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REGULATION OF ADULT HIPPOCAMPAL NEUROGENESIS AND AFFECTIVE BEHAVIORS BY TACTILE STIMULATION IN RATS

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MPhil

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Regulation of Adult Hippocampal Neurogenesis and Affective Behaviors by Tactile Stimulation in Rats

Chow Ka Wing

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

January 2019

CERTIFICATE OF ORIGINALITY

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

_____(signed)

_____CHOW KA WING_____(Name of student)

ABSTRACT

Tactile stimulation (TS) is a commonly employed treatment strategy for different clinical conditions, including developmental and psychiatric conditions. It was shown to have potential neurogenic effects by promoting cell proliferation in the spinal cord, however, the theoretical basis and underlying biological mechanisms of tactile stimulation in the brain remain unknown. Brushing, a specific type of tactile stimulation, has been employed as a treatment to decrease sensory over-responsiveness and enhance the recovery of cognitive function and emotions in human. For further understanding of the mechanisms, this study aims to investigate the effect of single and repeated tactile stimulation on affective behaviors, adult hippocampal neurogenesis and dendritic growth in the rat brain. The present study was divided into two experiments.

First, young male adult Sprague-Dawley (SD) rats were treated with TS for 1 ,14 and 28 days to assess the effect of novel and repeated TS on hippocampal neurogenesis, depression- and anxiety-like behaviors. TS was applied by a brush treatment over the back, forelimbs, hind limbs and whisker pads of rats. The results reveal that both novel and repeated TS may enhance dendritic growth but not neurogenesis in the hippocampus. Behavioral tests indicated that TS induced stress in rats.

In experiment two, young male adult SD rats were treated with high-dose corticosterone to induce suppression of neurogenesis, depression-like and anxiety-like behaviors. Repeated brush treatment (14 days) reduced the depression-like and anxiety-like behaviors in corticosterone (cort)-treated rats. In addition, the rate of neuronal cell differentiation in the hippocampus was enhanced by brush treatment. Moreover, the suppression of dendritic complexity in the hippocampal DG of cort-treated rats was reversed by repeated TS. Affective behaviors were also improved by TS in cort-treated rats. The findings indicate that repeated tactile stimulation has anxiolytic and antidepressant-like effects and promoted neurogenesis in hypercortisolemic animal model.

In conclusion, novel and repeated TS enhanced hippocampal dendritic growth but induced stress in healthy rats. In addition, the habituation effect was found, and affective behaviors were improved after rats receiving 28-days TS. In hypercortisolemic rat model, repeated TS reversed the neurogenic impairment in the hippocampus and improved affect behaviors. The result not only suggests that neurogenesis may be the underlying mechanism of TS, but also provides evidence to support the clinical practice of treatments incorporating TS.

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LIST OF ABBREVIATIONS

Brush treatment/intervention (Brush/Tx)

Bromodeoxyuridine (BrdU)

Control group (Ctrl)

Corticosterone (Cort)

Diaminobenzidine (DAB)

Doublecortin (DCX)

Forced swimming test (FST)

Open-field test (OFT)

Paraformaldehyde (FPA)

Social interaction test (SIT)

Tactile stimulation (TS)

Chapter 1

Introduction

1.1 Overview

An overview of the theoretical background of the study is provided in this chapter. It provides background information and outlines the relation between adult hippocampal neurogenesis, tactile stimulation and affective behaviors. Moreover, justification of the study and the organization of the thesis is provided.

1.2 Statement of purpose

Adult neurogenesis is defined as a process of new functional neurons production in the central nervous system (CNS) during adulthood. The processes include cell proliferation from neural precursor cells, cell survival, neuronal fate differentiation, migration and integration into the existing neural connection. Adult neurogenesis mainly occurs in two neurogenic regions in CNS, including the subgranular zone in hippocampal dentate gyrus and the subventricular zone at the lateral ventricles (Zhao, Deng & Gage, 2008, Ruan et al., 2014). As the hippocampus is responsible for emotional regulation and memory formation, adult neurogenesis in the hippocampus has been extensively studied with the relation to spatial memory, learning and

affective behaviors (Bannerman et al., 2004). Neurogenesis, as a mean of neuroplasticity, has been proposed as a new therapeutic target for the improvement of memory and emotional regulation (Campbell & Macqueen, 2004; Nestor, Graham, Bozeat, Simons, & Hodges, 2002; Shrager, Bayley, Bontempi, Hopkins, & Squire, 2007).

Several studies have reported that adult neurogenesis in the hippocampus can be enhanced by different environmental stimuli, for example enriched environment, which involved tactile stimulation (TS) and other type of sensory stimulations (Monteiro, Moreira, Massensini, Moraes & Pereira, 2014, Shechter et al., 2011, Nilsson, Perfilieva, Johansson, Orwar & Eriksson, 1999, Brown et al., 2003, Kempermann, Kuhn & Gage, 1997). Due to multiple sensory stimulations were applied in enriched environment, the effect of single sensory stimulation on neurogenesis in the hippocampus requires further investigation. Recently, researchers reported that TS promoted neurogenesis in the spinal cord (Shechter et al., 2011). Moreover, tickling stimulation, which is a form of TS that is related with positive emotion, enhanced adult neurogenesis in the rat's hippocampus (Yamamuro et al., 2010). Collectively, the evidence suggested that TS, as a pro-neurogenic stimulus, might promote hippocampal neurogenesis and thus influence emotional behaviors.

TS has been applied in rehabilitation for treating hypersensitivity clinically. A brushing protocol with tactile and pressure sensation, called Wilbarger's protocol, has been employed in rehabilitation for treating patients with anxiety (Kimball et al., 2007). Moreover, massage, another form of TS, reduced the level of anxiety in patients (Gurol, Polat, & Akcay, 2010). As TS serves as a potential rehabilitation method that is inexpensive, non-invasive, and easy to apply, the understanding of the underlying mechanisms of TS is essential to provide information for treatment development.

Based on the evidence that TS enhanced neurogenesis in the hippocampus in animal studies and improved affective behavior in both pre-clinical and clinical studies, it is hypothesized that TS may promote adult hippocampal neurogenesis and thus improve affective behaviors. This study aims to investigate the effect of TS on adult hippocampal neurogenesis and affective behaviors under controlled experiments.

1.3 Organization of chapters

This thesis is mainly divided into six parts. After the introduction, next chapter provides a summary of literature review with an overview of studies in the field of tactile stimulation, adult neurogenesis and affective behaviors. In chapter three, the

detailed experimental designs, procedures and materials are provided and discussed. Chapter four presents the results from experiments with charts and graphs and chapter five provides the discussion on experimental results. The ending chapter provides an overall conclusion for the project.

Chapter 2

Literature review

2.1 Overview

This chapter presents a literature review for hippocampal neurogenesis, and its relationship with affective behaviors and tactile stimulation. Moreover, this chapter summarizes and compares the current studies that are investigating tactile stimulation, neurogenesis and animal behaviors. The study hypothesis and objectives are provided at the of this chapter.

2.2 Adult hippocampal neurogenesis

Adult neurogenesis is a physiological process that generates new functional neurons in the adult mammalian central nervous system. The neurogenic process includes neural stem/ progenitor cells proliferation, progeny survival, neuronal differentiation and maturation, cell migration and circuit integration (Zhao, Deng &

Gage, 2008, Ruan et al., 2014, Ruan et al., 2014). Adult neurogenesis mainly takes place in two mammalian brain regions, namely, neurogenic regions, which are 1) the subgranular zone (SGZ) in the hippocampal dentate gyrus (DG) and 2) the subventricular zone (SVZ) of the lateral ventricles (Ruan et al., 2014). This study is focused on hippocampal neurogenesis.

The hippocampus is a structure located in the medial temporal lobe of each side of the brain. It is responsible for memory consolidation, spatial memory formation and mood regulation (Campbell & Macqueen, 2004; Nestor, Graham, Bozeat, Simons, & Hodges, 2002; Shrager, Bayley, Bontempi, Hopkins, & Squire, 2007). The signaling circuitry of the hippocampus is consisted of the connection from DG to CA1 and CA3, which formed a trisynaptic loop. Signal input from entorhinal cortex to DG granule cells via perforant pathway, and then transduces to CA3 by mossy fiber. Signal send from CA3 to CA1 by Schaffer collateral projection and eventually to the subiculum and entorhinal cortex (Christian, Song, & Ming, 2014). Also, a study demonstrated that the neurogenesis is critical for hippocampus-related functions (Aimone, Deng, & Gage, 2011).

In the adult hippocampal DG, new functional granule cells are produced by neurogenesis. Radial glia-like cells possess stem cell properties of self-renewal and cell proliferation in the SGZ. Proliferative cells convert to neuroblasts, which is a type

of cells that will be committed as neuron after migrating to granule cell layer (GCL) in the DG (Ruan et al., 2014). The exact functions of newly generated granule cells remain unknown. However, numerous studies have examined the functions of neurogenesis in brain disorders and affective emotions. In the following part, the relation between mental disorders and adult hippocampal neurogenesis is discussed.

2.3 Adult hippocampal neurogenesis and affective disorders

According to American Psychiatric Association and World Health Organization, mental disorders are defined as the negative alternation of thinking, emotion or behavior. Several disorders have been identified, for example: depression, dementia, anxiety disorders and schizophrenia. Patients with mental disorders show different psychological and biological dysfunction in the brain (American Psychiartric Association, 2015; World Health Organization, 2017).

The association of adult neurogenesis and mental disorders has been reported. The regulation of adult hippocampal neurogenesis has been reported to associate with different physiological and pathological situations (Ma, Kim, Ming, & Song, 2009). In this section, evidence for the interconnection of adult neurogenesis and depression and anxiety disorder is reviewed and discussed.

2.3.1 Adult neurogenesis and depression

Depression is a type of mental disorders that occur commonly around the world (World Health Organization, 2017). Approximately, three hundred million people are suffered from depression. It can be long-lasting in patients' lifespan. Symptoms include sadness, feeling of guilt, anhedonia, lack of concentration, insomnia and increased thoughts of death. Antidepressant is a possible treatment for patients with moderate and severe depression (World Health Organization, 2017). It is a group of drugs involving several classes of medication, for instant, tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (MAOIs).

A considerable number of studies have revealed that antidepressants promote neurogenesis. A study from Malberg et al. reported that prolonged administration of antidepressant significantly promotes neural proliferation in hippocampal DG but not in SVZ in adult rodent (Malberg, Eisch, Nestler, & Duman, 2000). Another study also demonstrated that antidepressants have pro-neurogenic properties by regulating the function of glucocorticoid receptor (Anacker et al., 2011). In order to investigate the neurogenic effect of antidepressants, researchers applied x-irradiation to eliminate the hippocampal neurogenesis in adult rodents (Santarelli et al., 2003). The ablation of hippocampal neurogenesis inhibits the anxiolytic effect of fluoxetine, a type of SSRI

antidepressant drug. These results suggested that adult neurogenesis in hippocampus is essential for antidepressants to improve mood disorders.

Although antidepressants require neurogenesis to exert therapeutic effects, the causal relationship between adult neurogenesis and depression remains unclear. Several studies have showen that treatment with high-dose corticosterone (more than 20 mg/kg), a stress hormone in animals, suppressed neurogenesis and induced depression-behaviors (Brummelte & Galea, 2010; Iijima, Ito, Kurosu, & Chaki, 2010). In contrast, studies from Airan et al. and David et al. reported that animals without neurogenesis do not cause depression (Airan et al., 2007; David et al., 2009).

2.3.2 Adult neurogenesis and anxiety disorders

Anxiety disorder is a class of mental disorders that patient suffering from extreme or prolonged anxiety and fear. It consists of different subtypes of disorders, for example, generalized anxiety disorder, panic disorder and social anxiety disorder. Treatment for anxiety disorders include psychotherapy, like cognitive behavioral therapy, and medication, such as antidepressants and anxiolytics. It is not able to cure anxiety disorders but symptoms can be relieved by medications (The National Institute of Mental Health, 2016). Several pre-clinical studies have investigated the relation between adult hippocampal neurogenesis and anxiety disorders.

In the review from Ruan et al., researchers summarized the evidence from the research of anxiolytics: 1) pro-neurogenic effects was found in serotonin reuptake inhibitors (SSRIs), 2) a synthetic anxiolytic drug, HU210, was found to be neurogenesis-dependent and 3) the improvement of behaviour by SSRIs administration was associated with neurogenesis. Also, neurogenesis in the hippocampus is enhanced and depression-like behaviours is prevented by anticonvulsant, which is prescribed for patients with generalized anxiety disorder. In addition, Ruan et al. pointed out that voluntary running and enriched environment, which have been reported as non-pharmacological antidepressants, can exert anxiolytic effects in animals with neurogenesis inhibition (Ruan et al., 2014, Monteiro et al., 2014, Naylor et al. 2008). The evidence suggested that pharmacological and non-pharmacological anxiolytics require adult neurogenesis in the hippocampus to exert its therapeutic effect, however, the interconnection of anxiety disorders and neurogenesis remain to be further confirmed.

2.4 The association of tactile stimulation with hippocampus

The hippocampus, as an important part of the mammalian brain, has been reported that involves in tactile sensation processing (Pereira et al., 2007). Researchers demonstrated the role of the hippocampus in navigation (Ekstrom et al.,

2003) and novelty detection (Ranganath, Esposito, & Wills, 2001) and proposed the underlying pathway. For example, rat, which is an excellent model for studying sensory neuroscience, uses their distinct sensory organ, whiskers (or facial vibrissae) for navigating and receiving tactile stimulation surrounding them. When an animal receives sensory stimulation from whiskers, information passes through the trigeminal nerve and reaches the brain. Information reached sensory cortical area S1 is then transferred to the perirhinal cortex and entorhinal cortex. In entorhinal cortex, sensory information is transfer to hippocampus by perforant path, and reaches hippocampal CA3 and CA1. The new information in CA1 is compared with information stored in CA3. This match and mismatch mechanism allows animals to discriminate novel stimulation from familiar stimulation (Pereira et al., 2007).

2.5 An overview of current studies on tactile stimulation, adult neurogenesis and affective behaviors

Tactile stimulation (TS) is defined as kinesthetic or mechanosensory stimulation which generate sensory input through the sense of touch (Poletto, 2006). TS occur in our daily life simultaneously, for example, walking barefoot on different textures and combing hair. In occupational therapy, tactile stimulation is employed for treating hypersensitivity. Moreover, a brushing protocol (Wibarger protocol) has been

employed for treating patients with anxiety and emotional liability by providing tactile and pressure sensation (Kimball et al., 2007). Researchers reported that repeated TS in the form of massage can reduce anxiety level in patients with burn injuries (Gurol, Polat, & Akcay, 2010) . In animal studies, TS showed an anxiolytic effect in rodents (Boufleur et al., 2012; Imanaka et al., 2008). The evidence suggested that TS, as a non-invasive and non-pharmacological treatment, can relieve negative affective emotions. However, the underlying therapeutic mechanism remains unknown.

A limited number of pre-clinical research assessed the effect of tactile stimulation on adult neurogenesis and animal behaviors. After a data base search of PUBMED, with key words “tactile stimulation” or “tactile” and “neurogenesis”, only 4 related articles were found by title and abstract screening at 21st Dec, 2018. Three studies were related to hippocampal neurogenesis, while one study was related to spinal cord neurogenesis (Table 1). The studies from Yamamuro et al., Angeles et al. and Shechter et al. reported that TS promotes adult neurogenesis. However, the emotional behaviors of the animals were not assessed in all the studies (de los Angeles, del Carmen, Wendy, & Socorro, 2016; Leslie, Guinsburg, Euge, & Sa, 2008; Shechter, Baruch, Schwartz, & Rolls, 2011; Yamamuro et al., 2010). The following table provides a summary of current studies focusing on TS, hippocampal neurogenesis and

behaviors in animal model.

Table 1: Summary of the studies from data base search of PUBMED

	Focus of the study				
Reference	**Animal model**	**Disease/Stressed**	**Trigger TS by**	**Hippocampal neurogenesis**	**Behavioral assessment**
(de los Angeles et al., 2016)	Wistar rats	Prenatally stressed	Fingers sliding on the back	Increased	Spatial learning and memory
(Yamamuro et al., 2010)	Wistar rats	Normal	Tickling by fingers	Significantly increased	Chirp of rats was recorded to indicate positive emotion
(Shechter et al., 2011)	C57BL/6J mice	Normal	Providing sensory-enriched environment	Not assessed, but increased spinal cord adult neurogenesis	Not assessed
(Leslie et al., 2008)	Newborn Wistar rats	Normal	Touching paws with cotton swabs	Not altered	Not assessed

2.6 Hypothesis

Based on the evidence that: 1) TS can relieve anxiety, 2) anxiolytics require neurogenesis to exert therapeutic effect and 3) TS promotes adult hippocampal neurogenesis, it is hypothesized that TS may enhance adult hippocampal neurogenesis and consequently improve affective behaviors.

2.7 Aims and objectives

This project aims to 1) examine the effect of single and repeated TS on adult hippocampal neurogenesis and affective behaviors, 2) determine whether TS alters hypothalamic-pituitary-adrenal axis and 3) examine whether TS improves or prevents the suppression of neurogenesis and affective behavioral disturbances in hypercortisolemic animals.

In order to achieve the objectives, this study was divided into 2 experiments, including: 1) The effects of single and repeated tactile stimulation on hippocampal neurogenesis and affective behaviors and 2) The potential therapeutic effects of repeated tactile stimulation on hippocampal neurogenesis and affective behaviors in the hypercortisolemic animal model. The first experiment mainly focused on the time effect of TS on adult neurogenesis in the hippocampus and the second experiment investigated the therapeutic effect of TS on rats with neurogenic impairment and

affective behaviors disturbances induced by chronic high-dose corticosterone administration. Outcome measures were the levels of neurogenesis in the hippocampus and depression- and anxiety-like behaviors. In order to assess neurogenesis in the hippocampus, proliferative and neuronal differentiation biomarkers were targeted by immunohistochemistry and neuronal morphology were analyzed. For affective behaviors, animal behavioral tests including open field test, social interaction test and forced swimming test were employed. The details of experimental designs, materials and procedures are provided in chapter 3.

Chapter 3

Methodology

3.1 Overview

This chapter provides the methodology of the project. The project divided into two experiments for investigating the effect of tactile stimulation on adult hippocampal neurogenesis and affective behaviors. The experimental design is provided at the beginning followed by materials used and procedures for applying TS, assessing animal behaviors and hippocampal neurogenesis.

3.2 Experimental designs

3.2.1 Experiment 1: The effects of single and repeated exposure of tactile stimulation on hippocampal neurogenesis and affective behaviors: a time-dependent study

This experiment aims to investigate the effect of novel and repeated exposure effect of tactile stimulation (TS) on hippocampal neurogenesis and affective behaviors in rat and to examine the time-dependent effect of TS. Sprague-Dawley (SD) rats aged from 6-7 weeks, weighted 250±20 g, were used for the experiment. Rats were obtained from the Centralized Animal Facilities of the Hong Kong Polytechnic University. They were housed three per cage in a 12/12-h light-dark cycle at 22°C and

were fed food and water *ad libitum*. The rats were treated according to the guidelines provided by the Animal Subjects Ethics Subcommittee of the Hong Kong Polytechnic University.

Thirty-six rats were randomly allocated into 6 groups (n=6 per group), including control (Ctrl) and treatment group (Tx) with 3 different treatment periods (1, 14 and 28 days, Fig. 1). Tx rats received TS on their back, forelimbs, hindlimbs and whiskers by a conventional toothbrush connected with a force detector. Force applied on rat was monitored by experimenter. TS was lasted for 5 minutes per day. The details of TS treatment are provided in part 3.2. In order to assess the level of neurogenesis in the hippocampal DG, bromodeoxyuridine (BrdU, 50mg/kg, intraperitoneally), an analog of thymidine, was injected at the last 3 days of treatment period for 14- and 28-days experiments to label proliferative cells. After the 14 days and 28 days treatment, behavioral assessments were conducted and rats were then euthanized. For novel treatment experiment (1day of TS), BrdU was injected 4 hours before TS and behavioral tests were conducted after 2 hours of TS. The brain was dissected for immunohistochemical analysis.

3.2.2 Experiment 2: The potential therapeutic effects of tactile stimulation on neurogenic impairment and affective disturbances in the hypercortisolemic animal

model

This experiment aims to investigate whether TS would prevent the suppression of neurogenesis and affective disturbances induced by hypercotisolemia. Sprague-Dawley (SD) rats aged from 6-7 weeks, weighted 250±20 g, were used for the experiment. Rats were obtained from the Centralized Animal Facilities of the Hong Kong Polytechnic University. They were housed three per cage in a 12/12-h light-dark cycle at 22°C and were fed food and water *ad libitum*. The rats were treated according to the guidelines provided by the Animal Subjects Ethics Subcommittee of the Hong Kong Polytechnic University.

Twenty-four rats were randomly divided into 4 groups (n = 6), including 1) control (Ctrl), 2) brush alone (Brush), 3) Corticosterone-treated (Cort) and 4) Co-treatment group (Brush+Cort). The treatment period was last for 14 days. High-dose corticosterone was injected daily (40mg/kg/day, subcutaneously) to the rats to induce affective disturbances and neurogenic suppression. TS treatment was applied to Brush and Brush+Cort groups for 14 days. At the last 3 days of treatment, BrdU (50mg/kg, intraperitoneally) was injected to label proliferative cells in the hippocampal DG. After the 14 days treatment, behavioral assessments were conducted followed by euthanasia. The brain was dissected for immunohistochemistry to examine the morphological changes in the hippocampal DG (Fig. 2).

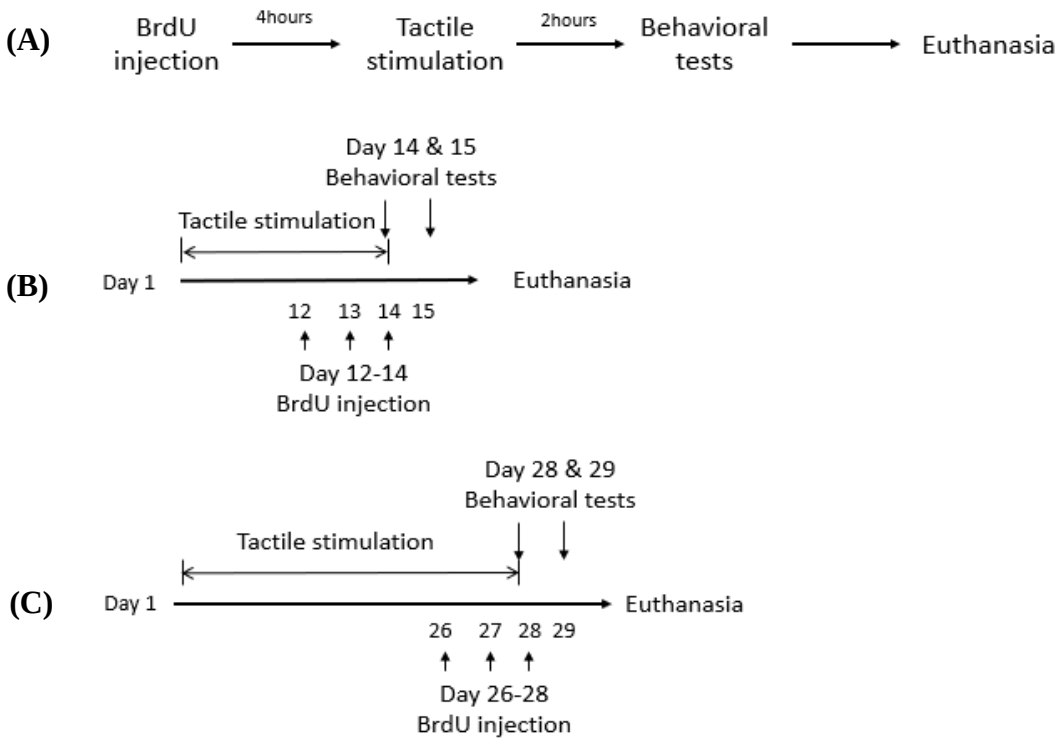
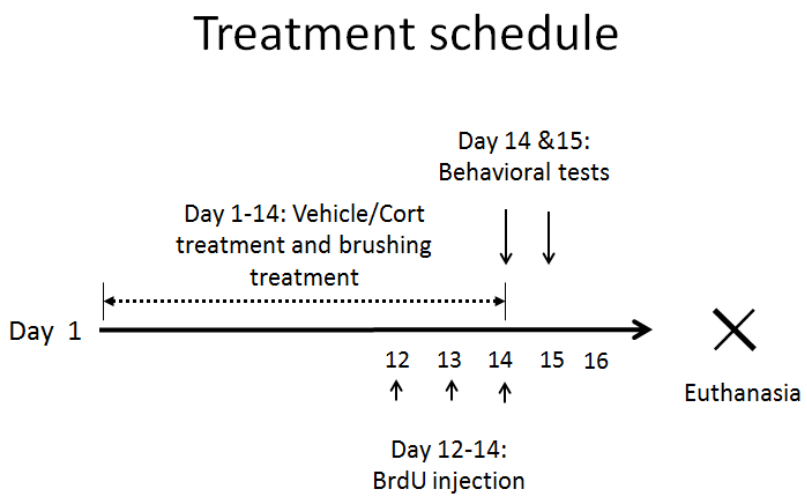


Figure 1. Design of experiment 1. The experiment divided into 3 parts, including TS for **(A)** 1, **(B)** 14 and **(C)** 28days. Rats were randomly divided into control and treatment groups (n=6/ group) in 3 parts of experiment. Treatment group received TS while control received daily handling. For one-day-treatment experiment, BrdU was injected four hours before TS.



Brush Treatment

	n=24	+	-
Corticosterone injection	+	Brush+Cort	Cort
	-	Brush	Ctrl

Figure 2. Design of experiment 2. Fourteen days of treatment. Vehicle (saline) was injected to Ctrl and Brush group, while corticosterone was injected to Cort and Brush+Cort group to suppress neurogenesis and induce affective disturbances. Tactile stimulation (brush treatment) was applied to Brush and Brush+Cort group for 14 days. At the least 3 days of treatment, BrdU was injected to label proliferative cells. Behavioral tests were conducted at day 14 & 15. Rats were then euthanized, and tissues were collected for biological analysis.

3.2 Tactile stimulation

Brush treatment was applied on the back, limbs and whiskers of the rats by using conventional toothbrush to achieve the tactile stimulation. The toothbrush (Slimsoft, Colgate) was connected to a loadcell which is linked to a force monitor. Force applied to the targeted areas (~200mN) was continually monitored by experimenter. TS was applied in the direction from anterior to posterior. This protocol is modified from the rehabilitative tactile stimulation protocol. The whisker pad was included because it is a highly sensitive tactile sensory organ in rats that is comparable to fingertips in primates and humans (Jadhav, Wolfe, & Feldman, 2009). TS treatment was applied to different targeted regions once per day, with duration of 5 minutes. In order to minimize the effect of TS triggered by handling, rats without treatment were received a daily handling method.

3.3 Behavioral tests

3.3.1 Open Field Test (OFT)

OFT was employed for assessing the exploratory anxiety level of rats. Animals were placed in an open arena (with 72cm long, 72cm wide and 40cm tall) individually for 10 minutes. The exploratory activity of each rat was video recorded by a camera mounted above the arena. For data analysis, the arena was divided into 16 equal

squares and the time spent for entering and staying at the central 4 squares were counted for each rat. Anxiety-like behavior is indicated by the decreased total time spent in the center area of the arena, defined as a36 x 36cm square.

3.3.2 Social Interaction Test (SIT)

Social anxiety of the rats was assessed by conducting SIT. Two unfamiliar rats were placed in the same arena for 15 minutes. In order to eliminate the anxious effect induced by novel environment, rats were placed into the arena which was for OFT previously. The social interaction was video recorded by a camera mounted above the arena. The numbers of positive social interaction, including sniffing, crossing, grooming and social play, were counted. Social anxiety is indicated by decreased number of positive social interaction.

3.3.3 Forced Swimming Test (FST)

FST was conducted for measuring the depression-like behavior. This assessment was conducted in transparent cylinder filled water with 30cm depth at room temperature. Before conducting the test, 15 min pre-test was allowed for rats to induce learned helplessness. Twenty-four hours later, the test was performed and their activities were recorded for 10 min. The level of depression-like behavior was

indicated by immobile time during the test. Increased immobility indicates a higher level of depression-like behavior. Immobility is defined as minimal limb movements and floating on the water.

3.4 Tissue processing

The rats were anesthetized with overdose sodium pentobarbital (100 mg/kg, intraperitoneal injection). Transcardial perfusion with 4% paraformaldehyde (PFA) was performed. The brain was then dissected from each rat and post-fixed with 4% PFA overnight at 4°C, and followed by cryoprotection using 30% sucrose. Coronal brain sectioning was performed by cryostat to prepare 40μm thick (1-in-12 series) sections for morphological and immunohistochemical studies.

3.5 Immunohistochemistry

The Brain sections were fixed onto glass slides coated with gelatin and then treated with 0.1M sodium citrate buffer (pH = 6.0) at 90°C for 20min for antigen retrieval. The sections were incubated with 2N hydrochloric acid for 25min at 37°C and then neutralized by using 0.1 M sodium borax buffer (pH = 8.5) for 10 min at room temperature. Primary antibodies were applied for targeting BrdU and doublecortin (DCX). The slides were incubated with 1st antibodies overnight at room

temperature. The details of antibodies used are shown in Table 2. In the next part, immunohistochemical assay was divided into 2 parts.

3.5.1 Peroxidase staining

For peroxidase staining, biotinylated secondary antibodies were applied and incubated with slides for 2 hours at room temperature. The process followed by incubating the slides with avidin-biotin couple (Vector) with 2 hours at room temperature to amplify the signals. After incubation, diaminobenzidine (DAB, Sigma-aldrich) was added onto the slides for signal visualization. The details of antibodies used are shown in Table 2.

3.5.2 Immunofluorescence

For immunofluorescence, specific 2nd antibodies were used for producing fluorescent signals. The details of antibodies used are shown in Table 2. The slides were incubated with secondary antibodies for 2 hours at room temperature.

Table 2: List of antibodies applied in immunohistochemistry

	Primary Antibodies	**Supplier**	**Dilution**
Peroxidase staining	Rabbit anti-DCX	Cell Signaling Technology	1:300
	Mouse anti-BrdU	Roche	1:300
	Biotinylated Goat anti-rabbit	Vector Laboratories	1:200
	Biotinylated Goat anti-mouse	Dako	1:200
Immunofluorescence	Rabbit anti-DCX	Cell Signaling Technology	1:300
	Rat anti-BrdU	Abcam	1:100
	Goat anti-rabbit Alexa Fluor 488	Life Technologies	1:200
	Goat anti-rat Alexa Fluor 568	Life Technologies	1:200

3.6 Proliferative cells and immature neurons quantification in the hippocampal dentate gyrus

In order to measure the effect of brush treatment on hippocampal neurogenesis, BrdU and DCX was target by immunostaining, as described in the pervious section. BrdU is an exogenous proliferative marker that can incorporate into newly synthesized DNA during DNA duplication. It has been commonly used to detect cell proliferation in the hippocampus. DCX is a protein marker that expressed in immature neurons. Quantification of BrdU or DCX-positive cells was performed. Six right hippocampal sections were observed for each rat and the numbers of BrdU+ or DCX+ cells in hippocampal DG were counted on every 12th section under 400x magnification. The quantification of BrdU-positive cells, the newly formed cells in hippocampal DG, was counted by using unbiased stereology with StereoInvestigatior (MicroBrightField, Williston, VT, USA). Total cell counts per rat were multiplied by 12 to estimate the total numbers of newly proliferative cells and immature neurons in the right hippocampal DG.

3.7 Phenotypic analysis of new cells in hippocampal DG

In this part, the data from immunofluorescent staining of BrdU+/DCX+ co-labeling was used for the analysis. Co-labeled BrdU+/DCX+ cells were counted under an eqifluorescent microscope. At least 25 BrdU+ cells were randomly selected for analysis. Results were presented as the proportion of BrdU+ cells co-expressed

DCX. This data indicates the level of neuronal differentiation form newly formed neural progenitor cells in the hippocampal DG.

3.8 Dendritic complexity assessment in the hippocampal DG

In the interest of dendritic growth, which is one of the essential processes for generating newly functional neuron, the complexity of immature neurons was analyzed by Sholl analysis. Ten DCX-expressing cells with tertiary dendrites (3'DCX+ cells) in the hippocampal DG were randomly selected for the analysis. Concentric circles were drawn on the soma of 3'DCX+ cells with increasing radii of 10μm. The numbers of intersection between dendrites and concentric circles were counted and measured with the distance from soma. Sholl analysis was performed with software ImageJ. The intersections are for the measurement of dendritic complexity. Greater number of intersections indicated a great level of dendritic growth.

3.9 Statistical analysis

Data was analyzed by SPSS. For experiment 1, One-way analysis of variance (ANOVA) followed by Tukey post-hoc test was employed for comparing the mean difference among groups. Student's t-test was used to compare the mean between Ctrl

and Tx groups in 3 timelines respectively.

For experiment 2, Two-way ANOVA and simple main effect analysis were employed. For pairwise comparison, Tukey Multiple Comparisons were employed. Data was presented as mean ± SEM. Statistical significance is defined as *p*-value less than or equate to 0.05.

Chapter 4

Results

4.1 The effect of novel tactile stimulation on affective behaviors and adult hippocampal neurogenesis

4.1.1 Novel tactile stimulation enhanced dendritic maturation

The effect of novel tactile stimulation was examined by applying one-day-treatment to the rats in experiment 1. Sholl analysis was conducted to analyze the dendritic complexity of DCX-positive (DCX+) cells in the DG of hippocampus. Student's t-test was conducted to compare the means between Ctrl and Tx groups. The results showed that TS significantly enhanced dendritic complexity in distance from soma 120-160μm (Fig. 3, $p < 0.05$, marked as *).

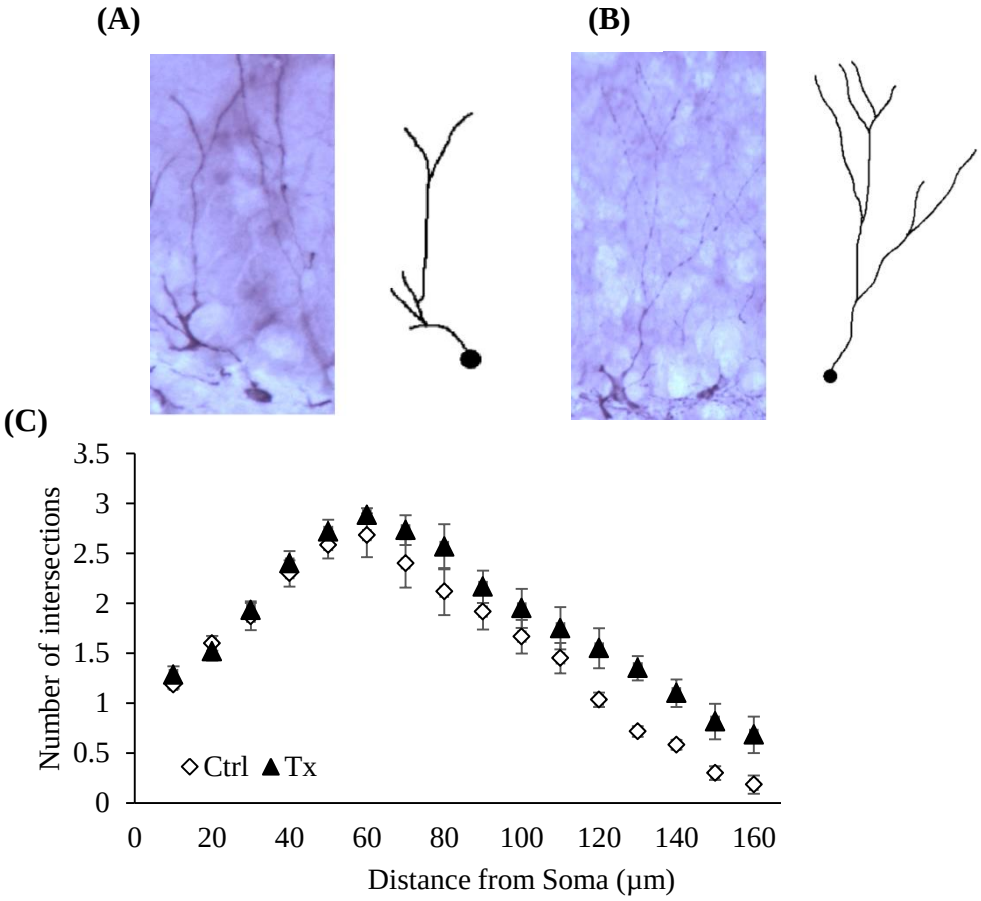


Figure 3. Novel tactile stimulation increased dendritic complexity. (A) & **(B)** are the representative images of DCX+ immature neurons and the corresponding tracing for Sholl analysis in the hippocampal DG of Ctrl and Tx groups respectively. **(C)** Tx group with novel tactile stimulation increased dendritic complexity. The result is presented as mean ± SEM. * $p<0.05$, Student's t-test.

4.1.2 Cell proliferation and neuronal differentiation was not significantly enhanced by novel tactile stimulation

Newly generated cells in the hippocampal DG were labeled by BrdU and differentiated neurons were labeled by DCX. The number of BrdU-positive (BrdU+) and DCX+ cells were quantified (Fig. 4 and 5, respectively). Student's t-test was employed to compare the mean between Ctrl and Tx groups. The result indicated that TS may not significantly increase the number of newly proliferative cells and differentiated immature neurons in the hippocampus.

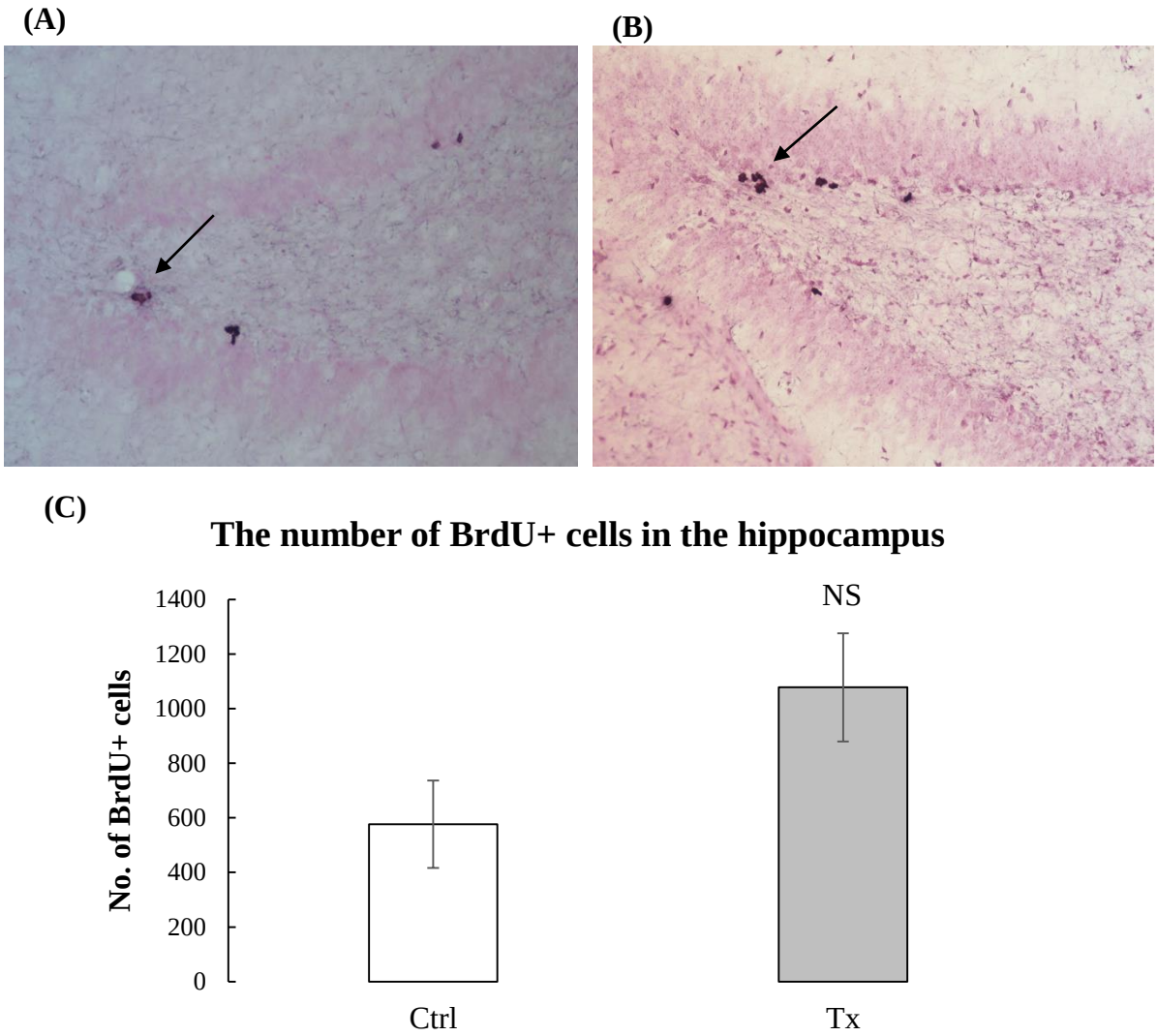


Figure 4. Cell proliferation np significantly altered in the hippocampal DG by novel tactile stimulation. The number of newly proliferative cells was evaluated and total number of cells in right hippocampal DG was estimated. **(A)** and **(B)** are the representative images (200x) of BrdU-positive (BrdU+, indicated by arrow) cells in the DG. Student's t-test showed that the numbers of BrdU+ cells were not significantly altered by novel tactile stimulation **(C)**. Result is presented as mean ± SEM. NS indicates no statistically significant.

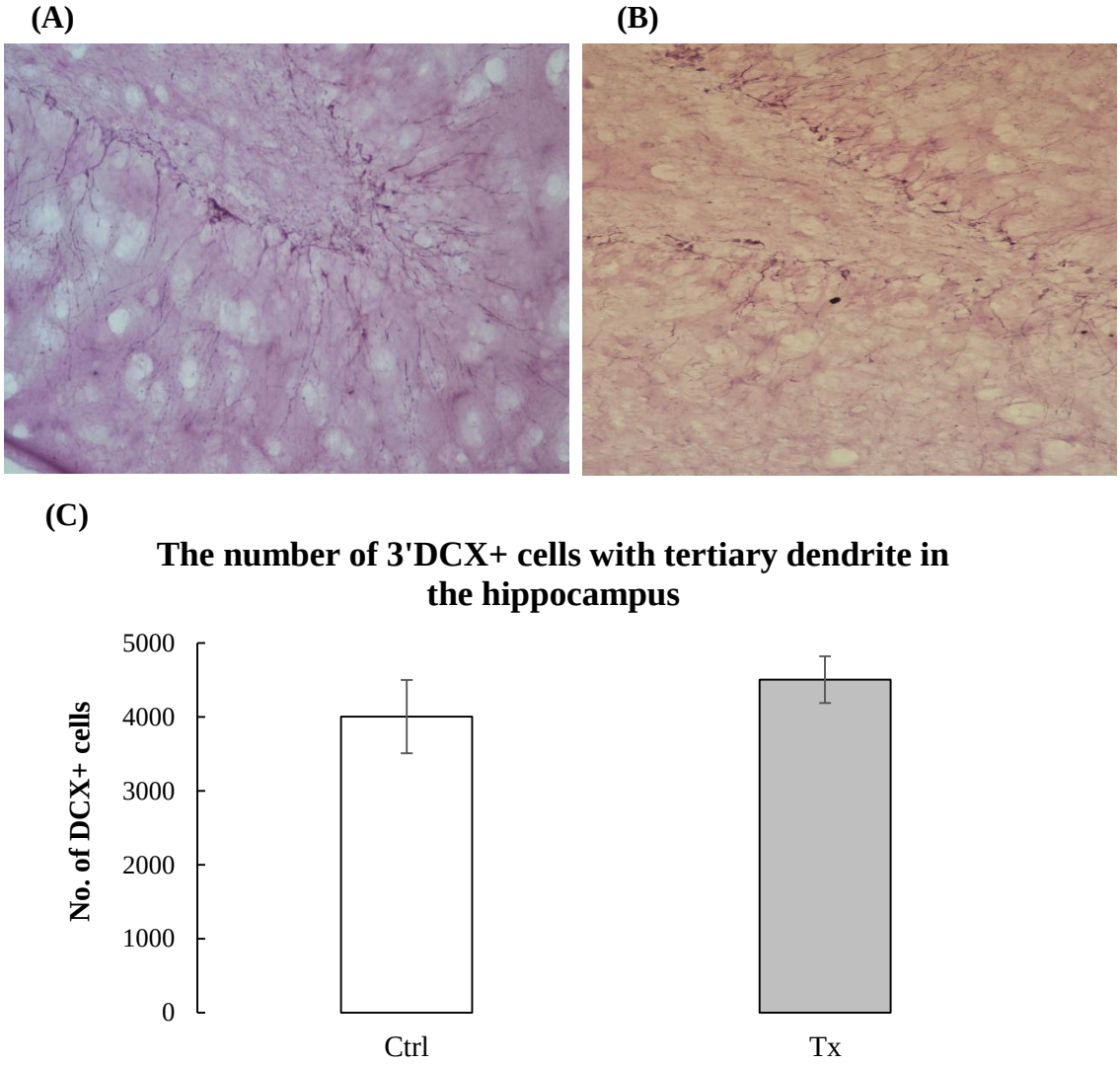


Figure 5. Doublecortin-expressing immature neurons in the hippocampal DG was not significantly increased by novel tactile stimulation. The level of neuronal differentiation is indicated by the number of DCX+ cells. The representative image (200x) of DCX+ cells in hippocampal DG of **(A)** Ctrl and **(B)** Tx group respectively. The bar chart is showing the number of DCX+ cells with tertiary dendrite (3' DCX+) in the hippocampal dentate gyrus **(C)**. Student's t-test showed no statistically

significant difference between Ctrl and Tx groups. Result is presented as mean ± SEM.

4.1.3 Novel tactile stimulation increased the level of depression-like behavior in rats

Depression-like behavior was assessed by the forced swimming test and indicated by total immobile time during the test. Student's t-test showed that novel TS significantly increased the immobile time in rats with brush intervention (p = 0.026, Fig. 6). These results indicated that the novel stimulation may induce stress and thus increased depression-like behavior in rats.

4.1.4 Novel tactile stimulation did not affect the level of social anxiety and exploratory anxiety level in rats

Open-field test (OFT) and social interaction test (SIT) were conducted to assess the level of exploratory and social anxiety, respectively. The total time spent in the center of OFT and greater numbers of positive social interactions in SIT indicated a greater level of anxiety of rat. Student's t-test indicated that the level of both social and exploratory anxiety is not significantly affected in rats after exposure to novel TS (Fig. 7).

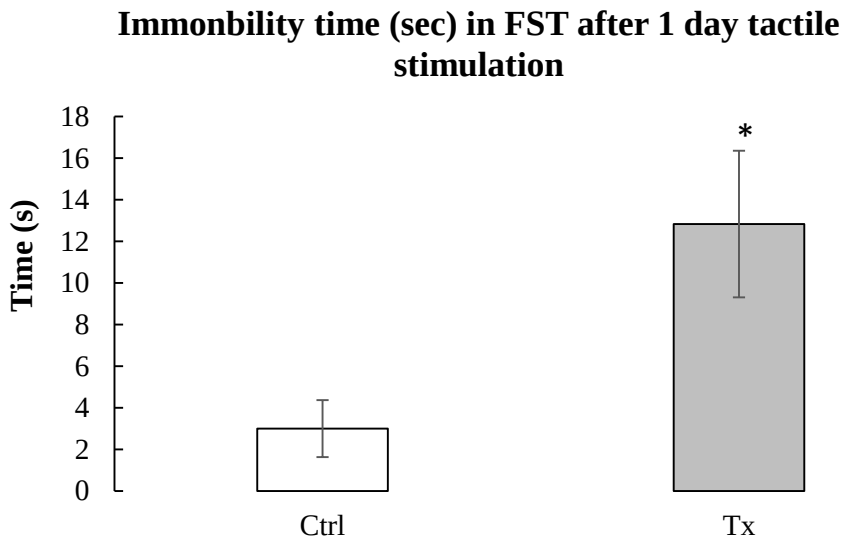


Figure 6. Novel tactile stimulation significantly increased depression-like behavior. Depression-like behavior is indicated by immobile time spent in FST. Student's t-test indicated that a novel tactile stimulation increased the level of depression-like behavior in rats (p = 0.026). Results are presented as mean ± SEM, *: $p < 0.05$. Student's t-test.

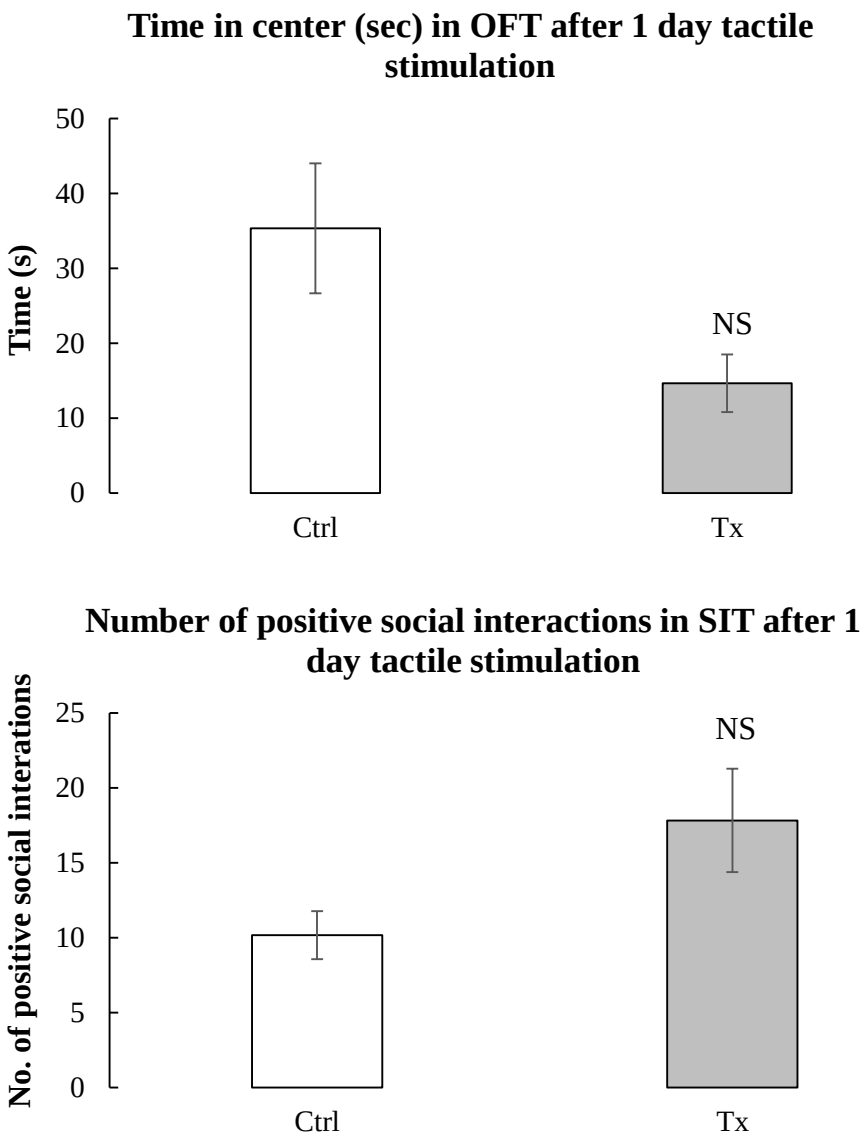


Figure 7. Anxiety-like behaviors were not affected by novel tactile stimulation. Anxiety-like behaviors were assessed by OFT and SIT. Student's t-test indicated that novel tactile stimulation not significantly affected the level of anxiety-like behavior in rats in both OFT and SIT. Results are presented as mean ± SEM. NS indicates not statistically significance. Student's t-test.

4.2 Time-dependent effect of tactile stimulation on affective behaviors and hippocampal neurogenesis

4.2.1 Prolonged tactile stimulation may relieve anxiety-like behavior but not depression-like behavior in rats

In order to assess the time-dependent effect of TS on affective behaviors, rats were subjected to TS for 1 day, 14 days and 28 days respectively. One-way ANOVA was conducted to compare the means of 1) the total immobile time in FST, 2) the total time spent in the open area of OFT and 3) the numbers of positive social interactions among 3 time points, respectively. The results indicated that prolonged exposure to TS may reduce the exploratory anxiety level in OFT (1-day Tx vs. 28-days Tx, p = 0.002, Fig 9). Repeated tactile stimulation (14 days) increased anxiety-like behaviors (p = 0.025). However, for FST and SIT, the results showed that the anxiety- and depression-like behaviors were not influenced by prolonged brushing intervention (Fig. 8 and 10, respectively).

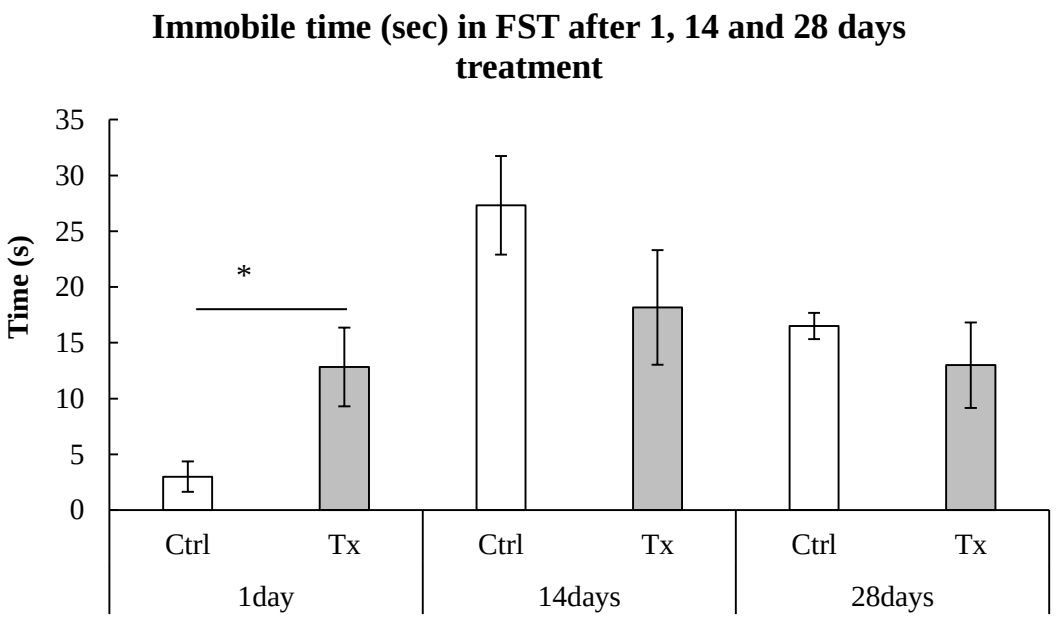


Figure 8. Repeated tactile stimulation did not affect depression-like behavior in rats.

FST was employed to assess the level of depression-like behavior after receiving 1, 14 and 28 days of tactile stimulation. The longer time of immobile time indicated the higher level of depression-like behavior. Novel tactile stimulation (1 day) significantly increased depression-like behavior (p = 0.026) while repeated tactile stimulation (14 and 28 days) not significantly affected depression-like behavior in rats. One-way ANOVA showed no significant difference among 1, 14 and 28 days Tx groups. Results are presented as mean ± SEM. * $p < 0.05$, Student's t-test.

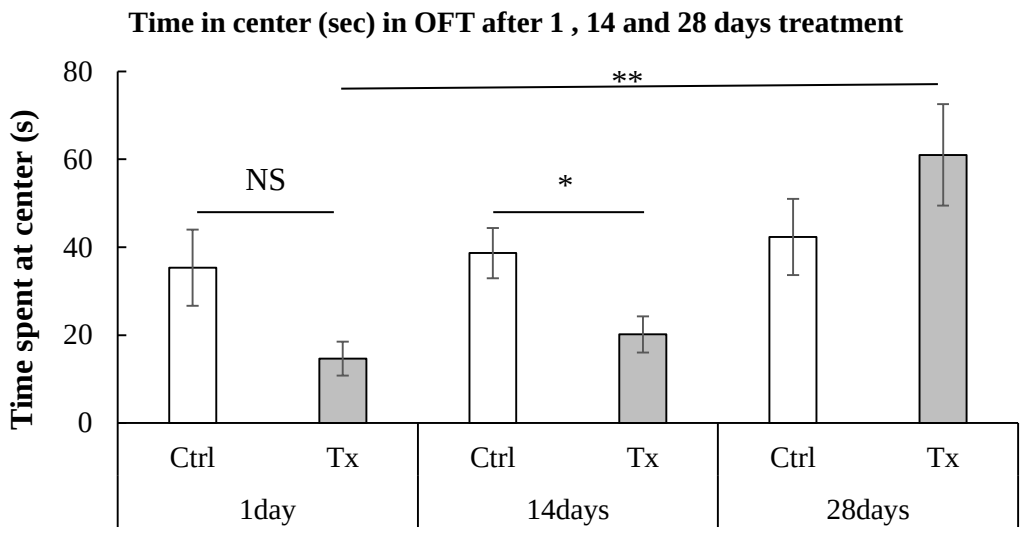


Figure 9. Repeated tactile stimulation affected anxiety-like behavior in OFT in a time dependent manner. Exploratoy anxiety were assessed by OFT after receiving 1, 14 and 28 days of tactile stimulation. The longer time spent at the center indicated the lower level of anxiety-like behavior. Novel tactile stimulation (1 day) showed a trend to increase anxiety-like behavior but not in a statistically signifance (NS). Repeated tactile stimulation (14 days) increased anxiety-like behaviors (p = 0.025). Prolonged tactile stimulation (28 days) showed no difference. One-way ANOVA followed by Tukey post-hoc test showed a significant difference between 1 day and 28 days Tx groups (p = 0.002). Results are presented as mean ± SEM. * $p < 0.05$, Student's t-test. ** $p < 0.01$, one-way ANOVA with Tukey post-hoc comparison.

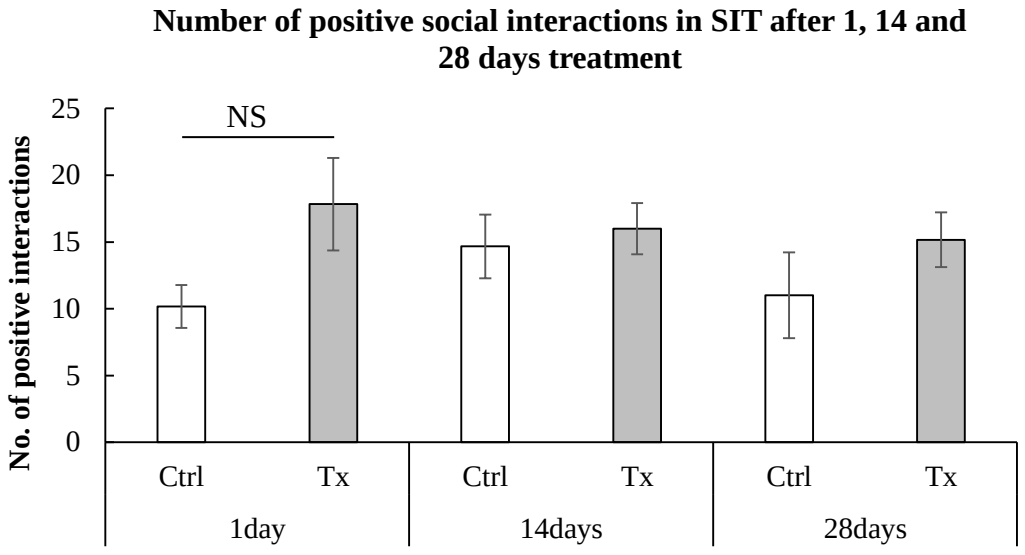
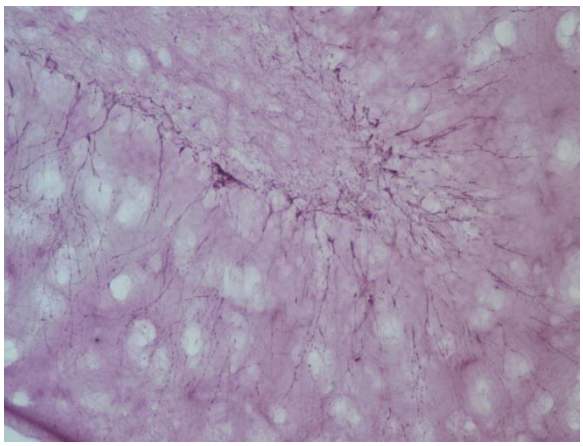
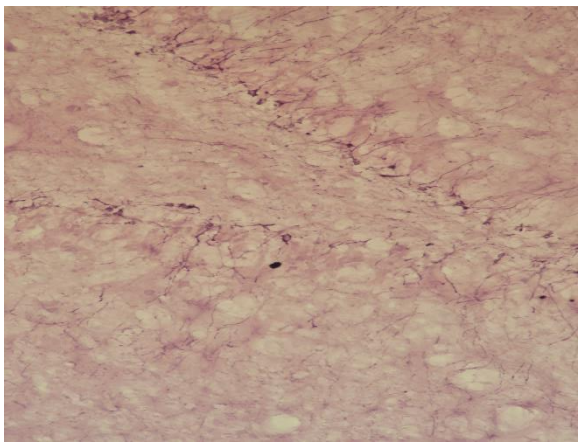
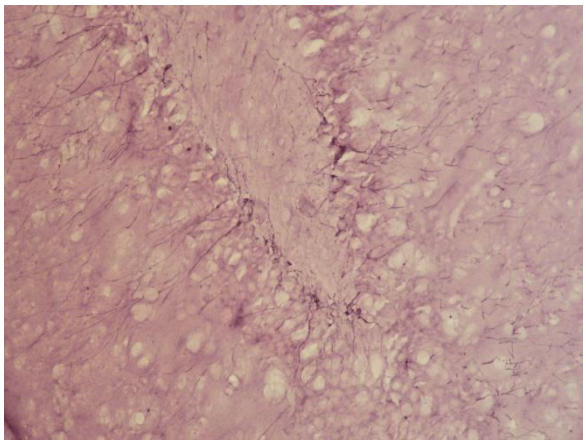
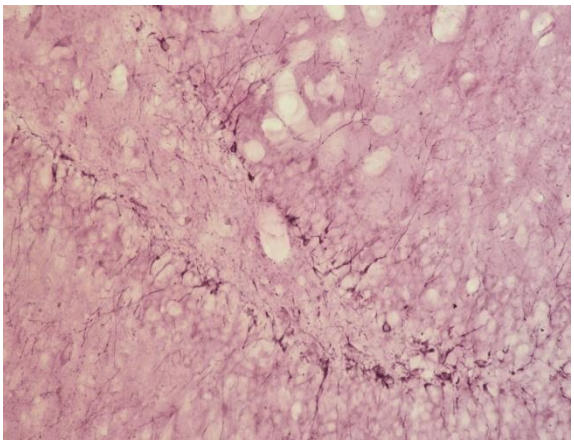
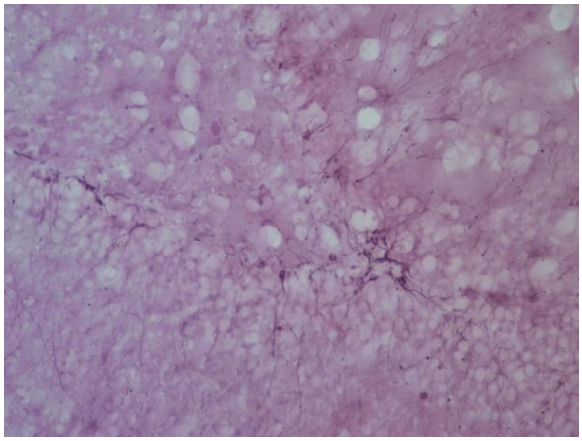
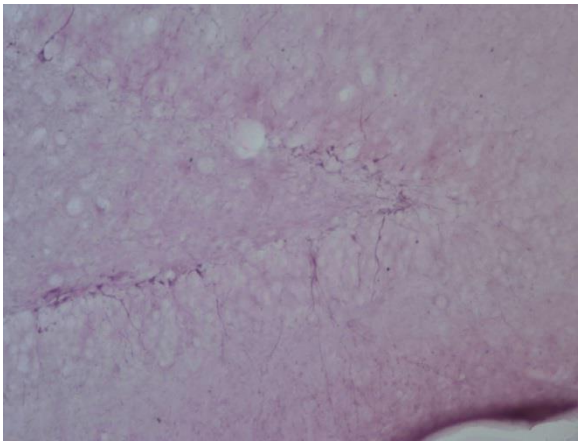


Figure 10. Repeated tactile stimulation did not affect anxiety-like behavior in SIT. Social anxiety were assessed by SIT after receiving 1, 14 and 28 days of tactile stimulation. The greater numbers of positive social interactions indicated the lower level of social anxiety-like behavior. Novel tactile stimulation (1 day) showed a trend to decrease social anxiety but not reach statistically signifance (NS). Repeated tactile stimulation (14 days) and prolonged tactile stimulation (28 days) showed no difference when comparing to Ctrl. One-way ANOVA showed no significant difference among groups. Results are presented as mean ± SEM. NS indicates not statistically significance, Student's t-test.

4.2.2 Prolonged tactile stimulation may not influence hippocampal neurogenesis

One-way ANOVA was conducted to compare the means of BrdU+ and DCX+ cells among 3 time points after rat received tactile stimulation. The results indicated that the numbers of BrdU+ and DCX+ cells were not increased in all time points significantly (Fig. 11 and 12, respectively). Moreover, student's t-test showed no significant difference between Ctrl and Tx groups in three different treatment periods respectively. These results revealed that brushing intervention may not affect the neurogenesis in the hippocampal DG in rats.

(A)

	Ctrl	Tx
1 Day		
14 Days		
28 Days		

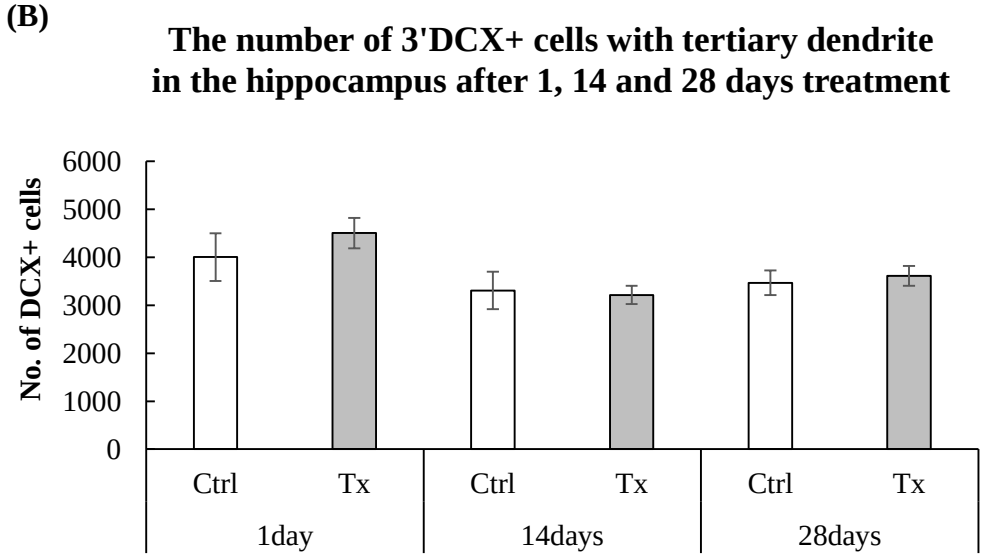
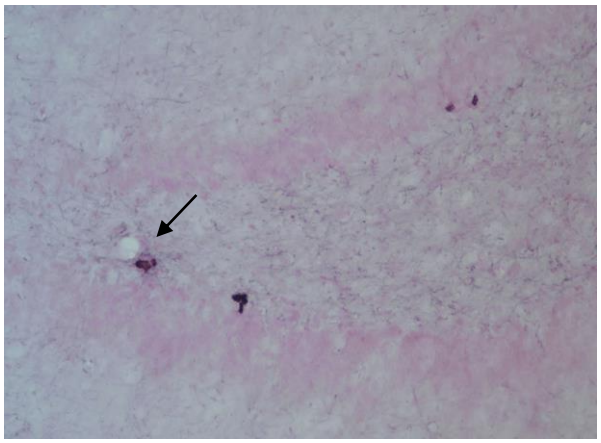
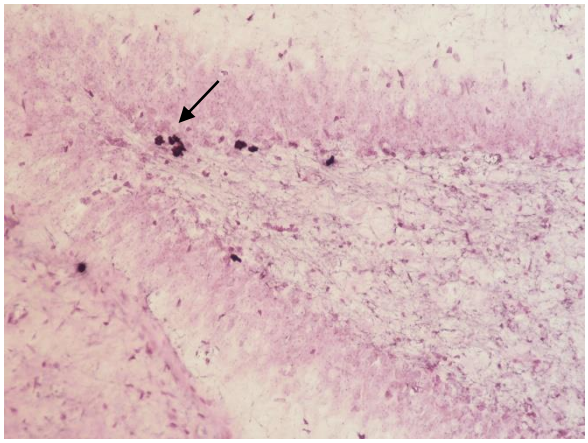
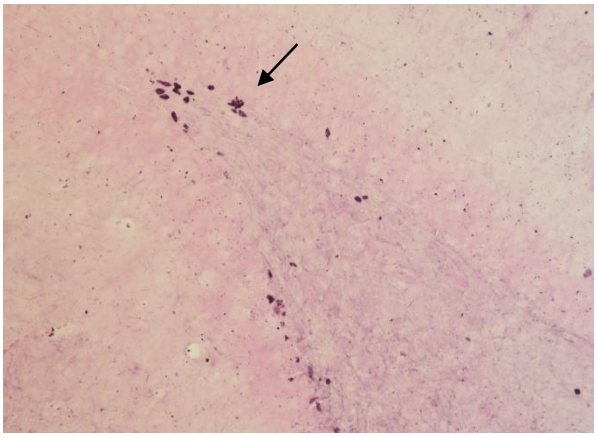
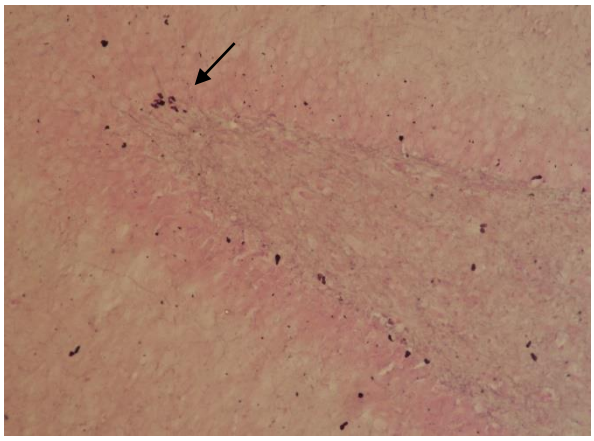
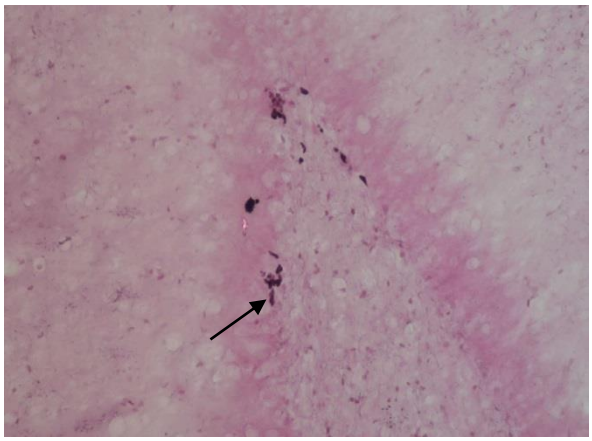
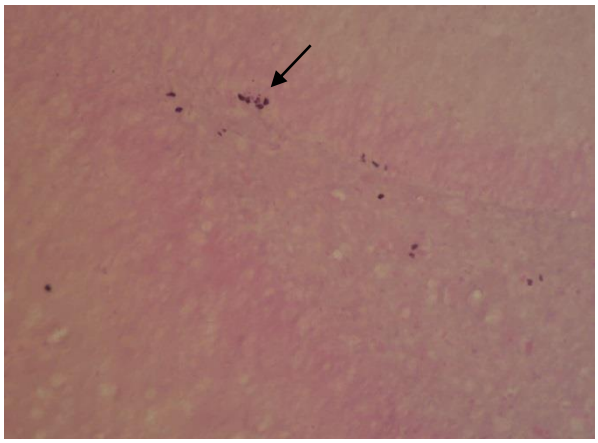


Figure 11. Doublecortin-expressing immature neurons in the hippocampal DG were not significantly increased by novel and repeated tactile stimulation. The level of neuronal differentiation in the hippocampus is indicated by the number of DCX+ cells. **(A)** The representative image (200x) of DCX+ cells in hippocampal DG of Ctrl and Tx groups in 3 treatment periods, respectively. **(B)** The number of DCX+ cells with tertiary dendrite (3' DCX+) in the hippocampal dentate gyrus (DG) is showing in the bar chart. Student's t-test showed no statistically significant difference between Ctrl and Tx groups in 1, 14 and 28 days intervention. Result is presented as mean ± SEM.

(A)

	Ctrl	Tx
1 Day		
14 Days		
28 Days		

(B)

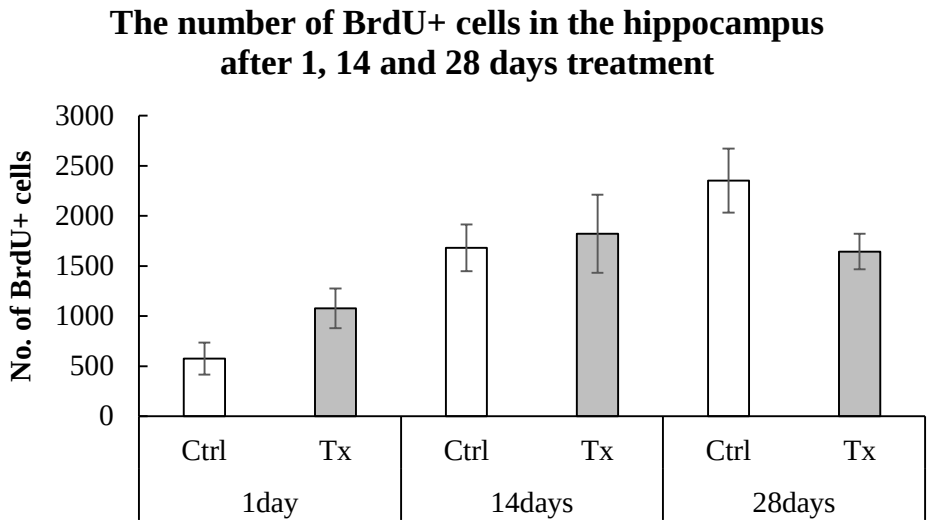
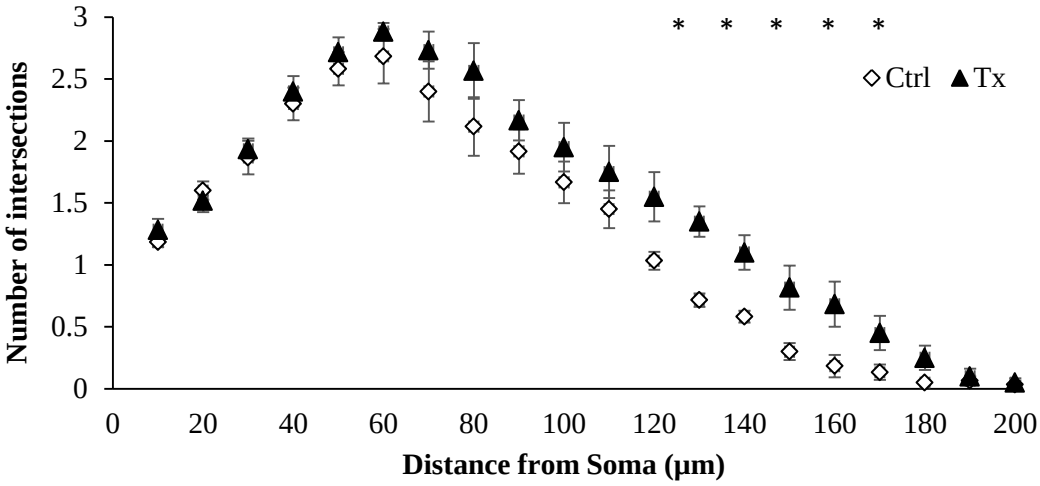


Figure 12. Cell proliferation was not enhanced in the hippocampal DG by both novel and repeated tactile stimulation. The number of newly proliferative cells was evaluated and the total number of cells in right hippocampal DG was estimated. **(A)** The representative images (200x) of BrdU-positive (BrdU+) cell in the DG. **(B)** Student's t-test showed that the numbers of BrdU+ cells were not significantly altered by 1-, 14- and 28- days tactile stimulation. One-way ANOVA showed no significant difference among 3 Tx groups. Result is presented as mean ± SEM.

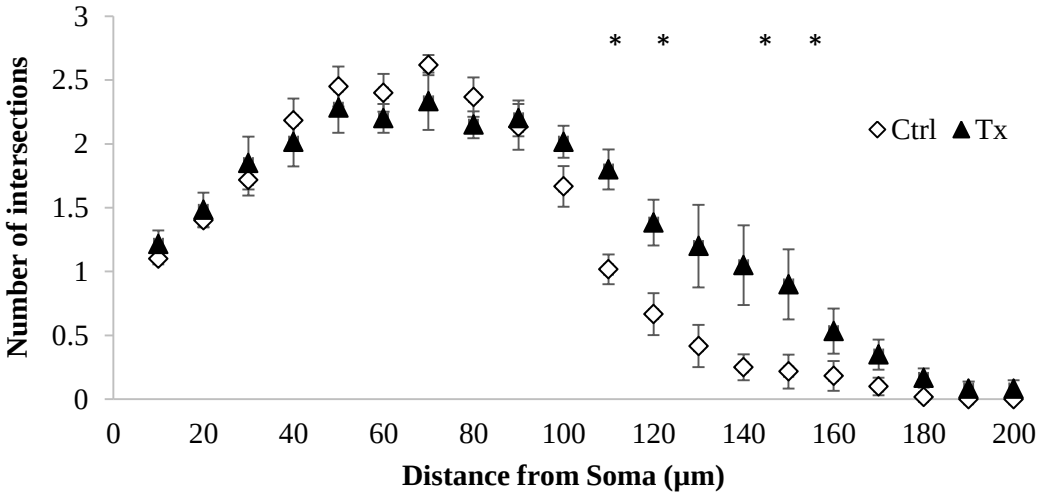
4.2.3 Dendritic complexity was influenced by both novel and repeated tactile stimulation

Sholl analysis was used to assess the dendritic complexity in the hippocampal DG. Student's t-test was employed to compare the means in Sholl analysis between Ctrl and Tx groups in 3 time points respectively. The results showed that 1-day and 14-days treatment significantly increased dendritic complexity while 28-days treatment showed no significant increase in dendritic complexity (Fig. 13). For 1-day-treatment, the differences mainly found in the distance from soma 120-160μm. For 14-day-treatment, the differences mainly found in the distance from soma 110-120 and 140-150μm. One-way ANOVA showed that no significant differences among 3 Tx groups (Fig. 14). The results indicated that the dendritic complexity might be influenced by TS in time-dependent manner.

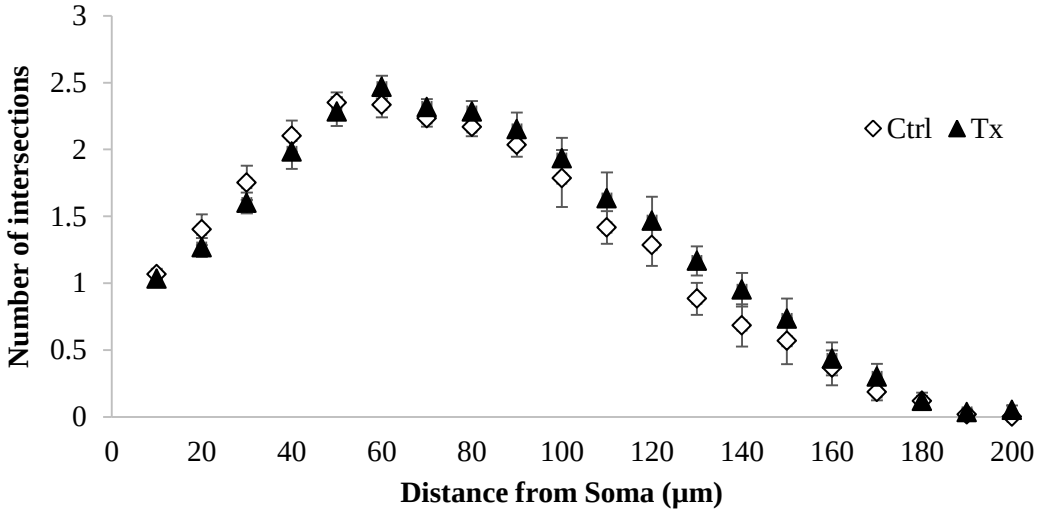
(A) **Dendritic complexity after 1 day tactile stimulation**



(B) **Dendritic complexity after 14 days tactile stimulation**



(C) **Dendritic complexity after 28 days tactile stimulation**



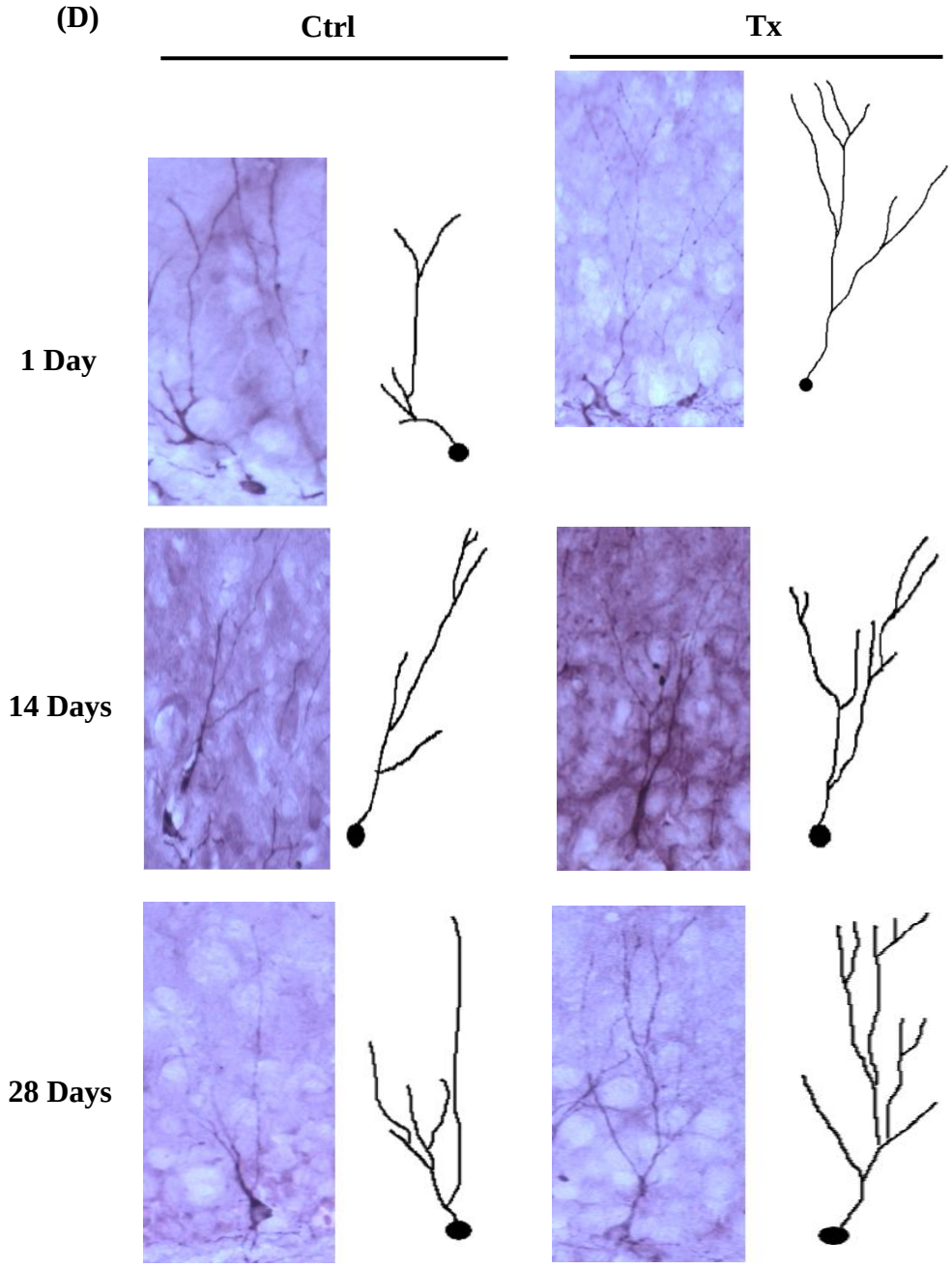


Figure 13. Novel and repeated tactile stimulation increased dendritic complexity. **(A)**, **(B)** and **(C)** indicated the level of dendritic complexities after rats receiving tactile stimulation for 1, 14 and 28 days respectively. The results showed that 1-day and 14-days treatment enhanced dendritic complexities in the rats' hippocampal DG. However, 28 days Tx showed no significant changes in dendritic complexities.

Results are presented as mean ± SEM. * $p < 0.05$, Student's t-test. **(D)** are representative images

of DCX+ immature neurons and the corresponding tracing for Sholl analysis in the hippocampal DG of Ctrl and Tx groups in 3 different time points study, respectively.

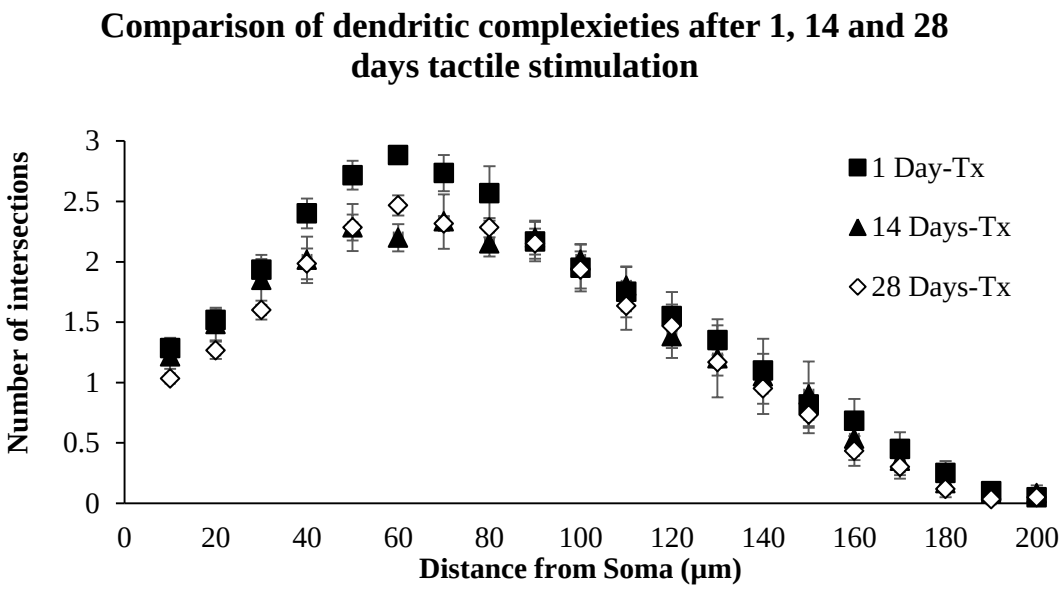


Figure 14. Similar dendritic complexities were found in the rats' hippocampal DG after receiving 1, 14- and 28-days tactile stimulation. This graph shows the comparison of dendritic complexities among 3 Tx groups, including 1, 14- and 28-days brush intervention. One-way ANOVA showed that no significant differences were found among groups.

4.3 The effects of repeated tactile stimulation on affective behaviors and adult hippocampal neurogenesis in hypercortisolemic animal model

4.3.1 High-dose corticosterone administration reduced body weight, while TS not affected body weight change

The changes of body weight of the rats were measured after 14 days of treatment (Fig 15). Two-way ANOVA was used to evaluate the effect of corticosterone and brush treatment on body weight change. No significant interaction was found between corticosterone and brush treatment on body weight change. The weights of rats were significantly reduced by chronic corticosterone injection (Ctrl vs. Cort, p = 0.042; Brush vs. Brush+Cort, p = 0.009). Brush treatment showed no significant effect on body weight change in both normal and hypercortisolemic rats.

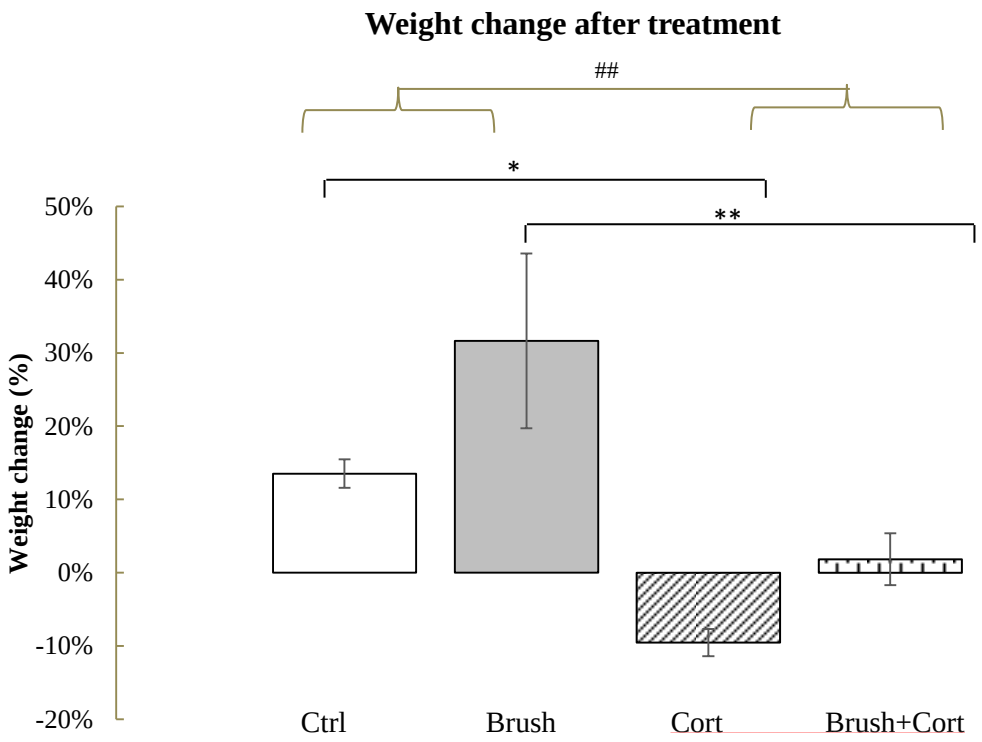


Figure 15. Body weight change after 14 days of treatment. Body weights of the rats were significantly reduced by chronic corticosterone treatment, indicated as ##. Pairwise comparison showed a significant difference between Ctrl and Cort; Brush and Brush+Cort. *: $p < 0.05$; **/##: $p < 0.01$, two-way ANOVA followed by Tukey post-hoc comparison.

4.3.2 Tactile stimulation not altered hypothalamic-pituitary-adrenal axis

In order to examine the effect of brush treatment on hypothalamic-pituitary-adrenal (HPA) axis, the weights of adrenal glands were measured after the rats were sacrificed (Fig. 16). Two-way ANOVA revealed a significant interaction effect between chronic corticosterone and brush treatment ($F(1,15) = 8.127$, $p = 0.012$). The weights of adrenal glands were significantly decreased by corticosterone administration (shown as ### in Fig.16, $p < 0.001$), but not altered by brush treatment. Ctrl vs. Cort, $p < 0.001$; Brush vs. Brush+Cort, $p < 0.001$.

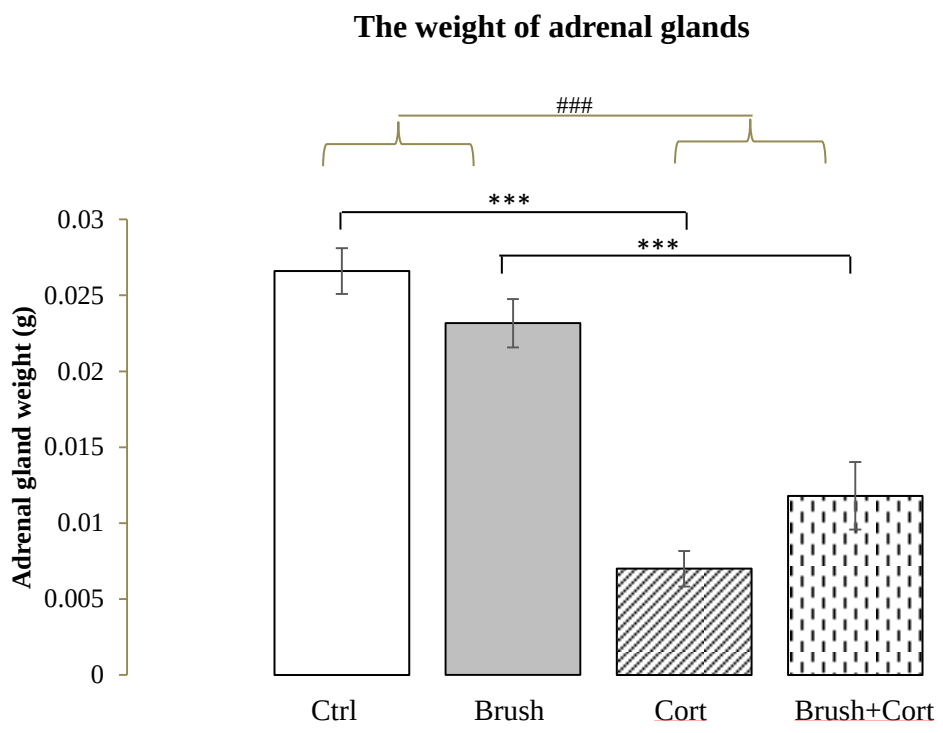
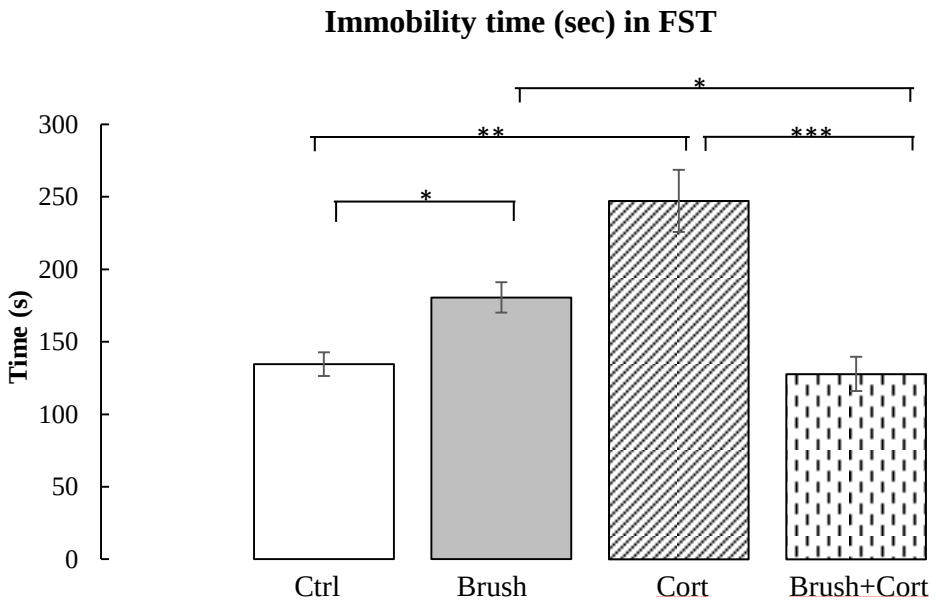


Figure 16. The weight of adrenal gland after 14 days of treatment. The adrenal glands of the rats were dissected and weighted after transcardial perfusion. Chronic corticosterone injection induced adrenal gland reduction, indicated as ###; $p < 0.001$. Pairwise comparison showed a significant between Ctrl and Cort; Brush and Brush+Cort. ***: $p < 0.001$, two-way ANOVA followed by Tukey post-hoc comparison.

4.3.3 Repeated tactile stimulation reduced depression-like behavior in hypercortisolemic animal model

Depression-like behavior was indicated by the total immobile time in forced swimming test (FST, Fig. 17). Two-way ANOVA showed a significant interaction effect between brush and corticosterone treatment on FST ($F(1,15) = 32.68$, $p < 0.001$).

Brush treatment showed a significant effect on the results of FST ($F(1, 15) = 6.412$, $p = 0.023$), differential effects were found. In depressive disease model, chronic corticosterone treatment induced a higher level of depression-like behavior (Ctrl vs. Cort, $p < 0.001$), but brush treatment greatly reduced the level of depression-like behavior (Cort vs. Brush+Cort, $p < 0.001$). On the other hand, the brush treatment induced depression-like behavior in normal rats (Ctrl vs. Brush, $p = 0.045$).



Repeated TS reduced depression-like behavior in cort-treated rats. Depression-like behavior is indicated by immobile time spent in FST. Two-way ANOVA revealed a significant interaction effect between brush treatment and corticosterone administration (F (1,15) = 32.68, $p < 0.001$). Chronic corticosterone administration significantly induced depression-like behavior (Ctrl vs. Cort) and tactile stimulation significantly reversed the condition (Cort vs. Brush+Cort, $p < 0.001$). Brush treatment slightly increased the immobile time in normal control rats (Ctrl vs. Brush, $p = 0.045$). Results are presented as mean ± SEM, *: $p < 0.05$, **: $p < 0.01$ and *: $p < 0.001$, two-way ANOVA followed by Tukey post-hoc comparison.

4.3.4 Repeated tactile stimulation reduced social anxiety and exploratory anxiety in hypercortisolemic animal model

Anxiety-like behaviors of the rats were measured by social interaction test (SIT) and open-field test (OFT). Social anxiety was measured by SIT and exploratory anxiety was evaluated by OFT.

In SIT, there was no significant interaction effect of corticosterone and brush treatment (Fig. 19). No main effect was found in these treatments. Chronic corticosterone treatment significantly induced social anxiety (Ctrl vs Cort, $p = 0.03$), which is indicated by decreased number of positive social interactions. However, when applying brush treatment to Cort-treated animals, the level of social anxiety was remarkably reduced to normal level (Cort vs. Brush+Cort, $p = 0.023$), similar as shown in Ctrl. Brush treatment exhibited no beneficial effect on SIT in normal rats.

From the results of OFT, brush treatment demonstrated a differential effect in control and depressive animals. The result is presented as total time spent in the center. Two-way ANOVA revealed an interaction effect between corticosterone and brush treatment ($F(1,37) = 6.896$, $p = 0.012$). In control rats, brush treatment induced anxiety-like behavior (Ctrl vs. Brush, $p = 0.028$). On the other hand, brushing treatment seems to reduce the anxiety level in depressive model, but not in a statistically significance.

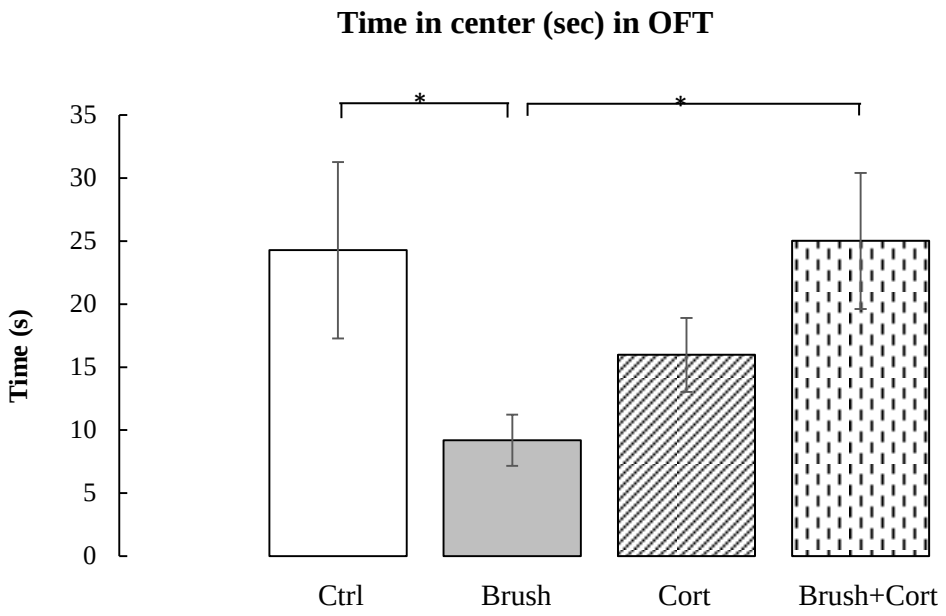


Figure 18. Exploratory anxiety improved by TS in cort-treated rats. Exploratory anxiety behavior is indicated by total time spent in the center of arena. Two-way ANOVA showed an interaction effect between corticosterone and brush treatment ($F(1,37) = 6.896$, $p = 0.012$). In control rats, tactile stimulation induced anxiety-like behavior (Ctrl vs. Brush, $p = 0.028$) while brushing treatment slightly reduced the anxiety level in depressive model, but not in a statistically significance (Cort vs. Brush+Cort). Result is presented as mean ± SEM, *: $p < 0.05$, two-way ANOVA followed by Tukey post-hoc comparison.

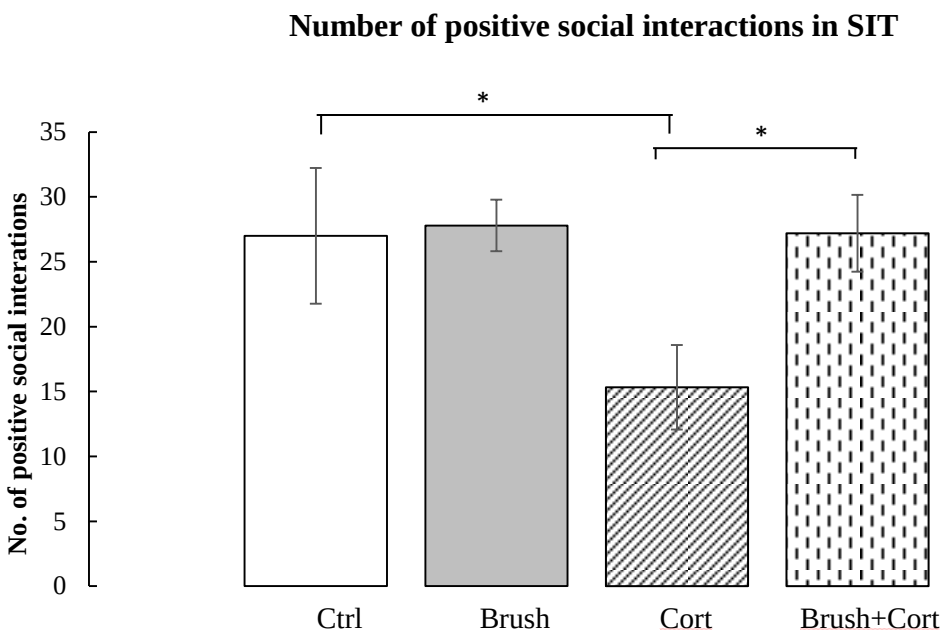


Figure 19. Social anxiety improved by TS in cort-treated rats. The numbers of positive social interactions are indexed the level of social anxiety. Two-way ANOVA revealed no significant interaction effect between corticosterone administration and brush treatment. Chronic corticosterone treatment induced social anxiety (Ctrl vs. Cort, $p = 0.03$) but tactile stimulation reversed this condition (Cort vs. Brush+Cort, $p = 0.023$). Result is presented as mean ± SEM, *: $p < 0.05$, two-way ANOVA followed by Tukey post-hoc comparison.

4.3.5 Repeated tactile stimulation promoted hippocampal neurogenesis in hypercortisolemic animal model by enhancing neuronal differentiation

Cell proliferation, which is an essential process for hippocampal neurogenesis, was assessed by the quantification of BrdU+ cells in the dentate gyrus. BrdU was injected at the least three days of treatment. Based on the statistical analysis, no interaction effect between corticosterone and brush treatment was found. The numbers of BrdU+ cells were not affected by brushing treatment significantly, but it was significant influenced by chronic corticosterone treatment, BrdU+ cells were decreased in depressive model ($F(1,18) = 7.541$, $p = 0.014$). No significant beneficial effects were found in both normal and depressive rats after applying tactile stimulation (Fig. 20).

In order to examine the effect of tactile stimulation on neuronal differentiation, the expression of doublecortin was measured. Doublecortin (DCX) is an immature neuronal protein marker which has been remarkably employed for studying the level of neuronal differentiation (Fig. 21). The results are displayed in the number of DCX+ cells with tertiary dendrite (3' DCX+) in the hippocampal dentate gyrus (DG). The results revealed a significant interaction effect between tactile stimulation and chronic corticosterone administration ($F(1,17) = 5.78$, $p = 0.028$). Depressive rats (Cort) showed a significant reduction of 3' DCX+cells in DG (Ctrl vs. Cort, $p < 0.001$).

Tactile stimulation counteracted the suppression effect of chronic corticosterone administration, enhanced neuronal differentiation in disease rat model (Cort vs. Brush+Cort, p = 0.007). This beneficial effect was not displayed in normal control rats and the expression level of 3’ DCX+ cells was not affected.

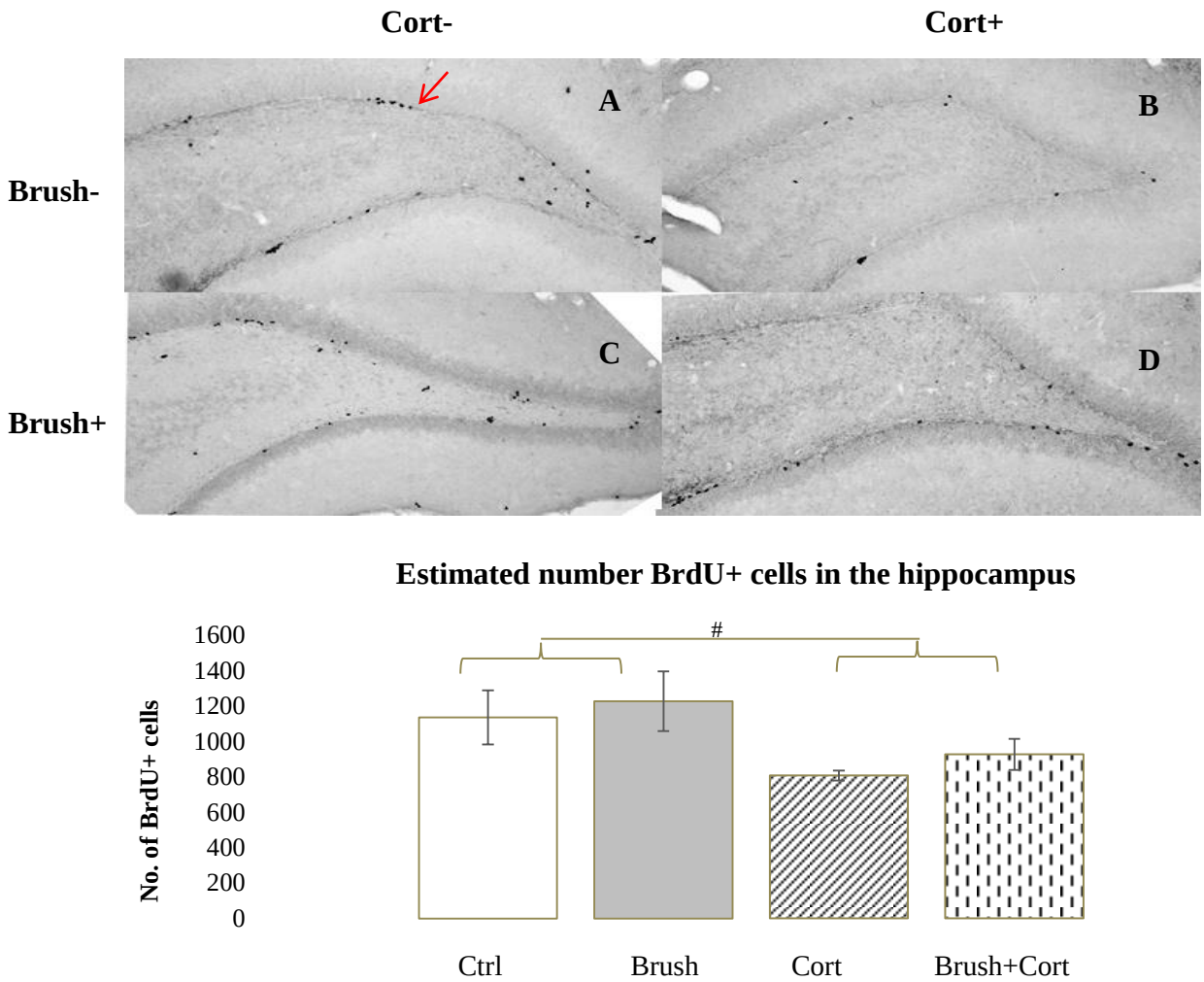
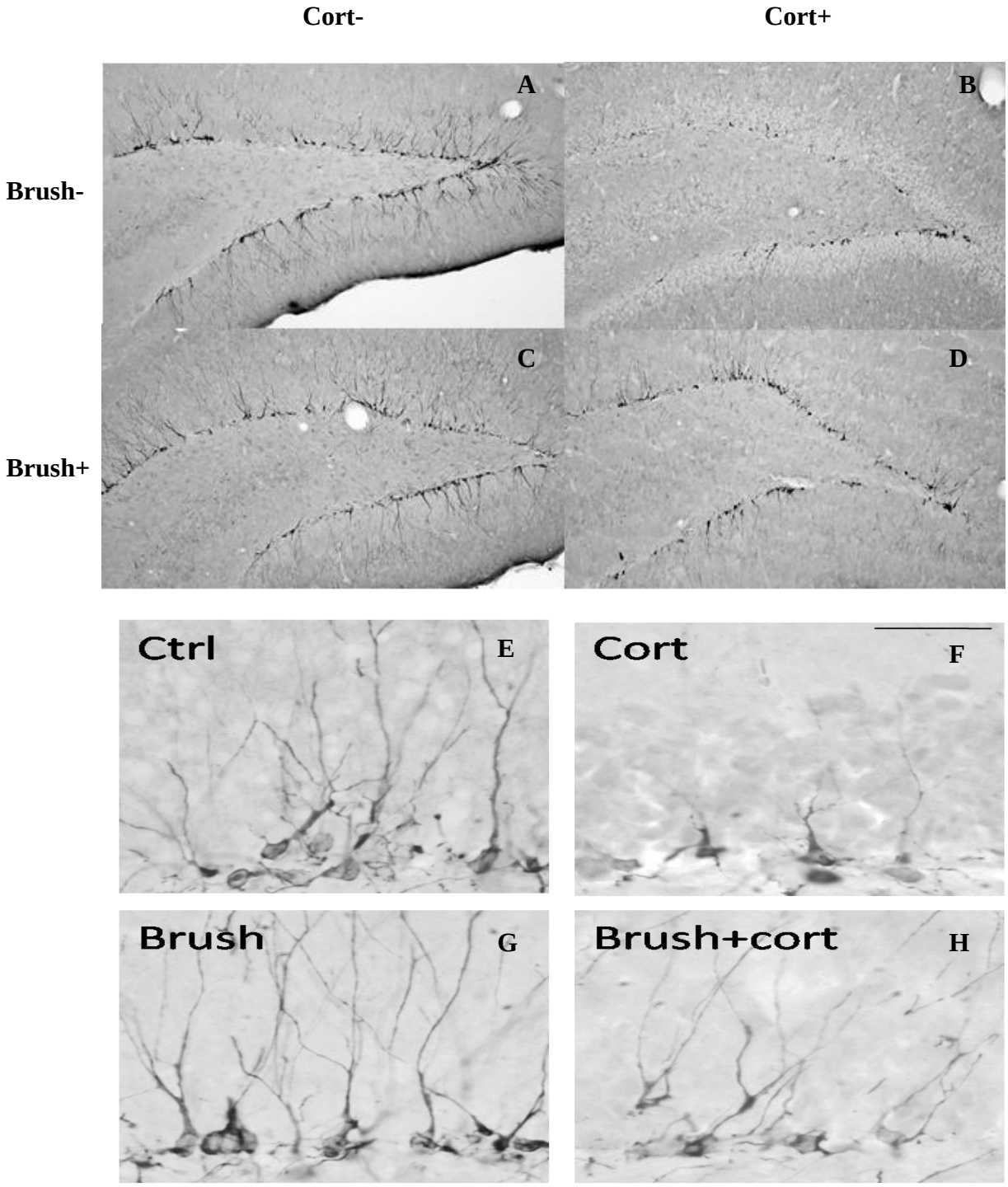


Figure 20. TS did not enhance cell proliferation in the hippocampus in normal control and cort-treated rats. The number of newly formed cells was evaluated and total number of cells in right hippocampal DG was estimated. The representative image (400x) of BrdU+ cells in DG; (A) Ctrl, (B) Cort, (C) Brush and (D) Brush+Cort. The numbers of BrdU+ cells were not altered by brushing treatment but it was significant influenced by chronic corticosterone treatment, BrdU+ cells were decreased ($F(1,18) = 7.541$, #: $p = 0.014$). No beneficial effects were found in both normal and depressive rats after applying tactile stimulation. Result is presented as mean ± SEM, #: $p < 0.05$.


Cort-
Cort+
Brush-
Brush+
A
B
C
D
Ctrl
E
Cort
F
Brush
G
Brush+cort
H

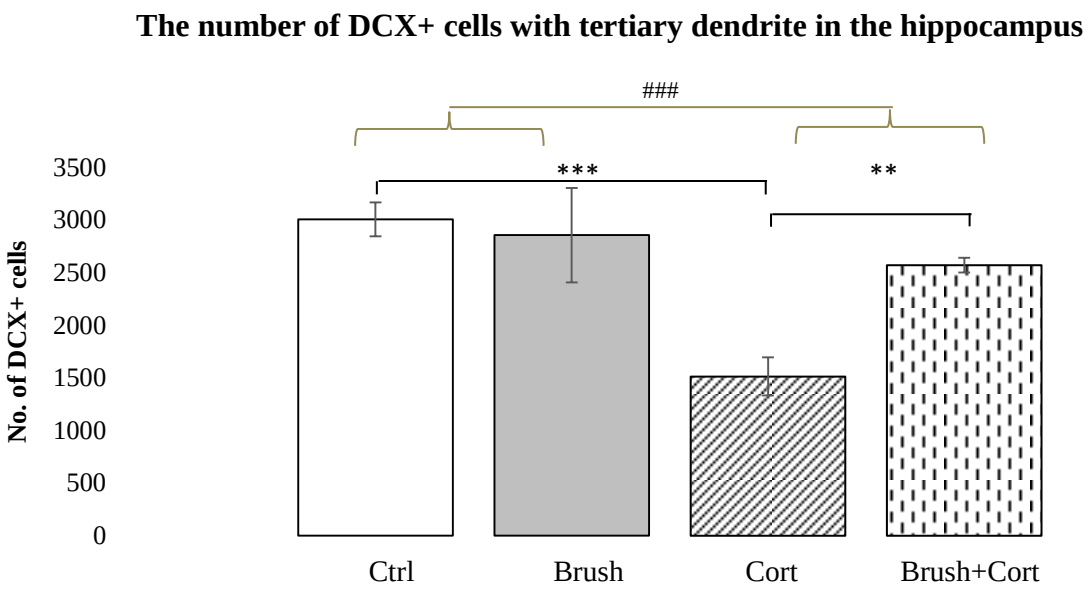


Figure 21. TS enhanced neuronal differentiation in the hippocampus in cort-treated rats. The level of neuronal differentiation is indicated by the number of DCX+ cells. The representative images (200x) of DCX+ cells in hippocampal DG of (A) Ctrl, (B) Cort, (C) Brush and (D) Brush+Cort, respectively. (E-H) are enlarged image (400x) for each group. The bar chart is showing the number of DCX+ cells with tertiary dendrite (3' DCX+) in the hippocampal dentate gyrus (DG). Two-way ANOVA revealed a significant interaction effect between tactile stimulation and chronic corticosterone administration ($F(1,17) = 5.78$, $p = 0.028$). Corticosterone administration suppressed DG neuronal differentiation. Depressive rats (Cort) showed a significant reduction of 3' DCX+ cells in DG (Ctrl vs. Cort, $p < 0.001$), but tactile stimulation reversed this condition and enhanced neuronal differentiation (Cort vs. Brush+Cort, $p = 0.007$). Result is presented as mean ± SEM, ##/**: $p < 0.01$; ***: $p < 0.001$.

4.3.6 The rate of neuronal differentiation in the hippocampal DG is enhanced by repeated tactile stimulation

The rate of neuronal differentiation of newly generated cells in hippocampus was analyzed by BrdU co-labeling with DCX (Fig. 22). Co-expression of the markers indicated an immature neuron differentiating from a newly divided cell. Two-way ANOVA revealed no interaction effect from corticosterone administration and tactile stimulation. Corticosterone and brush treatment displayed the main effects respectively, ($F(1, 16) = 7.962$, $p = 0.021$; $F(1, 16) = 20.506$, $p = 0.004$). TS enhanced neuronal differentiation in both control (Ctrl vs. Brush, $p = 0.001$) and corticosterone-treated rats (Cort vs. Brush+Cort, $p = 0.024$).

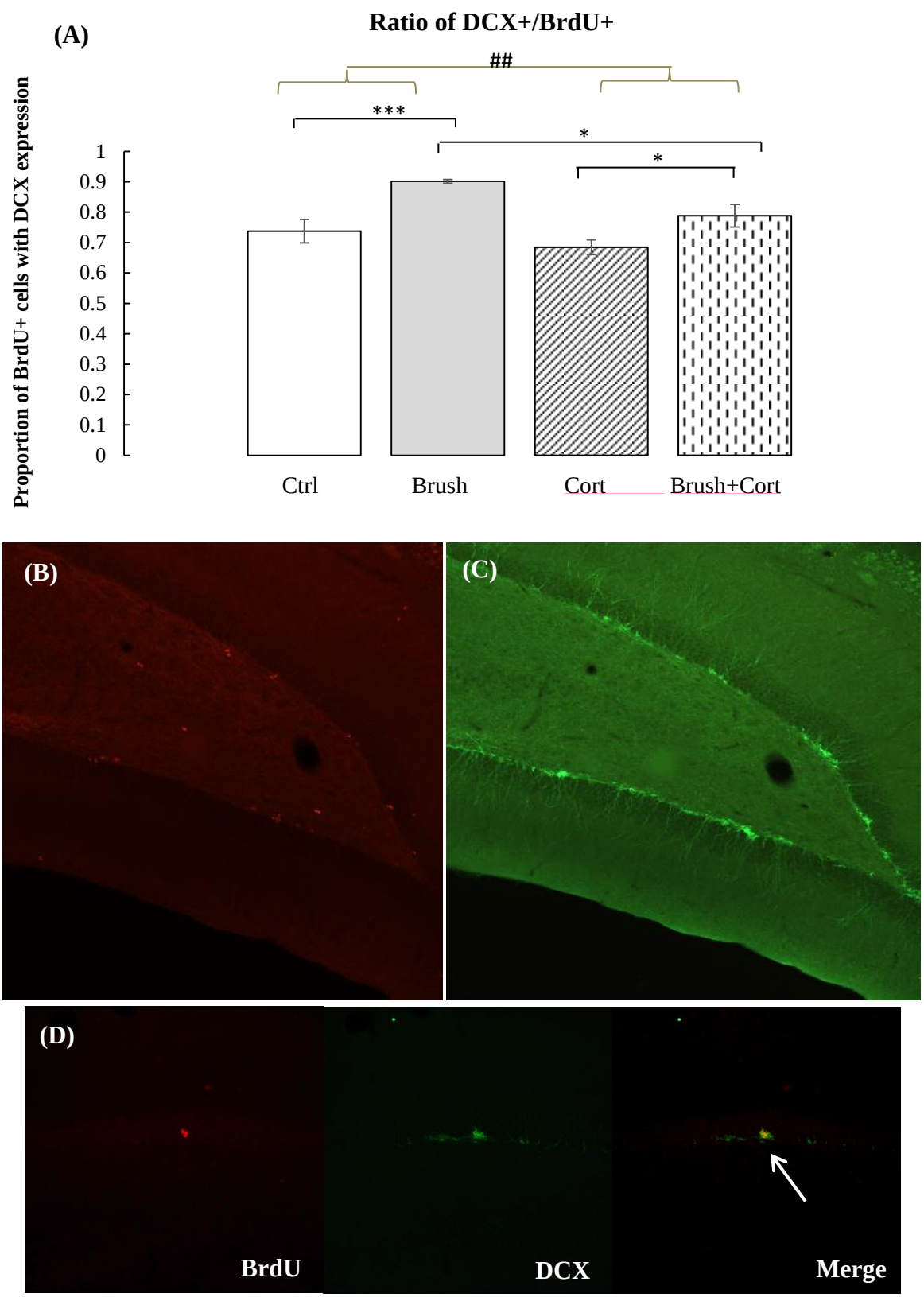

(A)
Ratio of DCX+/BrdU+
##
***
*
*
Proportion of BrdU+ cells with DCX expression
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ctrl
Brush
Cort
Brush+Cort
(B)
(C)
(D)
BrdU
DCX
Merge

Figure 22. The ratio of differentiation in newly generated cells in the hippocampus is enhanced by TS. (A) The result is presented as the proportion of BrdU+ cell co-expressed DCX. TS promoted neuronal differentiation in both control (Ctrl vs. Brush, p = 0.001) and corticosterone-treated rats (Cort vs. Brush+Cort, p = 0.024). Representative images of the hippocampal dentate gyrus stained with BrdU+ cells (fluorescent red, **B**) and DCX+ cells (fluorescent green, **C**) respectively. BrdU+/DCX+ cell is indicated by merging red and green (**D**, arrow).

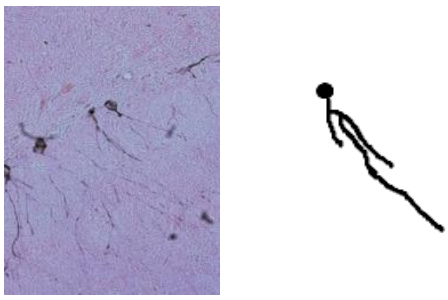
4.3.7 Repeated tactile stimulation enhanced dendritic growth in hypercortisolemic rats

Dendritic complexity, indicated the growth of the neuronal, was measured by Sholl analysis. This assessment evaluated the effect of tactile stimulation on the growth of dendrites in hippocampal DG. Ten 3' DCX+ cells in DG were randomly selected and traced with ImageJ. Concentric circles were drawn from the cell body with gradual increasing the diameter. The number of intersections from dendrites and circles were counted (Fig 23). Corticosterone administration and brush treatment showed an interaction effect (distance from soma 50-130μm, marked as *). Chronic corticosterone injection greatly suppressed the dendritic development in DG (Ctrl vs. Cort, labeled as #). The results demonstrated differential effects that brush treatment enhanced dendritic maturation in depressive rats (Cort vs. Brush+Cort, marked as +), while suppressed dendritic growth in normal rats (Ctrl vs. Brush, labeled as z).

(A)

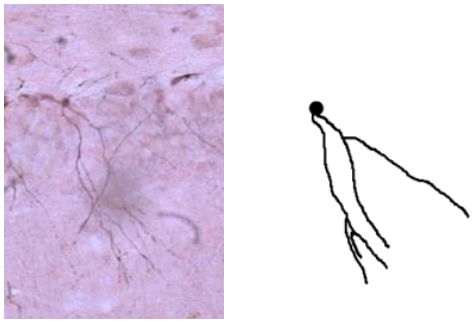
Ctrl

Cort

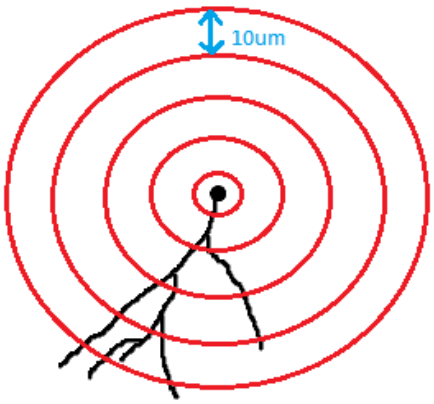


Brush

Brush+Cort



(B)



(C)

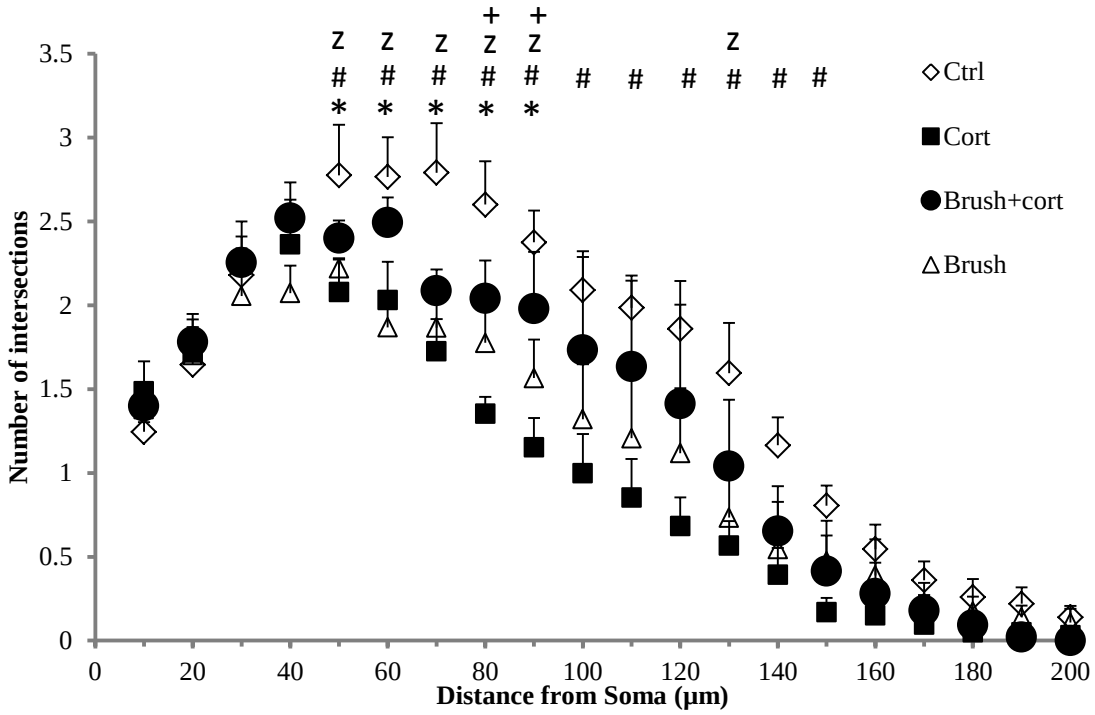


Figure 23. TS reversed the suppression of dendritic complexity in cort-treated rats. The analysis was carried out by tracing the dendrites from each cell body and concentric circles were drawn to measure the number of intersections between dendrites and circles **(B)**. Representative images of DCX+ immature neurons and the corresponding tracing for Sholl analysis in the hippocampal DG of are shown in **(A)**. The numbers of intersection are measured in different distance, 10-200 μm from soma, shown in **(C)**. Tactile stimulation greatly enhanced the dendritic complexity in corticosterone-treated animals, while reduced in control animals. * indicated the interaction effect of TS and corticosterone administration, two-way ANOVA. #: Ctrl

vs. Cort, +: Cort vs. Cort+Brush and z: Ctrl vs. Brush, $p < 0.05$, Tukey post-hoc comparison.

4.4 Conclusion

The chapter provides results obtained from experiment 1 and 2. Experiment 1 mainly focused on the time-dependent effects of tactile stimulation. It demonstrated differential effects between novel and repeated tactile stimulation on affective behaviors and adult hippocampal neurogenesis in rats. Experiment 2 focused on the potential therapeutic effect of repeated tactile stimulation on a depression animal model induced by chronic corticosterone administration. It revealed that repeated tactile stimulation reversed the conditions induced by corticosterone in rats. The following tables summarized the finding in the experiments (Table 3 and 4). The next chapter provides further discussion on the results.

Table 3. Summary of results in the experiment 1: the effects of novel and repeated tactile stimulation on affective behaviors and adult hippocampal neurogenesis in rats

	Novel TS	**Repeated TS**	
	1 Day	**14 Days**	**28 Days**
Depression-like behavior (FST)	Increased	No change	No change
Exploratory anxiety (OFT)	No change	Increased	No change
Social anxiety (SIT)	No change	No change	No change
Cell proliferation (BrdU+ cells)	Not significantly increased	No change	No change
Neuronal differentiation (DCX+ cells)	No change	No change	No change
Dendritic complexities of immature neurons (Sholl analysis)	Increased	Increased	No change

Table 4. Summary of results in the experiment 2: the effects of repeated tactile stimulation on affective behaviors and adult hippocampal neurogenesis in hypercortisolemic animal model

	After repeated TS	
	Control rats (Cort-)	**Depressive rats (Cort+)**
Depression-like behavior (FST)	Increased	Decreased
Exploratory anxiety (OFT)	Increased	Decreased
Social anxiety (SIT)	No change	Decreased
Cell proliferation (BrdU+ cells)	No change	No change
Cell differentiation (DCX+/BrdU+)	Increased	Increased
Dendritic complexities of immature neurons (Sholl)	decreased	Increased

Chapter 5

Discussion

5.1 Overview

This chapter provides the discussion on the results from experiments. The chapter begins with the discussion of first experiment: the effects of novel and repeated tactile stimulation on neurogenesis and affective behaviors. The time dependent effects of TS are discussed. The potential therapeutic effects of TS are discussed in the second part of this chapter. Suggestion of further investigation is provided at the end of the chapter.

5.2 Experiment 1: The effects of novel and repeated tactile stimulation on affective behaviors and adult hippocampal neurogenesis

5.2.1 The effects of novel and single exposure of tactile stimulation

This study provided evidence that novel TS (1-day treatment) may have influence on affective behaviors and dendritic complexity in the hippocampal dentate gyrus (DG). This is the first study that investigated the effect of novel single tactile stimulation on hippocampal neurogenesis. The results revealed that single exposure to tactile stimulation in the form of brush has a trend to increase cell proliferation (not

reaching statistical significance, indicated by the numbers of BrdU+ cells) and not enhances neuronal differentiation (indicated by the numbers of DCX+ cells) in the rat's hippocampal DG. Similarly, the result in line with pervious study that single exposure to novel tactile stimuli can induce cell proliferation but not neuronal differentiation in the spinal cord of rodents (Shechter et al., 2011).

In addition, novel tactile stimulation promoted dendritic growth in the hippocampal DG when comparing to control group, which only received daily handling. This is an evidence that the structure of neurons, or neuronal plasticity, can be altered by TS. Moreover, depression-like behavior was induced in rats after receiving 1-day treatment. This may due to stress induced by involuntary brush treatment.

5.2.2 The effects of repeated and prolonged tactile stimulation

The effects of repeated (14 days) and prolonged (28 days) TS were assessed in experiment 1. The result indicated that 14-days treatment induced exploratory anxiety but not in 28-days treatment. In addition, sholl analysis showed a result that 14-days treatment increased dendritic complexity in the hippocampus while 28-days were not. These results reveal that dendritic complexity in the hippocampus may involve in the expression of behaviors.

5.2.3 The time dependent effects of tactile stimulation

Tactile stimulation improved anxiety-like behavior in rats in a time-dependent manner. The time effect of tactile stimulation was demonstrated in OFT. A significant improvement of exploratory anxiety was found in rats that received 28-days treatment when comparing to 1-days treatment.

Moreover, tactile stimulation influences dendritic complexity and affective behaviors in a time-dependent manner. From all the results in experiment 1, the data illustrated that affective behaviors were influenced when the dendritic complexity increased. For example, in 1-day and 14-day brush treatment, stress was induced in rats and dendritic complexities were increased. On the other hand, 28-days treatment showed no changes in dendritic growth and depression- and anxiety-like behaviors. These results further confirm that the changes of dendritic complexity induced by brush treatment may involve in the alternation of mood expression, as emotional behaviors may be associated with dendritic arborization (Wang, David, Monckton, Battaglia, & Hen, 2008).

5.2.4 The habituation effect on repeated tactile stimulation

A habituation effect on repeated TS has been shown in rat receiving 28-days treatment. Repeated stimulation showed no effect on affective behaviors, neurogenesis and dendritic growth in rats after 28-days TS treatment. This may due to the habituation effect after prolonged stimulation. Hippocampal neurons have been observed to diminish the response to repeated sensory stimulation and thus to avoid redundant information (Hershman, Freedman & Bickford, 1995).

5.3 Experiment 2: The potential therapeutic effect of repeated tactile stimulation on hypercortisolemic animal model

This study provided evidence that repeated tactile stimulation (TS) relieved depression-like behavior and anxiety-like behavior in chronic corticosterone-treated rats, which is used as a model for depression (Iijima et al., 2010). However, repeated TS induced affective behaviors in healthy control rats, which showed similar results as in experiment 1 (14-day Tx group). Neuronal differentiation of newly generated (DCX+/BrdU+) cells in hippocampal dentate gyrus was enhanced by TS in both control and disease model. Interestingly, the dendritic growth, which indicated by dendritic complexity, showed a negative relationship with affective behaviors.

5.3.1 Repeated tactile stimulation not affected hypothalamic-pituitary-adrenal axis

In order to assess the effect of TS on hypothalamic-pituitary-adrenal (HPA) axis, the weight of adrenal gland was measured. HPA axis is a physiological mechanism that regulates the body for coping stress. The results demonstrated that repeated tactile stimulation not greatly influenced significant changes in HPA axis. Reduction of adrenal gland was observed in disease model. Significant changes are mainly because of high-dose corticosterone administration. This result is in line with previous studies (Donner et al., 2012). The reduction is the result of negative feedback in HPA axis induced by high corticosterone level (Karten, Nair, van Essen, Sibug, & Joëls, 1999).

5.3.2 Repeated tactile stimulation demonstrated antidepressant- and anxiolytic effect in hypercortisolemia animal model

Anxiolytic effect of repeated tactile stimulation was assessed by OFT and SIT. After receiving 14-days brush treatment, the levels of exploratory anxiety, indicated in OFT, in depressive model were decreased. From SIT, repeated TS also showed an anxiolytic effect in depressive model. In addition to FST, TS reversed the conditions in corticosterone-treated rats. The present study provides evidence that repeated tactile stimulation may exert anxiolytic and antidepressant effects in hypercortisolemia rats.

All the results from behavioral tests demonstrated that TS increased the level of depression-like and anxiety-like behaviors in normal rats. This may cause by stress

generated from restriction during the treatment or brush treatment on rat's highly sensitive sensory organ. Further investigation is required for answering the question of depressiongenic or anxiogenic effects in control animals.

Several studies have revealed that neonatal tactile stimulation promotes anxiolytic effects in rodents (Boufleur et al., 2012, 2013; Freitas et al., 2015; Imanaka et al., 2008; Wei et al., 2013). Another study demonstrated that anxiety level was reduced when adult rats received TS from 8-14 postnatal days, but no change was found when TS was applied from 15-21 postnatal days. However, these studies may not be comparable to the present study because former ones focused on neonatal TS but not adult TS. The present study is the first to display anxiolytic and antidepressant effects in hypercortisolemic rat model.

5.3.3 Repeated tactile stimulation improved hippocampal neurogenesis and dendritic growth in hypercortisolemia animal model

In neurogenic assay, TS showed a beneficial effect on neuronal differentiation of newly generated cells (the number of DCX+/BrdU+ cells) in DG, but no effect was found on cell proliferation (only BrdU+ cells). The current study is not in line with pervious study that tactile stimulation can promote cell proliferation in hippocampal DG (de los Angeles et al., 2016; Yamamuro et al., 2010). The research from de los

Angeles et al. (2016), reported that postnatal TS promotes cell proliferation and differentiation in the dentate gyrus in both stressed and normal rats. The study from Yamamuro et al, (2010) showed that tackling stimulation also promotes cell proliferation in the adult rat's hippocampus. The differences may due to different position, force, duration or mean of stimulus employed in the studies. In the mentioned studies, researcher gave TS by finger sliding on the animals, which may not comparable to the traditional tooth brush. In recent study, whisker was included for TS. Whisker tactile stimulation may influence the changes in the hippocampus since it also receives signals from whisker pad stimulation (Bellistri et al., 2013).

Dendritic growth, or dendritic maturation which is a process of neurogenesis, was assessed by Sholl analysis. The results revealed that the level of dendritic complexity in normal animals was reduced by TS while the complexity was increased in disease model. Taken together with behavioral tests, a negative relationship was found between the dendritic complexity and affective behaviors. Emotional behaviors is reported to associate with dendritic arborization (Wang, David, Monckton, Battaglia, & Hen, 2008). Based on the evidence, it is suggested that TS or brush treatment may affect neuronal growth in DG and induce stress in normal control animals. In contrast, TS or brush treatment may counteract or balance the physiological changes in depressive rat model and therefore relieve the affective behaviors. The exact

mechanism of TS in this aspect remains unknown. Further investigation in the molecular changes may be helpful to answer the question.

5.3.4 The possible underlying mechanism of tactile stimulation

It is proposed that tactile stimulation may enhance the release of oxytocin and therefore reversed the conditions in hypercortisolemic animal model. Low intensity cutaneous tactile stimulation has been reported to induce the release of oxytocin, enhance social motivation and improve the behaviors to cooing with stress (Walker et al., 2017). Oxytocin has been reported to reverse the neurogenic impairment and affective disturbances in chronic corticosterone animal model (Sanchez-Vidana et al., 2016). In addition, oxytocin has been reported to promote neurogenesis in the hippocampus by activating the receptors in CA3 neurons (Lin et al.,2017). However, the results indicated that brush treatment only exert therapeutic effect on hypercortisolemia model but not on health normal rats. A further study may require on this aspect.

5.4 Suggestion on further investigation

The relation between tactile stimulation, hippocampal neurogenesis and affective behaviors was illustrated in this study. However, the underlying mechanism remains unknown. For further understanding, it should move on to investigate the biological changes in the animals after receiving TS. The proteins expression related to neurogenesis should be investigated, such as Brain-derived neurotrophic factor

(BDNF). It is because whisker stimulation may promote BDNF mRNA expression in barrel cortex in rodents and BDNF has been reported to regulate neurogenesis by activating PI3-kinase pathways and MAP kinase (Binder & Scharfman, 2004, Rocamora et al., 1996, Barmabe-Heider & Miller, 2003).

In order to investigate whether neurogenesis is essential for the beneficial effect of tactile stimulation, another experiment should be conducted. It is suggested that the experiment should involve the inhibition of hippocampal neurogenesis by cytosine arabinoside (Lau et al., 2012). Brush treatment can be provided after the blockage of neurogenesis. This may answer a research gap that whether adult hippocampal neurogenesis is essential for TS to relieve depression-like or anxiety-like behavior in animals.

5.5 Overall conclusion

Collectively, the results indicated that TS promoted hippocampal neurogenesis and improved affective behaviors in hypercortisolemic rat model. HPA axis is not altered and involved in the behavioral changes after receiving TS.

Also, the alternation of dendritic complexity in the hippocampus may involve in the response and habituation to TS.

Chapter 5

Conclusion

The present study illustrates the effects of novel and repeated tactile stimulation on adult hippocampal neurogenesis and the regulation of affective behaviors. The results showed a time dependent effects of TS. A habituation effects were found when rats receiving 28-days brush treatment. The morphological changes of neurons in the hippocampus may help to respond and habituate to repeated TS. When rats habituated to TS, neurogenesis was not induced. This is the first study for investigating novel TS on hippocampal neurogenesis.

Furthermore, TS exerted the therapeutic effects on hypercortisolemic animal model to reverse the conditions such as neurogenic impairment and affective disturbances. This study provide evidence that TS may improve affective behaviors in stressed animals. TS showed a pro-neurogenic effect by enhancing neuronal differentiation in the hippocampal dentate gyrus. It is suggested that TS can relieve anxiety- and depression-like behaviors in depressive animal model by promoting neuronal differentiation and maturation.

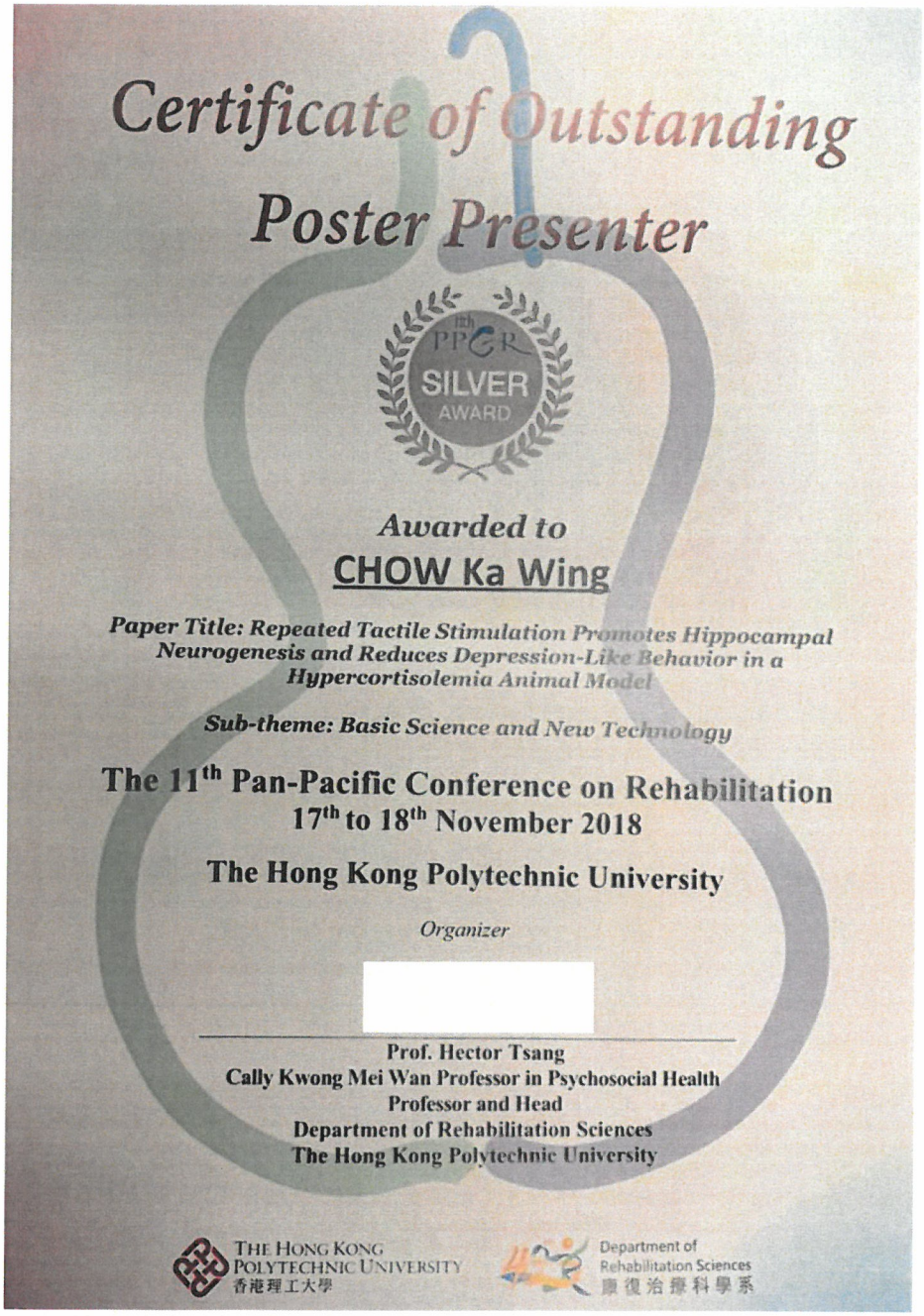
The present study has proven the hypothesis that tactile stimulation can improve affective behaviors by enhancing hippocampal neurogenesis, the effects only exerted

in hypercortisolemic animals but not in normal animals. Moreover, this study provided evidence that TS not altered hypothalamic-pituitary-adrenal axis.

In order to further understand the underlying mechanisms of TS on hippocampal neurogenesis and affective behaviors, it is suggested to investigate the levels of biological changes of neurogenic factors in the body, for example BDNF and Oxytocin. Moreover, for assessing the essential role of hippocampal neurogenesis in the effects of TS, blocking neurogenesis in further experiment is suggested.

APPENDIX

Certificate of Outstanding Poster Presentation at 11th Pan-Pacific Conference on Rehabilitation (2018), The Hong Kong Polytechnic University:



Certificate of Outstanding Poster Presenter

11th PPCR SILVER AWARD

Awarded to

CHOW Ka Wing

Paper Title: Repeated Tactile Stimulation Promotes Hippocampal Neurogenesis and Reduces Depression-Like Behavior in a Hypercortisolemia Animal Model

Sub-theme: Basic Science and New Technology

The 11th Pan-Pacific Conference on Rehabilitation
17th to 18th November 2018

The Hong Kong Polytechnic University

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The Hong Kong Polytechnic University

The Hong Kong Polytechnic University 香港理工大學

Department of Rehabilitation Sciences 康復治療科學系

REFERENCES

Aimone, J. B., Deng, W., & Gage, F. H. (2011). Perspective : Point / Counterpoint Resolving New Memories : A Critical Look at the Dentate Gyrus , Adult Neurogenesis , and Pattern Separation. *Neuron*, *70*(4), 589–596. https://doi.org/10.1016/j.neuron.2011.05.010

Airan, R. D., Meltzer, L. A., Roy, M., Gong, Y., Chen, H., & Deisseroth, K. (2007). High-Speed Imaging Reveals Neurophysiological Links to Behavior in an Animal Model of Depression. *Science*, *317*(August), 819–824.

American Psychiartric Association. (2015). What is mental illness. Retrieved December 5, 2017, from https://www.psychiatry.org/patients-families/what-is-mental-illness

Anacker, C., Zunszain, P. A., Cattaneo, A., Carvalho, L. A., Garabedian, M. J., Thuret, S., & Price, J. (2011). Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Molecular Psychiatry*, *16*(7), 738–750. https://doi.org/10.1038/mp.2011.26

Bannerman, D. M., Rawlins, J. N. P., McHugh, S. B., Deacon, R. M. J., Yee, B. K., Bast, T., ... & Feldon, J. (2004). Regional dissociations within the hippocampus—memory and anxiety. Neuroscience & biobehavioral

reviews, 28(3), 273-283.

Barnabé-Heider, F., & Miller, F. D. (2003). Endogenously produced neurotrophins regulate survival and differentiation of cortical progenitors via distinct signaling pathways. Journal of Neuroscience, 23(12), 5149-5160.

Bellistri, E., Aguilar, J., Brotons-mas, J. R., Foffani, G., Menendez, L., & Prida, D. (2013). Basic properties of somatosensory-evoked responses in the dorsal hippocampus of the rat, *10*, 2667–2686. https://doi.org/10.1113/jphysiol.2013.251892

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived Neurotrophic Factor. Growth factors, 22(3), 123-131.

Boufleur, N., Antoniazzi, C. T. D., Pase, C. S., Benvegnú, D. M., Barcelos, R. C. S., Dolci, G. S., … Bürger, M. E. (2012). Neonatal tactile stimulation changes anxiety-like behavior and improves responsiveness of rats to diazepam. *Brain Research*, *1474*, 50–9. https://doi.org/10.1016/j.brainres.2012.08.002

Boufleur, N., Antoniazzi, C. T. D., Pase, C. S., Benvegnú, D. M., Dias, V. T., Segat, H. J., … Bürger, M. E. (2013). Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: behavioral and oxidative parameters. *Stress (Amsterdam, Netherlands)*, *16*(3), 321–30. https://doi.org/10.3109/10253890.2012.723075

Brown, J., Cooper-Kuhn, C. M., Kempermann, G., Van Praag, H., Winkler, J., Gage, F. H., & Kuhn, H. G. (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. European Journal of Neuroscience, 17(10), 2042-2046.

Brummelte, S., & Galea, L. A. M. (2010). CHRONIC HIGH CORTICOSTERONE REDUCES NEUROGENESIS IN THE DENTATE GYRUS OF ADULT MALE AND FEMALE RATS. *NSC*, *168*(3), 680–690. https://doi.org/10.1016/j.neuroscience.2010.04.023

Campbell, S., & Macqueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression, *29*(6), 417–426.

Christian, K. M., Song, H., & Ming, G. (2014). Function and Dysfunctions of Adult Hippocampal Neurogenesis. *Annu Rev Neurosci.*, *37*, 234–262. https://doi.org/10.1146/annurev-neuro-071013-014134.Functions

David, D. J., Samuels, B. A., Rainer, Q., Wang, J., Marsteller, D., Mendez, I., … Leonardo, E. D. (2009). Article Effects of Fluoxetine in an Animal Model of Anxiety / Depression. *Neuron*, *62*(4), 479–493. https://doi.org/10.1016/j.neuron.2009.04.017

de los Angeles, G. A., del Carmen, R. O., Wendy, P. M., & Socorro, R.-M. (2016). Tactile stimulation effects on hippocampal neurogenesis and spatial learning and

memory in prenatally stressed rats. *Brain Res Bull*, *124*, 1–11. https://doi.org/10.1016/j.brainresbull.2016.03.003

Ekstrom, A. D., Kahana, M., Caplan, J., Fields, T., Isham, E., Newman, E., & Fried, I. (2003). Cellular networks underlying human spatial navigation. *Nature*, *425*(September), 184–188. https://doi.org/10.1038/nature01955.1.

Freitas, D., Antoniazzi, C. T. D., Segat, H. J., Metz, V. G., Vey, L. T., Barcelos, R. C. ., … Burger, M. E. (2015). Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats. *International Journal of Developmental Neuroscience*, *47*, 192–197. https://doi.org/10.1016/j.ijdevneu.2015.09.010

Gurol, A., Polat, S., & Akcay, M. (2010). Itching , Pain , and Anxiety Levels Are Reduced With. *Journal of Burn Care & Research*, *31*(3), 429–432. https://doi.org/10.1097/BCR.0b013e3181db522c

Iijima, M., Ito, A., Kurosu, S., & Chaki, S. (2010). Pharmacological characterization of repeated corticosterone injection-induced depression model in rats. *Brain Research*, *1359*, 75–80. https://doi.org/10.1016/j.brainres.2010.08.078

Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., & Yamawaki, S. (2008). Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male

and female adult Sprague-Dawley rats. *Behavioural Brain Research*, *186*(1), 91–7. https://doi.org/10.1016/j.bbr.2007.07.039

Jadhav, S. P., Wolfe, J., & Feldman, D. E. (2009). Sparse temporal coding of elementary tactile features during active whisker sensation. *Nature Neuroscience*, *12*(6), 792–800. https://doi.org/10.1038/nn.2328

Karten, Y. J., Nair, S. M., van Essen, L., Sibug, R., & Joëls, M. (1999). Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampal CA1 neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(23), 13456–61.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. Nature, 386(6624), 493.

Kimball, J. G., Lynch, K. M., Stewart, K. C., Williams, N. E., Thomas, M. A., & Atwood, K. D. (2007). Using salivary cortisol to measure the effects of a Wilbarger protocol-based procedure on sympathetic arousal: a pilot study. *The American Journal of Occupational Therapy : Official Publication of the American Occupational Therapy Association*, *61*(4), 406–13.

Lau, B. W. M., Chia-Di Lee, J., Li, Y., Fung, S. M. Y., Sang, Y. H., Shen, J., ... & So, K. F. (2012). Polysaccharides from wolfberry prevents corticosterone-induced inhibition of sexual behavior and increases neurogenesis. PLoS One, 7(4),

e33374.

Leslie, A. N. A. T. F. S., Guinsburg, R., Euge, L., & Sa, U. F. De. (2008). Repetitive Nociceptive Stimuli in Newborn Rats Do Not Alter the Hippocampal Neurogenesis, *63*(2), 154–157.

Lin, Y. T., Chen, C. C., Huang, C. C., Nishimori, K., & Hsu, K. S. (2017). Oxytocin stimulates hippocampal neurogenesis via oxytocin receptor expressed in CA3 pyramidal neurons. Nature communications, 8(1), 537.

Ma, D. K., Kim, R., Ming, G., & Song, H. (2009). Activity-dependent Extrinsic Regulation of Adult Olfactory Bulb and Hippocampal Neurogenesis, *673*, 664–673. https://doi.org/10.1111/j.1749-6632.2009.04373.x

Malberg, J. E., Eisch, A. J., Nestler, E. J., & Duman, R. S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(24), 9104–10. https://doi.org/20/24/9104 [pii]

Monteiro, B. M., Moreira, F. A., Massensini, A. R., Moraes, M. F., & Pereira, G. S. (2014). Enriched environment increases neurogenesis and improves social memory persistence in socially isolated adult mice. Hippocampus, 24(2), 239-248.

Naylor, A. S., Bull, C., Nilsson, M. K., Zhu, C., Björk-Eriksson, T., Eriksson, P. S., ...

& Kuhn, H. G. (2008). Voluntary running rescues adult hippocampal neurogenesis after irradiation of the young mouse brain. Proceedings of the National Academy of Sciences, 105(38), 14632-14637.

Nestor, P. J., Graham, K. S., Bozeat, S., Simons, J. S., & Hodges, J. R. (2002). Memory consolidation and the hippocampus : further evidence from studies of autobiographical memory in semantic dementia and frontal variant frontotemporal dementia, *40*, 633–654.

Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O., & Eriksson, P. S. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. Journal of neurobiology, 39(4), 569-578.

Pereira, A., Ribeiro, S., Wiest, M., Moore, L. C., Pantoja, J., Lin, S., & Nicolelis, M. A. L. (2007). Processing of tactile information by the hippocampus, *104*(46).

Poletto, C. J. (2006). Tactile Stimulation. In *Encyclopedia of Medical Devices and Instrumentation*. Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/0471732877.emd319

Ranganath, C., Esposito, M. D., & Wills, H. (2001). Medial Temporal Lobe Activity Associated with Active Maintenance of Novel Information, *31*, 865–873.

Rocamora, N., Welker, E., Pascual, M., & Soriano, E. (1996). Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory

stimulation. Journal of Neuroscience, 16(14), 4411-4419.

Ruan, L., Lau, B. W.-M., Wang, J., Huang, L., Zhuge, Q., Wang, B., … So, K.-F. (2014). Neurogenesis in neurological and psychiatric diseases and brain injury: from bench to bedside. *Progress in Neurobiology*, *115*, 116–37. https://doi.org/10.1016/j.pneurobio.2013.12.006

Sánchez-Vidaña, D. I., Chan, N. M. J., Chan, A. H., Hui, K. K., Lee, S., Chan, H. Y., ... & Lau, B. W. M. (2016). Repeated treatment with oxytocin promotes hippocampal cell proliferation, dendritic maturation and affects socio-emotional behavior. Neuroscience, 333, 65-77.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., … Belzung, C. (2003). Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants, *301*(August), 805–810.

Shechter, R., Baruch, K., Schwartz, M., & Rolls, A. (2011). Touch gives new life: mechanosensation modulates spinal cord adult neurogenesis. *Molecular Psychiatry*, *16*(3), 342–52. https://doi.org/10.1038/mp.2010.116

Shrager, Y., Bayley, P. J., Bontempi, B., Hopkins, R. O., & Squire, L. R. (2007). Spatial memory and the human hippocampus, *104*(8).

The National Institute of Mental Health. (2016). Mental Health Information: Anxiety Disorders. Retrieved December 5, 2017, from

https://www.nimh.nih.gov/health/topics/anxiety-disorders/index.shtml

Walker, S. C., Trotter, P. D., Swaney, W. T., Marshall, A., & Mcglone, F. P. (2017). C-tactile afferents: Cutaneous mediators of oxytocin release during affiliative tactile interactions?. Neuropeptides, 64, 27-38.

Wang, J.-W., David, D. J., Monckton, J. E., Battaglia, F., & Hen, R. (2008). Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(6), 1374–84. https://doi.org/10.1523/JNEUROSCI.3632-07.2008

Wei, B., Tai, F., Liu, X., Ma, L., Yang, X., Jia, R., & Zhang, X. (2013). Neonatal tactile stimulation alleviates the negative effects of neonatal isolation on novel object recognition, sociability and neuroendocrine levels in male adult mandarin voles (Microtus mandarinus). *Physiology & Behavior*, *112–113*, 14–22. https://doi.org/10.1016/j.physbeh.2013.02.005

World Health Organization. (2017). Mental disorders: fact sheet. Retrieved December 5, 2017, from http://www.who.int/mediacentre/factsheets/fs396/en/

Yamamuro, T., Senzaki, K., Iwamoto, S., Nakagawa, Y., Hayashi, T., Hori, M., … Urayama, O. (2010). Neurogenesis in the dentate gyrus of the rat hippocampus enhanced by tickling stimulation with positive emotion. *Neuroscience Research*,

68(4), 285–9. https://doi.org/10.1016/j.neures.2010.09.001

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. Cell, 132(4), 645-660.