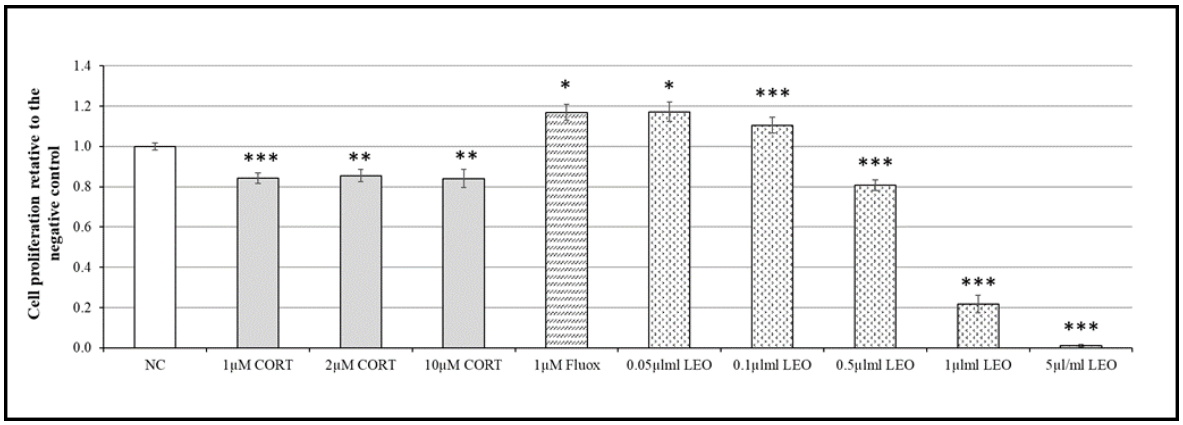


Figure 37. Dose-dependent effect of LEO on cell proliferation. *p < 0.05, ***p* < 0.01, and ****p* < 0.001 when compared to the control group.

The inhibitors, LY294002 and U0126, were used to block the PI3K/Akt/mTOR and MAPK/ERK signaling pathways respectively in order to identify whether the cell proliferation effect of LEO is the result of its activity on any of those pathways. The results of the signaling pathway experiment on LEO is shown in Figure 38. Treatment with CORT decreased cell proliferation while treatment with LEO and Fluox alone increased cell proliferation. Interestingly, treatment with LEO and CORT together reverted the decreased cell proliferation induced by CORT treatment alone (p = 000). Similar results were observed in the CORT + Fluox group (p = 000). No change in the decreased cell proliferation induced by LY294002 and U0126 was observed when treated with LEO and Fluox. This indicates that both LEO and Fluox act on both

signaling pathways. The findings demonstrated that LEO reverted the inhibited cell proliferation induced by CORT at a concentration of 0.05 µl/ml. Also, the protective effect of LEO is blocked by the inhibition of the PI3K/Akt/mTOR and MAPK/ERK signaling pathways, indicating that both pathways may be involved in the molecular mechanism of LEO to stimulate cell proliferation.

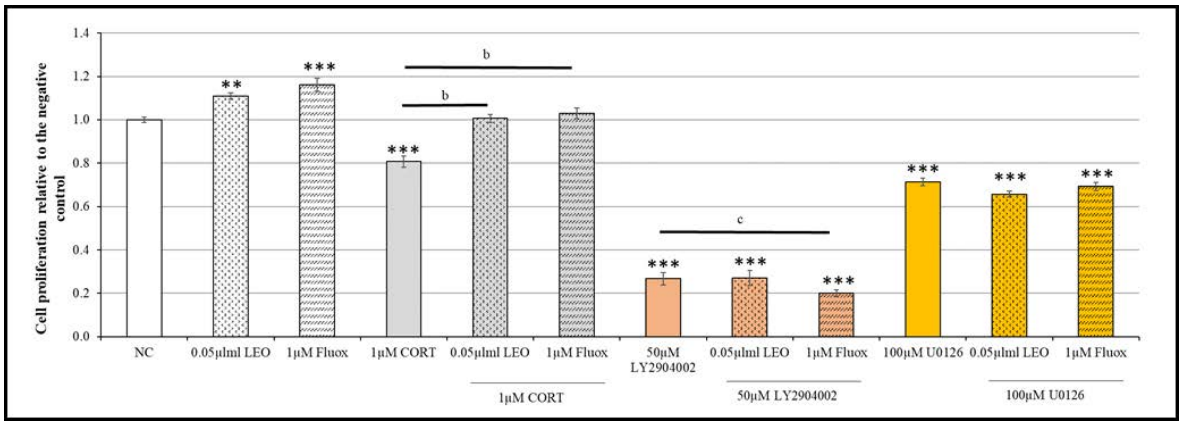


Figure 38. Effect of LEO on PI3K/Akt/mTOR and MAPK/ERK signaling pathways. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 when compared to the control group; **b** *p* < 0.05 when compared to the CORT group; **c** *p* < 0.05 when compared to the LY294002 group.

<u>Effect of B7C on cell proliferation and identification of the signaling pathways</u>

The dose-dependent effect of B7C on cell proliferation is presented in Figure 39. Treatment with 1µM CORT decreased cell proliferation while CORT at 2 and 10 µM did not show any inhibitory effect on cell proliferation. As 1 µM CORT showed inhibitory effect on cell proliferation, this concentration was used in the signaling pathway experiment. Conversely, treatment with 1 µM Fluox significantly increased

cell proliferation when compared with the NC group. Also, 0.1, 0.3 and 0.5 µM B7C stimulated cell proliferation while 1 µM B7C showed no effect and 5 µM B7C had an inhibitory effect on cell proliferation. Therefore, B7C at a concentration of 0.1 µM was used in the signaling pathway study.

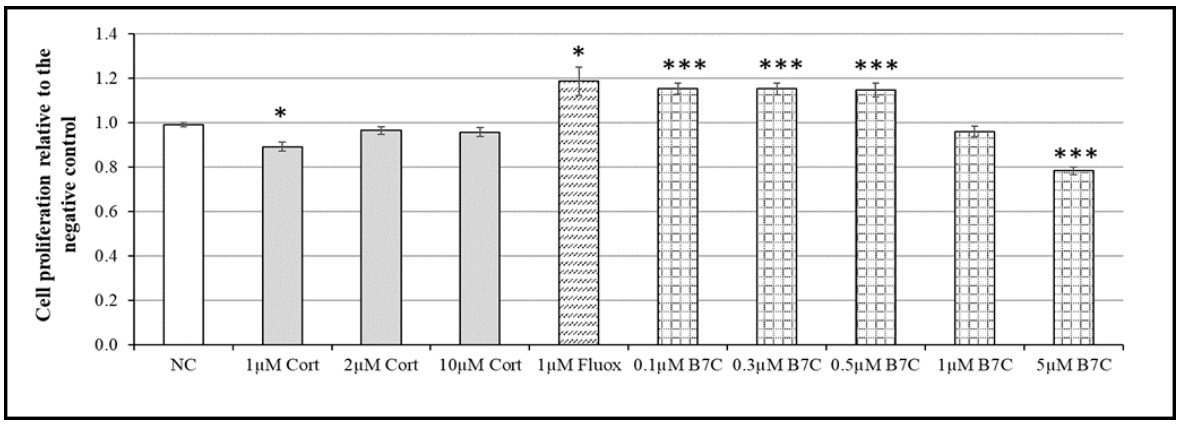


Figure 39. Dose-dependent effect of B7C on cell proliferation. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ when compared to the control group

The PI3K/Akt/mTOR and MAPK/ERK signaling pathways were inhibited using the inhibitors LY294002 and U0126 to block each pathway respectively and identity whether B7C acted on any of those pathways. The findings of the signaling pathway experiment are presented in Figure 40. As previously observed, treatment with 1 µM CORT decreased cell proliferation while treatment with 0.1 µM B7C and Fluox alone showed an opposite effect on cell proliferation. Although the CORT-B7C and CORT-Fluox groups showed decreased cell proliferation levels when compared with the NC, a protective effect could be observed. The reduced cell proliferation induced by CORT treatment was reverted by B7C ($p = 0.031$), but not by Fluox ($p = 0.059$). Treatment with Fluox showed a tendency to increase cell proliferation in the cotreated group, although it was not significantly different when compared with the CORT

group (p = 0.059). Treatment with LY294002 blocked the cell proliferation effect of B7C and Fluox and a similar effect was observed when using the U0126 in combination with B7C and Fluox. The results showed that B7C reverted the toxic effect of CORT. Also, blockade of the PI3K/Akt/mTOR and MAPK/ERK signaling pathways inhibited the neuroprotective effect of B7C, indicting the involvement of those pathways as underlying mechanisms of the effect of B7C.

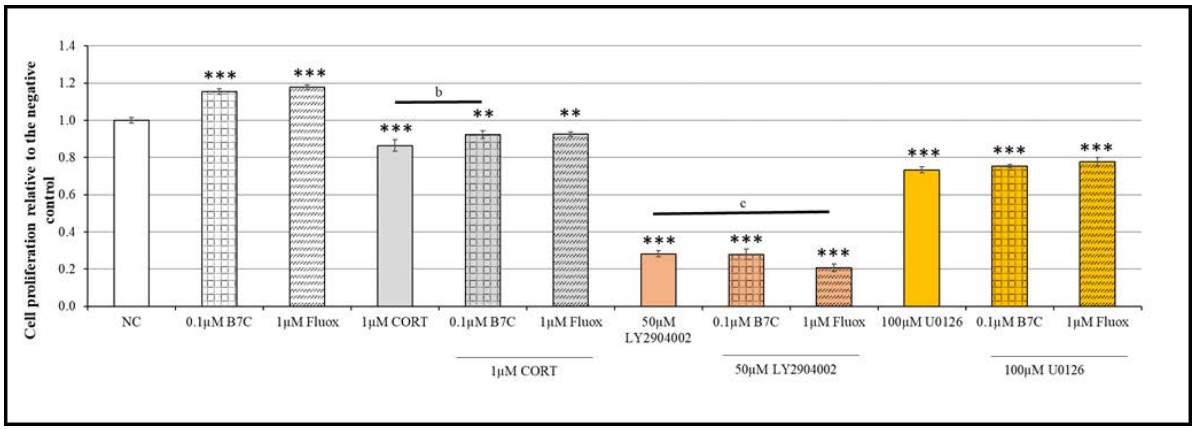


Figure 40. Effect of B7C on PI3K/Akt/mTOR and MAPK/ERK signaling pathways. *p < 0.05, **p < 0.01, and ***p < 0.001 when compared to the control group; **b** p < 0.05 when compared to the CORT group; **c** p < 0.05 when compared to the LY294002 group.

7.4. Discussion

PC12 cells have been used as a cell-based experimental model for depression since high levels of glucocorticoids lead to cellular damage (Gao et al., 2009; Wang et al., 2013). Also, classical antidepressants have been demonstrated as having neuroprotective effect on cytotoxicity in PC12 cells. Therefore, CORT-induced cytotoxicity in PC12 cells is not only used to screen drugs with neuroprotective

actions but also to explore the potential molecular mechanisms involved (Wang et al., 2013).

Previous studies have demonstrated a positive effect of LEO to decrease depression-like behavior *in vivo* (Seol et al., 2010; Guzmán-Gutiérrez et al., 2012; Coelho et al., 2013). However, the molecular mechanism behind the antidepressant effect of LEO has not been fully explored. Chronic stress is one of the most significant factors that increase the susceptibility to develop depressive symptoms (Duman et al., 2016). In rodents, high levels of corticosterone lead to neuronal atrophy in the hippocampus and inhibit cell proliferation in the adult dentate gyrus (Yu et al., 2004). Increased neurogenesis has demonstrated a positive effect on depression-like behavior (Apple et al., 2016) while high glucocorticoid levels result in aberrant functioning of the hippocampus (Berton and Nestler, 2006; Lee et al., 2010) thereby leading to depressive symptoms. Hippocampus malfunctioning has been associated with some of the symptoms observed in depression such as cognitive impairment, memory deficits and anhedonia (MacQueen and Frodl, 2011). Therefore, using CORT-based screening models of potential treatment options for depression appears to be a useful tool.

The present study is the first of its kind evaluating the effect of LEO and B7C *in vitro* to elucidate their effect on cell proliferation and to study the molecular mechanism underlying their effect. The results of the present study showed that LEO at concentrations of 0.05 and 0.1 µl/ml LEO and B7C at 0.1, 0.3 and 0.5 µM stimulated cell proliferation. A previous study showed the neuroprotective effect of LEO against hydrogen peroxide toxicity in SK-SY5Y cells (López et al., 2017). The findings of

López et al. (2017) and the results of the current study, thus, support the neuroprotective properties of LEO. A very important finding in the present study is the restorative effect of LEO observed in cells co-treated with CORT + LEO at a concentration of 0.05 µl/ml LEO. Fluox at a concentration of 1 µM also showed neuroprotective effects as observed in the cells treated with CORT+ Fluox. Further, the results of the study demonstrate the neuroprotective effect of LEO against CORT-induced decreased cell proliferation which is comparable to the neuroprotective effect of the antidepressant Fluox. In previous studies, the neuroprotective effect of B7C has been explored. For instance, B7C inhibited the glutamate-induced cell death in retinal ganglion cells (Fang et al., 2010) and cerebellar ganglion neurons (Fu et al., 2007). Also, B7C showed protective effect against H_2O_2-induced cell toxicity in PC12 cells (Xiao et al., 2000). The cell proliferation effect of B7C in a CORT-induced decreased cell proliferation *in vitro* model was explored for the first time in the present study. The findings suggest that treatment with 0.1 µM B7C protected the cells against the CORT-induced reduction in cell proliferation.

Previous evidence demonstrated the inhibition of cell proliferation caused by CORT in a dose-dependent manner in rat hippocampal progenitors cells (Yu et al., 2004). Another study demonstrated the CORT-induced decreased cell proliferation in PC12 cells which was reverted after treatment with Fluox (Zeng et al., 2016). Additional evidence also showed the neuroprotective effect of the antidepressant venlafaxine against CORT-induced decreased cell proliferation in PC12 cells (Wang et al., 2013). Taken altogether, the effects of LEO and B7C demonstrate their promising neuroprotective action.

The molecular mechanism underlying the cell proliferation effect of LEO and B7C was also explored. The results of the present study showed that the protective effects of LEO and B7C were blocked by the inhibition of the PI3K/Akt/mTOR and MAPK/ERK signaling pathways. The findings indicate that both pathways may be involved in the molecular mechanism of both LEO and B7C to stimulate cell proliferation.

The cell proliferation effects of LEO, B7C and Fluox observed in the present study were greatly attenuated by the inhibitor LY294002, indicating the involvement of the PI3K/Akt pathway. The PI3K/Akt pathway is responsible for cell survival and activation of Akt by Fluox has been previously implicated in the neuroprotective effect of Fluox against CORT-induced decreased cell proliferation (Zeng et al., 2016). Inhibition of Akt phosphorylation has been demonstrated to increase the number of apoptotic cells (Devesa et al., 2014). The decreased cell proliferation caused by the LY249002-mediated inhibition of Akt phosphorylation was not reverted by treatment with LEO or B7C. This indicates that LEO and B7C may activate the PI3K/Akt pathway to stimulate cell proliferation.

It is well established that degenerating neurons showed activation of the MAPK/ERK pathway indicating the relationship between activation of the MAPK/ERK pathway and induced decreased cell proliferation (Gao et al., 2009). Previous evidence has demonstrated that CORT increased the levels of ERK1/2 phosphorylation and inhibition of the CORT-induced ERK1/2 phosphorylation resulted in neuroprotection of PC12 cells (Gao et al., 2009). U0126 is a highly specific inhibitor of the MAPK/ERK signaling pathway by blocking the phosphorylation of ERK (Devesa et

al., 2014). The results of the present study showed that the cell proliferation effects of LEO, B7C and Fluox were blocked by the inhibition of the MAPK/ERK signaling pathway.

7.5. Conclusions

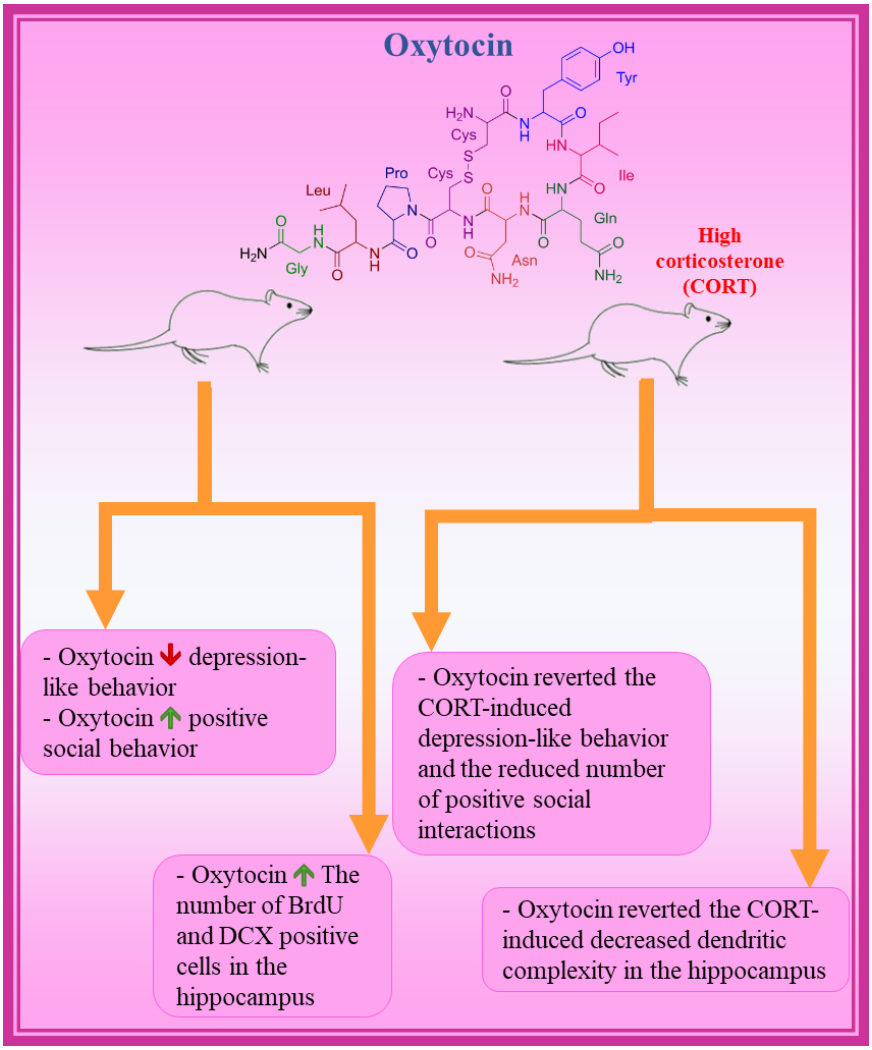
The present study pioneers the exploration of the effect of both LEO and B7C on cell proliferation in a CORT-induced reduction in cell proliferation in the *in vitro* model. Both Leo and B7C stimulated cell proliferation and reverted the CORT-induced reduction in cell proliferation. Furthermore, the findings of the present study indicate that the mechanism underlying the cell proliferation effects of LEO and B7C involves the regulation of the PI3K/Akt and MAPK/ERK signaling pathways. LEO and B7C have been demonstrated as having promising effects, thereby supporting the need for their further investigation as potential treatment options for depression.

CHAPTER 8

The content of his chapter was published in Neuroscience.

REPEATED TREATMENT WITH OXYTOCIN PROMOTES HIPPOCAMPAL CELL PROLIFERATION, AND DENDRITIC MATURATION AND AFFECTS SOCIO-EMOTIONAL BEHAVIOR

Sánchez-Vidaña DI, Chan JN-M, Chan AHL, Hui KKY, Lee S, Chan H-YH, Law YS, Sze MY, Tsui W-CS, Fung TKH, Lau BW-M, Lai CYY (2016). *Neuroscience* 333:65–77.



Abstract

Background: Adult neurogenesis is regulated by rewarding social behavior such as positive social interactions and sexual behaviors. Oxytocin is a hormone secreted during social or sexual behavior. Oxytocin plays a critical role in establishing social bonding and modulating of emotional distress. Although the acute effect of oxytocin has been previously studied, more research is required on the effect of oxytocin on behavior after repeated treatment.

Aims: The aim of this chapter was to evaluate the effect of repeated administration of oxytocin treatment on socio-emotional behavior, hippocampal cell proliferation, and dendritic maturation of new born neurons.

Materials and methods: Adult male Sprague-Dawley rats, either received vehicle or high dose corticosterone treatment, were administered with either vehicle or oxytocin (1 mg/kg) for 14 consecutive days. Behavioral tests were then carried out. Brains were dissected after perfusion of the animals and the immunohistochemistry assay was carried out to evaluate cell proliferation and differentiation.

Results: Treatment with oxytocin increased social behavior and reduced both depression- and anxiety-like behavior. Cell proliferation, differentiation and the dendritic complexity of new-born neurons in the hippocampus increased after oxytocin treatment. Oxytocin reversed the corticosterone-induced depression and anxiety-like behavior. Oxytocin reverted the reduced cell proliferation and reduced dendritic complexity induced by corticosterone.

Conclusions: Treatment with oxytocin enhanced positive emotional and social behaviors. Oxytocin promoted cell proliferation and differentiation. The findings suggest that treatment with oxytocin has potential therapeutic effect for the treatment of emotional and social dysfunction.

8.1. Introduction

Oxytocin (OXT) is a hormone released at central and peripheral levels as a result of rewarding social stimulation, including positive social interactions, sex, and suckling (McNeilly and Ducker, 1972). Oxytocin is synthesized in the hypothalamic supraoptic and paraventricular nuclei; it is then released into the bloodstream through the connections within the hypothalamus and other limbic regions (Neumann and Landgraf, 2012). Oxytocin strongly regulates social and emotional behaviors. For example, administration of oxytocin has a positive effect on social attachment and an anxiolytic effect on human that may confer protection against the negative consequence of stress. Therefore, oxytocin represents a potential therapeutic option in emotion-related disorders (Landgraf and Neumann, 2004; Leuner et al., 2012). Despite the significant role of oxytocin in the regulation of social and emotional behavior, the exact mechanism of action is still unknown.

Adult neurogenesis is a complex process regulated by internal and external environmental factors that intervene at different stages of the process such as cell proliferation, migration, differentiation and integration into the existing neural circuitry (Ming and Song, 2005; Song et al., 2012). Neurogenesis occurs in limited regions of the brain including the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) (Song et al., 2012). Previous research has demonstrated the importance of neurogenesis in the regulation of learning, sexual and social behaviors (Leuner et al., 2006; Deng et al., 2010).

Since it has been shown that both neurogenesis and oxytocin intervene in the regulation of social and emotional behaviors, an interaction between the two processes to regulate socio-emotional behavior might be possible. There is evidence to the effect that the administration of oxytocin for 7 consecutive days increased cell proliferation in the hippocampus under induced stress (Leuner et al., 2012).

In the present study, the effect of oxytocin on behavior and cell proliferation was evaluated. Also, the effect of oxytocin on behavior and neurogenesis in a high-corticosterone animal model (Gregus et al., 2005; Brummelte et al., 2006) was examined.

8.2. Materials and methods

Animals

Adult male Sprague-Dawley rats (31 rats) weighing 200 to 220 g were housed in pairs in polycarbonate cages and fed *ad libitum*. The room was given a 12-hour alternating light-dark cycle and the temperature was kept between 23 and 25°C. The housing and experimental procedures were approved by the Animal Subject Ethnics Sub-Committee of the Hong Kong Polytechnic University.

Experimental design

Experiment 1

The animals were randomly assigned into 2 groups (n= 6 rats/group): (1) the control group which received an i.p. injection of vehicle (saline) and (2) the oxytocin group (1 mg/kg/day; Bachem Americas). The dose of oxytocin administered has been

demonstrated to stimulate neurogenesis in the hippocampus in previous studies (Leuner et al., 2012). The treatment was administered for 14 consecutive days. On days 12, 13, and 14, 50 mg/kg/day of BrdU was i.p. injected to label the proliferative cells. Behavioral tests were carried out on day 14 and 15 and perfusion was performed on day 16.

Experiment 2

The animals were randomly assigned into three groups (n= 6-7 rats/group): (1) the control group (n = 6) received an i.p. injection of vehicle (saline) and a s.c. injection of propylene glycol, (2) the corticosterone group (CORT) (n = 7) received a s.c. injection of CORT (40 mg/kg) and (3) the CORT + Oxytocin group (n = 6) received both a s.c. injection of CORT (40 mg/kg) and an i.p. injection of oxytocin (1 mg/kg). The co-administration of oxytocin and CORT was performed around 17:00 every day for 14 days. In previous studies, repeated administration of CORT at 40 mg/kg was found to reliably induce depressive-like behavior in rats (Kalynchuk et al., 2004; Gregus et al., 2005; Brummelte et al., 2006). In addition, a dose of 40 mg/kg CORT to reduce cell proliferation in the hippocampus and SVZ (Cameron and Gould, 1994; Leuner et al., 2012; So et al., 2012). Behavioral analysis was carried out on day 14 and 15, followed by transcardial perfusion on day 16.

Behavioral tests

Forced Swimming Test (FST): The FST was carried out as described in CHAPTER 4.

Open Field Test (OFT): The OFT was carried out as described in CHAPTER 6.

Social Interaction Test (SIT): The FST was carried out as described in CHAPTER 4.

Animal perfusion and tissue processing

On day 16, animal perfusion was carried out as described in CHAPTER 4. Brains and serum samples were collected and processed as described in CHAPTER 4.

Immunoperoxidase staining and quantification of BrdU and DCX positive cells

Immunoperoxidase staining of BrdU and DCX positive cells, quantification of BrdU and DCX positive cells were carried out as described in CHAPTER 4.

Immunofluorescence staining of BrdU and DCX positive cells

Immunofluorescence staining was carried out as previously reported to identify the BrdU positive cells which express the DCX marker (Meshi et al., 2006; Ziv et al., 2006; Lau et al., 2011a). Antigen retrieval was carried out as previously described for the immunostaining of BrdU cells mentioned above. Slides were incubated with both rat anti-BrdU antibody (1:100, Abcam,) and rabbit anti-DCX antibody (1:300, Cell Signaling Technology) overnight at room temperature. Incubation with the secondary antibodies goat anti-rat and goat anti-rabbit antibodies (Alexa Fluor 568 and 488 respectively, 1:200, Life Technologies) was performed for 2 hours at room temperature. The fluorescent signal was analyzed under a fluorescence microscope Nikon series Eclipse H600L.

Quantification of proliferative cells and immature neurons expressing DCX marker

A StereoInvestigator system (version 11, MBF Bioscience) was used to count the number of BrdU positive cells expressing the DCX marker. The DCX positive cells

with tertiary dendrites or above order were counted. They were counted using the 40X objective. A total of 50 randomly selected BrdU positive cells were screened for the presence of the DCX signal by switching the filter on the microscope to detect the fluorescence signal corresponding to the DCX label (Meshi et al., 2006). The results were expressed as 'Differentiation Index' which can be interpreted as the proportion of DCX-positive cells over 50 BrdU+ cells (Plümpe et al., 2006; Hattiangady and Shetty, 2010; Song et al., 2012).

Dendritic complexity of immature neurons

Dendritic complexity of immature neurons was carried out as described in CHAPTER 4.

Biochemical analyses in serum

The concentration of CORT in serum was carried out in Experiment 2. The corticosterone level was determined using a corticosterone ELISA kit following the instructions of the manufacturer (Enzo Life Sciences).

Statistical analysis

Data were analyzed with the SPSS software. Student's t-test was used to compare the difference between the control and oxytocin-treated groups. To compare the three treatment groups in Experiment 2, one-way ANOVA was used and followed by Tukey post-hoc test. Kruskal-Wallis test followed by Mann-Whitney U post-hoc test was carried out in case the assumptions of normality and homogeneity of variance were not met. Statistical significance was defined as $p < 0.05$. All data were presented as Mean ± SEM.

8.3. Results

Experiment 1

Behavioral tests

The FST showed a statistically significant difference between the oxytocin (113.16 s ± 15.18) and the control group (192.33 s ± 24.43) in the time spent immobile (p = 0.020, Figure 41 A). The lesser time spent immobile of the oxytocin group is an indication of decreased depression-like behavior. The number of positive social interactions in the SIT showed a statistically significant difference (p = 0.014, Figure 41 B) was found between control (11.9 ± 2.3) and the oxytocin group (24.9 ± 3.71). A higher number of positive social interactions were observed in the oxytocin group. In the oxytocin, the time spent in the center of the arena showed a statistically significant difference (p = 0.037, Figure 41 C). The oxytocin group (22.50 ± 3.07) showed a longer time spent in the center of the open field when compared with the control group (13.83 s ± 1.88), thereby demonstrating the anxiolytic effect of oxytocin treatment. In the OFT, a statistically significant difference was observed in the distance traveled (p = 0.003, Figure 41 D). A longer distance was travelled by the animals in the oxytocin group (27.97 m ± 2.81) when compared with the control group (13.98 m ± 1.40).

.

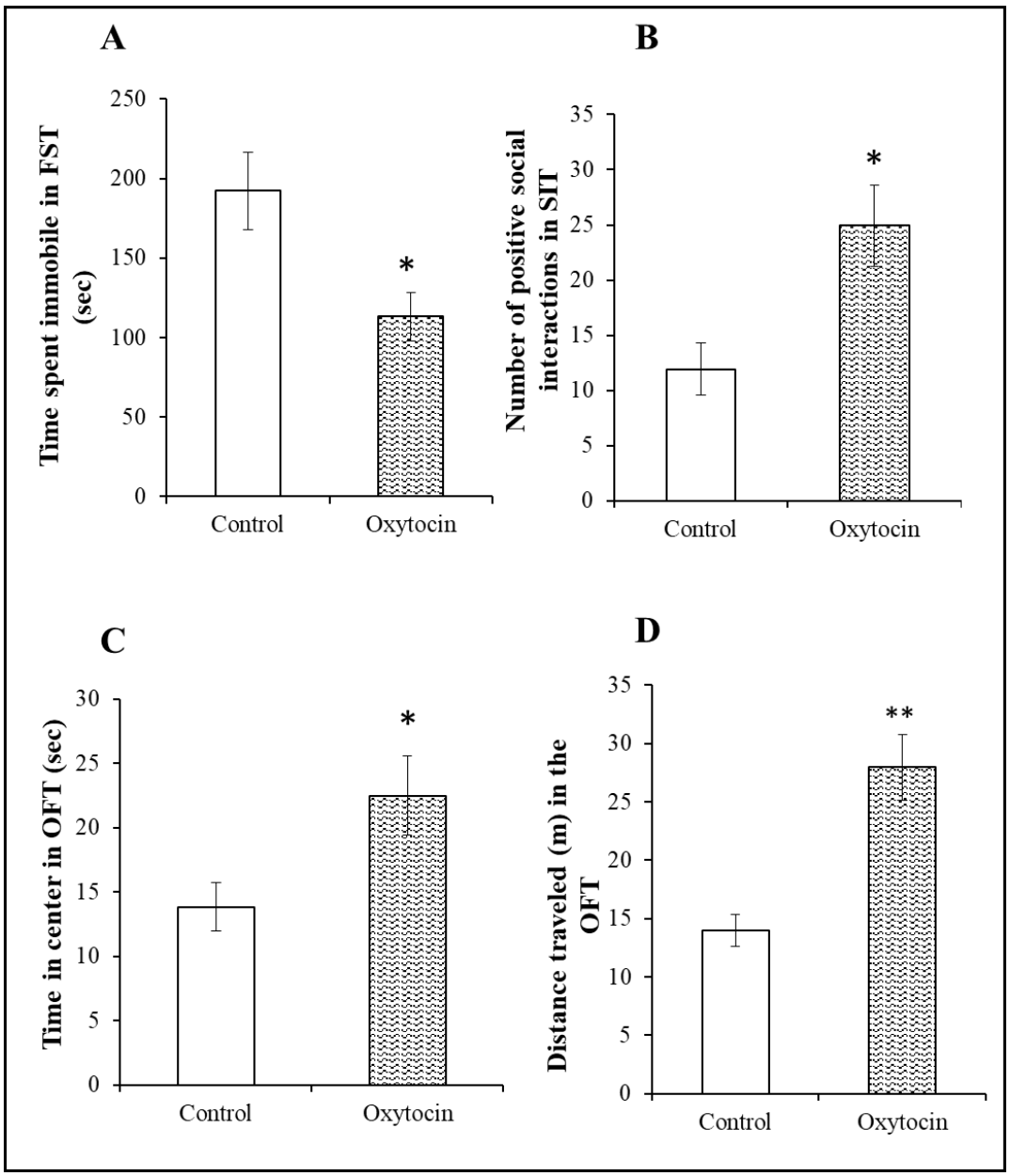


Figure 41. Results from the forced swimming test, social interaction test, and the open field test. **(A)** Results from the FST showing the time spent floating. **(B)** Total number of positive social interactions in the SIT. **(C)** Time spent in the center of the arena in the OFT. **(D)** Distance traveled in the OFT. Results are expressed as Mean ± SEM. *$p < 0.05$ and **$p < 0.01$ when compared to the control group.

BrdU and DCX positive cells

A significant difference was observed in the number of BrdU positive cells between the control (6470.33 ± 832.22) and oxytocin group (10496.33 ± 1348.58, p = 0.029, Figure 42 A). Also, treatment with oxytocin (11030.75 ± 323.57) increased the number of DCX positive cells when compared with the control group (7438.75 ± 1100.701, p = 0.042, Figure 42 A). No significant difference was observed in the proportion of BrdU positive cells with DCX expression (Figure 42 B). There was a significant difference between the control and the oxytocin group in the number of intersections at various distances from soma (Figure 42 C). A higher number of intersections were observed in the DCX positive cells of the oxytocin group.

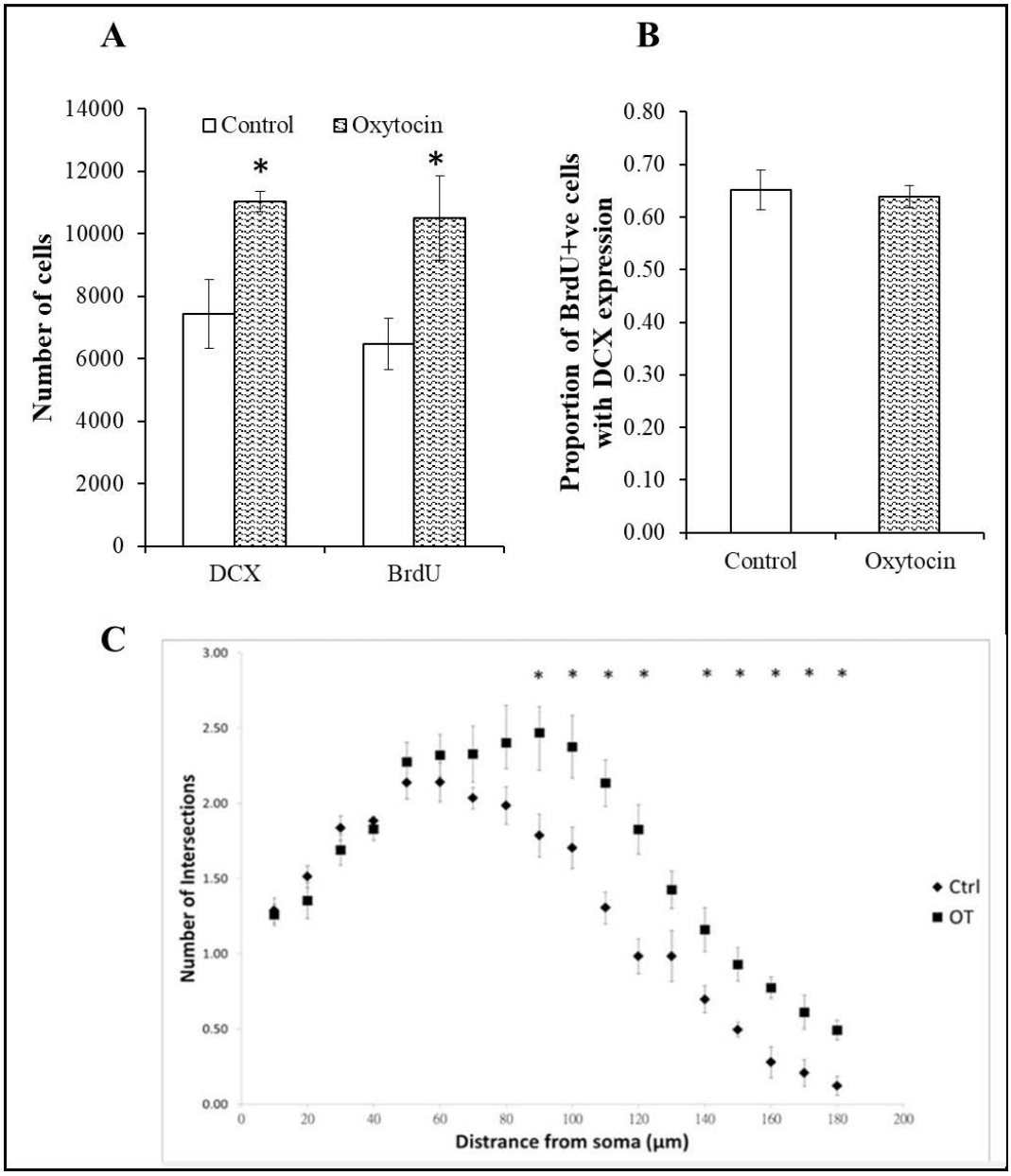


Figure 42. Number of BrdU and DCX positive cells in the hippocampus, number of BrdU positive cells with DCX expression, and analysis of the dendritic complexity. Abbreviations: Ctr, control; OT, Oxytocin. Figure C was modified from (Sánchez-Vidaña et al., 2016)

Experiment 2

Behavioral tests

Treatment with oxytocin showed antidepressant and anxiolytic effect in the behavioral tests in which oxytocin reversed the depression and anxiety caused by high dose CORT treatment. The time spent floating showed a statistically significant difference among the three groups evaluated in the FST (p = 0.001, Figure 43 A). The total floating time in the CORT group (p = 0.002) was significantly higher than the time spent floating in the control and the CORT + Oxytocin group (p = 0.007). This demonstrates the restorative effect of oxytocin. In the SIT, the CORT showed a lower number of positive social interactions than the control group (p = 0.004). On the other hand, the CORT+ Oxytocin group showed a higher number of positive social interactions when compared with the control group (p = 0.002, Figure 43 B) thereby suggesting an improvement of the anxiety-like behavior. In the OFT, a significantly longer time spent in the center of the arena was observed in the CORT + Oxytocin group when compared with the control and CORT group (p = 0.032, p = 0.027, respectively, Figure 43 C). No significant difference was observed between the control and the CORT group. Also, no significant difference was observed in the distance traveled in OFT (Figure 43 D).

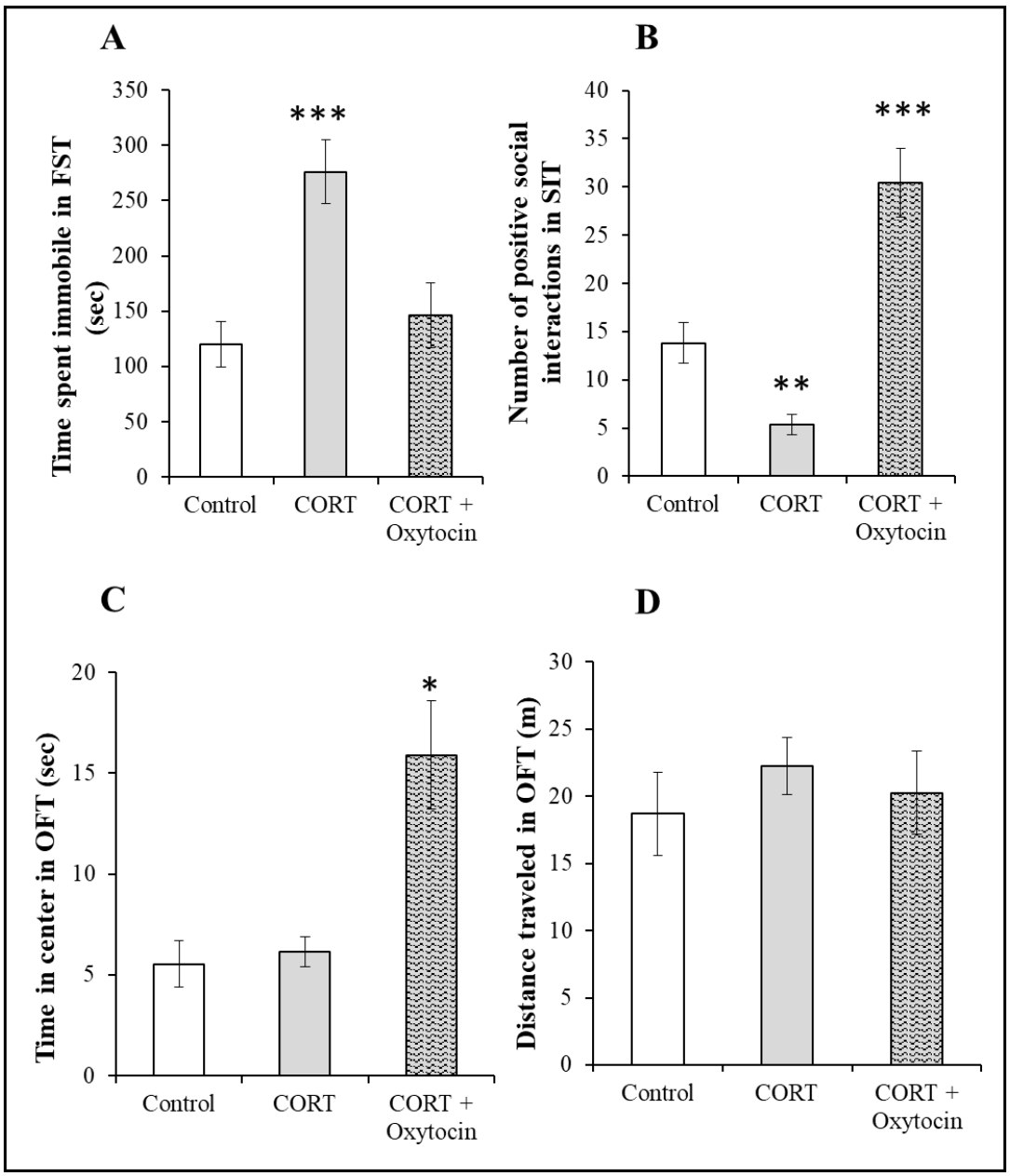


Figure 43. Results from the forced swimming test, social interaction test, and the open field test. **(A)** Results from the FST showing the time spent floating. **(B)** Total number of positive social interactions in the SIT. **(C)** Time spent in the center of the arena in the OFT. **(D)** Distance traveled in the OFT. Results are expressed as Mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ when compared with the control group.

DCX positive cells

A significant difference in the number of DCX cells was observed (p = 0.008, Figure 44 A). Treatment with CORT significantly reduced the number of DCX-positive cells in the hippocampus ($p = 0.004$). The Sholl analysis of the DCX positive cells showed a lower number of intersections in the CORT group when compared with both the control and the oxytocin group (Figure 44 B). These findings reveal that oxytocin can prevent the suppression of hippocampal cell proliferation caused by corticosterone in the hypercortisolemia treatment model.

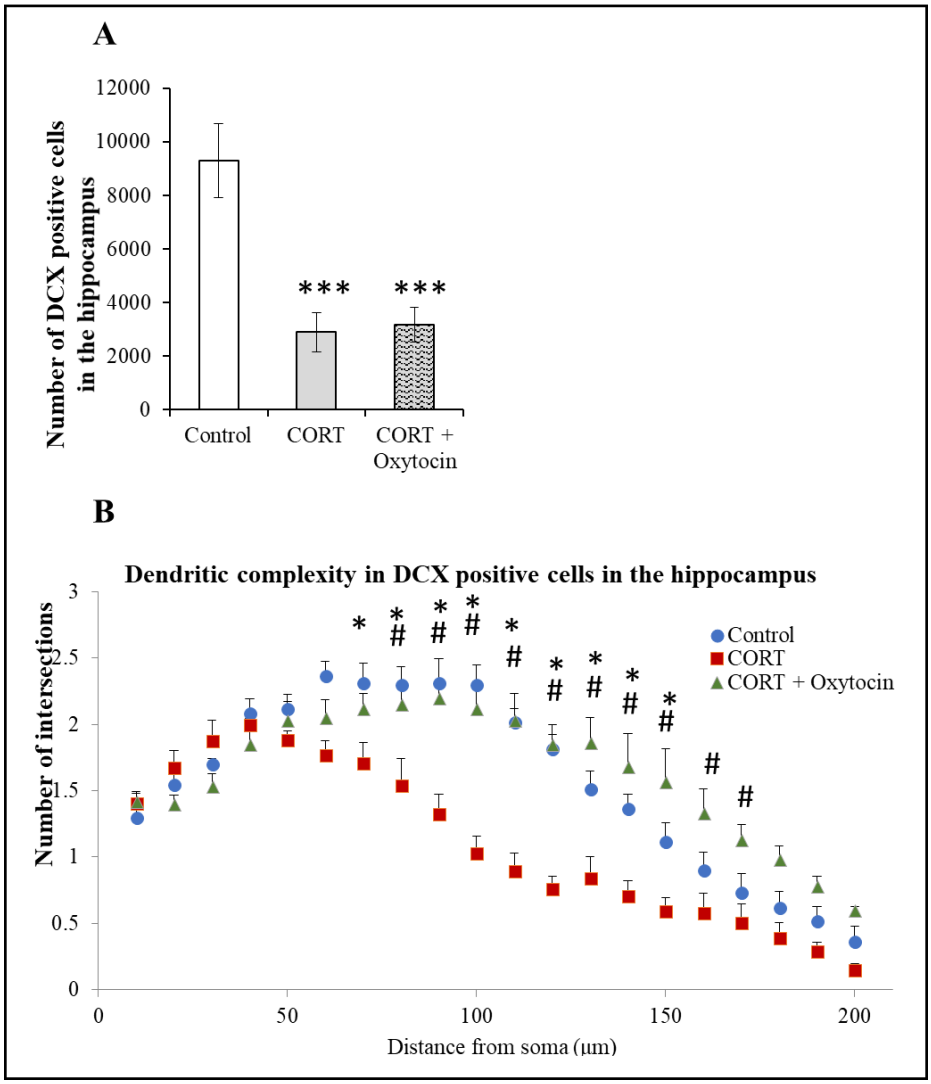


Figure 44. Number of DCX positive cells in the hippocampus and analysis of the dendritic complexity. **(A)** Number of DCX cells in the hippocampus. **(B)** Dendritic

complexity in the DCX positive cells in the hippocampus. *: $p < 0.05$ comparison between the control and the CORT group; # $p < 0.05$ Comparison between the control and the CORT + Oxytocin group.

Concentration of CORT in serum

The control group showed high levels of corticosterone when compared with CORT and the CORT + Oxytocin group ($p = 0.000$, Figure 45). The control and the CORT + Oxytocin showed a statistically significant difference ($p = 0.000$). Also, a significant difference was observed between the control and the CORT group ($p = 0.001$).

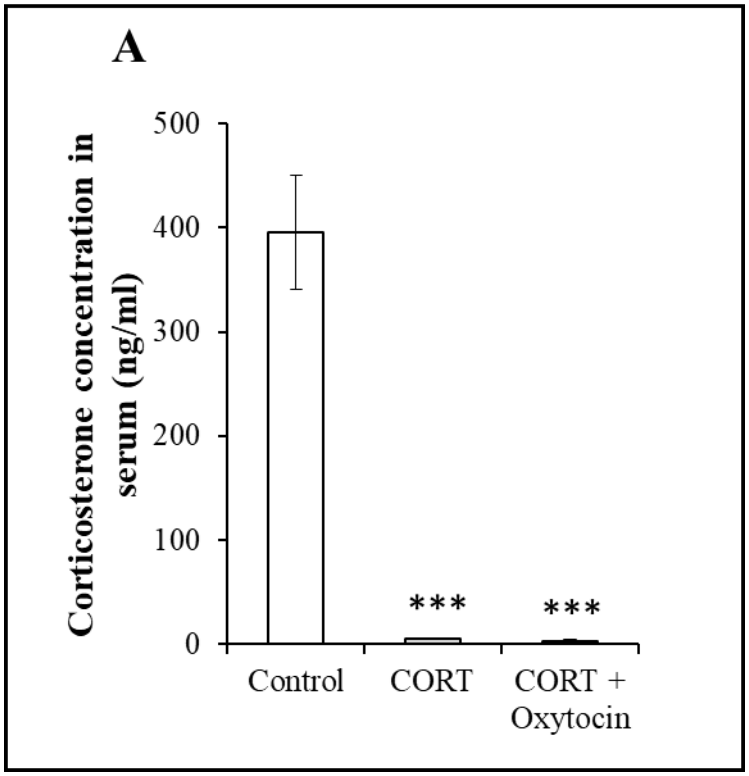


Figure 45. Corticosterone level in serum

8.4. Discussion

The present study evaluated the repeated administration of oxytocin for 14 days. As the half-life of oxytocin is 2 minutes in blood and 20 minutes in the cerebrospinal fluid in rats the chronic effect of oxytocin was evaluated in the present study (Ludwig and Leng, 2006; Leuner et al., 2012). The results revealed that the repeated administration of oxytocin significantly stimulated cell proliferation, promoted dendritic maturation of newborn neurons and reduced depression- and anxiety-like behaviors. Additionally, treatment with oxytocin prevented reduced cell proliferation and depression and anxiety-like behaviors induced by the administration of a high dose of CORT.

Previous studies reported suppression of the HPA axis after exogenous administration of CORT and the same effect was observed in Experiment 2. The negative feedback of the HPA axis observed was caused by the long exposure to a high concentration of CORT which exceeded the concentration required for physiological homeostasis (Young et al., 1995; Andrews et al., 2012). The dendritic complexity of DCX positive cells and the improved social interactions observed in the oxytocin treated groups were found to be associated. The results observed suggest that oxytocin has antidepressant and anxiolytic effect.

Hippocampal cell proliferation was previously reported in a study conducted by Leuner et al. (2012). The authors observed an increased cell proliferation in the ventral hippocampus after acute or repeated (7 days) administration of oxytocin. The present study provides additional information about Leuner et al.'s study, using a different

experimental design. For instance, Leuner et al. (2012) did not find any significant difference in the number of BrdU positive cells after oxytocin treatment, unlike the findings of the present study. This discrepancy can be attributed to the longer treatment period of the present study. Treatment with oxytocin, in the present study, also showed a stimulating effect on the dendritic complexity of immature neurons. A more complex dendritic arborization allows an increased number of possible synaptic connections within the molecular layer of the dentate gyrus of the hippocampus (Lau et al., 2013). Treatment with CORT suppressed cell proliferation and the dendritic growth of immature neurons which was corrected by co-treatment with oxytocin. It is well known that stress is a crucial trigger in depression and causes reduction in dendritic length and dendritic branching in immature neurons in the hippocampus (Sousa et al., 2000; Bessa et al., 2008). It has been suggested by previous studies the loss of synaptic stemmed from dendritic degeneration that could lead to behavioral abnormalities observed in stressed animals (Sousa et al., 2000). Furthermore, the negative effect of stress on dendritic length and complexity can be countered with antidepressants such as fluoxetine and imipramine (Lussier et al., 2013). In this regard, oxytocin was found in this study having an effect similar to that of antidepressants on dendritic complexity as observed when correcting the CORT-induced dendritic degeneration. The findings showed that oxytocin stimulated cell proliferation and higher dendritic complexity of immature neurons. Furthermore, oxytocin was found to be useful in the regulation of emotional and social behaviors, thereby exhibiting a positive therapeutic effect in animals with CORT-induced emotional disturbances.

The effect of oxytocin on emotional behaviors in both normal and stressed animals was carried out using the FST, OFT and SIT. The effect of oxytocin on social behavior,

anxiety and the HPA axis has been previously established (Parker et al., 2010; Ring et al., 2010). Also, dysregulation of the levels of oxytocin has been linked to emotional distress and impairment in social interactions which are features frequently observed in patients with depression (Parker et al., 2010; Yan et al., 2014). In the current study, reduction of depression and anxiety-like behaviors was observed after oxytocin treatment. The same findings have been reported in previous studies. For example, acute and repeated (10 days) treatment with oxytocin at doses of 0.25-1 mg/kg/day reduced depression-like behaviors in animals (Arletti and Bertolini, 1987). Furthermore, the central (0.3 µg) and systemic (30 mg/kg) administration of oxytocin caused a reduction in the time spent immobile in the FST and in the tail suspension test, and thus, demonstrating improved depression-like behavior after oxytocin treatment (Ring et al., 2010). Acute administration of oxytocin (1 µg/kg) demonstrated its antidepressant activity in rats (Nowakowska et al., 2002). In addition, Slattery and Neumann (2010a)'s study reported that oxytocin treatment attenuated the high anxiety-related behaviors of female rats after 6 days of treatment. The results of the present study are in sync with previous studies which support the therapeutic effect of oxytocin to alleviate depression and anxiety-like behavior and to increase cell proliferation.

On the other hand, some studies have reported conflicting results. For example, Yan et al., 2014 evaluated the acute central administration of oxytocin (1, 2 and 4 mg) and found improvement in the depression-like behavior in the FST. However, no effect was observed in acute peripheral administration of oxytocin in the FST. Similar results were reported by Slattery and Neumann, (2010b). The authors found that neither the

acute (1μg) nor the chronic (10 ng/h, 6 days) central administration of oxytocin had any effect on the time of immobility in the FST.

Although several studies have shown the antidepressant effect of oxytocin, other studies did not observe any effect. The conflicting results are an invitation for more in-depth exploration of the regulation of the oxytocinergic system in depression-like behavior (Rotzinger et al., 2010; Slattery and Neumann, 2010a, 2010b). Also, the mechanism of action of oxytocin on both central and peripheral levels should be further explored (Slattery and Neumann, 2010b).

Emotion is a pivotal modulator of social behavior (Blair et al., 2004). The aims of the SIT are to assess the anxiety-like behavior in a social situation and to assess the positive or negative social behaviors. The findings of the present study showed that oxytocin stimulated social interaction, confirming previous findings (Witt et al., 1992). Interestingly, the dendritic complexity was found to be associated with the display of pro-social behaviors and thus the maturation of new neurons may exert a positive effect on social interaction. A previous study on female prairie voles showed that decreased social interaction could lead to decreased neurogenesis (Lieberwirth et al., 2012). Although the causal relationship between cell proliferation, dendritic maturation and social interaction could not be fully established, it can be suggested that neurogenesis may have a reciprocal effect with the positive social behavior observed in the present study (Mak et al., 2007; Lau et al., 2011b).

8.5. Conclusions

As shown in previous chapters, it was suggested that oxytocin could be a mediator in the pro-neurogenic effect of B7C and LEO. Therefore, the present study was carried out to evaluate the effect of oxytocin on behavior and neurogenesis alone and in a high corticosterone model. The present study has shown that oxytocin has a positive effect on depression and anxiety-like behavior, stimulates neurogenesis, and increases dendritic complexity in immature neurons. The findings from the present study support the therapeutic use of oxytocin for the treatment of mood or social disorders (Chen et al., 2015b). However, further research is needed to evaluate the causal relationship between neurogenesis, social interaction and emotion.

CHAPTER 9

GENERAL CONCLUSIONS

Painting by Wei Ho Chan, 2018 (permission to use of the painting in this thesis was granted by the artist)

The present study is the first of its kind evaluating the effect of LEO and B7C at behavioral, cellular and molecular levels, taking neurogenesis as the main physiological mechanism investigated. Although the antidepressant effect of LEO has been previously studied in clinical studies, *in vivo* and *in vitro*, previous research on B7C has mainly focused on neurodegenerative disorders. Therefore, this is the first study exploring the antidepressant effect of B7C.

9.1. Lavender essential oil

Literature review

An extensive literature review was carried out in CHAPTER 3 part A to address the question "Is aromatherapy effective to reduce depressive symptoms?". The analysis of RCT studies focused on the effect of aromatherapy on depressive symptoms demonstrated the promising effect of aromatherapy in alleviating depressive symptoms. From the whole variety of essential oils evaluated to study the effect of aromatherapy on depressive symptoms in RCTs, LEO was the most frequently used, thereby supporting the need for it to be further investigated. Despite the clinical evidence supporting the positive effect of aromatherapy on depressive symptoms, further investigation is needed as most of the studies included had low scores in the quality assessment.

Because of the promising effect of aromatherapy to alleviate depressive symptoms found in CHAPTER 3 part A, another systematic review was carried out to address the following questions: "Is LEO efficacious in reducing depressive symptoms in preclinical and clinical studies? What is the mechanism of action of LEO involved in

the reduction of depressive symptoms?". The systematic review carried out in CHAPTER 3 part B was the first of its kind to analyze the preclinical and clinical evidence of LEO and some of its most abundant compounds on depressive symptoms. The animal studies selected in the systematic review clearly demonstrated the positive effect of LEO and linalool to decrease depression-like behavior compared with the effect of commercial antidepressants. Furthermore, the systematic review showed that the mechanism of action of LEO, linalool and linalyl acetate on relevant targets for depression was previously investigated demonstrating promising effects. Despite the solid evidence shown in the animal studies included in the systematic review, no conclusive evidence was demonstrated in the clinical studies. Therefore, the potential antidepressant effect of LEO needs further investigation in clinical settings.

Evaluation of LEO *in vivo*

The need to explore the mechanism of action behind LEO antidepressant effect was evident from the analysis of the preclinical evidence in CHAPTER 3 part B. However, no studies have explored the effect of LEO in animal models for depression and there were no animal studies that had reported on the mechanism of action involved in the effect of LEO. Therefore, the study carried out in CHAPTER 4 was the first of its kind to address the following questions: "Does LEO decrease depression-like behavior in an animal model for depression? Is neurogenesis involved in the antidepressant effect of LEO?". To answer these questions, the effect of LEO on behavior and neurogenesis was evaluated in a high corticosterone model in rats. The results demonstrated that treatment with LEO decreased the CORT-induced depression like behavior. Also, neurogenesis was found to be the physiological progress involved in the antidepressant effect of LEO, as treatment with LEO increased the number of positive

BrdU cells in the hippocampus and SVZ. Additionally, LEO reverted the CORT-induced dendritic branching atrophy in DCX positive cells in the hippocampus. The overall results of the evaluation of LEO in a high dose-CORT animal model for depression demonstrated that LEO is a promising treatment option for the treatment of depression as it has positive effects on mood and neurogenesis. Finally, treatment with LEO alone increased the concentration of oxytocin in serum. Since oxytocin has demonstrated a positive effect on behavior and neurogenesis, further investigation on the relationship between LEO and oxytocin should be carried out.

Since oxytocin was found to increase after consecutive exposure to LEO for 14 days, an animal experiment was designed to evaluate the effect of oxytocin alone on neurogenesis. The present study demonstrated that oxytocin decreased depression-like behavior and stimulated positive social interactions. Also, treatment with oxytocin increased the number of BrdU and DCX positive cells in the hippocampus. When evaluating the effect of oxytocin in a high dose-CORT animal model, oxytocin reverted the CORT-induced depression-like behavior and improved the reduced number of positive social interactions. Finally, oxytocin reverted the CORT-induced decreased dendritic complexity of DCX positive cells in the hippocampus. Taken all together, treatment with oxytocin demonstrated a positive effect on mood and neurogenesis and it should be considered as a key modulator when evaluating the effect of potential treatment options for depression such as LEO.

Evaluation of LEO *in vitro*

A positive effect on behavior and neurogenesis of LEO was demonstrated in CHAPTER 4. Therefore, the following step was to investigate the underlying molecular mechanism *in vitro* to address the question "What is the molecular mechanism involved in the cell proliferation effect of LEO?". In CHAPTER 7, a CORT-induced decreased cell proliferation model was set up using PC12 cells. Treatment with different concentrations of LEO and Fluox (as cell proliferation positive control) allowed the identification of the optimal dose of LEO that increased cell proliferation to be used in a signaling pathway experiment. The results from the *in vitro* experiments demonstrated that 0.05 µl/ml LEO reverted the CORT-induced decreased cell proliferation. Also, it was demonstrated that the mechanism underlying the cell proliferation effects of LEO involved the regulation of the PI3K/Akt and MAPK/ERK signaling pathways.

9.2. Recommendations for future research on LEO

In future RCT studies evaluating inhalation aromatherapy, a pre-test that will confirm the normal olfactory function should be included. Additionally, a longer exposure time and a higher number of sessions should be considered. When evaluating aromatherapy massage, at least 8 sessions once or twice per week should be included. Also, evaluation of pure single LEO is highly recommended in order to identify the essential oil that contributes to the biological effect observed in clinical studies. Finally, inclusion of a massage group without aromatherapy would be highly recommended when carrying out clinical studies on aromatherapy in order to discriminate the effect induced by massage alone from the effect of the LEO.

In animal studies, it is recommended that a longer treatment (more than 14 days) is used. Also, evaluation of the BDNF and oxytocin levels in the hippocampus could contribute to the understanding of the mechanism of action involved when evaluating the antidepressant effect of LEO.

9.3. Limitations of the study on LEO

The main limitation of the study on LEO is that only one dose was evaluated in the animal study. Several doses covering a wide range, for example low, medium and high dose of LEO, could have been evaluated in a dose-dependent *in vivo* experiment. Furthermore, a group treated with an antidepressant (e.g. fluoxetine) could have been included to evaluate the antidepressant effect of LEO in comparison with an antidepressant.

9.4. Bis-(7)-cognitin

Literature review

A large body of evidence on the effect of B7C on neurological disorders was collected and analyzed in CHAPTER 5 to address the question "What are the molecular targets and mechanism of action of B7C on neurological disorders?". A series of molecular targets relevant in the pathogenesis of neurological disorders were identified as targets of B7C. Therefore, the multi-target properties of B7C were clearly stated in the literature review. The analysis of the *in vitro* and *in vivo* studies on B7C clearly stated the superior activity of B7C when compared with its basic structure tacrine. Also, the lack of antidepressant evaluation of B7C was evident which greatly supported the novelty of the evaluation of B7C carried out in CHAPTER 6 and CHAPTER 7.

Evaluation of B7C *in vivo*

The effect of B7C was evaluated *in vivo* in order to address the following research questions: Does B7C improve depression-like and anxiety like behavior? Does B7C affect neurogenesis? What is the optimal concentration of B7C that increases neurogenesis? Does B7C decrease depression-like behavior and affect neurogenesis in an animal model for depression? The study of the behavioral and neurogenic effect of B7C was presented in CHAPTER 6. The study was divided into two phases. The first phase included a dose-dependent experiment to (1) evaluate whether B7C affected neurogenesis and (2) to identify the dose of B7C that showed a positive effect on behavior and stimulated neurogenesis. The second phase of this study was to evaluate the effect of B7C on rats with CORT-induced depression-like behavior and reduced neurogenesis. The experimental design used in the second phase included a comparison with the antidepressant Fluox to evaluate whether the effect of B7C was equal to or better than the effect of a widely used antidepressant. B7C was found to have a positive effect on behavior and neurogenesis. Also, the optimal concentration of B7C was 0.3mg/kg which was further evaluated in a high-CORT animal model for depression. Treatment with B7C showed improvement in the CORT-induced anxiety-like behavior and decreased neurogenesis. The evidence of the present study supports further investigation of B7C on mood and the molecular mechanism underlying its effect. The study carried out in the present project was the first evaluating the effect of B7C on behavior and neurogenesis in an animal model for depression and the findings of the study support B7C promising multitarget effect that can be highly valuable for the treatment of both neurodegenerative and mood disorders.

Evaluation of B7C *in vitro*

The effect of B7C in a CORT-induced decreased cell proliferation *in vitro* model was discussed in CHAPTER 7. Although the effect of B7C has been extensively studied *in vitro*, no previous study focused on evaluating the effect of B7C in a depression model. The findings of the *in vitro* experiments clearly demonstrated the effect of B7C in increasing cell proliferation. Also, B7C showed a restorative effect on cell proliferation in a CORT-induced decreased cell proliferation *in vitro* experiment. Finally, the findings of the present study indicate that the mechanism underlying the cell proliferation effects of B7C involves the regulation of the PI3K/Akt and MAPK/ERK signaling pathways. Therefore, the evidence from the *in vitro* study showed that B7C has demonstrated promising effects, necessitating its further investigation as potential treatment option for depression.

9.5. Recommendations for future research on B7C

Further evaluation of the molecular mechanism underlying the antidepressant effect of B7C should be carried out as B7C has clearly showed a positive effect on mood, neurogenesis and neuroprotection against CORT-induced decreased cell proliferation. Other molecular targets relevant to depression should also be explored. B7C has clearly showed to act on a wide variety of targets, therefore, characterization of the proteome is highly recommended. The proteome characterization can be done by using proteomic analysis to identify the signaling pathways involved in the effect of B7C using different cell-based platforms relevant in neurological disorders.

9.6. Limitations of the study on B7C

The main limitation of the study on B7C is that the doses tested in the dose-dependent experiment did not cover a wide range to have a better picture of the dose-effect of B7C. It would be better to include a low, medium and high dose at least 5x apart from each other.

APPENDICES

APPENDIX 1. Publications arising from the thesis

Neuroscience 333 (2016) 65–77

REPEATED TREATMENT WITH OXYTOCIN PROMOTES HIPPOCAMPAL CELL PROLIFERATION, DENDRITIC MATURATION AND AFFECTS SOCIO-EMOTIONAL BEHAVIOR

DALINDA ISABEL SÁNCHEZ-VIDAÑA, NGAI-MAN JACKIE CHAN,[†] ALAN H. L. CHAN,[†] KATY K. Y. HUI,[†] SYLVIA LEE,[†] HOI-YI CHAN,[†] YUEN SHAN LAW,[†] MEI YI SZE,[†] WAI-CHING SARAH TSUI,[†] TIMOTHY K. H. FUNG, BENSON WUI-MAN LAU[*] AND CYNTHIA Y. Y. LAI

Rehabilitation Sciences, The Hong Kong Polytechnic University, Hong Kong, China

Abstract—Rewarding social behaviors including positive social interactions and sexual behaviors are shown to regulate adult neurogenesis, but the underlying biological mechanisms remain elusive. Oxytocin, a neurohypophysial hormone secreted after exposure to social interaction or sexual behaviors, has a profound role in the formation of social bonding and regulation of emotional distress. While the acute effect of oxytocin was usually studied, relatively scarce evidence showed the behavioral consequence of repeated oxytocin treatment. The purpose of the current study was to investigate the effect of repeated oxytocin treatment on hippocampal cell proliferation, dendritic maturation of new born neurons and social/emotional behaviors. Adult male Sprague–Dawley rats received treatment with either vehicle or oxytocin (1 mg/kg) daily for two weeks. Behavioral tests revealed that oxytocin increased social behaviors and reduced the anxiety- and depression-like behaviors. Cell proliferation, differentiation and the dendritic complexity of new born neurons in the hippocampus were promoted by oxytocin treatment. Depression- and anxiety-like behaviors were induced by repeated treatment of corticosterone (40 mg/kg) for two weeks while oxytocin treatment reversed the behavioral disturbances. Suppression of cell proliferation caused by corticosterone was reverted by oxytocin treatment in which cell proliferation, cell differentiation, and dendritic complexity increased. The present findings reveal that oxytocin not only enhances cell proliferation, but also promotes the development of the new neurons which is associated with the induction of positive emotional and social behaviors. The results also suggest that oxytocin may be a potential therapeutic agent for treatment of emotional and social dysfunction.

Key words: oxytocin, hippocampal cell proliferation, neurogenesis, dendritic complexity, depression-like behaviors, anxiety-like behaviors.

INTRODUCTION

The neurohypophysial hormone oxytocin (OXT) is released both centrally and peripherally under rewarding social stimulation such as social interaction with desirable individuals, sexual activity, and suckling (McNeilly and Ducker, 1972). After being synthesized in magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei, oxytocin is released into the bloodstream via neuronal connections within hypothalamic and limbic regions (Neumann and Landgraf, 2012). Oxytocin is also released from dendrites and perikarya as a neuromodulator and reaches the oxytocin receptors (OXTR) through diffusion via extracellular fluid and ligand binding (Landgraf and Neumann, 2004). As suggested by the regulators of oxytocin secretion, oxytocin is closely associated with social and emotional behaviors. For instance, administration of oxytocin was shown to improve social attachment, show anxiolytic effect on human subjects and may protect an individual from the negative consequence of stress. which implicates the potential therapeutic value of oxytocin in emotion-related disorders (Landgraf and Neumann, 2004; Leuner et al., 2012). Despite the established roles of oxytocin in regulation of social and emotional behavior, the biological mechanism of its influence remains obscure.

Adult neurogenesis, which describes the production of new functional neurons in the adult central nervous system (CNS), is a complex dynamic process. Internal and external environmental factors can affect the regulation of neurogenesis at different stages including proliferation, migration, differentiation and integration into the existing neural circuitry (Ming and Song, 2005; Song et al., 2012). Neurogenesis can be found predominantly in two distinct regions in the CNS, namely, the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus and the subventricular zone (Song et al., 2012). Cell proliferation, the first step in the neurogenesis process, refers to one complete cell division cycle that can be detected using BrdU which is a marker for DNA synthesis. Once new cells have been born, they differentiate into mature neuronal phenotype and make synaptic connections in the existing circuitry (Malberg, 2004). External factors such as physical exercise and stress

*Corresponding author.
E-mail address: benson.lau@polyu.edu.hk (B. W.-M. Lau).
[†] Equal contribution.
Abbreviations: CNS, central nervous system; DG, dentate gyrus; FST, Forced Swimming Test; OFT, Open Field Test; SIT, Social Interaction Test; PBS, phosphate-buffered saline.

http://dx.doi.org/10.1016/j.neuroscience.2016.07.005
0306-4522/

have shown to increase and decrease cell proliferation respectively; therefore affecting neurogenesis (Song et al., 2012). Different lines of evidence demonstrate the functional significance of neurogenesis including learning, sexual behaviors and social behaviors (Leuner et al., 2006; Deng et al., 2010). Since neurogenesis has an impact on different emotional and cognitive behaviors, it is expected that the understanding of neurogenesis at different stages of the neurogenesis process would shed light on pathophysiology of emotional and cognitive disorders, and may provide insight on developing novel clinical treatment (Winner et al., 2011).

As both neurogenesis and oxytocin are shown to regulate social and emotional behaviors, it is possible that they may have intricate interaction to regulate the socio-emotional behaviors. One of the evidence to support the relationship between neurogenesis and oxytocin is that postnatal neuronal growth was observed in the vasopressin and oxytocin-containing nucleus of the pig hypothalamus (Rankin et al., 2003). Recently it was revealed that acute and sub chronic (7 day) treatment of oxytocin increases ventral hippocampal cell proliferation, and the neurogenesis-stimulating effect could be found under stressful situation (Leuner et al., 2012). Meanwhile another *in vitro* study showed that oxytocin treatment promotes neuronal differentiation of adipocyte-derived stem cells (Jafarzadeh et al., 2014). To further explore the effect of oxytocin on cell proliferation and the display of emotional and social behavior, the present study tested (1) the effect of repeated exposure to oxytocin on cell proliferation and dendritic maturation of new neurons; (2) the effect of prolonged exposure to oxytocin on social interaction and depression/anxiety-like behaviors and (3) the potential therapeutic effect of oxytocin in a corticosterone-induced depression- and anxiety-like behavior animal model (Gregus et al., 2005; Brummelte et al., 2006). Since oxytocin signaling may play key roles in the underlying mechanisms of pro-neurogenic effect of socially rewarding behaviors, the molecular mechanisms triggered to promote neurogenesis should be further investigated.

EXPERIMENTAL PROCEDURES

Experimental design

Young adult male Sprague–Dawley (SD) rats (31 rats in total, 7–8 weeks of age, 200–220 g) were housed in pairs in polycarbonate cages. They were fed *ad libitum* and the room was maintained on a 12-h alternating light–dark cycle and at 23–25 °C. The housing and behavioral procedures were approved by the Animal Subject Ethics Sub-Committee of the Hong Kong Polytechnic University.

Experiment 1. To study the effect of repeated oxytocin treatment on emotional behaviors and cell proliferation, SD rats ($n = 6$ rats/group) were injected intraperitoneally with oxytocin (1 mg/kg/day; Bachem Americas) or equal volume of vehicle (normal saline). Treatment was given around 17:00 every day for 14 consecutive days. The dose of oxytocin used has shown to stimulate neurogenesis in the DG in previous studies (Leuner et al., 2012). At day 12–14, 50 mg/kg/day of BrdU was intra-peritoneally injected to label proliferative cells. Behavioral tests were performed at days 14 and 15, which was followed by perfusion at day 16.

Experiment 2. Animals were divided into three groups ($n = 6$–7 rats/group): (1) Control group ($n = 6$) with intraperitoneal injection of vehicle (normal saline) and subcutaneous injection of propylene glycol which was the vehicle used to dilute the corticosterone in groups 2 and 3; (2) Corticosterone treatment group ($n = 7$) with subcutaneous injection of corticosterone (40 mg/kg) and (3) Oxt + Cort group ($n = 6$) which received both corticosterone (40 mg/kg) subcutaneously and intraperitoneal injection of oxytocin (1 mg/kg). The co-administration of oxytocin and corticosterone was performed around 17:00 every day for 14 days. In previous studies, repeated administration of corticosterone at 40 mg/kg showed to reliably induce depression-like behavior in rats (Kalynchuk et al., 2004; Gregus et al., 2005; Brummelte et al., 2006). In addition, a dose of 40 mg/kg corticosterone has been reported to diminished cell proliferation in the DG and subventricular zone (Cameron and Gould, 1994; Leuner et al., 2012; So et al., 2012). Behavioral analysis was carried out at days 14 and 15, followed by transcardial perfusion at day 16.

BEHAVIORAL TESTS

Forced Swimming Test (FST)

Swimming sessions were conducted in individual transparent cylinders (40 cm height × 30 cm diameter) filled with water at a depth of 30 cm at room temperature. The cylinders were deep enough to avoid that the rats touch the bottom of the cylinder to support themselves. Two swimming sessions were conducted: an initial 15-min pretest for habituation, followed 24 h later by a 10-min test. Test sessions were video recorded from a front view for scoring later by an observer blinded to the treatment. The behaviors scored in the FST included: (1) time spent immobile – floating in the water without struggling, minimal movement to keep from drowning, with only the necessary movement to keep the head above the water; (2) time spent swimming – making active motions, such as moving around in the cylinder, more than necessary to merely keep the head above water and less movements than those shown when climbing/struggling; and (3) time spent climbing/struggling – showing vigorous active movements with the forelimbs and hind limbs breaking the surface of the water in an clear attempt to get out of the cylinder. The depression-like behavior is exhibited when rats spent more time immobile during the test (Gregus et al., 2005; Lau et al., 2011b). After each session, the rats were removed from the cylinders, dried with cloth towels and returned to their home cages.

Open Field Test (OFT)

An arena (72 cm length × 72 cm width × 40 cm depth; lighting of 550 lux) was used for the OFT to evaluate anxiety in rats when exposed to an unfamiliar environment. A video camera was mounted above the open field adjusted to cover the whole test area. The test was video recorded for scoring later by an observer blinded to the treatment. In the test, each of the animals was put into the arena for 10 min and was allowed to explore the arena freely.

For the analysis, the arena was divided into 16 equal squares on a screen and the numbers of squares crossed by the rat's neck were counted to measure the locomotor activity. The measurements recorded included: (1) peripheral locomotor – the time spent walking close to the walls of the test field (thigmotaxis); (2) central locomotor activity – time spent in the central 36 cm × 36 cm area of the field. Increased time spent in the central area or decreased time of latency to enter the central area are indications of anxiety-like behavior (Prut and Belzung, 2003; Gregus et al., 2005; Airan et al., 2007; Gamberini et al., 2015).

Social Interaction Test (SIT)

The test was conducted similar to previous studies (Becker et al., 1999; Gregus et al., 2005). Briefly, an open-field arena of 72 cm length × 72 cm width × 40 cm depth, with an open top was used. A camera was mounted above the open field arena and adjusted to cover the whole test arena. One day before the test, the animals were familiarized with the arena through a 10-min exposure. In the test, pairs of unfamiliar rats under the same treatment and with a weight difference no larger than 20 g were introduced at the same time into opposite side corners of the arena. The test was video recorded for later analysis by an observer blinded to the treatment. The total number of social interactions was scored and the interactions were categorized into: (1) non-aggressive behavior – sniffing, following, crawling, social play, grooming; (2) aggressive/defensive behavior – kicking, boxing, wrestling, biting. An indication of increase anxiety is reflected in decreased social interaction between the rats.

Animal perfusion

On day 16, rats were administered with a lethal dose of anesthetic drug (sodium pentobarbital, 200 mg/kg, intraperitoneal injection) and transcardially perfused with 4% paraformaldehyde. Before perfusion, 1 ml blood was collected to measure the corticosterone plasma level. Brains were post-fixed overnight at 2–8 °C. After post-fixation, brains were transferred to 30% sucrose solution in 0.1 M phosphate-buffered saline (PBS) for cryoprotection and stored at 2–8 °C (Hillerer et al., 2014). The brain tissue was sectioned into 40-μm-thick coronal sections in 1-in-12 series by a cryostat (Shandon Cryotome E, Thermo Electron Corporation). The sections were stored in cryoprotectant at −20 °C until immunostaining was performed.

IMMUNOHISTOCHEMISTRY

Immunoperoxidase staining

Immunostaining of DCX-positive cells was performed according to previously published reports (Lau et al., 2011a). In brief, affixed brain sections were rehydrated with 0.1 M PBS and subjected to antigen retrieval at 80 °C for 20 min in sodium citrate buffer (pH 6.0). After being rinsed in PBS in three charges, the sections were incubated with rabbit anti-DCX antibody (1:300, Cell Signaling Technology) at room temperature overnight. Then the sections were washed thrice with PBS and incubated in biotinylated goat anti-rabbit antibody (1:200, Vector Laboratories) for 2 h at room temperature. Staining signal was visualized with Avidin–biotin complex solution (Vector Laboratories) and diaminobenzidine (Dako). DCX-positive cells were stained in brown.

The immunostaining of BrdU cells was conducted similarly as the immunostaining protocol for DCX cells, but the antigen retrieval steps were modified as follows. Antigen retrieval was carried out by incubating the slides at 80 °C for 25 min in preheated sodium citrate buffer. After that, the slides were incubated in 2 N HCl at 40 °C for 30 min to allow DNA denaturation. Neutralization of the acid was carried out by incubating the slides with 0.1 M borax buffer for 15 min at room temperature. After neutralization, the slides were washed with PBS before incubation with the primary antibody. The primary and secondary antibodies used included 1:300 mouse anti-BrdU antibody (Roche) and 1:200 goat anti-mouse antibody (Dako) respectively (Lau et al., 2011a).

Immunofluorescence

Immunofluorescence staining was applied to detect the presence of BrdU and DCX-positive cells. BrdU serves as a marker of proliferative cells while DCX is expressed in immature neurons. The co-immunostaining was performed based on the protocols reported in previous studies (Meshi et al., 2006; Ziv et al., 2006; Lau et al., 2011a). In order to allow anti-BrdU antibody to access the incorporated BrdU, the affixed brain sections were rehydrated in PBS first and incubated in 2 M HCl for 30 min at 37 °C. Following rehydration, the sections were incubated in both rat anti-BrdU antibody (1:100, Abcam), and rabbit anti-DCX antibody (1:300, Cell Signaling Technology) overnight at room temperature. Subsequently, goat anti-rat and goat anti-rabbit antibodies (Alexa Fluor 568 and 488 respectively, 1:200, Life Technologies) were applied on the sections and incubated for 2 h. The fluorescent signal was observed and analyzed under a fluorescence microscope Nikon series Eclipse Hl600L.

Cell counting

Slides were coded prior quantification of cells and analyzed by an experimenter blinded to the treatment. The stereology method used was the fractionator method since the number of cells can be estimated more precisely. This method is more efficient statistically and no estimation of the global volume of the brain

region of interest is needed. In the fractionator method, the number of cells is counted within all unbiased counting spaces and multiplied by the reciprocal value of the sampling probability (Schmitz and Hof, 2005; Altunkaynak et al., 2011). Therefore, the estimated number of cells was reported as an absolute number.

Quantification of proliferative cells and immature neurons expressing DCX marker: To measure the number of hippocampal cells that showed proliferation and the number of cells expressing the immature neuron marker DCX, the total number of BrdU-positive cells and DCX-positive cells in dorsal area of the DG was counted on every 12th section of the brains using the StereoInvestigator system (version 11, MBF Bioscience) which consisted of a camera interfaced with a Nikon series Eclipse H600L microscope coupled to a motorized stage. Only the DCX expressing cells with tertiary dendrites or above order were counted as DCX-positive cells. Labeled cells were identified using a 40× objective, the optical fractionator probe and dissector height of six 25 μm sections per rat were used to count the cells in the dorsal hippocampal area of the DG. The number of BrdU-positive cells and DCX-positive cells was presented as mean ± SEM.

Phenotypic analysis: Brain sections co-expressed with anti-BrdU and DCX were observed at 40× magnification using an epifluorescent microscope Nikon series H600L. To determine neuronal differentiation, the number of DCX-positive cells out of 50 randomly selected BrdU-positive cells was counted. When a BrdU-positive cell was observed, screening for DCX-positive signal was performed by switching the filter on the microscope to detect the fluorescence signal (Meshi et al., 2006). The results were expressed as 'Differentiation Index', which was the proportion of DCX-positive cells over the 50 BrdU + cells (Plümpe et al., 2006; Hattiangady and Shetty, 2010; Song et al., 2012).

Plasma corticosterone level

The concentration of corticosterone in plasma was measured in the animals from experiment 2. The corticosterone level was determined using a corticosterone ELISA kit following the instructions of the manufacturer (Enzo Life Sciences).

Sholl analysis

In order to measure the spatial distribution and complexity of the dendritic tree, Sholl analysis by tracing the selected neurons under 20× magnification using NeurphologyJ plugin for ImageJ software was carried out (Gutierrez and Davies, 2007; Meijering, 2010). Ten DXC-positive cells per animal were randomly chosen to have 60 neurons per treatment group (Ramirez-Rodriguez et al., 2011). The criteria followed to select the cells for Sholl analysis included: cells exhibiting tertiary or higher arborization, relatively untruncated dendrites and perpendicularly oriented (Meijering, 2010; Lau et al., 2011a). Series of concentric circles were created around the soma, which was identified on the image in the software. The starting radius, step size and ending radius were set as 10 micrometer, 10 micrometer and 200 micrometers respectively. The number of times that the dendrites intersected the traced circles, known as the intersection number, was counted and the distance from the soma to each intersection was measured. The Sholl analysis was carried out by an experimenter blinded to the treatment. A high intersection number is an indication of a more complex dendritic branching (Lau et al., 2011a; Xiao et al., 2015).

Data analysis

Data were analyzed by SPSS software. Student's *t*-test was used to compare difference between control and oxytocin-treated groups. To compare the three treatment groups in experiment 2, a one-way ANOVA was used and followed by Tukey post hoc test. When the assumptions of normality and homogeneity of variance were not met, non-parametric tests were used in experiment 2 namely Kruscal-Wallis test followed by Mann–Whitney *U* post hoc test were carried out. Statistical significance was defined as $p < 0.05$. All data were illustrated by mean ± SEM.

RESULTS

Experiment 1: Oxytocin reduced depression and anxiety-like behavior, and promoted cell proliferation

In the statistical analysis of the behavioral tests, the assumptions of normal distribution and homogeneity of variance were met and t-student was carried out without any adjustment except for the locomotion analysis in the OFT in which the homogeneity of variance was not met; therefore, adjustment was required in the analysis. FST showed a statistically significant difference between treatment (mean = 113.16 s ± SEM = 15.18) and control group (mean = 192.33 s ± SEM 24.43) in the time spent in floating ($t(10) = 2.752$, $p = 0.020$, Fig. 1A). The climbing behavior between the control treatment (mean = 105.50 s ± SEM = 31.71) and oxytocin group (mean = 128.33 s ± SEM = 30.48) did not showed any statistically significant difference ($t(10) = -0.519$, $p = 0.615$, Fig. 1B). Furthermore, the swimming behavior in the control (mean = 302.16 s ± SEM = 18.07) and treatment group (mean = 358.50 s ± SEM = 22.22) did not show any statistically significant difference ($t(10) = -1.966$, $p = 0.078$, Fig. 1C). When compared to the control group, less time was spent on floating in the treatment group, which indicates a decrease in depression-like behavior. Statistically significant difference in number of positive social behavior ($t(10) = -2.956$, $p = 0.014$, Fig. 1D) was found between treatment (mean = 24.9 ± SEM = 3.71) and control group (mean = 11.9 ± SEM = 2.3). When compared to control group, treatment group showed a higher frequency of positive social behavior. No aggressive social behavior was observed in all animals. Results from OFT indicated statistically significant difference in time spent in center of the OFT arena ($t(10) = -2.403$, $p = 0.037$, Fig. 1E) between the treatment (mean = 22.50 ± SEM = 3.07) and control group

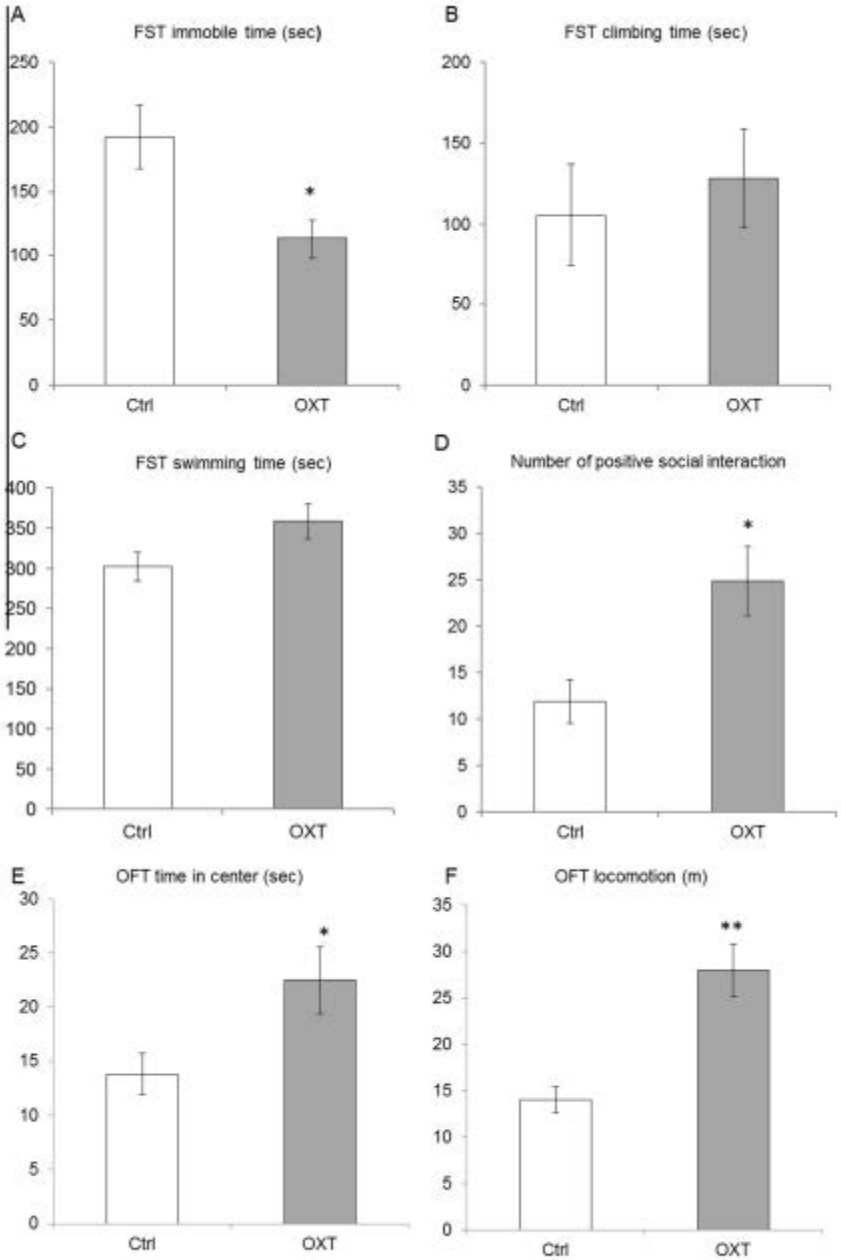


Fig. 1. Oxytocin decreased depression-like and anxiety-like behavior. (A) Oxytocin significantly reduced the time spent on floating in the forced swimming test. (B) Oxytocin group shows no difference in climbing behavior in the forced swimming test. (C) Oxytocin group shows no difference in the time spent swimming in the forced swimming test. (D) Oxytocin group shows significantly higher number of social interaction than control group. (E) Anxiety-like behavior is reduced by oxytocin, which is indicated by the significant increase in time spent in the central arena in the open field test and increased locomotion (F). Values expressed in mean ± SEM, *: $p < 0.05$, **: $p < 0.01$, when compared to control group. Student's *t*-test.

(mean = 13.83 ± SEM = 1.88). When compared to control group, treatment group spent significantly longer time in the center, which showed the anxiolytic effect of oxytocin. Similarly, statistically significant difference was observed in the distance traveled in the OFT (t(7.352) = −4.451, p = 0.003, Fig. 1F). The animals in the OXT-treated group traveled more distance (mean = 27.97 ± SEM 2.81) than the animals in the control group (mean = 13.98 ± SEM 1.40) which also demonstrate the anxiolytic effect caused by oxytocin.

In the statistical analysis of the cell proliferation and cell differentiation data, the assumptions of normal distribution and homogeneity of variance were met for the BrdU data and BrdU-immunoreactive cells with DCX expression, but homogeneity of variance for the DCX data was not met. Therefore, the t-student test for the DCX data was carried out adjusting for equal variances not assumed. Significant difference was found between control and experimental group (mean = 6470.33 ± SEM = 832.22 and mean = 10496.33 ± SEM = 1348.58, respectively) in terms of the number of BrdU-positive cells (t(10) = −2.541, p = 0.029, Fig. 2A–C) and the number of DCX-positive cells (mean = 7438.75 ± SEM = 1100.70 and mean = 11030.75 ± SEM = 323.57, respectively; t(3.51) = −3.131, p = 0.042, Fig. 2A–C), which indicated that oxytocin treatment promoted cell proliferation of proliferative cells in the hippocampus and the production of new neuroblasts. However, there was

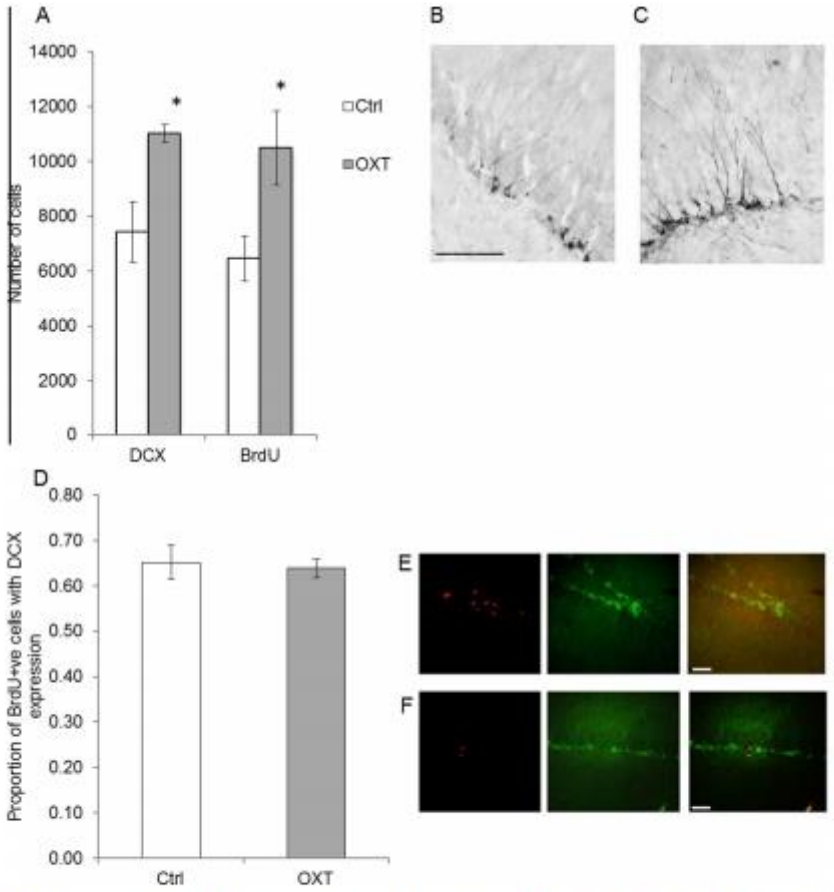


Fig. 2. Oxytocin enhances cell proliferation, but not cell differentiation in hippocampus. (A) Rats with OXT treatment had significantly higher number of BrdU-positive and DCX-positive cells in the dentate gyrus. Photomicrographs of control (B) and oxytocin-treated (C) animals showed that the number of DCX-positive cells in the dentate gyrus is increased by oxytocin. (D) No effect of Oxytocin treatment was found on the differentiation of newly proliferative cells. (E) Immunofluorescent photomicrographs showing the co-labeling of cells with BrdU (red) and DCX (green). (F) A BrdU-positive cell did not co-label with DCX in the dentate gyrus. Values expressed in mean ± SEM, *: $p < 0.05$ when compared to control group. Student's t-test. Scale bar = 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

no significant difference ($t(10) = 0.056$, $p = -2.160$, Fig. 2D–F) in the proportion of BrdU-immunoreactive cells with DCX expression of the treatment group (mean = 0.6367 ± SEM = 0.02) and control group (mean = 0.6517 ± SEM = 0.03).

Dendritic complexity of immature neurons was analyzed by Sholl analysis. There was a significant difference between the two groups in the number of intersections at various distances from soma (Fig. 3A–C). The number of intersections of oxytocin group was significantly higher than that of control group. This indicated that oxytocin treatment significantly promotes the dendritic maturation of new neurons.

Experiment 2: Oxytocin reversed the behavioral disturbance and suppression of cell proliferation induced by hypercorticosteronemia

In the statistical analysis, the assumptions of normal distribution and homogeneity of variance were checked. When the assumptions were not met, Kruskal–Wallis followed by Mann–Whitney post hoc test was carried out and the results of the non-parametric statistical analysis were expressed accordingly. Animals from the corticosterone group and the Oxt-Cort-treated group did not show any sickness behavior that might be related to the drugs administered. In addition, the mortality rate in

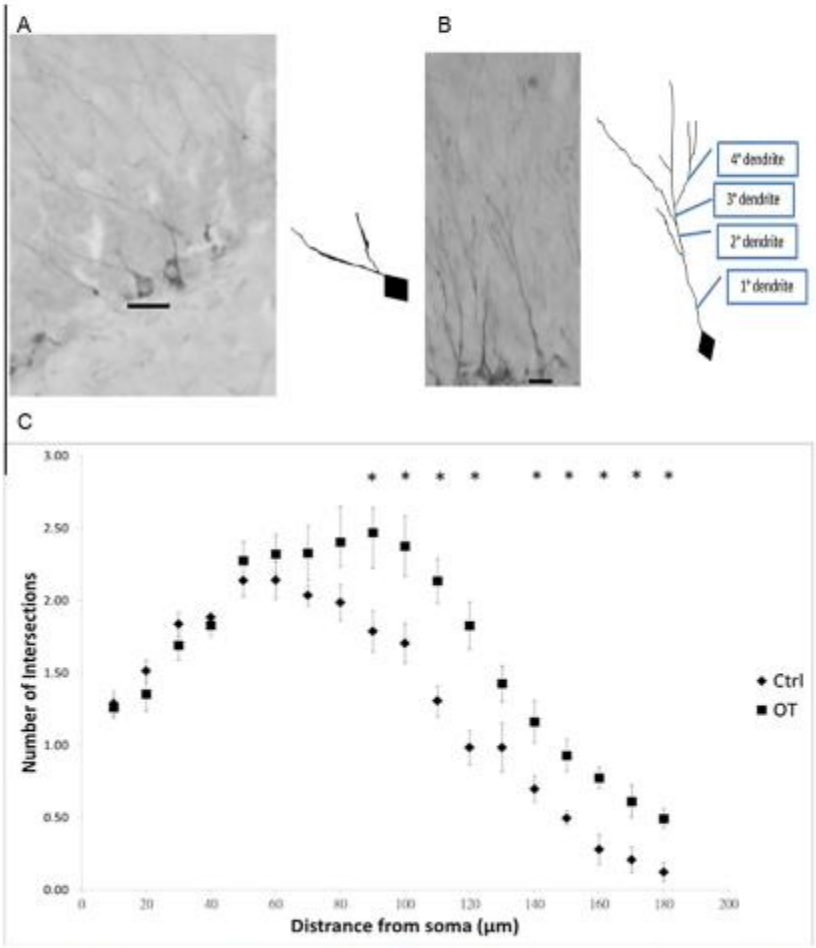


Fig. 3. Oxytocin promotes dendritic growth of immature neurons. (A, B) Photomicrographs and corresponding tracing of DCX-positive new neurons traced by ImageJ from control (A) and oxytocin-treated (B) rats. (C) Sholl analysis of DCX-positive cells of rats from control and oxytocin group. Oxytocin significantly promotes dendritic arborization at the distance 90–180 µm from the soma. Values expressed in mean ± SEM, *: $p < 0.05$ when compared to control group. Student's *t*-test.

all treatment groups was zero. Behavioral tests showed that the antidepressant and anxiolytic effect of oxytocin could reverse the depression and anxiety caused by high dose corticosterone treatment. In FST, the time spent floating showed statistically significant difference among the three groups evaluated ($F(2,16) = 10.645$, $p = 0.001$, Fig. 4A). The total floating time of rats with corticosterone treatment was significantly higher than the control ($p = 0.002$) and the group received both corticosterone and oxytocin treatment ($p = 0.007$). The climbing behavior showed no statistically significant difference among the three groups ($X^2(2) = 2.559$, $p = 0.278$, Fig. 4B). Statistically significant difference was observed in the swimming behavior among all the groups ($F(2,16) = 6.161$, $p = 0.008$, Fig. 4C). The control and corticosterone groups showed statistically significant difference ($p = 0.016$). No significant difference was observed between the control and Oxt + Cort group. Similarly, the total number of positive social interactions in SIT of corticosterone group was shown to be significantly lower than the control ($U < 0.001$, $p = 0.004$), and the co-treatment group showed a higher number of positive social interactions compared to the control group ($U = 1.50$, $p = 0.009$; $X^2(2) = 12.767$, $p = 0.002$, Fig. 4D). The results showed that oxytocin reversed the induced depression- and anxiety-like behaviors. In OFT, animals received both oxytocin and corticosterone treatment showed a significantly longer time spent in the center of the arena than the control and corticosterone-treated rats ($U = 1.00$, $p = 0.032$; $U = 12.00$, $p = 0.788$, respectively) ($X^2(2) = 7.192$, $p = 0.027$, Fig. 4E). However, no significant difference was found between the control and corticosterone group. Furthermore, no statistically significant difference was observed in the locomotion behavior among the groups in the OFT ($F(2,16) = 0.376$, $p = 0.693$, Fig. 4F). Regarding the concentration of corticosterone in plasma, the control group showed high levels of corticosterone when compared with the co-treatment and corticosterone group (detection limit of 26.99 pg/ml, intra-assay variability: low, 8.0%CV; medium, 8.4%CV; high 6.6% CV; inter-assay variability: low, 13.1%CV; medium 8.2% CV; high 7.8%CV; Enzo, catalog No. ADI-900–097). Statistical significant difference was observed among all the groups ($F(2,13) = 37.502$, $p < 0.001$, Fig. 4G). The control group (mean = 395.88 ± SEM = 54.84) and the Oxt-Cort group (mean = 5.32 ± SEM = 1.40) showed statistically significant difference ($p < 0.001$) as well as the Cort group (mean = 3.34 ± SEM = 0.34) when compared with the control group ($p < 0.001$).

Statistically significant difference was observed in the number of DCX-positive cells among all the groups ($X^2(2) = 9.609$, $p = 0.008$). Corticosterone treatment significantly reduced the number of DCX-positive cells in the hippocampus ($U < 0.001$, $p = 0.004$, Fig. 5A) while the rats co-treated with oxytocin and corticosterone has a significantly higher number of DCX-positive cells ($U = 1.50$, $p = 0.009$, Fig. 5A). Sholl analysis of the DCX-positive cells in corticosterone-treated rats showed a significantly lower number of intersections from both the control and oxytocin groups at various distances from soma (Fig. 5B–H). These findings revealed that oxytocin could prevent the suppression of hippocampal cell proliferation caused by corticosterone in the hypercortisolemia treatment model.

DISCUSSION

Taking into account that the half-life of oxytocin is 2 min in blood and 20 min in the cerebrospinal fluid in rats and the administration regimen for 14 consecutive days used in the present study, the observations made in the oxytocin treatment reflected the repeated effects rather than acute effects of oxytocin (Ludwig and Leng, 2006; Leuner et al., 2012). The present study revealed that repeated exposure to oxytocin could significantly enhance cell proliferation, promote dendritic maturation of new neurons and reduce depression- and anxiety-like behaviors. Furthermore, oxytocin could prevent the disruption in cell proliferation and behaviors caused by high dose corticosterone treatment.

As expected and reported in previous studies, suppression of the hypothalamo-pituitary-adrenal (HPA) axis was observed in experiment 2 after repeated exogenous administration of corticosterone which leads to low concentration of corticosterone in plasma. The negative feedback observed after treatment is due to the prolonged exposure to high concentration glucocorticoid that exceeds the concentration required for physiological homeostasis (Young et al., 1995; Andrews et al., 2012).

A significant correlation was also found between the dendritic complexity of DCX-positive cells and social interaction. These findings, collectively, suggest the antidepressant and anxiolytic effect of exogenous oxytocin and the involvement of the neurohypophysial hormone in the regulation of emotional behaviors.

The stimulatory effect of oxytocin on hippocampal cell proliferation was shown by a study conducted by Leuner et al. (2012), in which cell proliferation in the ventral hippocampus was stimulated after acute or repeated (7 days) administration of oxytocin. The present study provides further information by using different experimental design from Leuner's study. First, since the animals in the previous study were sacrificed three weeks after the treatment, it is possible that the BrdU labeling of cells reflect a mixed effect of oxytocin on both proliferation and survival. In the present study, we sacrificed the rats immediately after the behavioral test, which may better reflect the effect of oxytocin on cell proliferation. Second, as differentiation and maturation of new neurons is another important stage in neurogenesis, the dendritic morphology of new neurons (indicated by DCX staining) was studied in the present study. Third, the oxytocin treatment was maintained for a prolonged period (two weeks), which will reflect the effect of oxytocin in a repeated treatment manner.

Interestingly, the present study shows that repeated oxytocin treatment increases cell proliferation in the dorsal hippocampus, which is different from the previous finding (Leuner et al., 2012) and the difference may be

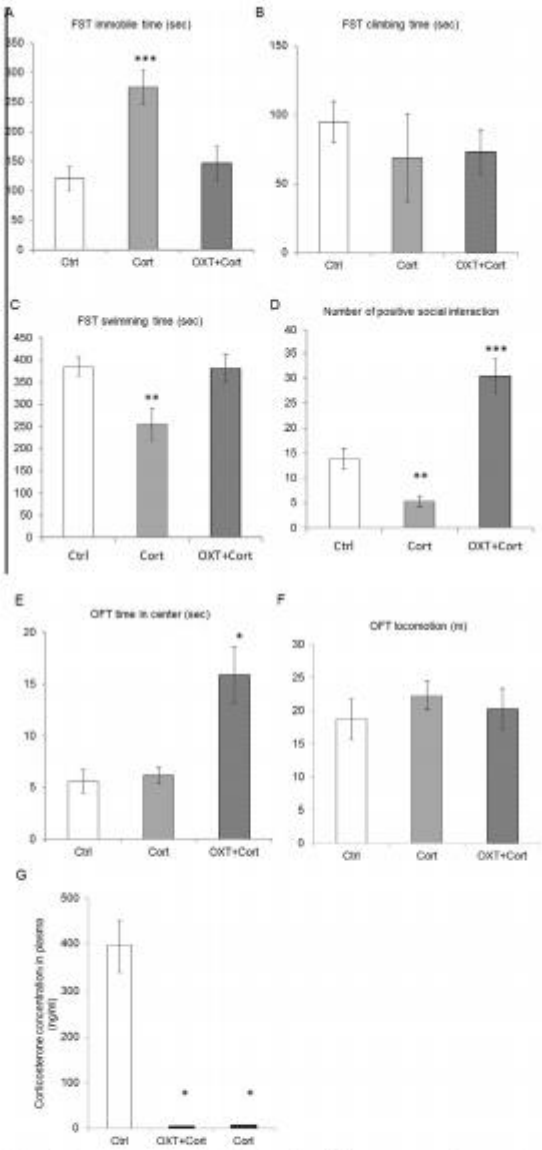


Fig. 4. Corticosterone treatment induced depression- and anxiety-like behavior while co-treatment with oxytocin reversed the behavioral deficit and suppression of HPA axis after administration of corticosterone treatments. (A) Corticosterone-treated animals showed significantly longer duration of floating, which was prevented by co-treatment with oxytocin. (B) No difference is seen in the climbing behavior among the treatments in the forced swimming test. (C) Corticosterone-treated group spent less time swimming which was reverted by the co-treatment with oxytocin. (D) Number of positive social interactions in corticosterone-treated animals was significantly lower than the control and co-treatment groups. (E) Co-treatment group shows significantly longer time spent in the central arena of OFT, but not significant difference was found between the control and corticosterone-treatment groups. (F) No statistically significant difference was observed in the locomotion behavior in the OFT. (G) Low concentration of corticosterone in plasma was observed in the corticosterone group and co-treatment when compared to the control group which is due to suppression of the HPA axis by exogenous administration of corticosterone. Values expressed in mean ± SEM, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, when compared to the other two groups; **: $p < 0.01$ when compared to the other two groups. One-way ANOVA with Tukey post hoc test; Kruskal–Wallis with Mann-Whitney U post hoc test.

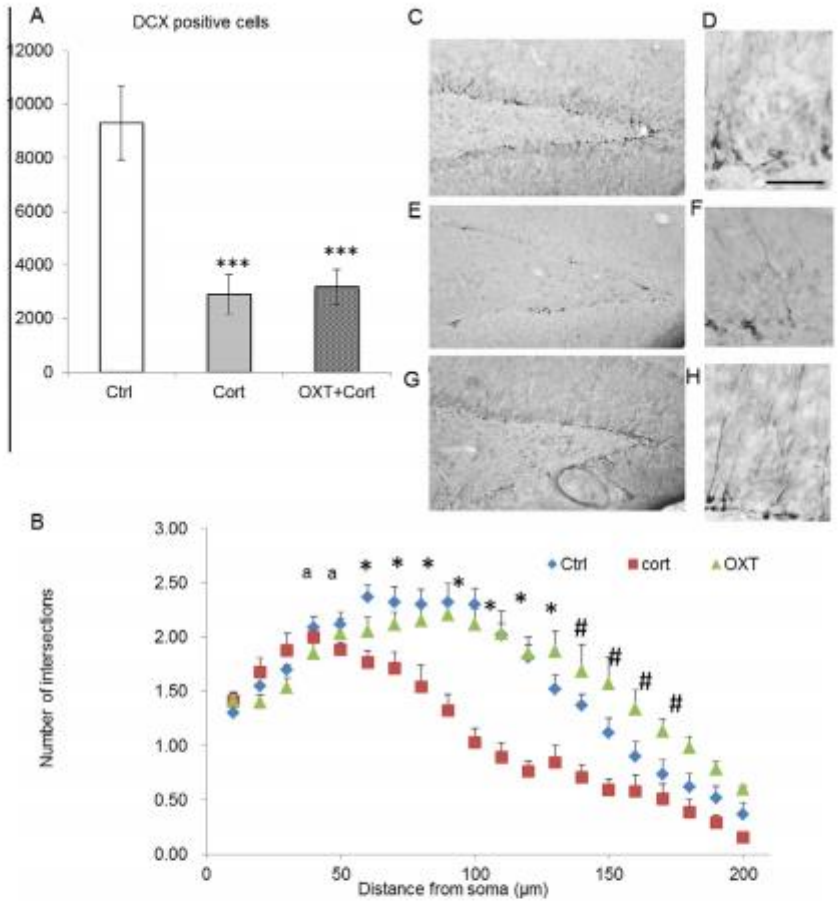


Fig. 5. Oxytocin reversed the suppression of cell proliferation and dendritic maturation of new neurons induced by corticosterone. (A) Corticosterone treatment reduced the number of DCX-positive cells with tertiary dendrites in the hippocampus, while co-treatment with oxytocin could reverse the change. (B) Sholl analysis showed that the dendritic complexity of immature neurons is significantly lower in corticosterone group when compared to the control and co-treatment groups. (C–H) Representative photomicrographs of DCX-positive cells in control (C, D), corticosterone (E, F) and co-treatment (G, H) group animals. (C, E, G) 100× magnification; (D, F, H) 400× magnification. Scale bar = 100 μm. a: $p < 0.05$ between corticosterone (Cort) and control group; *: $p < 0.05$ when Cort group is compared to Ctrl and OXT groups; #: $p < 0.05$ when comparing Cort group with OXT group; One-way ANOVA with Tukey post hoc test; Kruskal–Wallis with Mann-Whitney U post hoc test.

due to the prolonged treatment period. Furthermore, it is shown that OXT has a stimulating effect on dendritic development of new neurons. With elaborated dendrites, the number of possible synaptic connection within the molecular layer of the DG can be increased, which are essential for the integration of the new neurons into the existing hippocampal neural circuit (Lau et al., 2013). The effect of oxytocin on cell proliferation was also shown in corticosterone-treated animals which displayed suppressed cell proliferation and dendritic growth of immature neurons. Stress, which is a pivotal trigger in depression, has shown to cause reduction of dendritic length and distal dendritic branching density in immature granule cells in the hippocampus (Sousa et al., 2000; Bessa et al., 2008). In previous studies, it was suggested that synaptic loss as a consequence of dendritic degeneration could be the cause of behavioral deficits observed in stressed animals (Sousa et al., 2000). Furthermore, it has been shown that the effect of stress on dendritic length and complexity can be reverted by antidepressants such as fluoxetine and imipramine (Lussier et al., 2013). Therefore, the effect of oxytocin on dendritic complexity shows antidepressant-like effect also at dendritic developmental level when reverting the dendritic degeneration induced by corticosterone. Taken together, these results suggest that OXT stimulates cell proliferation with higher dendritic

complexity and may have a positive influence on integration, the last stage of neurogenesis. With the effect to increase the number of new cells and promote dendritic growth, oxytocin may regulate emotional and social behaviors, and exert the therapeutic effect on animals with emotional disturbance. However, as the net increase of new neurons will be affected by the survival rate, future study of oxytocin on neuronal survival would provide further understanding on its neurogenic effect.

Since hippocampus plays important roles in emotion, hippocampal neurogenesis was hypothesized to be involved in emotional regulation and mood disorders (Ruan et al., 2014). Animal studies showed that rats with impaired adult hippocampal neurogenesis had increased depression-like behavior, elevated anhedonia level (Snyder et al., 2011), and increased anxiety-like behaviors (Revest et al., 2009). Impaired neurogenesis was also found to decelerate the mice's recovery of circulating glucocorticoid level after exposing to stress (Snyder et al., 2011). Furthermore, several animal studies have shown that therapeutic effect of antidepressants was related to neurogenesis (Santarelli et al., 2003). To elucidate the effect of oxytocin on emotional behaviors in both normal and stressed animals, we used FST, OFT and SIT in this study. The role of oxytocin in the regulation of social behavior, anxiety and the HPA axis has been well established (Parker et al., 2010; Ring et al., 2010). In addition, dysregulation of oxytocin has been associated with emotional distress and impairment in social interaction which are frequently observed characteristics of depression (Parker et al., 2010; Yan et al., 2014). The results of the present study showed that repeated oxytocin treatment exerted effect on emotions by reducing depression-like and anxiety-like behaviors. These findings were in line with previous findings on the antidepressant and anxiolytic effect of oxytocin. For instance, an animal study showed that acute and repeated treatments (10 days) of oxytocin at 0.25–1 mg/kg/day were effective in reducing depression-like behaviors in normal animals (Arletti and Bertolini, 1987). In another study, central (0,3 μg) and systemic (30 mg/kg) administration oxytocin significantly reduced the immobility time in mice in the tail suspension test showing improvement in depression-like behavior (Ring et al., 2010). Furthermore, acute i.p. administration of oxytocin (1 μg/kg) showed antidepressant activity in rats (Nowakowska et al., 2002). Another animal study showed attenuated high anxiety-related behaviors of female rats after 6 days of oxytocin treatment (Slattery and Neumann, 2010a). Our results were in line with these studies, and suggested that oxytocin has antidepressant and anxiolytic effect by increasing cell proliferation.

However, some studies carried out on the effect of oxytocin on depression-like behavior show conflicting results. In a study carried out by Yan et al., 2014, acute central administration of oxytocin improved depression-like behavior while acute peripheral administration of oxytocin (1, 2 and 4 mg) did not have any effect on the depression-like behavior in the FST (Yan et al., 2014). Similar results were found in the study carried out by Slattery and Neumann, 2016 in rats. They found that neither acute (1 μg) nor chronic (10 ng/h, 6 days) central administration of OXT showed any effect on the forced swimming test meaning that the depressive state was not attenuated by OXT exposure (Slattery and Neumann, 2010a). As shown in the studies mentioned above, OXT has shown to display antidepressant properties in several preclinical studies while some other studies have shown no alteration in depression-like behavior in the forced swimming test either in acute or chronic administration as well as when administered centrally or peripherally. The discrepancy in the studies opens an invitation to explore further the regulation of the oxytocinergic system in depression-like behavior since no consistency in the role of OXT has been found in animal studies (Rotzinger et al., 2010; Slattery and Neumann, 2010a, 2010b). In addition, the mechanistic pathways activated by oxytocin at central and peripheral level should be explored more in detail to provide more conclusive evidence of the role of oxytocin and its anti-depressant properties (Slattery and Neumann, 2010b).

Emotion was found to be an important regulator of social behavior (Blair et al., 2004), we tested whether positive social behavior is induced by repeated oxytocin treatment. The purposes of SIT are two fold: assessing anxiety in a social situation and assessing positive/negative social behaviors. The results showed that oxytocin enhanced social interaction, which is in-line with a previous study (Witt et al., 1992), in which the social contact of rats with oxytocin infusion was doubled. Interestingly, strong correlation between dendritic complexity and social interaction was found in the present study. In other words, the dendritic maturation is associated with the display of prosocial behaviors and thus the maturation of new neurons may exert effect on social interaction. Another study on female prairie voles showed that decrease in social interaction could lead to decreased neurogenesis (Lieberwirth et al., 2012). Taken together, although causal relationship between cell proliferation, dendritic maturation and social interaction could not be established in the present study, it is possible that neurogenesis may have a reciprocal interaction with social interaction, which is shown in reproductive behaviors and neurogenesis (Mak et al., 2007; Lau et al., 2011b). Furthermore, OXT may alter social interaction indirectly by altering the emotional state of animals, as emotion mediates social behaviors.

The finding of the present study would provide evidence to support the use of oxytocin in improving positive social behavior, decreasing anxiety-like behavior and increasing cell proliferation. Therefore, oxytocin may have therapeutic value in psychiatric illnesses (Matsuzaki et al., 2012). On the other hand, as rewarding social interaction and sexual stimulus could increase the release of oxytocin and promote neurogenesis, oxytocin may have a role in mediating the neurogenic effect of rewarding social stimulation. If this is the case, it may be able to explain why pro-neurogenic stimuli are usually rewarding to the individual. The rewarding effect of OXT has been previously studied using low doses of MDMA (ecstasy) in which increased plasma levels of OXT in rodents were observed. The results suggest that OXT could also play a role in the rewarding effects of MDMA which supports the action of OXT in reward and

positive hedonic states (Slattery and Neumann, 2010b). Chronic peripheral administration of oxytocin has led to positive hedonic state. Nevertheless, no positive hedonic state was observed with intracerebral OXT administration. Lack of consistency in the effects of OXT in animal studies requires more thoroughly investigation to elucidate the mechanism of action of OXT. In this sense, Eliava et al. (2016), studied the OXT signaling pathway of OXT releasing neurons and identified a new population of OXT neurons modulating different biological processes (Eliava et al., 2016). Furthermore, Jurek et al. (2015) investigated the regulatory signaling cascade of OXT in stress response. They found that gene transcription of Crf gene was regulated by OXT via the translocation of CRTC3 (Jurek et al., 2015). Despite the effort to elucidate the signaling pathways involved in the effects caused by OXT at central and peripheral level, more studies are needed to construct a clearer picture of the biological role of OXT and its potential benefit in the treatment of depression.

The current finding might provide an alternate explanation for therapeutic value of social interaction in human. Studies showed that positive social stimuli and social experience increased the level of circulating oxytocin (Uvnäs-Moberg, 1998; Kikusui et al., 2006; Heinrichs and Domes, 2008). The stimulation in neurogenesis by oxytocin may benefit patients with mood or social disorders by reducing negative emotion and promoting social interaction or cognition, which are common problems to be encountered in treatment of mood disorder patients (Chen et al., 2015). The potential involvement and application value of neurogenesis in rehabilitation of clients with psychiatric illnesses has gained increasing attentions (Eisch et al., 2008). Further investigation is needed to explore the causal relationship between neurogenesis, social interaction and emotion, while thorough understanding of the relationships would inform the clinical practice on effective psychiatric treatment for patients with mood disorders.

Acknowledgments—The study was supported by the Large Equipment Fund, The Hong Kong Polytechnic University (1.51.27.DD6B), and ECS, University Grant Council, H.K. (0112876) to Lau B.W.

REFERENCES

Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K (2007) High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317:819–823.

Altunkaynak BZ, Onger ME, Altunkaynak ME, Ayrancı E, Canan S (2011) A brief introduction to stereology and sampling strategies: basic concepts of stereology. NeuroQuantology 10.

Andrews MH, Wood SA, Windle RJ, Lightman SL, Ingram CD (2012) Acute glucocorticoid administration rapidly suppresses basal and stress-induced hypothalamo-pituitary-adrenal axis activity. Endocrinology 153:200–211.

Arletti R, Bertolini A (1987) Oxytocin acts as an antidepressant in two animal models of depression. Life Sci 41:1725–1730.

Becker A, Grecksch G, Bernstein HG, Höllt V, Bogerts B (1999) Social behaviour in rats lesioned with ibotenic acid in the hippocampus: quantitative and qualitative analysis. Psychopharmacology 144:333–338.

Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OFX, Sousa N (2008) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry 14:764–773.

Blair KA, Denham SA, Kochanoff A, Whipple B (2004) Playing it cool: temperament, emotion regulation, and social behavior in preschoolers. J Sch Psychol 42:419–443.

Brummelte S, Pawluski JL, Galea LAM (2006) High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: a model of post-partum stress and possible depression. Horm Behav 50:370–382.

Cameron HA, Gould E (1994) Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 61:203–209.

Chen Y-L, Pan A-W, Hsiung P-C, Chung L (2015) Quality of life enhancement programme for individuals with mood disorder: a randomized controlled pilot study. Hong Kong J Occup Ther.

Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 11:339–350.

Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming G-L, Overstreet-Wadiche LS (2008) Adult neurogenesis, mental health, and mental illness: hope or hype? J Neurosci 28:11785–11791.

Eliava M et al (2016) A new population of parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing. Neuron 89:1291–1304.

Gamberini MT, Rodrigues DS, Rodrigues D, Pontes VB (2015) Effects of the aqueous extract of Pimpinella anisum L. seeds on exploratory activity and emotional behavior in rats using the open field and elevated plus maze tests. J Ethnopharmacol 168:45–49.

Gregus A, Wintink AJ, Davis AC, Kalynchuk LE (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behav Brain Res 156:105–114.

Gutierrez H, Davies AM (2007) A fast and accurate procedure for deriving the Sholl profile in quantitative studies of neuronal morphology. J Neurosci Methods 163:24–30.

Hattiangady B, Shetty AK (2010) Decreased neuronal differentiation of newly generated cells underlies reduced hippocampal neurogenesis in chronic temporal lobe epilepsy. Hippocampus 20:97–112.

Heinrichs M, Domes G (2008) Neuropeptides and social behaviour: effects of oxytocin and vasopressin in humans. Prog Brain Res 170:337–350.

Hillerer KM, Neumann ID, Couillard-Despres S, Aigner L, Slattery DA (2014) Lactation-induced reduction in hippocampal neurogenesis is reversed by repeated stress exposure. Hippocampus 24:673–683.

Jafarzadeh N, Javeri A, Khaleghi M, Taha MF (2014) Oxytocin improves proliferation and neural differentiation of adipose tissue-derived stem cells. Neurosci Lett 564:105–110.

Jurek B, Slattery DA, Hiraoka Y, Liu Y, Nishimori K, Aguilera G, Neumann ID, van den Burg EH (2015) Oxytocin regulates stress-induced Crf gene transcription through CREB-regulated transcription coactivator 3. J Neurosci 35:12248–12260.

Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS (2004) Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behav Neurosci 118:1365–1377.

Kikusui T, Winslow JT, Mori Y (2006) Social buffering: relief from stress and anxiety. Philos Trans R Soc London, B Biol Sci 361:2215–2228.

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25:150–176.

Lau BW, Lee JC, So K (2013) Neurogenic hypothesis and psychiatric disorders. Chinese Sci Bull 58:3188–3198.

Lau BW-M, Ren C, Yang J, Yan SWL, Chang RC-C, Pu M, So K-F (2011a) Light deprivation induces depression-like behavior and

suppresses neurogenesis in diurnal mongolian gerbil (Meriones unguiculatus). Cell Transplant 20:871–881.
Lau BW-M, Yau S-Y, Lee TMC, Ching Y-P, Tang S-W, So K-F (2011b) Effect of corticosterone and paroxetine on masculine mating behavior: possible involvement of neurogenesis. J Sex Med 8:1390–1403.
Leuner B, Caponiti JM, Gould E (2012) Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. Hippocampus 22:861–868.
Leuner B, Gould E, Shors TJ (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16:216–224.
Lieberwirth C, Liu Y, Jia X, Wang Z (2012) Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. Horm Behav 62:357–366.
Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 7:126–136.
Lussier AL, Lebedeva K, Fenton EY, Guskjolen A, Caruncho HJ, Kalynchuk LE (2013) The progressive development of depression-like behavior in corticosterone-treated rats is paralleled by slowed granule cell maturation and decreased reelin expression in the adult dentate gyrus. Neuropharmacology 71:174–183.
Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, Huhtaniemi I, Weiss S (2007) Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. Nat Neurosci 10:1003–1011.
Malberg JE (2004) Implications of adult hippocampal neurogenesis in antidepressant action. J Psychiatry Neurosci 29:196–205.
Matsuzaki M, Matsushita H, Tomizawa K, Matsui H (2012) Oxytocin: a therapeutic target for mental disorders. J Physiol Sci 62:441–444.
McNeilly AS, Ducker HA (1972) Blood Levels of oxytocin in the female goat during coitus and in response to stimuli associated with mating. J Endocrinol 54:399–406.
Meijering E (2010) Neuron tracing in perspective. Cytometry A 77:693–704.
Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R (2006) Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. Nat Neurosci 9:729–731.
Ming G, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28:223–250.
Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35:649–659.
Nowakowska E, Kus K, Bobkiewicz-Kozowska T, Hertmanowska H (2002) Role of neuropeptides in antidepressant and memory improving effects of venlafaxine. Pol J Pharmacol 54:605–613.
Parker KJ, Kenna HA, Zeitzer JM, Keller J, Blasey CM, Amico JA, Schatzberg AF (2010) Preliminary evidence that plasma oxytocin levels are elevated in major depression. Psychiatry Res 178:359–362.
Plümpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Römer B, Rodriguez GR, Kronenberg G, Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. BMC Neurosci 7:77.
Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33.
Ramírez-Rodríguez G, Ortíz-López L, Domínguez-Alonso A, Benítez-King GA, Kempermann G (2011) Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 50:29–37.
Rankin SL, Partlow GD, McCurdy RD, Giles ED, Fisher KRS (2003) Postnatal neurogenesis in the vasopressin and oxytocin-containing nucleus of the pig hypothalamus. Brain Res 971:189–196.
Revest J-M, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza P-V, Abrous DN (2009) Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry 14:959–967.
Ring RH, Schechter LE, Leonard SK, Dwyer JM, Platt BJ, Graf R, Grauer S, Pulicicchio C, Resnick L, Rahman Z, Sukoff Rizzo SJ, Luo B, Beyer CE, Logue SF, Marquis KL, Hughes ZA, Rosenzweig-Lipson S (2010) Receptor and behavioral pharmacology of WAY-267464, a non-peptide oxytocin receptor agonist. Neuropharmacology 58:69–77.
Rotzinger S, Lovejoy DA, Tan LA (2010) Behavioral effects of neuropeptides in rodent models of depression and anxiety. Peptides 31:736–756.
Ruan L, Lau BW-M, Wang J, Huang L, Zhuge Q, Wang B, Jin K, So K-F (2014) Neurogenesis in neurological and psychiatric diseases and brain injury: from bench to bedside. Prog Neurobiol 115:116–137.
Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809.
Schmitz C, Hof PR (2005) Design-based stereology in neuroscience. Neuroscience 130:813–831.
Slattery DA, Neumann ID (2010a) Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. Neuropharmacology 58:56–61.
Slattery DA, Neumann ID (2010b) Oxytocin and major depressive disorder: experimental and clinical evidence for links to aetiology and possible treatment. Pharmaceuticals 3:702–724.
Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476:458–461.
So KF, Tang SW, Lee TMC (2012) Neuroprotection in steroid therapy: a rodent model. Hong Kong Med J 18(Suppl 3):20–23.
Song J, Christian KM, Ming G, Song H (2012) Modification of hippocampal circuitry by adult neurogenesis. Dev Neurobiol 72:1032–1043.
Sousa N, Lukoyanov NV, Madeira MD, Almeida OFX, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97:253–266.
Uvnäs-Moberg K (1998) Oxytocin may mediate the benefits of positive social interaction and emotions. Psychoneuroendocrinology 23:819–835.
Winner B, Kohl Z, Gage FH (2011) Neurodegenerative disease and adult neurogenesis. Eur J Neurosci 33:1139–1151.
Witt DM, Winslow JT, Insel TR (1992) Enhanced social interactions in rats following chronic, centrally infused oxytocin. Pharmacol Biochem Behav 43:855–861.
Xiao F, Xu J-M, Jiang X-H (2015) CX3 chemokine receptor 1 deficiency leads to reduced dendritic complexity and delayed maturation of newborn neurons in the adult mouse hippocampus. Neural Regen Res 10:772–777.
Yan Y, Wang Y-L, Su Z, Zhang Y, Guo S-X, Liu A-J, Wang C-H, Sun F-J, Yang J (2014) Effect of oxytocin on the behavioral activity in the behavioral despair depression rat model. Neuropeptides 48:83–89.
Young EA, Kwak SP, Kottak J (1995) Negative feedback regulation following administration of chronic exogenous corticosterone. J Neuroendocrinol 7:37–45.
Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 9:268–275.

(Accepted 2 July 2016)
(Available online 11 July 2016)

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 5869315, 21 pages
https://doi.org/10.1155/2017/5869315

Review Article

The Effectiveness of Aromatherapy for Depressive Symptoms: A Systematic Review

Dalinda Isabel Sánchez-Vidaña, Shirley Pui-Ching Ngai, Wanjia He, Jason Ka-Wing Chow, Benson Wui-Man Lau, and Hector Wing-Hong Tsang

Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong

Correspondence should be addressed to Benson Wui-Man Lau; benson.lau@polyu.edu.hk

Received 25 August 2016; Revised 25 October 2016; Accepted 14 November 2016; Published 4 January 2017

Academic Editor: Arndt Büssing

Background. Depression is one of the greatest health concerns affecting 350 million people globally. Aromatherapy is a popular CAM intervention chosen by people with depression. Due to the growing popularity of aromatherapy for alleviating depressive symptoms, in-depth evaluation of the evidence-based clinical efficacy of aromatherapy is urgently needed. *Purpose*. This systematic review aims to provide an analysis of the clinical evidence on the efficacy of aromatherapy for depressive symptoms on any type of patients. *Methods*. A systematic database search was carried out using predefined search terms in 5 databases: AMED, CINHAL, CCRCT, MEDLINE, and PsycINFO. Outcome measures included scales measuring depressive symptoms levels. *Results*. Twelve randomized controlled trials were included and two administration methods for the aromatherapy intervention including inhaled aromatherapy (5 studies) and massage aromatherapy (7 studies) were identified. Seven studies showed improvement in depressive symptoms. *Limitations*. The quality of half of the studies included is low, and the administration protocols among the studies varied considerably. Different assessment tools were also employed among the studies. *Conclusions*. Aromatherapy showed potential to be used as an effective therapeutic option for the relief of depressive symptoms in a wide variety of subjects. Particularly, aromatherapy massage showed to have more beneficial effects than inhalation aromatherapy.

1. Introduction

Depression is a life-threatening mood disorder manifested as a combination of cognitive and physical symptoms that leads to decreased interest in daily life activities [1] which imposes significant negative impact on people's quality of life and work performance due to disability, suffering, and high risk of perpetrating self-harm [2, 3].

Depression is reported as the largest health concern in the 21st century [4]. About 350 million people are currently suffering from depression [5]. Major depressive disorder has been projected to be the highest cause of years of life lived with disability by 2030 [6, 7]. The prevalence of depression has increased dramatically at a global level and one million people with depression commit suicide yearly [6, 8, 9]. In USA, an annual economic loss around USD 210 billion is associated with depression, which is one of the diseases with highest economic burden [10, 11]. Depressive symptoms include feelings of guilt, sadness, worthlessness and desperation, inability to experience pleasure, changes in appetite and sleep patterns, lack of energy, poor concentration and memory, motor retardation, fatigue, and recurrent suicidal and death ideation which are experienced for more than 2 weeks [1, 9, 12, 13].

Diagnosis of depressive symptoms is carried out using validated tools [14]. One of the oldest and most widely used diagnostic tools is the Hamilton Depression Rating Scale [15] which comprises a clinical-rated and a self-reported assessment. Another tool included the Beck Depression Inventory that uses a patient self-reporting tool of depressive symptoms [14]. These tools demonstrated high sensitivity and specificity and therefore are the best validated scales used for assessing the degree of severity of depressive symptoms [14, 16, 17].

Nowadays, the first-line treatment for major depressive disorder is antidepressants including monoamine oxidase

inhibitors, tricyclic antidepressants, and serotonin-norepinephrine and selective serotonin reuptake inhibitors (SNRIs/SSRIs) [13, 30, 31]. Despite the wide variety of antidepressants available in the market, a significant proportion of patients cannot reach full remission or experience side effects [13, 32, 33]. For instance, it has been reported that nearly 30% of the patients do not respond [8]. Side effects including nauseas, insomnia, agitation, weight gain, somnolence, sexual dysfunction, and cardiovascular adverse events have been reported [9, 31, 34]. Another downside of the use of antidepressants is the long treatment period needed to experience the beneficial antidepressant effect. Due to the ineffectiveness of the treatment in some patients or intolerability to the side effects, a large number of patients do not comply with the treatment and search for other therapeutic options [13, 31, 35]. Hence, increasing number of people with depressive symptoms explored other nonpharmacological interventions including psychotherapy and counseling, psychoeducation, exercise, problem solving therapy, guided self-help and behavioral activation treatments [36], or even complementary and alternative medicine (CAM) [3, 31].

CAM is defined as a broad set of healing resources, such as medical products and practices, for the prevention, diagnosis, and treatment of diseases that functions as a complement to the mainstream medicine system [37]. In USA, about 53.6% of the patients suffering from depression have reported to use CAM as an adjuvant therapy for the treatment of depression [31, 38]. One of the CAM options that patients with depressive symptoms choose is aromatherapy. Aromatherapy is defined as the therapeutic use of plant-derived concentrated essences which are extracted by distillation [39–41]. Aromatherapy is an inexpensive and noninvasive modality of CAM used to improve the psychological health and wellbeing [40, 42, 43]. Essential oils contain volatile organic compounds that exert a pharmacological effect by penetrating the body by oral, dermal (aromatherapy massage or topical application of aromatherapy) [44, 45], or olfactory administration (inhalation aromatherapy) [46–48]. The classification of essential oils is based on the botanical classification of the plant from which the essential oils are extracted [44]. The use of chemotypes is another classification of essential oils based on the subspecies of a plant with the same morphological characteristics that produces essential oils with different chemical profile, for example, type and quantity of chemical components [45]. The chemotype describes the main compound within certain essential oil [45]. Frequently, essential oils are used at different concentrations depending on the route of administration: (1) for aromatherapy massage, 1–5% essential oil is used, (2) for oral administration, 8–50% essential oil is used, and (3) concentrated essential oil is used in inhalation aromatherapy [48]. However, the dosage and dilution of essential oil chosen are not standardized in practice [48]. The most potent and effective administration method is oral administration in which the components of the essential oil reach the bloodstream [44]. Since essential oils are lipophilic, they can easily be carried to all organs in the body [44]. In inhalation aromatherapy, the inhaled air containing essential oils can not only reach the circulation system via the blood capillary network in the nose and the bronchi in the lungs but also stimulate brain areas directly via the olfactory epithelium [44, 48]. Essential oils trigger mechanisms in the brain via the olfactory system. The mechanism of action of essential oils administered by inhalation involves stimulation of the olfactory receptors cells in the nasal epithelium, about 25 million cells, connected to the olfactory bulb. After stimulation, the signal is transmitted to the limbic system and hypothalamus in the brain through the olfactory bulb and olfactory tract. Once the signals reach the olfactory cortex, release of neurotransmitters, for example, serotonin, takes place which results in the expected effect on emotions related to essential oil use [49–51].

Increasing popularity of aromatherapy has been reported in the UK as one of the most commonly used CAM therapies [52]. Due to the increasing popularity of aromatherapy this modality of CAM was chosen to carry out a systematic review on its effectiveness [53].

There was one published systematic review evaluating the effects of aromatherapy for people with depressive symptoms which included studies from 2000 to 2008 [2]. Since 2009 to date, 10 new RCT studies have been carried out to evaluate the effectiveness of aromatherapy on depressive symptoms thereby raising the need to update the discussion on the new findings taking into account all the evidence reported up to date on the topic. Therefore, this systematic review aims to provide an updated analysis of the evidence of the efficacy of aromatherapy for depressive symptoms.

2. Methods

2.1. Search Strategy. An extensive literature search was carried out in the following databases: Allied and Complementary Medicine Database (AMED), Cochrane Central Register of Controlled Trials (CCRCT), Cumulative Index to Nursing and Allied Health (CINAHL), MEDLINE, and PsycINFO. The predefined search strategy used to obtain the reference list of potential articles in the present study is shown in Table 1. Only studies in English were included and the search was carried out by 2 independent authors having a third author to consult when discrepancy occurred. The present study included randomized clinical trials involving adult subjects of both genders. There was no age restriction.

2.2. Inclusion and Exclusion Criteria for Study Selection and Outcome Measures. The studies included in the present review comprise RCTs with any kind of study design (e.g., double blind, single blind, and crossover study). No time restriction on the publishing year was considered for the study selection and studies that fulfill the inclusion and exclusion criteria up to date were included. Studies in which depressive symptoms were evaluated using any standardized assessment tool for depressive symptoms were included disregarding the type of clinical condition studied. Studies that assessed depressive symptoms by anxiety scales or any other assessments for depressive symptoms, for example, Profile of Mood States rating scale (POMS) or Hospital Anxiety and Depression Scale (HADS), were included. Eligible studies had to include the use of essential oils administered by inhalation or topical administration. Any study combining

Table 1: Search terms and database search strategy.

ID	Disease search terms
1	Depress*
2	Major depress*
3	Mood disorder
4	Depressive disorder
5	1 OR 2 OR 3 OR 4
ID	RCT search terms
6	Controlled clinical trial*
7	Random* controlled trial*
8	6 OR 7
ID	Aromatherapy search terms
9	Aroma
10	Aromatherapy
11	Aromatic therapy
12	Essential oil*
13	Fragrance
14	Fragrant oil*
15	Scent
16	Massage therapy
17	Medical massage
18	Massage
19	9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
20	5 AND 8 AND 19

*Truncation symbol for database search.

aromatherapy and massage was included regardless of the application method of the massage. There was no restriction in the duration of the treatment and number of sessions used. Systematic reviews and meta-analyses on aromatherapy and depression, mood disorders, or depressive symptoms were not included.

2.3. Selection of Relevant Studies. After the article search and removal of duplicates, the titles of the articles retrieved in the database search were screened. The abstracts of the preselected articles were screened to make a selection for further analysis. In case of doubt to include any study in the second screening, the full article was reviewed. Two independent authors carried out the search and selection of relevant studies for the present review. Disagreement was resolved by discussion.

2.4. Data Extraction. The data extracted included the reference, type of study, total number of subjects, number of subjects per treatment condition, brief description of the subjects, and the inclusion criteria. Regarding the intervention, information about the comparison group, type of aromatherapy, duration of the study, frequency of the treatment, outcome measures, and conclusion were extracted from the selected studies.

2.5. Quality Assessment. The quality of the studies included was assessed using the Jadad scale whose rating criteria take into account randomization, double blinding, withdrawals, and dropouts [54]. The scoring range in the Jadad scale goes from 0 to 5 in which a higher score represents higher quality of the study.

3. Results

3.1. Description of the Study Selection Scheme. The combined database search was carried out from inception to May 2016 and resulted in 875 studies identified using the predefined search terms (Figure 1). After removal of duplicates (n = 207), the title of 668 studies was screened. Most of the studies excluded were not concerned with depression and/or aromatherapy (n = 552). In addition, 84 studies were excluded because they were not RCTs, they were not in English, and/or no depressive symptoms were measured. After title screening, 32 studies remained for further full text screening. A total number of 20 studies were excluded at this stage, 7 studies did not assess depressive symptoms, 1 study was a commentary, 6 were not RCTs, 1 study assessed colognes which are not essential oils, and 5 studies could not be accessed. From the 5 studies that could not be accessed, 1 study was not detected as duplicate before since it appeared with a short title in the database search; therefore, only 4 studies could not be accessed. The abstract of 2 of those studies was available while no abstract was available of the other 2 studies. The authors of those 4 studies were contacted via email requesting them the full studies. We could not get access to four studies whose title suggested that the studies could be included in the systematic review. However, the studies were nor provided by the author due to the following reasons: the study was still unpublished, the authors did not reply, or the author could not be reached. Therefore, those studies were excluded. A total number of 12 RCTs [18–29] that met the inclusion and exclusion criteria were selected.

3.2. Description of the Subjects. Detailed information on the subjects is stated in Table 2. The total number of subjects from all the studies was 1226 from which 984 were female (80.3%) and 224 (18.3%) were male participants. The study conducted by Lemon [23] did not specify the number of female and male subjects in the control group; therefore the gender of 18 subjects (1.4%) was not taken into account in the above mentioned percentages. The mean subject age included in the studies was 47 with mean average age range of 21–73. The participants included in the studies selected are people with cancer (n = 682), pregnant women (n = 333), women in menopause phase (n = 90), patients diagnosed with depression and/or anxiety (n = 32), postpartum women (n = 28), women whose children were diagnosed with attention deficit/hyperactivity disorder (n = 25), healthy female volunteers (n = 20), and patients diagnosed with idiopathic environmental intolerance (n = 16).

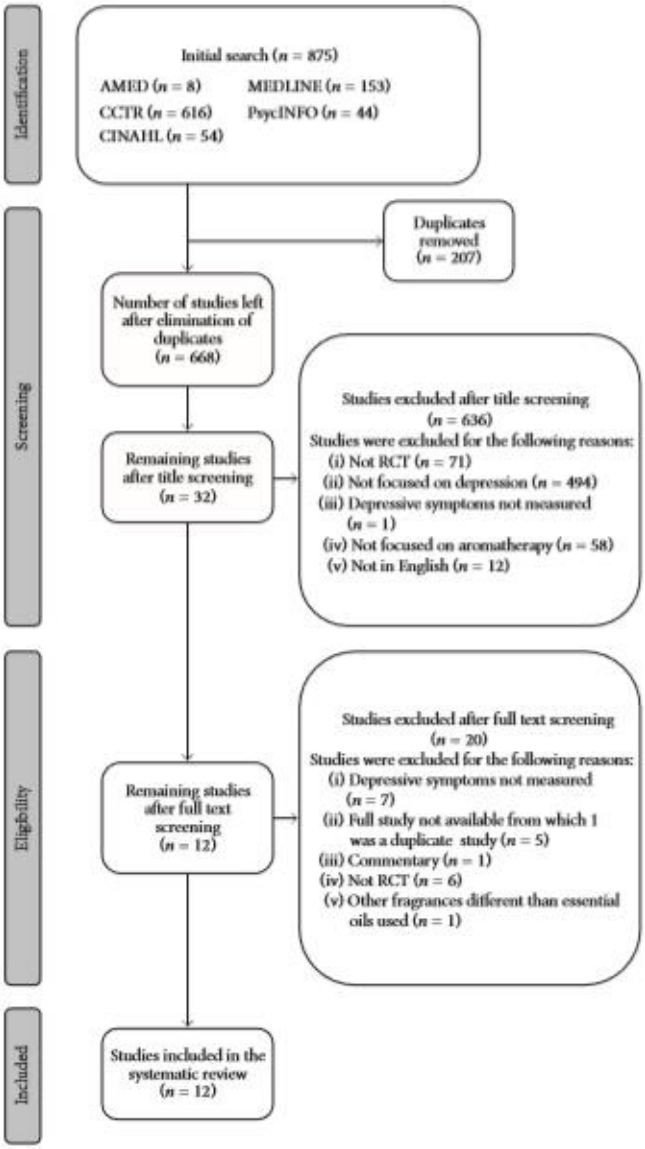


FIGURE 1: Study selection flowchart.

3.3. Intervention

3.3.1. Control Group. The comparison groups used in the studies included no intervention group ($n = 6$) [20, 22, 24, 26, 28, 29], vehicle group ($n = 4$, received vehicle such as carrier oil or water) [18, 19, 21, 23], and active control group ($n = 2$; usual supportive care and cognitive behavior therapy, well known treatments with positive effect on the outcome measures) [25, 27].

3.3.2. Intervention Group. Two administration methods for aromatherapy identified in the studies selected include aromatherapy via inhalation (inhalation aromatherapy, $n = 5$) [18–22] and aromatherapy with massage (aromatherapy massage, $n = 7$ plus 1 study from Conrad and Adams, 2012, which also used inhalation aromatherapy) [19, 23–29]. No RCT study included involved aromatherapy administered orally. Details of the intervention adopted in the 12 included studies were summarized in Table 3.

Table 2: Characteristics of the participants included in the selected studies.

Ref.	Type of study	Total number of subjects	Subjects			Diagnostic systems/inclusion criteria	Baseline score for depressive symptoms
			Mean subject age (range)	Gender (*n*)	Type of subject		
Inhalation aromatherapy							
[18]	Placebo-controlled randomized double blind RCT	313	65 (33–90)	Female (150) Male (163)	Individuals with cancer receiving radiotherapy treatment	Patients prescribed with 8 or more fractions of radiotherapy	Baseline depression status: odds ratio of 29 using HADS.
[19]*	Randomized observational pilot study with repeated measures	28	32 (25–43)	Female (28)	Postpartum women	0–18-month postpartum women with scores of 10 or higher on either the Edinburgh Postnatal Depression Scale or the Generalized Anxiety Disorder Scale	The baseline score using the Edinburgh Postnatal Depression Scale for the control group was 15.9 and 16.1 for the intervention group.
[20]	Prospective RCT	13	27.3 for the control group (NA) 29.3 for the treatment group (NA)	Female (13)	Pregnant women	28-week-pregnant women, singleton pregnancy	Depression-dejection scale baseline score using POMS was 2.7 in the control group and 1.6 in the treatment group.
[21]	Randomized controlled crossover study	20	20.5	Female (20)	College students	Healthy volunteers	The depression-dejection scale baseline score using POMS was not provided, but the change difference between pre- and posttreatment was reported. The change in depression-dejection score was lower than −1 in the treatment group and statistically significant when compared to the change in the control group.
[22]	Controlled double-blinded RCT	320	20–30, average age NA	Female (320)	Pregnant women	Women between 18–35 years, with a pregnancy age between 38 and 42 weeks, a score of 12 or less in the Edinburgh test	Depression grade baseline in the Edinburgh test was 6.3 in the control group and 6.1 in the intervention group.

Table 2: Continued.

Ref.	Type of study	Total number of subjects	Mean subject age (range)	Gender (n)	Subjects Type of subject	Diagnostic systems/inclusion criteria	Baseline score for depressive symptoms
Aromatherapy massage							
[23]	RCT	32	32.9 (23–53) in the treatment group	Female (10 in the treatment group) Male (4 in the treatment group) No information provided on the number of female and male subjects in the control group	Patients with depression and/or anxiety	Patients scoring more than 7 in the Montgomery-Asberg Depression Rating Scale and/or the Tyrer Brief Anxiety Scale	The baseline using the Montgomery-Asberg Depression Rating Scale was 19.8 in the control group and 30 in the treatment group. The baseline using the HADS was 14.6 and 15.3 in the control and treatment group, respectively.
[24]	Double blind RCT	42	73, (44–85)	Female (32), male (10)	Individuals with cancer	Individuals with a wide variety of levels of physical and psychological symptoms	Baseline score using HADS was not stated. Only the median change in HADS was provided being 0 for the aromatherapy group, −1.5 for the massage group, −0.5 for the aromatherapy massage group, and 0.5 for the control.
[25]	RCT	288	52.1; 52.8 for the usual care group; and 51.5 for the usual care plus aromatherapy group	Female (250), male (38)	Individuals with cancer	Patients diagnosed with cancer, a prognosis of more than 3 months, with clinical anxiety or depression	The baseline score using the Center for Epidemiological Studies Depression Scale was 26.1 for the aromatherapy group and 26 for the group receiving usual care (control).
[26]	Nonblinded randomized crossover trial	16	46.1 (37.9–54.3)	Female (15), male (1)	Patients diagnosed with idiopathic environmental intolerance	Clinical examination by a physician and scoring above 26 for men and 30 for women in the Chemical Odor Sensitivity Scale	Depression subscale baseline score using POMS was around 2.8 in the control period.
[19]*	Randomized observational pilot study with repeated measures	28	NA	Female (28)	Postpartum women	0–18-month postpartum women with scores of 10 or higher on either the Edinburgh Postnatal Depression Scale or the Generalized Anxiety Disorder Scale	The baseline score using the Edinburgh Postnatal Depression Scale for the control group was 15.9 and 16.1 for the intervention group.

Table 2: Continued.

Ref.	Type of study	Subjects: Total number of subjects	Subjects: Mean subject age (range)	Subjects: Gender (*n*)	Subjects: Type of subject	Diagnostic systems/inclusion criteria	Baseline score for depressive symptoms
[27]	Single blind RCT	39	52.5; 51.1 for the aromatherapy group; and 54 for the cognitive behavior therapy group	Female (31), male (8)	Individuals with cancer	Patients diagnosed for at least one month, who also had at least a predicted survival of 6 months and score 11 or more in the HADS for anxiety or depression	The baseline score in the depression-dejection subscale of POMS was 11.2 for the aromatherapy massage group and 13.4 for the control group.
[28]	RCT	90	53.70 for the control group (49.42–57.98), 52 (47.12–56.88) for the massage therapy group, and 53.35 (49.01–57.69)	Female (90)	Women who entered their menopausal period naturally	Woman, age between 45 and 60 years, with amenorrhea for at least 1 year	At baseline, according to the Menopause Rating Scale, the frequency of the severity of the depressive mood was reported as mild (14.9%), moderate (36.8%), severe (20.7%), and very severe (2.3%). No difference was found among the groups at baseline.
[29]	RCT	25	34–48, average age NA	Female (25)	Women with children	Women whose children were diagnosed with attention deficit hyperactivity disorder	Baseline using the Beck Depression Inventory was 8.6 in the control group and 10.8 in the treatment group.

*In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. NA, not available; HADS, Hospital Anxiety and Depression Scale; POMS, Profile of Mood States.

In the study carried out by Sehhatie et al. [22], a combination of nonpharmacological interventions for pain relief in labor, including aromatherapy, was used in the intervention group. The contribution of aromatherapy in the combined intervention cannot be discriminated in this study. Therefore, caution should be taken when discussing the results of this study.

3.4. Selection of Essential Oils. Essential oils were mainly used pure, diluted, or in a mixture of 2 or more essential oils at a particular ratio. The selection of the essential oils used was determined by the aromatherapist, the effect on physical and physiological states, subject's preference, or safety for use during pregnancy while other studies did not mention in the methodology section the rationale behind the essential oils chosen nor specify the type of essential oils used. The most commonly used essential oils were lavender in 8 studies [18–20, 22–24, 28, 29].

3.4.1. Inhalation Aromatherapy. The essential oils that were more commonly used in the inhalation aromatherapy studies were lavender and bergamot either as a single essential oil or in a mixture with other essential oils [18–20, 22–24, 28, 29]. One mixture of fractionated essential oils was used, but the type of essential oils contained in the mixture was not specified [18]. In addition, the purity of the fractionated mixture was unknown. Other essential oils utilized were petitgrain [20] and Yuzu [21] essential oil alone while cedarwood [18] and rose otto [19] were used in combination with a mixture of lavender and bergamot, and lavender, respectively.

3.4.2. Aromatherapy Massage. A set of 20 different essential oil options were used in two of the studies from which the therapists chose the most suitable essential oil for each one of the subjects [25, 27]. However, the type of essential oils used provided to the participants was not specified in those studies. On the other hand, in the study conducted by Lemon [23], the essential oils used were selected from a list of 9 options and the author specified the type of essential oil used in each subject of the intervention group. The essential oils used in the other studies comprise lavender, a mixture of 2–4 different essential oils, and rose otto combined with lavender.

3.5. Administration Protocol

3.5.1. Inhalation Aromatherapy. The method of administration of inhalation aromatherapy also differed among the studies [18–22]. The main differences in the administration methods rely on the distance between the aroma source and the subject's nose. In one study, cotton impregnated with essential oil placed in a diffuser was set in the nostrils of the subjects [21]. In the other two studies the source of aroma was placed approximately 30 cm away from the nose of the subjects [18, 20]. In other two studies, the essential oil was applied to a bib or to a cloth that were worn by the participants [18, 22]. The volume of essential oil used in the inhalation aromatherapy studies varies from 10 μL to 1 mL or 3, 5, or 8 drops. The exposure time to the aroma ranged from 5 to 20 minutes, with the number of sessions from 1 to 56 sessions [18–21] (Table 3). In one study, the total duration of the exposure to the aroma was not specified since the intervention was carried out during the active phase of the labor process that pregnant women underwent [22]. Furthermore, the frequency of the treatment in the 5 studies differs greatly. For example, the frequency of treatment in the studies varies from once [20, 22], twice [21], and twice a week [19] to daily [18]. The duration of the treatment in the inhalation aromatherapy studies allowed the evaluation of acute and long term effect due to the duration of the treatment from 1-2 days to 4–8 weeks, respectively.

3.5.2. Aromatherapy Massage. The types of massage are performed with standardized protocols [24–27] in which 3 of the studies [25–27] did not describe the areas of the body for the delivery of the massage while another study specified the target body areas to deliver the massage such as back massage [24]. Other massage target areas were also described in three studies [19, 28, 29]. Taavoni et al. [28] focused the massage on the abdomen, thighs, and arms while Wu et al. [29] focused on the neck, shoulders, arms, back, and legs with effleurage, friction, petrissage, and vibration at a moderate pressure. In the study of Conrad and Adams [19], the essential oil mixture was applied on both hands (hand aromatherapy massage) using the well m'technique which involves gently stroking movements applied in a set sequence with structured strokes, sequence, number, and pressure [45]. The duration of the studies was 4, 8, 10, and 12 weeks and 2 years. Weekly sessions were carried out in most of the studies [19, 24, 25, 27–29]; only in one study the frequency was once every 2 weeks [26]. The number of sessions per week also varied from once or twice weekly to once every 2 weeks. The duration of the treatments was 15, 30 min, and 40 min to 1 h and the total number of sessions varied from 4 and 6 to 8 sessions.

3.6. Outcome Measures. A summary of the outcome measures is shown in Table 4. The most frequently used instruments were HADS and POMS [18, 20, 21, 24, 26, 27] followed by EPDS which was used in 2 studies [19, 22]. Other assessment tools include the MADRS [23], MRD [28], BDI [29], and CES-D [25].

3.7. Efficacy of Aromatherapy

3.7.1. Inhalation Aromatherapy. Two out of 5 studies evaluating the effect of inhalation aromatherapy reported beneficial effects to improve depressive symptoms in the subjects [19, 21]. The subjects in the study of Conrad and Adams [19] were postpartum women exposed to two different aromatherapy interventions, inhalation aromatherapy and topic application of aromatherapy, for 8 sessions. At baseline, control and treatment groups showed similar levels of depressive symptoms (EPDS: $p = 0.8$). At the end of the study, the treatment group with aromatherapy showed a significant reduction of depressive symptoms (EPDS: $p = 0.01$), but the improvement was lesser than using m'technique. The study conducted by Matsumoto et al. [21] showed improvements in negative emotional stress after 2 sessions of 10 min on healthy volunteers. The TMD score ($p < 0.001$) and the

Table 3: Description of the interventions and protocols used in the selected studies.

Reference	Comparison group (*n*)	Treatment group(s) (*n*)	Intervention and protocol			Treatment frequency	Duration per session	Total number of sessions
			Type of essential oil used	Duration of the study	Administration method			
Inhalation aromatherapy								
[18]	Control with sweet almond cold-pressed pure vegetable oil with no fragrance (NA)	(i) Carrier oil with fractionated low (NA) grade essential oil (ii) Pure essential oil (NA)	(i) Fractionated oils of unknown purity diluted 1 : 3 in carrier oil (ii) Mixture of lavender, bergamot, and cedarwood (2 : 1 : 1)	8 weeks	3 drops of oil applied to a bib worn during the administration of the treatment	Daily	15–20 min	56
[19]*	Control, jojoba oil (14)	(i) 2% dilution of a mixture of essential oils (6)	(i) 0.25 rose otto essential oil and 0.75 lavender, 2% dilution of the essential oil mixture	4 weeks	8 drops of oil applied to a cotton pad. Subjects were instructed to smell the cotton pad for 15 min	Twice a week	15 min	8
[20]	Control, no intervention (6)	(i) Pure essential oil (7)	(i) Lavender (ii) Petitgrain (iii) Bergamot	1 day	5 drops of oil applied on a filter placed in a diffuser	Once	5 min	1
[21]	Control, water (20)	(i) Pure essential oil (20)	(i) Yuzu	2 days	10 *μ*L oil in a cotton pad used in a diffuser set in the subject's nostrils	Twice	10 min (sessions separated in intervals around 2.6 days)	2
[22]	Control group which did not receive any nonpharmacological method for pain relief of labor (160)	(i) Nonpharmacological methods for pain relief of labor including showering, being in upright position, aromatherapy, and soft music without words (160)	(i) 20% lavender essential oil	During labor	10 × 10 cm cloth impregnated with 1 mL 20% lavender essential oil which was attached to the mother's breast at the beginning of the active phase. The aromatherapy intervention was combined with other nonpharmacological interventions	Once	Duration of the active phase of labor	1

Table 3: Continued.

Reference	Comparison group (n)	Treatment group(s) (n)	Intervention and protocol		Administration method	Treatment frequency	Duration per session	Total number of sessions
			Type of essential oil used	Duration of the study				
Aromatherapy massage								
[23]	Control, grape seed oil (16)	(i) Diluted essential oil (16)	(i) 9 essential oils (bergamot, lemon clary sage, lavender, roman chamomile, geranium, rose otto, sandalwood, and jasmine). A combination of essential oils chosen by the aromatherapist on each treatment session (16)	12 weeks	15 mL grape seed carrier oil with (4 drops) or without essential oils applied in a full body massage using gentle effleurage and petrissage	Once a fortnight	40 min	6
[24]	Control, no intervention (13)	(i) Aromatherapy massage (16) (ii) Massage with inert carrier oil (13)	(i) 1% lavender essential oil diluted in sweet almond oil	2 years	Back massage	Weekly	30 min	4
[25]	Usual supportive care	(i) Usual supportive care and aromatherapy massage	(i) 20 essential oils	10 weeks	Standardized massage agreed by the therapists	Weekly	1 h	4
[26]	Control, no intervention	(i) Aromatherapy massage	(i) 1% massage oil containing melissa, juniper, and rosemary essential oils mixed into jojoba oil (1 : 2 : 2 ratio)	8 weeks	Standardized massage on the back, shoulders, arms, hands, lower legs, and feet using 20–30 mL massage oil	Every two weeks	1 h	4
[19]*	Control, essential oil blend unscented white lotion (14)	(i) 2% dilution of a mixture of essential oils (8)	(i) 0.25 rose otto essential oil and 0.75 lavender, 2% dilution of the essential oil mixture	4 weeks	Topic application of the oil or lotion on both hands with gentle strokes of homogeneous pressure and speed	Twice a week	15 min	8
[27]	Cognitive behavior therapy (19)	(i) Aromatherapy massage (20)	(i) 20 essential oils	2 years	Standardized massage combined with treatment as usual (routine support)	Weekly	1 h	Up to 8 sessions in 10 weeks
[28]	Control, no intervention (30)	(i) Aromatherapy massage (30) (ii) Massage (30)	(i) 3% oil mixture containing lavender, geranium, rose, and rosemary (4 : 2 : 1 : 1 ratio) in almond and evening primrose oil	4 weeks	Massage in the abdomen, tights, and arms using massage oil containing essential oils or odorless liquid petrolatum. Massage was applied with clockwise circular movements and light pressure	Twice a week	30 min	8

Table 3: Continued.

Reference	Comparison group (n)	Treatment group(s) (n)	Intervention and protocol			Treatment frequency	Duration per session	Total number of sessions
			Type of essential oil used	Duration of the study	Administration method			
[29]	Control, no intervention (12)	(i) Aromatherapy massage (13)	(i) Jojoba oil containing 2% lavender and 2% geranium essential oils	4 weeks	Massage on the neck, shoulders, arms, back, and legs including effleurage, friction, petrissage, and vibration at a moderate pressure using 20 mL of massage oil	Twice per week	40 min	8

*In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. NA, not available; min, minutes; h, hour.

Table 4: Description of the measurement tools, outcomes, and conclusions.

Reference	Outcome measures				Improvement of depressive symptoms
	Scale	Comparison group	Intervention group(s)	Outcome	
Inhalation aromatherapy					
[18]	(i) HADS	Control group	(i) Carrier oil with fractionated low grade essential oil (ii) Pure essential oil	Increased outcome measurement when compared to the baseline (18%–22% in HADS) using multivariate analysis. No statistically significant difference observed.	No
[19]*	(i) EPDS	Control group	(i) 2% dilution of a mixture of essential oils	The mean difference in EPDS scores between the control group and the intervention group at end point was −3.981 but no statistically significant difference was observed. Combined analysis of inhalation aromatherapy and massage aromatherapy showed statistically significant difference with a mean difference of −4.8.	Yes
[20]	(i) POMS	No intervention	(i) Pure essential oil	Depression-dejection subscale scores before and after test were 2.7 and 1.2 in the comparison group and 1.6 and 0.6 in the intervention group, respectively. No statistically significant difference was observed.	No
[21]	(i) POMS in TMD	Control group	(i) Pure essential oil	Change in TMD score was 0.5 ± 2.2 in the control group and −1.28 ± 2.6 in the intervention group (statistically significant difference).	Yes
[22]	(i) EPDS	No intervention	(i) Nonpharmacological methods for pain relief of labor including showering, being in upright position, aromatherapy, and soft music without words	Depression grades (0–30) before and after delivery were 6.3 and 8.8 in the no intervention group and 6.1 and 7.8 in the intervention group, respectively. No statistically significant difference was observed.	No

Table 4: Continued.

Reference	Outcome measures			Improvement of depressive symptoms
	Scale	Comparison group	Intervention group(s)	Outcome
Aromatherapy massage				
[23]	(i) MADRS	Control group	(i) Diluted essential oil	The scores in the MADRS at baseline and end point were 19.8 and 21.1 in the control group and 30 and 18.1 in the treatment group. Statistically significant difference was observed between the test and control group.	Yes
[24]	(i) HADS	No intervention	(i) Aromatherapy massage (ii) Massage with inert carrier oil	The median change in HADS at baseline and end point was 0.5 in the no intervention group, 0 in the aromatherapy massage group, and −1.5 in the massage group. No statistically significant difference among the groups.	No
[25]	(i) CES-D	Active control (usual supportive care)	(i) Usual supportive care and aromatherapy massage	The scores using CES-D at baseline and end point were 26.0 and 4.6 in the active control group and 25.9 and 6.2 in the intervention group, respectively. No statistically significant difference observed between the 2 groups.	No
[26]	(i) POMS	No intervention	(i) Aromatherapy massage	Statistically significant difference between the 2 groups when comparing the pre- and postsessions in all the POMS subscales including depression-dejection.	Yes
[19]*	(i) EPDS	Control group	(i) m'technique (hand massage)	The mean difference between the baseline and end point using EPDS was −6.031.	Yes
[27]	(i) POMS-TMS	Active control (cognitive behavior therapy)	(i) Aromatherapy massage	The POMS-TMS decreased in both groups after intervention from 46.3 to 26.5 in the active control group and 44.5 to 29 in the intervention group.	Yes
[28]	(i) MRS	No intervention	(i) Aromatherapy massage (ii) Massage	The mean difference in psychological symptoms (including depressive mood) was −0.379 in the no intervention group (no statistically significant difference), −3.49 in the aromatherapy massage group (statistically significant difference), and −1.20 in the massage group (statistically significant difference).	Yes

Table 4: Continued.

Reference	Outcome measures: Scale	Comparison group	Intervention group(s)	Outcome	Improvement of depressive symptoms
[29]	(1) BDI	No intervention	(1) Aromatherapy massage	The BDI score before and after test was 8.6 and 8.5 in the control group and 10.8 and 6.5 in the intervention group (statistically significant difference).	Yes

*In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. CES-D, Center of Epidemiological Studies Depression (self-reported depression); BDI, Beck Depression Inventory; DSM-IV, modified Diagnostic and Statistical Manual of Mental Disorder criteria; EPDS, Edinburgh Postnatal Depression Scale; HADS, Hospital Anxiety and Depression Scale; MADRS, Montgomery-Asberg Depression Rating Scale; MRS, Menopause Rating Scale; POMS, Profile of Mood States; TMD, Total Mood Disturbance; TMS, Total Mood Score (shortened version of the profile of mood states).

depression-dejection ($p = 0.041$), tension-anxiety ($p < 0.018$), anger-hostility ($p = 0.002$), and confusion ($p = 0.019$) subscores decreased significantly after inhalation of yuzu, respectively.

The other 3 studies in which no beneficial effect of inhalation aromatherapy was observed included Graham et al. [18], Igarashi [20], and Sehhatie et al. [22]. According to the study conducted by Graham et al. [18], the percentage of individuals with cancer showed an increase in the outcome measurement when compared to the initial baseline, from 18 to 22% using HADS. Graham et al. [18] concluded that inhalation aromatherapy did not reduce the levels of depressive symptoms in people with cancer undergoing radiotherapy and receiving inhalation aromatherapy.

Two studies evaluated the acute effect of inhalation aromatherapy on pregnant women, but no statistically significant difference (p value not given) was observed [20, 22]. In the study by Igarashi [20], inhalation aromatherapy showed no decrease in the depression-dejection mood state in the intergroup comparison in the POMS scale. The acute effect of inhalation aromatherapy assessed in the study carried out by Igarashi [20] showed an improvement in profile of mood states among the subjects. However, no difference was found when comparing the scores of the inhalation aromatherapy group and the control group. On the other hand, tension-anxiety and anger-hostility mood states showed a significant decrease ($p < 0.05$; $p < 0.05$, resp.) in the aromatherapy group [20]. Aromatherapy showed to be effective in POMPS in intragroup comparison, but no difference was observed when comparing the groups in pregnant women.

Sehhartie et al. [22] found no statistically significant difference ($p = 0.610$) in the reduction of the degree of postpartum depression between the control and the intervention group. They concluded that nonpharmacological interventions, including inhalation aromatherapy, for pain relief in labor did not reduce the degree of postpartum depression.

3.7.2. Aromatherapy Massage. Five out of 8 studies showed positive effects of aromatherapy when the intervention was carried out in combination with massage [23, 26–29]. In the study of Lemon [23], the assessment of depressive symptoms using MDRS showed slight improvement in the control group which was expected and attributed to the effect of massage alone. On the other hand, statistically significant difference was observed in the treatment group when comparing the baseline and end point scores. Lemon [23] concluded that aromatherapy massage had beneficial effect on subjects who showed mild or higher degree of depressive symptoms. Araki et al. [26] observed a statistically significant difference ($p < 0.001$) in the depression subscale of the POMS when comparing the baseline and end point scores. Although aromatherapy did not show to have any beneficial effect for the management of idiopathic environmental intolerance, the data reported showed that aromatherapy massage improved all the subscales of the POMS including depression. Conrad and Adams [19] who evaluated the effect of the application of aromatherapy on both hands in addition to the effect of inhalation aromatherapy showed a significant improvement (EPDS, $p = 0.025$) in depressive symptoms which was superior to the improvement observed when using inhalation aromatherapy. At the end of the study, a significant difference (EPDS, $p = 0.003$) was observed between the control and treatment group. Inhalation aromatherapy showed an improvement in the reduction of depressive symptoms but only when the data was presented as a combination of aromatherapy intervention including inhalation aromatherapy and aromatherapy massage. Therefore, caution should be taken when discussing these results. Another study that showed beneficial effects of aromatherapy massage was the study of Serfaty et al. [27]. The depression-dejection and tension-anxiety scores were similar in both interventions using the POMS-TMS. Aromatherapy massage showed to be advantageous in terms of general feeling in the last week and the cognitive behavior therapy seems to be more effective and the effect is sustained for a longer period according to the POMS-TMS and subscales scores. Serfaty et al. [27] concluded that both interventions showed the improvements and the effects of cognitive behavior therapy on depression showed to be sustained over time. Taavoni et al. [28] measured a series of psychological symptoms including depressive mood using the MRS. A statistically significant difference ($p < 0.001$) was found among the three groups evaluated. No difference was found between the pre- and posttest in the control group (Table 4). However, both massage and aromatherapy massage showed an improvement in the outcome measures. The conclusion of this study was that massage aromatherapy was more effective than massage to reduce the psychological scores. Finally, the study conducted by Wu et al. [29] showed a statistically significant difference ($p = 0.04$) in the treatment group when comparing the pre- and posttest depression assessment scores using the BDI while no significant difference (no p value given) was observed in the control group. Wu et al. [29] concluded that aromatherapy massage improves depressive symptoms.

The studies on aromatherapy massage that showed no beneficial effect of the intervention include Soden et al. [24] and Wilkinson et al. [25]. Soden et al. [24] did not find any significant difference in HADS among the control and aromatherapy massage groups. Similarly, Wilkinson et al. [25] reported no statistically significant difference in the levels of depression in people with cancer receiving aromatherapy massage combined with the usual supportive care intervention when compared with the usual supportive care intervention alone. Improvement in clinical depression was observed in 63% of the patients, but no difference in improvement was observed when comparing the two treatment groups. No significant difference in the self-reported depression was observed between the two groups. Wilkinson et al. [55] concluded that individuals with cancer receiving aromatherapy massage did not experience the reduction in the level of depressive symptoms.

3.8. Quality Assessment. Quality assessment of the included studies was graded by Jadad scale (Table 5). Jadad scale is a scoring system that assesses the quality of methodology of randomized controlled trials (RCTs). Jadad scale ranges from 0 to 5, with 5 comprising description of randomization

Table 5: Quality assessment of studies included according to the Jadad scale.

Reference	Quality assessment of methodology based on Jadad scale							
	Randomization	Appropriate method of randomization and description	Blinding			Appropriate method of double blinding and description	Description of dropouts/withdrawals	Jadad score (score out of 5)
			No blinding	Single blind	Double blind			
Inhalation aromatherapy								
[18]	Yes	Yes	No	No	Yes	Yes	No	4
[19]*	Yes	No	Yes	No	No	NA	Yes	2
[20]	Yes	Yes	Yes	No	No	NA	Yes	3
[21]	Yes	No	No	Yes	No	No	No	1
[22]	Yes	Yes	No	No	Yes	No	Yes	4
Aromatherapy massage								
[23]	Yes	No	Yes	No	No	NA	No	1
[24]	Yes	Yes	No	No	Yes	Yes	Yes	5
[25]	Yes	Yes	No	No	Yes	No	Yes	3
[26]	Yes	Yes	Yes	No	No	NA	NA	2
[19]*	Yes	No	Yes	No	No	NA	Yes	2
[27]	Yes	Yes	No	Yes	No	No	Yes	3
[28]	Yes	Yes	Yes	No	No	No	No	2
[29]	Yes	No	Yes	No	No	NA	No	1

*In this study, both aromatherapy modalities were tested, inhalation aromatherapy and aromatherapy massage. Therefore, the study was included in both categories in the table. NA, not applicable.

(2 points), double blinding (2 points), and withdrawals and it is a frequently used tool for assessing the risk of bias in the studies in which low scores are associated with high effect estimates [54, 56]. Out of 12 studies, one study was rated 5 [24], two studies were rated 4 [18, 22], three studies were rated 3 [20, 25, 27], three studies were rated 2 [19, 26, 28], and three studies were rated 1 [21, 23, 29]. All the studies included were RCTs. Among 12 articles, 4 studies did not describe the method of randomization [19, 21, 23, 29]. In 6 studies, no blinding was used [19, 20, 23, 26, 28, 29]. Single blinding was used in 2 studies [21, 27]. Three studies were double blind studies [18, 22, 24, 25] and the description and appropriate double blinding procedure was provided in 2 studies [18, 24]. Proper description of dropouts and withdrawals was provided in 6 studies [19, 20, 22, 24, 25, 27].

4. Discussion

The objective of this systemic review was to analyze the clinical evidences on the efficacy of aromatherapy for depressive symptoms and to update the previously published systematic review by Yim et al. [2].

4.1. Comparison with the Previous Systematic Review. The objective of this systemic review was to analyze the clinical evidences on the efficacy of aromatherapy for depressive symptoms. Regarding the comparison between the previously published systematic review on the effectiveness of aromatherapy on patients with depressive symptoms and the present systematic review, an updated detailed analysis of the evidence on the topic is provided in the present analysis. In the limitations of the systematic review, the authors mentioned that the sample size of the studies selected was small and they highlighted the only 2 RCTs were included. From the date of publication of the systematic review from Yim et al. in 2009 [2] to the date that the present systematic review was carried out, a higher number of RCTs have been published and some of the limitations described by Yim et al. [2] were addressed in the present systematic review as discussed below. The difference in the number of RCTs included in both systematic reviews suggests that in recent years the need for high quality clinical evaluation of the effectiveness of aromatherapy has been of increasing interest.

Although the search terms used in both systematic reviews were similar, our main focus was on RCTs. Two studies that fulfilled the inclusion and exclusion criteria established in the present systematic review were also included in the previous systematic review carried out by Yim et al. [2]. Those 2 studies selected in both systematic reviews are Soden et al. [24] and Wilkinson et al. [25]. One limitation reported by Yim et al. [2] was the small sample size used in the studies included. However, the sample size of the studies included in the present systematic review was larger.

4.2. Effectiveness of Aromatherapy to Relieve Depressive Symptoms. The systematic review included 12 studies with a diverse type of subjects such as pregnant women, postpartum women, women in menopause phase, women with

children diagnosed with attention deficit hyperactivity disorder (ADHD), healthy female volunteers, patients diagnosed with cancer, depression/anxiety, and idiopathic environmental intolerance. Discrepancy on the effectiveness of aromatherapy in both inhalation aromatherapy and aromatherapy massage was found. The mixed results could be related to the differences in the administration protocol or the diverse type of subjects included in the RCTs. In addition, caution should be taken when aromatherapy is administered by inhalation since proper olfactory function should be confirmed before starting the trial and the degree of the beneficial effect of aromatherapy when combined with massage should be properly studied to evaluate whether the added effect of aromatherapy is large enough to surpass the effect of massage alone.

Another important aspect to take into account to analyze the effectiveness of aromatherapy is the chemical nature of the different essential oils used in the studies. The chemical composition and mechanism of action of the essential oils used have shown beneficial effects on mood parameters such as anxiety, depression, and sedation which support their use in the clinical studies included [42, 57–59]. For instance, lavender [57], bergamot [58], and sandalwood [42] have shown to improve depressive symptoms while yuzu alleviates negative emotional stress [59]. The rest of the essential oils used contain chemicals such as limonene, linalool, and linalyl acetate that have been widely studied and have showed anxiolytic and sedative properties. On the other hand, 2 studies [25, 27] used a set of 20 essential oils but the type of essential oils used was not stated. Therefore, it is not possible to make an analysis on the nature of the essential oils used in those studies. Most of the studies used single, mixed, or diluted essential oils. Also, all the essential oils used, except the ones not specified in 2 studies, have shown to have anxiolytic, antidepressant, and sedative properties as stated above. Due to the differences in administration method, duration of the treatment session, frequency of the treatment, total number of sessions, and forms of essential oils (i.e., single, mixed, or diluted form), it is complicated to make a comparison of the treatment efficacy across different studies only taking into account the type of essential oil.

4.2.1. Inhalation Aromatherapy. In the present systematic review, 5 out of 12 studies used inhalation therapy as a modality of aromatherapy. However, only the studies carried out by Matsumoto et al. [21] and Conrad and Adams [19] found beneficial effects of the essential oil yuzu and a mixture of rose otto and lavender, respectively, to relieve negative emotional stress and depressive symptoms.

Inhalation aromatherapy given to people with cancer showed no effect although the number of sessions used was high (56 sessions). The lack of efficacy could be due to the quality of the essential oils used since Graham et al. [18] reported that one intervention group was administered with unknown purity essential oils and the other group was administered with a mixture of 3 pure essential oils at different concentration ratio.

Two studies carried out on pregnant women were also included. The results from the study carried out by Igarashi [20] showed acute beneficial effect of inhalation aromatherapy to decrease depressive symptoms in pregnant women. However, the difference observed was the result of within group comparison. Furthermore, the duration of the treatment and frequency of the aromatherapy intervention in pregnant women were too short to allow a full evaluation of the effectiveness of the intervention. Another study carried out on pregnant women did not show any significant benefit from inhalation aromatherapy intervention [22]. Both studies on pregnant women only used one session of inhalation aromatherapy of 5 min in one study [20] and unknown exposure time in the other study [22]. Therefore, the lack of effectiveness reported could be associated with the short intervention exposure time in the treatment groups.

Another population in the present systematic review was postpartum women including a group receiving inhalation aromatherapy and another group receiving aromatherapy massage applied to both hands [19]. This study showed improvement in depressive symptoms of both interventions. However, the authors combined the data of both interventions to make the statistical analysis and concluded that both aromatherapy interventions together were effective. The data presented only supported the effectiveness of aromatherapy massage but not that of inhalation aromatherapy. However, no group receiving only hand massage without aromatherapy was included to assess the contribution of the hand massage alone on the effectiveness of the intervention. Hence, it is not possible to conclude that the effect was attributed to aromatherapy massage.

In the inhalation aromatherapy intervention on healthy volunteers, beneficial effect was observed. When comparing the protocols of studies with positive findings with the protocols of the studies that did not show positive effect, the lack of effectiveness may be due to distance between nostrils and aroma. In the study from Matsumoto et al. [21], which shows positive effect of aromatherapy, the intervention was administered using a diffuser placed in the subject nostrils. The proximity of the source of aroma to the nasal mucous may have enhanced the interaction between the volatile compounds of the essential oil and the olfactory receptors. In addition, Matsumoto et al. [21] carried out 2 sessions of 10 minutes prior to the RCT study to make sure that the olfactory ability of the subjects had normal olfaction. The presence of a pretest and short distance between nostrils and aroma might be the reason behind the difference of efficacy observed in different studies involving inhalation aromatherapy. Both cotton impregnation and diffuser were effective methods to bring beneficial effect [49, 60, 61].

4.2.2. Aromatherapy Massage. Aromatherapy massage is another modality employed in 8 out of the 12 studies selected in which 5 studies showed positive effect of the intervention. Aromatherapy massage is a combination of aromatherapy and massage that offers the health benefits of both therapies and is commonly used by healthy individuals particularly for stress management [29]. Massage has been reported to be a popular therapy for the management of depression in which about 2.1% of the patients with severe depression undergo massage therapy for the relief of depressive symptoms [62]

and this therapy has been used in palliative care settings and individuals with cancer [29].

In aromatherapy massage, difficulties to separate the effects due to aromatherapy from the health benefits due to massage bring concerns. Both therapies alone have shown to be effective in the alleviation of psychological symptoms. It is clear that each therapy has a complex multitarget approach which leads to the effect observed. The aromatherapy massage studies included in the systematic review comprised different massage techniques and some of the important information is missing. For instance, the area of the body part to massage may not be reported [25]. Furthermore, the catalogue of massage techniques is diverse and the decision on the type of massage to be used may be elusive. Some examples of massage techniques include (1) effleurage, (2) kneading, (3) petrissage, (4) friction, (5) tapotement, and (6) vibrations and shaking [63]. The type of massage used has been linked to different physiological effect, for example, increase of the blood flow, enhancement of venous return flow, increased cardiac stroke volume, and even production of shirt-lived analgesia [63]. Therefore, the details of the massage technique used are very important to analyze the evidence of the aromatherapy massage studies. On the other hand, not much information about the massage technique was provided in these studies thereby limiting a deeper analysis of these results.

The study that showed effectiveness of the intervention [27] involved twice the number of sessions than the massage therapy protocol of the studies with no effect of the intervention [24, 25]. Based on the results reported on people with cancer, aromatherapy massage is effective compared to the active control when the number of sessions is at least 8 and administered weekly. In addition, studies carried out on the effect of massage therapy in the relief of symptoms experienced by individuals with cancer showed a positive effect of massage alone which contributed to the alleviation of the symptoms [32]. The lack of efficacy observed in the aromatherapy massage studies included in the systematic review might be due to the effect of massage itself without added beneficial effect of aromatherapy when used in combination with aromatherapy [32, 64–66]. Furthermore, caution should be taken regarding the interpretation of massage therapies since the positive effects on symptom relief in people with cancer are not compelling [67]. On the other hand, Fellowes et al. [68] reported a positive but limited effect of aromatherapy massage for alleviation of symptoms in people with cancer which is a clear example of the careful interpretation of the results that has to be done and the need of more studies to overcome the mixed evidence involving aromatherapy massage. Furthermore, Serfaty et al. [27] reported positive results in the aromatherapy massage group. However, it should be noted that aromatherapy massage was not given alone but in combination with cognitive behavior therapy. As direct effect of aromatherapy massage alone was not assessed in the study of Serfaty et al. [27], the evidence suggests that aromatherapy massage is effective when given in combination with cognitive behavior therapy.

The other two studies involving women in the menopause phase [28] and women with children diagnosed with ADHD [29] showed positive results in aromatherapy massage. Both studies used similar protocols in which the intervention was administered in a total number of 8 sessions twice a week for 30–40 minutes. Other two studies with a different subject population in which the effect of aromatherapy massage was evaluated used lower frequency of treatment (once a week) and lesser number of sessions (4) and no effect of the intervention was observed [24, 25]. When comparing the protocol settings with the rest of the aromatherapy massage studies carried out on different subject populations, it is noted that a higher number of sessions (6–8 sessions for 40 min to 1 h per session) were used in the studies in which beneficial effect of aromatherapy massage was observed [23, 27]. Only in one study [26] with low frequency of treatment (every two weeks) and 4 sessions of 1 hour per session, beneficial effects of aromatherapy massage were observed. In summary, the data presented in aromatherapy massage suggest that at least more than 4 sessions weekly or twice a week for at least 30 minutes would provide good administration settings to increase the likelihood of observing positive results in aromatherapy massage. However, it is also important to take into account other factors such as the comparison group, massage technique, and essential oils used to draw a conclusion on the effectiveness of aromatherapy massage.

In other two studies on aromatherapy massage, the subject population corresponded to patients diagnosed with depression and/or anxiety [23] and patients with idiopathic environmental intolerance [26]. Both studies showed positive results in the relief of depressive symptoms and the comparison groups were massage without aromatherapy and no intervention with 4–6 sessions of aromatherapy massage for 40 minutes to 1 hour. In the study of Lemon [23] the improvement in the depression level in the control group was attributed to the effect of massage alone. However, the aromatherapy massage group also showed positive effect on the relief of depressive symptoms. In the study carried out by Araki et al. [26], significant beneficial effect was observed in the aromatherapy massage intervention.

5. Limitations

Six out of the 12 studies included in the systematic review scored low (score of 1 or 2) in the Jadad scale. Therefore, the poor blinding in those studies could have contributed to the perceived effect of the treatment and have a positive impact on the effectiveness of the treatment. In addition, the administration protocol varied considerable among the studies. Particularly, in aromatherapy massage, the massage technique used is not fully described in some of the studies while in other studies it is briefly described. In order to analyze the contribution of the massage alone in the aromatherapy massage intervention, understanding of the benefits of the massage technique applied is crucial. However, in some studies the massage protocol is not described or no massage comparison group was used to make the discrimination of the effect between massage alone and aromatherapy massage. Also, different assessment tools were used across the studies; therefore, comparison of the evidence among the studies is difficult. Finally, differences regarding changes or

developments in the field of aromatherapy for the treatment of depressive symptoms when comparing both systematic reviews are difficult to highlight due to the diversity of the studies included in terms of subjects and intervention protocols.

6. Clinical Recommendation

When using inhalation aromatherapy, inclusion of a pretest is important to ensure that subjects have adequate olfactory function before the commencement of the treatment. Furthermore, a longer exposure time and higher number of sessions should be considered in the inhalation aromatherapy treatment since positive results were observed when a higher number of sessions and longer exposure times were used. Based on the protocols presented from the included studies, at least 8 sessions in the treatment are needed to assess the effectiveness of aromatherapy massage and beneficial effects to relieve depressive symptoms. In addition, it is suggested to apply aromatherapy massage treatment once or twice per week.

7. Conclusions

When Yim et al. [2] concluded that there was no sufficient evidence suggesting that aromatherapy could be used as complementary and alternative medicine for depression, the analysis of the evidence up to date presented in our systematic review showed otherwise. The present systematic review offers an overview of the most current evidence. Particularly, aromatherapy massage showed to be more efficacious than inhalation aromatherapy to alleviate depressive symptoms. However, inhalation aromatherapy also showed to be effective, but further studies will be needed to have more conclusive evidence on this aromatherapy modality. In the overall analysis carried out, aromatherapy showed potential to be used as an effective therapeutic option for the relief of depressive symptoms in a wide variety of subjects.

Disclosure

The funding source had no role in the preparation of the present article.

Competing Interests

The authors declare no conflict of interests.

Acknowledgments

The authors would like to acknowledge the Early Career Scheme, University General Council, Ref. 0112876, for providing the funding to carry out the work of the article.

References

[1] M. Baquero and N. Martín, "Depressive symptoms in neurodegenerative diseases," *World Journal of Clinical Cases*, vol. 3, no. 8, pp. 682–693, 2015.

[2] V. W. C. Yim, A. K. Y. Ng, H. W. H. Tsang, and A. Y. Leung, "A review on the effects of aromatherapy for patients with depressive symptoms," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 2, pp. 187–195, 2009.

[3] E. Ernst, J. L. Rand, and C. Stevinson, "Complementary therapies for depression: an overview," *Archives of General Psychiatry*, vol. 55, no. 11, pp. 1026–1032, 1998.

[4] WHO, *WHO — Sixty-Fifth World Health Assembly*, WHO, Geneva, Switzerland, 2013.

[5] WHO, *WHO—Depression*, WHO, Geneva, Switzerland, 2016.

[6] A. Bhattacharya, N. C. Derecki, T. W. Lovenberg, and W. C. Drevets, "Role of neuro-immunological factors in the pathophysiology of mood disorders," *Psychopharmacology*, vol. 233, no. 9, pp. 1623–1636, 2016.

[7] A. J. Ferrari, F. J. Charlson, R. E. Norman et al., "Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010," *PLoS Medicine*, vol. 10, no. 11, Article ID e1001547, 2013.

[8] F. Caraci, A. Copani, F. Nicoletti, and F. Drago, "Depression and Alzheimer's disease: neurobiological links and common pharmacological targets," *European Journal of Pharmacology*, vol. 626, no. 1, pp. 64–71, 2010.

[9] Y. Tizabi, "Duality of antidepressants and neuroprotectants," *Neurotoxicity Research*, vol. 30, no. 1, pp. 1–13, 2016.

[10] A. K. Wittenborn, H. Rahmandad, J. Rick, and N. Hosseinichimeh, "Depression as a systemic syndrome: mapping the feedback loops of major depressive disorder," *Psychological Medicine*, vol. 46, no. 3, pp. 551–562, 2016.

[11] P. E. Greenberg, A.-A. Fournier, T. Sisitsky, C. T. Pike, and R. C. Kessler, "The economic burden of adults with major depressive disorder in the United States (2005 and 2010)," *Journal of Clinical Psychiatry*, vol. 76, no. 2, pp. 155–162, 2015.

[12] A. Qaseem, M. J. Barry, and D. Kansagara, "Nonpharmacologic versus pharmacologic treatment of adult patients with major depressive disorder: a clinical practice guideline from the American College of Physicians," *Annals of Internal Medicine*, vol. 164, no. 5, pp. 350–359, 2016.

[13] Y.-Y. Chan, W.-Y. Lo, S.-N. Yang, Y.-H. Chen, and J.-G. Lin, "The benefit of combined acupuncture and antidepressant medication for depression: a systematic review and meta-analysis," *Journal of Affective Disorders*, vol. 176, pp. 106–117, 2015.

[14] J. E. Anderson, E. E. Michalak, and R. W. Lam, "Depression in primary care: tools for screening, diagnosis, and measuring response to treatment," *British Columbia Medical Journal*, vol. 44, no. 8, pp. 415–419, 2002.

[15] M. Hamilton, "A rating scale for depression," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 23, pp. 56–62, 1960.

[16] L. K. Kerr and L. D. Kerr, "Screening tools for depression in primary care: the effects of culture, gender, and somatic symptoms on the detection of depression," *Western Journal of Medicine*, vol. 175, no. 5, pp. 349–352, 2001.

[17] A. M. Carneiro, F. Fernandes, and R. A. Moreno, "Hamilton depression rating scale and montgomery-asberg depression rating scale in depressed and bipolar I patients: psychometric properties in a Brazilian sample," *Health and Quality of Life Outcomes*, vol. 13, no. 1, article 42, 2015.

[18] P. H. Graham, L. Browne, H. Cox, and J. Graham, "Inhalation aromatherapy during radiotherapy: results of a placebo-controlled double-blind randomized trial," *Journal of Clinical Oncology*, vol. 21, no. 12, pp. 2372–2376, 2003.

[19] P. Conrad and C. Adams, "The effects of clinical aromatherapy for anxiety and depression in the high risk postpartum woman—a pilot study," *Complementary Therapies in Clinical Practice*, vol. 18, no. 3, pp. 164–168, 2012.

[20] T. Igarashi, "Physical and psychologic effects of aromatherapy inhalation on pregnant women: a randomized controlled trial," *Journal of Alternative and Complementary Medicine*, vol. 19, no. 10, pp. 805–810, 2013.

[21] T. Matsumoto, H. Asakura, and T. Hayashi, "Effects of olfactory stimulation from the fragrance of the Japanese citrus fruit yuzu (Citrus junos Sieb. ex Tanaka) on mood states and salivary chromogranin A as an endocrinologic stress marker," *Journal of Alternative and Complementary Medicine*, vol. 20, no. 6, pp. 500–506, 2014.

[22] F. Sehhatie, M. Mirgafourvand, and Z. V. Niri, "The effect of non-pharmaceutical methods of labor pain relief on mothers' postpartum depression: a randomized controlled trial," *International Journal of Women's Health and Reproduction Sciences*, vol. 3, no. 1, pp. 48–55, 2015.

[23] K. Lemon, "An assessment of treating depression and anxiety with aromatherapy," *International Journal of Aromatherapy*, vol. 14, no. 2, pp. 63–69, 2004.

[24] K. Soden, K. Vincent, S. Craske, C. Lucas, and S. Asley, "A randomized controlled trial of aromatherapy massage in a hospice setting," *Palliative Medicine*, vol. 18, no. 2, pp. 87–92, 2004.

[25] S. M. Wilkinson, S. B. Love, A. M. Westcombe et al., "Effectiveness of aromatherapy massage in the management of anxiety and depression in patients with cancer: a multicenter randomized controlled trial," *Journal of Clinical Oncology*, vol. 25, no. 5, pp. 532–539, 2007.

[26] A. Araki, K. Watanabe, Y. Eitaki, T. Kawai, and R. Kishi, "The feasibility of aromatherapy massage to reduce symptoms of Idiopathic Environmental Intolerance: a pilot study," *Complementary Therapies in Medicine*, vol. 20, no. 6, pp. 400–408, 2012.

[27] M. Serfaty, S. Wilkinson, C. Freeman, K. Mannix, and M. King, "The ToT Study: Helping with Touch or Talk (ToT): a pilot randomised controlled trial to examine the clinical effectiveness of aromatherapy massage versus cognitive behaviour therapy for emotional distress in patients in cancer/palliative care," *Psycho-Oncology*, vol. 21, no. 5, pp. 563–569, 2012.

[28] S. Taavoni, F. Darsareh, S. Joolaee, and H. Haghani, "The effect of aromatherapy massage on the psychological symptoms of postmenopausal Iranian women," *Complementary Therapies in Medicine*, vol. 21, no. 3, pp. 158–163, 2013.

[29] J.-J. Wu, Y. Cui, Y.-S. Yang et al., "Modulatory effects of aromatherapy massage intervention on electroencephalogram, psychological assessments, salivary cortisol and plasma brain-derived neurotrophic factor," *Complementary Therapies in Medicine*, vol. 22, no. 3, pp. 456–462, 2014.

[30] C. Hiemke, "Why do antidepressant therapies have such a poor success rate?" *Expert Review of Neurotherapeutics*, vol. 16, no. 6, pp. 597–599, 2016.

[31] W.-F. Yeung, K.-F. Chung, K.-Y. Ng et al., "Prescription of Chinese herbal medicine in pattern-based traditional Chinese medicine treatment for depression: a systematic review," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 160189, 12 pages, 2015.

[32] S. Wilkinson, "Aromatherapy and massage in palliative care," *International Journal of Palliative Nursing*, vol. 1, no. 1, pp. 21–

[33] B. Arroll, S. Macgillivray, S. Ogston et al., "Efficacy and tolerability of tricyclic antidepressants and SSRIs compared with placebo for treatment of depression in primary care: a meta-analysis," *Annals of Family Medicine*, vol. 3, no. 5, pp. 449–456, 2005.

[34] M. H. Nezafati, M. Vojdanparast, and P. Nezafati, "Antidepressants and cardiovascular adverse events: a narrative review," *ARYA Atherosclerosis*, vol. 11, no. 5, pp. 295–304, 2015.

[35] N. A. Qureshi and A. M. Al-Bedah, "Mood disorders and complementary and alternative medicine: a literature review," *Neuropsychiatric Disease and Treatment*, vol. 9, pp. 639–658, 2013.

[36] J. Dirmaier, M. Steinmann, T. Krattenmacher et al., "Non-pharmacological treatment of depressive disorders: a review of evidence-based treatment options," *Reviews on Recent Clinical Trials*, vol. 7, no. 2, pp. 141–149, 2012.

[37] E. Ernst, K. L. Resch, S. Mills et al., "Complementary medicine—a definition," *British Journal of General Practice*, vol. 45, no. 398, article 506, 1995.

[38] G. van der Watt, J. Laugharne, and A. Janca, "Complementary and alternative medicine in the treatment of anxiety and depression," *Current Opinion in Psychiatry*, vol. 21, no. 1, pp. 37–42, 2008.

[39] N. Perry and E. Perry, "Aromatherapy in the management of psychiatric disorders: clinical and neuropharmacological perspectives," *CNS Drugs*, vol. 20, no. 4, pp. 257–280, 2006.

[40] B. Cooke and E. Ernst, "Aromatherapy: a systematic review," *British Journal of General Practice*, vol. 50, no. 455, pp. 493–496, 2000.

[41] R. S. Herz, "Aromatherapy facts and fictions: a scientific analysis of olfactory effects on mood, physiology and behavior," *International Journal of Neuroscience*, vol. 119, no. 2, pp. 263–290, 2009.

[42] W. N. Setzer, "Essential oils and anxiolytic aromatherapy," *Natural Product Communications*, vol. 4, no. 9, pp. 1305–1316, 2009.

[43] D. H. Ndao, E. J. Ladas, B. Cheng et al., "Inhalation aromatherapy in children and adolescents undergoing stem cell infusion: results of a placebo-controlled double-blind trial," *Psycho-Oncology*, vol. 21, no. 3, pp. 247–254, 2012.

[44] S. Carke, *Essential Chemistry for Aromatherapy*, Churchill Livingstone Elsevier, 2nd edition, 2008.

[45] J. Buckle, *Clinical Aromatherapy: Essential oils in Practice*, Churchill Livingstone Elsevier Science, New York, NY, USA, 2nd edition, 2003.

[46] M. Louis and S. D. Kowalski, "Use of aromatherapy with hospice patients to decrease pain, anxiety, and depression and to promote an increased sense of well-being," *The American journal of hospice & palliative care*, vol. 19, no. 6, pp. 381–386, 2002.

[47] A. C. de Groot and E. Schmidt, "Essential oils, Part I," *Dermatitis*, vol. 27, no. 2, pp. 39–42, 2016.

[48] R. Tisserand and T. Balacs, *Essential Oil Safety: A Guide for Health Care Professionals*, Churchill Livingstone Elsevier, 1st edition, 1995.

[49] B. Ali, N. A. Al-Wabel, S. Shams, A. Ahamad, S. A. Khan, and F. Anwar, "Essential oils used in aromatherapy: a systemic review," *Asian Pacific Journal of Tropical Biomedicine*, vol. 5, no. 8, pp. 601–611, 2015.

[50] L. Price and S. Price, *Aromatherapy for Health Professionals*, vol. 11, Elsevier Health Sciences, 2011.

[51] K. M. Burnett, L. A. Solterbeck, and C. M. Strapp, "Scent and mood state following an anxiety-provoking task," *Psychological*

[52] M.-H. Hur, J.-A. Song, J. Lee, and M. S. Lee, "Aromatherapy for stress reduction in healthy adults: a systematic review and meta-analysis of randomized clinical trials," *Maturitas*, vol. 79, no. 4, pp. 362–369, 2014.

[53] D. Joswiak, M. E. Kinney, J. R. Johnson et al., "Development of a health system-based nurse-delivered aromatherapy program," *Journal of Nursing Administration*, vol. 46, no. 4, pp. 221–225, 2016.

[54] A. R. Jadad, R. A. Moore, D. Carroll et al., "Assessing the quality of reports of randomized clinical trials: is blinding necessary?" *Controlled Clinical Trials*, vol. 17, no. 1, pp. 1–12, 1996.

[55] S. Wilkinson, J. Aldridge, I. Salmon, E. Cain, and B. Wilson, "An evaluation of aromatherapy massage in palliative care," *Palliative Medicine*, vol. 13, no. 5, pp. 409–417, 1999.

[56] A. Lundh and P. C. Gøtzsche, "Recommendations by Cochrane Review Groups for assessment of the risk of bias in studies," *BMC Medical Research Methodology*, vol. 8, article no. 22, 2008.

[57] A. Kageyama, T. Ueno, M. Oshio, H. Masuda, H. Horiuchi, and H. Yokogoshi, "Antidepressant-like effects of an aqueous extract of lavender (*Lavandula angustifolia* Mill.) in rats," *Food Science and Technology Research*, vol. 18, no. 3, pp. 473–479, 2012.

[58] G. Bagetta, L. A. Morrone, L. Rombolà et al., "Neuropharmacology of the essential oil of bergamot," *Fitoterapia*, vol. 81, no. 6, pp. 453–461, 2010.

[59] T. Matsumoto, T. Kimura, and T. Hayashi, "Aromatic effects of a Japanese citrus fruit—yuzu (Citrus junos Sieb. ex Tanaka)—on psychoemotional states and autonomic nervous system activity during the menstrual cycle: a single-blind randomized controlled crossover study," *BioPsychoSocial Medicine*, vol. 10, no. 1, 2016.

[60] S. Stea, A. Beraudi, and D. De Pasquale, "Essential oils for complementary treatment of surgical patients: state of the art," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 726341, 6 pages, 2014.

[61] A. S. Lillehei and L. L. Halcon, "A systematic review of the effect of inhaled essential oils on sleep," *Journal of Alternative and Complementary Medicine*, vol. 20, no. 6, pp. 441–451, 2014.

[62] H. F. Coelho, K. Boddy, and E. Ernst, "Massage therapy for the treatment of depression: a systematic review," *International Journal of Clinical Practice*, vol. 62, no. 2, pp. 325–333, 2008.

[63] G. C. Goats, "Massage—the scientific basis of an ancient art: part 1. The techniques," *British Journal of Sports Medicine*, vol. 28, no. 3, pp. 149–152, 1994.

[64] J. Corner, N. Cawley, and S. Hildebrand, "An evaluation of the use of massage and essential oils in the well-being of cancer patients," *International Journal of Palliative Nursing*, vol. 1, no. 2, pp. 67–73, 1994.

[65] N. C. Russell, S.-S. Sumler, C. M. Beinhorn, and M. A. Frenkel, "Role of massage therapy in cancer care," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 2, pp. 209–214, 2008.

[66] W.-H. Hou, P.-T. Chiang, T.-Y. Hsu, S.-Y. Chiu, and Y.-C. Yen, "Treatment effects of massage therapy in depressed people: a meta-analysis," *Journal of Clinical Psychiatry*, vol. 71, no. 7, pp. 894–901, 2010.

[67] E. Ernst, "Massage therapy for cancer palliation and supportive care: a systematic review of randomised clinical trials," *Supportive Care in Cancer*, vol. 17, no. 4, pp. 333–337, 2009.

[68] D. Fellowes, K. Barnes, and S. Wilkinson, "Aromatherapy and massage for symptom relief in patients with cancer," *Cochrane database of systematic reviews (Online)*, no. 2, p. CD002287, 2004.

APPENDIX 2. Conference proceedings

Abstract ID: PP-006

and neuropsychological test, and blood test for microparticles using flow cytometer method.

Results: Among 60 individuals; 23 of them have WMH, whereby most of them are older individuals (40's to 60's). Age does significantly correlates ($p<0.05$) with QRISK2 cardiovascular risk prediction. Age was significantly correlated with the outcomes of neuropsychological test. Cognitive and memories ability did correlate with the prevalence of WMH with respect to the location of the lesion. Participants with WMH+ found to have lower neuropsychological performances and higher microparticles counts when compared with the WMH- participant.

Conclusion: Finally, further data collection and analysis are still in progress, which include a repeat MRI brain at one-year follow-up for all the subjects, as well as the establishment of blood microparticles testing. In addition, further DTI analysis will be conducted to better correlate the degree of white matter damage with serial of haemostatic biomarkers and neuropsychological test.

ABSTRACT ID: PP-005

Association Study of SCN Polymorphism in Epilepsy Refractoriness in Malaysia

C. K. Chan[1, 2], K. S. Lim[1], M. H. Tsai[3,4], C. C. Ng[2], C. T. Tan[1]

[1]Department of Neurology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Kuala Lumpur, Malaysia

[2]Unit of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Kuala Lumpur, Malaysia

[3]Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Kaohsiung, Taiwan

[4]Department of Nursing, Meiho University, Neipu Township, Pingtung County, Taiwan

Refractory epilepsy, also known as drug resistant epilepsy is a condition where seizures remain uncontrolled despite antiepileptic drug therapy. Studies have shown that polymorphisms in genes encoding for sodium voltage-gated channel alpha subunits are associated with antiepileptic (AED) drug responsiveness. This study aims to discover the association between sodium ion channel gene polymorphisms and AED responsiveness in a multi-ethnic Malaysian population. A total of 118 unrelated Malaysians (38 Malay, 54 Chinese and 26 Indian) with epilepsy were recruited in this study. Among the patients, 81 of them were drug resistant and 37 of them were drug responsive. Genomic DNA was extracted from peripheral blood or buccal swap samples followed by targeted re-sequencing of SCN1A, SCN1B and SCN9A genes in these patients. 15 polymorphic variants were identified and tested for their associations with drug response. We found a significant association ($p < 0.05$) between SCN1A variant (rs2298771) and drug resistant in Chinese. This result is consistent with previous studies conducted in Han Chinese from China. On the other hand, a variant in SCN1B (rs55742440) was significantly ($p < 0.05$) associated with drug resistant in Malay. No significant association was detected in Indian patients. In conclusion, our findings indicate that specific genetic variants in sodium channel genes might influence AED responsiveness in Malaysian patients.

ABSTRACT ID: PP-006

Effect of lavender essential oil on behavior and neurogenesis in an animal model for depression

D. I. Sánchez-Vidaña[1], K. K. T. Po, H. W. H. Tsang, B. W. M. Lau

(Affiliations not provided)

Background: Depression is a major health problem that affects about 350 million people worldwide. Patients with depressive symptoms frequently make use of complementary and alternative therapies such as aromatherapy. Lavender essential oil (LEO) is one of the most commonly used essential oils in aromatherapy for the treatment of sleeping disorders, anxiety and depression. However, the mechanism of action by which LEO exerts its antidepressant effect is still unknown. Neurogenesis is a physiological process that occurs in restricted areas of the adult brain (hippocampus and subventricular zone), has been linked to the regulation of emotional behaviour and is a promising target process for the study of psychiatric disorders such as major depression.

Aim: The aim of the present study is to evaluate the effect of LEO on behaviour and neurogenesis in an

animal model for depression.

Materials and methods: Young adult male Sprague-Dawley rats were grouped into 4 groups: (1) Control (vehicle), (2) corticosterone, (3) LEO, (4) LEO + Corticosterone. Animals were exposed to the vehicle and LEO in a chamber containing a cotton impregnated with the correspondent treatment. After 14 days of treatment, behavioural tests were carried out to measure depression-like behaviour and anxiety-like behaviour. On day 16, perfusion was carried out and brains were collected for immunohistochemical analysis targeting newborn neurons (BrdU positive cells) and immature neurons (DCX positive cells).

Results: LEO treatment prevented the corticosterone-induced depression-like and anxiety-like behaviour. Furthermore, LEO stimulated neurogenesis.

Conclusions: The present study contributes to the understanding of the antidepressant effect of LEO at a behavioural and cellular level.

ABSTRACT ID: PP-007

Toll-like Receptor 4 (TLR4) agonist causes motor behaviour deficits through modulation of neuronal morphology, dopamine and glutamate receptors in striatum and cerebellum of mice

D. Y. Abg Abd Wahab[1], N. H. Abd Aziz[1], G. H. Chuang1, K. Sayed[1], M. Mustapha[1], J. M. Abdullah[1,2], Z. Idris[1,2], Z. Mustafa[1], Z. Abdullah[2], M. Sangu[1]

[1]*Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu, Kelantan, Malaysia*

[2]*Center for Neuroscience Services and Research (P3Neuro), Universiti Sains Malaysia, Kubang Kerian, Kota Bharu, Kelantan, Malaysia*

[3]*Biomedicine Program, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu, Kelantan, Malaysia*

The alcohol addiction is one of the possible factors to stimulate brain microglia activation and leads to neuroinflammation through toll-like receptor which is present in microglia. In fact, alcohol addiction ultimately caused motor deficits through neuroinflammation. However, the underlying mechanisms of neuroinflammation inducing motor behaviour through activation of TLR4 receptors have not yet been elucidated. Toll-like receptors (TLR) are always found to be associated or involved in the induction of neuroinflammation in neurodegenerative diseases. Activation of TLR4 is stimulated by TLR4 Agonist, LPS, and the TLR4-LPS interaction has been found to result in physiological and behavioural changes including retardation of motor activity in the mouse model. Therefore, the present study aimed to investigate the neuronal morphological, dopamine receptors (Dopamine D1 receptor and Dopamine D2 receptor) and glutamate (EAAT1 and EAAT4) in the striatum and cerebellum following treatment with toll-like receptor 4 agonist. The animals were divided into four groups; (1) Control (n=6), (2) LPS treatment (0.83mg/kg) (n=6) and (3) LPS-RS (0.25mg/kg) (n=6). After treatment, behaviour studies were carried out in Rota rod, hanging method, wooden beam walking, open field test and hole board method. Following behaviour test, animal's brains were harvested for morphological changes and gene expression studies. The results showed that there were morphological changes in striatum and cerebellum motor neurons. The gene expression studies suggested that there were significant changes in dopamine receptors (Dopamine D1 receptor and Dopamine D2 receptor) and glutamate (EAAT1 and EAAT4) in the striatum and cerebellum along with motor deficits. In conclusion, toll-like receptor 4 causes motor deficits through regulation of dopamine and glutamate receptors in striatum and cerebellum.

ABSTRACT ID: PP-008

Dysregulations in metabolic activity of neurospheres derived from embryonic Ts1Cje mouse model for Down syndrome

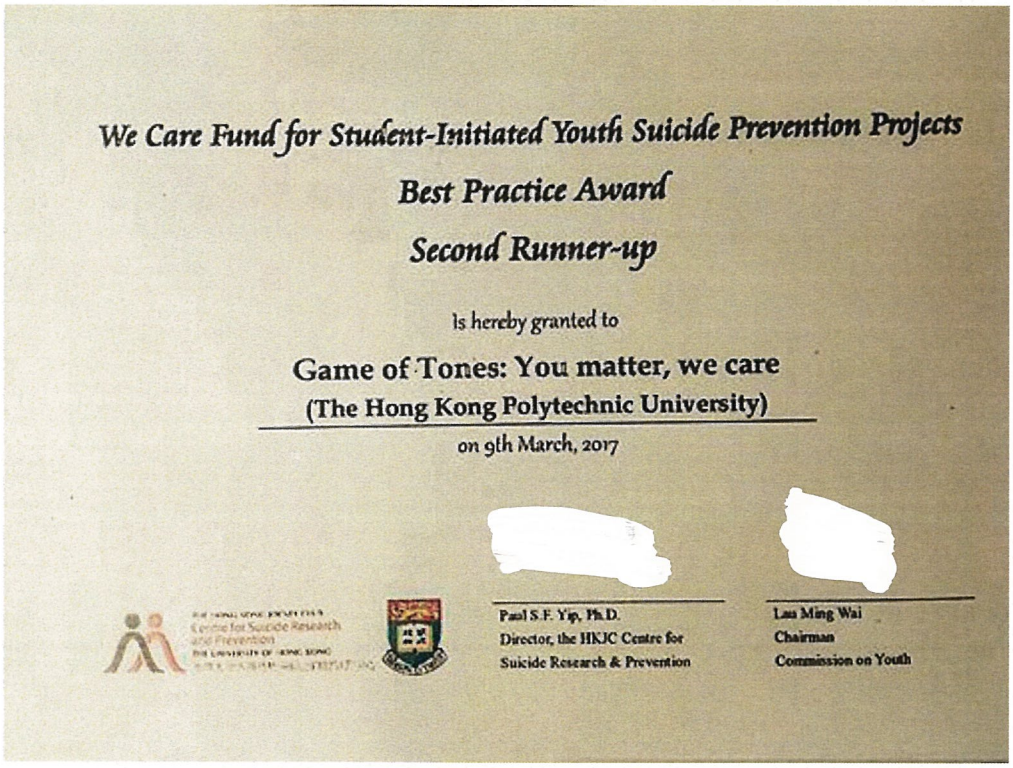
E. A. Seth[1,2], H. C. Lee[1,2], H. H. Yusof[1,2], Y. K. Cheah[1,2], T. W. Ho[4], K. H. Ling[1,2], P. S. Cheah[1,2]

[1]*Genetics and Regenerative Medicine Centre (GRMRC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[2]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[3]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

APPENDIX 3. Awards

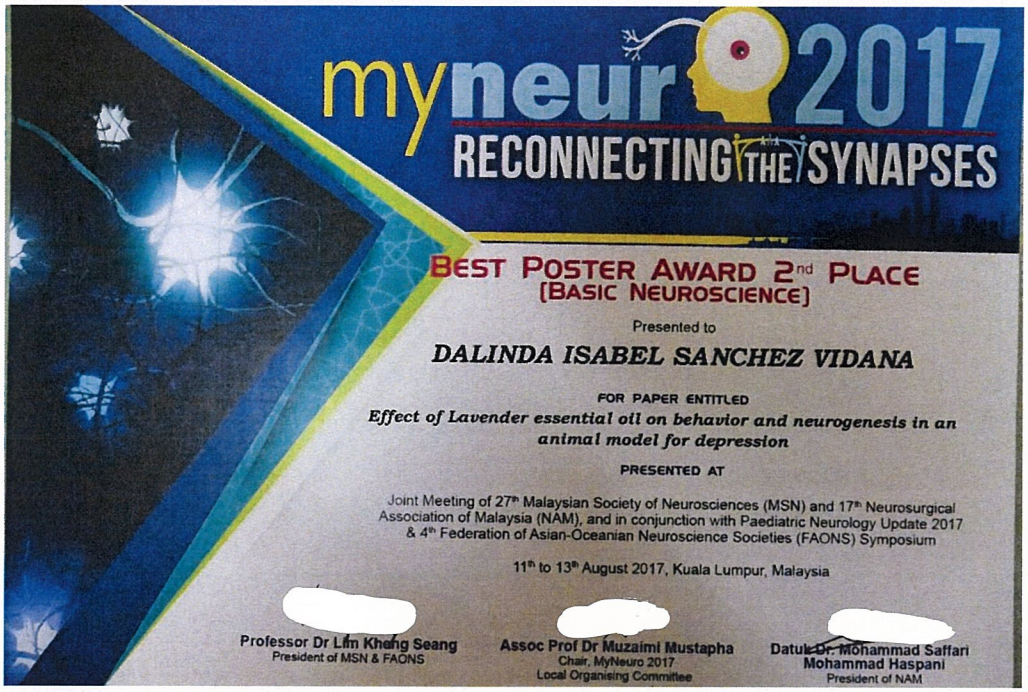
Awards

Best Practice Award, WeCare Fund, 2nd runner up position. Student-initiated suicide prevention project "Game of tones: You matter, we care" implemented at The Hong Kong Polytechnic University from July to October 2016. **Team leader**



Best Poster Award, 2nd place (Basic Neuroscience). Sánchez-Vidaña DI, Po KK-T, Tsang HW-H, Lau BW-M (2017). Effect of lavender essential oil on behavior and neurogenesis in an animal model for depression. My Neuro 2017: Reconnecting the Synapses. Joint meeting of 27th Malaysian Society of Neurosciences (MSN)and 17th Neurosurgical Association of Malaysia (NAM) and in conjunction with Paedriatric Neurology update 2017 and 4th Federation

of Asian-Oceaninan Neuroscience Societies (FAONS) Symposium. 11th to 13th August, Kuala Lumpur, Malaysia.


myneur 2017
RECONNECTING THE SYNAPSES

BEST POSTER AWARD 2nd PLACE
(BASIC NEUROSCIENCE)

Presented to

DALINDA ISABEL SANCHEZ VIDANA

FOR PAPER ENTITLED

Effect of Lavender essential oil on behavior and neurogenesis in an animal model for depression

PRESENTED AT

Joint Meeting of 27th Malaysian Society of Neurosciences (MSN) and 17th Neurosurgical Association of Malaysia (NAM), and in conjunction with Paediatric Neurology Update 2017 & 4th Federation of Asian-Oceanian Neuroscience Societies (FAONS) Symposium

11th to 13th August 2017, Kuala Lumpur, Malaysia

Professor Dr Lim Kheng Seang
President of MSN & FAONS

Assoc Prof Dr Muzaimi Mustapha
Chair, MyNeuro 2017
Local Organising Committee

Datuk Dr. Mohammad Saffari Mohammad Haspani
President of NAM

APPENDIX 4. Other publications and grants during PhD study

Publications

He WJ, Ngai SPC, **Sánchez-Vidaña Dalinda Isabel**, Lau BWM, Pang MYC. Impacts of Cigarette Smoke on Muscle Derangement: A Systematic Review (co-author). **In preparation**

Ahorsu Kwasi Daniel, **Sánchez Vidaña Dalinda Isabel**, Shah Parth Bharat, Cruz González Pablo, Lipardo Donald, Shende Sachin, Shilpa Gurung, Venkatesan Harun, Duongthuothewa Anchalee, Ansari Talha Qasim, Veronika Schoeb. De-mythifying mental health taboo: Can an educational program in combination with workshops change the perspective towards mental health and help-seeking behavior among university students in Hong Kong? **In preparation**

Lingyi Liao BS, Benson Wui-Man Lau, **Dalinda Isabel Sánchez-Vidaña**, Qiang Gao. Transplantation of Exogenous Neural Stem Cells in the Treatment of Cerebral Ischemia. **Under review**

Conference

Leung Joseph Wai Hin, Lee Jada Chi Di, Chan Jackie Ngai Man, Fung Timothy Kai Hang, Chow Jason K.W., Sánchez-Vidaña, D. I., So Kwok-Fai, Lau Benson W.M. (2017). An animal model of simulated predator odor exposure: Exploring the potential role of neurogenesis in specific phobia extinction. *Annual Scientific Meeting of the Hong Kong Society of Simulation in Healthcare*. Hong Kong, 2017.09.09-09.10 (Poster).

GRANTS

WeCare Fund grant (20,000 HKD) (2017-2018). Grant awarded after obtaining the 2nd runner up position for "Best Practice Award" of the student-initiated suicide prevention project "Game of tones: You matter, we care" which was implemented at The Hong Kong Polytechnic University from July to October 2016. Grant awarded by the Center for Suicide Research and Prevention at the University of Hong Kong. 9th March to continue implementing the project from April 2017 to April 2018. **Position: Team leader**

WeCare Fund grant (50,000 HKD) (2016). Grant awarded by the Center for Suicide Research and Prevention at the University of Hong Kong to run the student-initiated suicide prevention project "Game of tones: You matter, we care" which was implemented at The Hong Kong Polytechnic University from July to October. **Position: Team leader**

REFERENCES

Ache BW, Young JM (2005) Review Olfaction: Diverse Species, Conserved Principles. Neuron 48:417–430.

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH (2014) Regulation and Function of Adult Neurogenesis: From Genes to Cognition. Physiol Rev 94:991–1026.

Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K (2007) High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317:819–823.

Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F (2015) Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed 5:601–611.

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457.

Alzheimer's Association (2015) 2015 Alzheimer's disease facts and figures. Alzheimers Dement 11:332–384.

Anacker C, Cattaneo A, Luoni A, Musaelyan K, Zunszain PA, Milanesi E, Rybka J, Berry A, Cirulli F, Thuret S, Price J, Riva MA, Gennarelli M, Pariante CM (2013) Glucocorticoid-Related Molecular Signaling Pathways Regulating Hippocampal Neurogenesis. Neuropsychopharmacology 38:872–883.

Anderson JE, Michalak EE, Lam RW (2002) Depression in primary care: Tools for screening, diagnosis, and measuring response to treatment. BCMJ 44:415–419.

Andrade LB, Echeverrigaray S, Fracaro F, Pauletti GF, Rota L (1999) The effect of growth regulators on shoot propagation and rooting of common lavender (Lavandula vera DC). Plant Cell Tissue Organ Cult 56:79–83.

Andreescu C, Mulsant BH, Emanuel JE (2008) Complementary and alternative medicine in the treatment of bipolar disorder--a review of the evidence. J Affect Disord 110:16–26.

Andrews MH, Wood SA, Windle RJ, Lightman SL, Ingram CD (2012) Acute glucocorticoid administration rapidly suppresses basal and stress-induced hypothalamo-pituitary-adrenal axis activity. Endocrinology 153:200–211.

Apple DM, Fonseca RS, Kokovay E (2016) The Role of Adult Neurogenesis in Psychiatric and Cognitive Disorders. Brain Res 1655:270–276.

Araki A, Watanabe K, Eitaki Y, Kawai T, Kishi R (2012) The feasibility of aromatherapy massage to reduce symptoms of Idiopathic Environmental Intolerance: a pilot study. Complement Ther Med 20:400–408.

Arisi GM, Foresti ML, Mukherjee S, Shapiro LA (2012) The role of olfactory stimulus in adult mammalian neurogenesis. Behav Brain Res 227:356–362.

Arletti R, Bertolini A (1987) Oxytocin acts as an antidepressant in two animal models of depression. Life Sci 41:1725–1730.

Arroll B, Macgillivray S, Ogston S, Reid I, Sullivan F, Williams B, Crombie I (2005) Efficacy and tolerability of tricyclic antidepressants and SSRIs compared with

placebo for treatment of depression in primary care: a meta-analysis. Ann Fam Med 3:449–456.

Ates-Alagoz Z, Adejare A (2013) NMDA Receptor Antagonists for Treatment of Depression. Pharmaceuticals (Basel) 6:480–499.

Bagetta G, Morrone LA, Rombolà L, Amantea D, Russo R, Berliocchi L, Sakurada S, Sakurada T, Rotiroti D, Corasaniti MT (2010) Neuropharmacology of the essential oil of bergamot. Fitoterapia 81:453–461.

Bai-fang Z, Fang-fang P, Jiang-zhou Z, Dong-cheng W (2001) Bis(7)-Tacrine, a promising anti-Alzheimer's agent, attenuates glutamate-induced cell injury in primary cultured cerebrocortical neurons of rats. Wuhan Univ J Nat Sci 6:737–741.

Bailey KR, Crawley JN (2009) Anxiety-Related Behaviors in Mice. CRC Press/Taylor & Francis.

Baquero M, Martín N (2015) Depressive symptoms in neurodegenerative diseases. World J Clin cases 3:682–693.

Barati F, Nasiri A, Akbari N, Sharifzadeh G (2016) The Effect of Aromatherapy on Anxiety in Patients. Nephrourol Mon 8:e38347.

Bartocci M, Winberg J, Ruggiero C, Bergqvist LL, Serra G, Lagercrantz H (2000) Activation of Olfactory Cortex in Newborn Infants After Odor Stimulation: A Functional Near-Infrared Spectroscopy Study. Pedriatric Res 48:18–23.

Bartsch T (2012) The clinical neurobiology of the hippocampus : an integrative view. Oxford University Press.

Bátiz LF, Castro MA, Burgos P V., Velásquez ZD, Muñoz RI, Lafourcade CA, Troncoso-Escudero P, Wyneken U (2016) Exosomes as Novel Regulators of Adult Neurogenic Niches. Front Cell Neurosci 9:501.

Becker A, Grecksch G, Bernstein HG, Höllt V, Bogerts B (1999) Social behaviour in rats lesioned with ibotenic acid in the hippocampus: quantitative and qualitative analysis. Psychopharmacology (Berl) 144:333–338.

Bekinschtein P, Kent BA, Oomen CA, Clemenson GD, Gage FH, Saksida LM, Bussey TJ (2014) Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories. Hippocampus 24:905–911.

Bergmann O, Liebl J, Bernard S, Alkass K, Yeung MSY, Steier P, Kutschera W, Johnson L, Landén M, Druid H, Spalding KL, Frisén J (2012) The Age of Olfactory Bulb Neurons in Humans. Neuron 74:634–639.

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151.

Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OFX, Sousa N (2008) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry 14:764–773.

Best JG, Stagg CJ, Dennis A (2014) Other Significant Metabolites: Myo-Inositol, GABA, Glutamine, and Lactate. Magn Reson Spectrosc:122–138.

Bhattacharya A, Derecki NC, Lovenberg TW, Drevets WC (2016) Role of neuro-

immunological factors in the pathophysiology of mood disorders. Psychopharmacology (Berl).

Blackburn L, Achor S, Allen B, Bauchmire N, Dunnington D, Klisovic R, Naber S, Roblee K, Samczak A, Tomlinson-Pinkham K, Chipps E (2017) The Effect of Aromatherapy on Insomnia and Other Common Symptoms Among Patients With Acute Leukemia. Oncol Nurs Forum 44:E185–E193.

Blair KA, Denham SA, Kochanoff A, Whipple B (2004) Playing it cool: Temperament, emotion regulation, and social behavior in preschoolers. J Sch Psychol 42:419–443.

Bolognesi ML, Bartolini M, Mancini F, Chiriano G, Ceccarini L, Rosini M, Milelli A, Tumiatti V, Andrisano V, Melchiorre C (2010) Bis(7)-tacrine Derivatives as Multitarget-Directed Ligands: Focus on Anticholinesterase and Antiamyloid Activities. ChemMedChem 5:1215–1220.

Brann JH, Firestein SJ (2014) A lifetime of neurogenesis in the olfactory system. Front Neurosci 8:182.

Brennan PA (2010) Pheromones and Mammalian Behavior. CRC Press/Taylor & Francis.

Brummelte S, Galea ALAM, Galea LAM (2010) Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. NSC 168:680–690.

Brummelte S, Pawluski JL, Galea LAM (2006) High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation

and behavior of offspring: A model of post-partum stress and possible depression. Horm Behav 50:370–382.

Buchbauer G, Jirovetz L, Jäger W, Dietrich H, Plank C (1991) Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. Z Naturforsch C 46:1067–1072.

Buckle J (2003) Clinical Aromatherapy: Essential oils in practice (Buckle J, ed)., 2nd ed. USA: Churchill Livingstone Elsevier Science.

Burke SM, Graf R, Ochsenbein-Kölble N, Hummel T (2015) Oxytocin Effects on Chemosensory Function in a Clinical Setting—a Preliminary Study. Chemosens Percept 8:159–166.

Burnett KM, Solterbeck LA, Strapp CM (2004) Scent and mood state following an anxiety-provoking task. Psychol Rep 95:707–722.

Calabrese F, Molteni R, Racagni G, Riva MA (2009) Neuronal plasticity: A link between stress and mood disorders. Psychoneuroendocrinology 34:S208–S216.

Cameron HA, Gould E (1994) Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 61:203–209.

Campbell S, Macqueen G (2004) The role of the hippocampus in the pathophysiology of major depression. J Psychiatry Neurosci 29:417–426.

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012a) The mouse forced swim test. J Vis Exp:e3638.

Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD (2012b) The tail

suspension test. J Vis Exp:e3769.

Caraci F, Copani A, Nicoletti F, Drago F (2010) Depression and Alzheimer's disease: neurobiological links and common pharmacological targets. Eur J Pharmacol 626:64–71.

Carke S (2008) Essential chemistry for aromatherapy (Clarke S, ed)., 2nd ed. Churchill Livingstone Elsevier.

Cavanagh HMA, Wilkinson JM (2002) Biological activities of Lavender essential oil. Phyther Res 16:301–308.

Chan Y-Y, Lo W-Y, Yang S-N, Chen Y-H, Lin J-G (2015) The benefit of combined acupuncture and antidepressant medication for depression: A systematic review and meta-analysis. J Affect Disord 176:106–117.

Chatzopoulou PS, Goliaris AH (2003) Contribution to the Analysis of the Volatile Constituents from some Lavender and Lavandin Cultivars Grown in Greece. Sci Pharm (Sci Pharm) 71:229–234.

Chen H-H, Zhang N, Li W-Y, Fang M-R, Zhang H, Fang Y-S, Ding M-X, Fu X-Y (2015a) Overexpression of brain-derived neurotrophic factor in the hippocampus protects against post-stroke depression. Neural Regen Res 10:1427–1432.

Chen S-L, Chen C-H (2015) Effects of Lavender Tea on Fatigue, Depression, and Maternal-Infant Attachment in Sleep-Disturbed Postnatal Women. Worldviews evidence-based Nurs 12:370–379.

Chen Y-L, Pan A-W, Hsiung P-C, Chung L (2015b) Quality of Life Enhancement Programme for Individuals with Mood Disorder: A Randomized Controlled Pilot

Study. Hong Kong J Occup Ther 25:23–31.

Chen Y, Huang X, Zhang Y, Rockenstein E, Bu G, Golde TE, Masliah E, Xu H (2012) Alzheimer's β-secretase (BACE1) regulates the cAMP/PKA/CREB pathway independently of β-amyloid. J Neurosci 32:11390–11395.

Chesnokova V, Pechnick RN, Wawrowsky K (2016) Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. Brain Behav Immun 58:1–8.

Coburn-Litvak PS, Tata DA, Gorby HE, McCloskey DP, Richardson G, Anderson BJ (2004) Chronic corticosterone affects brain weight, and mitochondrial, but not glial volume fraction in hippocampal area CA3. Neuroscience 124:429–438.

Coelho HF, Boddy K, Ernst E (2008) Massage therapy for the treatment of depression: a systematic review. Int J Clin Pract 62:325–333.

Coelho V, Mazzardo-Martins L, Martins DF, Santos ARS, da Silva Brum LF, Picada JN, Pereira P (2013) Neurobehavioral and genotoxic evaluation of (-)-linalool in mice. J Nat Med 67:876–880.

Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM (2013) Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr Neuropharmacol 11:315–335.

Conrad CD, Ortiz JB, Judd JM (2017) Chronic stress and hippocampal dendritic complexity: Methodological and functional considerations. Physiol Behav 178:66–81.

Conrad P, Adams C (2012) The effects of clinical aromatherapy for anxiety and depression in the high risk postpartum woman - a pilot study. Complement Ther

Clin Pract 18:164–168.

Cooke B, Ernst E (2000) Aromatherapy: a systematic review. Br J Gen Pract 50:493–496.

Corner J, Cawley N, Hildebrand S (1994) An evaluation of the use of massage and essential oils on the wellbeing of cancer patients. Int J Palliat Nurs 1:67-73. Int J Palliat Nurs 1.

Culpepper L, Muskin PR, Stahl SM (2015) Major Depressive Disorder: Understanding the Significance of Residual Symptoms and Balancing Efficacy with Tolerability. Am J Med 128:S1–S15.

David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux J-P, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R (2009) Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62:479–493.

de Groot AC, Schmidt E (2016) Essential Oils, Part I. Dermatitis 27:39–42.

Dean L (2012) Imipramine Therapy and CYP2D6 and CYP2C19 Genotype. National Center for Biotechnology Information (US).

Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 11:339–350.

Devesa P, Agasse F, Xapelli S, Almengló C, Devesa J, Malva JO, Arce VM (2014) Growth hormone pathways signaling for cell proliferation and survival in

hippocampal neural precursors from postnatal mice. BMC Neurosci 15:100.

Diego MA, Jones NA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, McAdam V, Galamaga R, Galamaga M (1998) Aromatherapy positively affects mood, EEG patterns of alertness and math computations. Int J Neurosci 96:217–224.

Dirmaier J, Steinmann M, Krattenmacher T, Watzke B, Barghaan D, Koch U, Schulz H (2012) Non-pharmacological treatment of depressive disorders: a review of evidence-based treatment options. Rev Recent Clin Trials 7:141–149.

Donner NC, Montoya CD, Lukkes JL, Lowry CA (2012) Chronic non-invasive corticosterone administration abolishes the diurnal pattern of tph2 expression. Psychoneuroendocrinology 37:645–661.

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 22:238–249.

Duman RS, Li N (2012) A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. Philos Trans R Soc Lond B Biol Sci 367:2475–2484.

Duman RS, Voleti B (2012) Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends Neurosci 35:47–56.

Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming G-L, Overstreet-Wadiche LS (2008) Adult neurogenesis, mental health, and mental illness: hope or hype? J Neurosci 28:11785–11791.

Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317.

Ernst E (2009) Massage therapy for cancer palliation and supportive care: a systematic review of randomised clinical trials. Support Care Cancer 17:333–337.

Ernst E, Rand JI, Stevinson C (1998) Complementary therapies for depression: an overview. Arch Gen Psychiatry 55:1026–1032.

Ernst E, Resch K, Mills S, Hill R, Mitchell A, Willoughby M, White A (1995) Complementary medicine — a definition. Br J Gen Pract 45:506.

Fang JH, Wang XH, Xu ZR, Jiang FG (2010) Neuroprotective effects of bis(7)-tacrine against glutamate-induced retinal ganglion cells damage. BMC Neurosci 11:31.

Fellowes D, Barnes K, Wilkinson S (2004) Aromatherapy and massage for symptom relief in patients with cancer. Cochrane database Syst Rev:CD002287.

Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJL, Vos T, Whiteford HA (2013) Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS Med 10:e1001547.

Fißler M, Quante A (2014) A case series on the use of lavendula oil capsules in patients suffering from major depressive disorder and symptoms of psychomotor agitation, insomnia and anxiety. Complement Ther Med 22:63–69.

Fu H, Dou J, Li W, Cui W, Mak S, Hu Q, Luo J, Lam CSC, Pang Y, Youdim MBH, Han Y (2009) Promising multifunctional anti-Alzheimer's dimer bis(7)-Cognitin

acting as an activator of protein kinase C regulates activities of α-secretase and BACE-1 concurrently. Eur J Pharmacol 623:14–21.

Fu H, Li W, Lao Y, Luo J, Lee NTK, Kan KKW, Tsang HW, Tsim KWK, Pang Y, Li Z, Chang DC, Li M, Han Y (2006) Bis(7)-tacrine attenuates β amyloid-induced neuronal apoptosis by regulating L-type calcium channels. J Neurochem 98:1400–1410.

Fu H, Li W, Liu Y, Lao Y, Liu W, Chen C, Yu H, Lee NTK, Chang DC, Li P, Pang Y, Tsim KWK, Li M, Han Y (2007) Mitochondrial Proteomic Analysis and Characterization of the Intracellular Mechanisms of Bis(7)-tacrine in Protecting against Glutamate-Induced Excitotoxicity in Primary Cultured Neurons. J Proteome Res 6:2435–2446.

Fu H, Li W, Luo J, Lee NTK, Li M, Tsim KWK, Pang Y, Youdim MBH, Han Y (2008) Promising anti-Alzheimer's dimer bis(7)-tacrine reduces β-amyloid generation by directly inhibiting BACE-1 activity. Biochem Biophys Res Commun 366:631–636.

Fujiwara M, Izuishi K, Sano T, Akram Hossain M, Kimura S, Masaki T, Suzuki Y (2008) Modulating effect of the PI3-kinase inhibitor LY294002 on cisplatin in human pancreatic cancer cells.

Fung JKKM, Tsang HWH, Chung RCK (2012) A systematic review of the use of aromatherapy in treatment of behavioral problems in dementia. Geriatr Gerontol Int 12:372–382.

Gage GJ, Kipke DR, Shain W (2012) Whole animal perfusion fixation for rodents. J Vis Exp:3564.

Gamberini MT, Rodrigues DS, Rodrigues D, Pontes VB (2015) Effects of the aqueous extract of Pimpinella anisum L. seeds on exploratory activity and emotional behavior in rats using the open field and elevated plus maze tests. J Ethnopharmacol 168:45–49.

Gao M, Zhou H, Li X (2009) Curcumin Protects PC12 Cells from Corticosterone-Induced Cytotoxicity: Possible Involvement of the ERK1/2 Pathway. Basic Clin Pharmacol Toxicol 104:236–240.

Gersner R, Gal R, Levit O, Moshe H, Zangen A (2014) Inherited behaviors, BDNF expression and response to treatment in a novel multifactorial rat model for depression. Int J Neuropsychopharmacol 17:945–955.

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81:629–683.

Goats GC (1994) Massage--the scientific basis of an ancient art: Part 1. The techniques. Br J Sports Med 28:149–152.

Graham PH, Browne L, Cox H, Graham J (2003) Inhalation aromatherapy during radiotherapy: results of a placebo-controlled double-blind randomized trial. J Clin Oncol 21:2372–2376.

Greenberg MJ, Slyer JT (2017) Effectiveness of Silexan oral lavender essential oil compared to inhaled lavender essential oil aromatherapy on sleep in adults. JBI Database Syst Rev Implement Reports 15:961–970.

Greenberg PE, Fournier A-A, Sisitsky T, Pike CT, Kessler RC (2015) The economic burden of adults with major depressive disorder in the United States (2005 and

2010). J Clin Psychiatry 76:155–162.

Gregus A, Wintink AJ, Davis AC, Kalynchuk LE (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behav Brain Res 156:105–114.

Gu X, Wang Y, Xiang J, Chen Z, Wang L, Lu L, Qian S (2013) Interferon- γ triggers hepatic stellate cell-mediated immune regulation through MEK/ERK signaling pathway. Clin Dev Immunol 2013:389807.

Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, Hickie IB (2010) Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry 67:692–694.

Gutierrez H, Davies AM (2007) A fast and accurate procedure for deriving the Sholl profile in quantitative studies of neuronal morphology. J Neurosci Methods 163:24–30.

Guzmán-Gutiérrez SL, Gómez-Cansino R, García-Zebadúa JC, Jiménez-Pérez NC, Reyes-Chilpa R (2012) Antidepressant activity of Litsea glaucescens essential oil: Identification of β-pinene and linalool as active principles. J Ethnopharmacol 143:673–679.

Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62.

Han R, Zhang R, Chang M, Peng Y, Wang P, Hu S, Choi C, Yin M, Wang R, Han Y (2012) Reversal of scopolamine-induced spatial and recognition memory deficits in mice by novel multifunctional dimers bis-cognitins. Brain Res 1470:59–68.

Han X, Gibson J, Eggett DL, Parker TL (2017) Bergamot (Citrus bergamia) Essential

Oil Inhalation Improves Positive Feelings in the Waiting Room of a Mental Health Treatment Center: A Pilot Study. Phytother Res 31:812–816.

Han Y-F, Wu D-C, Xiao X-Q, Chen PMY, Chung W, Lee NTK, Pang Y-P, Carlier PR (2000) Protection against ischemic injury in primary cultured astrocytes of mouse cerebral cortex by bis(7)-tacrine, a novel anti-Alzheimer's agent. Neurosci Lett 288:95–98.

Hancianu M, Cioanca O, Mihasan M, Hritcu L (2013) Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia via anti-oxidative activities in rats. Phytomedicine 20:446–452.

Hansen HH, Sánchez C, Meier E (1997) Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? J Pharmacol Exp Ther 283:1333–1341.

Hanson ND, Owens MJ, Nemeroff CB (2011) Depression, antidepressants, and neurogenesis: a critical reappraisal. Neuropsychopharmacology 36:2589–2602.

Hansson AC, Sommer WH, Metsis M, Stromberg I, Agnati LF, Fuxe K (2006) Corticosterone Actions on the Hippocampal Brain-Derived Neurotrophic Factor Expression are Mediated by Exon IV Promoter. J Neuroendocrinol 18:104–114.

Hattiangady B, Shetty AK (2010) Decreased neuronal differentiation of newly generated cells underlies reduced hippocampal neurogenesis in chronic temporal lobe epilepsy. Hippocampus 20:97–112.

Herz RS (2009) Aromatherapy facts and fictions: a scientific analysis of olfactory

effects on mood, physiology and behavior. Int J Neurosci 119:263–290.

Hiemke C (2016) Why do antidepressant therapies have such a poor success rate? Expert Rev Neurother 16:597–599.

Hill RA, Du X, Wu Y-WC (2014) BDNF–TrkB signaling as a therapeutic target in neuropsychiatric disorders. J Receptor Ligand Channel Res Volume 7:61.

Hillerer KM, Neumann ID, Couillard-Despres S, Aigner L, Slattery DA (2014) Lactation-induced reduction in hippocampal neurogenesis is reversed by repeated stress exposure. Hippocampus 24:673–683.

Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC (2004) Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 7:726–735.

Horii Y, Nagai K, Nakashima T (2013) Order of exposure to pleasant and unpleasant odors affects autonomic nervous system response. Behav Brain Res 243:109–117.

Hossain E, Matsuzaki K, Katakura M, Sugimoto N, Al A, 1¤ M, Islam R, Hashimoto M, Shido O (2017) Direct exposure to mild heat promotes proliferation and neuronal differentiation of neural stem/progenitor cells in vitro.

Hou W-H, Chiang P-T, Hsu T-Y, Chiu S-Y, Yen Y-C (2010) Treatment effects of massage therapy in depressed people: a meta-analysis. J Clin Psychiatry 71:894–901.

Hritcu L, Cioanca O, Hancianu M (2012) Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats.

Phytomedicine 19:529–534.

Hu M-K, Wu L-J, Hsiao G, Yen M-H (2002) Homodimeric tacrine congeners as acetylcholinesterase inhibitors. J Med Chem 45:2277–2282.

Hu S-Q, Cui W, Mak S-H, Choi C-L, Hu Y-J, Li G, Tsim KW-K, Pang Y-P, Han Y-F (2015a) Robust Neuritogenesis-Promoting Activity by Bis(heptyl)-Cognitin Through the Activation of alpha7-Nicotinic Acetylcholine Receptor/ERK Pathway. CNS Neurosci Ther 21:520–529.

Hu S-Q, Wang R, Cui W, Mak S-H, Li G, Hu Y-J, Lee M-Y, Pang Y-P, Han Y-F (2015b) Dimeric bis (heptyl)-Cognitin Blocks Alzheimer's β-Amyloid Neurotoxicity Via the Inhibition of Aβ Fibrils Formation and Disaggregation of Preformed Fibrils. CNS Neurosci Ther 21:953–961.

Hu S, Cui W, Mak S, Xu D, Hu Y, Tang J, Choi C, Lee M, Pang Y, Han Y (2015c) Substantial Neuroprotective and Neurite Outgrowth-Promoting Activities by Bis(propyl)-cognitin via the Activation of Alpha7-nAChR, a Promising Anti-Alzheimer's Dimer. ACS Chem Neurosci 6:1536–1545.

Hur M-H, Song J-A, Lee J, Lee MS (2014) Aromatherapy for stress reduction in healthy adults: a systematic review and meta-analysis of randomized clinical trials. Maturitas 79:362–369.

Igarashi T (2013) Physical and psychologic effects of aromatherapy inhalation on pregnant women: a randomized controlled trial. J Altern Complement Med 19:805–810.

Imoto Y, Kira T, Sukeno M, Nishitani N, Nagayasu K, Nakagawa T, Kaneko S,

Kobayashi K, Segi-Nishida E (2015) Role of the 5-HT4 receptor in chronic fluoxetine treatment-induced neurogenic activity and granule cell dematuration in the dentate gyrus. Mol Brain 8:29.

Iñiguez SD, Alcantara LF, Warren BL, Riggs LM, Parise EM, Vialou V, Wright KN, Dayrit G, Nieto SJ, Wilkinson MB, Lobo MK, Neve RL, Nestler EJ, Bolaños-Guzmán CA (2014) Fluoxetine exposure during adolescence alters responses to aversive stimuli in adulthood. J Neurosci 34:1007–1021.

Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, McQuay HJ (1996) Assessing the quality of reports of randomized clinical trials: Is blinding necessary? Control Clin Trials 17:1–12.

Jessberger S, Gage FH (2014) Adult neurogenesis: bridging the gap between mice and humans. Trends Cell Biol 24:558–563.

Joswiak D, Kinney ME, Johnson JR, Kolste AK, Griffin KH, Rivard RL, Dusek JA (2016) Development of a Health System-Based Nurse-Delivered Aromatherapy Program. J Nurs Adm 46:221–225.

Kageyama A, Toshio U, Oshio M, Masuda H, Horiuchi H, Yokogoshi H (2012) Antidepressant-like Effects of an Aqueous Extract of Lavender (Lavandula angustifolia Mill.) in Rats. Food Sci Technol Res 18:473–479.

Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS (2004) Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behav Neurosci 118:1365–1377.

Keller-Wood M (2015) Hypothalamic-Pituitary-Adrenal Axis-Feedback Control. In:

Comprehensive Physiology, pp 1161–1182. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Kempermann G, Song H, Gage FH (2015) Neurogenesis in the Adult Hippocampus. Cold Spring Harb Perspect Biol 7:a018812.

Khairul M, Baharudin A, Hamid SA, Susanti D (2015) Chemical Composition and Antibacterial Activity of Essential Oils from Three Aromatic Plants of the Zingiberaceae Family in Malaysia. J Phys Sci 26:71–81.

Kim YS, Yoon B-E (2017) Altered GABAergic Signaling in Brain Disease at Various Stages of Life. Exp Neurobiol 26:122–131.

Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493.

Kitagishi Y, Kobayashi M, Kikuta K, Matsuda S (2012) Roles of PI3K/AKT/GSK3/mTOR Pathway in Cell Signaling of Mental Illnesses. Depress Res Treat 2012:1–8.

Köhler CA, Evangelou E, Stubbs B, Solmi M, Veronese N, Belbasis L, Bortolato B, Melo MCA, Coelho CA, Fernandes BS, Olfson M, Ioannidis JPA, Carvalho AF (2018) Mapping risk factors for depression across the lifespan: An umbrella review of evidence from meta-analyses and Mendelian randomization studies. J Psychiatr Res 103:189–207.Kohli P, Soler ZM, Nguyen SA, Muus JS, Schlosser RJ (2016) The Association Between Olfaction and Depression: A Systematic Review. Chem Senses 41:479–486.

Komiya M, Takeuchi T, Harada E (2006) Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. Behav Brain Res 172:240–249.

Koo M (2017) A bibliometric analysis of two decades of aromatherapy research. BMC Res Notes 10:46.

Kotasová H, Veselá I, Kučera J, Houdek Z, Procházková J, Králičková M, Pacherník J (2012) Phosphoinositide 3-Kinase Inhibition Enables Retinoic Acid-Induced Neurogenesis in Monolayer Culture of Embryonic Stem Cells. J Cell Biochem 113:563–570.

Kott JM, Mooney-Leber SM, Shoubah FA, Brummelte S (2016) Effectiveness of different corticosterone administration methods to elevate corticosterone serum levels, induce depressive-like behavior, and affect neurogenesis levels in female rats. Neuroscience 312:201–214.

Koutmani Y, Karalis KP (2015) Neural stem cells respond to stress hormones: distinguishing beneficial from detrimental stress. Front Physiol 6:77.

Krogsgaard-Larsen P (1992) GABA and Glutamate Receptors as Therapeutic Targets in Neurodegenerative Disorders. Pharmacol Toxicol 70:95–104.

Krugers HJ, Hoogenraad CC, Groc L (2010) Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. Nat Rev Neurosci 11:675–681.

Krusemark EA, Novak LR, Gitelman DR, Li W (2013) When the sense of smell meets emotion: anxiety-state-dependent olfactory processing and neural circuitry adaptation. J Neurosci 33:15324–15332.

Kuhn HG (2015) Control of Cell Survival in Adult Mammalian Neurogenesis. Cold Spring Harb Perspect Biol 7:a018895.

Lakhan SE, Sheafer H, Tepper D (2016) The Effectiveness of Aromatherapy in Reducing Pain: A Systematic Review and Meta-Analysis. Pain Res Treat 2016:8158693.

Lamers F, van Oppen P, Comijs HC, Smit JH, Spinhoven P, van Balkom AJLM, Nolen WA, Zitman FG, Beekman ATF, Penninx BWJH (2011) Comorbidity patterns of anxiety and depressive disorders in a large cohort study: the Netherlands Study of Depression and Anxiety (NESDA). J Clin Psychiatry 72:341–348.

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25:150–176.

Lang UE, Borgwardt S (2013) Molecular Mechanisms of Depression: Perspectives on New Treatment Strategies. Cell Physiol Biochem 31:761–777.

Lau BW-M, Lee JC-D, Li Y, Fung SM-Y, Sang Y-H, Shen J, Chang RC-C, So K-F (2012) Polysaccharides from Wolfberry Prevents Corticosterone-Induced Inhibition of Sexual Behavior and Increases Neurogenesis Borlongan C V., ed. PLoS One 7:e33374.

Lau BW-M, Ren C, Yang J, Yan SWL, Chang RC-C, Pu M, So K-F (2011a) Light deprivation induces depression-like behavior and suppresses neurogenesis in diurnal mongolian gerbil (Meriones unguiculatus). Cell Transplant 20:871–881.

Lau BW-M, Yau S-Y, Lee TMC, Ching Y-P, Tang S-W, So K-F (2011b) Effect of corticosterone and paroxetine on masculine mating behavior: possible involvement of neurogenesis. J Sex Med 8:1390–1403.

Lau BW, Lee JC, So K (2013) Neurogenic hypothesis and psychiatric disorders. Chinese Sci Bull 58:3188–3198.

Lebedeva KA, Caruncho HJ, Kalynchuk LE (2017) Cyclical corticosterone administration sensitizes depression-like behavior in rats. Neurosci Lett 650:45–51.

Lee B-H, Kim Y-K (2010) The Roles of BDNF in the Pathophysiology of Major Depression and in Antidepressant Treatment. Psychiatry Investig 7:231.

Lee B, Sur B, Kwon S, Yeom M, Shim I, Lee H, Hahm D-H (2013) Chronic administration of catechin decreases depression and anxiety-like behaviors in a rat model using chronic corticosterone injections. Biomol Ther (Seoul) 21:313–322.

Lee S, Jeong J, Kwak Y, Park SK (2010) Depression research: where are we now? Mol Brain 3:8.

Lemon K (2004) An assessment of treating depression and anxiety with aromatherapy. Int J Aromather 14:63–69.

Leuchtweis J, Boettger MK, Niv F, Redecker C, Schaible H-G (2014) Enhanced neurogenesis in the hippocampal dentate gyrus during antigen-induced arthritis in adult rat--a crucial role of immunization. PLoS One 9:e89258.

Leuner B, Caponiti JM, Gould E (2012) Oxytocin stimulates adult neurogenesis even

under conditions of stress and elevated glucocorticoids. Hippocampus 22:861–868.

Leuner B, Glasper ER, Gould E (2009) Thymidine analog methods for studies of adult neurogenesis are not equally sensitive. J Comp Neurol 517:123–133.

Leuner B, Gould E, Shors TJ (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16:216–224.

Li C, Carlier PR, Ren H, Kan KKW, Hui K, Wang H, Li W, Li Z, Xiong K, Clement EC, Xue H, Liu X, Li M, Pang Y, Han Y (2007a) Alkylene tether-length dependent γ-aminobutyric acid type A receptor competitive antagonism by tacrine dimers. Neuropharmacology 52:436–443.

Li CY, Wang H, Xue H, Carlier PR, Hui KM, Pang YP, Li ZW, Han YF (1999) Bis(7)-tacrine, a novel dimeric AChE inhibitor, is a potent GABA(A) receptor antagonist. Neuroreport 10:795–800.

Li W, Lee NTK, Fu H, Kan KKW, Pang Y, Li M, Tsim KWK, Li X, Han Y (2006) Neuroprotection via inhibition of nitric oxide synthase by bis(7)-tacrine. Neuroreport 17:471–474.

Li W, Mak M, Jiang H, Wang Q, Pang Y, Chen K, Han Y (2009) Novel anti-Alzheimer's dimer bis(7)-Cognitin: Cellular and molecular mechanisms of neuroprotection through multiple targets. Neurotherapeutics 6:187–201.

Li W, Pi R, Chan HHN, Fu H, Lee NTK, Tsang HW, Pu Y, Chang DC, Li C, Luo J, Xiong K, Li Z, Xue H, Carlier PR, Pang Y, Tsim KWK, Li M, Han Y (2005) Novel Dimeric Acetylcholinesterase Inhibitor Bis(7)-tacrine, but Not Donepezil,

Prevents Glutamate-induced Neuronal Apoptosis by Blocking *N* -Methyl-d-aspartate Receptors. J Biol Chem 280:18179–18188.

Li W, Xue J, Niu C, Fu H, Lam CSC, Luo J, Chan HHN, Xue H, Kan KKW, Lee NTK, Li C, Pang Y, Li M, Tsim KWK, Jiang H, Chen K, Li X, Han Y (2007b) Synergistic Neuroprotection by Bis(7)-tacrine via Concurrent Blockade of N-Methyl-D-aspartate Receptors and Neuronal Nitric-Oxide Synthase. Mol Pharmacol 71:1258–1267.

Li X-Y, Zhang J, Dai J-P, Liu X-M, Li Z-W (2010) Actions of bis(7)-tacrine and tacrine on transient potassium current in rat DRG neurons and potassium current mediated by KV4.2 expressed in Xenopus oocyte. Brain Res 1318:23–32.

Lieberwirth C, Liu Y, Jia X, Wang Z (2012) Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. Horm Behav 62:357–366.

Lillehei AS, Halcón LL, Savik K, Reis R (2015) Effect of Inhaled Lavender and Sleep Hygiene on Self-Reported Sleep Issues: A Randomized Controlled Trial. J Altern Complement Med 21:430–438.

Lim DA, Alvarez-Buylla A (2016) The Adult Ventricular–Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. Cold Spring Harb Perspect Biol 8:a018820.

Lin Y-T, Chen C-C, Huang C-C, Nishimori K, Hsu K-S (2017) Oxytocin stimulates hippocampal neurogenesis via oxytocin receptor expressed in CA3 pyramidal neurons. Nat Commun 8:537.

Linck V., da Silva ALL, Figueiró M, Caramão E. B, Moreno PRHRH, Elisabetsky E, Linck V. M, da Silva ALL, Figueiró M, Caramão E. B, Moreno PRHRH, Elisabetsky E (2010) Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice. Phytomedicine 17:679–683.

Lipton SA (2004) Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. NeuroRx 1:101–110.

Liu H, Song N (2016) Molecular Mechanism of Adult Neurogenesis and its Association with Human Brain Diseases. J Cent Nerv Syst Dis 8:5–11.

Liu J, Ho W, Lee NT, Carlier PR, Pang Y, Han Y (2000) Bis(7)-tacrine, a novel acetylcholinesterase inhibitor, reverses AF64A-induced deficits in navigational memory in rats. Neurosci Lett 282:165–168.

Liu S-H, Lin T-H, Chang K-M (2013) The physical effects of aromatherapy in alleviating work-related stress on elementary school teachers in taiwan. Evid Based Complement Alternat Med 2013:853809.

Liu Y-W, Li C-Y, Luo J-L, Li W-M, Fu H-J, Lao Y-Z, Liu L-J, Pang Y-P, Chang DC, Li Z-W, Peoples RW, Ai Y-X, Han Y-F (2008a) Bis(7)-tacrine prevents glutamate-induced excitotoxicity more potently than memantine by selectively inhibiting NMDA receptors. Biochem Biophys Res Commun 369:1007–1011.

Liu Y-W, Luo J-L, Ren H, Peoples RW, Ai Y-X, Liu L-J, Pang Y-P, Li Z-W, Han Y-F, Li C-Y (2008b) Inhibition of NMDA-gated ion channels by bis(7)-tacrine: Whole-cell and single-channel studies. Neuropharmacology 54:1086–1094.

Liu Y, Li C (2012) Inhibition of N-methyl-D-aspartate-activated current by bis(7)-tacrine in HEK-293 cells expressing NR1/NR2A or NR1/NR2B receptors. J Huazhong Univ Sci Technol [Medical Sci 32:793–797.

Lopes J, da Costa J, Ceschi M, Gonçalves C, Konrath E, Karl A, Guedes I, Dardenne L, Lopes JPB, Costa JS da, Ceschi MA, Gonçalves CAS, Konrath EL, Karl ALM, Guedes IA, Dardenne LE (2017) Chiral Bistacrine Analogues: Synthesis, Cholinesterase Inhibitory Activity and a Molecular Modeling Approach. J Braz Chem Soc 28:2218–2228.

López-Muñoz F, Alamo C (2009) Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr Pharm Des 15:1563–1586.

López-Muñoz F, Álamo C (2016) History of the Discovery of Antidepressant Drugs. In: Melatonin, Neuroprotective Agents and Antidepressant Therapy, pp 365–383. New Delhi: Springer India.

López V, Nielsen B, Solas M, Ramírez MJ, Jäger AK (2017) Exploring Pharmacological Mechanisms of Lavender (Lavandula angustifolia) Essential Oil on Central Nervous System Targets. Front Pharmacol 8:280.

Louis M, Kowalski SD (2002) Use of aromatherapy with hospice patients to decrease pain, anxiety, and depression and to promote an increased sense of well-being. Am J Hosp Palliat Care 19:381–386.

Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 7:126–136.

Luo J-L, Zhang J, Guan B-C, Pang Y-P, Han Y-F, Li Z-W (2004) Inhibition by bis(7)-tacrine of 5-HT-activated current in rat TG neurons. Neuroreport 15:1335–1338.

Luo J, Li W, Liu Y, Zhang W, Fu H, Lee NTK, Yu H, Pang Y, Huang P, Xia J, Li Z-W, Li C, Han Y (2007) Novel dimeric bis(7)-tacrine proton-dependently inhibits NMDA-activated currents. Biochem Biophys Res Commun 361:505–509.

Lussier AL, Lebedeva K, Fenton EY, Guskjolen A, Caruncho HJ, Kalynchuk LE (2013) The progressive development of depression-like behavior in corticosterone-treated rats is paralleled by slowed granule cell maturation and decreased reelin expression in the adult dentate gyrus. Neuropharmacology 71:174–183.

MacQueen G, Frodl T (2011) The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry 16:252–264.

Magni LR, Purgato M, Gastaldon C, Papola D, Furukawa TA, Cipriani A, Barbui C (2013) Fluoxetine versus other types of pharmacotherapy for depression. In: Cochrane Database of Systematic Reviews (Barbui C, ed). Chichester, UK: John Wiley & Sons, Ltd.

Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, Huhtaniemi I, Weiss S (2007) Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. Nat Neurosci 10:1003–1011.

Malberg JE, Duman RS (2003) Cell Proliferation in Adult Hippocampus is Decreased by Inescapable Stress: Reversal by Fluoxetine Treatment. Neuropsychopharmacology 28:1562–1571.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110.

Manion CR, Widder RM (2017) Essentials of essential oils. Am J Heal Pharm 74:e153–e162.

Marais L, Stein DJ, Daniels WMU (2009) Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. Metab Brain Dis 24:587–597.

Marks W, Fournier NM, Kalynchuk LE (2009) Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. Physiol Behav 98:67–72.

Matsumoto T, Asakura H, Hayashi T (2014) Effects of olfactory stimulation from the fragrance of the Japanese citrus fruit yuzu (Citrus junos Sieb. ex Tanaka) on mood states and salivary chromogranin A as an endocrinologic stress marker. J Altern Complement Med 20:500–506.

Matsumoto T, Kimura T, Hayashi T (2016) Aromatic effects of a Japanese citrus fruit-yuzu (Citrus junos Sieb. ex Tanaka)-on psychoemotional states and autonomic nervous system activity during the menstrual cycle: a single-blind randomized controlled crossover study. Biopsychosoc Med 10:11.

Mattson MP (2000) Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 1:120–130.

McEwen BS, Magarinos AM (2001) Stress and hippocampal plasticity: implications

for the pathophysiology of affective disorders. Hum Psychopharmacol Clin Exp 16:S7–S19.

McNeilly AS, Ducker HA (1972) Blood Levels of oxytocin in the female goat during coitus and in response to stimuli associated with mating. J Endocrinol 54:399–406.

Meijering E (2010) Neuron tracing in perspective. Cytometry A 77:693–704.

Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R (2006) Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. Nat Neurosci 9:729–731.

Ming G, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28:223–250.

Mirescu C, Gould E (2006) Stress and adult neurogenesis. Hippocampus 16:233–238.

Mouly A-M, Sullivan R (2010) Memory and Plasticity in the Olfactory System: From Infancy to Adulthood. CRC Press/Taylor & Francis.

Murray F, Smith DW, Hutson PH (2008) Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. Eur J Pharmacol 583:115–127.

Mutunga JM, Boina DR, Anderson TD, Bloomquist JR, Carlier PR, Wong DM, Lam PC-H, Totrov MM (2013) Neurotoxicology of bis(n)-tacrines on Blattella germanica and Drosophila melanogaster acetylcholinesterase. Arch Insect Biochem Physiol 83:180–194.

National Collaborating Centre for Mental Health (2010) The classification of depression and rating scales/questionnaires. Br Psychol Scociety.

Ndao DH, Ladas EJ, Cheng B, Sands SA, Snyder KT, Garvin JH, Kelly KM (2012) Inhalation aromatherapy in children and adolescents undergoing stem cell infusion: results of a placebo-controlled double-blind trial. Psychooncology 21:247–254.

Negoias S, Croy I, Gerber J, Puschmann S, Petrowski K, Joraschky P, Hummel T (2010) Reduced olfactory bulb volume and olfactory sensitivity in patients with acute major depression. Neuroscience 169:415–421.

Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35:649–659.

Newcomer JW, Farber NB, Olney JW (2000) NMDA receptor function, memory, and brain aging. Dialogues Clin Neurosci 2:219–232.

Nezafati MH, Vojdanparast M, Nezafati P (2015) Antidepressants and cardiovascular adverse events: A narrative review. ARYA Atheroscler 11:295–304.

Nie H, Yu W-J, Li X-Y, Yuan C-H, Pang Y-P, Li C-Y, Han Y-F, Li Z-W (2007) Inhibition by bis(7)-tacrine of native delayed rectifier and KV1.2 encoded potassium channels. Neurosci Lett 412:108–113.

Nowakowska E, Kus K, Bobkiewicz-Kozłowska T, Hertmanowska H (2002) Role of neuropeptides in antidepressant and memory improving effects of venlafaxine. Pol J Pharmacol 54:605–613.

Nowakowski RS, Hayes NL (2008) 2 Numerology of Neurogenesis: Characterizing the Cell Cycle of Neurostem Cells. Cold Spring Harb Monogr Arch 52:7–23.

Numakawa T, Odaka H, Adachi N (2017) Actions of Brain-Derived Neurotrophic Factor and Glucocorticoid Stress in Neurogenesis. Int J Mol Sci 18:2312.

Nunciato AC, Meirelles EL, Gardino P (2013) From Micro to Macro: Regulation Up- and Down-Adult Neurogenesis Process. J Behav Brain Sci 3:489–496.

Nuss P (2015) Anxiety disorders and GABA neurotransmission: a disturbance of modulation. Neuropsychiatr Dis Treat 11:165–175.

O'Connor TM, O'Halloran DJ, Shanahan F (2000) The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. QJM 93:323–333.

O'Neill KM, Akum BF, Dhawan ST, Kwon M, Langhammer CG, Firestein BL (2015) Assessing effects on dendritic arborization using novel Sholl analyses. Front Cell Neurosci 9:285.

Olescowicz G, Neis VB, Fraga DB, Rosa PB, Azevedo DP, Melleu FF, Brocardo PS, Gil-Mohapel J, Rodrigues ALS (2018) Antidepressant and pro-neurogenic effects of agmatine in a mouse model of stress induced by chronic exposure to corticosterone. Prog Neuro-Psychopharmacology Biol Psychiatry 81:395–407.

Owens MJ, Nemeroff CB (1994) Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 40.

Pan S-Y, Zhang Y, Guo B, Han Y-F, Ko K-M (2011) Tacrine and Bis(7)-Tacrine Attenuate Cycloheximide-Induced Amnesia in Mice, with Attention to Acute

Toxicity. Basic Clin Pharmacol Toxicol 109:261–265.

Pan SY, Yu ZL, Dong H, Lee NTK, Wang H, Fong WF, Han YF, Ko KM (2007) Evaluation of acute bis(7)-tacrine treatment on behavioral functions in 17-day-old and 30-day-old mice, with attention to drug toxicity. Pharmacol Biochem Behav 86:778–783.

Pangalos MN, Schechter LE, Hurko O (2007) Drug development for CNS disorders: strategies for balancing risk and reducing attrition. Nat Rev Drug Discov 6:521–532.

Pariante CM (2006) The glucocorticoid receptor: part of the solution or part of the problem? J Psychopharmacol 20:79–84.

Pariante CM, Kim RB, Makoff A, Kerwin RW (2003) Antidepressant fluoxetine enhances glucocorticoid receptor function *in vitro* by modulating membrane steroid transporters. Br J Pharmacol 139:1111–1118.

Pariante CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. Trends Neurosci 31:464–468.

Parker KJ, Kenna HA, Zeitzer JM, Keller J, Blasey CM, Amico JA, Schatzberg AF (2010) Preliminary evidence that plasma oxytocin levels are elevated in major depression. Psychiatry Res 178:359–362.

Pérez-Hernández J, Zaldívar-Machorro VJ, Villanueva-Porras D, Vega-Ávila E, Chavarría A (2016) A Potential Alternative against Neurodegenerative Diseases: Phytodrugs. Oxid Med Cell Longev 2016.

Perry N, Perry E (2006) Aromatherapy in the management of psychiatric disorders:

clinical and neuropharmacological perspectives. CNS Drugs 20:257–280.

Perry R, Terry R, Watson LK, Ernst E (2012) Is lavender an anxiolytic drug? A systematic review of randomised clinical trials. Phytomedicine 19:825–835.

Petrik D, Lagace DC, Eisch AJ (2012) The neurogenesis hypothesis of affective and anxiety disorders: Are we mistaking the scaffolding for the building? Neuropharmacology 62:21–34.

Piepmeier AT, Etnier JL (2015) Brain-derived neurotrophic factor (BDNF) as a potential mechanism of the effects of acute exercise on cognitive performance. J Sport Heal Sci 4:14–23.

Pignatelli A, Belluzzi O (2010) Neurogenesis in the Adult Olfactory Bulb. CRC Press/Taylor & Francis.

Pilar-Cuéllar F, Vidal R, Díaz A, Castro E, dos Anjos S, Pascual-Brazo J, Linge R, Vargas V, Blanco H, Martínez-Villayandre B, Pazos Á, Valdizán EM, Mart&#xed, nez-Villayandre B, Pazos &#xc1, ngel, Valdiz&#xe1, n EM (2013) Neural plasticity and proliferation in the generation of antidepressant effects: hippocampal implication. Neural Plast 2013:537265.

Pilar Muñoz-Ruiz §, Laura Rubio §, Esther García-Palomero §, Isabel Dorronsoro §, María del Monte-Millán §, Rita Valenzuela §, Paola Usán §, Celia de Austria §, Manuela Bartolini ‡, Vincenza Andrisano ‡, Axel Bidon-Chanal #, Modesto Orozco †, F. Javier Luque #, Miguel Medina § and, Ana Martínez* § (2005) Design, Synthesis, and Biological Evaluation of Dual Binding Site Acetylcholinesterase Inhibitors: New Disease-Modifying Agents for Alzheimer's Disease.

Plümpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Römer B, Rodriguez GR, Kronenberg G, Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. BMC Neurosci 7:77.

Polacchini A, Metelli G, Francavilla R, Baj G, Florean M, Mascaretti LG, Tongiorgi E (2016) A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. Sci Rep 5:17989.

Preskorn SH (1995) Comparison of the tolerability of bupropion, fluoxetine, imipramine, nefazodone, paroxetine, sertraline, and venlafaxine. J Clin Psychiatry 56 Suppl 6:12–21.

Price L, Price S (2011) Aromatherapy for Health Professionals. Elsevier Health Sciences.

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33.

Qaseem A, Barry MJ, Kansagara D, Clinical Guidelines Committee of the American College of Physicians (2016) Nonpharmacologic Versus Pharmacologic Treatment of Adult Patients With Major Depressive Disorder: A Clinical Practice Guideline From the American College of Physicians. Ann Intern Med 164:350–359.

Qiu G, Helmeste DM, Samaranayake AN, Lau W-M, Lee TMC, Tang S-W, So K-F (2007) Modulation of the suppressive effect of corticosterone on adult rat hippocampal cell proliferation by paroxetine. Neurosci Bull 23:131–136.

Qureshi NA, Al-Bedah AM (2013) Mood disorders and complementary and alternative medicine: a literature review. Neuropsychiatr Dis Treat 9:639–658.

Ramirez-Rodriguez G, Ortíz-López L, Domínguez-Alonso A, Benítez-King GA, Kempermann G (2011) Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 50:29–37.

Ren Y, Zhu C, Wu J, Zheng R, Cao H (2015) Comparison between herbal medicine and fluoxetine for depression: a systematic review of randomized controlled trials. Complement Ther Med 23:674–684.

Richelson E (2013) Multi-modality: a new approach for the treatment of major depressive disorder. Int J Neuropsychopharmacol:1–10.

Ring RH, Schechter LE, Leonard SK, Dwyer JM, Platt BJ, Graf R, Grauer S, Pulicicchio C, Resnick L, Rahman Z, Sukoff Rizzo SJ, Luo B, Beyer CE, Logue SF, Marquis KL, Hughes ZA, Rosenzweig-Lipson S (2010) Receptor and behavioral pharmacology of WAY-267464, a non-peptide oxytocin receptor agonist. Neuropharmacology 58:69–77.

Rizzo S, Bisi A, Bartolini M, Mancini F, Belluti F, Gobbi S, Andrisano V, Rampa A (2011) Multi-target strategy to address Alzheimer's disease: design, synthesis and biological evaluation of new tacrine-based dimers. Eur J Med Chem 46:4336–4343.

Rolls ET, Kringelbach ML, de Araujo IET (2003) Different representations of pleasant and unpleasant odours in the human brain. Eur J Neurosci 18:695–703.

Ros E, Aleu J, Gomez De Aranda I, Cantí C, Pang Y-P, Marsal J, Solsona C (2001) Effects of Bis(7)-Tacrine on Spontaneous Synaptic Activity and on the Nicotinic ACh Receptor of *Torpedo* Electric Organ. J Neurophysiol 86:183–189.

Rotzinger S, Lovejoy DA, Tan LA (2010) Behavioral effects of neuropeptides in rodent models of depression and anxiety. Peptides 31:736–756.

Russell NC, Sumler S-S, Beinhorn CM, Frenkel MA (2008) Role of massage therapy in cancer care. J Altern Complement Med 14:209–214.

Sah A, Schmuckermair C, Sartori SB, Gaburro S, Kandasamy M, Irschick R, Klimaschewski L, Landgraf R, Aigner L, Singewald N (2012) Anxiety- rather than depression-like behavior is associated with adult neurogenesis in a female mouse model of higher trait anxiety- and comorbid depression-like behavior. Transl Psychiatry 2:e171–e171.

Saltiel PF, Silvershein DI (2015) Major depressive disorder: mechanism-based prescribing for personalized medicine. Neuropsychiatr Dis Treat 11:875–888.

Sánchez-Vidaña DI, Chan JN-M, Chan AHL, Hui KKY, Lee S, Chan H-YH, Law YS, Sze MY, Tsui W-CS, Fung TKH, Lau BW-M, Lai CYY (2016) Repeated treatment with oxytocin promotes hippocampal cell proliferation, dendritic maturation and affects socio-emotional behavior. Neuroscience 333:65–77.

Sánchez-Vidaña DI, Ngai SP-C, He W, Chow JK-WJ, Lau BW-M, Tsang HW-H (2017) The Effectiveness of Aromatherapy for Depressive Symptoms: A Systematic Review. Evidence-Based Complement Altern Med 2017:21.

Sandi C, Haller J (2015) Stress and the social brain: behavioural effects and

neurobiological mechanisms. Nat Rev Neurosci 16:290–304.

Sarafoleanu C, Mella C, Georgescu M, Perederco C (2009) The importance of the olfactory sense in the human behavior and evolution. J Med Life 2:196–198.

Scherer IJ, Holmes P V, Harris RBS (2011) The importance of corticosterone in mediating restraint-induced weight loss in rats. Physiol Behav 102:225–233.

Schmidt HD, Shelton RC, Duman RS (2011) Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. Neuropsychopharmacology 36:2375–2394.

Scuteri D, Morrone LA, Rombolà L, Avato PR, Bilia AR, Corasaniti MT, Sakurada S, Sakurada T, Bagetta G (2017) Aromatherapy and Aromatic Plants for the Treatment of Behavioural and Psychological Symptoms of Dementia in Patients with Alzheimer's Disease: Clinical Evidence and Possible Mechanisms.

Sehhatie F, Mirgafourvand M, Niri ZV (2015) The Effect of Non-Pharmaceutical Methods of Labor Pain Relief on Mothers' Postpartum Depression: A Randomized Controlled Trial. IJWHR 3:48–55.

Seol GH, Shim HS, Kim P-J, Moon HK, Lee KH, Shim I, Suh SH, Min SS (2010) Antidepressant-like effect of Salvia sclarea is explained by modulation of dopamine activities in rats. J Ethnopharmacol 130:187–190.

Serfaty M, Wilkinson S, Freeman C, Mannix K, King M (2012) The ToT study: helping with Touch or Talk (ToT): a pilot randomised controlled trial to examine the clinical effectiveness of aromatherapy massage versus cognitive behaviour therapy for emotional distress in patients in cancer/palliative care.

Psychooncology 21:563–569.

Setzer WN (2009) Essential oils and anxiolytic aromatherapy. Nat Prod Commun 4:1305–1316.

Shellie R, Marriott P, Cornwell C (2000) Characterization and Comparison of Tea Tree and Lavender Oils by Using Comprehensive Gas Chromatography. J High Resolut Chromatogr 23:554–560.

Shellie R, Mondello L, Marriott P, Dugo G (2002) Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. J Chromatogr A 970:225–234.

Shen J, Niijima A, Tanida M, Horii Y, Maeda K, Nagai K (2005) Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 383:188–193.

Shirsat K, Prasad PD, Dhake AS (2013) Research Journal of Topical and Cosmetic Sciences. A & V Publications.

Sholl DA (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. J Anat 87:387–406.

Shu X-J, Liu W, Zhang L, Yang R, Yi H-L, Li C-L, Ye Y-J, Ai Y-X (2012) Effect of bis(7)-tacrine on cognition in rats with chronic cerebral ischemia. Neurosci Lett 512:103–108.

Sierksma ASR, van den Hove DLA, Steinbusch HWM, Prickaerts J (2010) Major depression, cognitive dysfunction and Alzheimer's disease: is there a link? Eur J

Pharmacol 626:72–82.

Singh P, Chaturvedi A (2015) Complementary and alternative medicine in cancer pain management: a systematic review. Indian J Palliat Care 21:105–115.

Slattery DA, Cryan JF (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc 7:1009–1014.

Slattery DA, Neumann ID (2010a) Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. Neuropharmacology 58:56–61.

Slattery DA, Neumann ID (2010b) Oxytocin and Major Depressive Disorder: Experimental and Clinical Evidence for Links to Aetiology and Possible Treatment. Pharmaceuticals 3:702–724.

Smith CA, Hay PP, Macpherson H (2010) Acupuncture for depression. Cochrane database Syst Rev:CD004046.

Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476:458–461.

So KF, Tang SW, Lee TMC (2012) Neuroprotection in steroid therapy: a rodent model. Hong Kong Med J 18 Suppl 3:20–23.

Soden K, Vincent K, Craske S, Lucas C, Ashley S (2004) A randomized controlled trial of aromatherapy massage in a hospice setting. Palliat Med 18:87–92.

Song J, Christian KM, Ming G, Song H (2012) Modification of hippocampal circuitry by adult neurogenesis. Dev Neurobiol 72:1032–1043.

Soudry Y, Lemogne C, Malinvaud D, Consoli S-M, Bonfils P (2011) Olfactory system and emotion: Common substrates. Eur Ann Otorhinolaryngol Head Neck Dis 128:18–23.

Sousa-Ferreira L, Aveleira C, Botelho M, Álvaro AR, Pereira de Almeida L, Cavadas C (2014) Fluoxetine Induces Proliferation and Inhibits Differentiation of Hypothalamic Neuroprogenitor Cells In Vitro Alquier T, ed. PLoS One 9:e88917.

Sousa N, Lukoyanov NV, Madeira MD, Almeida OFX, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97:253–266.

Staní D, Plé Caš-Solaroví B, Mirkoví D, Jovanoví P, Dana Dronjak S–, Markoví B, Đor T, Deví C –, Ignjatoví S, Peší V (2017) Oxytocin in corticosterone-induced chronic stress model: Focus on adrenal gland function. Psychoneuroendocrinology 80:137–146.

Strawbridge R, Young AH, Cleare AJ (2017) Biomarkers for depression: recent insights, current challenges and future prospects. Neuropsychiatr Dis Treat 13:1245–1262.

Sun Y, Liu W-Z, Liu T, Feng X, Yang N, Zhou H-F (2015) Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J Recept Signal Transduct Res 35:600–604.

Taalman H, Wallace C, Milev R (2017) Olfactory Functioning and Depression: A Systematic Review. Front psychiatry 8:190.

Taavoni S, Darsareh F, Joolaee S, Haghani H (2013) The effect of aromatherapy massage on the psychological symptoms of postmenopausal Iranian women. Complement Ther Med 21:158–163.

Taupin P (2006) Neurogenesis and the effect of antidepressants. Drug Target Insights 1:13–17.

Taupin P (2007) BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198–214.

Teipel S, Grothe MJ, Zhou J, Sepulcre J, Dyrba M, Sorg C, Babiloni C (2016) Measuring Cortical Connectivity in Alzheimer's Disease as a Brain Neural Network Pathology: Toward Clinical Applications. J Int Neuropsychol Soc 22:138–163.

Tisserand R, Balacs T (1995) Essential oil safety: A guide for health care professionals, 1st ed. Churchill Livingstone Elsevier.

Tizabi Y (2015) Duality of Antidepressants and Neuroprotectants. Neurotox Res 30:1–13.

Tsang HWH, Lo SCL, Chan CCH, Ho TYC, Fung KMT, Chan AHL, Au DWH (2013) Neurophysiological and behavioural effects of lavender oil in rats with experimentally induced anxiety. Flavour Fragr J 28:168–173.

Vadodaria KC, Gage FH (2014) SnapShot: Adult Hippocampal Neurogenesis. Cell 156:1114–1114.e1.

Vadodaria KC, Jessberger S (2013) Maturation and integration of adult born hippocampal neurons: signal convergence onto small Rho GTPases. Front

Synaptic Neurosci 5:4.

Valera E, Masliah E (2016) Therapeutic approaches in Parkinson's disease and related disorders. J Neurochem 139:346–352.

van der Watt G, Laugharne J, Janca A (2008) Complementary and alternative medicine in the treatment of anxiety and depression. Curr Opin Psychiatry 21:37–42.

Vassar R, Kovacs DM, Yan R, Wong PC (2009) The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. J Neurosci 29:12787–12794.

Ventola CL (2010) Current Issues Regarding Complementary and Alternative Medicine (CAM) in the United States: Part 1: The Widespread Use of CAM and the Need for Better-Informed Health Care Professionals to Provide Patient Counseling. P T 35:461–468.

Vilar M, Mira H (2016) Regulation of Neurogenesis by Neurotrophins during Adulthood: Expected and Unexpected Roles. Front Neurosci 10:26.

Wacker DW, Ludwig M (2012) Vasopressin, oxytocin, and social odor recognition. Horm Behav 61:259–265.

Wainwright SR, Galea LAM, Wainwright SR, Galea LAM (2013) The neural plasticity theory of depression: assessing the roles of adult neurogenesis and PSA-NCAM within the hippocampus. Neural Plast 2013:805497.

Wang H, Carlier PR, Ho WL, Wu DC, Lee NT, Li CP, Pang YP, Han YF (1999a) Effects of bis(7)-tacrine, a novel anti-Alzheimer's agent, on rat brain AChE.

Neuroreport 10:789–793.

Wang H, Carlier PR, Ho WL, Wu DC, Lee NTK, Li CPL, Pang YP, Han YF (1999b) Effects of bis(7)-tacrine, a novel anti-Alzheimer's agent, on rat brain AChE. Neuroreport 10:789–793.

Wang H, Zhou X, Huang J, Mu N, Guo Z, Wen Q, Wang R, Chen S, Feng Z-P, Zheng W (2013) The role of Akt/FoxO3a in the protective effect of venlafaxine against corticosterone-induced cell death in PC12 cells. Psychopharmacology (Berl) 228:129–141.

Wang Y, Cui X-L, Liu Y-F, Gao F, Wei D, Li X-W, Wang H-N, Tan Q-R, Jiang W (2011) LPS inhibits the effects of fluoxetine on depression-like behavior and hippocampal neurogenesis in rats. Prog Neuro-Psychopharmacology Biol Psychiatry 35:1831–1835.

Waters P, McCormick CM (2011) Caveats of chronic exogenous corticosterone treatments in adolescent rats and effects on anxiety-like and depressive behavior and hypothalamic-pituitary-adrenal (HPA) axis function. Biol Mood Anxiety Disord 1:4.

WHO (1993) The ICD-10 Classification of Mental and Behavioural Disorders Diagnostic criteria for research.

WHO (2013) WHO | Sixty-fifth World Health Assembly. WHO.

WHO (2016) WHO | Depression. WHO.

Wieland LS, Manheimer E, Berman BM (2011) Development and classification of an operational definition of complementary and alternative medicine for the

Cochrane collaboration. Altern Ther Health Med 17:50–59.

Wilkinson S (1995) Aromatherapy and massage in palliative care. Int J Palliat Nurs 1:21–30.

Wilkinson SM, Love SB, Westcombe AM, Gambles MA, Burgess CC, Cargill A, Young T, Maher EJ, Ramirez AJ (2007) Effectiveness of aromatherapy massage in the management of anxiety and depression in patients with cancer: a multicenter randomized controlled trial. J Clin Oncol 25:532–539.

Witt DM, Winslow JT, Insel TR (1992) Enhanced social interactions in rats following chronic, centrally infused oxytocin. Pharmacol Biochem Behav 43:855–861.

Wittenborn AK, Rahmandad H, Rick J, Hosseinichimeh N (2015) Depression as a systemic syndrome: mapping the feedback loops of major depressive disorder. Psychol Med 46:1–12.

Wolkowitz OM, Reus VI, Mellon SH (2011) Of sound mind and body: depression, disease, and accelerated aging. Dialogues Clin Neurosci 13:25–39.

Wong EYH, Herbert J (2005) Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. Eur J Neurosci 22:785–792.

Woolley JD, Lam O, Chuang B, Ford JM, Mathalon DH, Vinogradov S (2015) Oxytocin administration selectively improves olfactory detection thresholds for lyral in patients with schizophrenia. Psychoneuroendocrinology 53:217–222.

Wu C, Sun D (2015) GABA receptors in brain development, function, and injury. Metab Brain Dis 30:367–379.

Wu J-J, Cui Y, Yang Y-S, Kang M-S, Jung S-C, Park HK, Yeun H-Y, Jang WJ, Lee S, Kwak YS, Eun S-Y (2014) Modulatory effects of aromatherapy massage intervention on electroencephalogram, psychological assessments, salivary cortisol and plasma brain-derived neurotrophic factor. Complement Ther Med 22:456–462.

Wu P, James W (2011) Lavender. Dermatitis 22:344–347.

Wuwongse S, Chang RC-C, Law ACK (2010) The putative neurodegenerative links between depression and Alzheimer's disease. Prog Neurobiol 91:362–375.

Xiao XQ, Lee NT, Carlier PR, Pang Y, Han YF (2000) Bis(7)-tacrine, a promising anti-Alzheimer's agent, reduces hydrogen peroxide-induced injury in rat pheochromocytoma cells: comparison with tacrine. Neurosci Lett 290:197–200.

Yan Y, Wang Y-L, Su Z, Zhang Y, Guo S-X, Liu A-J, Wang C-H, Sun F-J, Yang J (2014) Effect of oxytocin on the behavioral activity in the behavioral despair depression rat model. Neuropeptides 48:83–89.

Yeung W-F, Chung K-F, Ng K-Y, Yu Y-M, Zhang S-P, Ng BF-L, Ziea ET-C (2015) Prescription of Chinese Herbal Medicine in Pattern-Based Traditional Chinese Medicine Treatment for Depression: A Systematic Review. Evid Based Complement Alternat Med 2015:160189.

Yim VWC, Ng AKY, Tsang HWH, Leung AY (2009) A review on the effects of aromatherapy for patients with depressive symptoms. J Altern Complement Med 15:187–195.

Young EA, Kwak SP, Kottak J (1995) Negative feedback regulation following

administration of chronic exogenous corticosterone. J Neuroendocrinol 7:37–45.

Young SN, Moskowitz DS, aan het Rot M (2014) Possible role of more positive social behaviour in the clinical effect of antidepressant drugs. J Psychiatry Neurosci 39:60–65.

Yu H, Li W-M, Kan KKW, Ho JMK, Carlier PR, Pang Y-P, Gu Z-M, Zhong Z, Chan K, Wang Y-T, Han Y-F (2008) The physicochemical properties and the in vivo AChE inhibition of two potential anti-Alzheimer agents, bis(12)-hupyridone and bis(7)-tacrine. J Pharm Biomed Anal 46:75–81.

Yu IT, Lee S-H, Lee Y-S, Son H (2004) Differential effects of corticosterone and dexamethasone on hippocampal neurogenesis in vitro. Biochem Biophys Res Commun 317:484–490.

Zagorcheva T, Stanev S, Rusanov K, Atanassov I (2013) Comparative GC/MS analysis of lavender (Lavandula angustifolia Mill.) inflorescence and essential oil volatiles. J Agric Sci Technol 5:459–462.

Zeng B, Li Y, Niu B, Wang X, Cheng Y, Zhou Z, You T, Liu Y, Wang H, Xu J (2016) Involvement of PI3K/Akt/FoxO3a and PKA/CREB Signaling Pathways in the Protective Effect of Fluoxetine Against Corticosterone-Induced Cytotoxicity in PC12 Cells. J Mol Neurosci 59:567–578.

Zhang Z-H, Liu Y-W, Jiang F-G, Tian X, Zhu Y-H, Li J-B, Wang Q, Fang J-H (2011) Bis(7)-tacrine protects retinal ganglion cells against excitotoxicity via NMDA receptor inhibition. Int J Ophthalmol 4:125–130.

Zhao C, Deng W, Gage FH (2008a) Mechanisms and Functional Implications of Adult

Neurogenesis. Cell 132:645–660.

Zhao Y, Li W, Chow PCY, Lau DTK, Lee NTK, Pang Y, Zhang X, Wang X, Han Y (2008b) Bis(7)-tacrine, a promising anti-Alzheimer's dimer, affords dose- and time-dependent neuroprotection against transient focal cerebral ischemia. Neurosci Lett 439:160–164.

Zhou L, Liu Y-W, Peoples RW, Yang M, Tian X, Ai Y-X, Pang Y-P, Li Z-W, Han Y-F, Li C-Y (2009) Mechanism of bis(7)-tacrine inhibition of GABA-activated current in cultured rat hippocampal neurons. Neuropharmacology 57:33–40.

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 9:268–275.

Biography of the author

By Sachin Shende

Picture by Harun Venkatesan

Born and raised near a small town called Texcoco around Mexico City. She grew up in Mexico, then moved to The Netherlands for her master's in Biomedical Sciences, eventually studying at The Hong Kong Polytechnic University for her PhD research study in Biological Sciences.

She attended National School of Biological Sciences from 2000-2004 and graduated with **Bachelor in Pharmaceutical Industrial Chemistry** in Biological Sciences. While at NSBS, she worked on the preparation of imidazo-oxazol compound with potential biological activity with Dr. María Elena Campos Aldrete. Here, she developed a passion for interdisciplinary approaches to solving the effective drug development challenges.

Dalinda received another national scholarship from the University of Belize to pursue her passion for language learning. She received her diploma in English as a Second Language from the Regional Language Centre of the University of Belize. From here, she started exploring the world of limitless potential. She had already covered about 20% of the global population. Now, mastering English, she is eagerly ready to commune with more than half of the world. And, here, Europe has already started paving the platform.

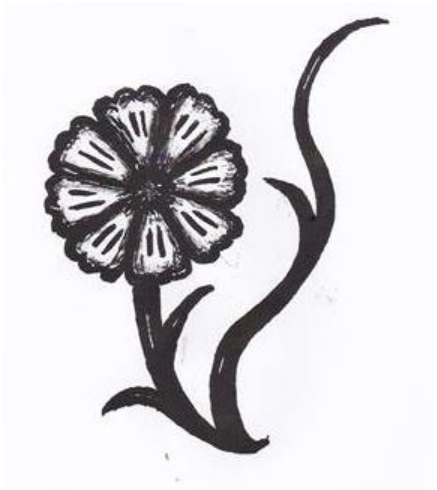
After undergraduate school, Dalinda, the 'author' of this thesis, moved to The Netherlands to fulfil her aspiration of higher studies. Of course, the biological sciences have always been a motivation. She attended Utrecht University and enrolled for a Master's program in Drug Innovation during 2009-2011. She was awarded a respected fellowship from Utrecht University, the Utrecht Excellence Scholarship. Her thesis research was based on the development of the biofilm formation and biofilm destruction *in vitro* assays for *Pseudomonas aeruginosa*. Medicinal plants were used in the treatment of bovine mastitis.

She was so much passionate about the European traditions and Dutch culture, she decided to a take the citizenship of The Netherlands. But, for those who are born to set up trends by pursuing the hardship; never take rest. Yes, she got through another scholarship, and this time; it's a continent of diverse culture. Asia. And that too, she landed to the mini world … Hong Kong.

In 2014, she entered the PhD program at The Hong Kong Polytechnic University. As a part of her PhD studies in the Department of Rehabilitation Sciences, she focused on drug development to reduce anxiety and depression. At PolyU, she published various articles on treatment options for depression and other related issues. The author becomes interested in the issues of the neurogenic and behavioural effect of Bis-7-cognition and lavender essential oil while working with Dr. Benson Wui-Man Lau, Professor Hector Tsang and Dr. Shirley Ngai. The selective perception towards her own research interest was a reason behind leaving her previous lab. She was blessed with the consistent supervision of Dr. Lau to groom her in such a profound way.

Dalinda communicated many of her significant research findings in reputed scientific journals. Her publications focused on vaccine development, quality control, drug discovery and proof of concept. She attracted various grants to lead the projects. She also contributed to various other institutional scientific, academic and University Campus Sustainability projects and lead to overall success. Before submitting her doctoral thesis, she was a research fellow at the Department of Rehabilitation Sciences in PolyU, Hong Kong. She would be starting her professional carrier as a postdoctoral fellow for the Department of Neurosciences and Physiology which is a part of the University of University of Gothenburg setup.

In December 2017, she got engaged with her longtime partner Kristofer Söderqvist. She finally decided to settle down in Sweden to devoting her rest of the life towards scientific contribution to the well-being of humankind and explore peace through meditation for ultimate liberation.



Cosmos flower / the love flower by Kristofer Söderqvist