CORTICOSTERONE INDUCED MORPHOLOGICAL CHANGES OF HIPPOCAMPAL AND AMYGDALOID CELL LINES ARE DEPENDENT ON 5-HT7 RECEPTOR RELATED SIGNAL PATHWAY

By

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To my Mom and Dad

ACKNOWLEDGMENTS

This project was accomplished within the range of one year, and my advisor Dr. William Ogle has been providing me with all support and guidance that could never be replaced. Also I am deeply impressed by his profound academic knowledge and original understanding of high quality research, which has been and will always be positively impacting my further study and occupational life.

I sincerely thank Dr. Ying Xu for bring me into the field of exploiting great interest while conducting steadfast research, as well as her brilliant ideas and problem solving techniques. I would like to thank all my labmates in Gene Dynamics Lab, Shan, Phil, Matt, Erin, Raj and Ling, for their kind assistance throughout the entire project.

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Abstract of Thesis Presented to the Graduate School
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Stress is an unavoidable life experience that can disturb emotional and cognitive processes, and neuroplasticity. This study observed two aspects of how stress hormone affects the hippocampus and amygdala. Firstly, we investigated whether administration of corticosterone to hippocampal and amygdaloid cell lines induced different changes in 5-HT sub-receptors. Secondly, we tested whether stress induced morphological changes in these two cell lines are involved in the 5-HT sub-receptors expression. We now show, using HT-22 and AR-5 cell lines, that 5-HT_7 receptor mRNA is significantly up-regulated in HT-22 cells, but down-regulated in AR-5 cells by exposure to stress level of corticosterone (50 μM) for 24h. Pretreatment of cells with 5-HT_7 antagonist SB-269970 and agonist LP-44 reversed corticosterone induced cell lesion in a dose-dependent manner in HT-22 and AR-5 cells, respectively. Moreover, corticosterone induced different changes of dendritic length in HT-22 and AR-5 cells were also reversed by pretreatment with SB-269970 and LP-44. These results support the hypothesis that serotonin may differentially modulate neuronal morphology in

hippocampus and amygdala depending on the expression levels of the 5-HT sub-receptors during stress hormone attacks.

CHAPTER 1
INTRODUCTION

Stress may be described as any environmental change, either internal or external, that disturbs the maintenance on homeostasis (Leonard, 2005). The stress response is to maintain homeostasis, which includes a series of physiological reactions such as modulation of neuroplasticity (limbic system), endocrine activation (especially of the hypothalamic-pituitary-adrenal axis, HPA axis), and cardiovascular changes (Sapolsky, 2003). The central feature of the limbic-HPA stress response is the synthesis and the secretion of glucocorticoids from the adrenal cortex. The excessive stress hormone often acts as a trigger to the onset of major depression and Alzheimer's disease (AD), which is associated with a decreased sensitivity to HPA axis feedback inhibition by cortisol in primates or corticosterone in rodents. In addition to the HPA axis, brain neuronal systems, including the monoaminergic systems and in particular the serotonin (5-HT) containing neuronal one, play critical roles in stress-related disorders (Lanfumey, Mongeau, Cohen-Salmon, & Hamon, 2008; Y. Xu et al., 2006). Numerous data have demonstrated the existence of reciprocal interactions between the central serotonin system and HPA axis in stress related depression, in which dysfunction of both the 5-HT and its sub-receptors and HPA axis have been evidenced (Kitamura, Araki, & Gomita, 2002).

The hippocampus and the amygdala are essential components of the neural circuitry mediating stress responses. The hippocampus is critical in its role controlling the limbic-HPA axis, mood and memory through excitatory inputs. Changes within this structure, synaptic loss and atrophy, is known to involve in prolonged elevated glucocorticoid levels, major depression and cognitive impairment (Lupien et al., 1998).

The amygdala is responsible for the detection of an environmental stressor (or threat) and controls the expression of the fear reaction, including behavioral, autonomic and endocrine responses via projects to downstream areas, such as hypothalamus and central gray, which in turn regulate the secretion of neurotransmitters, corticotropin-releasing hormone (CRH) and glucocorticoids (Fanselow & Poulos, 2005) (Rodrigues et al., 2009). However, recent studies show that enhanced hippocampal input would suppress the HPA axis, while enhanced amygdaloid input could have the opposite effect on HPA activity (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002). In rodents, corticosterone reduces response to serotonin in the hippocampus, which could contribute to the onset of symptoms of depression in predisposed individuals (Joels et al., 2004). But the amygdala activation leads to an increase in arousal and vigilance in response to the fear reaction after stress, which result in the release of neurotransmitters (5-HT, noradrenaline and dopamine) and their sub-receptors change (LeDoux, 2007; Leonard, 2005).

In view of the potentially contrasting impact of stress hormone on the hippocampus and amygdala at the behavioral level and neuroendocrine mechanism, it is important to study the molecular mechanism underlying how the stress hormone affects the hippocampal and amygdaloid neurons function when they are exposed to the stress hormone. Therefore, the present study was designed to investigate the morphological changes of hippocampal and amygdaloid cell lines under corticosterone exposure. We also tested if the mechanism was dependent on 5-HT sub-receptors and related signal pathway.

CHAPTER 2
MATERIALS AND METHODS

2.1 Materials

HT-22 cells were a generous gift from Dr. David Schubert (The Salk Institute for Biological Studies, La Jolla, CA, USA) (Y. Li, Maher, & Schubert, 1997). AR-5 cells were kindly provided by Dr. Rosalie Uht (University of North Texas Health Science Center, Fort Worth, TX, USA)(Lalmansingh & Uht, 2008). Culture plates were acquired from Nunc (A/S, Roskilde, Denmark). DMEM/F12 media were bought from Hyclone (Logan, UT). NeuroBasal medium, fetal Bovine Serum (FBS) and N2 nutrient supplement were from Invitrogen (Carlsbad, CA). Corticosterone was purchased from Sigma Chemical Co. (St. Louis, MO). MTT assay was obtained from Biotium, Inc. (Hayward, CA). 5-HT_{1A} receptor antagonist, NAN-190 (1-(2-methoxyphenyl)-4[-(2-phthalimido)butyl]piperazine), 5-HT_7 receptor agonist, LP-44 ((4-[2-(Methylthio)phenyl]-N-(1,2,3,4-tetrahydro-1-naphthalenyl)-1-piperazinehexanamide)) and antagonist SB-269970 ((2R)-1-[(3-Hydroxyphenyl)sulfonyl]-2-(2-(4-methyl-1-piperidinyl)ethyl)pyrrolidine) were purchased from Tocris (Avonmouth, UK). Other routine cell culture supplies and reagents were from Sigma, Invitrogen or Fisher.

2.2 Cell Culture

HT-22 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplied with 10% FBS, and grown at 37℃ in 5% CO_2, and differentiated in NeuroBasal medium containing 1× N2 supplement for 12h before treatment. AR-5 cells were cultured in DMEM/F12 media as described previously (Lalmansingh & Uht, 2008) and differentiated the same way as HT-22 were differentiated. Cells were plated at 10^5/ml

for MTT experiment and mRNA extraction, 5×10^4/ml for cell morphology tests. All treatments were performed in the differentiation media.

2.3 Drug Treatment

Corticosterone, 5-HT_7 receptor antagonist SB-269970 and agonist LP-44 were dissolved in dimethyl sulfoxide (DMSO), NAN-190 in ethanol and respectively, the vehicle concentrations did not exceed 0.1% of the total volume in the cell culture well. SB-269970 and LP-44 were added 2h before corticosterone application (doses and treatment schedules were presented in the results of each experiment), and cells were pretreated with NAN-190 (1μm) wherever LP-44 was used.

2.4 Cell Viability

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay based on the kit protocol. Briefly, 12 hr after differentiation on 96-well plates, cells were treated with corticosterone and/or other protective reagents at different concentrations and incubation time. Following the indicated treatments, 10μl of MTT solution was added to each well and incubated for another 3 hr. The dark blue formazan crystals formed in intact cells were dissolved with 200μl DMSO/well and absorbance at was measured using Synergy Multi-mode Microplate Reader (BioTek, USA).

2.5 mRNA Extraction and Real-time Reverse Transcriptase (RT)-PCR

Cultures were washed and total cellular RNA was isolated with TriZOL reagent (TriZOL® Invitrogen) according to the manufacture's protocol. For RT-PCR, 600ng total RNA was reverse transcribed using MJ Mini ™ Gradient Thermal Cycler (Bio-Rad, Hercules CA, USA) and PCR reaction was performed using iCycler Real-Time PCR machine (Bio-Rad, Hercules CA, USA). After cDNA synthesis, a PCR mixture

containing 50% v/v per sample of SYBER Green (iQ SYBER Green Supermix reagent, Bio-Rad) was tested with specific primers for β-actin (5'-CGTGCGTGACATTAAAGAG-3', 5'-GCCACAGGATTCCATACC-3'), RhoA (5'-TATTGAAGTGGACGGGAAGC-3', 5'-ACTATCAGGGCTGTCGATGG-3'), Rac1 (5'-GGGAACAAGAGCAAGTCTGC-3', 5'-CGATTCCCGTTCTCCTTCTA-3'), Cdc42 (5'-TTGTTGGTGATGGTGCTGTT-3', 5'-TCTCAGGCACCCACTTTTCT-3'), Synaptophysin (5'-CCCCCTTTTCCCATATCCTA-3', 5'-AGGTCTGGTTCCCTTCCTGT-3'), Gap-43 (5'-CGTGCGTGACATTAAAGAG-3', 5'-GGCATTTCCTTAGGTTTTGGT-3') and Synaptopodin (5'-AGTCCTCACCAAACCCTCCT-3', 5'-TGGACCTCACTTCCTCTGCT-3'). PCR products were amplified in the iCycler real-time PCR machine followed by melt curve analysis and gel electrophoresis to verify specificity and purity of product. All the data were normalized to the housekeeping gene, β-actin.

2.6 Image Collection and Data Analysis

Images were collected in a blinded manner using a Zeiss LSM510 Pascal confocal microscope (Carl Zeiss imaging systems, Germany). A stack of images was taken using a 20×objective and total lengths of neurite outgrowth were measured. Randomly selected HT-22 or AR-5 cells from each group were excluded if precise tracing was deemed to be questionable due to extensive overlapping with processes originating from analysis or if their morphology was not intact and possessed membrane varicosities. Fewer than 5% of the randomly selected neurons met one or more of these exclusion criteria. Digitized images were assembled offline using Photoshop 7.0 software (Adobe Systems, Mountainview, CA) and used for analyses without further manipulation (Jugloff, Jung, Purushotham, Logan, & Eubanks, 2005). Ten cells of each

group from at least two separate experiments were analyzed (Xie, Cahill, & Penzes, 2010).

2.7 Statistical Analysis

All data were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by a LSD test was used for statistical evaluation. Statistical significance was set at $p < 0.05$.

CHAPTER 3
RESULTS

3.1 Corticosterone Impairs Hippocampal and Amygdaloid Primary Cultures in a Dose- and Time- Dependent Manner

The ceoncentration- and time-course of corticosterone caused cytotoxicity were test in hippocampal and amygdaloid primary cultures with MTT assay. Primary neurons were seeded at a density of 10^6/ml in 0.1% (w/v) poly-L-lysine coated dishes. Corticosterone treatment at higher than 10μM for 24 hr caused significant cytotoxicity in the above two types of primary cultures ($p<0.01$) (Fig. 1A, B, C and D).

3.2 The Influence of Corticosterone on 5-HT Receptors mRNA Expression in Primary Hippocampal and Amygdaloid Cultures

Dysfunction of the 5-HT system is present in stress-related depression and anxiety. The present study investigated the influence of stress levels of corticosterone (10 μM) on seven types of 5-HT receptors, which are closely involved in stress-related mood disorders (Table 1). Results show that for hippocampus, 5-HT_{1A}, 5-HT_{2A} and 5-HT_4 and 5-HT_7 receptor mRNAs were significantly increased after corticosterone exposure for 24h ($p<0.05$ vs. vehicle-treated group without corticosterone, respectively). For amygdala, 5-HT_{1A}, 5-$HT_{2B,}$ 5-$HT_{4,}$ 5-HT_6 receptor mRNA was shown to increase following exposure to corticosterone, but 5-HT_7 mRNA was shown to decrease significantly ($p<0.05$ vs. vehicle-treated group).

3.3 Protective Effects of SB-269970 and LP-44 on HT-22 Cells and AR-5 Cells Respectively From the Lesion Induced by Corticosterone

HT-22 cells were exposed to 6.25, 12.5, 25, 50, 100μM corticosterone, and cell survival was quantified by MTT assay. Cell viability was markedly reduced after exposure to 50μM corticosterone for 24 hr ($p<0.01$) (Fig. 2A). Pretreatment of HT-22 cells with 2.5, 5, 10μM SB-269970 could dose-dependently protect against the cell

lesion induced by 50μM corticosteronn. The effect was significant at 5 and 10 μM ($p<0.05$ and $p<0.01$) (Fig. 2B). AR-5 cell survival was significantly at 50μM corticosterone for 24 hr ($p<0.01$) and the effect was reversed dose-dependently by LP-44 markedly at 0.1 and 0.2μM ($p<0.01$) (Fig. 3A and B).

3.4 SB-26970 and LP-44 Modulation of Cell Morphology Against Corticosterone Toxicity

Corticosteone has been proved to cause not only cell toxicity but also cell morphology. Firstly, differentiating HT-22 and AR-5 cells are morphologically different from proliferating cells, as they have less cell densities and longer neurite outgrowth (Fig. 4A and B, Fig. 5A and B). The results also showed significant reductions in total length of cell neurite outgrowth of HT-22 cells and increment in AR-5 cells respectively in corticosterone treated groups (Fig. 4C and E; Fig. 5C and E). These results were reversed by SB-269970 on HT-22 cells and LP-44 on AR-5 cells (Fig. 4D and E; Fig 5D and E).

3.5 SB-269970 and LP-44 Regulate Rho Family mRNA Changes Induced by Corticosterone in HT-22 Cells and AR-5 Cells

The Rho family genes are known to have impact on cell morphology. To determine if SB-269970 reverse effect of corticosterone-induced HT-22 cells morphological changes were dependent on Rho family small GTPase, Cdc-42, RhoA and Rac-1 mRNA levels were measured in the presence or absence of 50μM corticosterone, and also in the presence of 2.5, 5, 10μM SB-269970. Cdc-42 and RhoA mRNA levels were increased about 1.5- and 3-fold respectively in HT-22 cells, following exposure to 50μM corticosterone for 24 hr ($p<0.01$ and $p<0.05$ versus vehicle treated groups). These increases in mRNA levels were prevented by treating the cells with SB-269970 2 hr prior to corticosterone exposure, and the reversing effects were significant at 10μM for

Cdc42 mRNA and 5, 10μM for RhoA mRNA (Fig. 6A and B). Neither corticosterone nor SB-269970 had impact on Rac-1 mRNA expression (Fig. 6C). Similarly, 50 μM corticosterone exposure markedly increase AR-5 cells Cdc-42 mRNA levels by 1.5-fold and RhoA mRNA levels by 1.4 Fold ($p<0.05$). And these effects were prevented by pretreatment of AR-5 cells with 0.05, 0.1, 0.2μM LP-44 2 hr prior to corticosterone, among which 0.2μM LP-44 had significant effect ($p<0.05$ and $p<0.01$) (Fig. 7A and B). Rac-1 mRNA levels did not change either with corticosterone or LP-44 treatment (Fig. 7C). These results show that SB-269970 and LP-44 can prevent HT-22 cells and AR-5 cells morphological changes induced by corticosterone through Cdc-42 and RhoA, but not Rac-1.

3.6 The Effect of SB-269970 and LP-44 on Synaptic Markers mRNA Expression in Corticosterone-Treated HT-22 and AR-5 Cells

Synaptic markers such as Synaptopodin, Gap-43 and Synaptophysin are usually mentioned in studies regarding neuroplasticity. In order to determine whether SB-269970's protective effect against corticosterone on HT-22 cells toxicity and morphological changes involves these markers, mRNA levels of the three genes were detected using quantitative real-time PCR. Synaptopodin mRNA levels were increased about-fold in HT-22 cells after treatment with 50μM corticosterone, and 10μM SB-269970 significantly prevented this effect ($p<0.05$, $p<0.05$) (Fig. 8A). However, 50μM corticosterone did not affect Gap-43 expression, while the use of SB-269970 still increases it's mRNA levels significantly at 5 and 10μM ($p<0.05$, $p<0.01$) (Fig. 8B). On the other hand, Synaptopodin and synaptophysin levels were decreased with 50μM corticosterone treatment on AR-5 cells ($p<0.05$), and LP-44 raised synaptopodin levels significantly at 0.2μM ($p<0.05$ compared with vehicle group) (Fig. 9A), while only having

an increasing trend on Synaptophysin (Fig. 9C). Similar to HT-22 cells, Gap-43 expression did not change in AR-5 cells with treatment of 50μM corticosterone and LP-44 lifted its levels in an increasing trend (Fig. 9B).

Table 3-1. Corticosterone induced 5-HT receptors mRNA expression in rat hippocampal and amygdaloid neruons by real-time PCR

Target	Genbank	Position	Oligonucleotide sequence 5'-3'	Hippocampus		Amygdala	
				RT-PCR fold change	*P* value	RT-PCR fold change	*P* value
5-HT1A	NM_012585	623-642 719-738	TGTTGCTCATGCTGGTTCTC CCGACGAAGTTCCTAAGCTG	1.92	<0.05	2.50	<0.05
5-HT1B	NM_022225	502-521 593-612	CTGGTGTGGGTCTTCTCCAT GTAGAGGACGTGGTCGTGT	1.06	>0.05	1.55	<0.05
5-HT2A	NM_017254	504-523 715-734	GCGATCTGGATTTACCTGGA CCCCTCCTTAAAGACCTTCG	2.62	<0.05	0.85	>0.05
5-HT2B	NM_017250	466-485 601-620	GGAGAAAAGGCTGCAGTACG ATAACCAGGCAGGACACAGG	1.14	>0.05	0.71	>0.05
5-HT4	NM_012853	780-799 972-991	GAGACCAAAGCAGCCAAGAC AGGAAGGCACGTCTGAAAGA	3.00	<0.05	2.82	<0.05
5-HT6	NM_024365	110-119 296-315	ATCAGTACCCTCCCCAAAC GACTGGGTTGAGGACCAAGA	1.25	>0.05	1.56	<0.05
5-HT7	NM_022938	786-805 925-944	GGGCTCAGAATGTGAACGAT TGTGTTTGGCTGCACTCTTC	1.92	<0.05	0.49	<0.05

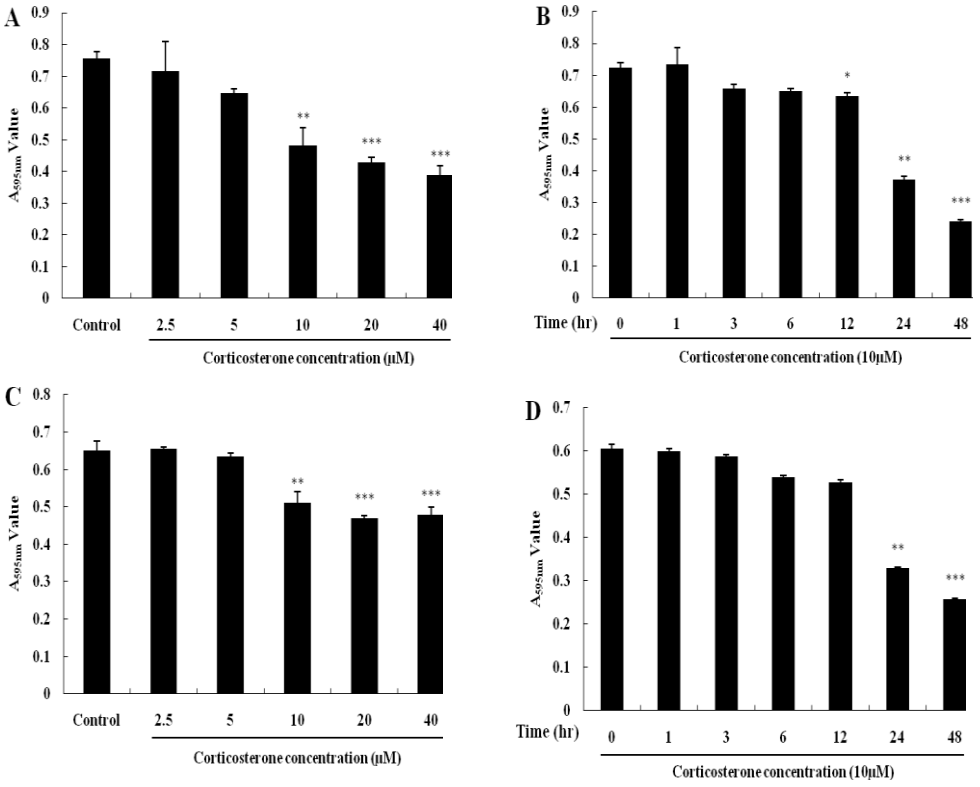

Figure 3-1. Corticosterone (CORT) impairs cell viability of primary hippocampal and amygdaloid cell cultures in a concentration- and time- dependent manner, measured by MTT assays. A) Primary hippocampal neurons were treated with various concentrations of CORT for 24 hr. B) Primary hippocampal neurons were treated with 10μM CORT for the indicated periods. C) Primary amygdaloid neurons were treated with various concentrations of CORT for 24 hr. D) Primary amygdaloid neurons were treated with 10μM CORT for the indicated periods. Results are expressed as mean ± SEM from at least three independent experiments (*p<0.05; **p<0.01; ***p<0.001 versus controls).

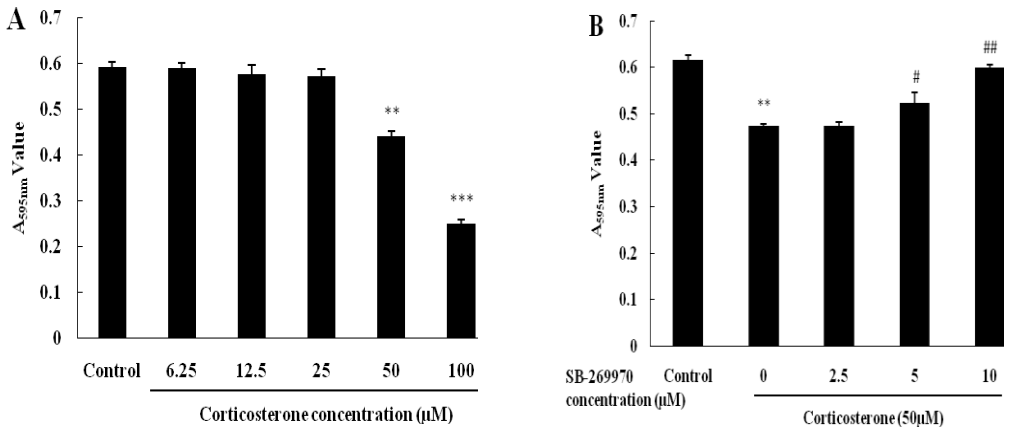

Figure 3-2. Corticosterone impairment of HT-22 cultures cell viability and the reverse effect of SB-269970 against it, measured by MTT assay. A) HT-22 cells were treated with indicated concentrations of CORT for 24 hr. B) SB-269970 protected HT-22 cells against CORT-induced decrease in cell viability dose-dependently. Results are expressed as mean ± SEM from at least three independent experiments (*, p<0.05; **, p<0.01; ***, p<0.001 as compared with controls by one-way ANOVA. #, p<0.05; ##, p<0.01; ###, p<0.001 as compared with corticosterone-treated group by one-way ANOVA).

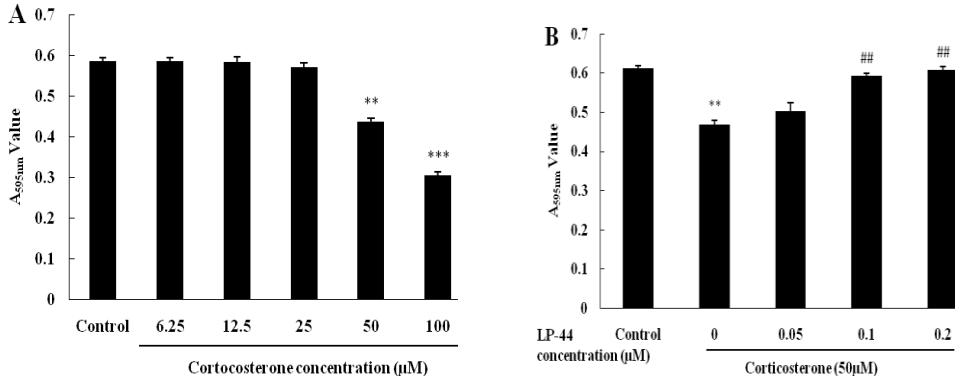

Figure 3-3. Corticosterone impairment of AR-5 cultures cell viability and the reverse effect of LP-44 against it, measured by MTT assay. A) AR-5 cells were treated with indicated concentrations of CORT for 24 hr. B) LP-44 protected HT-22 cells against CORT-induced decrease in cell viability dose-dependently. Results are expressed as mean ± SEM from at least three independent experiments (*, p<0.05; **, p<0.01; ***, p<0.001 as compared with controls by one-way ANOVA. #, p<0.05; ##, p<0.01; ###, p<0.001 as compared with corticosterone-treated group by one-way ANOVA).

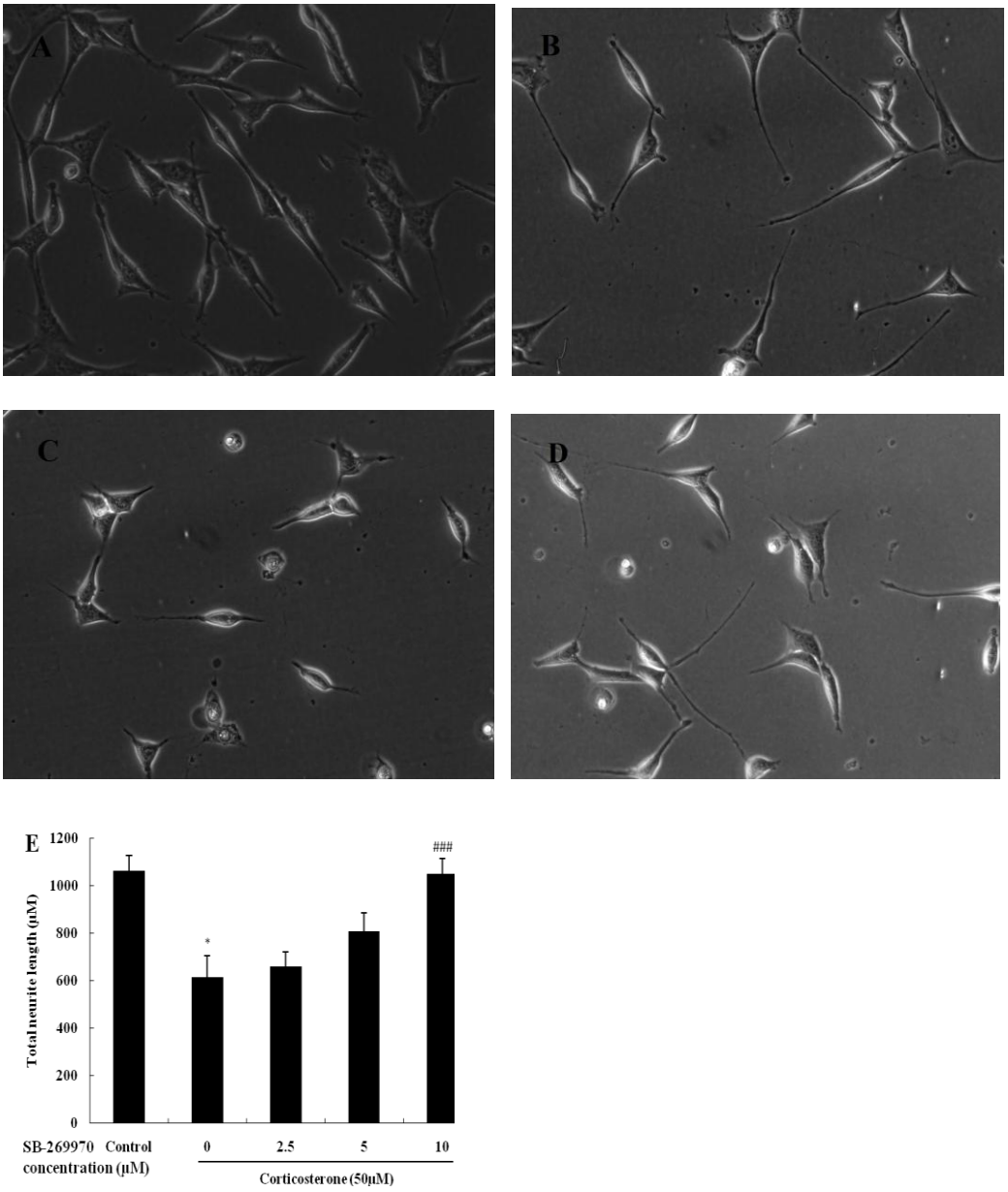

Figure 3-4. HT-22 cell morphological changes in proliferation, differentiation and corticosterone/SB-269970 treated conditions. A) Proliferating HT-22 cells at 3 day of cultrue. B) Differenitating HT-22 cells at 3 day of culture. C) HT-22 cells treated with 50μM corticosterone for 24 hr. D) HT-22 cells treated with 5μM SB-269970 prior to 50μM corticosterone. E) Quantification of HT-22 cells neurite outgrowth treated with corticosterone or SB-269970.

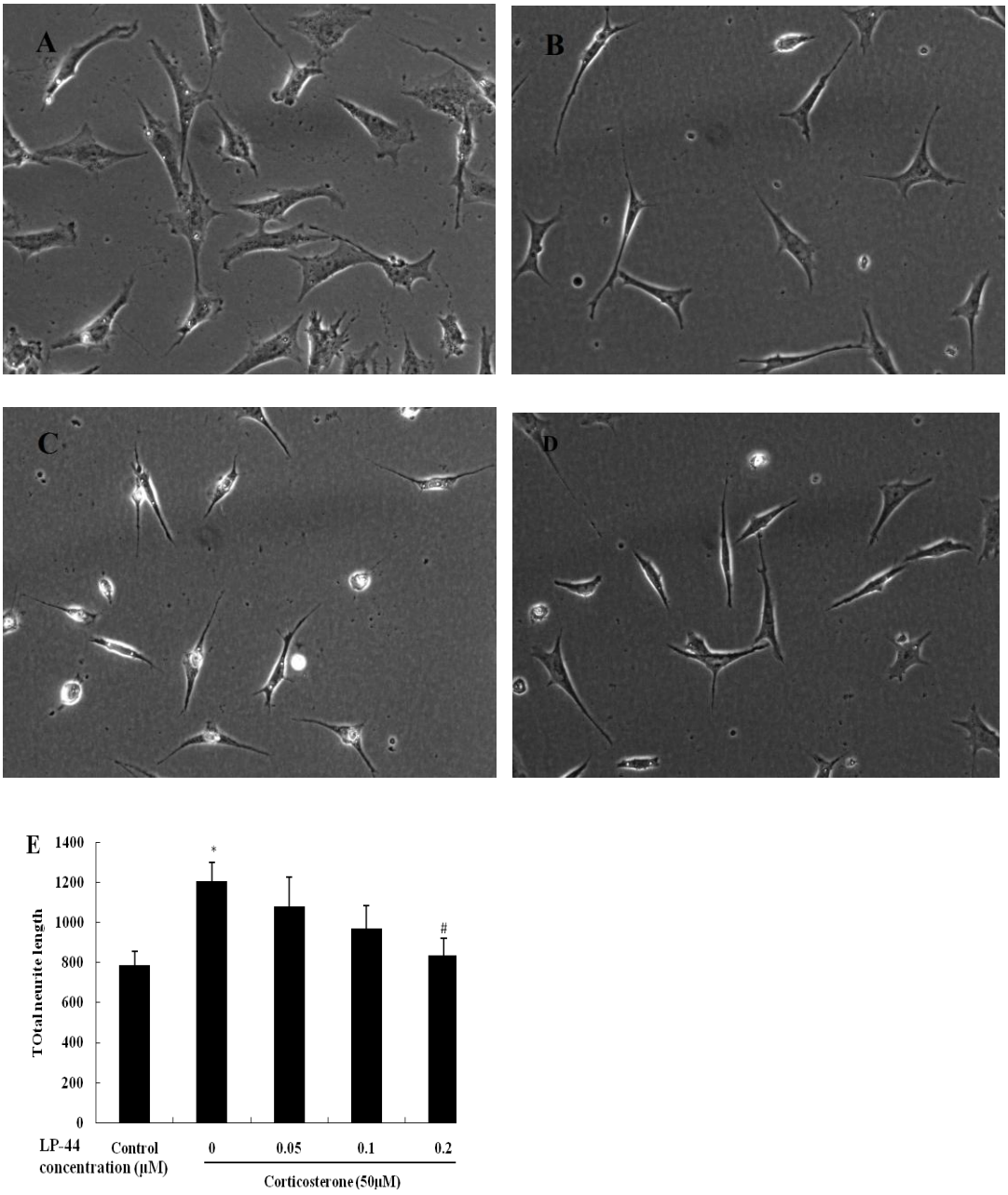

Figure 3-5. AR-5 cell morphological changes in proliferation, differentiation and corticosterone/LP-44 treated conditions. A) Proliferating AR-5 cells at 3 day of cultrue. B) Differenitating AR-5 cells at 3 day of culture. C) AR-5 cells treated with 50μM corticosterone for 24 hr. D) AR-5 cells treated with 0.2μM LP-44 prior to 50μM corticosterone. E) Quantification of AR-5 cells neurite outgrowth treated with corticosterone or LP-44.

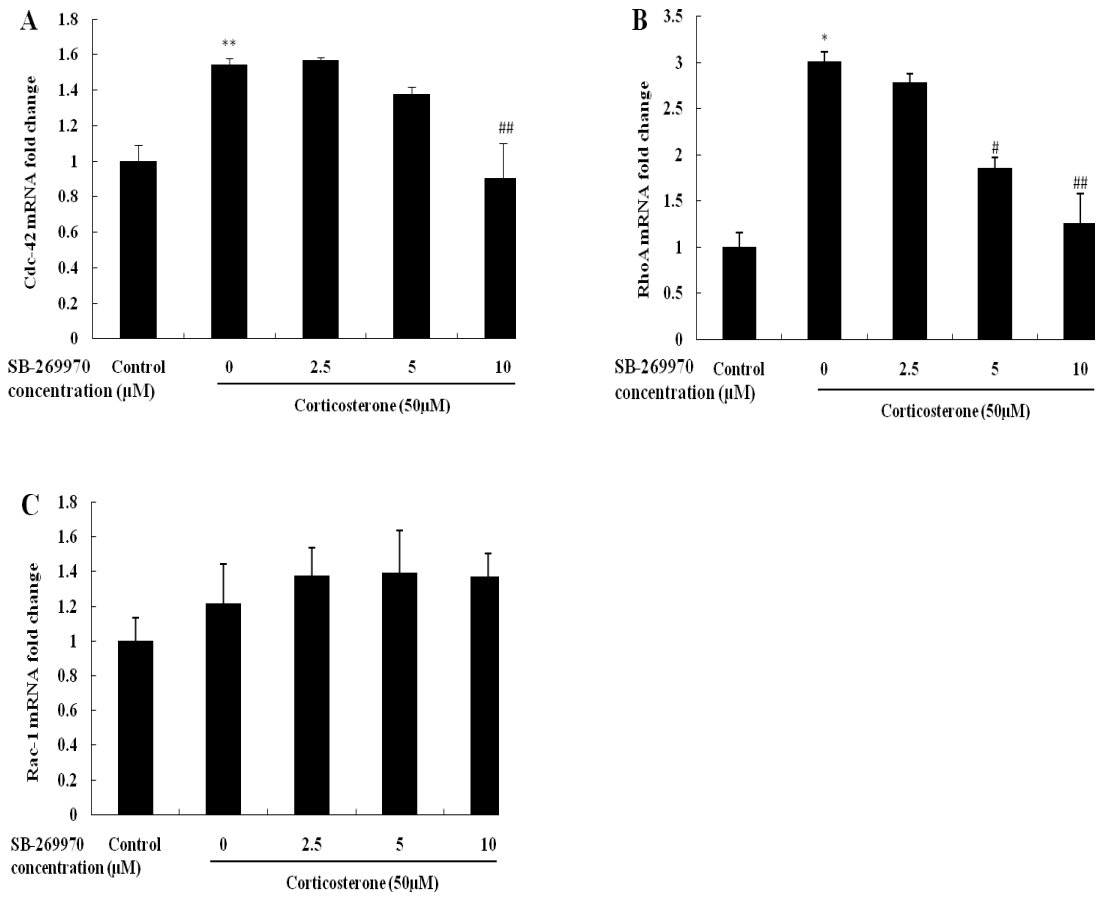

Figure 3-6. Effect of SB-269970 on Rho family small GTPase mRNA expression in corticosterone treated HT-22 cells, measured with quantitative real-time PCR. A) SB-269970 reverses corticosterone-induced Cdc-42 mRNA fold change in a dose-dependent manner. B) SB-269970 reverses corticosterone-induced RhoA mRNA fold change in a dose-dependent manner. C) Effects of corticosterone and SB-269970 on Rac-1 mRNA expression. . Results are expressed as mean ± SEM, n=6 (*, $p<0.05$; **, $p<0.01$ as compared with controls by one-way ANOVA. #, $p<0.05$; ##, $p<0.01$ as compared with the corticosterone-treated group by one-way ANOVA).

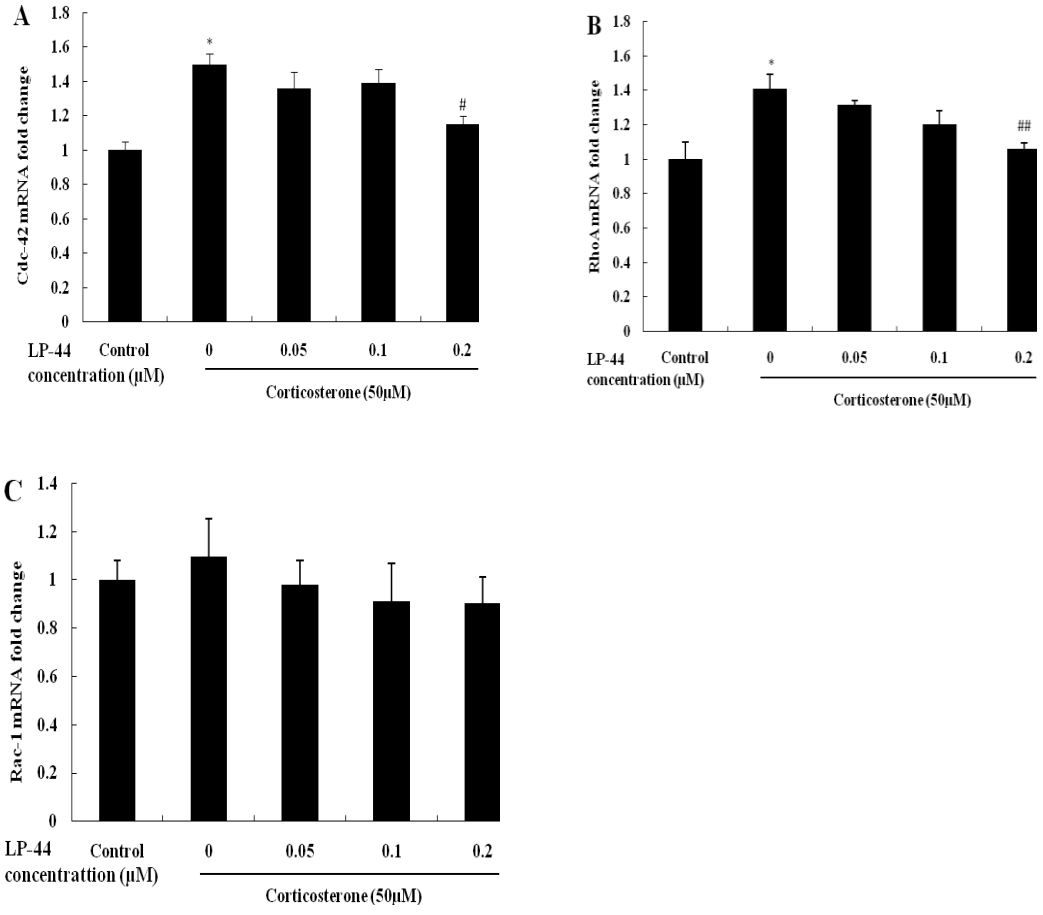

Figure 3-7. Effect of LP-44 on Rho family small GTPase mRNA expression in corticosterone treated AR-5 cells, measured with quantitative real-time PCR. A) LP-44 reverses corticosterone-induced Cdc-42 mRNA fold change in a dose-dependent manner. B) LP-44 reverses corticosterone-induced RhoA mRNA fold change in a dose-dependent manner. C) Effects of corticosterone and LP-44 on Rac-1 mRNA expression. Results are expressed as mean ± SEM, n=6 (*, $p<0.05$; **, $p<0.01$ as compared with controls by one-way ANOVA. #, $p<0.05$; ##, $p<0.01$ as compared with corticosterone-treated group by one-way ANOVA).

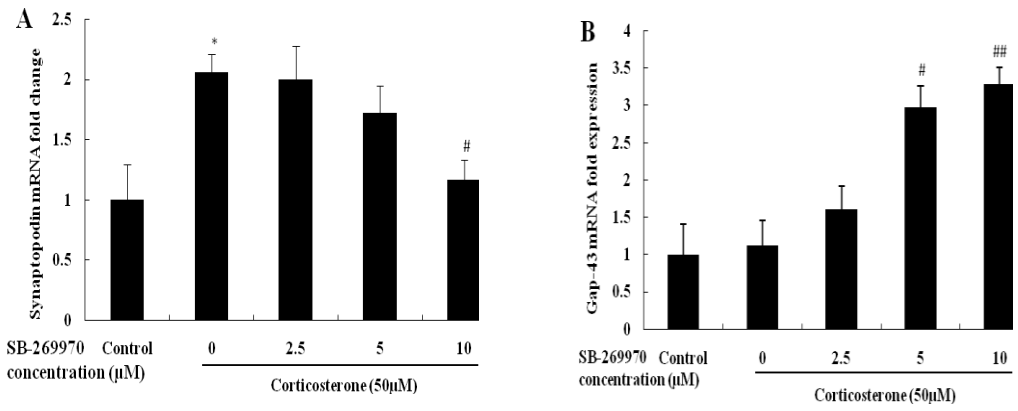

Figure 3-8. Effect of SB-269970 on synaptic protein mRNA expression in corticosterone treated HT-22 cells, measured with quantitative real-time PCR. A) SB-269970 reverses corticosterone-induced synaptopodin mRNA fold change in a dose-dependent manner. B) Effects of corticosterone and SB-269970 on Gap-43 mRNA expression. . Results are expressed as mean ± SEM, n=6 (*, $p<0.05$; **, $p<0.01$ as compared with controls by one-way ANOVA. #, $p<0.05$; ##, $p<0.01$ as compared with corticosterone-treated groupby one-way ANOVA).

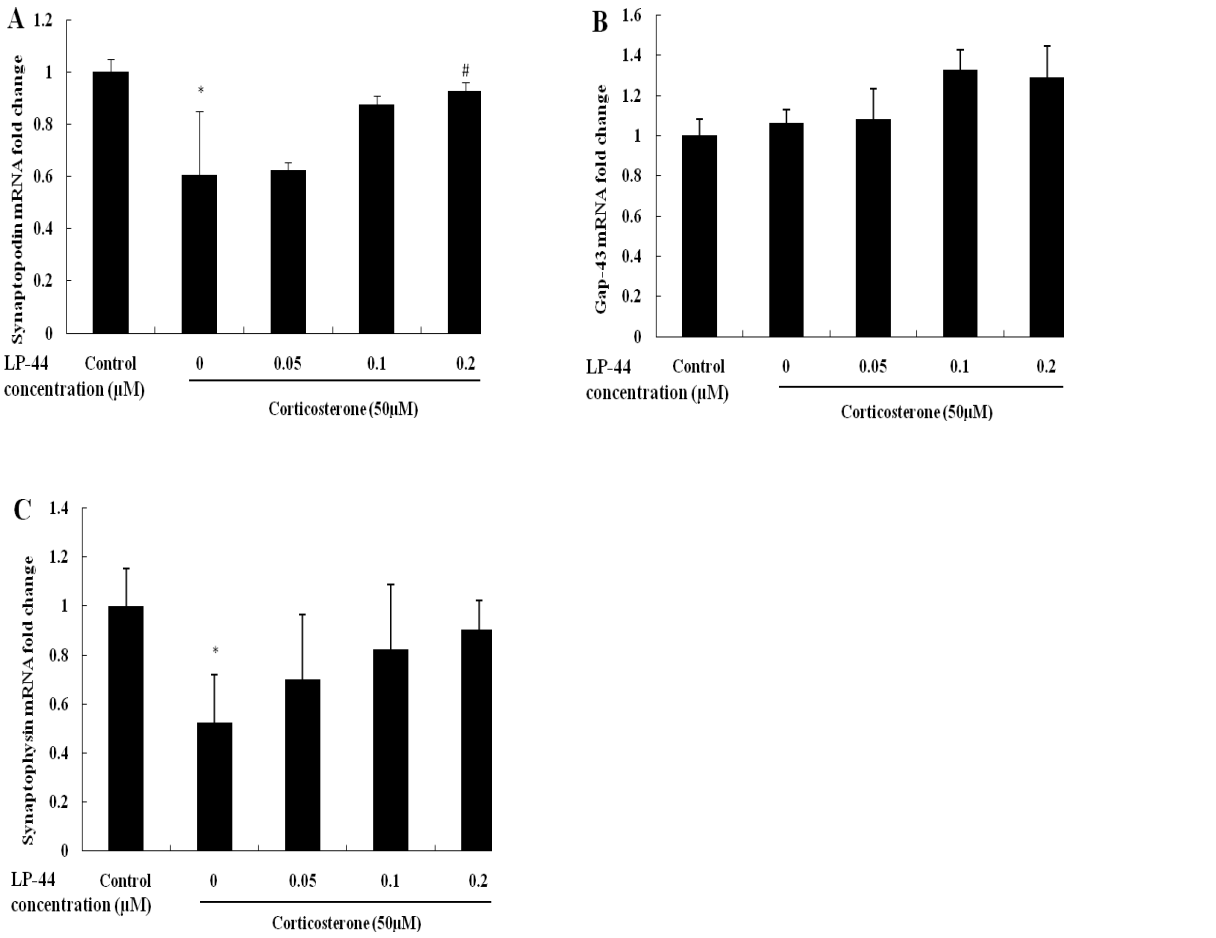

Figure 3-9. Effect of LP-44 on synaptic protein mRNA expression in corticosterone treated AR-5 cells, measured with quantitative real-time PCR. A) LP-44 reverses corticosterone-induced synaptopodin fold change in a dose-dependent manner. B) Effects of corticosterone and LP-44 on Gap-43 mRNA expression. C) Effects of corticosterone and LP-44 on synaptophysin mRNA expression. Results are expressed as mean ± SEM, n=6 (*, $p<0.05$; **, $p<0.01$ as compared with controls by one-way ANOVA. #, $p<0.05$; ##, $p<0.01$ as compared with corticosterone-treated group by one-way ANOVA).

CHAPTER 4
DISCUSSION

This study investigated two aspects of how stress hormone affects the hippocampus and amygdala. Firstly, we tested whether administration of corticosterone to hippocampal and amygdaloid cell lines induced different changes in 5-HT sub-receptors. Secondly, we tested whether stress induced morphological changes in these two cell lines are involved in the 5-HT sub-receptors expression. We now show, using HT-22 and AR-5 cell lines, that 5-HT_7 receptor mRNA is significantly up-regulated in HT-22 cells, but down-regulated in AR5 cells by exposure to stress level of corticosterone (50 μM) for 24h. Pretreatment of cells with 5-HT_7 antagonist SB-269970 and agonist LP-44 reversed corticosterone induced cell lesion in a dose-dependent manner in HT-22 and -AR5 cells, respectively. Moreover, corticosterone induced different changes of dendritic complexity in HT-22 and AR-5 cells were also reversed by pretreatment with SB-269970 and LP-44. This 5-HT_7 sub-receptor mRNA expression difference was confirmed by primary hippocampal and amygdaloid neuron cultures when they were exposed to corticosterone.

In the present study, we focused our interest on two distinct brain regions, the hippocampus and amygdala. The involvement of these brain regions in emotional, motivational, and mnemonic processes may be related to stress related depression and dementia (Butterweck, Bockers, Korte, Wittkowski, & Winterhoff, 2002). It is interesting that Kelly et al. (Kelly, Wrynn, & Leonard, 1997)suggested that impaired hippocampal function and/or dendritic atrophy may underlie memory deficits in stressed animals. In contrast, the pyramidal and stellate neurons of amygdala showed increased dendritic arborization (Vyas et al., 2002). This finding allows us to ask specific questions about

the impact of glucocorticoids on brain regions and the molecular basis on this pathway. In the central nervous system, immortalized cell lines from various brain regions which retain parental cell characteristics have been generated from neuron/glial precursors, astrocytes and microglia (Lendahl & McKay, 1990). HT-22 cells are immortalized mouse hippocampal neuronal precursor cells that were sub-cloned from their parent HT-4 cells (Liu, Li, & Suo, 2009). The retroviral-mediated transfer of the SV 40 large T antigen into rat embryonic amygdaloid cells has resulted in the production of an immortalized AR-5 cell line which is able to form stable monolayers in culture (Sheriff et al., 2001). Both HT-22 and AR-5 cell lines are valuable models for better understanding the cellular and molecular processes relevant to the hippocampus and amygdala, respectively.

Serotonin is an important neurotransmitter that exerts a wide influence over many brain functions through activating or inhibiting families and subtypes of its receptors. The interactions between HPA axis and the 5-HT system are of particular relevance when being subjected to stress, in which dysfunctioning actually concerns both of these two systems (Barden, 1999; Lesch et al., 1990). Among the 14 known 5-HT receptor subtypes, 5-HT_7 sub-receptor is one of the least well known. Recent attention has been received mainly due to its pivotal role in the pathogenesis of depression and the involvement in specific aspects of hippocampus-dependent contextual learning and memory processing (C. Roberts, Thomas, Bate, & Kew, 2004). In situ hybridization study reveals 5-HT_7 sub-receptor expression in cortex, hippocampus, amygdala and hypothalamus (M. Guscott, Bristow, Hadingham, Rosahl, Beer, Stanton, Bromidge, Owens, Huscroft, Myers, Rupniak, Patel, Whiting, Hutson, Fone, Biello, Kulagowski et al., 2005b). Animal study was shown that 5-HT_7 sub-receptor mRNA was up-regulated

in the hippocampus, but not cortex in rats after exposure to chronic stress (Y. C. Li et al., 2009). Recent evidences have shown a synergistic interaction between individually ineffective doses of the selective antagonist SB-269970 and antidepressants in forced swim test with an increase in 5-HT levels in the frontal cortex and hippocampus (Bonaventure et al., 2007; Wesolowska, Nikiforuk, & Stachowicz, 2006). In this study, in vitro HT-22 and AR-5 cell lines were tested to determine whether administration of corticosterone to them is related to changes in 5-HT sub-receptors. We chose the concentration of corticosterone in vitro (50 μM) that is equivalent to moderate to high stress levels in vivo (Sapolsky, Brooke, & Stein-Behrens, 1995). Analyses by real-time PCR in the present study revealed that 5-HT_7 sub-receptor was up-regulated in HT-22 cells, but it was down-regulated in AR-5 cells. Subsequent cell viability test showed that 5-HT_7 antagonist SB-269970 and agonist LP-44 reversed corticosterone induced cell lesion in HT-22 and AR-5 cells, respectively. This is the first time to get such an intriguing finding, which was also confirmed by hippocampal and amygdaloid neuron cultures in our study. These results imply that elevated corticosterone level may change some 5-HT receptor binding inculding 5-HT_7 sub-receptor, which affects the neural circuitry as an indirect mechanism (Raffa & Codd, 1996).

Dysfunction of neuronal plasticity is exhibited during chronic stress and in patients with depression and likely occurs when the brain fails to induce the appropriate adaptive response or remodeling phenomena (Reines et al., 2008). Hippocampus is one of the most intensely studied structures in the stress-inhibitory circuit, other limbic inputs, including amygdala have received less attention. Recent studies show that the hippocampus experienced synaptic loss and atrophy while the amygdala had increased

plasticity and synaptogenesis when rats subjected to chronic stress (Vyas et al., 2002). However, the sensitivities of hippocampal and amygdaloid neurons on stress hormone and 5-HT_7 sub-receptor have not been described. Neuronal soma size has been correlated with increased neural activity (Gorski, Zeiler, Tamowski, & Jones, 2003) and since dendritic trees act as the postsynaptic sites of excitatory input in the mammalian brain, structural alterations in them must affect network function (Alfarez et al., 2009). The present study, which quantified soma size and total dendritic length showed decreased dendritic complexity of hippocampal and amygdaloid cells after corticosterone exposure. Restoration of synaptic plasticity, reflected by increased neuron complexities in the face of corticosterone cytotoxicity, were found in the presence of SB-269970 and LP-44 in HT-22 and AR-5 cell lines, respectively. These results confirmed our hypothesis that regulation of synaptic function after glucocorticoid exposure was dependent on the amelioration of 5-HT neuronal function.

Molecular downstream mechanisms underlying such opposite effects of stress hormone/5-HT sub-receptor on neurite outgrowth are still poorly understood. Studies show that activation of the members of the Rho family of small GTPase (Rho, Rac-1 and Cdc-42) initiated pathway induces growth cone collapse and neurite retraction (Kranenburg et al., 1999; Kvachnina et al., 2005). Marked changes in morphology, motility and guidance of axons have been observed in response to activation of Rho family GTPases both in vitro and in vivo (Ruchhoeft, Ohnuma, McNeill, Holt, & Harris, 1999; Zipkin, Kindt, & Kenyon, 1997). We chose to focus our current study on Rho, Rac-1 and Cdc-42, which regulate cellular pathways involved in stress hormone and presynaptic regulation in hippocampal neurons (Owe-Larsson et al., 2005). The present

findings suggest that there were significant increases in the levels of RhoA and Cdc-42 when the HT-22 and AR-5 cells were exposed to corticosterone. These effects were prevented by SB-269970 in HT-22 cells and LP-44 in AR-5 cells, respectively. The results are agreement with the previous studies (Owe-Larsson et al., 2005) and demonstrate that the 5-HT_7 sub-receptor effectively communicates with Rho family when corticosterone exposure.

As plasticity-responsive elements, three synaptic markers including synaptophysin (presynaptic vesicle protein), Gap 43 (synaptic membrane protein) and synaptopodin (postsynaptic protein) are reported to be closely involved in neurotransmitter release and neuronal sprouting during stress (M. Nishi, Whitaker-Azmitia, & Azmitia, 1996; Reddy et al., 2005). However, they were shown to differentially respond to stress hormone and antidepressant treatment in our study. The present findings suggested that there were significant increase in Synaptopodin levels in HT-22 cells after corticosterone exposure, and decreased Synaptopodin and Synaptophysin levels in AR-5 cells, which were either reversed by SB-269970 and LP-44 respectively or having a reversing trend.

The most distinctive finding of the present study is the investigation that corticosterone-induced different expression of 5-HT_7 receptor in HT-22 and AR-5 cell lines which were generated from hippocampal and amygdaloid neurons. These data were confirmed by primary neuron cultures. Moreover, the distinct cell viability and morphological changes in HT-22 and AR-5 cells were also involved in activation and/or inhibition of 5-HT_7 receptor and the related genes expression in these cells after corticosterone exposure. Our results support the hypothesis that serotonin may

differentially modulate neuronal morphology in hippocampus and amygdala depending on the expression levels of the 5-HT sub-receptors during stress hormone attacks.

CHAPTER 5
LITERATURE REVIEW: THE ROLE OF 5-HT SYSTEM IN STRESS AND DEPRESSION

5.1 Introduction

In the 1930s, it has been established that any environmental changes, whether internal or external, that disturbs the maintenance on homeostasis can cause stress response, including psychological, neuronal, endocrine and immune system reactivity (Leonard, 2005). Chronic stress or long-term exposure to external stress hormone glucocorticosteroids induces the hyperactivity of the HPA axis (Hypothalamic-Pituitary-Adrenal axis), which produces an increase in plasma glucocorticoid level, and finally impairs the negative feedback mechanism, causing psychological disorders such as depression, anxiety and inhibition of learning and memory (Croes, Merz, & Netter, 1993; Henry, 1992). In normal physiological conditions, HPA axis and serotonergic system interact with each other to cross regulate body functions (Chaouloff, 1993). However, in stress conditions, serotonergic system exerts its self-regulatory functions. Pathological mechanisms of psychological disorders such as major depression that is caused by chronic stress has been implicated to be closely related to dysregulation of HPA axis, monoaminergic systems especially the 5-HT system (Barden, 1999; Porter, Gallagher, Watson, & Young, 2004).

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter in the central nervous system (CNS). Through activating or inhibiting families and subtypes of its receptors, 5-HT has been demonstrated to have multiple physiological functions, and dysregulation of serotonergic system can cause stress related diseases such as Alzheimer's Diseases (AD), anxiety, depression and cognitive disorders (Goddard et al., 2010; Ramanathan & Glatt, 2009). Animal models of chronic stress have revealed that,

the activation of HPA axis and elevated levels of adrenocortical hormone are the main characteristics of stress (Konakchieva, Mitev, Almeida, & Patchev, 1998).

Limbic system is a set of brain structures including the hippocampus, amygdala, anterior thalamic nuclei, and limbic cortex, which creates a closed loop by embracing part of the two brain hemispheres and support a variety of functions including emotion, behavior, long term memory, and olfaction. A great amount of serotonergic neurons can be found in this system. Chronic stress leads to long-term hyperactivity of HPA axis and elevated levels of glucocorticoids, causing impairment of brain regions in the limbic system, in conjunction with down-regulation of HPA negative feedback control. Abnormality of monoaminergic neurotransmitter secretion and continuous damage of neurons finally leads to cognitive dysfunction including gradual loss of the ability of learning and memory, which are the most important characteristics of affective disorders (e.g. anxiety, depression, schizophrenia) and neurodegenerative diseases (e.g. AD, Parkinson's Diseases) (Kaneda, 2009; Ribes, Colomina, Vicens, & Domingo, 2009; Uc et al., 2009).

5.2 The 5-HT System

5-HT is an important neurotransmitter in the mature central nervous system. The neurons of the raphe nuclei are the major source of 5-HT release in the brain, and it projects upon many other regions of the brain, exerting its regulatory function of physiology. 5-HT expression in the developing raphe nuclei neurons and the preferential generation of the nerve fiber projecting terminals during the formation of neuronal synapses, demonstrated that 5-HT plays a significant roles not only in the morphology and neural activity of embryonic neurons, but also in neurogenesis and neuroplasticity after maturation, including proliferation, translocation, differentiation and synapse built

(Benninghoff et al., 2010; Veenstra-VanderWeele, Anderson, & Cook, 2000). It's been proved that 5-HT is related to the development of cerebral cortex in mammals. During the early stages of sensory cortex development, temporary serotonergic fibers projections have been detected, indicating that 5-HT might be helpful in conjugation and integration of the developing cortex (Nayyar et al., 2009). Brain serotonin synthesis, packaging, transportation, release, as well as its action at targeting ligands, reuptake and degradation all affect the concentration of 5-HT and its function. Proteins and related genes that are involved in regulating these physiological functions include speed-limiting enzyme TPH-1 and TPH-2 (Illi et al., 2009), Vmat2 (Fukui et al., 2007; Zucker, Weizman, & Rehavi, 2005), serotonin transporter (5-HTT), monoamine oxidase A (MAO-A) and 5-HT pre and post synaptic receptors such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3}, 5-HT_{4}, 5-HT_{5}, 5-HT_{6}, 5-HT_{7} (Lesch & Gutknecht, 2005; Paaver et al., 2007).

Firstly, 5-HT precursor L-tryptophan passes through the blood-brain barrier and reaches the serotonergic neurons. Catalyzed by TPH, 5-HTP is generated from L-tryptophan, and after decarboxylation catalyzed by 5-HTPDC it turns into 5-HT. 5-HT is imported and stored at the vesicles from nerve terminals, and then released at presynaptic membrane via Vmat2 and 5-HTT, binds to different subtypes of receptors located on pre and post synaptic membranes and exerts its physiological function. 5-HT level in synaptic space is regulated by SERT mediated reuptake, and 5-HT in cell cytosol is metabolized to 5-HIAA by MAO-A (Holmes, 2008).

According to research regarding the molecular and functional properties of the 5-HT receptors, each of them are now assigned to one of seven receptor families, 5-HT1–7, comprising a total of 14 structurally and pharmacologically distinct mammalian 5-HT

receptor subtypes. With the exception of the 5-HT_3 receptor, a ligand-gated ion channel, all other serotonin receptors (5-HT_{1A-E}, 5-HT_{2A-C}, 5-HT_4, 5-HT_5, 5-HT_6, 5-HT_7) are G protein-coupled receptors that activate an intracellular second messenger cascade to produce an excitatory or inhibitory response. Activation of the specific G-protein can affect enzymes (such as adenylate cyclase, phospholipase A and C, mitogen-activated protein kinase and so on) and the function of cation channels especially K^+ and Ca^{2+} (Kushwaha & Albert, 2005). Recently, literatures has been reporting that in the intact brain the function of many 5-HT receptors can now be unequivocally associated with specific physiological responses, ranging from modulation of neuronal activity and transmitter release to behavioral change, especially psychological disorders like depression, anxiety, obsessive-compulsive disorder, panic disorder and migraine (add references here). Among the receptor subtypes, 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/2C}$, 5-HT_4, 5-HT_6, 5-HT_7 are considered related to chronic stress induced neural diseases, inhibition of learning and memory, and cognitive disorders (King, Marsden, & Fone, 2008; Meneses, 2007; Perez-Garcia, Gonzalez-Espinosa, & Meneses, 2006). Studies have shown that chronic unpredictable mild stress (CUMS) remarkably reduced 5-HT concentrations with over-expression of 5-HT_{1A} receptor in the hippocampus, cortex and hypothalamus, of 5-HT_{1B} receptors in the hypothalamus and of 5-HT_7 receptor in the hippocampus and hypothalamus in rats (Y. C. Li et al., 2009). Also, it was found that activation of presynaptic receptors enhanced stimulus-induced long-term depression (Bailey et al., 2008), and antidepressant medications could attribute the ability to change postsynaptic 5-HT_{1A} receptors and cAMP production (Hines, Tabakoff, &

WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators, 2005).

5.3 5-HT$_1$ Receptors

The 5-HT$_1$ subfamily consists of five G protein-coupled receptors (GPCRs) that are coupled to Gi/Go and mediate inhibitory neurotransmission, including 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, and 5-HT$_{1F}$. There is no 5-HT$_{1C}$ receptor, as it was reclassified as the 5-HT$_{2C}$ receptor.

5.3.1 5-HT$_{1A}$ Receptor

5-HT$_{1A}$ is the first successfully cloned subtype of 5-HT receptors, and knowledge of the pharmacology and function of the receptor have been quickly progressed. The 5-HT$_{1A}$ receptor is widely distributed in the central nervous system, existing in the cerebral cortex, hippocampus, septum, amygdala, and raphe nucelus in high densities, while low amounts also exist in the basal ganglia and thalamus, mediating the release of 5-HT (Cryan & Leonard, 2000).

Stimulation of 5-HT$_{1A}$ autoreceptors inhibits the activation of nucleus raphes dorsalis neurons and blocks the release of serotonin in nerve terminals. The self-regulatory function of 5-HT system is inhibited under chronic stress. Lanfumey and his colleagues have found that exogenous corticosterone exposure or chronic mild stress can desensitize the 5-HT$_{1A}$ autoreceptor and activate the postsynaptic 5-HT$_{1A}$ receptor, which further elevates serotonin levels. Postsynaptic 5-HT$_{1A}$ receptor regulates the release of 5-HT as well as other neurotransmitters such as acetyl choline, glutamate and γ-aminobutyric acid (Laaris, Le Poul, Laporte, Hamon, & Lanfumey, 1999; Lanfumey et al., 1999; Le Poul, Laaris, Hamon, & Lanfumey, 1997). Activation of 5-HT$_{1A}$ receptors has been demonstrated to impair cognition, learning, and memory by

inhibiting the release of glutamate and acetylcholine in various areas of the brain (Bhagwagar, Rabiner, Sargent, Grasby, & Cowen, 2004).

Studies show that long-term chronic stress or high levels of exogenous corticosterone can impair 5-HT_{1A} feedback system, induce helplessness behavior in animal models and psychological disorders in conjunction with deteriorated learning and memory in human and rodents (Martin, 1991). Current research has discovered that in chronic stress, 5-HT_{1A} receptor expression varies in different brain regions (Y. C. Li et al., 2009). In clinical reports, depression patients tend to have decreased postsynaptic 5-HT_{1A} receptors and increased presynaptic inhibitive autoreceptors (Zhou et al., 2008). In animal studies, post-synaptic 5-HT1A receptor antagonist manifests important antidepressant roles.

5.3.2 5-HT_{1B} Receptor

5-HT_{1B} receptors are expressed throughout the rodent central nervous system. These receptors are located in the axon terminals of both serotonergic and nonserotonergic neurons in basal ganglia, striatum and the frontal cortex, where they act as inhibitory autoreceptors or heteroreceptors. 5-HT_{1B} receptors inhibit the release of a range of neurotransmitters, including serotonin, GABA, acetylcholine, and glutamate (Moret & Briley, 1997; Moret & Briley, 2000; Morikawa, Manzoni, Crabbe, & Williams, 2000). Knockout mice lacking the 5-HT_{1B} gene has shown an increase of aggression and a higher preference for alcohol (Clark et al., 2002; Clark, Vincow, Sexton, & Neumaier, 2004; Kaiyala, Vincow, Sexton, & Neumaier, 2003). Selective serotonin reuptake inhibitors can reverse these outcomes (de Boer & Koolhaas, 2005). In open-field test and elevated plus maze test, behavior of the rats in chronic stress group is always accompanied by 5-HT_{1B} overexpression (Lin & Parsons, 2002), in which

receptor agonist PC-94,253 can reverse the effect. However in forced swimming test and shuttle box test, the same group of rats has low level expression of 5-HT_{1B} receptors (Bolanos-Jimenez et al., 1995; Lin & Parsons, 2002).

5.4 5-HT_2 Receptors

The 5-HT_2 receptors are a subfamily of 5-HT receptors that bind the endogenous serotonin. The 5-HT_2 subfamily consists of three GPCRs which are coupled to G_q/G_{11} and mediate excitatory neurotransmission. The 5-HT_2 receptor family currently accommodates three receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which are similar in terms of their molecular structure, pharmacology and signal transduction pathways.

5.4.1 5-HT_{2A} Receptor

5-HT_{2A} is expressed widely throughout the central nervous system (CNS). It is expressed near most of the areas rich of serotoninergic terminals, including neocortex (mainly prefrontal, parietal, and somatosensory cortex) and the olfactory bulb. High densities of this receptor on the apical dendrites of pyramidal cells in layer V of the cortex may modulate cognitive processes (Miner, Backstrom, Sanders-Bush, & Sesack, 2003; T. Xu & Pandey, 2000). Studies have shown that 5-HT_{2A} receptor is sensitive to glucocoticoids. Chronic stress, isolated raising and long-term exposure to exogenous corticosterone cause hyperactivities of HPA axis and desensitization of 5-HT_{2A} receptor, indicating that this receptor has important modulatory function in central nervous system diseases (Lee, Redila, Hill, & Gorzalka, 2009). In patients with depression and AD, which is usually accompanied by impaired learning and memory, 5-HT_{2A} receptor mRNA level in cortex is lower than normal condition (Lai et al., 2005; Lanctot, Herrmann, & Rothenburg, 2008). More and more evidence have revealed that the loss

of 5-HT_{2A} receptor function is not only critical in schizophrenia, but also depression and anxiety (Dawson & Watson, 2009; Weisstaub et al., 2006).

5.4.2 5-HT_{2C} Receptor

5-HT_{2C} receptors are distributed in basal ganglia regions such as striatum, substantia nigra pars reticulata (SNr), and subthalamic nucleus (Q. Li et al., 2003; Lopez-Gimenez, Tecott, Palacios, Mengod, & Vilaro, 2002). 5-HT_{2C} receptor angonitst increases ACTH and corticosterone/cortisol levels in animal models and human (Klaassen, Riedel, van Praag, Menheere, & Griez, 2002). 5-HT_{2C} knockout mice do not show depressive behavior in tale suspension test and forced swimming test. Moreover, in tale suspension test, 5-HT_{2C}-R gene knockout mice and those treated with 5-HT_{2C} antagonist SB242084 or RS102221 both exhibited increased antidepressive effects of SSRI and increased the 5-HT level in cortex and hippocampus (Cremers et al., 2004; Cremers et al., 2007). However, on the other hand, 5-HT_{2C} receptor agonist WAY 163909, RO 600175 also have some antidpressive effects (Cryan & Lucki, 2000; Rosenzweig-Lipson et al., 2007). Schmauss and his colleagues found that up-regulated 5-HT_{2C} receptor expression in chronic stress can be reversed by SSRI, indicating that psychological disorders caused by chronic stress is related to 5-HT_{2C} receptor function change (Schmauss, 2003).

5.5 5-HT_4 Receptors

The 5-HT_4 receptor was initially identified in cultured mouse colliculi neurones and guinea pig brain by Bockaert and co-workers using a functional assay stimulation of adenylate cyclase activity, which exerts domaminergic function and regulating glutamatic and cholinergic neurotransmitter release (Matsumoto et al., 2001). The fact that 5-HT_4 receptor is mostly distributed in the limbic system implies it relation with

learning and memory, emotion and stress. Studies show that patients with schizophrenia, attention-deficit hyperactivity disorder or neurodegenerative diseases such as AD, have down-regulated levels of 5-HT_4 receptor expression, as was shown previously in rat models with learning and memory disabilities (Reynolds et al., 1995; Wong, Reynolds, Bonhaus, Hsu, & Eglen, 1996). In numerous depression models such as forced swimming test, olfactory bulb removal model and chronic bandaged-stress model, 5-HT_4 receptor agonist shows difference levels of antidepressant effect (G. Lucas et al., 2007). 5-HT_4 receptor knockout mice and antagonist treated mice show anxiety, along with ameliorated abnormal behavior caused by chronic stress (Compan et al., 2004; Conductier et al., 2006; Smriga & Torii, 2003). On the other hand, 5-HT_4 receptor agonist increases neuronal activities in hippocampus and cortex, remodels neuroplasticity such as densities of spines and length of dendrites (Restivo et al., 2008), which is possibly the critical mechanism in improving cognition and treating CNS diseases.

5.6 5-HT_6 Receptors

5-HT_6 receptor appears in CNS of rodents, mainly on corpus striatum, olfactory bulb, limbic and forebrain regions including hippocampus and cortex. It is usually involved in glutamatergic and cholinergic neuronal activity. It is a potential target for drugs treating cognitive diseases, schizophrenia, anxiety and obesity. This receptor binds to G_s protein, and couples to the stimulation of adenylate cyclase. 5-HT_6 receptor antagonist (such as SB-399885) improves learning and memory, ameliorate depression and anxiety as well as the behavior disorder and microbiological changes caused by chronic stress (Mitchell & Neumaier, 2005; Svenningsson et al., 2007; Wesolowska & Nikiforuk, 2008; Wesolowska, 2008). On the other hand, receptor agonist (such as

WAY-208466, WAY-181187) can reduce 5-HT and dopamine release in cortex and corpus striatum, inhibit glutamate release in hippocampus induced by potassium, and finally lead to cognitive disorders (Burnham et al., 2010).

5.7 5-HT$_7$ Receptors

The 5-HT$_7$ receptor was first identified from brain cDNA libraries screened to identify novel sequences showing homology to known 5-HT receptors. In situ hybridization, immunohistochemistry and autoradiographical studies have demonstrated the presence of 5-HT$_7$ receptors throughout the CNS, mainly in the hypothalamus, thalamus, hippocampus, amygdala and cortex, in both terminal fields and serotonergic nuclei (J. J. Lucas & Hen, 1995).

Several studies have indicated a possible involvement of the 5-HT$_7$ receptor in mood, emotion, and other neuropsychiatric disorders. In recent studies, 5-HT$_7$ receptor mRNA level was showed upregulated in the hippocampus and hypothalamus, but not in the cortex in rats after exposure to chronic unpredictable stress (Y. C. Li et al., 2009), while strong evidence have been supporting the involvement of 5-HT7 receptor in hippocampus- and cortex-dependent contextual learning and memory processing (Eriksson, Golkar, Ekstrom, Svenningsson, & Ogren, 2008; Gasbarri, Cifariello, Pompili, & Meneses, 2008; A. J. Roberts et al., 2004; Sarkisyan & Hedlund, 2009). Data collected from the use of 5-HT$_7$ receptor antagonists SB269970 or SB656104 have showed that this receptor is involved in 5-HT mediated hypothermia and sleep patterns which are normally seen altered in depressed patients (M. R. Guscott et al., 2003; Hagan et al., 2000). In both of the forced swim test and tail suspension test, pharmacological blockade of the 5-HT$_7$ receptor or inactivation of the receptor gene leads to an antidepressant-like behavioral profile (Bonaventure et al., 2007; M. Guscott,

Bristow, Hadingham, Rosahl, Beer, Stanton, Bromidge, Owens, Huscroft, Myers, Rupniak, Patel, Whiting, Hutson, Fone, Biello, Kulagowski et al., 2005a; Hedlund, Huitron-Resendiz, Henriksen, & Sutcliffe, 2005b; Wesolowska, Nikiforuk, Stachowicz, & Tatarczynska, 2006). A drug originally believed to be selective for the 5-HT_{1A} receptor, 8-OH-DPAT, was shown to act on the 5-HT_7 receptor when inducing phase resetting within the suprachiasmatic nucleus (SCN) of the hypothalamus (Ehlen, Grossman, & Glass, 2001; Horikawa et al., 2000; Sprouse, Reynolds, Li, Braselton, & Schmidt, 2004).

Neuroimaging studies have shown that structural changes such as volumetric reductions in the frontal cortex, amygdale, caudate and putamen, along with concomitant increase in volume of the lateral ventricles are observed in depressed patients (Sheline, 2003). Since disruptions to the sleep cycle is a form of chronic stress which has been shown to suppress hippocampal neurogenesis (Hairston et al., 2005), and decreases in REM sleep have been produced in mice using 5-HT_7 receptor antagonist (Hedlund, Huitron-Resendiz, Henriksen, & Sutcliffe, 2005a), the relationship between 5-HT_7 receptor and neurogenesis has been brought into discussion (Kvachnina et al., 2005). The existing conflicting results about 5-HT_7 receptor, such as an increasing mRNA expression of this receptor as well as glucocorticoid levels in restraint stress paradigm (Laplante, Diorio, & Meaney, 2002), and opposite effect in a chemical adrenalectomy study (Yau, Noble, Widdowson, & Seckl, 1997), have revealed our preliminary understanding of the complexity of this system and yet more in-depth research into this area.

5.8 5-HT Receptor Agonist and Antagonist

Many of the effective or specific 5-HT receptor ligands have either been discovered or synthesized, mediating 5-HT receptor subtypes and the whole 5-HT

system. 5-HT neurons show distinct function under receptor agonist or antagonist, many of which have been widely utilized in preclinical and clinical studies, as listed in Table 5-1.

5.9 Signal Transduction in 5-HT Receptor System

5-HT as a neurotransmitter exerts its function through relative subtypes of receptors and diverse signaling pathways, which commonly involves second messengers and other receptors. With the exception of the 5-HT_3 receptor, a ligand-gated ion channel, all other serotonin receptors are G protein-coupled receptors that activate an intracellular second messenger cascade to produce an excitatory or inhibitory response. G proteins, short for guanine nucleotide-binding proteins, are a family of proteins involved in second messenger cascades. It carries information from the cell surface to its target receptors, including adenylate cyclase (AC), phospholipase C and ion channels. 5-HT increases the phosphorylation in DARPP-32, Thr34 (PKA) and Ser137 (CK-1), and decreases the phosphorylation in Thr75 (Cdk5). DARPP-32 is a dual functional protein, which works as an inhibitor of phosphatase (such as PP-1), as well as an inhibitor of protein kinase (such as PKA); that is, DARPP-32 exerts its bidirectional regulatory function in both phosphorylation and dephosphorylation, through self-phosphorylation on different spots (A. Nishi, Snyder, & Greengard, 1997).

The 5-HT_1 receptor subfamily is coupled to inhibitory pathways. Through the biding to G_i protein it inhibits adenylyl cyclase and rapidly decreases cAMP level. They are also coupled to stimulate phospholipase C and mitogen-activated protein kinase (MAPK) growth signaling pathway (Noda, Higashida, Aoki, & Wada, 2004). 5-HT_2 receptors increase the activity of casein kinase through activating G_q, PLC, followed by PKA, and phosphorylating at Ser137. Studies show that Chronic stress impede the

cAMP-CREB signal transduction, inhibit CREB phosphorylation and decreases brain-derived neurotrophic factor (BDNF) expression (Nair et al., 2007; Y. Xu et al., 2006). In contrast, the 5-HT receptors that are positively coupled to adenylyl cyclase are a heterogenous group, including the 5-HT_4, 5-HT_6, and 5-HT_7 receptor subtypes. 5-HT_4, 5-HT_6, and 5-HT_7 receptors mainly activates G_s and AC to boost cAMP dependent protein kinase A, phosphorylation at Thr34 and CREB, and increases BDNF expression and neurogenesis, which finally ameliorate the inhibition of learning and memory caused by chronic stress.

In summary, chronic stress induces the hyperactivity of the HPA axis, elevates the adrenal hormone levels in body, which further increases the glucocorticoid level and impairs the 5-HT system function. High levels of glucocorticoids cause atrophy and death in hippocampal neurons, which leads to inhibition in learning and memory in patients with neuropsychological diseases. 5-HT plays a critical role in regulating CNS diseases because of the large number of receptors locating in the limbic system. We assume that relative agonists or antagonists of different 5-HT receptors exert it protective or destructive function through various pathways. Research into the relationship between different subtypes of 5-HT receptors and stress provides theoretical backgrounds in drug development, clinical diagnosis and assessment of clinical efficacy in the area of central nervous system disorders.

5.10 Future Directions

Current antidepressant drugs are mostly focusing on inhibiting the plasma membrane transporters for serotonin and/or noradrenaline, such as SSRIs, SNRIs, NRIs as well as MAOIs. However, one of the major drawbacks of these medications is that it takes at least several weeks for their antidepressant effects to become manifest.

Moreover, only about half of the depression patients show full remission to these mechanisms and some of these medications put potential risks upon liver metabolism. Thus studies focusing on other mechanisms such as CFR antagonist and 5-HT receptors highly selective ligands provide new ideas for future antidepressant development.

Table 5-1. 5-HT subreceptors agonist and antagonist

5-HT receptor	Agonist	Antagonist
5-HT_{1A}	8-OH-DAPT	WAY-100135
5-HT_{1B}	Ergotamine	Risperidone
5-HT_{1D}	5-(Nonyloxy)tryptamine	Yohimbine
5-HT_{1E}	BRL-54443	Methiothepin
5-HT_{1F}	LY-344	Methiothepin
5-HT_{2A}	Ketanserin	APD-125
5-HT_{2B}	α-Methyl-5-HT	Yohimbine
5-HT_{2C}	α-Methyl-5-HT	Fluoxetine
5-HT_{3}	2-methyl-5-HT	Alosetron
5-HT_{4}	Cisapride	GR-113
5-HT_{5A}	LSD	SB-699
5-HT_{6}	EMD-386088	SB-399885
5-HT_{7}	5-CT/LP-44	SB-269970

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BIOGRAPHICAL SKETCH

Chong Zhang was born in 1985 in a physicians family, in Ningbo, China. At an early age, she was introduced to in the impact of current medical technology, with which she gradually developed her interest in biomedical research later in her academic study. She grew up in Ningbo and graduated from Xiaoshi High School in 2001and earned the Bachelor of Engineering degree later in Biopharmaceutical Engineering from Zhejiang University of Technology in 2004. After that she continued with her master's study in University of Florida Biomedical Engineering department, with a specialization in the field of neuropharmacology under the mentorship of Dr. William Ogle. She earned the Degree of Master of Science from University of Florida in the summer of 2010. Chong intends to pursue further study in neuropsychopharmacology and contributes fundamentals for the current pharmaceutical industry.