THE AVERSIVE EFFECTS OF COUGH SUPPRESSANTS ON NEUROGENESIS AND EMOTIONAL BEHAVIOUR: CHRONIC EXPOSURE TO A COMMON ACTIVE INGREDIENT IN COUGH SUPPRESSANTS CAUSES EMOTIONAL DISTURBANCES THROUGH THE SUPPRESSION OF ADULT NEUROGENESIS, AND THEIR PREVENTION BY WOLFBERRY POLYSACCHARIDE IN RATS

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Po Kai Ting

A thesis submitted in partial fulfilment of the requirements for

the degree of Master of Philosophy

June 2016

CERTIFICATE OF ORIGINALITY

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Po Kai Ting
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Abstract of thesis entitled "The Aversive Effects of Cough Suppressants on Neurogenesis and Emotional Behaviour: Chronic exposure to a common active ingredient in cough suppressants causes emotional disturbances through the suppression of adult neurogenesis, and their prevention by wolfberry polysaccharide in rats" submitted by Po Kai Ting for the degree of Master of Philosophy at the Hong Kong Polytechnic University in June 2016

ABSTRACT

Cough syrup abuse is a worldwide problem with increasing prevalence, causing chronic disability to abusers. Clinical studies have revealed that abusive consumption of the cough medicine can lead to various psychiatric signs and symptoms including delusion, hallucination, anxiety and depression. These persistent psychiatric symptoms not only disturb the abusers, but are also heavy burdens to the healthcare system. However, the underlying mechanisms of these adverse effects remain elusive. In order to investigate the mechanisms as well as the possible adjunctive treatments, the objectives of the present study are: 1. To explore the effect of repeated, high-dose

dextromethorphan (DXM, a common antitussive component in cough syrup) treatment on adult hippocampal neurogenesis; 2. to study the dose-response effect of DXM on neurogenesis, and 3. to investigate the potential effect of wolfberry (a traditional Chinese herb) polysaccharide on reducing emotional distress caused by repeated treatment of DXM.

The results revealed that high-dose DXM treatment induced mood disturbances and suppressed neurogenesis, while wolfberry polysaccharide could reverse the undesirable effects. These findings suggest that neurogenesis may be an underlying mechanisms of emotional symptoms induced by DXM, and the result also provided insights for using wolfberry polysaccharide, an herbal component, as a possible adjunctive treatment for the rehabilitation of cough syrup abusers suffering from emotional signs and symptoms.

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Kevin Po Kai Ting

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Antigen retrieval (AR)

Blood–brain barrier (BBB)

Brain microvessel endothelial cells (BMECs)

Bromodeoxyuridine (BrdU)

Diaminobenzidine (DAB)

Dentate gyrus (DG)

Dextromethorphan (DXM)

Doublecortin (DCX)

Electroconvulsive therapy (ECT)

Forced swimming test (FST)

Gastrointestinal (GI)

Ginseng total saponins (GTS)

N-methyl-d-aspartate (NMDA)

Open field test (OFT)

Over-the-counter (OTC)

Paraformaldehyde (PFA)

Social interaction test (SIT)

Tail suspension test (TST)

CHAPTER 1. INTRODUCTION

1.1 Dextromethorphan (DXM): abuse and pharmacology

Cough syrup is one of the most commonly abused drugs for recreational purposes, although it is regarded as relatively safe over the counter (OTC) medication. Being highly affordable and readily accessible, the drug has become very popular worldwide for abusive consumption. Abuse of cough syrup can lead to persistent psychiatric symptoms, including delusion, hallucination, anxiety and depression, which not only cause disturbances for abusers, but also create a heavy burden on healthcare systems.

This study focused on affective symptoms induced by dextromethorphan (DXM). Being similar to other psychiatric diseases, the biological mechanism of DXM induced affective symptoms remains controversial and there are contradictory theories about the cause of the symptoms. One of the hypotheses, neurogenesis hypothesis, was studied in the present study.

An active component of cough syrup, DXM (Amaladoss & O'Brien, 2011) is a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Werling, Lauterbach, & Calef, 2007) that acts on the cough centre in the

medulla oblongata to exert an antitussive effect (Bem & Peck, 1992). However, DXM in supratherapeutic doses can affect other parts of the brain and cause psychiatric symptoms. Since abundant NMDA receptors could be found in the hippocampus and these receptors involved in affective behaviours, the hippocampus was chosen as a target site for this study. The hippocampus is known as a site of neurogenesis, and rich in NMDA receptors (Mochizuki et al., 2007).

1.2 Neurogenesis hypothesis of depression and neurogenesis-suppressing DXM

Neurogenesis is hypothesised to be related to the normal functioning of the hippocampus and the suppression of neurogenesis has been suggested to be associated with affective disorders. The neurogenic hypothesis of depression, prompted by the discovery of the pro-neurogenic effect of antidepressants, suggests that impaired adult neurogenesis may be responsible for the pathobiology of depression (Malberg, Eisch, Nestler, & Duman, 2000).

The neurogenesis hypothesis was the focus of this study. According to the hypothesis, there may be a causal relationship between the suppression of

neurogenesis and the induction of depressive-like behaviour. Ketamine, a compound with a chemical structure and NMDAR antagonistic property similar to DXM, induces depression-like behaviour and suppresses neurogenesis when rodents were treated with repeated high doses (Tung, Herrera, Fornal, & Jacobs, 2008). Studies revealed that repeated administrations of ketamine can lead to a hallucinogenic effect in humans, which is very similar to the effect of prolonged exposure to DXM. Due to the similar molecular structure of DXM and ketamine, all these findings suggest that DXM may induce depression and anxiety by suppressing adult neurogenesis in the hippocampus.

As there are similarities between DXM and ketamine, so DXM may induce psychological effects via similar pathway which ketamine does. However few studies have investigated the underlying mechanisms of DXM-induced psychological effects. This study therefore aimed to explore whether DXM, at abusive doses, would suppress neurogenesis and trigger depressive disorder. To gain a better understanding of DXM's mechanism, this study also focused on whether neurogenesis is affected by different DXM dose.

1.3 Wolfberry extract with neuroprotecting effect

In addition to revealing the possible mechanism of the DXM-induced adverse effect on moods, this study explored possible adjunctive treatments for DXM-induced depression-like behaviour. Wolfberry, a component of Chinese herbal medicine that has been in use for over one thousand years was investigated to determine whether it can attenuate the adverse effects of DXM. Recent studies have revealed that the active component of wolfberry is *Lycium barbarum* polysaccharides (LBP) (Yu et al., 2007), a complex mixture of highly branched polysaccharides and proteoglycans (Lim, 2012). LBP has been investigated for its activities in promoting neurogenesis and treating affective disorders in various animal disease models with suppressed neurogenesis (Chen et al., 2014; Gao et al., 2015; Lau, Leung, Po, Chang, & So, 2015; Wen, Yang, & Ren, 2010; Zhang, Chang, & So, 2009). With its known effect on promoting neurogenesis, it is hypothesised that LBP can alleviate depression- and anxiety-like behaviour found in rats associated with impaired neurogenesis. Although depression symptoms can be reduced by prescribed antidepressants, these medications seem ineffective in treating depression in last decade (40–60%) (Masand, 2003) and produce side effects including headache and abdomen discomfort. Even though new antidepressant with

improved effectiveness and fewer side effects, they may still cause extra burden to patients. Therefore developing an adjunctive treatment with LBP might possibly prevent these undesirable side-effects by reducing the number of prescription for antidepressant.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

This chapter begins with a review of the literature on DXM, covering its chemical proprieties, biological activities and pharmacological mechanism. Studies on the severity and current situation of DXM abuse are also presented, followed by a discussion on the neurogenic hypothesis of depression and a possible adjunctive treatment for DXM abuse.

2.2 Chemical proprieties, biological activity and pharmacological mechanism of DXM

2.2.1 Chemical properties and biological activity of DXM

Cough syrup usually contains DXM as an active component (Amaladoss & O'Brien, 2011). DXM is a non-competitive *N*-methyl-d-aspartate (NMDA) receptor antagonist that acts on the medulla oblongata to produce its antitussive effect (Werling et al., 2007). However, its specificity to the medulla is limited. At high doses, it interacts with other parts of the brain and induces acute and chronic psychotic symptoms.

2.2.2 Pharmacological mechanism of DXM

After oral administration, DXM is absorbed into the bloodstream via the gastrointestinal (GI) tract. Part of the drug is then metabolised by the liver to dextrorphan, which is also an active cough suppressor. After binding to glutamic acid carriers on the brain microvessel endothelial cells (BMECs), DXM is able to pass through the blood–brain barrier (BBB) by passive diffusion (Shi, Cavitt, Bailey, Malick, & Audus, 1993). P-glycoprotein also plays an important role in the uptake of DXM into the brain (Uhr, Namendorf, Grauer, Rosenhagen, & Ebinger, 2004). At therapeutic doses, DXM has an antitussive effect by interacting with the cough centre in the medulla oblongata and increasing the threshold needed for the cough reflex.

2.2.3 Adverse effects and current situation of DXM abuse

The abusive consumption of DXM can lead to elevated mood in acute intoxication, from euphoria to out-of-body experience depending on the dosage (DEA & US DOJ, 2011). However, after the pleasurable feelings wane, side effects begin to surface, including hyperactivity, hallucination and anxiety. A clinical study revealed that according to medical reports, over 60% of chronic

DXM abusers suffered from depressive symptoms and some of them were diagnosed with psychosis-related disorders (Dickerson, Schaepper, Peterson, & Ashworth, 2008). Recent DXM abusers show an even higher rate (>90 %) of depression and suicide attempts when compared with abusers who have stopped the abuse. Consumption of high doses of DXM was shown to cause mood alteration, including anxiety and a sense of nervousness in the acute phase (Reissig et al., 2012). Besides the effect on CNS, DXM is also a sigma agonist, a nicotinic acetylcholine receptor, an opioid receptor agonist and serotonin transporter inhibitor (Boyer & Burns, 2013; Codd, Shank, Schupsky, & Raffa, 1995; Damaj, Flood, Ho, May, &Martin, 2005). Some of the mentioned receptors are also present peripherally. For example, nicotinic acetylcholine receptor found on skeletal muscle and serotonin transports in the alimentary canal. This receptor distribution pattern may be responsible for the adverse effects of DXM at supratherapeutic doses like muscle spasms, vomiting and diarrhoea as the normal functions of these receptors are disrupted.

According to a study conducted by the Substance Abuse and Mental Health Services Administration (2008) in 2008, 5.3 % of adolescents aged between 12

and 25 in the US had used OTC cough and cold medication to achieve an elevated mood. In 2013, 7.9 % of the 5,997 reported cases of drug abuse in Hong Kong were related to DXM. The high incidence of DXM abuse in adolescents and its severe psychiatric effects suggest that a better understanding of the mechanism of the affective disturbances caused by DXM might aid the research on rehabilitation of DXM abuser and consequently relieve pressure on public healthcare systems.

Among the reported cases associated with affective disturbances, a depressive status is often found in DXM abusers (Hinsberger, Sharma, & Mazmanian, 1994; Miller, 2005). The daily dosage of DXM consumption could fall between 34 to 51 mg/kg (Miller, 2005). A survey on DXM abusers in the US discovered that more than 25 % had reported different types of mood disorder in the sub-acute phases (Ziaee et al., 2005). A clinical report also found that over 60 % of admissions with a history of chronic DXM abuse suffered from depressive- or psychosis-related disorders (Dickerson et al., 2008). However, the mechanism of DXM-induced mood disorder remains unclear, especially with respect to the hypothesis of the etiology of depression.

2.2.4 Suppression effect on neurogenesis by NMDA receptor antagonist

There are several similarities between DXM, phencyclidine and ketamine. Not only do NMDA antagonists produce a hallucinogenic effect, they also induce various psychiatric symptoms in both acute and chronic phases if repeated supratherapeutic doses are administered (Reissig et al., 2012). Furthermore, repeated administrations of ketamine in rats suppresses adult neurogenesis in the hippocampus (Tung et al., 2008). The suppression of neurogenesis has been found to be associated with depression (Malberg et al., 2000) and other psychological diseases (Lau, Yau, Po, & So, 2016).

2.3 Discovery and importance of adult neurogenesis

The continuous production of new functional neurons in the adult brain is known as adult neurogenesis. Using a ^{3}H-thymidine autoradiographic technique, Altman et al. (1962) found adult-generated neurons scattered in the neocortex in rats (Altman, 1962). Adult neurogenesis has been observed in birds, rodents, non-human primates and humans (Duman, Malberg, & Nakagawa, 2001; Kempermann & Song, 2008).

Neurogenesis in the olfactory bulb is essential for female mice's offspring recognition (Lau, Yau, & So, 2011), and neurogenesis in the hippocampus is associated with mood disorders (Malberg et al., 2000). It has been observed that suppressed neurogenesis exists in patients with various types of psychotic symptom (Reif et al., 2006), suggesting that DXM-induced disruption of affective and psychotic symptoms may result from altered neurogenesis.

2.4 *Lycium barbarum* polysaccharides

2.4.1 Origin and chemical properties

Lycium barbarum polysaccharides (LBP) is a complex extracted from the fruit of *Lycium barbarum*, commonly known as wolfberry or goji, which has been used in Chinese herbal medicine for more than one thousand years. The plant has been widely cultivated in China, especially in Ningxia (Lim, 2012).

The most important compounds found in *L. barbarum* are in LBP (Potterat, 2010), which is a complex mixture of highly branched polysaccharides and proteoglycans (Lim, 2012), that protects neurons and

neurogenesis in various animal disease models, as discussed later in this section.

2.4.2 Effect of LBP on neurogenesis and depression in different disease models

It has been suggested that LBP protects adult neurogenesis from several types of intoxication and disease in animal models, in addition to improving results in tests for depression- and anxiety- behaviour. Behavioural dysfunction induced by scopolamine poisoning (Chen et al., 2014), manganese intoxication (Wen et al., 2010) Post-traumatic stress (Gao et al., 2015), corticosterone-induced depression (Lau et al., 2015; Zhang et al., 2009) and some other diseases involving impaired adult neurogenesis can be improved by LBP (Table 1).

Given that LBP can protect against deteriorating behaviour and protect neurogenesis in different disease models, DXM-induced depression may also be prevented by the administration of LBP.

Table 1. Summary of studies that found that LBP can protect adult neurogenesis from several types of poisoning and disease models in animals and improve behavioural test results.

Author	Disease model	LBP daily dosage	LBP administration period	Histological assessment	Behavioural tests
Chen et al., 2014	Scopolamine intoxication	0.2–1 mg/kg	14 days before SCO treatment	ki-67, DCX	Water maze/ object recognisation
Gao et al., 2015	Post-traumatic stress	20 mg/kg	3 days before or 28 days after	Silver impregnation, Nissl, HE	OFT, water maze, resistance to capture
Wen et al., 2010	Manganese poisoning	1.5 mg/kg	14 days simultaneously with manganese administration	BrdU	Water maze
Zhang et al., 2012	Corticosterone-induced depression	1 mg /kg	7 days before corticosterone treatment	BrdU, BCX, Golgi	FST

2.5 Neurogenic Hypothesis of depression

There is evidence from human and animal studies that neurogenesis contributes to the etiology of depressive disorder. A decrease in the volume of the hippocampus in patients with depression has been revealed by magnetic resonance imaging (Fotuhi, Do, & Jack, 2012). The size of the hippocampus increases significantly after electroconvulsive therapy (ECT), which has been suggested as the most potent biological therapy for depression by some researchers (Tendolkar et al., 2013). The association between hippocampal atrophy and depressive disorder implies that neurogenesis might be involved in the disorder.

The faster atrophy rate deviated from the normal ageing implied a higher chance of developing neurological diseases. A longitudinal study has revealed that the hippocampus of senior adults older than 55 years old shrinks 1-2% annually (Jack et al., 1998). The same study also reported that adults with Alzheimer disease are even having a faster hippocampal atrophy rate (3-5% annually) while a faster atrophy rate usually means a higher chance to develop dementia (Erickson, Miller, &Roecklein, 2012).

Furthermore, Banasr and Duman (2007) suggested that the time lag between the onset of the therapeutic effect of antidepressants and the beginning of drug administration is the result of delayed neurogenesis increase, since the time period required by the onset is similar to that required for the increased neurogenesis (Banasr & Duman, 2007).

Malberg et al. (2000) suggested that the 'neurogenesis hypothesis' of depression is supported by the pro-neurogenic effect of antidepressants. According to this hypothesis, neurogenic changes are key to the mechanism underlying the pathology of mood disorder, and neural stem cells could be a target for curing mood disorders and some kinds of psychiatric disease, including schizophrenia, as part of a multidisciplinary approach (Ruan et al., 2014).

Suppressed hippocampal neurogenesis may impair the resistance of hypothalamic–pituitary–adrenal (HPA) axis against stress hormone. Increased glucocorticoid levels (a common factor in depression animal and patient) suppress activity-dependent increases in brain-derived neurotrophic factor (BDNF) levels (Cosi, Spoerri, Comelli, Guidolin, &Skaper, 1993), possibly desensitise the hippocampal neurons morphologically to stimuli and inhibiting

dendritic arborization. This phenomenon is common in patients with depression that suffering from impaired resistance to glucocorticoid-induced negative feedback control and elevated HPA axis activity lead to the rising in glucocorticoid levels (Campbell &Macqueen, 2004; Maes, deRuyter, &Suy, 1987; Young et al., 1994). Although the detailed explanations of pathobiology between the adult hippocampal neurogenesis and depression are still needed further investigations, the stated evidence above shed light on the investigations that using neurogenesis as a treatment target.

Risk factors for depression, such as stress and other psychiatric diseases, have been found to suppress neurogenesis (Gould & Tanapat, 1999) and suppression or blocking hippocampal neurogenesis inactivates the therapeutic effect of antidepressants in mice (Dranovsky & Hen, 2006). These observations further strengthen the linkage of depressive disorder and adult neurogenesis.

Many neurodevelopmental and neurodegenerative disorders are associated with cognitive impairment, emotional disturbances and altered adult hippocampal neurogenesis. Indeed, evidence for altered neurogenesis has been found in animal models for Alzheimer's disease (Haughey et al.,

2002), Parkinson's disease (Crews et al., 2008), Huntington's disease (Gil-Mohapel, Boehme, et al., 2011), foetal alcohol syndrome (Gil-Mohapel, Simpson, Ghilan, & Christie, 2011) and Fragile X syndrome (Eadie et al., 2009). The changes in neurogenesis may hinder the normal functioning of the hippocampus and result in the impairments mentioned above. However, as increasing new findings have been discovered in the past decade, Lucassen et al (2012) suggested that the mechanism of depression is unlikely to be fully explained by neurogenesis alone (Lucassen et al., 2013). The contradictory evidence is discussed in Chapter 5 (Discussion).

2.6 Animal behaviour tests

Mood disorders such as depression are a complicated series of pathological consequences that cannot be measured and reproduced in animals directly (Petit-Demouliere, Chenu, & Bourin, 2005). Only specific behaviour relevant to human mood disorder can be assayed since the certain behaviour do not present in rodents (Holmes, 2003). In this study, the forced swim test and tail suspension test were used to assess depression-like

behaviour, and the open field test and social interaction test were used to assess anxiety-like behaviour.

2.6.1 Forced swim test

The forced swim test (FST) was first introduced by Porsolt et al. in 1979 when they performed the water maze test (Petit-Demouliere et al., 2005) and inadvertently discovered that most rats were able to find the exit within 10 minutes; one remained in the maze, stopped struggling altogether and remained passively floating (Porsolt, Bertin, Blavet, Deniel, & Jalfre, 1979). The team further developed the test and discovered that it was able to detect the effect of a number of antidepressants by the reduction of immobility time in rats (Porsolt et al., 1979; Porsolt, Bertin, & Jalfre, 1977). The test was found to possess strong interrater reliability across laboratories and strong predictive validity to differentiate antidepressants from neuroleptics and anxiolytics (Borsini & Meli, 1988).

2.6.2 Tail suspension test

Similar to the FST, tail suspension test (TST) is used to assess depression-like behaviour in rodents. Mice (majority) , rats and gerbils are species can be the subjects to be tested by the TST (Cryan, Mombereau, &

Vassout, 2005). During the test, the animals are held by their tail and remain in a passive or immobile posture after a series of escape attempts. Immobile time in the TST can be reduced by a range of antidepressants (Porsolt et al., 1987). Compared to the TST, forced swimming is an uncontrollable, inescapable and stressful condition in which hypothermal stress is induced. The TST is therefore a desirable test of choice if the additional stress of FST is to be avoided in the testing condition. The TST and the FST have very similar conceptual designs in which stress is induced in an inescapable condition, and both are able to detect depression-like behaviour. However, behavioural changes found in the tests might be due to the activation or suppression of different biological pathways (Porsolt, 2000). In this study, the differences in the tests allowed the results to be cross-validated when performing both tests to assess depression-like behaviour.

2.6.3 Open field test

The open field test is a common animal model for assessing anxiety-like behaviour. Calvin S. Hall (1932) was the first investigator to use the open field test to assess rats' emotions (Hall & Ballachey, 1932). In the test, the animal is

put into an inescapable environment surrounded by walls (Walsh & Cummins, 1976). Originally, the test assessed animal emotionality by scoring the defecation and urination of the tested animal (Hall, 1934), and animals with food restriction became more 'emotional' (the term previously used to describe the depressed state) and had a lighter level of defecation and fewer entries to the central part of the field (Prut & Belzung, 2003). The test has since been refined and now focuses on the animal's willingness of to move. Animals staying longer in the central region of the field are considered less anxious than the animals that stay longer in the peripheral area (Stanford, 2007). However the difference of the animal's locomotor activity could be a major thread to the validity of the test (Stanford, 2007). The details of confounding controls will be discussed in section 5.1.4.

2.7 Histology analysis: Immunostaining

Immunostaining was a key technique applied in this study to obtain quantitative data on the changes in neurogenesis among different treatment groups. The technique was first described by Dr Albert Hewett Coons (1912–1978) in 1941. He labelled type III pneumococci by applying rabbit

antibodies labelled with fluorophores, β-anthryl-carbamido (Coons, Creech, & Jones, 1941). Dr Coons is regarded as the father of immunohistochemistry because he was the first researcher to label specific antigens with the aid of antibodies.

Immunostaining is an application of antigen–antibody interactions. The antigen–antibody bond is a combination of van der Waals forces and electrostatic interactions (Absolom & van Oss, 1986). The interaction is so specific (Ramos-Vara, 2005) and dyes like chromophores or fluorophores are added to antibodies to allow visualisation of specific antigens (Coons et al., 1941).

2.7.1 Direct and indirect detection methods

The mechanisms of detection systems can be broadly divided into 2 types: direct and indirect methods. The direct method is a one-step, simple and quick reaction process. Primary antibodies are conjugated with a reporter molecule. This first method described by Coons was an example of this method. However, the method lacks sufficient sensitivity to detect antigens in small quantities (Coons, 1949). An improved or indirect method was subsequently invented by

Coons et al. (Coons, 1955). The sensitivity of this method is significantly greater because the primary antibodies are not labelled and do not undergo any chemical modification so their binding of the antigen is retained. Another mechanism to further increase the sensitivity of the indirect method is to increase the number of labels per molecule of primary antibodies (Ramos-Vara, 2005) because labelled secondary antibodies are raised against the first antibodies. Multiple conjugated secondary antibodies bind to the primary antibodies, resulting in a stronger signal.

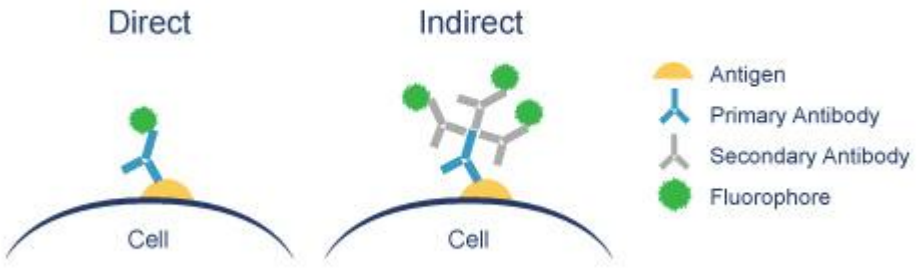


Figure 1. Direct and indirect detection of antigens.

Adapted from invitogen.com

2.7.2 Avidin–biotin complex (ABC) methods

Avidin–biotin complex (ABC) methods are a specific type of indirect method applied in this study for cell proliferation and survival assays. With this method, a further increase in the sensitivity of staining is achieved by introducing the interaction of avidin and biotin into the detection system. Avidin is a glycoprotein derived from egg white and its affinity with biotin is very high. In this system, secondary antibodies are biotinylated instead of being conjugated with fluorophores. By cooperating with the labelled peroxidase avidin, the cascade is further enhanced as several avidin molecules can bind to a single biotinylated secondary antibody (Polak & Van Noorden, 1983). Thus, large numbers of label molecules are attached to a primary antibody (Elias, Margiotta, & Gaborc, 1989). The peroxidase from the dark precipitate with 3,3’-diaminobenzidine (DAB) is continuously used as a label in the reaction. Colour intensity can be adjusted according to the amount of DAB and reaction time.

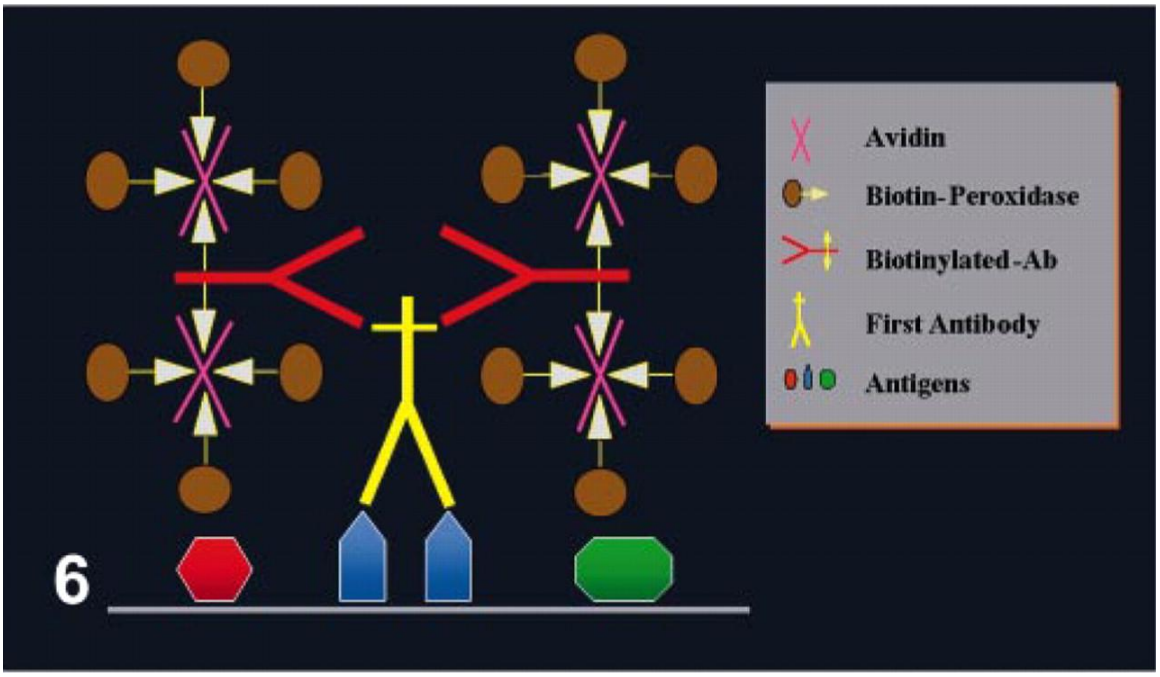


Figure 2. The ABC immunohistochemical method

Adapted from J. A. Ramos-Vara *Vet Pathol* 2005; 42:405-426

2.8 Conclusion

2.8.1 DXM, neurogenesis and mood regulation

The hippocampus, which is rich in NMDA receptors, is responsible for mood regulation and characterised by adult neurogenesis. Given that altered mood has been observed in DXM abusers, DXM is hypothesised to affect neurogenesis, suggesting a link to mood regulation.

2.8.2 Justification of using LBP as a protective agent

Although the incidence of mood disorder of DXM abusers is high (Dickerson et al., 2008), the current treatment are rather ineffective. Only supportive treatments are available, including counselling and antidepressants. However, antidepressants have a high rate of failure (40–60%) (Masand, 2003) and their side effects can be intolerable. In contrast, LBP has been reported to have very few side effects and is non-toxic (Potterat, 2010). Only a few cases of drug interaction (with warfarin) have been reported (Rivera, Ferro, Bursua, & Gerber, 2012) so it can be considered a very safe herbal drug component and suitable for development as a novel treatment for DXM abuse. Because the

herbal extract can allay the fatigue and stress associated with neurogenesis impairment (Lim, 2012), LBP may be a potent treatment for DXM abuse.

2.8.3 Dosage of LBP used in this study

In previous research, LBP dosage at 1 mg/kg has shown the highest protective effect in different disease model, including intoxication of scopolamine and manganese and cortisol induced stress models (Chen et al., 2014; Wen et al., 2010; Endong Zhang et al., 2012). The maximum daily dosage can be administrated in animals without causing obvious side effects is 20 mg/kg (Gao et al., 2015).

2.9 Hypothesis

It is hypothesised that repeated high doses of DXM treatment can alter hippocampal neurogenesis and cause emotional disturbance. Based on this hypothesis, this study further hypothesised that LBP, which can protect against suppressed neurogenesis and its related depression-like behaviour, can also protect against adverse consequences induced by DXM.

2.10 Aim of the study

The aim of this study was to investigate the effect of DXM treatment on hippocampal neurogenesis and affective behaviours, and to explore if LBP could be a potential adjunctive treatment. The following research questions were addressed:

- How does DXM affect hippocampal neurogenesis and affective behaviours?
- Can LBP, a neuroprotective agent, protect against the adverse effects on mood and neurogenesis induced by DXM?

With regards to the research questions above, the following specific objectives were defined:

- To investigate whether repeated, high-dose DXM treatment can modify mood regulation in rats
- To investigate whether repeated, high-dose DXM treatment can suppress neurogenesis in the hippocampus
- To compare the differences in induced neurogenesis between different doses of DXM

- To investigate whether LBP can be an adjunctive treatment for DXM abuse by protecting neurogenesis

In the following chapters, the detailed methodology and results are presented. Chapter 3 provides details of the study design and animal treatment. The treatment schedule and the administration method are described and the protocols for the behaviour tests and histological assessment of neurogenesis are explained. The findings of the study are presented in Chapter 4. The effect of DXM on different aspects of neurogenesis and the protective effect exerted by LBP are illustrated in Chapter 5. A comprehensive summary of the findings is given in Chapter 6.

CHAPTER 3. METHODOLOGY

3.1 Introduction

This chapter describes the research paradigm and methodology for the three experiments mentioned in the introduction. First, the paradigm applied in the different experiments is described. As the technique and methodology applied were consistent across the three experiments, the methodology is described in the same section. Finally, the data analysis is described.

3.2 Study design

3.2.1 Experiment 1: Examine the effect of repeated, high-dose DXM on mood and neurogenesis

This experimental study investigated the effects of repeated and high-dose DXM treatment on affective behaviour and hippocampal neurogenesis.

3.2.1.1 Animal treatment and grouping

Animals were randomly assigned to four treatment groups: (1) control group for proliferation assay, (2) DXM group for proliferation assay, (3) control group for cell survival assay, and (4) DXM group with bromodeoxyuridine

(BrdU; Sigma-Aldrich, St Louis, MO, USA) for cell survival assay. Body weight, behavioural test results, immunohistochemistry and histomorphology were recorded. Changes in mood regulation and neurogenesis were evaluated based on the results obtained.

Twenty-two adult male Sprague-Dawley rats weighing between 200 to 240 g were used for the study. The rats were kept under a 12:12 light-dark cycle at 22 °C. For the proliferation study, they were randomly assigned to either the control group (n = 5) or the DXM group (n = 5). The control group received vehicle (distilled water) intraperitoneal injection and the DXM group received repeated treatment of 40 mg/kg/day DXM (Sigma-Aldrich, St Louis, MO, USA) by intraperitoneal injection for 2 weeks. To perform the cell proliferation assay, BrdU was injected intraperitoneally in the last three days of treatment in a daily dose of DXM at 50 mg/kg, divided into 2 doses of 25 mg/kg and injected every 12 hours, to label dividing cells.

For the cell survival assay, another two groups of animals (n = 6 each group) were injected intraperitoneally with BrdU on the first day of the treatment to determine the effect of DXM on the survival of new cells.

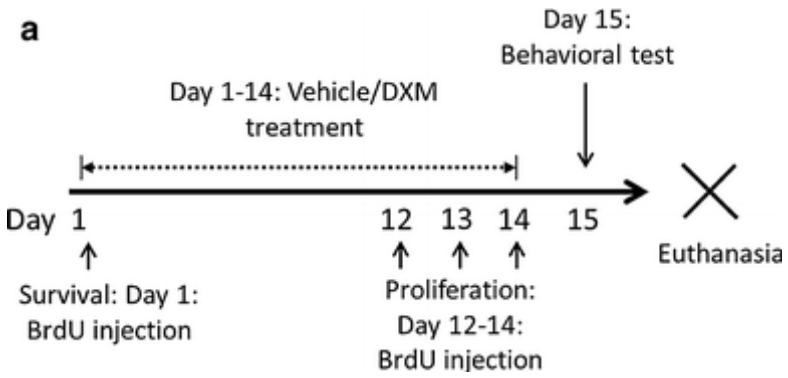


Figure 3. Paradigm for the experiment to investigate the effect of DXM on neurogenesis and mood

Table 2. Group allocation of rats

Group	**Number of rats**	**DXM injection**	**BrdU injection**
Ctrl, proliferation	5	Vehicle only	last 3 days
DXM, proliferation	5	40 mg/kg/daily for 14 days	last 3 days
Ctrl, survival	6	Vehicle only	1st day
DXM, survival	6	40 mg/kg/daily for 14 days	1st day

3.2.1.2 Data analysis

The data obtained from the behaviour tests and histological assessments on the control and DXM treatment groups were compared by Student's t-test using SPSS software (IBM, SPSS Statistics 20). A *p*-value <0.05 was defined as statistically significant.

3.2.2 Experiment 2: Dose-response relationship between daily DXM dosage and neurogenesis

The second experiment aimed to characterise the dose-dependent effect of DXM. SD rats were divided into 4 groups (n = 6 per group) and intraperitoneally injected daily with 0, 5, 20, or 60 mg/kg of DXM before being subjected to histological analysis.

Twenty-four young adult male SD rats weighing 220 ± 20 g, were used for the study. The rats were kept under a 12:12 light-dark cycle at 22 °C. The control group received intraperitoneal injections of the vehicle (distilled water) and the DXM group received repeated intraperitoneal injection treatment with 5, 20 or 60 mg/kg/day DXM (Sigma-Aldrich, St Louis, MO, USA) for 2 weeks. To

perform the cell proliferation assay, BrdU (Sigma-Aldrich, St Louis, MO, USA) was injected intraperitoneally at a dose of 50 mg/kg daily in the last three days of treatment to label dividing cells.

3.2.3 Experiment 3: Protective effect of LBP on DXM-induced suppression on neurogenesis and affective disorder

Twenty-four adult male SD rats (225 ± 25 g) from the Central Animal Facility of HK PolyU were used for the study. They were kept at a constant temperature (~22 °C) in a 12:12 light-dark cycle. According to different treatments, the rats were randomly assigned to 4 treatment groups (n = 6 per group): 1) normal control group, using distilled water as a vehicle for intraperitoneal and intragastric administration, which aimed at providing comparable the handling stress caused in other treatment groups to prevent confounding results, because injections and restraints are known stressors to the animals; 2) DXM group, which received daily intraperitoneal injections of DXM but were administrated with the vehicle by the intragastric route; 3) LBP group, which received a vehicle as an injection but received intragastric administration of the LBP solution; and 4) co-treatment group, which received

repeated DXM and LBP n via intraperitoneal and intragastric administration respectively.

DXM was administrated as a daily dose of 40 mg/kg and its corresponding vehicle was administered by intraperitoneal injection. LBP was applied in a daily dose of 1 mg/kg and its vehicle was administered by the intragastric route with a stainless steel feeding tube. Bromodeoxyuridine (Sigma-Aldrich, St Louis, MO, USA) was administered at a dose of 50 mg/kg/day via intraperitoneal injection during the last 3 days (days 12–14) of the treatment period for cell proliferation assay in the dentate gyrus of the hippocampus.

The data collected from behavioural tests and immunohistochemistry assessments were analysed by a researcher blind to the treatment condition. Changes in mood-related behaviour and adult hippocampal neurogenesis were assessed. The treatment schedule is summarised in Figure 4.

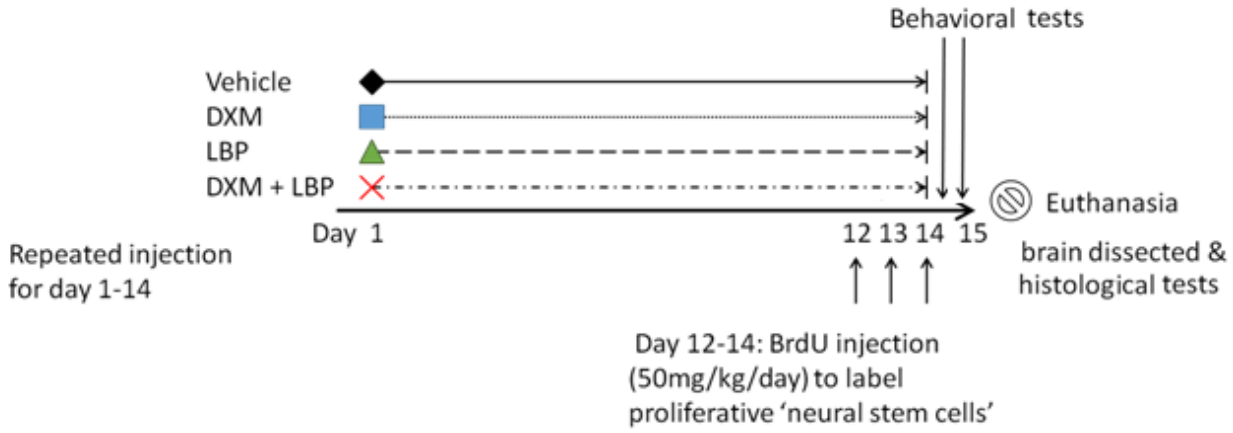


Fig 4. Treatment schedule to investigate the protective effect of LBP on DXM-induced suppression of neurogenesis and affective disorder

3.2.3.1 Preparation of LBP from wolfberry

The preparation of LBP used in this study was the same as that described in a previous study conducted by our collaborator (Yu et al., 2007). Fructus lycii, the harvested fruits of *Lycium barbarum* from mainland China, were continuously stirred in 95% ethanol for 120 hours. The residue was then dried, followed by 2 extractions with hot water. The extraction solution was filtered and concentrated. The processed extract was incubated with trichloroacetic acid by dialysis. After washing with ethanol, the precipitate was collected for lyophilisation and future uses. The preparation of LBP is summarised in Figure 5.

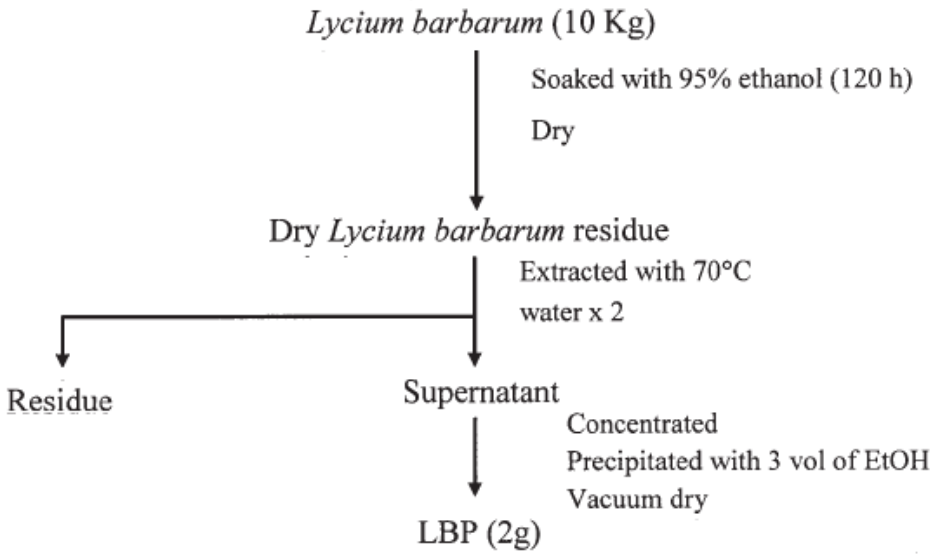


Figure 5. Production of LBP used in this study.

(Adapted from 'Characterization of the effects of anti-aging medicine fructus lycii on β-amyloid peptide neurotoxicity' Int. J. Mol. Med., vol. 20, no. 2, pp. 261–268, Aug. 2007. (Yu et al., 2007))

3.2.3.2 Data analysis

The data obtained from the behaviour tests and histological assessments on different treatment groups were compared by one-way ANOVA, followed by LSD post-hoc tests. Statistical analyses were performed using SPSS Statistics version 20 (IBM Corp., Armonk, NY). A *p*-value <0.05 was defined as statistically significant. The dependent measurements obtained in this study are expressed as mean ± SEM.

3.3 Behaviour tests

3.3.1 Tail suspension test

On the last day of treatment, the animals were suspended by the tail for 6 min and the process was videotaped. The immobile time indicated depression-like behaviour, which was scored by an observer who was blind to the treatment condition.

3.3.2 Forced swim test

The test was performed 4 hours after the last administration of the drug on the 14th day. The rats were placed separately in a clear cylinder filled

with water 30 cm deep for 15 min in an attempt to induce learned helplessness.

The temperature of the water was maintained at a relatively constant room temperature (23 ± 2 °C). The animals were placed in the same cylinder for another 10 min the next day and videotaped. The taped experiment was scored by an experimenter who was blind to the treatment condition. The immobile time, which indicated depression-like behaviour, was defined as minimal limb movement to keep the animal floating.

3.3.3 Social interaction test

In this test, different pairs of unfamiliar rats from different treatments groups in the same experiment were randomly put together in an arena (72 × 72 × 40 cm) and their interaction observed. The 15 min test was video-recorded for later analysis by an investigator blind to the treatment. The viedo was assessed for twice, which one animal was scored for each time. The total number of positive social acts, including sniffing, following and grooming partners, and social play, was measured. A higher number of positive social interactions indicated a lower level of social anxiety.

3.3.4 Open field test

The open field test examined the anxiety-like behaviour of the animals. The animals were introduced to an open arena of 72 × 72 × 40 cm. Their locomotor activity was video-recorded over a duration of 10 min. The video was later analysed by an investigator blind to the treatment. In the analysis, the arena was divided into 16 equal squares, which the 4 central square was defined as the centre of the arena. The time the animals spent at the centre of the arena was an indicator of anxiety-like behaviour.

3.4 Tissue processing and immunohistochemistry

An overdose of sodium pentobarbital (100 mg/kg) by intraperitoneal injection was given for deep anaesthesia. Immediately after the onset of anaesthesia, the rats were transcardially perfused with normal saline to remove blood from the circulation followed by a perfusion with 4 % paraformaldehyde (PFA).

Brain tissue is delicate and vulnerable to hypoxia and hypercapnia conditions during dissection (Gage, Kipke, & Shain, 2012). Fixation by submersion of the whole brain in fixative does not allow fixative to reach all

regions of the brain evenly. Transcardial perfusion of fixative was therefore applied to the experimental animals to achieve fixation by utilising the circulation system as drainage for the fixative. After perfusion, the rats' brains were removed and post-fixed in 4% PFA for 24 hr after the perfusion. The fixation was crucial for the staining procedure as it allowed tissues to withstand harsh retrieval methods and prevent the loss of antigenicity. However, as fixation can also modify epitopes recognised by antibodies (Arnold et al., 1996; Boenisch, 2005) over-fixation should be prevented.

After post-fixation, the brains were submerged in a 30 % sucrose solution for cryoprotection to prevent the occurrence of freezing artifacts during cryosectioning. Coronal brain sections 40 µm thick were sectioned 1-in-12 series on a cryostat. The preparation for the section was handled with care, especially temperature optimisation and dust prevention, which can create unnecessary artifacts (Gerfen, 2003). The prepared sections were mounted onto gelatin-coated slides.

Antigen retrieval (AR) was done before the incubation of antibodies to break down cross-linkages induced by PFA. PFA fixation changes the conformation of antigen epitopes, resulting in a loss of electrostatic charges in

antigen detection for antibodies (Boenisch, 2006). In this series of experiments, the sections were subjected to an antigen retrieval process by heating in a 0.1 M sodium citrate buffer (pH 6.0 at 90 °C) for 20 min after being mounted onto gelatin-coated slides.

Various mechanisms of AR have been reported by other research groups, such as breaking of cross-linkages, removal of diffusible blocking proteins, removal of precipitated proteins, and removal of hydrolysis of Schiff's bases (Morgan, Navabi, & Jasani, 1997; Shi, Cote, & Taylor, 2001). AR aims to prevent artifacts introduced through the preparation. However, the significance of the chemical characteristics of the retrieval solution is still elusive (Ramos-Vara et al., 2008). To expose the antigens in cell nuclei, the mounted sections were soaked in 2 M dilute hydrochloric acid at 37 °C for 25 min.

The pH of the retrieval solution applied is critical to the procedure. A low pH buffer (pH 1.0–2.0) appears to be especially useful for nuclear antigens (Shi et al., 2001). The pH of the sections was adjusted by incubation in 0.1 M sodium borax buffer (pH 8.5) at room temperature for 10 min.

Two primary antibodies were used to the labelling of target cells. The first primary antibody was rat anti-BrdU (1:300, Abcam, Cambridge, MA), which labels proliferative cells. The other antibody was rabbit anti-doublecortin (Cell Signaling Technology, Beverly, MA), which is a marker of immature neurons. Both antibodies were applied at a concentration of 1:300. Peroxidase staining of BrdU and DCX were performed by applying biotinylated goat anti-rabbit and rat secondary antibodies (Vector Laboratories, Inc., Burlingame, CA) to the slides after primary antibodies incubation at room temperature overnight. The signal intensity was further improved by avidin–biotin couples (Vectastain elite ABC kit, Vector Laboratories, Inc., Burlingame, CA) and the visualisation was performed with diaminobenzidine (DAB; Sigma-Aldrich, St Louis, MO). For the double-labelling immunofluorescence to reveal differentiation changes, both anti-BrdU and anti-DCX antibodies were applied in the incubation of sections at room temperature for 12 hours. After incubation, the slides were rinsed and incubated in fluorophores conjugated with secondary antibodies (Alexa Fluor 488 goat anti-rat for labelling DCX-positive cells in green and Alexa Fluor 568

goat anti-rabbit for labelling BrdU-positive cells in red; Molecular Probes, Eugene, OR) at a concentration of 1:200 at room temperature for 2 hours.

3.5 Data collection

3.5.1 Neuronal differentiation, cell proliferation and survival assay

A computer aided, unbiased stereology protocol was used to quantify the number of BrdU and DCX positive cells in the dentate gyrus (DG) of the hippocampus (Leuner, Caponiti, & Gould, 2012). The two advantages of this analytical technique are that no labelled cell is counted twice and the areas chosen in each section are random and not overlapping. The sections were analysed by an investigator blinded to the treatments. The count of labelled cells (BrdU or DCX) in the DG was estimated in the every 12th section. BrdU-positive or DCX-positive cells in the hippocampus proper from 2200 to 4800 µm posterior to the bregma were quantified by unbiased stereology with a semi-automated stereo investigator (MicroBrightField, Williston, VT, USA) system. Six coronal brain sections from each animal were counted. To estimate the total number of proliferative cells (BrdU-positive cells) or new neurons (DCX-positive cells) in the DG, the cell counts were multiplied by 12.

3.5.2 Phenotyping of newly proliferative cells

To assess the percentages of BrdU-positive cells that express the marker of new neurons, BrdU and DCX double-labelling immunofluorescence staining was applied. This is because BrdU-positive labelling of new cells indicates not only those that differentiate into new neurons but also those that differentiate to different types of cell, such as astrocytes and oligodendrocytes. Therefore characterisation of newly born cells is required to determine whether the cells are new neurons.

After the incubation with primary and secondary antibodies, BrdU and DCX antigens on the sections were labelled. Each section was observed with an epifluorescent microscope; BrdU was labelled in red and DCX in green. A minimum of 25 BrdU-positive cells was selected at random to examine whether DCX were also expressed. The results of this comparison of different treatment groups was expressed as the proportion of BrdU-positive cells with DCX expression.

3.5.3 Sholl analysis

Sholl analysis to assess the dendritic complexity of DCX-positive cells has been described in previous studies (Wang, David, Monckton, Battaglia, & Hen, 2008). Ten DCX positive cells from each animal with tertiary dendrites in the DG were randomly chosen for analysis. The images of the labelled cells were taken under 400× magnification and then analysed with ImageJ software (NIH) and the Sholl analysis plug-in (The Ghosh Lab, http://labs.biology.ucsd.edu/ghosh/software/). The dendrites of the neuron were traced and concentric circles with increasing radii of 10 µm using the cell body as the centre were drawn automatically. The software automatically calculated the intersections formed by the dendrites and the circles. The number of intersections was an indicator of dendritic complexity; a higher number indicated more complex dendritic morphology.

3.6 Data analysis

As different experiments had differences in study design, different data analysis methods were applied, as discussed in the sub-section on study design (see Section 3.2).

CHAPTER 4. RESULTS

4.1 Effects of repeated, high-dose DXM on mood and neurogenesis

4.1.1 Dextromethorphan treatment induces depression- and anxiety-like behaviour

Weight is an indicator of stress-induced responses and general health. After 2 weeks of treatment, the weight gain of the DXM group was significantly lower than the control group (Figure 6, $p < 0.05$). The FST and TST, two tests that have been extensively used to evaluate depression-like behaviour in rats and mice (Chermat, Thierry, Mico, Steru, & Simon, 1986; Lau, Yau, Lee, et al., 2011), were also performed to assess whether depression-like behaviour was induced by repeated, high-dose DXM treatment. The DXM treatment group showed an increase in immobility time in both the FST (Figure 7a, $p < 0.05$) and TST (Figure 7b, $p < 0.05$) when compared to the animals in the control group. The results highlight that DXM treatment can lead to an increase in depression-like behaviour.

DXM also induced a decrease in positive social interaction in the SIT (Figure 7c, $p < 0.05$). A test related to anxiety-like behaviour, the OFT also revealed that DXM treatment significantly decreased the amount of time in

which the animals stayed in the centre area of the field when compared with the controls (Figure 7d, $p < 0.05$). These results indicate that anxiety-like behaviour can also be induced by DXM.

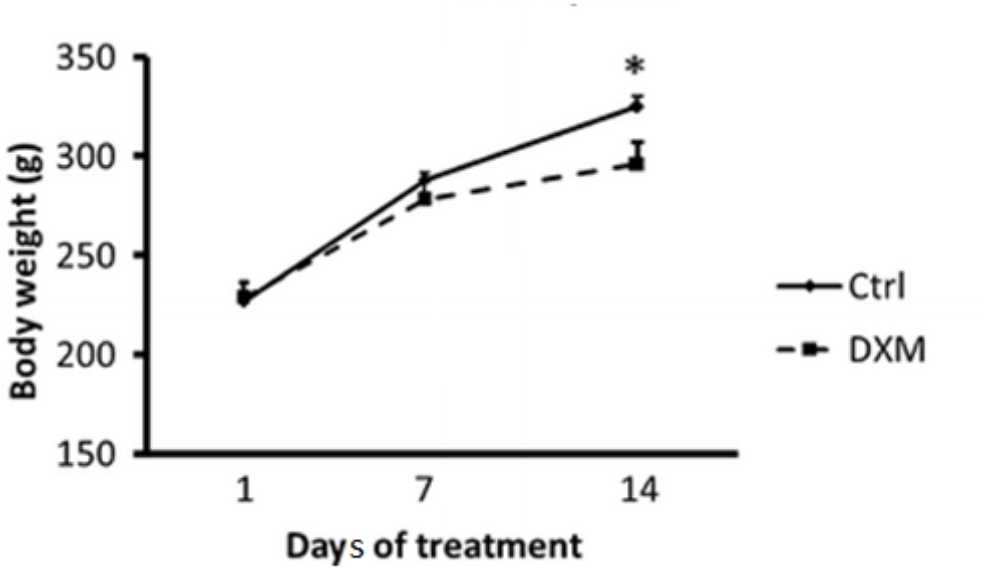


Figure 6. Repeated, high-dose DXM treatment reduced weight gain of rats significantly when compared with the control group

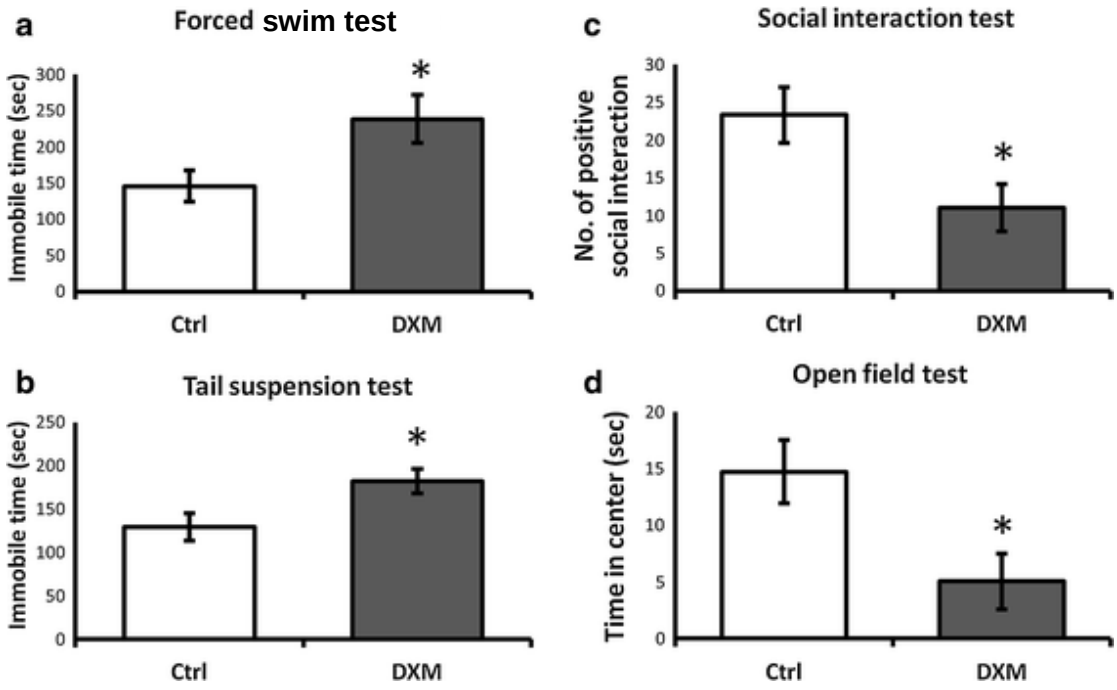


Figure 7. Repeated, high-dose DXM administration leads to depression-like behaviour with a significant increase in immobility time in the FST and TST. DXM treatment increased the immobile time of rats in **(a)** forced swimming test and **(b)** tail suspension test. DXM treatment also decreased the number of positive social interaction in **(c)** social interaction test and the time spend in centre in **(d)** open field test. The Student's t-test results are expressed in mean ± SEM. *p <0.05.

4.1.2 Dextromethorphan-suppressed neurogenesis

BrdU is a marker that incorporated by dividing cells. It was injected into the rats to label new cells in the DG and test whether repeated DXM administrations suppressed cell proliferation. The results indicated that DXM reduced cell proliferation in the DG (Figure 8a–c, $p < 0.05$) when compared with the control. However, the same treatment did not have a significant effect on the survival rate of neuronal precursor cells in the dentate gyrus (DG) of hippocampus (Figure 8d, $p = 0.33$).

Neuronal differentiation was also examined in the study. As DCX is only expressed in immature neurons, it is a marker labelled by anti-DCX antibodies. The DCX cell counts in the DG indicated that DXM can reduce the number of immature neurons (Figure 8e, f, h, $p < 0.05$).

To test whether neuronal differentiation is suppressed by DXM treatment, co-immunostaining of BrdU and DCX (Figure 8g) was performed. The results revealed a drop in the proportion of BrdU-immunoreactive cells to DCX expression (Figure 8i, $p < 0.05$). This decrease in co-expression shows that the suppression of neuronal differentiation of new cells in the DG was induced by DXM treatment.

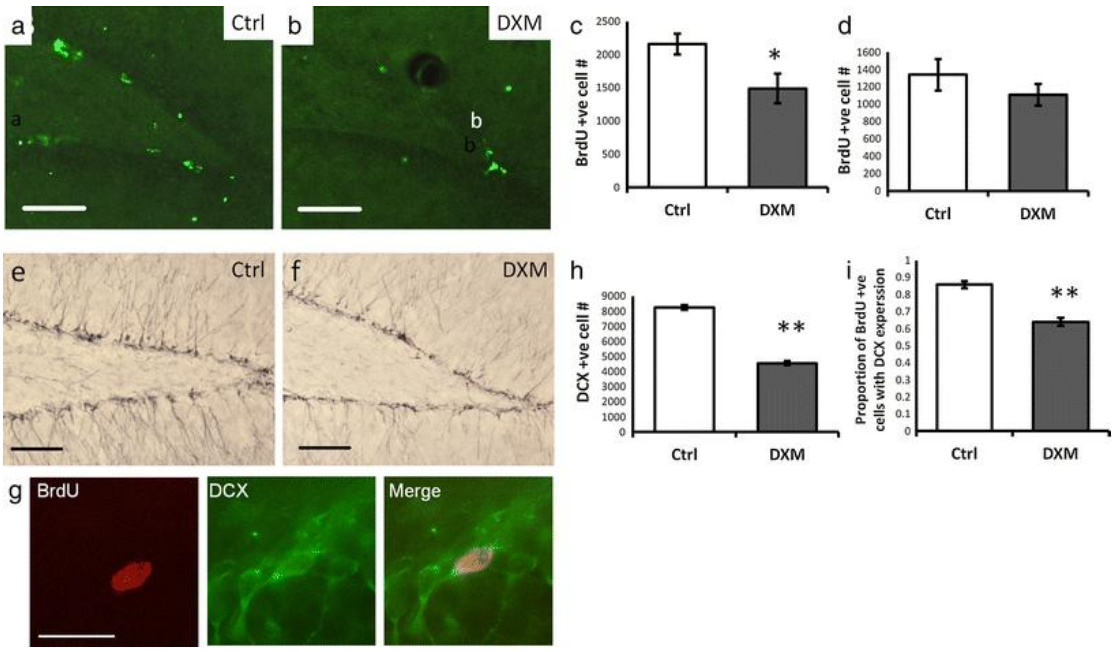


Figure 8. Repeated, high-dose DXM treatment suppresses neurogenesis in the DG. **(a** & **b)** Representative images of BrdU-positive cells in the DG of control and DXM groups. **(c)** DXM administration reduced the count of BrdU-positive cells, **(d)** but there was no effect on the survival rate of new cells. **(e** & **f)** Representative images of DCX-positive cells in the DG. **(h)** DXM administration reduced the count of DCX-positive cells significantly. **(g)** A cell co-expressing BrdU (red) and DCX (green). **(I)** DXM treatment reduced the ratio of BrdU-positive cells that also expressed DCX in DG significantly. The results are expressed in mean ± SEM. *p <0.05; **p <0.01, Student's t-test. Scale bars a, b, e, f: 100 µm; g: 20 µm.

4.1.3 Dextromethorphan-suppressed dendritic maturation of immature neurons

To examine whether the dendritic maturation of immature neurons was suppressed by DXM treatment, Sholl analysis was applied in this study. Representative images and tracings of DCX-positive cells from the control and DXM groups are shown in Figure 9a & b.

The results revealed that DXM treatment significantly reduced the complexity of dendrites when compared with the control. A higher number of intersections indicate more complex dendritic branching (Figure 9c). DXM treatment suppressed the dendritic maturation of immature neurons in the DG.

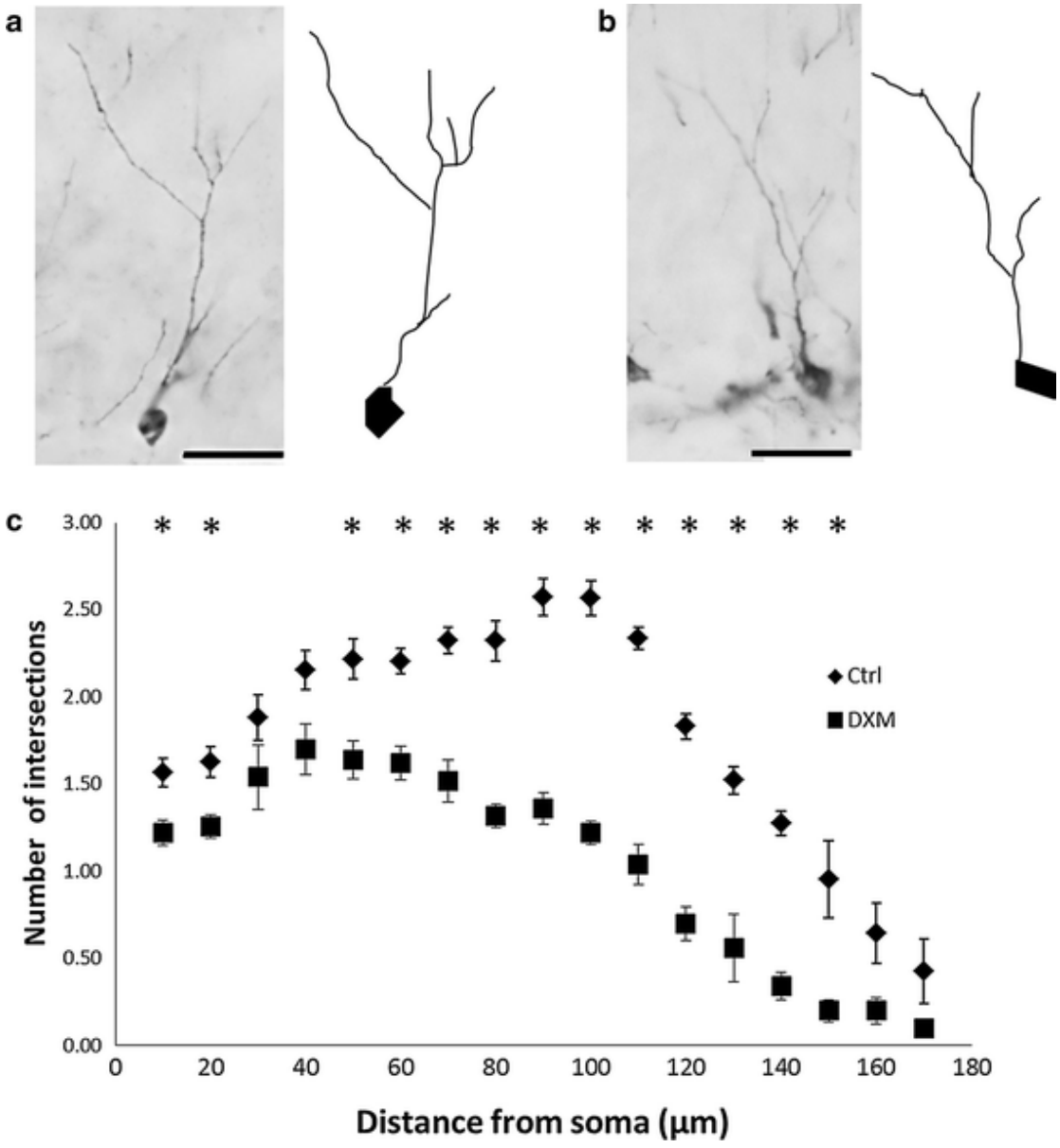


Figure 9. Dendritic complexity of new neurons in the DG is suppressed by high-dose DXM treatment. **(a** & **b)** Representative images of a DCX-positive immature neuron and its corresponding tracing for Sholl analysis in the DG of control and DCX groups, respectively. **(c)** DXM decreases the number of intersections between dendrites of new neurons and concentric circles of Sholl analysis. The results are expressed in mean ± SEM. **p* <0.05, Student's t-test. Scale bar 25 µm.

4.2 Dose-response relationship between daily DXM dosage and neurogenesis

4.2.1 Neurogenesis is suppressed starting from low dose of DXM (5 mg/kg)

To test how the adverse effect of DXM on cell proliferation varied with different dosages, an experiment on the effects of different dosages of DXM (0, 5, 20, and 60 mg/kg) on neurogenesis was conducted. After the treatment, the brain tissue of the rats was subjected to proliferation assay. To conduct this assay, BrdU was injected into the rat during the 12th–14th days of the treatment period to label proliferative neural precursor cells in the DG. The cell count with different dosages of DXM is characterised by a sharp drop at 5 mg/kg and reached a plateau in which a further increase in DXM does not have any additional suppressing effect on the mean value of the BrdU cell count (Figure 10, p <0.01).

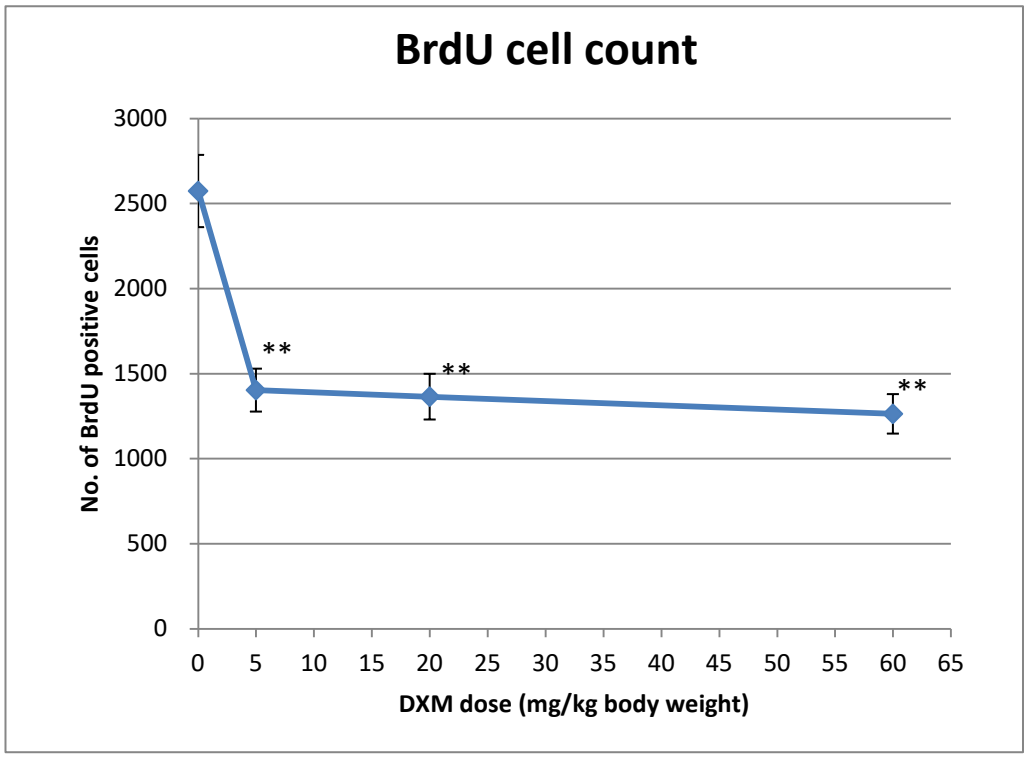


Figure 10. BrdU cell count in DG with 0, 5, 20 and 60 mg/kg of daily DXM after 14 days of treatment. The decrease in BrdU cell count reached a plateau after a sharp drop at 5mg. The results are expressed as mean ± SEM. $^{**}p < 0.01$ compared with control (0 mg), one-way ANOVA with LSD post-hoc tests.

To test how DXM affected neuronal differentiation at different doses, co-immunostaining of BrdU and DCX (Figure 11) was performed. The results reveal a decrease in the proportion of BrdU-immunoreactive cells with DCX expression at 5 mg/kg (Figure 11, $p = 0.155$). After a sharp drop, a gradual drop from 5 mg to 20 mg followed, and the proportion at 20 mg/kg daily is significantly lower than the control (Figure 11, $p < 0.05$). At 60mg/kg, a further mild drop was observed. These results illustrate the dose-response relationship between DXM and its suppressing effect on neuronal differentiation in the DG.

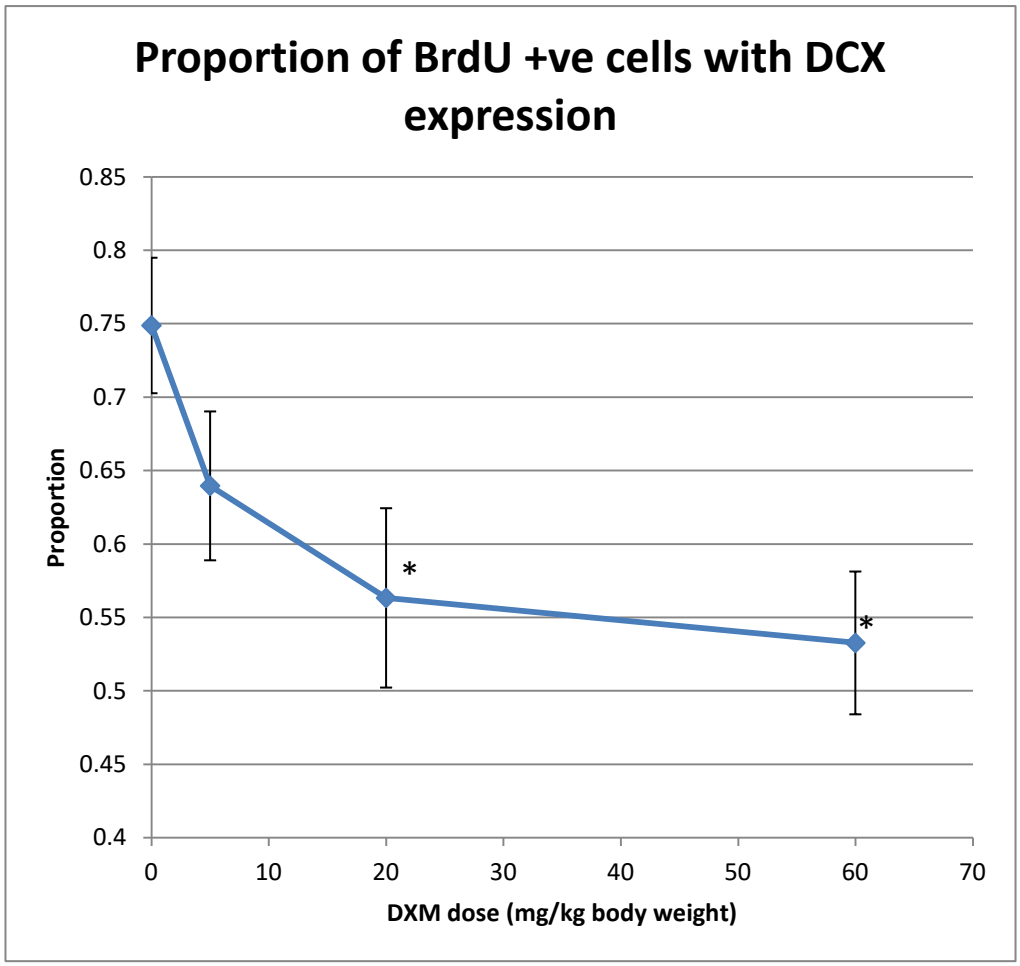


Figure 11. Proportion of BrdU +ve cells with DCX expression in the DG with 0, 5, 20 and 60 mg/kg of daily DXM dose after 14 days of treatment. The decreasing trend starts at 5 mg/kg daily and reaches a plateau at 20 mg/kg daily. The results are expressed as mean ± SEM. *p <0.05 compared with control (0 mg), one-way ANOVA with LSD post-hoc tests.

4.3 Protective effects of LBP on DXM-induced mood changes and neurogenesis suppression

4.3.1 LBP-induced protection against DXM-induced depression- and anxiety-like behaviour

Body weight can be an indicator of general health in response to stress. After treatment for 14 days, the body weight of all 4 groups increased. The rate of weight increases in the control, LBP-treated (1 mg/kg LBP) and DXM+LBP treated rats were similar. The rate of weight increase of the DXM-treated rats (40 mg/kg DXM) was marginally lower than that of the normal control group (Figure 12b, $p = 0.073$).

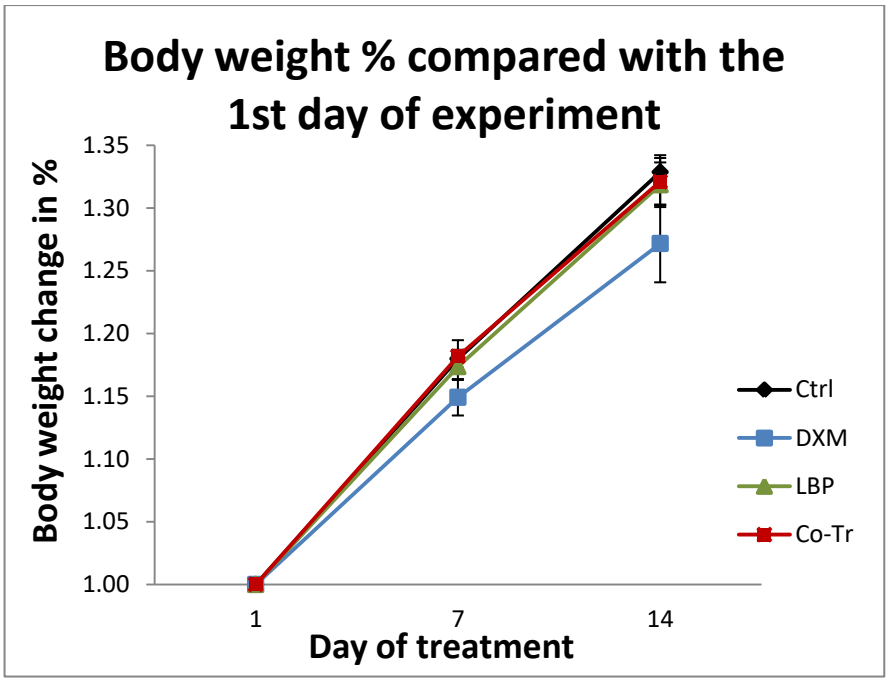


Figure 12. Body weight gains in %. The decrease in body weight gain of day 14 in rats treated with DXM when compared with the control group is of marginal significance (p = 0.073), and body weight changes in LBP and co-treatment groups are not affected. The results are expressed as mean ± SEM, one-way ANOVA with LSD post-hoc test.

The FST was applied here to examine whether LBP can protect rats from DXM-induced depression-like behaviour. The rats with DXM treatment had the longest immobility time among all the treatment groups, significantly longer than the normal control group (Figure 13, p <0.05). However, there was no significant difference between the immobile time of the co-treatment group (DXM and LBP) and that of the control.

To determine whether anxiety-like behaviour caused by DXM administration can be reversed by LBP treatment, the rats were subjected to a social interaction test (SIT). The results indicate that the number of positive social interactions was reduced in the DXM-treated rats when compared with the control and co-treatment groups (Figure 14b, p <0.05). Such a decrease was absent in the group co-treated with LBP and DXM when compared with the control (Figure 14b, p = 0.91). Interestingly, LBP treatment alone did not affect immobility time in the FST or the number of positive social interactions in the SIT. No significant beneficial or adverse effect was observed in this group in either test. These results suggest that DXM-induced anxiety- and depression-like behaviour can be alleviated by co-treatment with LBP.

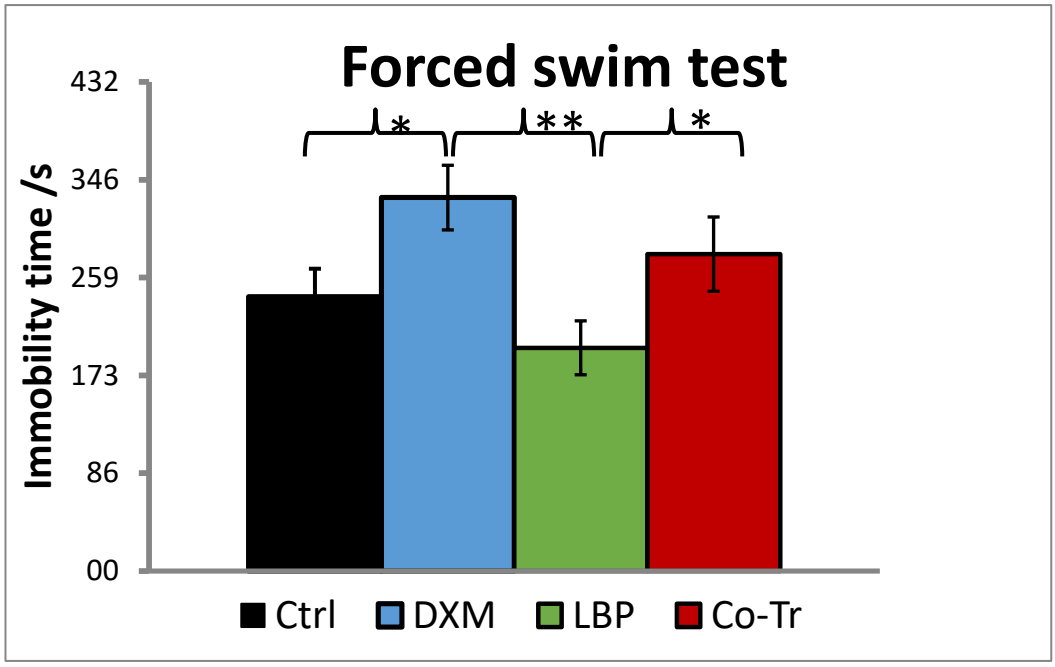


Figure 13. LBP protects against DXM-induced depression-like behaviour. DXM treatment increased immobility time significantly when compared with the normal control. When the rats were co-treated with LBP and DXM, the increase in immobility time induced by DXM was diminished. In contrast, LBP treatment alone did not have any effect. The results are expressed in mean ± SEM. *p <0.05, **p <0.01, one-way ANOVA with LSD post-hoc tests.

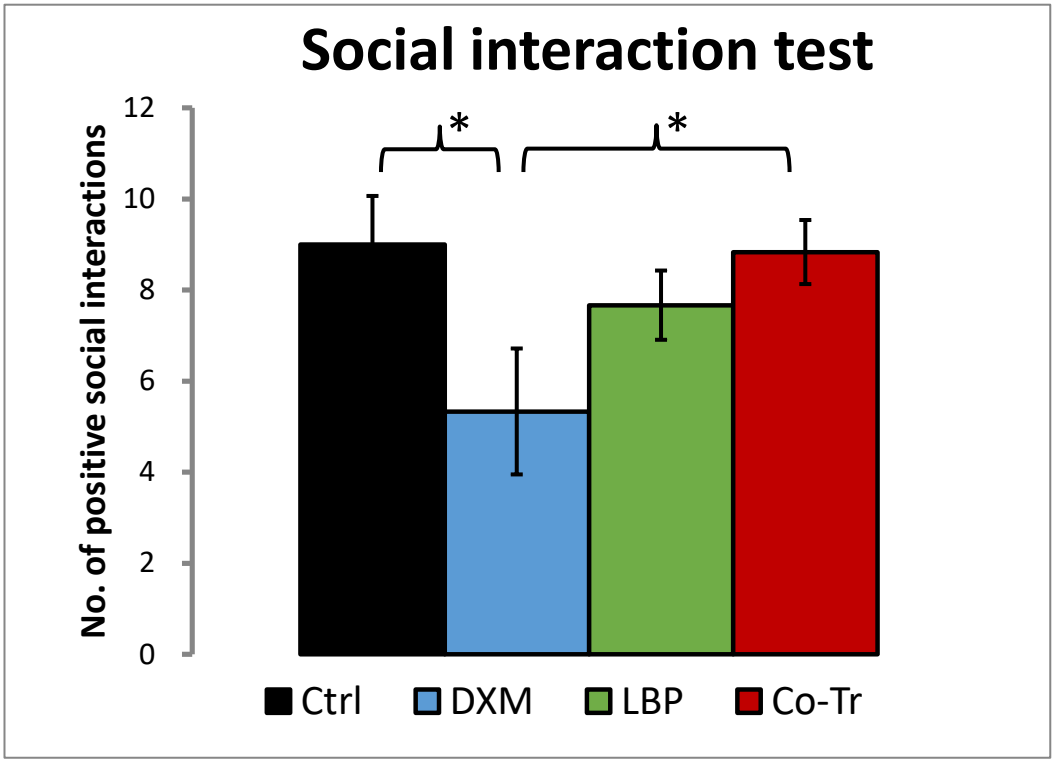


Figure 14. LBP protection against anxiety-like behaviour induced by repeated DXM treatment. Co-treatment of LBP and DXM significantly prevented a decrease in positive interactions in the SIT. The results are expressed in mean ± SEM. *p < 0.05, **p < 0.01, One-way ANOVA with LSD post-hoc tests.

4.3.2 LBP-induced restoration of dextromethorphan-suppressed neurogenesis

To test whether LBP had protected against the suppressed neurogenesis caused by DXM, the brain tissue of the rats was subjected to various histological assessments. To conduct a proliferation assay, BrdU was injected into the rats during days 12–14 of the treatment period to label the proliferative neural precursor cells in the DG. The DXM-treated rats had a significantly lower cell count than the control animals (Figure. 15i, p <0.01). When oral LBP was provided with the DXM injection, this decrease in cell proliferation was prevented. The number of BrdU-positive cells from the rats in the co-treatment group was not significantly different from that of the control.

Neuronal differentiation was also examined in the study. As DCX is only expressed in immature neurons, it is labelled by anti-DCX antibodies. The results indicate that the DCX-positive cells in the co-treatment group did not suffer from the suppression shown in the DXM group. The number of cells in the co-treatment group was significantly higher than in the DXM group (Figure. 15j, p <0.01) and was not significantly different from that of the control as the DXM group was (Figure. 15j, p <0.01). Interestingly, LBP treatment alone did

not have any significant beneficial or adverse effect on either cell proliferation or differentiation.

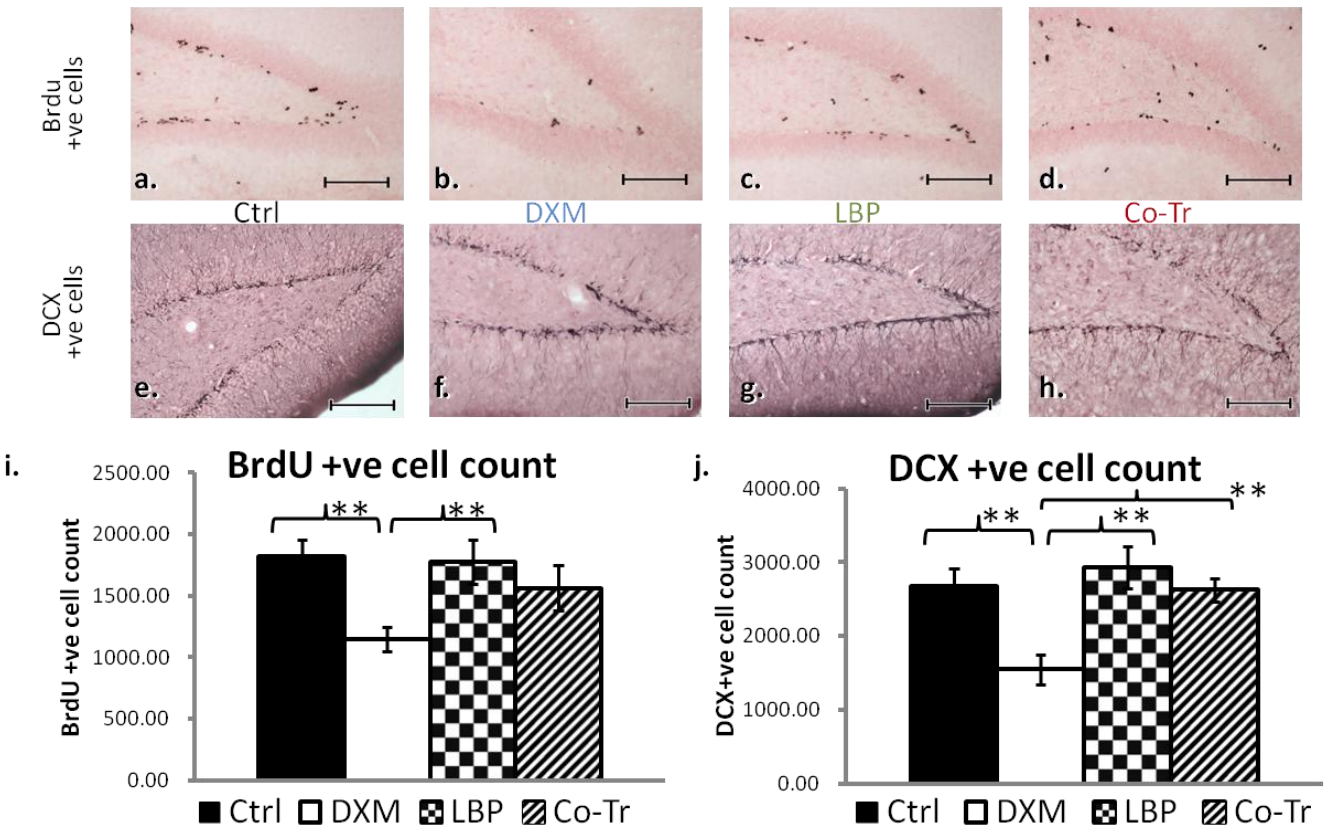


Figure. 15. LBP prevents suppression of neurogenesis in the DG due to repeated, high-dose DXM treatment. **(a-h)** Representative micrographs of BrdU- and DCX-immunoreactive cells in the DG of control, DXM, LBP and co-treatment groups, respectively. **(i)** Co-treatment of LBP and DXM significantly prevented a decrease in BrdU-immunoreactive cells, but LBP treatment alone had no effect. **(j)** Co-treatment with both drugs significantly prevented a decrease in DCX-immunoreactive cells. The results are expressed as mean ± SEM. **p* <0.05, ***p* <0.01, one-way ANOVA with LSD post-hoc tests. Scale bars: 100 µm.

4.3.3 LBP prevention of DXM-induced suppression of dendritic maturation of immature neurons

Sholl analysis is a quantitative method for analysing the dendritic complexity of immature neurons in the DG. A higher number of intersections represents higher dendritic complexity. Representative photomicrographs and the traces of DCX-positive neurons in the four treatment groups are shown in Figure 16.

The results indicate that the complexity of neurons in the DG in the DXM-treated rats decreased significantly compared with the other three groups over a broad range of distances. In contrast, only a single data point at 60 μm in the LBP group was found to be significantly different from the other three groups.

The results also indicate that DXM treatment suppressed dendritic maturation of immature neurons (

Figure 16e) and LBP treatment with DXM prevented the suppression. Interestingly, LBP treatment alone did not exert any adverse or beneficial

effect on dendritic complexity in the DG when compared with the normal control.

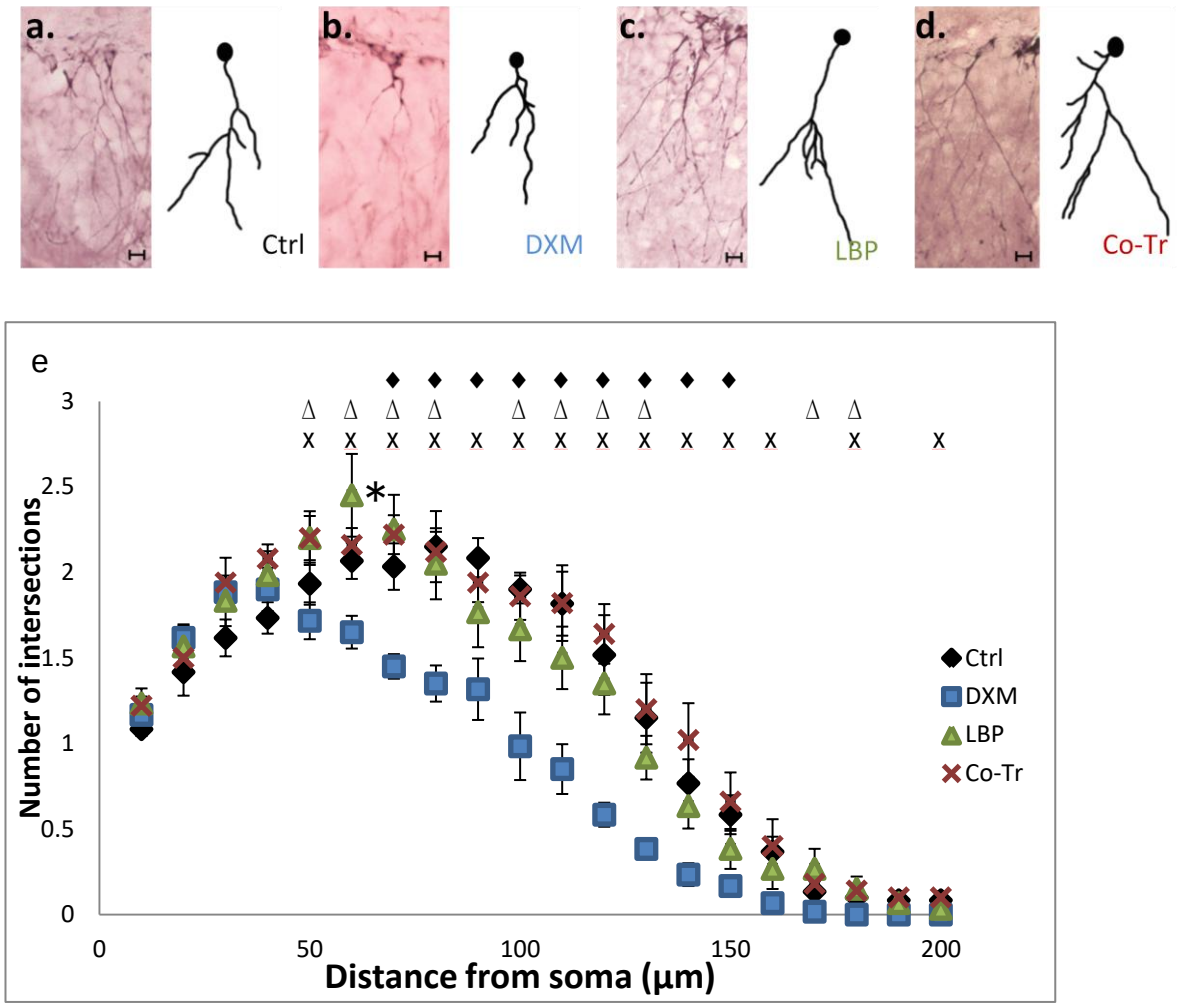


Figure 16. DXM treatment suppresses dendritic complexity and co-treatment prevents the suppression. Representative images of a DCX-immunoreactive immature neuron (left) and its corresponding tracking for Sholl analysis (right) in DG of (**a**) normal control, **b**) DXM-treated group, **c**) LBP-treated group, and **d**) co-treated group. Scale bar: 10 µm. **e**) DXM decreases the number of intersections compared with the other groups, and there are no significant differences except one single data point among the other groups. The results are expressed in mean ± SEM. * p <0.05 between control group and LBP group, ♦,Δ,x: p <0.05 for DXM group compared with control group, LBP group and co-treatment group, one-way ANOVA with LSD post-hoc test.

CHAPTER 5. DISCUSSION

5.1 Experiment 1: Effect of repeated, high-dose DXM on mood and neurogenesis

5.1.1 A 'safe' drug usually overlooked

Cough suppressants, particularly those formulated with DXM, which is regarded as one of the five key abused groups of OTC medicine in the world (Cooper, 2013), are commonly abused because they are easily accessible due to their OTC nature. The severity of the abuse is increasing, particularly among youngsters (Crouch, Caravati, & Booth, 2004; L. C. Lam, Lee, Shum, & Chen, 1996), and Desai et al. (2006) reported that DXM may be the most commonly abused substance that is not well recognised at present (Desai et al., 2006). Understanding the consequences of DXM abuse will aid in the search for effective treatments in clinical situations.

5.1.2 Pharmacology and mechanisms of DXM

DXM is taken up by the gastrointestinal (GI) tract after oral administration. Part of the drug is metabolised by the liver to dextrorphan, which is an active cough suppressant. By binding glutamic acid carriers with

brain microvessel endothelial cells (BMECs), DXM is able to cross the BBB by passive diffusion (Shi et al., 1993). P-glycoprotein is also a factor in the uptake of DXM into the brain (Uhr et al., 2004). In therapeutic doses, DXM acts mainly on the cough centre in the medulla oblongata, and raises the threshold required for a cough reflex (Bem & Peck, 1992) and hence exerts an antitussive effect. Previous studies have shown that the abuse of DXM can induce psychotic and other mood-related symptoms, including hallucination, mania, depressed mood and dysphoria (Amaladoss & O'Brien, 2011; Desai et al., 2006). The findings of this study agree with the observations from the clinical reports, because treatment with repeated supratherapeutic doses of DXM were able to induce depression-like behaviour in the animal model. Supratherapeutic dose of DXM suppresses cell proliferation, neuronal differentiation and the dendritic maturation of new neurons in the DG of the hippocampus, which may help to explain the mechanisms of dysregulated emotion and behaviour (Ruan et al., 2014).

5.1.3 Complex nature of depression is difficult to explain by neurogenesis alone

Various treatments, including antidepressants and physical exercise, have been found to be effective for depressive disorder. Interestingly, these treatments have been found to promote neurogenesis. Therefore, suppressed neurogenesis is assumed to be associated with depressive disorder (Ruan et al., 2014).

Evidence supports the existence of the relationship between neurogenesis and depressive symptoms. First, antidepressants have been found to be pro-neurogenic, and a 2-week time lag has been found between the beginning of treatment and the onset of therapeutic effect (Malberg et al., 2000). Second, stress from psychological or physiological sources (i.e., risk factors for depression) suppress hippocampal neurogenesis *in vivo* (DeCarolis & Eisch, 2010). Third, although with limitations of small sample size and various time point, the autopsy result of untreated depression patients are reported to have a lower rate of neurogenesis and a significantly smaller volume of DG compared with medicated patients (Boldrini et al., 2009). However, nothing to date has demonstrated a causal relationship between hippocampal neurogenesis and depressive disorder. There is also contradictory evidence challenging the neurogenesis theory. For example, without a source

of stress, ablation of neurogenesis alone did not induce depression-like behaviour in mice (Wang et al., 2008).

Besides neurogenic theory, there are also other theory trying to explain the underlying mechanism of depression. The investigation of the pathobiology of the neuroendocrine system in depressive disorder aided develop the monoamine theory of depression. This hypothesis proposes that the level of monoamines in the diseased brain are lowered (Duman, Heninger, &Nestler, 1997). And one possible underlying mechanism of this drop may due to the upregulated monoamine oxidase A Levels in the brain (Meyer et al., 2006). SSRI type antidepressant

Neurogenesis-independent mechanism also proposed for the effect of antidepressants. This mechanism is associated with the modification of the activity of the amygdala, prefrontal cortex and HPA axis (Sahay & Hen, 2007). As part of the HPA-axis, hypothalamus seems to be involved in the mechanism. Since prefrontal cortex and amygdala receive the input from hippocampus, any changes of hippocampal function are likely to modify the function of these highly emotion related structures (Maren &Hobin, 2007; O'Donnell &Grace, 1995; Seidenbecher, Laxmi, Stork, &Pape, 2003).

The neurogenic theory suggests that normal neurogenesis is crucial for antidepressants to exert their beneficial effect (Mahar, Bambico, Mechawar, & Nobrega, 2014) because the function of the hypothalamic–pituitary–adrenal (HPA) axis, which is responsible for mood regulation, is linked to new neurons.

The finding in the third experiment of this study that neurogenesis was restored with improvement of emotional behaviour in the co-treated group relative to the DXM group may be partly explained by this theory.

5.1.4 Normal control and confounding prevention

The control group was injected with vehicle in this experiment because injection stress caused by handling could be a confounding effect, which it is likely to suppress neurogenesis and deteriorate the behaviour tests without a proper control group. The control group protocol in this study minimises the possibility of obtaining a false positive result on behaviour tests and histological tests in the experimental groups because the stress from the injection itself induced depression- and anxiety-like behaviour and also the secretion of cortisol.

Difference of the animal's locomotor activity could be a major thread to the validity of the behaviour tests (Stanford, 2007). Strekalova et.al (2005) summarised that assessment of rodent anxiety-like behaviour and forced swim test can be modified randomly by chronic stress. Chronic stress can interfere these tests unspecifically by altering the locomotor activity of rodents. The locomotor activity of stressed animals is also sensitive to lighting conditions (Bertoglio & Carobrez, 2002; Igarashi & Takeshita, 1995). To prevent the confounding due to the alternation of locomotor activity by stress, some measures have been taken in the experiment. Firstly, empty vehicles were injected into the animals groups that did not assign for drug administration. For example, normal control was administrated with empty distilled water (vehicle of DXM in DXM groups). The purpose of the vehicle administration is to control the chronic stress exposure during the experiment period. Secondly, the experiments have done in a laboratory solely illuminated by artificial light sources to prevent the effect of lighting conditions. Therefore, the confounding effects on locomotor activity were minimised in this series of experiments.

5.1.5 Sholl analysis

Sholl analysis is a tool commonly used in neuroscience to quantify the dendritic complexity of neurons. The main principle of the analysis is to compare the radial distributions of dendritic branches with the distance from the cell body (Ristanović, Milosević, & Stulić, 2006). In the analysis, the number of intersections formed between dendrites and concentric circles with increasing radii centred on the cell body is calculated and compared. A higher number of intersections indicate more complex dendritic branching of a neuron.

Emotional behavioural improvement is associated with the development of immature granule cells and the promotion of dendritic arborisation, and these two effects are common in antidepressant administration and electroconvulsive therapy (Wang et al., 2008). In contrast, stressors suppress dendritic arborisation. For instance, maternal deprivation can suppress the dendritic complexity of young male rodents (Oomen et al., 2011). Sholl analysis is therefore a powerful tool to investigate neuronal maturation and mood regulation as these two factors are tightly linked.

5.1.6 Contradictory action of NMDA receptor antagonism

The contribution of NMDA receptors in neurogenesis is still a matter for debate because their exact function in terms of suppression and promotion of neurogenesis is still unclear. A high dose of ketamine (an NMDA receptor antagonist) suppresses the proliferation of neural progenitor cells isolated from the foetus *in vitro* with prolonged exposure (Dong, Rovnaghi, & Anand, 2012), and continuous exposure to ketamine can suppress neurogenesis in rats (Tung et al., 2008). Another NMDA receptor antagonist, MK-801, also suppresses the development of neurospheres *in vitro* (Mochizuki et al., 2007). NMDA antagonists also suppress increased cell proliferation which is inadduced by ischemia. Treatment with high doses of ketamine or MK-801 can also suppress the upregulation of neurogenesis in a cerebral ischemia condition induced by middle cerebral artery occlusion (Arvidsson, Kokaia, & Lindvall, 2001; Winkelheide et al., 2009). The findings of the present study agree with the above studies and show that repeated supratherapeutic dosings of DXM, a NMDA antagonist, suppress the proliferation of new neurons in the hippocampus.

5.1.7 Limitations

Because mice, rats and monkeys share many physiological and genetic similarities with humans (Monamy, 2009), animal experimentation using these animals can be tremendously helpful for furthering medical sciences, as animal studies can predict the consequences of various tests in humans without causing harm to humans. Although this study has clearly demonstrated the effects of DXM on adult rats' neurogenesis, extra care is required to translate the results to humans.

The dosage applied may not be easily translated to human studies. Pound et al (2004) made some suggestions about animal studies. First, different species can have variations in metabolism, differences in metabolite conversion and variations in efficacy and toxicity. The rats we used might metabolise DXM at different speeds and in different ways to humans. Second, the dosing schedule might not be the same for humans. In this study, the animals were administrated regularly with DXM with constant daily dosages for 2 weeks, while in reality abusers consume DXM irregularly several times a day (Pound, Ebrahim, Sandercock, Bracken, & Roberts, 2004). Another problem is that the administration route in this study was intraperitoneal

injection but abusers consume the drug orally (Mayhew, 2007). The difference in route of administration might lead to differences in effective dosage due to differences in bioavailability (Yeleswaram, McLaughlin, Knipe, & Schabdach, 1997).

The rats expressed depression- and anxiety-like behaviour after treatment with DXM, which agrees with observations of human abusers, and may provide some evidence that the physiology of rat is close enough to human's.

5.2 Experiment 2: Dose-response relationship between daily DXM dosage and neurogenesis

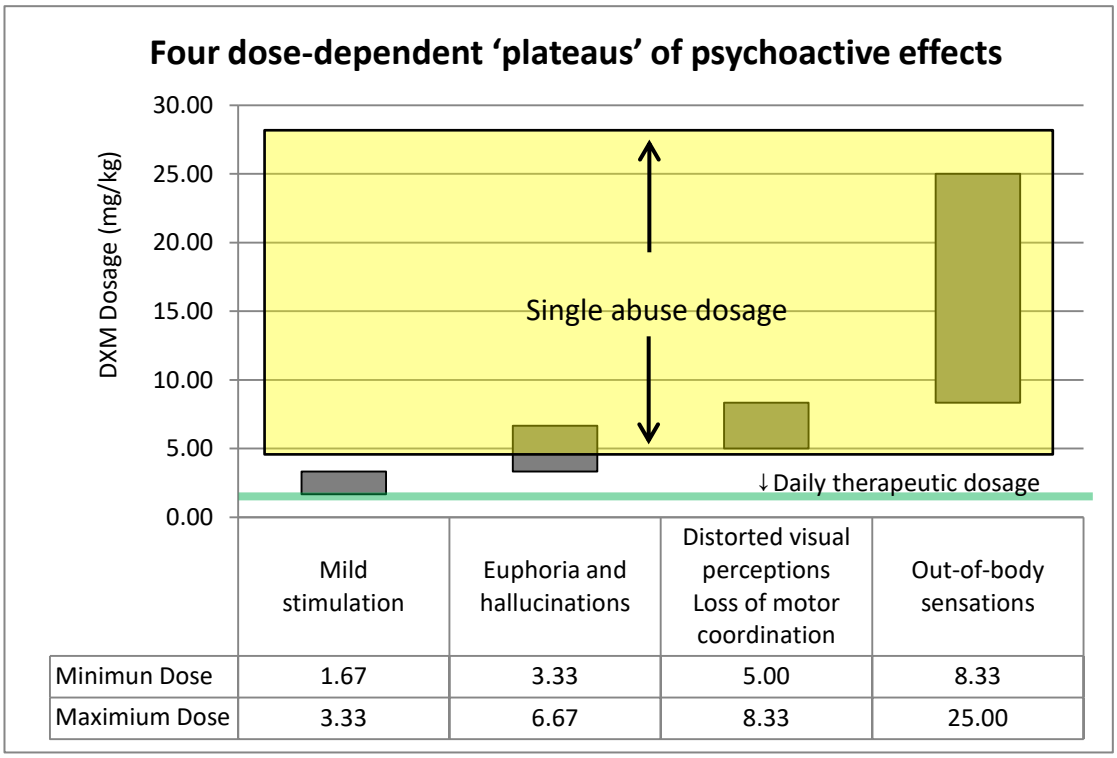
5.2.1 Suppression of neurogenesis from low dose limits DXM's application as antidepressant

The daily therapeutic dosage of DXM for a 60 kg adult is about 1 to 2 mg/kg (Mayhew, 2007). According to a drug fact sheet from the US government, the single abuse dosage ranges from 4.17 to 25 mg/kg (DEA & US DOJ, 2011) (Figure 17). The finding in this experiment indicates that the significantly adverse effects of DXM on cell proliferation in the DG occured at a

relatively low daily dosage of 5 mg/kg. Although the dose for the rats cannot be directly translated into human, this nevertheless suggests that neurogenesis would be suppressed in mild abusers with repeated consumption. This finding that DXM disrupts neurogenesis even in a low dose may indicate the danger of using DXM or NMDA antagonists for recreational purposes. As there is evidence that certain NMDA antagonists have shown antidepressant activity (Amaladoss & O'Brien, 2011; Arvidsson et al., 2001; Bem & Peck, 1992), some researchers have suggested that these types of the antagonist may have potential as antidepressants. Berman et al conducted a human study with oral treatment of 0.5 mg/kg/daily of ketamine, which successfully lowered the depression rate of the ketamine treatment group compared with the control group (Berman et al., 2000), It was suggested that NMDA receptor-modulating drugs may be used in the treatment of depression. Lauterbach (2011) even suggested that DXM should be tested in clinical trials for treatment-resistant depression (Lauterbach, 2011). However, the study of Berman et al. (2000) simply focused on the acute and subacute changes of ketamine administration and neither study discussed the possible chronic effects of NMDA antagonists on neurogenesis. Drugs that suppress

neurogenesis may not be suitable antidepressants because disrupted neurogenesis is associated with depression symptoms (Malberg et al., 2000). As mentioned in section 5.16, NMDA antagonists act in a contradictory manner. Suppression of neurogenesis occurs in high doses but promotion occurs in low doses. Therefore, detailed experiments concerning the dose-response relationship between DXM doses and neurogenesis should be conducted before introducing these antagonists as a treatment for depression.

Apart from cell proliferation, neuronal differentiation, which is another benchmark of neurogenesis, was also affected at the relatively low dose of 5 mg. However, neuronal differentiation plateaued at 20 mg.



	Mild stimulation	Euphoria and hallucinations	Distorted visual perceptions Loss of motor coordination	Out-of-body sensations
Minimun Dose	1.67	3.33	5.00	8.33
Maximium Dose	3.33	6.67	8.33	25.00

Figure 17. Therapeutic dose (labelled in green) and single abuse dose for abusers (labelled in yellow). Dosage to induce the psychoactive effect of mild stimulation is very close to a high therapeutic dosage. Secondary data adapted from Drug Fact Sheets, US Drug Enforcement Administration (DEA & US DOJ, 2011) and Mayhew et al. (2007).

5.2.2 Limitation

The limitation of this experiment is similar to that mentioned in section 5.1.7, the difficulty of translating the results into human terms.

5.3 Experiment 3: Protective effect of LBP against adverse effects induced by DXM on mood and neurogenesis

5.3.1 Possible usage of LBP to treat DXM abuse (including advantages over traditional antidepressants)

DXM is a common and active component in various cough suppressants (Amaladoss & O'Brien, 2011). DXM is a non-competitive *N*-methyl-d-aspartate (NMDA) receptor antagonist which exerts its antitussive effect by acting on the cough centre of the medulla oblongata (Bem & Peck, 1992; Werling et al., 2007). However, the NMDA antagonistic activity of DXM resembles the characteristics of phencyclidine and ketamine, which are well-known hallucinogens that also exhibit NMDA receptor antagonistic activity. The similarity between DXM and these two commonly abused recreational drugs might explain the psychiatric and hallucinogenic effects of DXM in

supratherapeutic doses in both acute and chronic situations (Krystal et al., 1994; Reissig et al., 2012).

Although this study did not fully explain the underlying mechanism of mood disorder in DXM abusers, it demonstrated that mood symptoms of drug abusers can be modelled in rodent models, and there was an association between DXM-induced mood symptoms and impairments of neurogenesis; therefore, neurogenesis protection could be a possible treatment target and *Lycium barbarum* polysaccharide (LBP) may be a suitable protective agent. LBP is a compound extracted from the fruit of *L. barbarum*. Commonly known as wolfberry or goji, *L. barbarum* has been a component of Chinese herbal medicine for more than a thousand years. The plant has been widely cultivated in China, especially in Ningxia (Lim, 2012). Quantitatively, the most important group of compounds in *L. barbarum* is LBP (Potterat, 2010), which is actually a complex mixture of highly branched polysaccharides and proteoglycans rather than a single chemical (Lim, 2012).

With its high prevalence, DXM abuse not only affects personal health, but also the public healthcare system because the long rehabilitation period of patients places a heavy burden on the healthcare system. Current medical treatments

for psychotic patients have limitations. For example, antidepressant treatments have a high rate of failure (40–60%) (Masand, 2003) and their side effects can be unpleasant for patients. In contrast, few side-effects have been reported for LBP. Extra precaution should be taken when prescription LBP to patients with blood clots problems. LBP has been reported to potentiate the effect of warfarin, a common blood thinner drug. There have been a few cases of drug interaction of LBP with warfarin (Rivera et al., 2012). In addition to a long medicinal history of wolfberry use in China, LBP can be regarded as a safe herbal component and suitable for a novel treatment of DXM abuse. As the herb can minimise general health problems such as fatigue and stress, which are symptoms of neurogenesis impairment (Lim, 2012), LBP could be a potent adjunctive therapeutic agent for DXM abuse.

5.3.2 Possible mechanisms of LBP

5.3.2.1 Neurogenesis-dependent and independent pathways of wolfberry

The causal relationship between depression and impaired neurogenesis is still a matter of debate. Previous research by our team revealed that normal cell–cell communication is affected by neurogenesis and

the blockage of neurogenesis induces impairment of prepulse inhibition (Lau et al., 2009). Prepulse inhibition and sensorimotor processes are two neurological phenomena regulated by the hippocampus. Prepulse inhibition is a phenomenon in which a reduction of the startle reflex response occurs when there is a weak prepulse 30–500 ms before the main startle stimulus (Bast & Feldon, 2003). It has been suggested as a mechanism protecting sensorimotor-gating. Prepulse inhibition deficits are linked with neurological conditions including schizophrenia and with the administration of NMDA receptor antagonists (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Perry, Minassian, & Feifel, 2004). These findings suggest that the behaviour changes observed in this study may be induced by the alteration of normal hippocampus functions caused by suppressed neurogenesis.

Another study explored which biochemical pathway is affected by LBP to produce its protective effect. In a study conducted by Chen et al. (2014), the protective effect of LBP in scopolamine intoxication was studied. The prevention of the adverse biochemical effects was observed. A decrease in the concentration of antioxidant enzymes and a rise in oxidative stress and

apoptosis due to SCO administration were prevented by LBP treatment (Chen et al., 2014).

In a vitro study involving primary neuronal culture, LBP was able to lower the level of activated protein kinase R Caspase-2 and 3 when these cells are treated with beta-amyloid peptide. Thu, this discovery has suggested the involvement of the mentioned caspase and kinase when LBP exert its protective effects against inflammation and apoptosis (Yu et al., 2007).

Despite the fact that LBP shows a protective effect on neurogenesis, there is evidence that its effects may be exerted by other mechanisms. A neurogenesis-independent pathway through which LBP exerts its protective effect was revealed by Zhang et al. (2012). In their study, rats with ablated hippocampal neurogenesis were given a corticosteroid to see if LBP could still exert its protective effect. Their study showed that LBP was able to reduce the immobility time of corticosteroid-treated rats with blocked neurogenesis and enhanced synaptic plasticity was observed in the rats (Zhang et al., 2012). The study thus revealed that a neurogenesis-independent pathway might be present in the protective effect of LBP. However, a neurogenesis-independent mechanism has been observed for antidepressants such as fluoxetine (Jedynak,

Kos, Sandi, Kaczmarek, & Filipkowski, 2014). Thus, this study may only explored the neurogenesis-dependent protective effects of LBP against repeated high dose of DXM administration and further study on neurogenesis-independent pathway is required for a full understanding of LBP's protective effect against DXM.

5.3.2.2 Another component of wolfberry stimulating neurogenesis: taurine

Taurine is also a major component of wolfberry that is reported to have certain pro-neurogenic effects. The addition of taurine to culture medium can enhance the neurogenesis of cultured neural stem cells of adult mice (Hernández-Benítez, Ramos-Mandujano, & Pasantes-Morales, 2012) and cultured neural precursors of foetal human brains (Hernández-Benítez, Vangipuram, Ramos-Mandujano, Lyman, & Pasantes-Morales, 2013). However, these related studies focused on *in vitro* developmental status, which limits their relevance to adult neurogenesis in the DG and mood disturbances.

The purification route of LBP in this study was the same as in the study conducted by Yu et al. (2007). There were alcohol precipitation steps in the purification and the precipitate was discarded. As taurine is polar and

insoluble in absolute alcohol, this compound is likely to be removed by the process. Therefore, taurine is unlikely to have been present in the LBP used in this study.

5.3.3 Justification for using LBP (compared with other traditional Chinese medicine)

Like wolfberry, ginseng has also been applied in different treatments in traditional Chinese medicine, including various neurological diseases and disturbances of mood and cognitive performance (Kennedy & Scholey, 2003). Although ginseng is famous for its all-round functions related to the nervous system, it is also expensive because it can only be cultivated in a few areas, and grows slowly when compared with wolfberry. Ginseng total saponins (GTS), the active component of ginseng, account for only 2.0–4.5 wt% in the root of mature *Panax ginseng* (Huang, 1998; Lui & Staba, 1980; Tang & Eisenbrand, 1992). In contrast, LBP can compose up to 23% of the dry fruit mass of wolfberry (Yin & Dang, 2008). In addition, 50–100 mg/kg of GTS is required in a 7-day treatment for significant reduction in the immobility time in the FST, (Dang et al., 2009) which is much more than the amount required for LBP in

different disease models (Chen et al., 2014; C. Lam et al., 2013; Lau et al., 2012). Therefore, compared with GTS, LBP seems to be a better candidate as an adjunctive treatment for DXM abusers because its effective dosage and much lower costs.

Although *Curcuma longa* can also minimise neurogenesis impairment in stress models (Xu et al., 2007), it has not been as extensively investigated in disease models as LBP or ginseng. Moreover, it does not have a long medicinal history of treating neurological diseases as ginseng and wolfberry do.

5.3.4 Limitations

This study only addressed the question of the neuroprotective effects of LBP against mood-related behavioural impairment and suppressed neurogenesis due to DXM abuse. However exploratory, this study offers some insights into the possibility of using LBP as an adjunctive treatment for DXM abuse. The animal study using LBP as post-treatment aimed to investigate whether rehabilitation could be improved by LBP in an attempt to shed more light on LBP's role in treatment for DXM abuse.

As physiological differences exist between humans and rats, the LBP dosage applied in the experiment might not be suitable for testing in human subjects.

CHAPTER 6. CONCLUSION

6.1 Experiment 1: effects of repeated, high-dose DXM on mood and neurogenesis

It can be concluded that repeated, high-dose DXM treatment can suppress hippocampal neurogenesis and induce depression-like behaviour in adult rats, as illustrated by the current study. As the development of the central nervous system can be modified by the abuse of cough syrup, future studies of the effect of repeated high dose of DXM administration in adolescent animals are suggested. Also, an investigation into the dose-dependent effects of DXM and the molecular mechanisms underlying its effects will provide further understanding of the damage caused by DXM abuse and suitable treatment, which may help develop a new clinical treatment for DXM abuse. Further studies on the potential beneficial effect of antidepressants on DXM-treated animals may increase our understanding of the underlying mechanism of DXM abuse; however, extra precaution is required for investigations involving selective serotonin reuptake inhibitor (SSRI) type antidepressants. Like SSRIs, DXM is also exhibit serotonin reuptake inhibition activity (Lane & Baldwin, 1997). Combined use of these inhibitors is

highly associated with serotonin syndrome, especially with the severe cases of the syndrome (Boyer & Shannon, 2005). For instance, drug interactions of DXM and SSRIs have been reported in humans as serotonin syndrome, which is a life-threatening condition with symptoms including fever, tremor and diarrhoea (Schwartz, Pizon, & Brooks, 2008).

6.2 Experiment 2: Dose-response relationship between daily DXM dosage and neurogenesis

The significant adverse effect of DXM on cell proliferation in the DG occurred from a relatively low dose of 5 mg/kg daily. This finding suggests that hippocampal neurogenesis may be suppressed even in mild DXM abusers with repeated consumption. As low doses of NMDA antagonists such as ketamine can alleviate depression symptoms in humans in acute and subacute phases, some research groups have suggested that DXM may be a suitable treatment for depression. However, the suppression of neurogenesis at low doses may limit DXM's application as an antidepressant. Further detailed experiments concerning dose-response relationship between DXM dose and neurogenesis

should be done before introducing these antagonists as a treatment for depression in humans.

6.3 Experiment 3: The protective effect of LBP against the adverse effect induced by DXM on mood and neurogenesis

To conclude, LBP not only shows a neuroprotective effect against the suppression of neurogenesis in repeated and high-dose DXM administration, but also relieves mood symptoms by reducing depression- and anxiety-like behaviour. This finding sheds light on the clinical application of this inexpensive herbal component as an adjunctive treatment for DXM abuse.

APPENDICES

Certificates of attendance of 21th PPCR

Certificate of Attendance

PPCR

This is to certify that

Kevin, Kai Ting PO

attended

The 9th Pan-Pacific Conference on Rehabilitation
cum 21st Annual Congress of Gerontology
第九屆泛太平洋康復會議暨第廿一屆老年學週年會議

Rewinding the Aging Clock
黃金歲月・活出精彩

29-30 November 2014, Hong Kong

Co-organizers

Prof. Gabriel Ng
Chair Professor of Rehabilitation Sciences
and Head
Department of Rehabilitation Sciences
The Hong Kong Polytechnic University

Dr. Edward M F Leung
President
Hong Kong Association of Gerontology

The Hong Kong Polytechnic University 香港理工大學

康復治療科學系 Department of Rehabilitation Sciences

Certificates of attendance of 25th IBNS Meeting

IBNS
International Behavioral Neuroscience Society

June 24, 2016

CERTIFICATE OF ATTENDANCE

This certificate confirms that Kai Ting Po attended and participated in the 25th International Behavioral Neuroscience Society (IBNS) Meeting held in Budapest, Hungary, June 7-12, 2016.

Marianne Van Wagner, IBNS Executive Coordinator

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