AN INVESTIGATION OF THE EFFECT OF CHRONIC UNPREDICTABLE STRESS ON HIPPOCAMPAL INTEGRITY AND SPATIAL LEARNING

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A Dissertation

Presented to

The Faculty of the Department

of Psychology,

University of Houston

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In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

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By

Darby F. Hawley, MA

May, 2012

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AN INVESTIGATION OF THE EFFECT OF CHRONIC UNPREDICTABLE STRESS ON HIPPOCAMPAL INTEGRITY AND SPATIAL LEARNING

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ABSTRACT

Chronic stress has been shown to induce neuroplastic changes in the hippocampus, decrease the survival of progenitor cells in the hippocampus, and impair hippocampal-dependent learning and memory. Recent evidence suggests that the hippocampus has two functionally distinct subregions. The dorsal portion appears to be primarily associated with spatial navigation, while the ventral region has been linked to anxiety-related functions. Regionally-specific neuroplastic changes in the dorsal and ventral subregions of the hippocampus suggest that the hippocampus may play a dual role in the stress response. We previously demonstrated that following chronic unpredictable stress (CUS), markers of neuroplasticity were preserved specifically in the dorsal dentate gyrus (DG) of the hippocampus. Considering that hippocampal integrity is imperative for learning and memory, we hypothesized that the increased markers of neuroplasticity observed in the dorsal DG of CUS-exposed animals would enhance performance on tests of spatial navigation ability. In the present investigation, spatial navigation on the radial arm water maze (RAWM) was assessed in rats following exposure to CUS, as well as neurogenesis, neuroprotective proteins, and synaptic plasticity in the dorsal and ventral DG of the hippocampus. Despite similarly elevated levels of corticosterone, stressed animals found the hidden platform faster and with fewer errors on the RAWM long-term memory trial compared to control animals. Furthermore, elevated coriticosterone in control and stressed animals exposed to the RAWM had decreased cell proliferation (CldU) and neurogenesis (DCX) in the ventral DG. Stressed animals also had decreased cell survival (IdU) in the ventral DG. Proteins proBDNF and PSD-95, which promote LTP and synaptic plasticity, were increased in the dorsal DG. Stressed animals had

increased neuroprotective proteins and preserved neuroplasticity in the dorsal DG, which may have contributed to the improved spatial navigation abilities on the RAWM. These regionally-specific neuroplastic changes suggest that the hippocampus does, in fact, play a dual role in response to chronic stress, and chronic stress does not impair spatial learning and memory.

ACKNOWLEDGMENTS

This research project was supported and guided under the direction of Dr J.L. Leasure and Dr. M. Hiscock from the Developmental Cognitive Neuroscience division of the Psychology Department and Dr. K. Alkadhi and Dr. J. Eriksen from the Department of Pharmacological and Pharmaceutical Sciences at the University of Houston. Gratitude is extended to all committee members for their support and guidance.

Additionally I would also like to thank Jennifer Parra, Jessica Holland, Aisha Shamsi, and Ana Alvarez from the Leasure Behavioral Neuroscience Laboratory for their assistance in the behavioral portions of the study as well as mounting tissue for stereology.

Gratitude is also extended to Munder Zagaar from Dr. Alkadhi's laboratory for taking the time to teach me the Radial Arm Water Maze and allowing me to observe his research with the RAWM.

Lastly, I would like to thank Dr. Brian Christie and members of his laboratory, Kristin Morch and Sarah de Rham, from the University of Victoria's Division of Medical Sciences for processing my tissue for protein analysis.

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LIST OF ABBREVIATIONS

BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
CldU	chlorodeoxyuridine
CMS	chronic mild stress
CRS	chronic restraint stress
CUS	chronic unpredictable stress
DCX	doublecortin
DG	dentate gyrus
GR	glucocorticoid receptor
HPA-axis	hypothalamic-pituitary-adrenal axis
IdU	iododeoxyuridine
IS	inescapable shock
LTP	long-term potentiation
MR	mineralcorticoid receptor
MWM	Morris water maze
NDMARs	N-methyl-D-asparte receptors
proBDNF	precursor of brain-derived neurotrophic factor
PSD-95	postsynaptic density protein 95

RAM	radial arm maze
RAWM	radial arm water maze
SD	standard deviation
SEM	standard error of the mean

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DEDICATION

I'm delighted to dedicate this dissertation to my husband, Philip Hawley. Throughout the years he continues to support me and often believes in me more than I do myself. Thank you Phil for your continued patience, priceless encouragement, and unconditional love. There has never been a woman who loved a man more than I love you. This and everything I do is dedicated to you.

Stress is a pervasive and unavoidable component of life (Sapolsky, 1998). People of all ages, genders, and socioeconomic status can fall victim to the feelings of stress (Baum, Garofalo, & Yali, 1999). Stress is often described as feeling overwhelmed, worried, dreadful, or anxious, and these feelings are frequently accompanied by predictable biochemical, physiological, and behavioral changes (Anderson, Johnson, Belar, Breckler, & Nordal, 2012; Baum, 1990). Beginning in 2007, the American Psychological Association started yearly tracking and reporting how people in the United States were experiencing stress in their daily lives and how that was impacting their health. Americans consistently report extreme levels of stress, and reported a 39% increase in chronic stress from 2010 to 2011 (Anderson et al., 2012). As the national level of stress increases, so does the occurrence of chronic illnesses associated with stress (Anderson et al., 2012).

The purpose of the body's response to acute stress is to maintain homeostasis when exposed to threatening stimuli (Sapolsky, 1998). Although the acute stress response is beneficial, it can become unhealthy when maintained for extended periods of time, eventually leading to the emergence of stress-related diseases (e.g. cardiovascular diseases or depression) (Baum & Posluszny, 1999; Lambert et al., 1998; Sapolsky, 1998). Further, prolonged periods of stress contribute to learning and memory impairments, which are common symptoms of depression and decreased neurogenesis (Gould & Tanapat, 1999; Hattiangady, Rao, Shetty, & Shetty, 2005; Mirescu & Gould, 2006). Chronic stress has been shown to induce neuroplastic changes in the hippocampus (Akirav & Richter-Levin, 1999; Gould & Tanapat, 1999; Hawley & Leasure, 2011; McGaugh, 2004), decrease the survival of progenitor cells in the hippocampus

(Brummelte & Galea, 2010; Czeh et al., 2007; Dagyte et al., 2009; Hawley & Leasure, 2011; Mirescu & Gould, 2006), and impair hippocampal-dependent learning and memory (Diamond, Park, Heman, & Rose, 1999; Luine, Martinez, Villegas, Magarinos, & McEwen, 1996; McHugh, Deacon, Rawlins, & Bannerman, 2004).

Recent evidence suggests that there are differing afferent and efferent projects to and from various subregions of the hippocampus (Bannerman, Rawlins, McHugh, Deacon, & Yee, 2004; Dolorfo & Amaral, 1998), and that these subregions have differing functions (E. Moser, Moser, & Andersen, 1993; M. B. Moser & Moser, 1998; Richmond et al., 1999). However, in previous investigations of stress-induced neuroplasticity, potential differences between hippocampal subregions have not been thoroughly investigated.

BIOLOGY OF THE STRESS RESPONSE

During stress, the sympathetic nervous system, a division of the autonomic nervous system, is activated by norepinephrine, a neurotransmitter causing an increase in heart rate, dilation of pupils, and preparation of the body to cope with a stressor (Sapolsky, 1998). Growth, reproduction, immune function, and digestion are decreased during stressful events so that the body can remobilize energy to cope more effectively with the challenge (De Kloet, Joels, & Holsboer, 2005; Sapolsky, 1998). The hypothalamic-pituitary-adrenal (HPA) axis is an additional stress-response system that is activated simultaneously with the sympathetic nervous system. The parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN), a basic brain area involved in motivational behaviors, secretes the hormone corticotrophin-releasing factor

(CRF) that activates the anterior pituitary, a gland that controls many of the body's hormones (Fediuc, Campbell, & Riddell, 2006; Sapolsky, 1998; Vreugdenhil, de Kloet, Schaaf, & Datson, 2001). Upon the activation of the anterior pituitary gland, adrenocorticotropic hormone (ACTH) is released into the bloodstream, activating the adrenal glands to release glucocorticoid hormones (cortisol in humans and corticosterone in rodents) (Campbell, Lin, DeVries, & Lambert, 2003; Cavigelli et al., 2005; Southwick, Vythilingam, & Charney, 2005; Vreugdenhil et al., 2001). When faced with a stressor and corticosterone is elevated, the amygdala activates cells in the bed nucleus of the stria terminalis (BNST), which produces a response to the stressor such as blood pressure elevation, respiratory distress, and increased attention (Salloway, Malloy, & Cummings, 1997). Corticosterone affects behavior through actions on multiple neurotransmitter systems in the brain, such as glutamate, norepinephrine, and GABA (Groeneweg, Karst, De Kloet, & Joels, 2012; Joels et al., 2004), resulting in the enhancement of behaviors necessary for survival in emergency situations (De Kloet et al., 2005). At the termination of a stressor, the parasympathetic nervous system, involved in growth, digestion, reproduction and the immune system, suppresses the stress response system and begins to repair the body (Sapolsky, 1998).

LABORATORY MODELS FOR INDUCING CHRONIC STRESS

Chronic stress has been investigated with rodents in the laboratory using various paradigms. Some of the more common chronic stress paradigms include an inescapable footshock, chronic restraint stress, social stress, chronic mild stress, and chronic unpredictable stress.

Inescapable shock (IS) is a severe chronic stressor that is frequently used as a model for post-traumatic stress disorder (PTSD) (Kikuchi, Shimizu, Nibuya, Hiramoto, & Nomura, 2008; Malberg & Duman, 2003) or depression (Ho & Wang, 2010). Animals are given an uncontrollable series of shocks, about 60 for 15 seconds. In this paradigm of chronic stress animals exhibit symptoms of learned helplessness, a loss of hope that proceeds the onset of depression (Ho & Wang, 2010; Kikuchi et al., 2008). IS has been shown to decrease cell proliferation and cell survival in the hippocampus (Cherng, Chang, Su, Tzeng, & Yu, 2012; Ho & Wang, 2010; Kikuchi et al., 2008; Vollmayr, Simonis, Weber, Gass, & Henn, 2003).

Chronic restraint stress (CRS) is a laboratory model of chronic stress where animals are immobilized in tight plastic tubes for hours at a time on a daily basis (Fediuc et al., 2006; Luine et al., 1996; Thorsell et al., 1998). In CRS paradigms, animals are generally immobilized for at least 2-6 hours daily for a week (Thorsell et al., 1998). Animals exposed to CRS have decreased body weight (Luine et al., 1996), elevated levels of circulating corticosterone (Hutchinson et al., 2012; Miyake et al., 2012), decreased hippocampal cell proliferation (Babic, Ondrejcakova, Bakos, Racekova, & Jezova, 2012) and decreased neurogenesis (Veena, Srikumar, Mahati, Raju, & Shankaranarayana-Roa, 2011).

Psychological and social stressors are thought to be the most common stressors that humans experience (Bjorkqvist, 2001; Iio, Matsukama, Tsukahara, Kohari, & Toyoda, 2011; Sapolsky, 1998). The use of social conflicts between species to generate tension is a natural way to examine chronic stress in a rodent model (Blanchard, McKittrick, & Blanchard, 2001; Iio et al., 2011; Koohlhaas, De Boer, De Rutter, Meerlo,

& Sgoifo, 1997). The resident-intruder paradigm is a laboratory model of social defeat stress which can be acute or chronic depending on the amount of repetitions (Blanchard et al., 2001). In this model of chronic stress, the stressor, an intruder animal, is placed in the home cage of a dominant resident animal. When the intruder is introduced to the resident, the subject is generally attacked and defeated by the resident. The intruder then exhibits signs of subordination such as vocalizations, jumping, freezing, and submissive postures (Miczek, Convington, Nikulina, & Hammer, 2004). Chronic social stress activates the HPA-axis, increases the expression of stress hormones, and decreases hippocampal cell survival (Blanchard et al., 2001; Iio et al., 2011). Results from chronic social stress paradigms vary depending on species, strain, gender, and age (Blanchard et al., 2001).

The chronic mild stress (CMS) paradigm was developed to examine the impact of relatively realistic stress conditions on anhedonia and symptoms of depression (Katz, 1981; Willner, 2005). In a CMS paradigm, animals are exposed to a variety of mildly intense stressors, such as, temperature reductions, food and water deprivation, soiled cages, tilted cages, and changed in cage mates for a period of weeks or months (Harro, Haidkind, Harro, Modrini, & Oreland, 1999; Jayatissa, Bisgaard, West, & Wiborg, 2008; Kim, Whang, Kim, Pyun, & Shim, 2003; Willner, 2005; Willner, Muscat, & Papp, 1992). Prolonged exposure to CMS reduces rodent sucrose consumption suggesting a decreased in hedonia (Katz, 1982). Additionally, animals exposed to CMS exhibit depressive-like behaviors which are synonymous with symptoms of chronic stress (i.e. decreased grooming, decreased weight gain, and decreased hippocampal volume) (Jayatissa et al., 2008; Kim et al., 2003; Willner, 2005).

The chronic unpredictable stress (CUS) paradigm is another model for inducing chronic stress (Hawley et al., 2010; Hawley & Leasure, 2011; Matthews, Forbes, & Reid, 1995). CUS consists of daily stressors that are ecologically relevant to the animal (e.g. predator odors and sounds) (Matthews et al., 1995). This chronic stress paradigm persists for two weeks with stressors varying in order to prevent the animal habituation. CUS is different from CMS because of the exclusion of food and water restrictions, the absence of exposure to extreme temperatures, and the unpredictable nature in which animals are exposed to stressors (Bielajew, Konkle, Kentner, Baker, & Fouriezos, 2003; Harris, Zhou, Youngblood, Smagin, & Ryan, 1997; Hawley et al., 2010; Jayatissa et al., 2008; Kim et al., 2003; Matthews et al., 1995; Willner, 2005). Since stressors are presented in an unpredictable fashion, this model is similar to the daily, mildly intense, unpredictable stressors experienced by humans (Hawley & Leasure, 2011; Matthews et al., 1995). Studies that investigated the effect of chronic stress on the HPA axis and the cardiovascular system in rats found that CUS raised the resting heart rate by 30% (Bhatnagar, Dallman, Roderick, Basbaum, & Taylor, 1998; Hawley et al., 2010; Lawler et al., 1985).

CHRONIC STRESS AND THE HIPPOCAMPUS

The Hippocampus

The hippocampus is a bilateral brain structure associated with learning and memory (Kempermann, 2006; McEwen, 1994; Steffenach, Witter, Moser, & Moser, 2005), information processing (Bannerman et al., 2004), regulation of behavior (Bannerman et al., 2004), and neurogenesis (the creation of neurons) (Brown et al., 2003;

Gould & Tanapat, 1999); integrity of the hippocampus is critical for these functions. The most prominent account of hippocampal functions is the report on a patient named Henry Gustav Molaison, famously known as H.M., who suffered from extreme epileptic seizures (Scoville & Milner, 1957). H.M. underwent surgery to remove his medial temporal lobe, including the hippocampus. Following the surgery and hippocampal damage, H.M. had extreme long term memory deficits (Scoville & Milner, 1957, 2000; Segal, Richter-Levin, & Maggio, 2010). While H.M. retained his memories from before the surgery, he lost the ability to form new implicit memories (Scoville & Milner, 1957). The research surrounding H.M. initiated a new era of study on the hippocampus and memory, because without the integrity of the hippocampus there are deficits in hippocampal functions.

The hippocampus is a highly organized structure and contains distinct anatomical subfields that have been shown to serve specific biological functions (Kempermann, 2006; Segal et al., 2010). There are two major areas of the hippocampus: Ammon's Horn (CA1-CA4 subfields) and the dentate gyrus (DG) (Storm-Mathisen, Zimmerm, & Ottersen, 1990). These areas are determined based on the cell shape, size, connectivity, and structural differences underlying functional differences (X. M. Zhao, Lein, He, Smith, & Gage, 2001). Whereas the DG contains mainly granule neurons, Ammon's horn contains mainly pyramidal neurons (X. M. Zhao et al., 2001). The CA1 contains smaller pyramidal neurons and CA2-CA4 contain larger pyramidal neurons (O'Keefe & Nadel, 1978). The DG can also be divided into two portions: the suprapyramidal blade and the infrapyramidal blade (Scharfman, Sollas, Smith, Jackson, & Goodman, 2002; Snyder, Radik, Wojtowicz, & Cameron, 2009).

The anatomically different subfields within the hippocampus also have unique afferent and efferent projections. The hippocampus is organized into a tri-synaptic circuit, through which the motion of information flows uni-directionally (O'Keefe & Nadel, 1978). The DG receives input from neurons in layer II of the entorhinal cortex via the perforant pathway, and then projects largely to CA3 via the moss fiber pathway. The CA3 neurons project to the CA1 via the Schaeffer collaterals. CA1 also receives afferent projections from the entorhinal cortex but from layer III by means of the perforant pathway (Kempermann, 2006; O'Keefe & Nadel, 1978; X. M. Zhao et al., 2001). The CA3 subfield also projects to the contralateral hippocampus by the commissural pathway (O'Keefe & Nadel, 1978). A critical function of the DG is the production of new neurons (Kempermann, Kuhn, & Gage, 1998; X. M. Zhao et al., 2001). After new granule neurons are formed in the DG, they form synapses onto CA3 pyramidal neurons (Hastings & Gould, 1999; Markakis & Gage, 1999) (See Figure 1).

Stress and the Hippocampus

When the HPA-axis is activated in response to a stressor, corticosterone, the rodent stress hormone, is elevated (Campbell et al., 2003; Cavigelli et al., 2005; Southwick et al., 2005). The effects of corticosterone are mediated by two intracellular types: Type I, mineralcorticoid receptor (MR) and Type II, glucocorticoid receptor (GR) (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998; Vreugdenhil et al., 2001). GR can be found almost all over the brain; whereas, MR is specific to limbic regions, including the hippocampus (De Kloet et al., 1998; Vreugdenhil et al., 2001). Both GR and MR are ligand-driven transcription factors (Groeneweg et al., 2012). When corticosterone binds to MR or GR in the cytoplasm, the receptors dissociate from proteins and form a complex

(Vreugdenhil et al., 2001). In this activated form, MR and GR are translocated to the nucleus where they act as transcription factors and influence gene transcription (Groeneweg et al., 2012; Vreugdenhil et al., 2001). GRs have an established function in the facilitation of the body's response to stress and recovery from stress (De Kloet et al., 1998; Groeneweg et al., 2012). Chronically high levels of circulating corticosterone activate GR, and ultimately lead to excitotoxicity and hippocampal atrophy due to an increased release of glutamate at the postsynaptic membrane (Groeneweg et al., 2012; Joels et al., 2004; McEwen, 1994; Vreugdenhil et al., 2001).

Hippocampal neurons express high levels of both GR and MR (Mirescu & Gould, 2006), therefore the hippocampus is extremely sensitive to circulating levels of corticosterone (De Kloet et al., 1998; Groeneweg et al., 2012; McEwen, 2000; Vreugdenhil et al., 2001; Wong & Herbert, 2005). When there is an imbalance in the level of circulating corticosterone, hippocampal physiology and functioning are altered (Gould, Woolley, & McEwen, 1990). Due to its sensitivity to corticosterone, functions associated with the hippocampus, such as learning and memory, are often impaired following exposure to stress (Groeneweg et al., 2012; McEwen, 1994; Mirescu & Gould, 2006). Furthermore, cell proliferation and neurogenesis are impaired with chronic stress (Jayatissa et al., 2008; McEwen, 2000; Mirescu & Gould, 2006). When the adrenal gland is removed, and corticosterone is no longer secreted, cell generation is not impaired following chronic stress (Gould & Tanapat, 1999; Joels, 2007).

Learning and memory are critical hippocampal functions which have been shown to be compromised by chronic stress (Conrad, 2010). After decades of researching the hippocampus and its critical role in spatial learning, investigators began exploring the

relationship between chronic stress and spatial abilities (Conrad, 2010). In food-reward tasks rats are motivated to complete a task in order to obtain food. However, when animals are exposed to chronic stress, such as CRS, social stress, or IS, animals do not learn the radial arm maze (RAM) or the T-maze, where the premises is for the animal to learn the goal arm containing the food (Luine, Villegas, Martinez, & McEwen, 1994; Ohl & Fuchs, 1999; Shankaranarayana-Roa & Raju, 2000; Veena et al., 2009). In aversively motivated tasks like the Morris water maze (MWM) and the radial arm water maze (RAWM) the potential for food motivation is removed from the paradigm. The animal is placed in a pool and escape from the water is the motivation for the animal to learn the location of the hidden platform. Studies investigating the impact of CRS and IS on spatial learning and memory using the MWM, found impaired spatial learning (Abidin et al., 2004; Conrad, 2010; Kitraki, Keremmyda, Youlatos, Alexis, & Kittas, 2004; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006; Venero et al., 2002). Chronic stress studies examining the effect of psychosocial stress, switching rodent cage mates, on spatial learning and memory using the RAWM found statistical similarities between control and stress animals on the initial learning trials; however, spatial memory impairments became evident in the chronically stressed groups following 15 to 30 minute delays (A.M. Aleisa, Alzoubi, Gerges, & Alkadhi, 2006; Alzoubi et al., 2009; Conrad, 2010; Gerges, Alzoubi, Park, Diamond, & Alkadhi, 2004; Srivareerat, Tran, Alzoubi, & Alkadhi, 2009). Chronic stress effects on spatial learning and memory appear to be task-specific.

Chronic stress impairs spatial learning on food motivated tasks, such as the RAM, a task that evokes a relatively mild arousal component. Conversely, under testing

conditions where there is a strong arousal component, such as RAWM, chronic stress appears to have minimal impairing effects or may even facilitate spatial learning (Conrad, 2010). However, the impact of CUS on spatial learning and memory on the RAWM has not been thoroughly investigated (Conrad, 2010).

Subregions of the Hippocampus

Recent evidence suggests that the hippocampus has two functionally distinct subregions (Bannerman et al., 2004; McNaughton & Gray, 2000). The dorsal portion appears to be primarily associated with spatial navigation (Eadie et al., 2009; M. B. Moser & Moser, 1998), while the ventral region has been linked to anxiety-related functions (Bannerman et al., 2004; Richmond et al., 1999) (See Figure 2).

The dorsal subregion receives afferent projections from the dorsolateral band, layers II and III, of the entorhinal cortex (a brain region required for spatial learning) (Dolorfo & Amaral, 1998; Fyhn, Molden, Witter, Moser, & Moser, 2004; Snyder et al., 2009; Steffenach et al., 2005), and association cortex (Bannerman et al., 2004), and then projects primarily to the neocortex (Naber & Witter, 1998; Segal et al., 2010). The dorsal subregion of the hippocampus engages in functions of spatial learning and navigation (Bannerman et al., 2004; Dolorfo & Amaral, 1998; Fyhn et al., 2004). When the dorsal subregion of the DG is completely lesioned in rodents, there are significant impairments in spatial learning and memory tasks (e.g. MWM, RAM, T-maze) (Bannerman et al., 2004; E. Moser et al., 1993).

The ventral subregion of the hippocampus receives afferent projections from the hypothalamus and amygdala (basal and lateral nuclei) (Brummelte & Galea, 2010; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000; Segal et al., 2010; Snyder et al.,

2009), and projects to the prefrontal cortex (Bannerman et al., 2004), BNST (Bannerman et al., 2004), and subcortical structures (e.g. amygdala and hypothalamus) (Naber & Witter, 1998; Segal et al., 2010). Following complete ventral DG lesions in rodents, no spatial navigation impairments are produced; however, anxiety related behaviors are significantly decreased (Bannerman et al., 2003; Kjelstrup et al., 2002; E. Moser et al., 1993; Richmond et al., 1999). Therefore, it appears that the ventral subregion is associated with affect-related functions (e.g. emotional, social, neuroendocrine processing) (Bannerman et al., 2004; Brummelte & Galea, 2010; Richmond et al., 1999).

CUS, Hippocampal Subregions, and Neuroplasticity

Neurogenesis, the creation of neurons, occurs in the ventral DG of the hippocampus (Brown et al., 2003; Couillard-Despres, Winner, Schaubeck, Aigner, & Aigner, 2005) and begins in gestation during the embryonic period and continues into adulthood (Bannerman et al., 2004; Couillard-Despres et al., 2005; Saito, 2000). In the mammalian brain there is a high demand for new neurons in the hippocampus, and to compensate, the hippocampus produces new neurons daily that are integrated into the hippocampal circuitry (van Praag et al., 2002; C. Zhao, Deng, & Gage, 2008). New granule neurons are physiologically functional, provide increased plasticity, and contribute to hippocampal dependent behaviors (Snyder et al., 2009; van Praag et al., 2002; C. Zhao et al., 2008). Neurogenesis is a firmly regulated process which can be mediated by genetic influence (Kempermann et al., 1998), age (Kuhn, Dickinson-Anson, & Gage, 1996; Kuhn, Winkler, Kempermann, Thal, & Gage, 1997), growth factors (e.g. brain-derived neurotrophic factor) (Duman, Malberg, Nakagawa, & D'Sa, 2000; Hattiangady et al., 2005), exercise (van Praag et al., 2002), alcohol (S. A. Morris, Eaves,

Smith, & Nixon, 2010; Redila et al., 2006), environmental enrichment (Kempermann et al., 1998), and stress (Mirescu & Gould, 2006). Unfortunately, since the health of neurons in the DG of the hippocampus is environmentally dependent, chronic stress can easily repress cell generation and survival (Mirescu & Gould, 2006; Saito, 2000). Because neurogenesis facilitates learning and memory, decreased cell proliferation as a result of chronic stress can have significant impairments on hippocampal functions (i.e. learning and memory) (Saito, 2000).

Markers of neuroplasticity, for example Neuropeptide Y (NPY) and ΔFosB, are increasingly expressed following chronic stress and have been implicated in adaptive responses to chronic stress (Hattiangady et al., 2005; Heilig, 2004; Kim et al., 2003; Perrotti et al., 2004). NPY is an abundant neuroactive peptide in the central nervous system (Thorsell et al., 1998). Hypothalamic NPY is involved in the activation of the HPA-axis and is closely associated with the corticotropin-releasing neurons (Kim et al., 2003; Small et al., 1997; Zukowska-Groject, 1995). Following stressors, such as CRS and CMS, there is an upregulated expression of NPY in both the hypothalamus and the hippocampus (Conrad & McEwen, 2000; Thorsell, Carlsson, Ekman, & Heilig, 1999). Increased levels of NPY following stressful events in both humans and rats (Boulenger et al., 1996; Kim et al., 2003; Thorsell et al., 1999) have been shown to play a critical role in the physiological regulation of the stress response and promote adaptation (Kim et al., 2003; Thorsell et al., 1999) at the Y1 receptor (Kask, Rago, & Harro, 1997; Thorsell et al., 1998).

ΔFosB is a transcription factor which is induced in various brain regions by a wide variety of stimuli, including drug exposure (Brenhouse & Stellar, 2006; Hiroi et al.,

1997; McClung et al., 2004), sexual activity (Wallace et al., 2008), electroconvulsive seizures (Hiroi, Marek, Brown, Ye, & Nestler, 1998; Hope, Kelz, Duman, & Nestler, 1994), exercise (Werme et al., 2002), and stress (Berton, Covington, Ebner, Tsankova, & Nestler, 2007; Perrotti et al., 2004). ΔFosB increases in the limbic regions with the onset of emotionally-arousing situations due to the increased need for plasticity, specifically learning and memory storage (McClung et al., 2004; Perrotti et al., 2004). Following exposure to chronic stress, such as CRS or cat odor, rats have increased expression of ΔFosB in various limbic regions, including the hippocampus. (Perrotti et al., 2004; Staples, McGregor, & Hunt, 2009). Rats exposed to IS with a higher expression of ΔFosB in the hippocampus have fewer symptoms of learned helplessness (failing to display escape behaviors) (Berton et al., 2003). This suggests that behavioral plasticity is mediated by ΔFosB in the hippocampus, and therefore, facilitates an adaptive coping mechanism in the stress response (Berton et al., 2007; McClung et al., 2004).

In a previous study, NPY and ΔFosB were preserved specifically in the dorsal DG of the hippocampus following CUS (Hawley & Leasure, 2011). While previous studies have demonstrated that the survival of neural progenitor cells in the hippocampus is diminished in animals exposed to chronic stress (Duman, 2009; Mirescu & Gould, 2006); we found a region-specific effect that animals exposed to CUS had fewer proliferating cells in the ventral DG, but not the dorsal DG of the hippocampus (Hawley & Leasure, 2011) (See Figure 3).

Region-specific neuroplastic changes in the dorsal and ventral subregions of the hippocampus suggest that the hippocampus may play a dual role in the stress response. Whereas previous studies have indicated that only the ventral subregion was impacted by

chronic stress (Bannerman et al., 2004; Richmond et al., 1999), it is possible that both hippocampal subregions are impacted by stress, but in different ways. The ventral subregion may be principally involved in the emotional response to the stressor, whereas the dorsal subregion may be mainly engaged in the behavioral aspects of the stress response, such as avoidance or amelioration of the stressor (Hawley & Leasure, 2011). In our previous research, since neuroplasticity was preserved in the dorsal, spatially oriented subregion of the DG in animals exposed to CUS, we predicted that stressed animals would perform better than controls on a spatial task.

CURRENT INVESTIGATION

Spatial Navigation

There are several established animal models to test various types of memory in order to evaluate specific brain area involvement. Three of the most common hippocampal-dependent tasks that evaluate spatial learning are the Morris water maze (MWM), radial arm maze (RAM), and the radial arm water maze (RAWM). These tasks require the animal to use spatial cues to learn a certain desirable goal (e.g. food or escape).

The RAM was designed to test spatial learning and memory (Olton, 1987). This learning task consists of eight arms diverging from a central location. Four of the eight arms are baited with food, and these arms remain constant throughout the learning trials. Following food deprivation, each animal is placed in the center of the maze where it may enter any of the eight arms. The animal remains in the apparatus until all of the food rewards are consumed, or 5 minutes have passed. Using the spatial cues the animal learns which arms contain the food reward; errors, entering an arm that is not baited or

entering an arm where the reward has already been consumed during a single trial, are noted on subsequent trials (Olton, 1987). Animals with dorsal hippocampal lesions have spatial learning and memory deficits on the RAM (Bannerman et al., 2004; Conrad, 2010). Odors and food deprivation as a motivator are limitations with this model of spatial learning and memory; however, this task allows the investigator to measure shore-term and long-term memory (Conrad, 2010).

The MWM is a spatial learning and memory task made from a pool of water containing a hidden platform located in one of the four quadrants (R. G. Morris, Garrud, Rawlins, & Okeefe, 1982). Animals are released from various points around the wall of the maze, and the animal must use visuospatial cues to escape the water on the hidden platform. The latency to find the platform decreases with increased exposure to the maze, suggesting that learning has occurred. In order to test spatial memory, there is a probe trial where the hidden platform is removed from the pool and the time the animal spends in the quadrant that previously contained the platform is recorded. Animals with dorsal hippocampal lesions have spatial learning and memory deficits on the MWM (Bannerman et al., 2004; Conrad, 2010). The MWM eliminates the limitation of food deprivation and odor found in the RAM (Buresova, Bures, Oitzl, & Zahalka, 1985; Hodges, 1996). However, swimming an appropriate fixed distance around the sides of the MWM as a strategy to located the hidden platform is the main MWM disadvantage (Hodges, 1996).

The radial arm water maze (RAWM) is a hippocampal-dependent task assessing spatial learning and memory performance (Buresova et al., 1985; Diamond et al., 1999). The well-established RAWM is a combination of the MWM and the RAM (Buresova et

al., 1985). The RAWM is advantageous because it combines spatial complexity and efficient learning associated with the RAM and the MWM, while eliminating the need for extra controls to determine strategy or motivation, and it does not require food deprivation (Berchtold, Castello, & Cotman, 2010; Conrad, 2010; Diamond et al., 1999; Shukitt-Hale, McEwen, Szprengiel, & Joseph, 2004). Furthermore, the RAWM maintains the advantage of the RAM in which short-term and long-term memory can be accessed (Buresova et al., 1985). In the current investigation, spatial navigation in the RAWM was used to assess adult Long-Evans rats following exposure to a two-week CUS paradigm (Hawley et al., 2010; Hawley & Leasure, 2011).

The hippocampus is an essential brain region for the encoding and consolidation of episodic memories (Steffenach et al., 2005), and hippocampal integrity is imperative for learning and memory (Conrad, 2010; Diamond et al., 1999). Given that markers of neuroplasticity (NPY and ΔFosB) were preserved in the dorsal subregion of the hippocampus, an area required for spatial learning (Hawley & Leasure, 2011; Snyder et al., 2009; Steffenach et al., 2005), I predicted that instead of spatial navigation impairments, rats exposed to CUS would have enhanced performance in tests of spatial learning and memory on the RAWM, as compared with control animals (Buresova et al., 1985; Conrad, 2010).

Corticosterone

For a physiological confirmation of stress, an endocrine (i.e. corticosterone) assessment was made prior to and after the CUS paradigm via the collection of fecal boli. Corticosterone can be accurately measured from fecal samples, which are easily collected non-invasively (Cavigelli et al., 2005). Fecal corticosterone analysis is ideal for

measuring the long-term corticosterone response to an on-going experimental manipulation such as chronic stress (Thanos et al., 2009). Consistent with previous investigations (Hawley et al., 2010), I expected that following exposure to CUS, stressed animals would have higher levels of corticosterone than control animals.

Neurogenesis

The health of neurons in the DG of the hippocampus is environmentally dependent, chronic stress can easily repress cell generation and survival (Mirescu & Gould, 2006; Saito, 2000). Because neurogenesis facilitates learning and memory, decreased cell proliferation as a result of chronic stress can have significant impairments on hippocampal functions (i.e. learning and memory) (Saito, 2000). Bromodeoxyuridine (BrdU), an exogenous marker of cell proliferation, is incorporated into the DNA of newly generated cells from the time of administration, and then tracks the survival of all newly generated cells (Brown et al., 2003). In a previous study from our lab, BrdU was used to quantify dividing cells at the beginning of CUS, and indicated a decrease in cell survival in the ventral DG of stressed animals after the 2-week paradigm (Hawley & Leasure, 2011) (See Figure 3). However, BrdU only labeled cells that were generated and survived through the end of CUS. In order to investigate both cell survival and cell proliferation in the present study two similar exogenous markers were used: iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU) (Conboy, Karasov, & Rando, 2007; Leuner, Glasper, & Gould, 2009; Vega & Peterson, 2005). IdU and CldU are also thymidine analogues that are integrated into cells undergoing DNA synthesis (Conboy et al., 2007; Kimoto, Yura, Kishino, Toyosawa, & Ogawa, 2008; Leuner et al., 2009). These analogs can be detected individually with different antibodies that have been

reported to specifically detect IdU+ and CldU+ cells (Kimoto et al., 2008; Leuner et al., 2009; Vega & Peterson, 2005).

Additionally, BrdU is not an indicator of a new cell's phenotype (e.g. neurons, glial cells). In order to further investigate region-specific neuroplasticity, in the present study, the synthesis of neurons was quantified. Type 2 cells which lack glial features express doublecortin (DCX)(Lucassen et al., 2010). The microtubule-binding phosphoprotein DCX is used to label newly generated post mitotic neuroblasts (Brown et al., 2003; Couillard-Despres et al., 2005; Lucassen et al., 2010; S. A. Morris et al., 2010; Rao & Shetty, 2004) (See Figure 5) . DCX is a specific marker of plasticity, or more specifically, adult neurogenesis, in the granule cell layer of the DG (Brandt, Jessberger, Steiner, Kronenberg, & Kempermann, 2003; Brown et al., 2003; Brummelte & Galea, 2010; Couillard-Despres et al., 2005). The expression of DCX decreases with age in accordance with a decrease in neurogenesis (Brown et al., 2003; Kuhn et al., 1996), and increases with exercise in accordance with neurogenesis (Brown et al., 2003; Couillard-Despres et al., 2005). Psychosocial stress (Van Bokhoven et al., 2011), CMS (Dagyte, Crescente, Postema, Sequin, & Koolhaas, 2011; Jayatissa et al., 2008; Parihar, Hattiangady, Kuruba, Shuai, & Shetty, 2011), and IS (Dagyte et al., 2009) reduce the expression of DCX in the DG.

The survival of neural progenitor cells in the hippocampus is diminished in animals exposed to chronic stress (Duman, 2009; Mirescu & Gould, 2006). Further, previous investigations indicated a region-specific effect such that animals exposed to CUS have fewer proliferating cells in the ventral, but not the dorsal subregion of the hippocampus (Hawley & Leasure, 2011). Additionally, not only were the neural

proliferating cells preserved in the dorsal subregion, but markers of neuroplasticity (NPY and ΔFosB) were as well; therefore, I hypothesized that cell proliferation (CldU), cell survival (IdU), and neurogenesis (DCX) would also be preserved in the granule cell layer of the dorsal DG in animals following CUS and the RAWM, but reduced in the ventral subregion of the DG compared to control animals.

Neuroprotective Proteins

Neurotrophic factors are critical proteins for cell growth and survival (Alhaider, Aleisa, Tran, & Alkadhi, 2010; Barde, 1989; Duman, 2009; McAllister, 2002). Given that the production of neurotrophins may enhance the integrity of the hippocampus (Raivich et al., 1999), the region-specific expression of neuroprotective proteins was investigated.

Neurotrophins play a critical role in the survival and function of neurons (Duman, 2009; McAllister, 2002). Brain-derived neurotrophic factor (BDNF) is a neurotrophin with various effects on the nervous system, such as neuronal growth, differentiation, and repair (Alonso et al., 2002; Aydemir et al., 2006; Kiprianova, Sandkuhler, Schwab, Hoyer, & Spranger, 1999). BDNF modulates the strength of existing synaptic connections and acts in the formation of new synaptic contacts (Kiprianova et al., 1999; Lipsky & Marini, 2007). Within the hippocampus, BDNF is essential for plasticity, neurogenesis, learning and memory, and hippocampal long-term potentiation (LTP) (Aydemir et al., 2006; Boehme et al., 2011; Duman, 2009; Kiprianova et al., 1999; Lessmann, Gottmann, & Heumann, 1994; Martinowich, Manji, & Lu, 2007). Endogenous BDNF is also essential for spatial learning and memory (Linnarsson, Bjorklund, & Ernfors, 1997). BDNF-knockout mice show impairments in spatial

learning and memory (Linnarsson et al., 1997). Additionally, the gene expression of BDNF has been reported to increase in the hippocampus after spatial learning tasks (Falkenberg et al., 1992; Mizuno, Yamada, He, Nakajima, & Nabeshima, 2003). Animals exposed to CUS (Nibuya, Takahashi, Russell, & Duman, 1999), CRS (Xu et al., 2004), or psychosocial stress (Pizzarro et al., 2004) have decreased expression of BDNF in the hippocampus (Duman & Monteggia, 2006). Following CUS, the expression of BDNF has been shown to be higher in the dorsal DG of the hippocampus compared to the ventral DG (Larsen, Mikkelsen, Hay-Schmidt, & Sandi, 2010). In our previous study, cell survival was preserved in the dorsal DG; therefore, in the present study it was predicted that following CUS and the RAWM, stressed animals would have significantly more BDNF in the dorsal subregion compared to the ventral subregion in order to promote cell survival.

Neurotrophins are synthesized as precursor forms (proneurotrophins) that dimerize after translation into mature proteins (Lu, Pang, & Woo, 2005; Martinowich et al., 2007; Teng et al., 2005). ProBDNF is the precursor of mature BDNF (Barker, 2009; Teng et al., 2005), and is cleaved via intracellular release (Barnes & Thomas, 2008; Holm et al., 2009; Martinowich et al., 2007; J. Yang et al., 2009). Similarly to BDNF, proBDNF is released from hippocampal neurons and mediates hippocampal functions, such as LTP (Arancio & Chao, 2007; Barker, 2009; Lu et al., 2005; Pang et al., 2004) and memory (Barnes & Thomas, 2008). Following chronic stress there is a decrease of proBDNF cleaved into mature BDNF leading to long-term deficits in hippocampal plasticity (Pang et al., 2004), such as decreased LTP (Martinowich et al., 2007; Pang et al., 2004). However, it is not yet known how proBDNF is impacted by CUS or the

RAWM in the dorsal and ventral DG. Considering that proBDNF is essential for BDNF, I hypothesized that the expression of proBDNF would be higher in the dorsal DG compared to the ventral DG following CUS and RAWM exposure in order to promote BDNF expression.

Synaptic Plasticity

N-methyl-D-asparte receptors (NMDARs) are widely expressed in the central nervous system and heavily involved in excitatory synaptic transmission, synaptic plasticity, synaptic regulation, and learning and memory (Alhaider et al., 2010; El-Husseini, Schnell, Chetkovich, Nicoll, & Bredt, 2000; Papadia & Hardingham, 2007; Zhu, Zhang, & Zhang, 2011). These glutamate-gated ion channels (Luo, Qiu, Zhang, & Shu, 2012) are embedded in the postsynaptic density, a structure associated with the postsynaptic membrane (Luo et al., 2012). Postsynaptic density-95 (PSD-95) is a postsynaptic scaffolding protein that is a critical regulator of synaptic development, strength, and plasticity (El-Husseini et al., 2000; Han & Kim, 2008), as well as a protein that affects neuronal survival and function (Luo et al., 2012; Zhu et al., 2011). PSD-95 also plays a central role in the NMDA receptor signaling and mediates LTP (Han & Kim, 2008; Zhu et al., 2011). Following chronic administration of corticosterone, there was a decrease in expression of PSD-95 in the DG of the hippocampus in mice; however, the dorsal and ventral subregions were not independently examined (Cohen, Louneva, Han, Hodes, & Arnold, 2011). Since PSD-95 promotes synaptic plasticity, learning, memory, and LTP, and markers of plasticity (NPY and ΔFosB) were preserved in the dorsal DG but not the ventral DG following CUS (Hawley & Leasure, 2011), I predicted that PSD-

95 would also be more abundant in the dorsal DG in order to preserve spatial learning following CUS.

In sum, this study examines the potential dual role of the hippocampus in response to chronic stress. The subregions of the hippocampus may respond differently to chronic stress in an effort to efficiently cope with the stressor. While the ventral subregion may be principally involved in the emotional coping response to chronic stress (Bannerman et al., 2004), the dorsal subregion may be more engaged in the behavioral coping aspects (i.e. avoidance or escape) of the stress response. Consequently, efficient behavioral responses to the RAWM following CUS may be facilitated by preserved neuroplasticity and neuroprotective proteins in the dorsal subregion of the hippocampus.

Method

Animals

All experimental procedures were conducted in accordance with the approved guidelines of the Institutional Animal Care and Use Committee at the University of Houston. Sixty-one male Long-Evans rats (Harlan, Indianapolis, IN) were housed individually in clear plexiglass cages lined with Kay Kob bedding, and received rat chow and water *ad libitum*. It has been shown that group housed animals established a social hierarchy which increases corticosterone and decreases neurogenesis in the DG in male and female rats (Kozorovitskiy & Gould, 2004). In an effort to control for stress associated with social status, animals were individually housed. Animals were approximately three months old, weighing 325-355 grams. Upon arrival, animals were given one week to habituate to the vivarium environment with a reversed light/dark cycle

with lights off at 9:00am and on at 9:00pm, and were randomly assigned to one of two conditions: control (*n*=31) or stress (*n*=30).

Design and Procedure

Figure 4 is a diagram of the investigation, specifically indicating when various treatments occurred for both stress and control conditions.

Chronic Unpredictable Stress (CUS)

The CUS paradigm was adapted from previously described protocols (Alfarez, Joels, & Krugers, 2003; Hawley & Leasure, 2011; Matthews et al., 1995), and expanded to include more ecologically relevant stressors encountered by an animal in daily life (i.e. predator odors, sounds, and simulated noninvasive insect-like "bites" using a tail clip) (Hawley et al., 2010; Hawley & Leasure, 2011; Matthews et al., 1995). The unpredictable exposure to stressors reduces the chance that animals will habituate to the paradigm (Hawley et al., 2010; Joels et al., 2004; Munhoz et al., 2006). Consistent with previous investigations, this chronic stress model produces typical signs of chronic stress exposure, including: elevated corticosterone, cardiovascular responses, decreased weight gain, and decreased neuroplasticity (Hawley et al., 2010; Hawley & Leasure, 2011; Kim et al., 2003; Larsen et al., 2010; Li et al., 2010; Lin et al., 2005; McFadden et al., 2011; McGuire, Herman, Horn, Sallee, & Sah, 2010).

Following a week of acclimation to the environment, the CUS paradigm lasted for 14 days, with two different daily stressors (See Table 1) determined by a random number generator, including: 1 ml of fox or raccoon urine (Wildlife Research Center; Anoka, MN) placed on a cotton ball in the front of each cage for 6 hours; exposure to novel objects; a strobe light (Xenon Strobe Light; Multi Media Electronics, Inc.; Farmingdale,

NY) for 2 hours to simulate environmental challenges (i.e. lightning in thunderstorms) (Hawley et al., 2010; Hawley & Leasure, 2011); vinegar-laced drinking water (10% vinegar for 6 hours); damp bedding (400ml of room temperature water added to the bedding of the animals cage for 4 hours); wildlife predator calls played for 1 hour (at approximately 90 decibels; Johnny Stewart Wildlife Calls; Waco, TX); non-invasive black clips (Conair Corporation: Scunci; East Winsor, NJ) placed at the base of the tail to simulate insect bites (Hawley et al., 2010; Hawley & Leasure, 2011); tilting the animal cages at a 45° angle for 6 hours; exposure to white noise for 4 hours; and a 5 minute open field task. All stressful conditions were conducted in a separate room from where the control animals were housed to prevent exposure to CUS.

Behavioral Assessments of Stressors. Throughout the CUS, behavioral data were collected for the tail clip task and open field (See Figure 6). During the tail clip task, which was conducted in the rat's home cage, a small clip was placed at the base of the tail for 5 minutes (Hawley et al., 2010). While the clip fit securely, it did not penetrate or injure the tail. New clips were used for each animal to prevent exposure of odors from other animals. The following behaviors were observed during the tail clip task: latency to contact the tail clip, duration of freezing (defined as no visible movement for at least 3 seconds), and the time the animal spent trying to remove the clip with the paws or mouth.

The open field task consisted of a box with a grid of 25 15 x 15 cm squares drawn on the bottom (See Figure 7). Each rat was placed in the open field apparatus at the same location and removed after 5 minutes. Between each animal, the open field was thoroughly cleaned. The behaviors observed during the task were the latency to enter the inner area and the length of time spent in the inner area.

Radial Arm Water Maze

Following CUS exposure, animals (control, *n*=15; stress, *n*=15) were tested for spatial learning and memory performance using a hippocampal-dependent task: the RAWM (See Figure 4) (Buresova et al., 1985; Diamond et al., 1999). In the RAWM, six stainless steel, V-shaped arms were inserted into a black circular tub filled with room temperature water (Diamond et al., 1999) made opaque with non-toxic paint (Crayola LLC, Easton, PA) to prevent the animal from seeing the platform during the task (Berchtold et al., 2010) (See Figure 8). In dim lighting, during the animals' dark cycle to prevent fatigue and sleep deprivation, animals were given 12 1-minute trials to find the "goal arm" where an escape platform was placed 1 cm below the surface (A. M. Aleisa et al., 2011). Available visual cues included variously shaped figures on the walls. For each trial, animals were gently placed in the entrance arm facing the wall of the pool. Starting location arms for each trial were randomized but did not include the goal arm; the goal arm remained the same throughout all 12 trials for each animal. During the 1 minute intertrial interval, the animal remained on the platform. If the animal could not find the platform within 60 seconds, the animal was guided to and allowed to sit on the platform for the 1 minute intertrial interval. The 12 acquisition trials were divided into two blocks of six consecutive trials, interspersed with a 5-minute break. Following all of the learning trials, the animals performed a short-term memory trial (30 minutes after the last learning acquisition trial) and a long-term memory trial (24 hours after the short-term memory trial). After each trial, animals were towel dried before being returned to their home cage.

The number of errors was recorded during the learning acquisition, short-term memory, and long-term memory tasks. Errors were operationally defined as anytime the animal's entire body entered an arm that was not the goal arm, as well as anytime an animal entered the goal arm but did not escape using the platform. Additionally, latency to find the platform was recorded in seconds. Errors and latency to find the platform were compared across conditions and time points (learning acquisition trials, short-term memory trial, and long-term memory trial).

Corticosterone Assessments

Measuring the rodent stress hormone, corticosterone, via fecal sampling is advantageous since fecal pellets are easily accessible and because fecal corticosterone is highly correlated with serum corticosterone (Cavigelli et al., 2005; Thanos et al., 2009). This non-invasive method for corticosterone sampling is ideal for longitudinal studies and minimizes stress to the animal (Cavigelli et al., 2005; Thanos et al., 2009). For a physiological confirmation of stress throughout the study, fresh fecal boli was collected before CUS began, the last day of CUS, and the last day of the experiment (the day following RAWM exposure) (See Figure 4). It was advantageous to individually house animals so that each sample of fecal boli was directly linked with the correct animal. All fecal samples were stored at -80º C until they are ready to be processed.

Corticosterone levels were quantified using a commercially available Enzyme Immunoassay Kit (EIA) (Assay Designs, Inc., Ann Arbor, MI). Prior to the assay, samples were weighed to 0.1g and added to 1ml of 100% methanol in a plastic centrifuge tube and homogenized using a metal stirring rod. Each sample was sonicated for 10 seconds at 4000ppm and placed in a centrifuge for 10 minutes at 2500rpm. The

supernatant was removed and put into a 13 x 10mm glass test tube using a transfer pipette, and then diluted (in MeOH) 1:20 using the tris buffered saline provided in the EIA kit. Using a 96-well donkey anti-sheep microplate provided in the kit, 100µL of each sample was placed into a single well. In each well, 50µL of the blue alkaline phosphatase corticosterone conjugate and 50µL of the yellow sheep polyclonal antibody, provided in the kit, were added each sample. The plate was then incubated at room temperature on a shaker for two hours at 500 rpm. The contents of the wells were emptied and washed three times with the provided wash buffer. After washing, the provided p-nitrophenyl phosphate substrate solution was added to each well and incubated for an hour at room temperature. To stop the reaction, the provided trisodium phosphate stop solution was added to each well. Microplates were then immediately placed on an absorption reader (Biotek Instruments, Inc., Winooski, Vermont) and analyzed at an optical density of 405nm to assess the amount of light absorption per sample using Gen5 software (Biotek Instruments, Inc., Winooski, VT). To determine the corticosterone absorption, the slope of the antibody binding to the fecal sample was compared with that of the standard curve of the provided five serially diluted standards (1:32 to 1:20000). Each sample was run in triplicate and averaged. Corticosterone levels were compared across conditions (stress and control) and time points (before CUS, after CUS, and at the end of the experiment).

CldU and IdU Administration

In order to quantify the impact of CUS on cell survival in the DG, control (*n*=18) and stress (*n*=18) animals were injected, with IdU (MP Biomedicals, Solon, OH, USA; 57.5 mg/kg, i.p.,) (Vega & Peterson, 2005) daily for the first 5 days of CUS. To assess

the effects of CUS on cell proliferation of hippocampal progenitor cells, rats were injected with CldU (Sigma-Aldrich, St. Louis, MO, USA; 42.5 mg/kg, i.p.) 2 hours prior to sacrifice (Vega & Peterson, 2005). Animals that were not injected with CldU or IdU (control *n*= 13, stress *n*=12), received a control injection of saline (See Figure 4).

Histology

In order to examine the impact of CUS on cell survival, cell proliferation, and neurogenesis, a subset of animals did not undergo learning in the RAWM, and instead were sacrificed following the last day of the CUS paradigm (control, *n*=9; stress, *n*=9). (See Figure 4). The rest of the animals were sacrificed the day after the long-term memory trial (control, *n*=9; stress, *n*=9). Animals were lethally anesthetized using 1cc, i.p. injection (with a 28 gauge, ½ inch needle) of a ketamine (100mg/ml), xylazine (20mg/ml), and acepromazine (10mg/ml) cocktail. Sensitivity to noxious stimuli (foot pinch) was assessed and more anesthetic was administered if necessary (in 0.5 cc increments) until the animals were rendered insensate. Once unresponsive, animals were transcardially perfused with 100-200ml 0.9% phosphate-buffered solution (PBS) followed by 200-300ml 4% paraformaldehyde until the upper body was stiff. After removal from the skull, brains were post-fixed for 24 hours in 4% paraformaldehyde and then refrigerated in 30% sucrose. Brains were cut in 50μm coronal sections at -20° C using a freezing-stage sliding microtome (Leica Microsystems, SM2000R, Nussloch, Germany) and stored in cryoprotectant in 96-well microliter plates at -20° C.

For CldU and IdU immunohistochemistry, every sixth section was pre-treated to inactivate the endogenous peroxidase for 10 minutes in 0.1 M tris-buffered saline (TBS) and 0.3% hydrogen peroxide (TBS-per). Sections were rinsed six times in TBS for 10

minutes each. Sections were then incubated in 2N HCl at 37° C for 10 minutes. Next, sections were washed in 0.1 M borate buffer for 10 minutes and then rinsed six times for 10 minutes each in TBS. IdU and CldU sections were then blocked for 2 hours with 3% normal donkey serum (Sigma-Aldrich, St. Louis, MO, USA), followed by incubation at 4° C for 72 hours in primary antibody (antibody (mouse anti-BrdU, Becton Dickenson, Franklin Lakes, NJ, USA; 1:100; rat anti-BrdU, Accurate Chemical & Scientific Corporation, Westbury, NY, USA; 1:250). After two TBS rinses for 15 minutes each, sections were blocked with 3% normal donkey serum twice for 30 minutes each. The tissue was then incubated overnight at room temperature in secondary antibody (biotinylated donkey anti-mouse, Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:250; biotinylated donkey anti-rat, Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:250). Next, sections were rinsed three times in TBS for 15 minutes each, treated for 60 minutes in avidin-biotin complex (ABC, Vector Labs, Burlingame, CA, USA), and then rinsed three times in TBS for 15 minutes each. Sections were reacted and visualized with diaminobenzidine (DAB) and then rinsed four times in TBS for 10 minutes each, before being mounted onto gelatinized slides and allowed to dry overnight (Vega & Peterson, 2005).

For DCX immunohistochemistry, every sixth serial section was rinsed with TBS three times at room temperature for 10 minutes each. Sections were quenched for 30 minutes at room temperature in 0.6% hydrogen peroxide (to exhaust the activity of endogenous peroxidases) followed by three 10 minute washes in TBS. DCX sections were then blocked for 60 minutes in 3% normal donkey serum (Sigma-Aldrich, St. Louis, MO, USA), followed by incubation at 4° C for 72 hours in primary antibody (goat anti-

DCX, Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:100) (S. A. Morris et al., 2010). After two TBS rinses for 15 minutes each and 15 minutes blocking in 3% normal donkey serum, sections were incubated overnight at room temperature in secondary antibody (biotinylated donkey anti-goat, Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:250). Next, sections were rinsed three times in TBS for 10 minutes each, then treated for 60 minutes in avidin-biotin complex (ABC, Vector Labs, Burlingame, CA, USA) and then rinsed three times in TBS for 10 minutes each. Sections were reacted and visualized with DAB and then rinsed four times in TBS for 10 minutes each, before being mounted onto gelatinized slides and allowed to dry overnight.

Once all sections, IdU, CldU, and DCX, were dry, sections were counterstained with methyl green, a counterstain which enhances nuclear staining. All sections were then cleared in xylene, cover slipped using Protexx, and coded so that when the tissue was viewed under the microscope, the investigator was blind to experimental condition.

Stereology

Through increased control and random-sampling, design-based stereology is an unbiased and reliable estimate of the total number of cells in a particular brain region. This automated system maximizes efficiency by decreasing the human errors that are associated with other methods of quantification, e.g., decisions of size, shape, and distribution of the cells under examination (Kulesza, Vinuela, Saldana, & Berrebi, 2002). In order to determine the population of IdU+, CldU+, or DCX+ cells in the 50μm coronally sectioned dorsal and the ventral hippocampus, the optical fractionator probe was applied using a semi-automated stereology system (StereoInvestigator,

MicroBrightField, Williston, VT, USA) as the means of unbiased estimation of the total population of cells (Ritchie, De Butte, & Pappas, 2004).

Using a Nikon Eclipse 80i upright microscope, the region of interest was traced, using a 10x objective. The program then creates a two-dimensional counting frame formed by two inclusion and two exclusion lines; this frame was used to systematically sample sites within each contour. The counting frame and grid size were determined at the specified magnification by the size of the chosen region and the distribution of the cells within that region by the optical fractionators (Hattiangady et al., 2005). Cells were counted within the two-dimensional counting frames using a 40x oil objective for IdU+ cells and CldU+ cells (Brandt, Maass, Kempermann, & Storch, 2010) and 100x oil objective for DCX (Rao & Shetty, 2004). The average mounted section thickness were approximately 37µm, thus top and bottom guard zones were set at 5µm each, for an optical dissector height of 27µm.

Within the optical dissector zone, only the cells that came into focus and did not intersect the exclusion lines of the counting frame were considered positively stained somata and counted (Eadie et al., 2009; Holmes, Galea, Mistlberger, & Kempermann, 2004; Kempermann, Gast, Kronenber, Yamaguchi, & Gage, 2003; Kulesza et al., 2002). In order to preserve a conservative and systematic estimation, groups of cells where an accurate number could not be determined were counted as a single cell.

The estimated total of positive cells by mean measured thickness was calculated by the following formula: N_{est}= section fraction X area fraction X dissector fraction X number of counted cells. The variables for the neuronal estimate (N_{est}) equation are calculated from: a selected section containing IdU+ cells, CldU+ cells, or DCX+ cells

sampling uniformly spaced sections determined by the size of the brain region (*section fraction*), the sample of each counting frame across the number of sites visited within the contour enclosing (*area fraction*), the tissue reserved as guard zones (*dissector fraction*), and the number of IdU+ cells, CldU+ cells, or DCX+ cells counted (*number of cells counted*) (Kulesza et al., 2002). The precision of individual estimations was expressed by the coefficient of error (CE). The CE expresses the intra-individual variation due to stereological estimations (i.e. sampling of sections and counting locations). The accepted individual CE should be under 0.10 (Keuker, Vollman-Honsdorf, & Fuchs, 2001). Using this parameter, the obtained CE for both the dorsal DG and ventral DG was 0.045 (Hedou, Jongen-Relo, Murphy, Heidbreder, & Feldon, 2002; Keuker et al., 2001).

The IdU+, CldU+, and DCX+ somata were quantified in the granule cell layer of the DG in the hippocampus (Brandt et al., 2010; Rao & Shetty, 2004). Each soma located in the granule cell layer and subgranular zone, defined as up to two cell bodies from the granule cell layer (S. A. Morris et al., 2010; Redila et al., 2006), were counted in every sixth section in a single hemisphere of the DG. The counting frame for the granule cells layer was set at 40 x 40 µm and the grid size was 60 x 60 µm (Hattiangady et al., 2005; Rao & Shetty, 2004). For analysis of hippocampal subregions, the dorsal and ventral portions were separately quantified for IdU+ somata, CldU+ somata, and DCX+ somata beginning at Bregma -1.88mm and ending at Bregma -4.30mm and beginning at Bregma -4.52mm and ending at Bregma -6.04mm for dorsal and ventral respectively (Hawley & Leasure, 2011; Wolf et al., 2002) (See Figure 9).

Proteins Assessments

Following the CUS paradigm (control, *n* = 7; stress, *n* = 6) and the RAWM exposure (control, *n* = 6; stress, *n* = 6), animals were sacrificed to generate a profile of BDNF, proBDNF, and PSD-95 expression in subregions of the DG (See Figure 4). Under deep anesthesia, hippocampal tissue was harvested and positioned on a filter paper soaked in 0.2 M sucrose (to avoid the brain from sticking to the filter paper) over a covered petri dish containing dry ice. The hippocampus was extracted and dissected into three sections: dorsal, ventral, and middle. A middle area was discarded to ensure that samples from the dorsal and ventral subregions did not overlap (Tran, Srivareerat, & Alkadhi, 2010). Using a light microscope, the dorsal and ventral subregions were then placed in a vertical position to dissect the DG (Tran et al., 2010). Subregions of the DG from both hemispheres were stored at -80° C until homogenized for processing (Alkadhi, Srivareerat, & Tran, 2010; Tran et al., 2010).

To prepare tissue from the right hemisphere for immunoblotting, protein extracts from the hippocampal tissue were homogenized separately in 200µl of lysis buffer cocktail (150mM NaCl, 10mM HEPES, 10nM EGTA) and supplemented with 100x protease and phosphatase inhibitors (ThermoScience, Rockford, IL) with a sonicator at a medium speed for 5 seconds, repeated four times. The homogenates were then centrifuged at 14,000g for 15 minutes at 4°C. The supernatant was then removed and stored at -80°C.

The total protein concentration was estimated using a bicinchoninic acid (BCA) assay (Pierce Chemical, BCA protein Assay Kit, cat#23227, Rockford, IL). Following the kit instructions in the microtiter format, samples were prepared in triplicate at a 1:50

dilution within a final volume of 200µl. After a 30 minute incubation at 37°C, the absorbance was measured at 562nm using a VersaMAX reader (Molecular Devises, LCC, Sunnyvale, CA) and analyzed with Softmax Pro 5.2 software (Molecular Devises, LCC, Sunnyvale, CA). The data analysis masked the individual values that were outliers and achieved and r-square value of >0.98 for the fit curve. Protein concentrations were then determined from the generated log-log fit standard curve. From the known protein concentration, protein concentrations were adjusted to 2µg/µl using distilled water and 1X loading buffer (2% SDS, 62.5 mM Tris, 10% glycerol, 1% β-mercaptoethanol, 0.01% bromophenol blue, pH 6.8). The resulting mixture was incubated at 95 ^{o}C for 5 min and then left at room temperature until cool. Once at room temperature, samples were stored at -20 ^{o}C until utilized (Boehme et al., 2011).

For mature BDNF and proBDNF, proteins were resolved on 17% SDS/PAGE gels (Milli Q H2O, 0.39 M Tris pH8, 10% w/v acrylamide, 0.2% w/v SDS, 0.1% w/v APS, 0.4 ul/mL TEMED). Gels were loaded with 7ul of Kaleidoscope molecular weight marker (BioRad, Hercules, CA) and 30ug of protein. Gels were run in SDS running buffer (25mM Tris-base, 192mM Glycine, 0.1% v/v SDS diluted in distilled water) at 90V for 10 minutes, followed by 120V for 2 hours. Proteins were then transferred onto a polyvinylidene fluoride (PVDF) membrane. Transfer was complete in transfer buffer for 1.5 hours at 45V at room temperature. Following transfer, membranes were stained with Ponceau S for visualization of total protein then rinsed in wash buffer (1X TBS with 0.05% Tween). Once rinsed, membranes were blocked for an hour at room temperature with continual mixing using 5% skim milk in TBS (Beckon Dickenson and Company, Sparks, MD, USA) with 0.05% Tween-20 (BDNF) and 5% skim milk in PBS with 0.05%

Tween-20 (Bactin). Membranes were subsequently washed 3 times for 5 minutes with wash buffer (TBS with 0.05% Tween for BDNF and PBS with 0.05% Tween for Bactin) with mixing. Following washing, membranes were incubated in primary antibody (polyclonal rabbit anti-BDNF, 1:1000, Chemicon; polyclonal mouse anti-B-actin, 1:20000, Millipore) diluted in 5% bovine serum albumin (BSA) with 0.05% Tween20 overnight at 4^{o}C. Following four 5 minute washes in TBS with 0.05% Tween-20 (BDNF) or in PBS with 0.05% Tween-20 (Bactin), membranes were incubated with secondary antibody (goat anti-rabbit, 1:15000, KHL and goat anti-mouse, 1:5000, KHL) in secondary antibody dilution buffer (5% skim milk in TBS with 0.05% Tween for BDNF and 5% skim milk in PBS with 0.05% Tween for Bactin) for 1 hour at room temperature. Blots were washed as above prior to development (Boehme et al., 2011).

For PSD-95, proteins were resolved on 10% SDS/PAGE gels (Milli Q H2O, 0.39 M Tris pH8, 10% w/v acrylamide, 0.2% w/v SDS, 0.1% w/v APS, 0.4 ul/mL TEMED). Gels were loaded with 7ul of Kaleidoscope molecular weight marker (BioRad, Hercules, CA) and 30ug of protein. Gels were run in SDS running buffer (25mM Tris-base, 192mM Glycine, 0.1% v/v SDS diluted in distilled water) at 90V for 10 minutes, followed by 120V for 2 hours. Proteins were then transferred onto a PVDF membrane. Transfer was complete in transfer buffer overnight at 40V at 4 ^{o}C. Following transfer, membranes were stained with Ponceau S for visualization of total protein then rinsed in water. Once rinsed, membranes were blocked for an hour at room temperature with continual mixing using 5% skim milk in TBS with 0.05% Tween-20 (PSD95) and 5% skim milk in PBS with 0.05% Tween-20 (Bactin). Membranes were subsequently washed 3 times for 5 minutes with wash buffer (TBS with 0.05% Tween for PSD95 and PBS with 0.05%

Tween for Bactin) with mixing. Following washing, membranes were incubated in primary antibody (mouse anti-PSD95, 1:500, Chemicon; mouse anti-Bactin, 1:2000, Millipore) diluted in 5% BSA with 0.05% Tween20 overnight at 4^{o}C. Following four washes in TBS with 0.05% Tween-20 (PSD85) or in PBS with 0.05% Tween-20 (Bactin), membranes were incubated with secondary antibody (goat anti-mouse, 1:5000, KHL) in secondary antibody dilution buffer (5% skim milk in TBS with 0.05% Tween for PSD95 and 5% skim milk in PBS with 0.05% Tween for Bactin) for an hour at room temperature. Blots were then washed as above prior to development.

For the development of all proteins, bands were visualized using an enhanced chemiluminescence (ECL Plus, GE Healthcare, Buckinghamshire, UK) detection method. Band intensity was assessed using a BioRad Gel Doc Imaging System with Quantity One software (BioRad, Hercules, CA). To produce an image, developed film was placed on a UV to White light converter screen and exposed to white light. Using the image, protein quantification was assessed from the adjusted band intensity using the volume rectangle analysis tools and linear regression methods. Each sample value was divided by the total protein loading value (the intensity of Bactin) and subtracting local background (Boehme et al., 2011). Samples were expressed as optical density and compared across conditions and time points.

Statistics

The number of animals proposed for this study was estimated with power analyses indicating an *n* of 30 required for each condition, for a total of 60 animals. Specifically, power (delta ≥ 0.80) to detect a moderate to large effect (Cohen's d f = 0.25-0.4) using an analysis of variance (ANOVA) requires an *n* of 15 animals per group for

behavioral (RAWM) and an *n* of 9 animals per group for stereology measures (CldU, IdU, and DCX), for a total of 24 animals per condition. However, separate animals were used for western blot analyses since brain tissue must be harvested fresh. Based on previous effect sizes, in order to detect a moderate to large effect (Cohen's d f = 0.25-0.4) using an ANOVA only requires an *n* of 6 animals per group to detect significance for western blot analysis (BDNF, proBDNF, PSD-95), bringing the total number of animals needed per group (stress and control) to 30, for a total of 60 animals for the study. An extra animal was sent, and the animal was randomly assigned to the control condition, bringing the total number of control animals to 31. Figure 4 illustrates the number of animals in each condition and learning experience.

Behavioral, endocrinological, and neuroanatomical data were analyzed using a factorial ANOVA. When appropriate, dependent measures were also subjected to a repeated measure ANOVA to determine the effects of CUS across time points (e.g. RAWM learning acquisition, short-term memory trial, long-term memory trial, and corticosterone). Statistical significance was predetermined (alpha ≤ 0.05). All statistics were calculated in SPSS Statistics 17.0 (IBM SPSS Statistics, IBM, Chicago, IL, USA).

Results

The Effect of CUS on Stress Behavior

Only animals in the stressed groups ($n = 30$) were exposed to the CUS. During CUS, stressed animals were exposed to the tail clip three times (See Table 1). Each time the non-invasive clip was placed at the base of the animal's tail for 5 minutes, the following behaviors were recorded: latency to attend to the clip, time spent attending to

the clip, and time spent freezing. A one-way repeated measure ANOVA was used to compare the tail clip measures across the three time points of exposure. There was not a significant difference in the amount of time that the animal spent freezing when exposed to the tail over the three time points of exposure, $F < 1$(See Figure 10). There was also not a significant difference on the latency to contact the tail clip between the three time points of exposure during CUS $F < 1$ (See Figure 11). There was a significant increase on the amount of time spent attending to the tail clip on the third exposure, $F(2,58) = 14.20$, $p < 0.001$ (See Figure 12).

Animals were also exposed to the open field on three different days (See Table 1). Each time the animal was placed in the open field for 5 minutes the following behaviors were recorded: latency to enter the inner area and time spent in the inner area. A one-way repeated measure ANOVA was used to compare the open field measures across the three time points of exposure. There was not a significant change in the latency to enter the inner area of the open field, $F < 1$ (See Figure 13) nor was there a difference on the time spent in the inner area of the open field, $F < 1$. (See Figure 14).

See Table 2 for a summary of these stress-induced behavioral changes on quantified CUS tasks.

The Effect of CUS on Corticosterone Levels

Fecal boli samples were collected to assess corticosterone concentrations in 12 randomly selected animals (control + RAWM, $n = 6$; stress + RAWM, $n = 6$). Baseline levels of corticosterone were collected from fecal boli after animals had acclimated to their environment for a week but before CUS commenced (See Figure 4). In order to see what impact CUS and the RAWM had on corticosterone concentrations, fecal boli

samples were collected 24 hours after the last stressor and again following the long-term memory trial for the RAWM (See Figure 4).

There was a significant main effect of time point, $F(2,20) = 18.47$, $p < 0.001$), significant main effect of group, $F(1,10) = 8.59$, $p < 0.025$, and a significant group by time point interaction, $F(2,20) = 7.54$, $p < 0.05$). Post hoc comparisons demonstrated that before CUS commenced, there was no significant corticosterone baseline difference between control and stressed groups, $p > 0.05$. At the end of CUS, stressed animals had significantly more corticosterone compared to control animals at the same time point, $p < 0.05$, and had more than doubled corticosterone levels compared to the stressed baseline, $p < 0.05$. Within the control group, from baseline to post CUS, there was no significant difference in corticosterone levels, $p > 0.05$. Following the RAWM, there was not a significant corticosterone difference between control and stressed animals, $p > 0.05$; however, corticosterone in the control condition significantly increased within-subjects from baseline to post RAWM, $p < 0.05$, , and from post CUS to post RAWM, $p < 0.05$. Within the stressed group, there was a significant increase from baseline to post RAWM, $p < 0.05$, but did not increase within the stressed condition from post CUS to post RAWM, $p > 0.05$(See Figure 15).

The Effect of CUS on Body Weight

Throughout the CUS paradigm body weights were monitored in both the control ($n = 31$) and stressed groups ($n = 30$), and body weights were analyzed before and after CUS (See Figure 4). For body weight, there was a significant main effect of group, $F(1,59) = 17.98$, $p < 0.001$, significant main effect of time, $F(1,59) = 22.59$, $p < 0.001$, and a significant group by time interaction, $F(1,59) = 4.75$, $p < 0.05$. Follow-up tests

indicated that at baseline, before CUS, there was no difference in body weight between groups, $p > 0.05$; however, following CUS control animals had gained significantly more weight than stressed animals, $p < 0.05$. Within the control group, animals gain weight from baseline to Post CUS, $p < 0.05$; whereas stressed animals did not, $p > 0.05$ (See Figure 16).

The Effect of CUS on the RAWM Task

Following CUS, control and stress animals were exposed to the RAWM to evaluate spatial learning and memory (control $n = 15$, stress $n = 15$) (See Figure 4). During the RAWM the latency to find the platform was recorded. During the learning acquisition phase, 12 learning trials, rats in both groups learned the task with latency to find the platform at equivalent rates, $p > 0.05$. A factorial ANOVA was used to analyzed group (control, stress) and time (12^{th} learning trial, short-term, long-term) differences. There was a significant main effect of group, $F(1,28) = 9.85$, $p < 0.005$, significant main effect of time, $F(2,56) = 5.43$, $p < 0.05$, and significant group by time interaction, $F(2,56) = 4.21$, $p < 0.05$. Post hoc comparisons illustrated that there was not a significant difference in the latency to find the platform between control and stressed animals at the end of the learning acquisition, the 12^{th} learning trial, $p > 0.05$, or 30 minutes later at the short-term memory trial, $p > 0.05$. At the long-term memory trial, 24 hours later, stressed animals found the platform significantly faster than control animals, $p < 0.05$. Within the control group, animals performed the same from the 12^{th} learning trial to the short-term memory trial, $p > 0.05$, and from the 12^{th} learning trial to the long-term memory trial, $p > 0.05$. From the short-term memory trial to the long-term memory trial control animals took significantly longer to find the platform, $p < 0.05$. Within the stressed group there

was not a significant difference in the latency to find the platform from the 12th learning trial to the short-term memory trial, $p > 0.05$the 12th learning trial to the long-term memory trial, $p > 0.05$, or from the short-term memory trial to the long-term memory trial, $p > 0.05$ (See Figure 17).

The number of errors an animal made during the RAWM was recorded. During the learning acquisition phase, 12 learning trials, both conditions learned the RAWM task at equivalent rates with respect to the number of errors, $p > 0.05$. A factorial ANOVA reveal a significant main effect of group, $F(1,28)$ 7.64, $p < 0.025$, significant main effect of time, $F(2,56) = 3.60$, $p < 0.05$, and a significant group by time interaction, $F(2,56) = 4.65$, $p < 0.025$. Post hoc comparisons demonstrated that there was not a significant difference in the number of RAWM errors between the control and stressed groups on the 12th learning trial, $p > 0.05$or at the shot-term memory trial, $p > 0.05$; however, control animals made more errors than stressed animals at the long-term memory trial, $p < 0.05$. Within the control group, animals made the same number of errors from the 12th learning trial to the short-term memory trial, $p > 0.05$from the 12th learning trial to the long-term memory trial, $p > 0.05$, and from the short-term memory trial to the long-term memory trial, $p > 0.05$. Within the stressed group there was not a significant difference in the number of RAWM errors from the 12th learning trial to the short-term memory trial, $p > 0.05$, the 12th learning trial to the long-term memory trial, $p > 0.05$, or from the short-term memory trial to the long-term memory trial, $p > 0.05$ (See Figure 18).

The Effect of CUS and RAWM Training on Neurogenesis

In order to harvest brain tissue and quantify CldU, IdU, and DCX, animals were sacrificed after CUS (control $n = 9$, stress $n = 9$) and after RAWM (control + RAWM $n = 9$, stress + RAWM $n = 9$) (See Figure 4).

After animals were sacrificed for IHC (See Figure 4), each brain was weighed. There were no significant differences across conditions on the weight of the brain, $F < 1$ (See Figure 19).

Cell Proliferation. In order to investigate cell proliferation in the dorsal and ventral DG, animals were injected 2 hours prior to sacrifice with CldU. There was a significant main effect of group, $F(3,32) = 9.43$, $p < 0.025$, significant main effect of subregion, $F(1,32) = 10.56$, $p < 0.001$, and significant group by subregion interaction, $F(3,32) = 12.00$, $p < 0.001$. Post hoc comparisons indicated that there was not a significant CldU difference between dorsal and ventral subregions in control animals, $p > 0.05$. The ventral DG had significantly less CldU than the dorsal DG in the control + RAWM, $p < 0.05$, stress, $p < 0.05$, and stress + RAWM groups, $p < 0.05$ (See Figure 20). Across conditions within the ventral DG, control animals had significantly more CldU than any other group, $p < 0.05$, and stressed animals had more CldU in the ventral DG compared to stress + RAWM animals, $p < 0.05$ (See Figure 21). Within the dorsal subregion, control animals had more cell proliferation, CldU+ cells, than any other group, $p < 0.05$ (See Figure 22 and 23).

Cell Survival. Animals were injected with IdU for the first 5 days of CUS in order for cell survival throughout CUS and RAWM to be quantified in the dorsal and ventral DG (See Figure 4). There was a significant group by subregion interaction, $F(3,32) =$

8.56, $p < 0.001$, and main effect of group, $F(3,32) = 11.05$, $p < 0.001$, but not a main effect of subregion $F < 1$. Post hoc comparisons revealed that there was no significant difference between subregions in the control group, $p > 0.05$, or control + RAWM group, $p > 0.05$. There were significantly fewer IdU+ cells in the ventral DG compared to the dorsal DG in stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ groups (See Figure24). Across groups within the ventral subregion of the DG, there was not a significant difference in the number of IdU+ cells between control and control + RAWM groups, $p > 0.05$, nor was there a difference between the stress and stress + RAWM groups, $p > 0.05$. However, there were significantly fewer IdU+ cells in both the stress, $p < 0.05$, and stress + RAWM, $p < 0.05$, groups compared to the control group(See Figure 25). Within the dorsal subregion of the DG, there was not a significant difference in the number of IdU+ cells between control and control + RAWM groups, $p > 0.05$, nor was there a difference in the stress and stress + RAWM groups, $p > 0.05$. However both control and control + RAWM groups had significantly more IdU+ cells in the dorsal subregion of the DG compared to the stress and stress + RAWM groups, $p < 0.05$ (See Figure 26 and 27).

Neurogenesis. DCX was quantified to examine neurogenesis in the dorsal and ventral DG. There was a significant main effect of group, $F(3,32) = 8.75$, $p < 0.025$, and a significant main effect of subregion, $F(1,32) = 8.62$, $p < 0.025$, but not a significant group by subregion interaction, $F < 1$. Post hoc comparisons illustrated that there was not a difference in DCX+ cells between the dorsal and ventral DG in control animals, $p > 0.05$. There were significantly fewer DCX+ cells in the ventral DG compared to the dorsal DG in control + RAWM, $p < 0.05$, stress, $p < 0.05$, and stress + RAWM, $p < 0.05$, groups (See Figure 28). Within the ventral DG, across groups, control animals had

significantly more DCX+ cells than any other group, $p < 0.05$. Between the two groups that experienced learning, the stress + RAWM had significantly more DCX+ cells than control + RAWM animals within the ventral DG, $p < 0.05$ (See Figure 29). Across groups within the dorsal DG, control animals had more DCX+ cells than any other group, $p < 0.05$. Within the dorsal DG, stress + RAWM animals had more DCX+ cells compared to control + RAWM animals, $p < 0.05$(See Figure 30 and 31).

The Effect of CUS and RAWM Training on Neuroprotective Proteins

In order to harvest brain tissue and quantify BDNF, proBDNF, and PSD-95, animals were sacrificed after CUS (control $n = 7$, stress $n = 6$) and after RAWM (control + RAWM $n = 5$, stress + RAWM $n = 6$) (See Figure 4). One animal from the control group and one animal from the stressed group were omitted from the protein data because there was too little protein to be detected. Mature BDNF, proBDNF, and PSD-95 were examined across groups (control, control + RAWM , stress, stress + RAWM) and subregions (dorsal, ventral).

BDNF is a critical neurotrophic factor which has been shown to regulate cell survival in the adult hippocampal DG (Barnabe-Heider & Miller, 2003; Lee, Duan, & Mattson, 2002). Through western blotting, mature BDNF was detected in both control and stress animals following CUS and following RAWM (See Figure 4). A factorial ANOVA revealed that there was not a significant main effect of group, $F < 1$, main effect of subregion, $F < 1$, or group by subregion interaction, $F < 1$ (See Figure 32). While there does seem to be a general trend with a decreased expression of mature BDNF in the ventral DG, this trend is not significant for any group. There was no significant

difference in the expression of BDNF across groups within the ventral subregion, $p > 0.05$(See Figure 33) or dorsal subregion, $p > 0.05$ (See Figure 34).

ProBDNF is the precursor of mature BDNF and was also quantified across groups in the dorsal and ventral DG through western blotting after CUS and after RAWM (See Figure 4). A factorial ANOVA revealed that there was a significant main effect of group, $F(3,18) = 4.56$, $p < 0.025$, significant main effect of subregion, $F(1,18) = 6.52$, $p < 0.05$, and a significant group by subregion interaction, $F(3,18) = 5.87$, $p < 0.001$ (See Figure 35). Post hoc comparisons revealed that control animals had a higher expression of proBDNF in the ventral DG compared to the dorsal DG, $p < 0.05$, but in the control + RAWM group the dorsal DG had a higher expression of proBDNF, $p < 0.05$. There was not a significant difference in proBDNF across subregions within the stress, $p > 0.05$, or stress + RAWM, $p > 0.05$, groups. Across groups within the ventral subregion of the DG, control animals had significantly more proBDNF than the control + RAWM animals, $p < 0.05$ (See Figure 36). Within the dorsal subregion, between-subjects, there was no significant difference in the expression of proBDNF between control and stressed animals, $p > 0.05$. However, control animals had significantly less proBDNF expression than the control + RAWM, $p < 0.05$, and stress + RAWM groups, $p < 0.05$ (See Figure 37).

The Effect of CUS and RAWM Training on Synaptic Plasticity

PSD-95 was assessed in the dorsal and ventral DG across conditions. A factorial ANOVA found a significant main effect of group, $F(3,18) = 7.06$, $p < 0.001$, and main effect of subregion, $F(1,18) = 5.34$, $p < 0.05$), but did not reveal a significant group by subregion interaction, $F < 1$. Post hoc analyses revealed that there were no significant

differences in PSD-95 expression between subregions in the control, $p > 0.05$, control + RAWM, $p > 0.05$, stress, $p > 0.05$, or stress + RAWM, $p > 0.05$ groups (See Figure 38). Within the ventral subregion of the DG, control animals had significantly less PSD-95 expression than the control + RAWM, $p < 0.05$, and stress, $p < 0.05$ groups, but was not significantly different from the stress + RAWM group, $p > 0.05$ (See Figure 39). Across groups within the dorsal DG, stress + RAWM animals had a significantly higher expression of PSD-95 than control, $p < 0.05$, control + RAWM, $p < 0.05$, and stress, $p < 0.05$animals (See Figure 40).

See Table 3 for a summary of the corticosterone, stereology, and western blot results from the present study.

Discussion

Although the stress response is adaptive when acute, it becomes toxic when stress is chronic (Dumas, Gillette, Ferguson, Hamilton, & Sapolsky, 2010). Investigators have examined the effects of chronic stress on learning and memory for decades (Bangasser & Shors, 2010; Conrad, 2010). When evaluating the effects of chronic stress on hippocampal-dependent functions, researchers have produced conflicting results on spatial tasks (Conrad, 2010). One possible reason for the conflicting evidence surrounding chronic stress and learning could be related to the observation that investigators have generally examined the hippocampus, specifically the DG, as a single unit. Considering the large size of the hippocampus and the differing afferent and efferent neural projections in the rodent brain, it is not surprising that the DG has multiple functions and that those functions may have various roles in the stress response.

Regionally-specific neuroplastic changes in the dorsal and ventral subregions of the hippocampus suggest that the hippocampus may play a dual role in the stress response. Whereas previous studies have indicated that only the ventral subregion was impacted by chronic stress (Bannerman et al., 2004; Richmond et al., 1999), it is possible that both hippocampal subregions are impacted by stress, but in different ways. The ventral subregion may be principally involved in the emotional response to the stressor, whereas the dorsal subregion may be mainly engaged in the behavioral aspects of the stress response, such as avoidance or amelioration of the stressor (Hawley & Leasure, 2011). In my previous research, since neuroplasticity was preserved in the dorsal, spatial oriented subregion of the DG in animals exposed to CUS, I predicted that chronically stressed animals would perform better than controls on a spatial task, such as the RAWM.

The Effect of CUS on Stress Behavior

CUS was the model of chronic stress used in the present study due to its ecologically relevant nature to the animal (Hawley et al., 2010; Hawley & Leasure, 2011; Matthews et al., 1995). Additionally, CUS is a model of chronic stress that mimics the unpredictable quality of chronic stressors that humans experience (Bielajew et al., 2003; Hawley & Leasure, 2011; Matthews et al., 1995). Animals in the stressed conditions were exposed to two tasks during CUS where behavioral responses were assessed: the tail clip task and the open field task. Animals were exposed to both tasks three times throughout the paradigm (See Table 1). During the tail clip task the latency to contact the clip, the time spent attending to the clip, and the time spent freezing were evaluated. There was not a difference on the amount of time spent freezing or the latency to contact the clip across all time points on the tail clip task. On the last time point, there was a

significant spike in the amount of time that stress animals spent attending to the tail clip. This spike does not indicate habituation to the paradigm, rather it suggests that the animals were increasingly irritated and actively attempting to cope with the tail clip (Hawley et al., 2010). If the animals were adapting to the paradigm there would be a significant decrease in the amount of time spent attending to the clip (Hawley et al., 2010; Matthews et al., 1995).

For the open field task, the latency to enter the inner, more vulnerable, area and the time spent in the inner area were recorded. Across all three exposures to the open field task, there were not differences on any of the dependent measures. If the animals were adapting to the open field they would have spent increasing amounts of time in the inner area across the three exposures. The tail clip data and the open field data indicate that the nature of the chronic stress paradigm in the present study was in fact unpredictable and animals were unable to adapt to the chronic stress paradigm (Harris et al., 1997; Jayatissa et al., 2008; Kim et al., 2003; Lawler et al., 1985).

The Effect of CUS on Corticosterone Levels

Following the activation of the HPA-axis in response to a stressor, corticosterone is released from the adrenal glands (Campbell et al., 2003; Cavigelli et al., 2005). The stress hormone corticosterone enhances behaviors necessary for survival in emergency situations (De Kloet et al., 2005). In the present study, animals were individually housed so that each sample of fecal boli was directly linked to the correct animal, and the stress of establishing social hierarchies within cages was removed (Kozorovitskiy & Gould, 2004; Thanos et al., 2009). Since all animals were given a full week to adapt to the housing conditions, and both groups, control and stress, experienced social isolation, it is

not likely that individual housing confounded the results from the present study (Cavigelli et al., 2005; Kozorovitskiy & Gould, 2004). Consistent with the literature, and as hypothesized, before CUS baseline levels of corticosterone were similar; however, following exposure to CUS, stress animals had significantly more corticosterone that control animals (Hawley et al., 2010). More specifically, stressed animals had more than double the baseline levels of corticosterone within-subjects following CUS exposure. High corticosterone was a physiological confirmation of the stressful nature of CUS in the present study (Cavigelli et al., 2005; Hawley & Leasure, 2011; Larsen et al., 2010; Thanos et al., 2009).

Interestingly, following RAWM experience, there was not a significant difference in levels of corticosterone from post CUS to post RAWM within stressed animals. Alternatively, within control animals, there was a significant increase in the amount of corticosterone with RAWM experience. However following the spatial learning task, there was not a statistical difference in corticosterone between control and stressed animals. The increase in corticosterone in control animals indicates that the spatial learning task itself was a stressful experience. Control animals that experience learning, also experienced acute stress.

MR and GR establish a balance and mediate stress responsiveness (Groeneweg et al., 2012). After a period of enhanced levels of corticosterone during CUS, it is probable that stressed animals established a new balance between MR and GR. Conversely, the RAWM experience disrupted the established balance in MR and GR in control animals, which may have enhanced the vulnerability to the stress associated with the spatial

learning task causing an increase in corticosterone (De Kloet et al., 2005; De Kloet et al., 1998; McEwen, 2008).

The Effect of CUS on Body Weight

CUS has been shown to produce typical signs of chronic stress exposure, including: elevated corticosterone, increased cardiovascular responses, decreased weight gain, and decreased neuroplasticity (Hawley et al., 2010; Hawley & Leasure, 2011; Kim et al., 2003; Larsen et al., 2010; Li et al., 2010; Lin et al., 2005; McFadden et al., 2011; McGuire et al., 2010). For an additional physiological confirmation of the nature of the chronic stress paradigm in the current study, body weights for control and stressed animals were recorded before and after CUS. At baseline, before the onset of CUS, control and stressed groups had statistically similar body weights. Consistent with the chronic stress literature, during the CUS paradigm, control animals continued to gain weight, whereas stressed animals did not (Hawley et al., 2010; Hawley & Leasure, 2011). The decreased weight gain and increased corticosterone in stressed animals are physiological confirmations of the stressful nature of CUS.

The Effect of CUS on the RAWM Task

In my previous study, markers of neuroplasticity, NPY and ΔFosB, were preserved specifically in the dorsal DG of the hippocampus following CUS (Hawley & Leasure, 2011). Since neuroplasticity was preserved in the dorsal, spatial oriented subregion of the DG in animals exposed to CUS, I predicted that chronically stressed animals would perform better than controls on a spatial task, such as the RAWM.

The RAWM is a hippocampal-dependent task assessing spatial learning and memory performance (Buresova et al., 1985; Diamond et al., 1999). The well-

established RAWM is a combination of the MWM and the RAM (Buresova et al., 1985). The RAWM is advantageous because it combines spatial complexity and efficient learning associated with the RAM and the MWM, while eliminating the need for extra controls to determine strategy or motivation and without food deprivation (Berchtold et al., 2010; Conrad, 2010; Diamond et al., 1999; Shukitt-Hale et al., 2004). Furthermore, the RAWM maintains the advantage of the RAM where short-term and long-term memory can be accessed (Buresova et al., 1985). In the current investigation, spatial navigation in the RAWM was used to assess adult rats following exposure to CUS (Hawley et al., 2010; Hawley & Leasure, 2011).

During the 12 learning acquisition trials, animals from both groups, control and stressed, learned the task at equivalent rates in respect to the latency to find the platform and the number of errors. On the last learning trial, there were no statistical differences between conditions on any dependent measure indicating that all animals had learned the task the same. Thirty minutes after the last learning trial, control and stressed animals performed statistically similar on the short-term memory trial. However, 24 hours later, control animals took longer to find the platform and made more errors compared to the stressed animals. It is possible that the increase in corticosterone from the acute stress of the RAWM, facilitated in decreased spatial memory in control animals.

While it has been shown that chronic stress impairs spatial learning and memory (Conrad, 2010), it appears that the spatial memory deficit following chronic stress is dependent on type of spatial memory task used, for example the RAWM or MWM. Chronic stress impairs spatial learning on food motivated tasks, such as the RAM, a task that evokes a relatively mild arousal component. However, under testing conditions

where there is a strong arousal component, such as RAWM, chronic stress appears to have minimal impairing effects or may even facilitate spatial learning (Conrad, 2010). The idea that the amount of arousal a learning tasks exerts directly mirrors the Yerkes-Dodson Law which suggests an inverted-U-shaped relationship between arousal and memory consolidation (Andreano & Cahill, 2006; Diamond, Campbell, Park, Halonen, & Zoladz, 2007; Yerkes & Dotdson, 1908) (See Figure 41). In relation to the Yerkes-Dodson Law, and considering the already established elevated levels of corticosterone in stressed animals, it is probable that they were physiologically trained to respond to another highly stressful task; whereas, control animals were physiologically unprepared for the spatial task with acutely elevated levels of corticosterone. This suggested that CUS enhances long-term spatial memory on the RAWM.

In addition to the specificity of the type of spatial learning and memory task, the memory performance is also dependent on the type and duration of the previous stressor (Conrad, 2010). For example chronic psychosocial stress decreases spatial abilities on the RAWM (A.M. Aleisa et al., 2006; Alzoubi et al., 2009); whereas, acute social defeat stress improves spatial abilities on the RAWM (Blanchard et al., 2001; Krugers et al., 1997). Furthermore, it should also be noted that within a chronic stressor, if the animal has the ability to freely respond to the stressor, then spatial memory may be enhanced (Miyake et al., 2012). Animals exposed to CRS have spatial learning and memory impairments, but if those animals are given the option to chew on a stick while in the restraint tube, they outperform control animals on the MWM (Miyake et al., 2012). The chewing stick serves as potential coping mechanism for the animal, which improves spatial learning and memory abilities. Similarly in the present study, stressed animals

were free to respond to or ignore the stressors. For example, since stressed animals were given the predator odor on a cotton ball in their home cage, animals could bury the cotton ball containing a predator odor or avoid it. Therefore, despite the high levels of corticosterone, CUS animals had a perceived sense of control during chronic stress, and outperformed control animals on tests of spatial navigation (Hutchinson et al., 2012; Miyake et al., 2012; Sapolsky, 1998). Stress where an animal has the ability to respond to stressors, in combination with stress being chronic enabling physiological adaptation or training to stress, enhanced long-term spatial memory in stressed animals.

The Effect of CUS and RAWM Training on Neurogenesis

The survival of neural progenitor cells in the hippocampus is diminished in animals exposed to chronic stress (Duman, 2009; Mirescu & Gould, 2006); further, previous investigations indicated a region-specific effect such that animals exposed to CUS have fewer proliferating cells in the ventral, but not the dorsal subregion of the hippocampus (Hawley & Leasure, 2011). Therefore, I hypothesized that cell proliferation (CldU), cell survival (IdU), and neurogenesis (DCX) would again be preserved in the granule cell layer of the dorsal DG in animals following CUS and the RAWM, but reduced in the ventral subregion of the DG compared to control animals.

Cell proliferation, marked by CldU, is the generation of cells. With the elevation of corticosterone via acute or chronic stress, there was a decrease in cell proliferation in both the dorsal and ventral DG. Since CldU was injected 2 hours prior to sacrifice, it is probable that these cells were highly sensitive the effects of corticosterone, thus both the acute stress of the RAWM experience and CUS exposure decreased cell proliferation.

However, there was a greater decrease of corticosterone on the ventral DG compared to the dorsal DG in both acutely and chronically stressed animals.

In the previous investigation, cell survival, marked by BrdU, was significantly lower in the ventral DG in stressed animals compared to control animals (Hawley & Leasure, 2011). Similarly, in the present study, cell survival, cells that were generated at the beginning of the study and survived to the end, were marked by IdU. It is likely that there was not a decrease in IdU in control + RAWM animals because IdU was less immediate compared to CldU and cells were more mature at the end of the 2 weeks. In stressed animals there was significantly less cell survival in the ventral DG compared to control animals. Additionally, in the present study, IdU in stressed animals was also lower in the dorsal DG compared to control animals. However, within the stressed conditions, there was a significant decrease in cell survival in the ventral DG compared to the dorsal DG, thus replicating the pattern of BrdU found in the previous study.

Consistent with the literature, neurogenesis (DCX) was decreased in CUS animals (Cameron & Gould, 1994; Gould & Tanapat, 1999; Mirescu & Gould, 2006), but also in acutely stressed animals in the control + RAWM group (Kutsuna, Suma, Takada, Yamashita, & Katayama, 2012). Within the stressed conditions, there were fewer DCX+ cells in the ventral, affect-related, subregion of the DG compared to the dorsal DG. The control animals that experienced the acute stress of RAWM, also had a significant decrease in DCX+ cells in the ventral DG compared to the dorsal DG, which is consistent with the decreased expression of DCX following acute stress literature (Ho & Wang, 2010; Kutsuna et al., 2012; D. Yang et al., 2011). It is probable that stress + RAWM animals had more DCX compared to control + RAWM animals because they had been

learning to cope with stress for two weeks, during CUS, prior and therefore were more prepared for the RAWM task.

I hypothesized that cell proliferation, cell survival, and neurogenesis would be preserved in the dorsal DG but reduced in the ventral DG. In stressed animals, with elevated levels of corticosterone, cell proliferation (CldU), cell survival (IdU), and neurogenesis (DCX) were decreased in both the dorsal and ventral DG compared to control animals. Within the stressed conditions, there was an apparent decreased in CldU, IdU, and DCX within the ventral DG compared to the dorsal DG. Within stressed conditions cell proliferation (CldU), cell survival (IdU), and neurogenesis (DCX) were higher in the dorsal, spatial oriented, DG. Following CUS and RAWM, cell proliferation, cell survival, and neurogenesis were not specifically preserved in the dorsal DG, but there was a greater decrease in the ventral DG compared to the dorsal DG supporting my dual role of the hippocampal response to chronic stress hypothesis.

The Effect of CUS and RAWM Training on Neuroprotective Proteins

Neurotrophic factors are critical proteins for cell growth, survival, and the differentiation of new neurons (Alhaider et al., 2010; Barde, 1989; Duman, 2009; Lewin & Barde, 1996; McAllister, 2002). BDNF is a neurotrophin with various effects on the nervous system, such as neuronal growth, differentiation, and repair (Alonso et al., 2002; Aydemir et al., 2006; Kiprianova et al., 1999). Within the hippocampus, BDNF is essential for plasticity, neurogenesis, learning and memory, and hippocampal LTP (Aydemir et al., 2006; Boehme et al., 2011; Duman, 2009; Kiprianova et al., 1999; Lessmann et al., 1994; Martinowich et al., 2007). BDNF has also been reported to increase in the hippocampus after spatial learning tasks (Falkenberg et al., 1992; Mizuno

et al., 2003). Following three weeks of CUS, the expression of BDNF has been shown to be higher in the dorsal DG of the hippocampus compared to the ventral DG (Larsen et al., 2010). Given that the production of neurotrophins may enhance the integrity of the hippocampus (Raivich et al., 1999), the region-specific expression of neuroprotective proteins was investigated. In the previous study, cell survival was preserved in the dorsal DG; therefore, in the present study it was predicted that following CUS and the RAWM, stressed animals would have significantly more BDNF in the dorsal subregion compared to the ventral subregion in order to promote cell survival.

Neurotrophins, like BDNF, are synthesized from proneurotrophins (Martinowich et al., 2007; Teng et al., 2005). ProBDNF is the precursor of mature BDNF (Barker, 2009; Barnes & Thomas, 2008; Holm et al., 2009; Martinowich et al., 2007; J. Yang et al., 2009). Similar to BDNF, proBDNF is released from hippocampal neurons and mediates hippocampal functions, such as LTP (Arancio & Chao, 2007; Barker, 2009; Lu et al., 2005; Pang et al., 2004) and memory (Barnes & Thomas, 2008). When proBDNF is not cleaved into mature BDNF there are long-term deficits in hippocampal plasticity (Pang et al., 2004), such as decreased LTP (Martinowich et al., 2007; Pang et al., 2004). Considering that proBDNF is essential for BDNF, I hypothesized that the expression of proBDNF would also be higher in the dorsal DG compared to the ventral DG following CUS and RAWM exposure in order to promote BDNF expression and ultimately, support cell survival.

While learning generally increases the expression of BDNF (Aydemir et al., 2006; Duman, 2009; Lessmann et al., 1994; Linnarsson et al., 1997), there was not a difference in the expression of BDNF across conditions or subregions of the DG in the present

study. It is possible that because there was high variance in BDNF, significance was not detected. However, more probable, since animals were sacrificed so quickly after the learning experience (24 hours), it is possible that there was not enough time for BDNF to be cleaved from proBDNF (Larsen et al., 2010; Nair et al., 2007). Additionally, a study that quantified BDNF in the hippocampus, found an increase in the dorsal DG following 3 weeks of CUS (Larsen et al., 2010). Since my animals were only exposed to CUS for 2 weeks, it is possible that had animals been exposed to chronic stress for another week, I could have replicated previous mature BDNF results in subregions of the hippocampus. Despite a nonsignificant increase of mature BDNF in the DG, one could speculate that the learners were in the process of cleaving proBDNF to mature BDNF in order to promote spatial learning due to the increase of proBDNF in the dorsal DG. Given the increased expression of proBDNF in the dorsal DG, I suspect that BDNF expression would be significantly higher in the dorsal DG if there was more time following RAWM before animals were sacrificed.

Similar to the stipulation that memory is dependent on the paradigm of chronic stress and type of spatial learning task (Conrad, 2010), BDNF expression is also dependent on the hippocampal region under investigation. In the present study there was not a significant increase in BDNF in either subregion of the DG following CUS or RAWM; however, it is possible that I could have found an increase in alternative hippocampal region (e.g. the CA1). Following psychosocial stress there is a decrease in BDNF expression in the CA1, but not the DG, as well as impaired long-term memory but not short-term memory on the RAWM (Alkadhi et al., 2010). Thus when investigating

the impact of chronic stress, the results are dependent on the type and duration of stress, the type of memory task, and the hippocampal brain region.

The Effect of CUS and RAWM Training on Synaptic Plasticity

PSD-95 is a postsynaptic scaffolding protein that is a critical regulator of synaptic development, strength, and plasticity (El-Husseini et al., 2000; Han & Kim, 2008). It is also a modulator of neuronal survival and function (Luo et al., 2012; Zhu et al., 2011). PSD-95 also plays a central role in the NMDA receptor signaling and mediates LTP (Han & Kim, 2008; Zhu et al., 2011). Since PSD-95 promotes synaptic plasticity, learning, memory, and LTP (Han & Kim, 2008; Zhu et al., 2011), and markers of plasticity (NPY and ΔFosB) were preserved in the dorsal DG but not the ventral DG following CUS (Hawley & Leasure, 2011), I hypothesized that PSD-95 would also be more abundant in the dorsal DG in order to preserve spatial learning following CUS. Within the ventral DG, PSD-95 increased significantly in conditions with elevated corticosterone (control and stressed animals), suggesting the increased need for synaptic plasticity in order to assess the affective component of stress. Within the dorsal DG, stressed learners had significantly more PSD-95 than any other condition. It is probable that the increase of PSD-95 in the dorsal DG encouraged synaptic plasticity and helped the stressed animals perform better than controls on the RAWM. Since there was not a region-specific effect of PSD-95, it could be assumed that both the dorsal and ventral DG were in need of synaptic plasticity in both conditions, control and stress, with elevated corticosterone.

Summary and Conclusions

Considering the pervasive nature of chronic stress in modern society, understanding how chronic stress impacts the hippocampus is crucial for the development

of effective coping strategies and pharmacological interventions. Regionally-specific neuroplastic changes in the dorsal and ventral subregions of the hippocampus suggest that the hippocampus may play a dual role in the stress response. Whereas previous studies have indicated that only the ventral subregion was impacted by chronic stress (Bannerman et al., 2004; Richmond et al., 1999), it is possible that both hippocampal subregions are impacted by stress, but in different ways. The ventral subregion may be principally involved in the emotional response to the stressor, whereas the dorsal subregion may be mainly engaged in the behavioral aspects of the stress response, such as avoidance or amelioration of the stressor (Hawley & Leasure, 2011).

Chronic stress has been shown to induce neuroplastic changes in the hippocampus, decrease the survival of progenitor cells in the hippocampus, and impair hippocampal-dependent learning and memory. In the present investigation, spatial navigation on the RAWM was assessed in rats following exposure to CUS, as well as neurogenesis and neuroprotective proteins in the dorsal and ventral DG of the hippocampus. Despite similarly elevated levels of corticosterone, stressed animals found the hidden platform faster and with fewer errors on the RAWM long-term memory trial compared to control animals. Furthermore, elevated coriticosterone in control and stressed animals exposed to the RAWM had decreased cell proliferation (CldU) and neurogenesis (DCX) in the ventral DG. Stressed animals also had decreased cell survival (IdU) in the ventral DG. Proteins proBDNF and PSD-95, which promote LTP and synaptic plasticity, were increased in the dorsal DG. Stressed animals had increased neuroprotective proteins and preserved neuroplasticity in the dorsal DG, which may have contributed to the improved spatial navigation abilities on the RAWM. These regionally-

specific neuroplastic changes suggest that the hippocampus does, in fact, play a dual role in response to chronic stress.

In addition to investigating how multiple hippocampal DG subregions are differentially affected by CUS, the current study suggests that chronic stress does not necessarily inhibit learning (Bangasser & Shors, 2010; Conrad, 2010). In contrast to previous research (Bangasser & Shors, 2010; Conrad, 2010), chronic stress may not impair all forms of hippocampal-dependent learning simultaneously (Conrad, 2010). During a challenging time (e.g. chronic stress), dorsal hippocampal functions (i.e. spatial-orienting and problem-solving) may be some of the later functions compromised by chronic stress, in order to efficiently engage in the behavioral aspects of the stress response.

The impact of the results from this study informs future investigators of chronic stress and learning at both the conceptual and methodological levels. This study supports that the hippocampus does respond to stress, acute and chronic, in a region-specific manner. Establishing the hippocampus as dual role responder to stress will likely encourage further investigations on hippocampal subregions in response to stress, such as LTP and prefrontal-dependent learning and memory tasks, such as object-recognition.

In summary, this study examines the potential dual role of the hippocampus in response to chronic stress. The subregions of the hippocampus may respond differently to chronic stress in an effort to efficiently cope with the stressor. While the ventral subregion may be principally involved in the emotional coping response to chronic stress (Bannerman et al., 2004), the dorsal subregion may be more engaged in the behavioral coping aspects (i.e. avoidance or escape) of the stress response. Consequently, efficient

behavioral responses to the RAWM following CUS may be facilitated by preserved neuroplasticity and neuroprotective proteins in the dorsal subregion of the hippocampus.

Table Captions

Table 1. Chronic Unpredictable Stress Schedule of Stressors.

Table 2. Stress-Induced Behavioral Changes on the Tail Clip and Open Field Tasks.

Table 3. Summary of Investigation Results.

Tables

Table 1. Chronic Unpredictable Stress Schedule of Stressors.

CUS Day	Time	Stressor
1	11am	vinegar water
	12pm	IdU/saline injections (57.5 mg/kg)
	1pm	white noise
2	11am	tail clip
	12pm	IdU/saline injections (57.5 mg/kg)
	2pm	predator sounds
3	6am	fox odor
	12pm	IdU/saline injections (57.5 mg/kg)
	2pm	strobe light
4	8am	white noise
	12pm	IdU/saline injections (57.5 mg/kg)
	6pm	tail clip
5	7am	altered light cycle
	12pm	IdU/saline injections (57.5 mg/kg)
	2pm	novel stimulus
6	10am	open field
	5pm	strobe light
7	5am	fox odor
	2pm	novel stimulus
8	10am	open field
	2pm	tilted cage
9	9am	vinegar water
	4pm	raccoon odor
10	6am	altered light cycle
	7pm	white noise
11	10am	tilted cage
	4pm	tail clip
12	12pm	open field
	6pm	damp bedding
13	9am	raccoon odor
	8pm	predator sounds
14	8am	vinegar water
	2pm	strobe light

Table 2. Stress-Induced Behavioral Changes on the Tail Clip and Open Field Tasks.

	Time Point 1	Time Point 2	Time Point 3
Tail Clip- latency to contact	30.77 ± 6.76	26.83 ± 4.13	20.5 ± 2.98
Tail Clip- time attending to clip	106.5 ± 8.58	107.0 ± 8.63	142.50 ± 11.60*
Tail Clip- time spent freezing	5.63 ± 2.19	6.23 ± 1.74	4.63 ± 1.79
Open Field- latency to enter inner area	93.83 ± 18.11	117.80 ± 21.96	88.53 ± 21.04
Open Field- time in inner area	3.30 ± 0.53	2.53 ± 0.70	3.03 ± 0.75

*$p < 0.05$ significantly different from Time Point 1

Table 3. Summary of Investigation Results.

	Pre CUS		Post CUS		RAWM	
	Control	Stress	Control	Stress	Control	Stress
Body Weight	365 ± 8.59	360 ± 12.22	413 ± 9.84	368 ± 7.71*	-	-
Corticosterone	570 ± 291	433 ± 157	610 ± 203	1389 ± 182*	1798 ± 258**	1607 ± 265**
Brain Weight	-	-	1.903 ± 0.13	1.811 ± 0.12	1.839 ± 0.09	1.895 ± 0.18
RAWM Latency-12th learning trial	-	-	-	-	16 ± 5	8 ± 4
RAWM Latency-Short-term memory trial	-	-	-	-	13 ± 4	11 ± 4
RAWM Latency-Long-term memory trial	-	-	-	-	27 ± 5	8 ± 1*
RAWM Errors-12th learning trial	-	-	-	-	1.2 ± 0.58	0.33 ± 0.34
RAWM Errors-Short-term memory trial	-	-	-	-	0.8 ± 0.46	0.67 ± 0.40
RAWM Errors-Long-term memory trial	-	-	-	-	2.67 ± 0.87	0.27 ± 0.15*

*p < 0.05 significantly different from control at the specified time point
**p < 0.05 significantly different from baseline within-subjects

	Dorsal				Ventral			
	Control	Control + RAWM	Stress	Stress + RAWM	Control	Control + RAWM	Stress	Stress + RAWM
CldU	3780 ± 216	2404 ± 247α	2656 ± 301α	2036 ± 264α	3744 ± 287	1873 ± 256*α	1998 ± 289*α	1428 ± 234*α
IdU	3541 ± 205	3252 ± 164	2311 ± 199α	2164 ± 178α	3847 ± 198	3605 ± 231	1759 ± 245*α	1653 ± 212*α
DCX	4577 ± 322	3319 ± 312α	3759 ± 269α	4104 ± 287α	4017 ± 336	2684 ± 245*α	3097 ± 312*α	3404 ± 264*α
BDNF	0.90 ± 0.04	1.23 ± 0.27	0.85 ± 0.20	0.90 ± 0.09	0.81 ± 0.13	0.80 ± 0.06	0.54 ± 0.19	0.72 ± 0.09
ProBDNF	0.48 ± 0.03	0.82 ± 0.14α	0.58 ± 0.10	0.78 ± 0.11α	0.64 ± 0.02*	0.50 ± 0.02*α	0.58 ± 0.04	0.63 ± 0.09
PSD-95	1.53 ± 0.15	1.79 ± 0.11	1.59 ± 0.11	2.37 ± 0.17α	1.28 ± 0.08	1.7 ± 0.09α	1.65 ± 0.08α	1.84 ± 0.32

*p < 0.05 significantly different from dorsal within-subjects
α < 0.05 significantly different from control within specified subregion

Figure Captions

Figure 1. Anatomically Distinct Subfields of the Hippocampus. There are five commonly accepted subfield of the hippocampus: CA1, CA2, CA3, CA4, and DG. This diagram illustrates the subfields and connecting pathways within the hippocampus. Image: (X. M. Zhao et al., 2001)

Figure 2. Diagram of the hippocampus in the rodent brain. The dorsal subregion is the more anterior portion of the hippocampus, while the ventral portion is more posterior. Diagram adapted from Eadie et al., 2009.

Figure 3. CUS Neuroplasticity in the Dentate Gyrus. Consistent with the literature, in our previous investigation (control $n = 17$, stress $n = 15$) CUS decreased the number of surviving cells (BrdU+ cells) in the DG of stressed animals compared to control animals. This decreased cell survival was specific to the ventral subregion (Panel A). Additionally, markers of neuroplasticity, NPY and ΔFosB, were significantly preserved in the dorsal subregion of the hippocampus (Panels B and C). BrdU, NPY, and ΔFosB are reported in mm^3 (Hawley & Leasure, 2011). *$p < 0.05$ significantly different from control.

Figure 4. Procedural Timeline for Current Investigation. A timeline of the procedure from the present study for all conditions: control, control + RAWM, stress, and stress + RAWM.

Figure 5. Diagram of the Neuronal Differentiation of Newborn Cells in the Adult Hippocampus. Type 2 cells, within the oval, lack glial features and express DCX. DCX is microtubule-binding phosphoprotein used to label newly generated post mitotic neuroblasts. Image adapted from Lucassen et al., 2010.

Figure 6. Measured Stressors During Chronic Unpredictable Stress. Animals in the stressed conditions were exposed to two tasks that observed various behaviors. A non-invasive tail clip was attached to the base of the animal's tail to measure the attention paid to the naturalistic stressor as the animal attempted to remove the clip (Panel A). In the open field task exploratory behaviors were measured (Panel B).

Figure 7. Open Field Diagram. A diagram of the grid drawn onto the bottom of the open field, where exploratory behaviors were measured in animals in stressed animals during the CUS paradigm.

Figure 8. Radial Arm Water Maze Diagram. The Radial Arm Water Maze which as used to examine spatial learning and memory in both control and stressed rats.

Figure 9. Coronal Sections of the Dorsal and Ventral Dentate Gyrus. The DG of the hippocampus was separately quantified for IdU+ somata, CldU+ somata, and DCX+ somata (via stereology), and BDNF, ProBDNF, and PSD-95 (via western blots), beginning at Bregma -1.88mm and ending at Bregma -4.30mm and beginning at Bregma -4.52mm and ending at Bregma -6.04mm, for the dorsal and ventral respectively (Hawley & Leasure, 2011; Paxinos & Watson, 1998).

Figure 10. Time Spent Freezing During the Tail Clip Task. A repeated measures one-way ANOVA within stressed animals (n = 30) revealed no significant difference between time points of exposure on the time spent freezing on the tail clip task during CUS, $F < 1$. Mean ±SD. Time point 1, 5.63 seconds ± 2.19; Time point 2, 6.23 seconds ± 1.74; Time point 3, 4.63 seconds ± 1.79.

Figure 11. Latency to Contact the Tail Clip. A repeated measures one-way ANOVA within stressed animals (n = 30) revealed no significant difference between time points on the latency to contact the tail clip during CUS, $F < 1$. Mean ± SD. Time point 1, 30.77 seconds ± 6.76; Time point 2, 26.83 seconds ± 4.13; Time point 3, 20.5 seconds ± 2.98.

Figure 12. Time Spent Attending to the Tail Clip During CUS. A repeated measures one- way ANOVA within stressed animals (n = 30) revealed that there was a significant increase in the time spent contacting the tail clip with the upper body on the third exposure to the tail clip during CUS, $F(2,58) = 14.20$, $p < 0.001$. Mean ± SD. Time point 1, 106.5 seconds ± 8.58; Time point 2, 107.0 seconds ± 8.63; Time point 3, 142.50 seconds ± 11.60.

*$p < 0.05$ significantly different from Time point 1.

Figure 13. Latency to Enter the Inner Area of the Open Field Task During CUS.A repeated measures one- way ANOVA within stressed animals (n = 30) revealed no significant difference on the latency to enter the inner are of the open field during CUS, $F < 1$. Mean ± SD. Time point 1, 93.83 seconds ± 18.11; Time point 2, 117.80 seconds ± 21.96; Time point 3, 88.53 seconds ± 21.04.

Figure 14. Time Spent in the Inner Area of the Open Field During CUS. A repeated measures one- way ANOVA within stressed animals (n = 30) revealed no significant difference on the time spent in the inner area of the open field during CUS, $F < 1$. Mean ± SD. Time point 1, 3.30 seconds ± 0.53; Time point 2, 2.53 seconds ± 0.70; Time point 3, 3.03 seconds ± 0.75.

Figure 15. Corticosterone Concentrations (pg/ml) Across Conditions and Time. A factorial ANOVA between control (n = 6) and stressed (n = 6) animals revealed that there was a significant main effect of time point, $F(2,20) = 18.47$, $p < 0.001$), significant main effect of group, $F(1,10) = 8.59$, $p < 0.025$, and a significant group by time point interaction, $F(2,20) = 7.54$, $p < 0.05$). Between groups, there was not a significant difference at baseline, $p > 0.05$, or post RAWM, $p > 0.05$, but there was a significant difference at post CUS, $p < 0.05$. Within the stressed group there was a significant increase in corticosterone from baseline to post CUS, $p < 0.05$, but not between post CUS and post RAWM, $p > 0.05$. Within the control group, there was not a significant difference in corticosterone levels from baseline to post CUS, $p > 0.05$, but there was a significant increase from post CUS to post RAWM, $p < 0.05$. Mean ± SEM. Control, Baseline 570 pg/ml ± 291, Post CUS 610 pg/ml ± 203, Post RAWM 1798 pg/ml ± 258. Stress, Baseline 433 pg/ml ±157, Post CUS 1389 pg/ml ±182, Post RAWM 1607 pg/ml ±265.

*$p < 0.05$ significantly different from control.

#$p < 0.05$ significantly different from Baseline within the stressed condition.

**$p < 0.05$ significantly different from Post CUS within the control condition.

Figure 16. Body Weights (g) Across Conditions and Time. A factorial ANOVA between control (n = 31) and stressed (n = 30) animals revealed that there was a significant main effect of group, $F(1,59) = 17.98$, $p < 0.001$, and significant main effect if time, $F(1,59) = 22.59$, $p < 0.001$, as well as a significant group by time interaction, $F(1,59) = 4.75$, $p < 0.05$. Post hoc comparisons demonstrated that at baseline there was not a difference in body weight between conditions, $p > 0.05$, but control animals were significantly heavier than stress animals post CUS, $p < 0.05$. Control animals continued to gain weight from baseline to post CUS, $p < 0.05$, whereas stressed animals did not, $p > 0.05$. Mean ± SD. Control animals Baseline 365 g ± 8.59, Post CUS 413 g ± 9.84. Stress animals Baseline 360 g ± 12.22, Post CUS 368 g ±7.71.

*$p < 0.05$ significantly different from baseline control.

Figure 17. The Latency to Find the Platform in the RAWM. During the learning acquisition, the first 12 trials of the RAWM, control (n = 15) and stressed (n = 15) animals learned the task at equivalent rates, $F < 1$, as indicated by the similar decrease in latency to find the platform in both groups. A factorial ANOVA between groups revealed that there was a significant main effect of group, $F(1,28) = 9.85$, $p < 0.005$, significant main effect of time, $F(2,56) = 5.43$, $p < 0.05$, and significant group by time interaction, $F(2,56] = 4.21$, $p < 0.05$. Post hoc comparisons revealed that there was no significant difference between groups at the 12^{th} learning trial, $p > 0.05$, or the short-term memory trial, $p > 0.05$. At the long-term memory trial, control animals took longer than stressed animals to find the platform, $p < 0.05$. Mean ± SD. Control 12^{th} learning trial 16 seconds ± 5, short-term memory trial 13 seconds ± 4, long-term memory trial 27 seconds ± 5. Stress 12^{th} learning trial 8 seconds ± 4, short-term memory trial 11 seconds ± 4, long-term memory trial 8 seconds ± 1.

*$p < 0.05$ significantly different from control.

Figure 18. The Number of Errors in the RAWM. During the learning acquisition, the first 12 trials of the RAWM, control (n = 15) and stressed (n = 15) animals learned the task at equivalent rates, $F < 1$, as indicated by the similar decrease in the number of errors in both groups. A factorial ANOVA between groups revealed that there was a significant main effect of group, $F(1,28)$ 7.64, $p < 0.025$, significant main effect of time, $F(2,56) = 3.60$, $p < 0.05$, and a significant group by time interaction, $F(2,56) = 4.65$, $p < 0.025$. Post hoc comparisons demonstrated that there was not a significant difference in the number of RAWM errors between the control and stressed groups on the 12^{th} learning trial, $p > 0.05$ or at the shot-term memory trial, $p > 0.05$; however, control animals made more errors than stressed animals at the long-term memory trial, $p < 0.05$. Mean ± SD. Control 12^{th} learning trial 1.2 errors ± 0.58, short-term memory trial 0.8 errors ± 0.46, long-term memory trial 2.67 errors ± 0.87. Stress 12^{th} learning trail 0.33 errors ± 0.34, short-term memory trial 0.67 errors ± 0.40, long-term memory trial 0.27 errors ± 0.15.

*$p < 0.05$ significantly different from control.

Figure 19. Brain Weights Across Conditions. After sacrificed for IHC, the brains of animals were weighed (control n = 9, control + RAWM n = 9, stress n = 9, stress + RAWM n = 9). A one-way ANOVA revealed that there was no significant difference in brain weights across groups, $F < 1$. Mean ± SD. Control 1.90 grams ± 0.13, Control + RAWM 1.84 grams ± 0.09, Stress 1.81 grams ± 0.12, Stress + RAWM 1.90 grams ± 0.18.

Figure 20. CldU+ Cells in the Dorsal and Ventral DG. A factorial ANOVA between control (n = 9), control + RAWM (n = 9), stressed (n = 9), and stressed +RAWM (n = 9) animals revealed that there was a significant main effect of group, $F(3,32) = 9.43$, $p < 0.025$, significant main effect of subregion, $F(1,32) = 10.56$, $p < 0.001$, and significant group by subregion interaction, $F(3,32) = 12.00$, $p < 0.001$. Post hoc comparisons indicated that there was not a significant CldU difference between dorsal and ventral subregions in control animals, $p > 0.05$, but that there were significantly fewer CldU+ cells in the ventral DG compared to the dorsal DG in the control + RAWM, $p < 0.05$, stress, $p < 0.05$, and stress + RAWM, $p < 0.05$, groups. Across groups within the ventral DG, control animals had significantly more CldU+ cells than any other group, $p < 0.05$. Additionally, stressed animals had more CldU+ cells compared to the stress + RAWM animals, $p < 0.05$, in the ventral DG. Within the dorsal subregion across groups, control animals had more CldU+ cells, than any other group, $p < 0.05$. Mean ± SEM. Control dorsal 3780 mm^3 ± 216, Control ventral 3744 mm^3 ± 287, Control + RAWM dorsal 2404 mm^3 ± 247, Control + RAWM ventral 1873 mm^3 ± 256, Stress dorsal 2656 mm^3 ± 301, Stress ventral 1998 mm^3 ± 289, Stress + RAWM dorsal 2036 mm^3 ± 264, Stress + RAWM ventral 1428 mm^3 ± 234.

*$p < 0.05$ significantly different subregion within condition.

Figure 21. CldU+ Cells in the Ventral DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the ventral DG, control animals had significantly more CldU+ cells than any other group, $p < 0.05$. Stressed animals had more CldU+ cells compared to the stress + RAWM animals, $p < 0.05$, in the ventral DG. Mean ± SEM. Control ventral 3744 mm^3 ± 287, Control + RAWM ventral 1873 mm^3 ± 256, Stress ventral 1998 mm^3 ± 289, Stress + RAWM ventral 1428 mm^3 ± 234

*$p < 0.05$ significantly different from control.

#$p < 0.05$ significantly different from stress.

Figure 22. CldU+ Cells in the Dorsal DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the dorsal DG, control animals had more CldU+ cells, than any other group, $p < 0.05$. Mean ± SEM, Control dorsal 3780 mm^3 ± 216, Control + RAWM dorsal 2404 mm^3 ± 247, Stress dorsal 2656 mm^3 ± 301, Stress + RAWM dorsal 2656 mm^3 ± 301.

*$p < 0.05$ significantly different from control.

Figure 23. CldU+ Cells. Following CUS and learning there was a decrease in the number of CldU+ cells in the ventral DG compared to the dorsal DG in all conditions except control animals. CldU+ cells in the DG of a control animal. Scale bar = 10μm.

Figure 24. IdU+ Cells in the Dorsal and Ventral DG. A factorial ANOVA between control (n = 9), control + RAWM (n = 9), stressed (n = 9), and stressed +RAWM (n = 9) animals revealed that there was a significant group by subregion interaction, $F(3,32) = 8.56$, $p < 0.001$, and main effect of group, $F(3,32) = 11.05$, $p < 0.001$, but not a main effect of subregion, $F < 1$. Post hoc comparisons revealed that there was no significant difference between subregions in the control group, $p > 0.05$, or control + RAWM group, $p > 0.05$; however, there were significantly fewer IdU+ cells in the ventral DG compared to the dorsal DG in stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ groups. Across groups within the ventral subregion of the DG there were significantly fewer IdU+ cells in both the stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ compared to the control group. Within the dorsal DG, there were significantly fewer IdU+ cells in both the stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ compared to the control group. Mean ± SEM. Control dorsal 3541 mm^3 ± 205, Control ventral 3847 mm^3 ± 198, Control + RAWM dorsal 3252 mm^3 ± 164, Control + RAWM ventral 3605 mm^3 ± 231, Stress dorsal 2311 mm^3 ± 199, Stress ventral 1759 mm^3 ± 245, Stress + RAWM dorsal 2164 mm^3 ± 178, Stress + RAWM ventral 1653 mm^3 ± 212.

*$p < 0.05$ significantly different subregion within condition.

Figure 25. IdU+ Cells in the Ventral DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the ventral DG there were significantly fewer IdU+ cells in both the stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ compared to the control group. Mean ± SEM. Control ventral 3847 mm^3 ± 198, Control + RAWM ventral 3605 mm^3 ± 231, Stress ventral 1759 mm^3 ± 245, Stress + RAWM ventral 1653 mm^3 ± 212.

*$p < 0.05$ significantly different from control.

Figure 26. IdU+ Cells in the Dorsal DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the dorsal DG , there were significantly fewer IdU+ cells in both the stress, $p < 0.05$, and stress + RAWM, $p < 0.05$ compared to the control group. Mean ± SEM. Control dorsal 3541mm^3 ± 205, Control + RAWM dorsal 3252 mm^3 ± 164, Stress dorsal 2311 mm^3 ± 199, Stress + RAWM dorsal 2164 mm^3 ± 178.

*$p < 0.05$ significantly different from control.

Figure 27. IdU+ Cells. CUS decreased the number of IdU+ cells in both the dorsal and ventral DG compared to control animals. IdU+ cells in the DG of a control animal. Scale bar = 10μm.

Figure 28. DCX+ Cells in the Dorsal and Ventral DG. A factorial ANOVA between control (n = 9), control + RAWM (n = 9), stressed (n = 9), and stressed +RAWM (n = 9) animals revealed that there was a significant main effect of group, $F(3,32) = 8.75$, $p < 0.025$, and a significant main effect of subregion, $F(1,32) = 8.62$, $p < 0.025$, but not a significant group by subregion interaction, $F < 1$. Post hoc comparisons illustrated that there was not a difference in DCX+ cells between the dorsal and ventral DG in control animals, $p > 0.05$; however, there were significantly fewer DCX+ cells in the ventral DG compared to the dorsal DG in control + RAWM, $p < 0.05$, stress, $p < 0.05$, and stress + RAWM, $p < 0.05$, groups. Across groups within the ventral DG control animals had significantly more DCX+ cells than any other group, $p < 0.05$. Between the two learning groups, the stress + RAWM had significantly more DCX+ cells than control + RAWM animals within the ventral DG, $p < 0.05$. Across groups within the dorsal DG, control animals had more DCX+ cells than any other group, $p < 0.05$, and stress + RAWM animals had more DCX+ cells compared to control + RAWM animals, $p < 0.05$. Mean ± SEM. Control dorsal 4577 mm^3 ± 322, Control ventral 4017 mm^3 ± 336, Control + RAWM dorsal 3319 mm^3 ± 312, Control + RAWM ventral 2684 mm^3 ±245, Stress dorsal 3759 mm^3 ± 269, Stress ventral 3097 mm^3 ± 312, Stress + RAWM dorsal 4104 mm^3 ± 287, Stress + RAWM ventral 3404 mm^3 ± 264.

*$p < 0.05$ significantly different subregion within condition.

Figure 29. DCX+ Cells in the Ventral DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the ventral DG control animals had significantly more DCX+ cells than any other group, $p < 0.05$. Between the two learning groups, the stress + RAWM had significantly more DCX+ cells than control + RAWM animals within the ventral DG, $p < 0.05$. Mean ± SEM. Control ventral 4017 mm^3 ± 336, Control + RAWM ventral 2684 mm^3 ±245, Stress ventral 3097 mm^3 ± 312, Stress + RAWM ventral 3404 mm^3 ± 264.

*$p < 0.05$ significantly different from control.

#$p < 0.05$ significantly different from control + RAWM.

Figure 30. DCX+ Cells in the Dorsal DG. Post hoc comparisons following a factorial ANOVA indicated that across groups within the dorsal DG, control animals had more DCX+ cells than any other group, $p < 0.05$, and stress + RAWM animals had more DCX+ cells compared to control + RAWM animals, $p < 0.05$. Mean ± SEM. Control dorsal 4577 mm^3 ± 322, Control + RAWM dorsal 3319 mm^3 ± 312, Stress dorsal 3759 mm^3 ± 269, Stress + RAWM dorsal 4104 mm^3 ± 287.

*$p < 0.05$ significantly different from control.

#$p < 0.05$ significantly different from control + RAWM.

Figure 31. DCX+ Cells. Following CUS and learning there was a decrease in the number of DCX+ cells in the ventral DG compared to the dorsal DG in all conditions except control animals. DCX+ cells in the DG of a control animal. Scale bar = 10μm.

Figure 32. Mature BDNF in Subregions of the Hippocampal DG. A factorial ANOVA between control (n = 7), control + RAWM (n = 6), stressed (n = 5), and stressed +RAWM (n = 6) animals revealed that there was not a significant main effect of group, $F < 1$, main effect of subregion, $F < 1$, or group by subregion interaction, $F < 1$. Additionally, post hoc comparisons indicated there was no significant difference in the expression of BDNF across groups within the ventral DG, p 0.05, or dorsal DG, p > 0.05. Mean ± SEM. Control dorsal 0.90 OD ± 0.04, Control ventral 0.81 OD ± 0.13, Control + RAWM dorsal 1.23 OD ± 0.27, Control + RAWM ventral 0.80 OD ± 0.06, Stress dorsal 0.85 OD ± 0.20, Stress ventral 0.54 OD ± 0.19, Stress + RAWM dorsal 0.90 OD ± 0.09, Stress + RAWM ventral 0.72 OD ± 0.09.

Figure 33. Mature BDNF in the Ventral DG of the Hippocampus. Post hoc comparisons following a factorial ANOVA indicated that there was no significant difference in the expression of BDNF across groups within the ventral DG. Mature BDNF in the ventral DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control ventral 0.81 OD ± 0.13, Control + RAWM ventral 0.80 OD ± 0.06, Stress ventral 0.54 OD ± 0.19, Stress + RAWM ventral 0.72 OD ± 0.09.

Figure 34. Mature BDNF in the Dorsal DG of the Hippocampus. Post hoc comparisons following a factorial ANOVA indicated that there was no significant difference in the expression of BDNF across groups within the dorsal DG. There was not a significant difference in the expression of mature BDNF across conditions within the dorsal DG ($F[3,47] = 1.72$, $p = 0.194$). Mature BDNF in the dorsal DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control dorsal 0.90 OD ± 0.04, Control + RAWM dorsal 1.23 OD ± 0.27, Stress dorsal 0.85 OD ± 0.19, Stress + RAWM dorsal 0.90 OD ± 0.09.

Figure 35. ProBDNF in Subregions of the Hippocampal DG. A factorial ANOVA between control (n = 7), control + RAWM (n = 6), stressed (n = 5), and stressed +RAWM (n = 6) animals revealed that there was a significant main effect of group, $F(3,18) = 4.56$, $p < 0.025$, significant main effect of subregion, $F(1,18) = 6.52$, $p < 0.05$, and a significant group by subregion interaction, $F(3,18) = 5.87$, $p < 0.001$. Post hoc comparisons revealed that control animals had a higher expression of proBDNF in the ventral DG compared to the dorsal DG, $p < 0.05$, but in the control + RAWM group the dorsal DG had a higher expression of proBDNF, $p < 0.05$, compared to the ventral DG. There was not a significant difference in proBDNF across subregions within the stress, $p > 0.05$, or stress + RAWM, $p > 0.05$, groups. Across groups within the ventral subregion of the DG, control animals had significantly more proBDNF than the control + RAWM animals, $p < 0.05$. Within the dorsal subregion, across groups, there was no significant difference in the expression of proBDNF between control and stressed animals, $p > 0.05$; however, control animals had significantly less proBDNF expression than the control + RAWM, $p < 0.05$, and stress + RAWM groups, $p < 0.05$. Mean ± SEM. Control dorsal 0.48 OD ± 0.03, Control ventral 0.64 OD ± 0.02, Control + RAWM dorsal 0.82 OD ± 0.14, Control + RAWM ventral 0.50 OD ± 0.02, Stress dorsal 0.58 OD ± 0.10, Stress ventral 0.58 OD ± 0.04, Stress + RAWM dorsal 0.78 OD ± 0.11, Stress + RAWM ventral 0.63 OD ± 0.09.
*$p < 0.05$ significantly different subregion within condition.

Figure 36. ProBDNF in the Ventral DG of the Hippocampus. Post hoc comparisons following a factorial ANOVA indicated that control animals had significantly more proBDNF than the control + RAWM animals, $p < 0.05$, in the ventral DG. ProBDNF in the ventral DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control ventral 0.64 OD ± 0.02, Control + RAWM ventral 0.50 OD ± 0.02, Stress ventral 0.58 OD ± 0.04, Stress + RAWM ventral 0.63 OD ± 0.09.

*$p < 0.05$ significantly different from control.

Figure 37. ProBDNF in the Dorsal DG of the Hippocampus Post hoc comparisons following a factorial ANOVA indicated that there was no significant difference in the expression of proBDNF in the dorsal DG between control and stressed animals, $p > 0.05$. Furthermore, control animals had significantly less proBDNF expression than the control + RAWM, $p < 0.05$, and stress + RAWM groups, $p < 0.05$. ProBDNF in the dorsal DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control dorsal 0.48 OD ± 0.03, Control + RAWM dorsal 0.82 OD ± 0.14, Stress dorsal 0.58 OD ± 0.10, Stress + RAWM dorsal 0.75 OD ± 0.11.

*$p < 0.05$ significantly different from control.

Figure 38. PSD-95 in Subregions of the Hippocampal DG. A factorial ANOVA between control (n = 7), control + RAWM (n = 6), stressed (n = 5), and stressed +RAWM (n = 6) animals revealed that there was a significant main effect of group, $F(3,18) = 7.06$, $p < 0.001$, and main effect of subregion, $F(1,18) = 8.34$, $p < 0.05$, but did not reveal a significant group by subregion interaction, $F < 1$. Post hoc comparisons revealed that there were no significant differences in PSD-95 expression between subregions in the control, $p > 0.05$, control + RAWM, $p > 0.05$, stress, $p > 0.05$, or stress + RAWM, $p > 0.05$ groups. Within the ventral DG, control animals had significantly less PSD-95 expression than the control + RAWM, $p < 0.05$, and stress, $p < 0.05$, groups, but was not significantly different from the stress + RAWM group, $p > 0.05$. Across groups within the dorsal DG, stress + RAWM animals had a significantly higher expression of PSD-95 than control, $p < 0.05$, control + RAWM, $p < 0.05$, and stress, $p < 0.05$ animals. Mean ± SEM. Control dorsal 1.53 OD ± 0.15, Control ventral 1.28 OD ± 0.08, Control + RAWM dorsal 1.79 OD ± 0.11, Control + RAWM ventral 1.7 OD ± 0.09, Stress dorsal 1.59 OD ± 0.11, Stress ventral 1.65 OD ± 0.08, Stress + RAWM dorsal 2.37 OD ± 0.17, Stress + RAWM ventral 1.84 OD ± 0.32.

Figure 39. PSD-95 in the Ventral DG of the Hippocampus. Post hoc comparisons following a factorial ANOVA indicated that within the ventral DG control animals had significantly less PSD-95 expression than the control + RAWM, $p < 0.05$, and stress, $p < 0.05$, groups, but was not significantly different from the stress + RAWM group, $p > 0.05$. PSD-95 in the ventral DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control ventral 1.28 OD ± 0.08, Control + RAWM ventral 1.7 OD ± 0.09, Stress ventral 1.65 OD ± 0.08, Stress + RAWM ventral 1.84 OD ± 0.32.

*$p < 0.05$ significantly different from control.

Figure 40. PSD-95 in the Dorsal DG of the Hippocampus. Post hoc comparisons following a factorial ANOVA indicated that within the dorsal DG stress + RAWM animals had a significantly higher expression of PSD-95 than control, $p < 0.05$, control + RAWM, $p < 0.05$, and stress, $p < 0.05$ animals. PSD-95 in the dorsal DG was detected as a 14-kDA band by western blotting. Mean ± SEM. Control dorsal 1.53 OD ± 0.15, Control + RAWM dorsal 1.80 OD ± 0.12, Stress dorsal 1.60 OD ± 0.12, tress + RAWM dorsal 2.37 OD ± 0.17.

*$p < 0.05$ significantly different from control.

Figure 41. A Model of the Yerkes-Dodson Law. The Yerkes-Dodson Law is an inverted-U-shaped curve representing how arousal interacts with cognition. The extreme ends of arousal or stress produce a weak cognitive performance; whereas, the optimal cognitive performance is a product of moderate arousal (image from Diamond et al. 2007).

Figures

Figure 1.

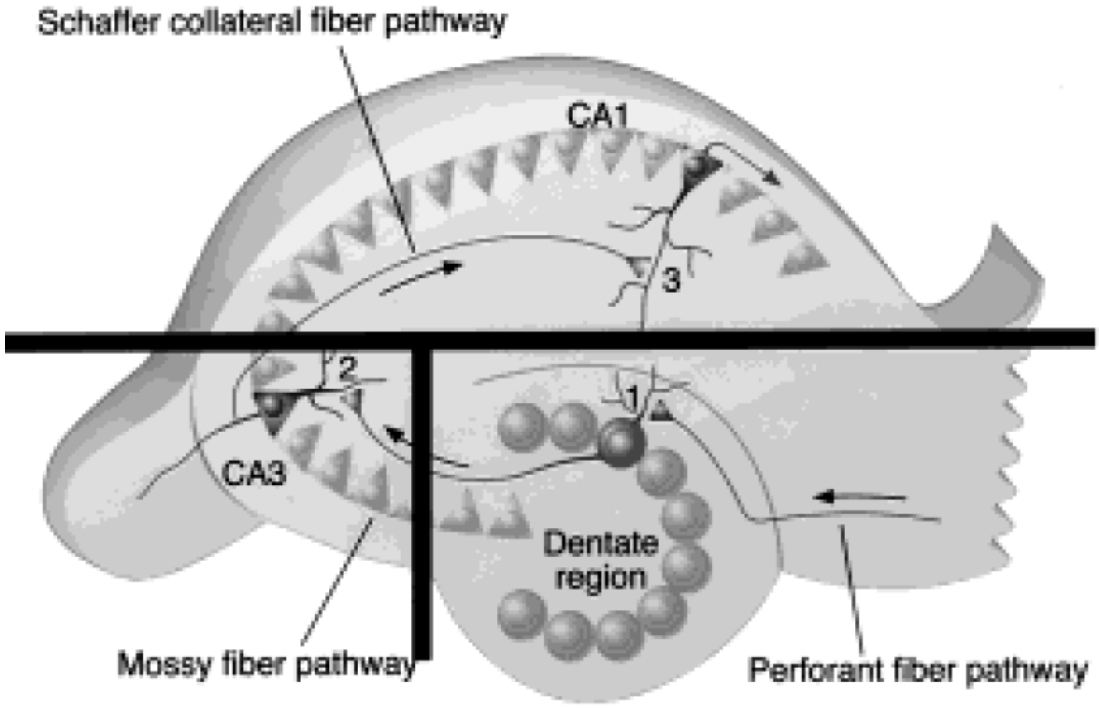
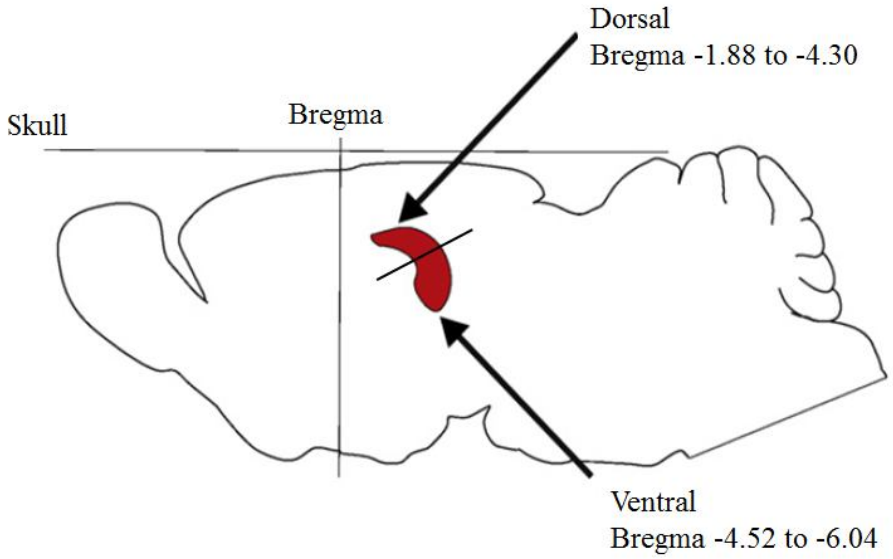


Figure 2.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Skull
Bregma
Ventral
Bregma -4.52 to -6.04

Figure 3.

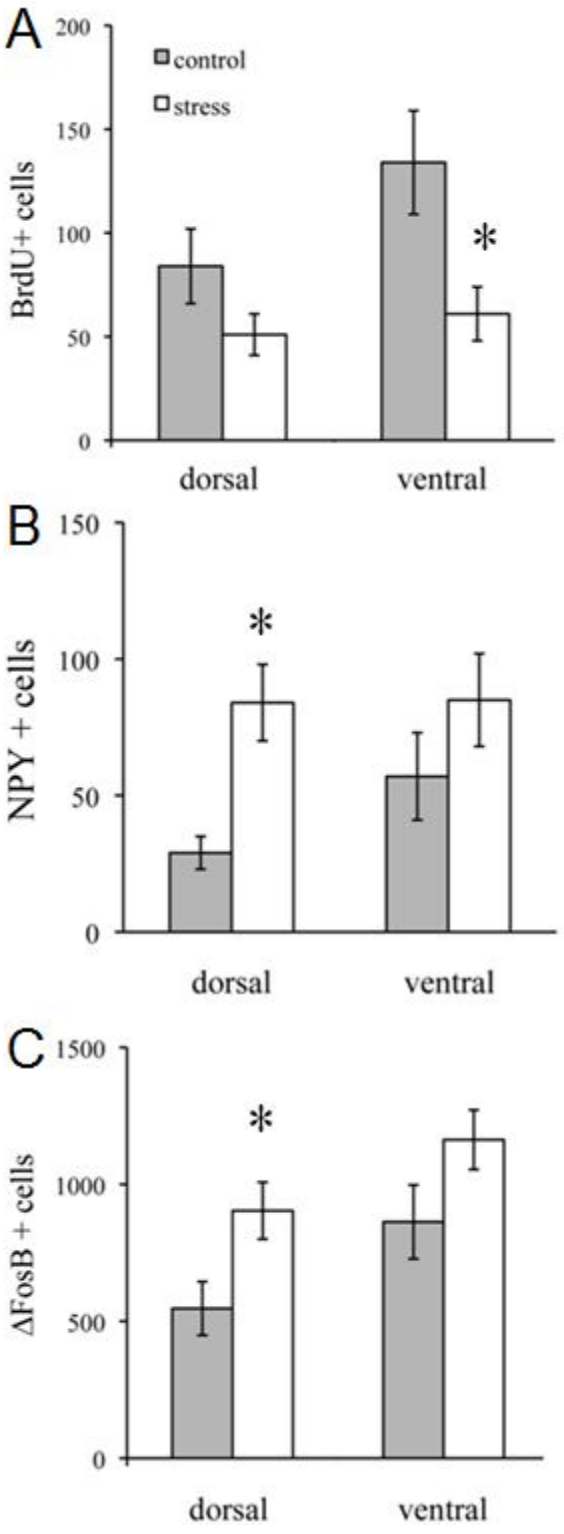
A
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Figure 4.

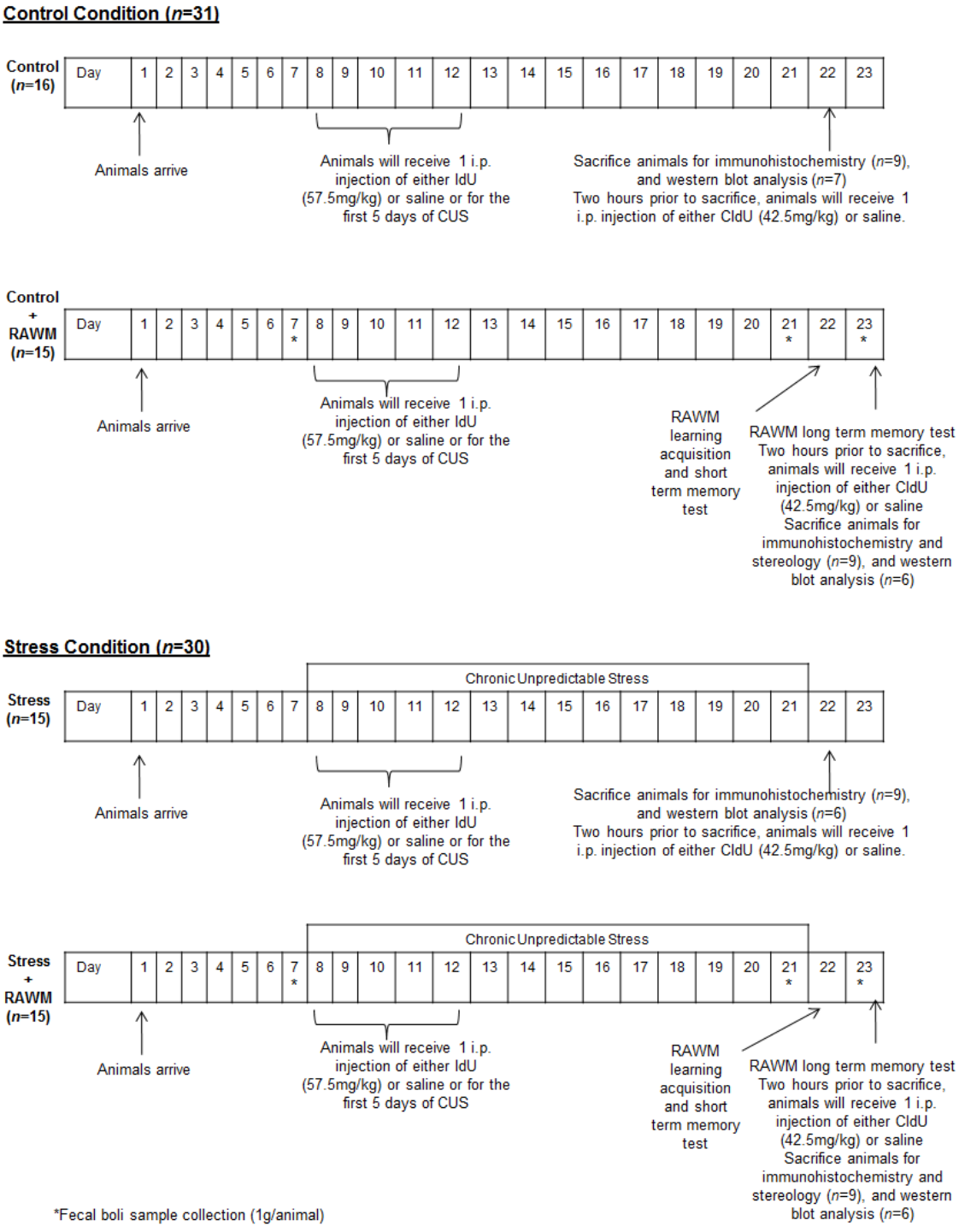

Control Condition (n=31)
Control (n=16)
Day 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Animals arrive
Animals will receive 1 i.p. injection of either IdU (57.5mg/kg) or saline or for the first 5 days of CUS
Sacrifice animals for immunohistochemistry (n=9), and western blot analysis (n=7)
Two hours prior to sacrifice, animals will receive 1 i.p. injection of either CldU (42.5mg/kg) or saline.
Control + RAWM (n=15)
Day 1 2 3 4 5 6 7* 8 9 10 11 12 13 14 15 16 17 18 19 20 21* 22 23*
Animals arrive
Animals will receive 1 i.p. injection of either IdU (57.5mg/kg) or saline or for the first 5 days of CUS
RAWM learning acquisition and short term memory test
RAWM long term memory test
Two hours prior to sacrifice, animals will receive 1 i.p. injection of either CldU (42.5mg/kg) or saline
Sacrifice animals for immunohistochemistry and stereology (n=9), and western blot analysis (n=6)
Stress Condition (n=30)
Chronic Unpredictable Stress
Stress (n=15)
Day 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Animals arrive
Animals will receive 1 i.p. injection of either IdU (57.5mg/kg) or saline or for the first 5 days of CUS
Sacrifice animals for immunohistochemistry (n=9), and western blot analysis (n=6)
Two hours prior to sacrifice, animals will receive 1 i.p. injection of either CldU (42.5mg/kg) or saline.
Chronic Unpredictable Stress
Stress + RAWM (n=15)
Day 1 2 3 4 5 6 7* 8 9 10 11 12 13 14 15 16 17 18 19 20 21* 22 23*
Animals arrive
Animals will receive 1 i.p. injection of either IdU (57.5mg/kg) or saline or for the first 5 days of CUS
RAWM learning acquisition and short term memory test
RAWM long term memory test
Two hours prior to sacrifice, animals will receive 1 i.p. injection of either CldU (42.5mg/kg) or saline
Sacrifice animals for immunohistochemistry and stereology (n=9), and western blot analysis (n=6)
*Fecal boli sample collection (1g/animal)

Figure 5.

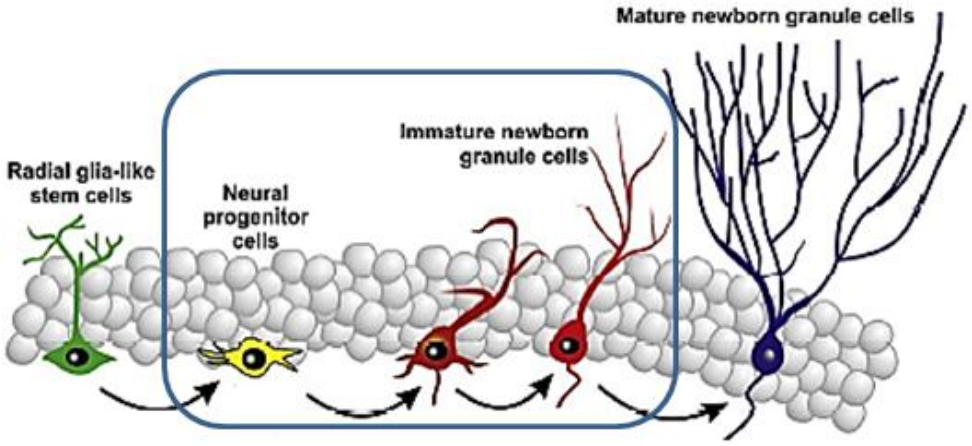

Mature newborn granule cells
Immature newborn granule cells
Radial glia-like stem cells
Neural progenitor cells

Figure 6.

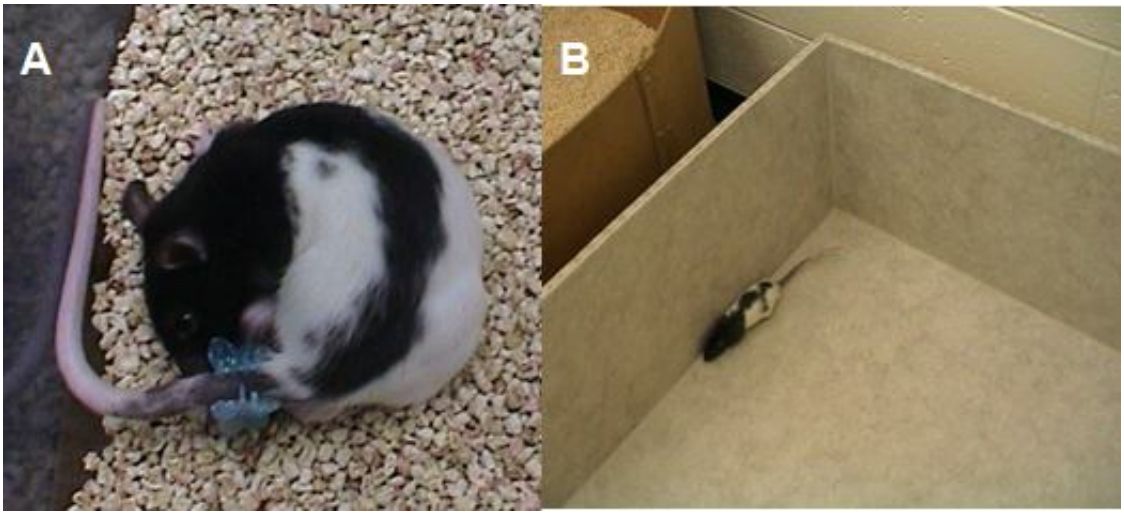


Figure 7.

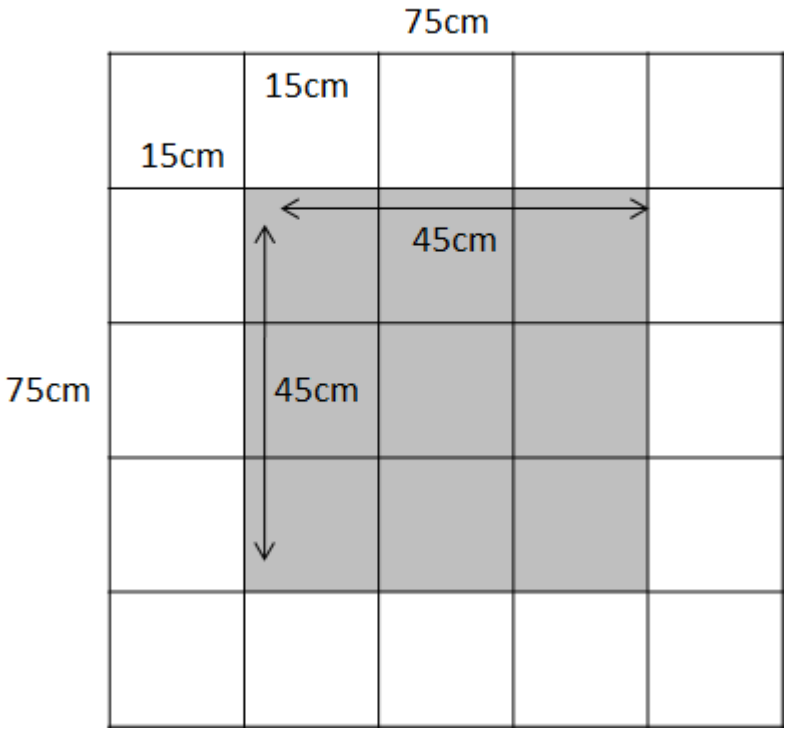


Figure 8.

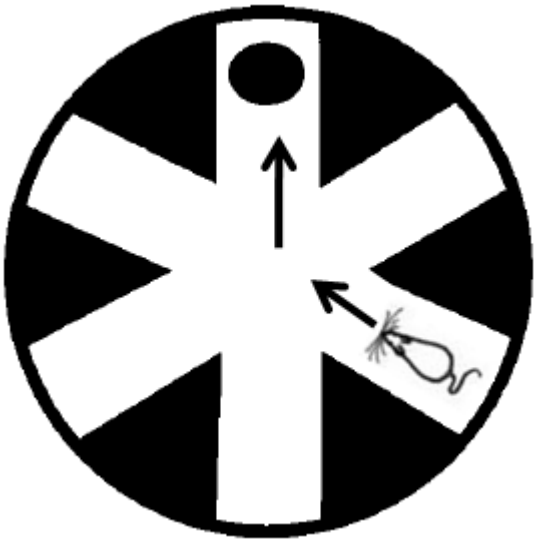


Figure 9.

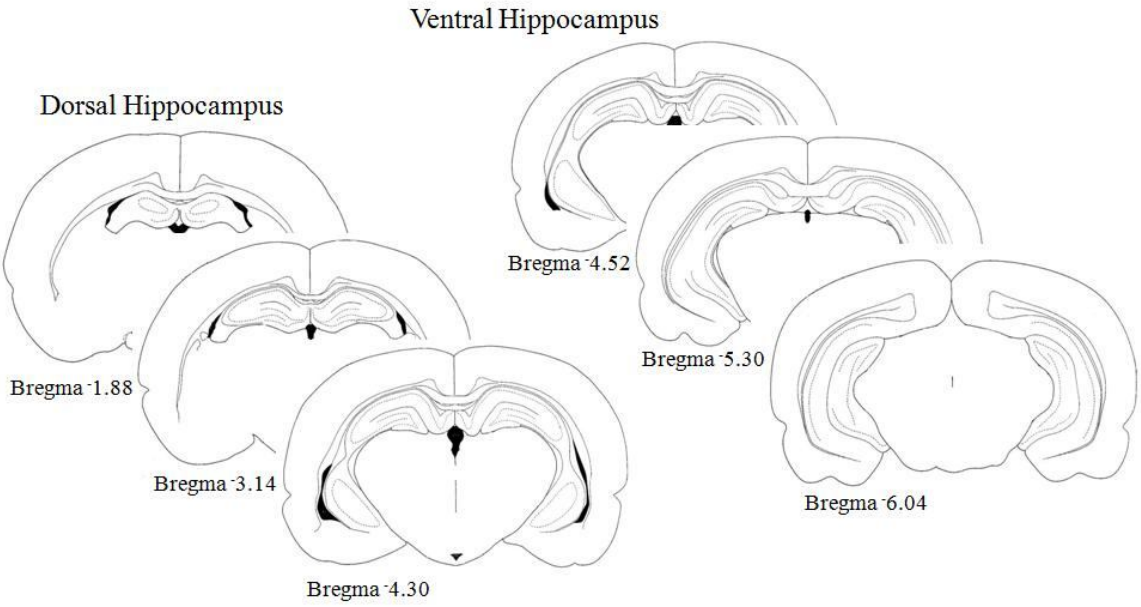

Ventral Hippocampus
Dorsal Hippocampus
Bregma -4.52
Bregma -5.30
Bregma -6.04
Bregma -1.88
Bregma -3.14
Bregma -4.30

Figure 10.

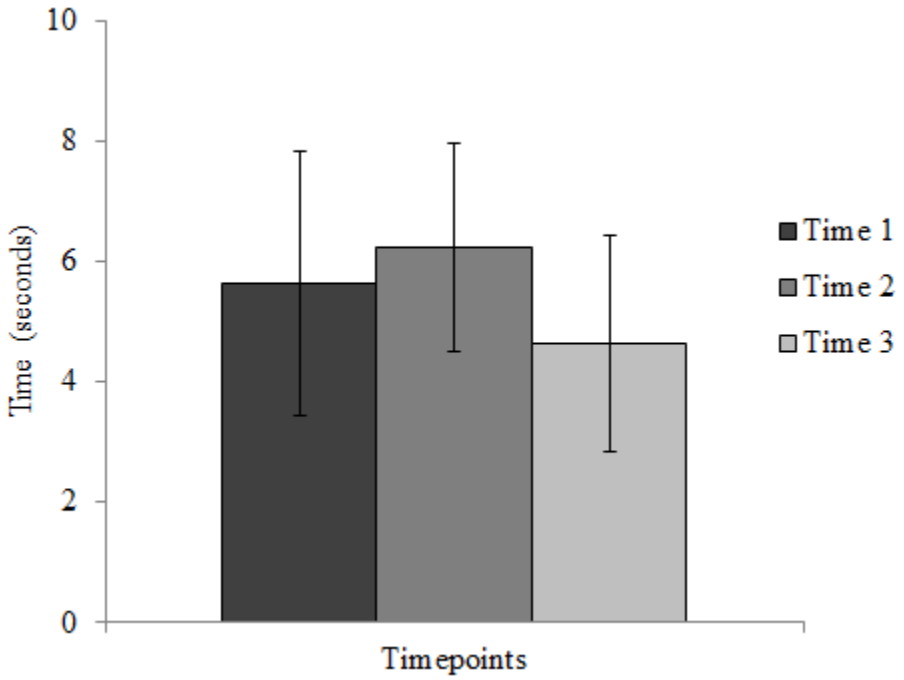


Figure 11.

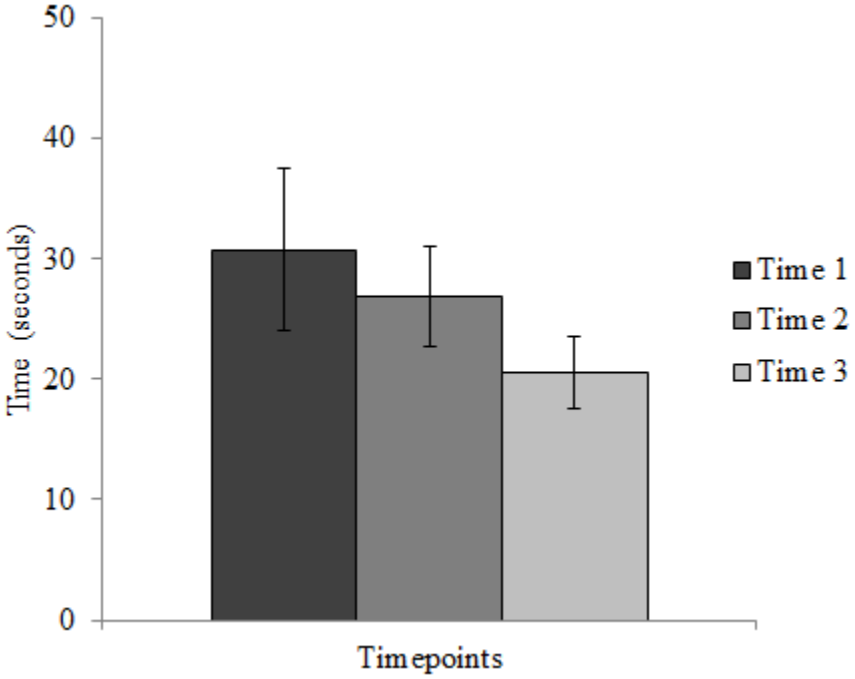


Figure 12.

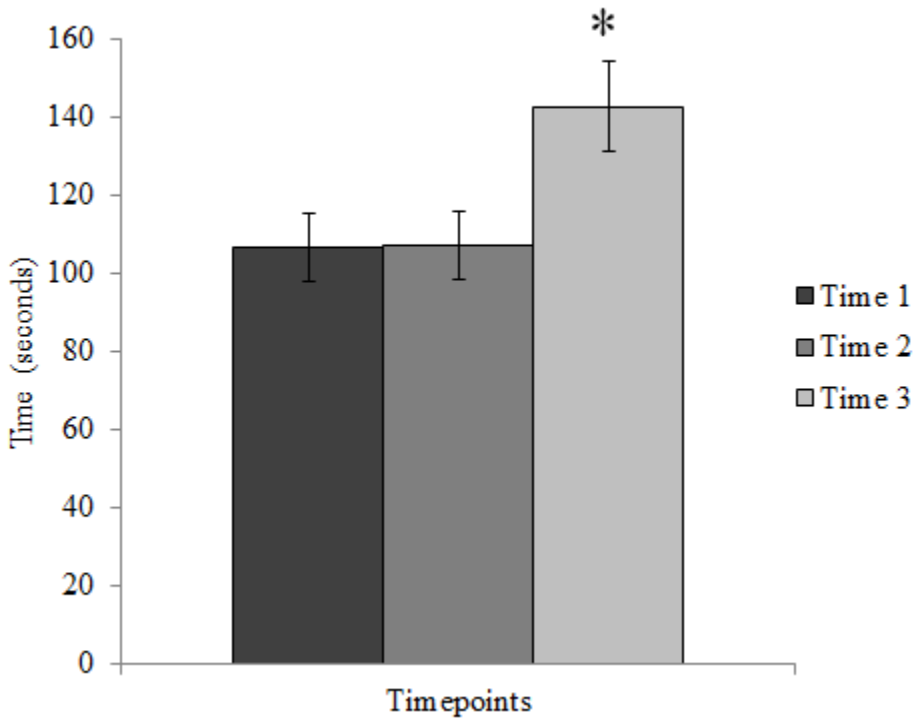

*
160
140
120
100
80
60
40
20
0
Time (seconds)
Timepoints
Time 1
Time 2
Time 3

Figure 13.

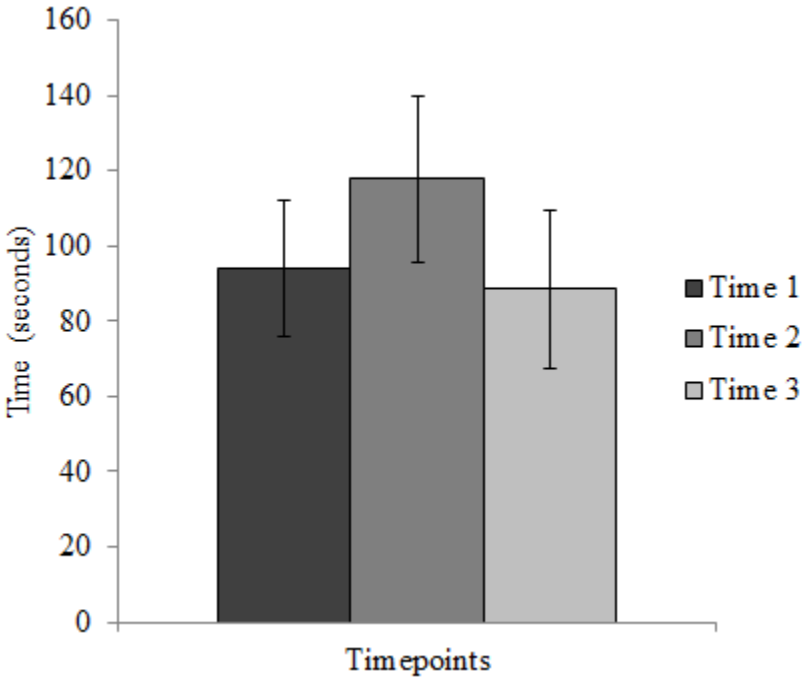

160
140
120
100
80
60
40
20
0
Time (seconds)
Timepoints
Time 1
Time 2
Time 3

Figure 14.

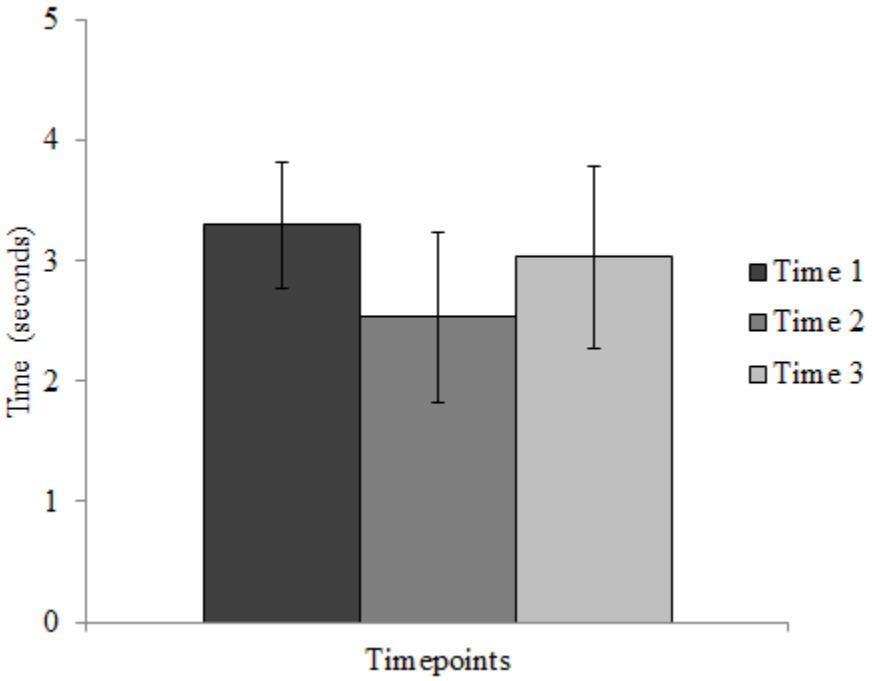


Figure 15.

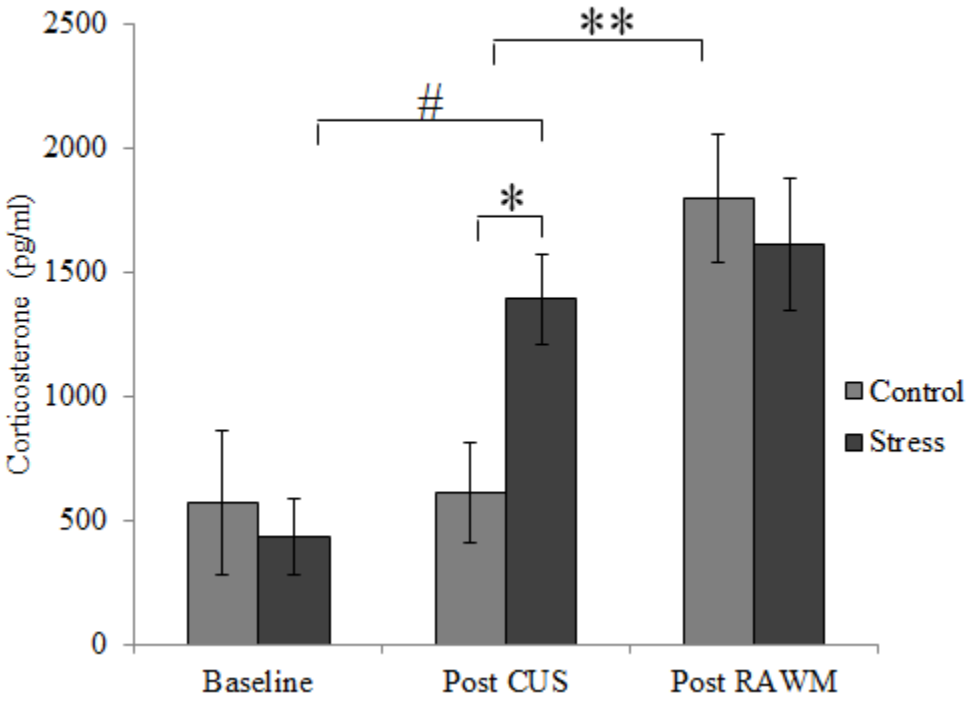

2500
2000
1500
1000
500
0
Corticosterone (pg/ml)
#
**
*
Control
Stress
Baseline
Post CUS
Post RAWM

Figure 16.

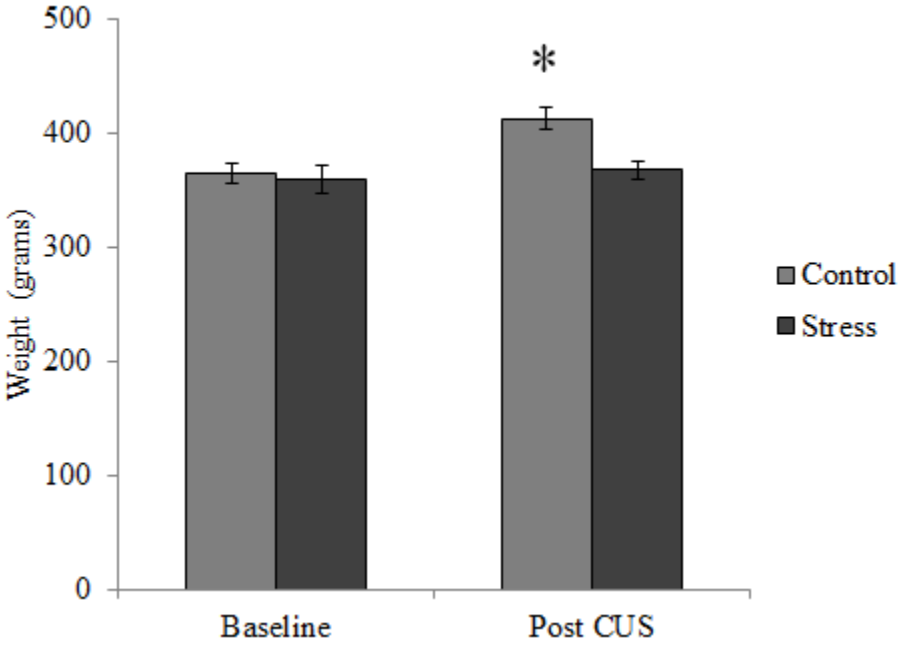

500
400
300
200
100
0
Weight (grams)
*
Baseline
Post CUS
Control
Stress

Figure 17.

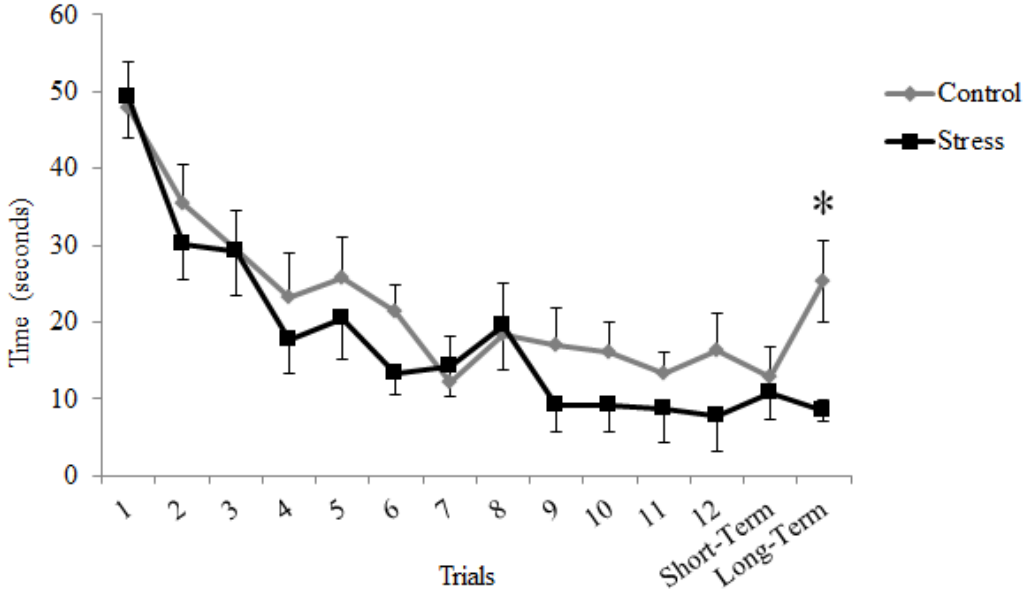

60
50
40
30
20
10
0
Time (seconds)
1
2
3
4
5
6
7
8
9
10
11
12
Short-Term
Long-Term
Trials
*
Control
Stress

Figure 18.

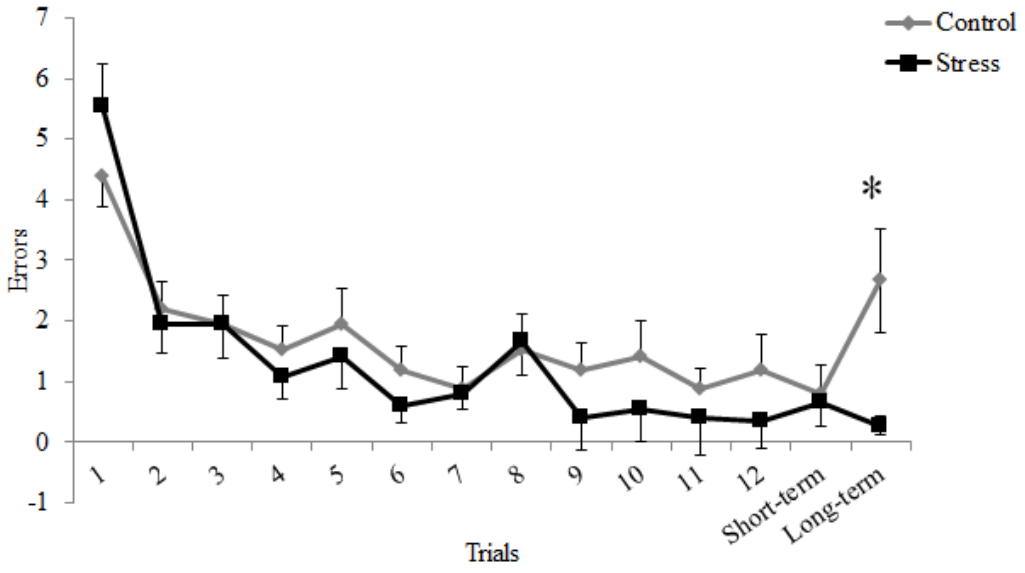

Control
Stress
Errors
Trials
*
1
2
3
4
5
6
7
8
9
10
11
12
Short-term
Long-term
7
6
5
4
3
2
1
0
-1

Figure 19.

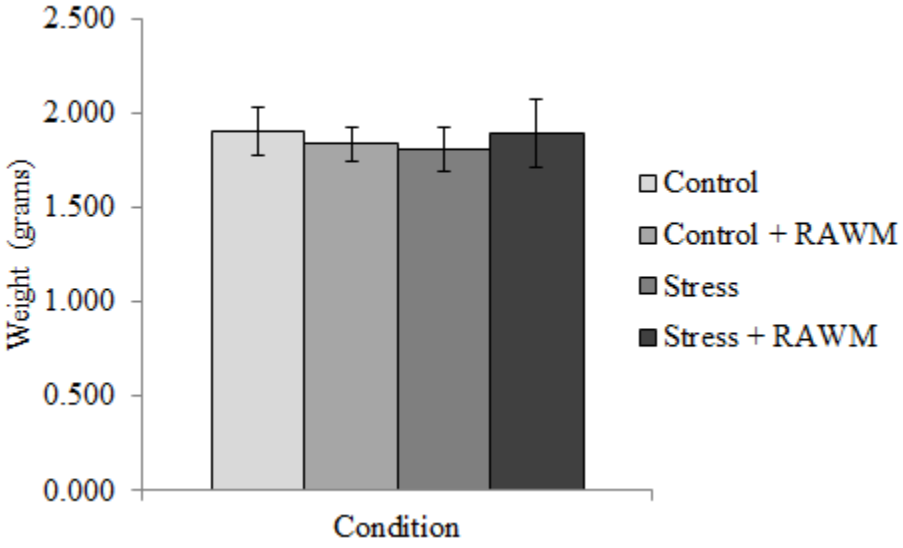

2.500
2.000
1.500
1.000
0.500
0.000
Weight (grams)
Condition
Control
Control + RAWM
Stress
Stress + RAWM

Figure 20.

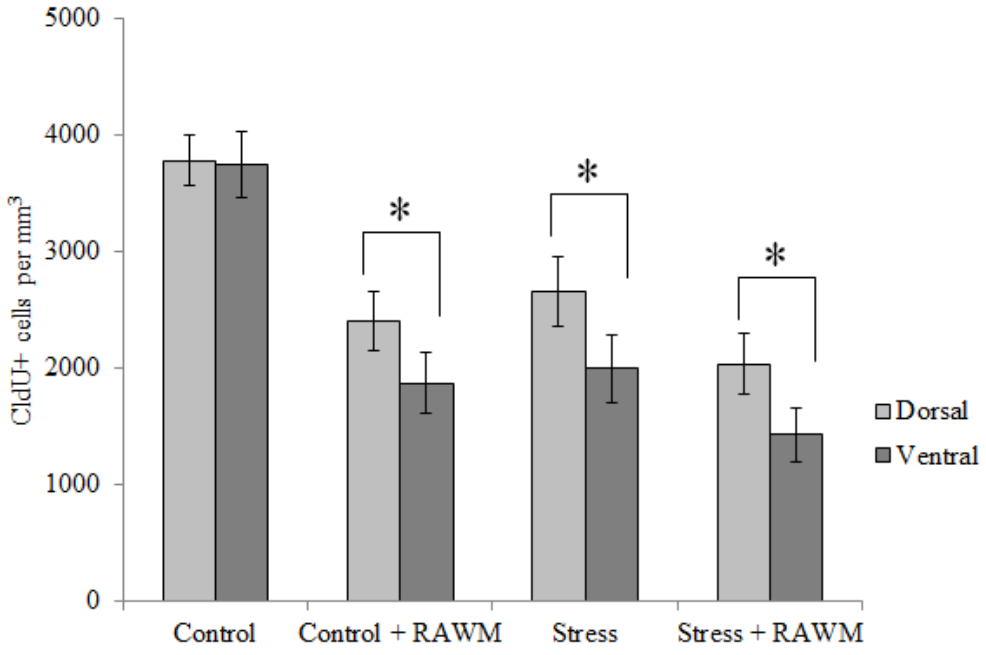

5000
4000
3000
2000
1000
0
CldU+ cells per mm3
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Dorsal
Ventral

Figure 21.

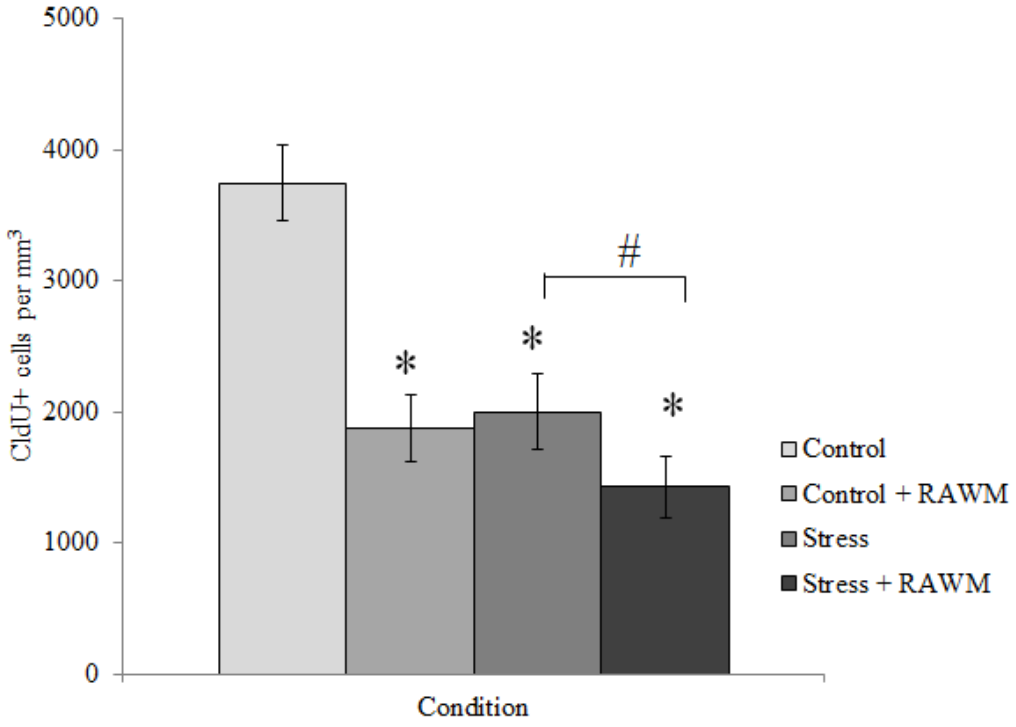

5000
4000
3000
2000
1000
0
CldU+ cells per mm3
Condition
#
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM

Figure 22.

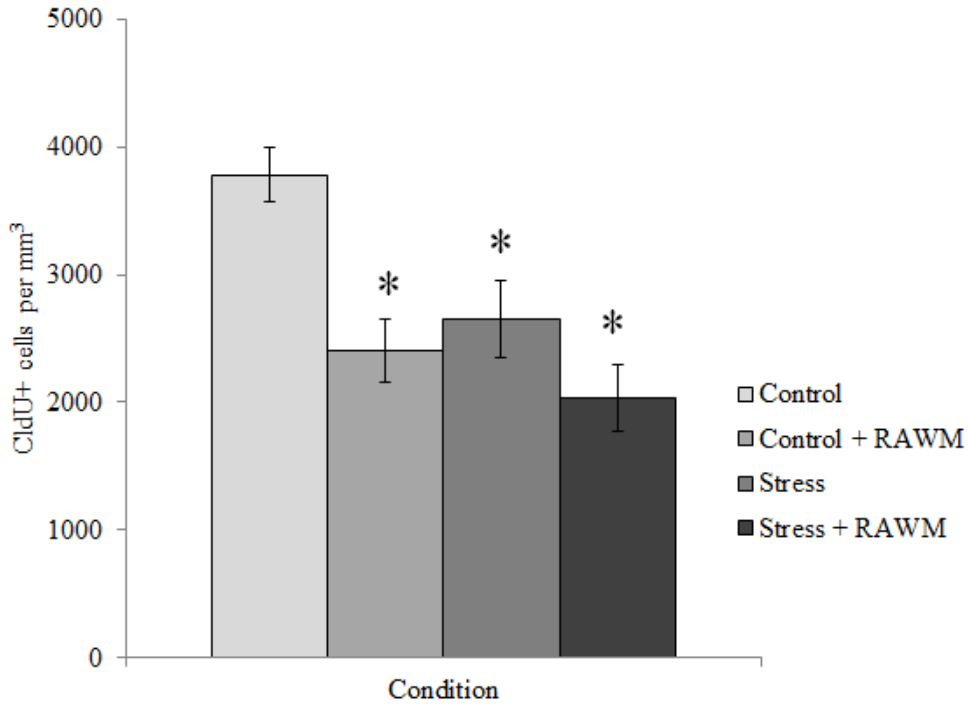

5000
4000
3000
2000
1000
0
CldU+ cells per mm3
Condition
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM

Figure 23.

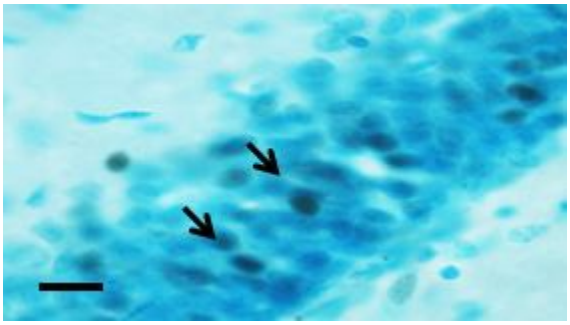


Figure 24.

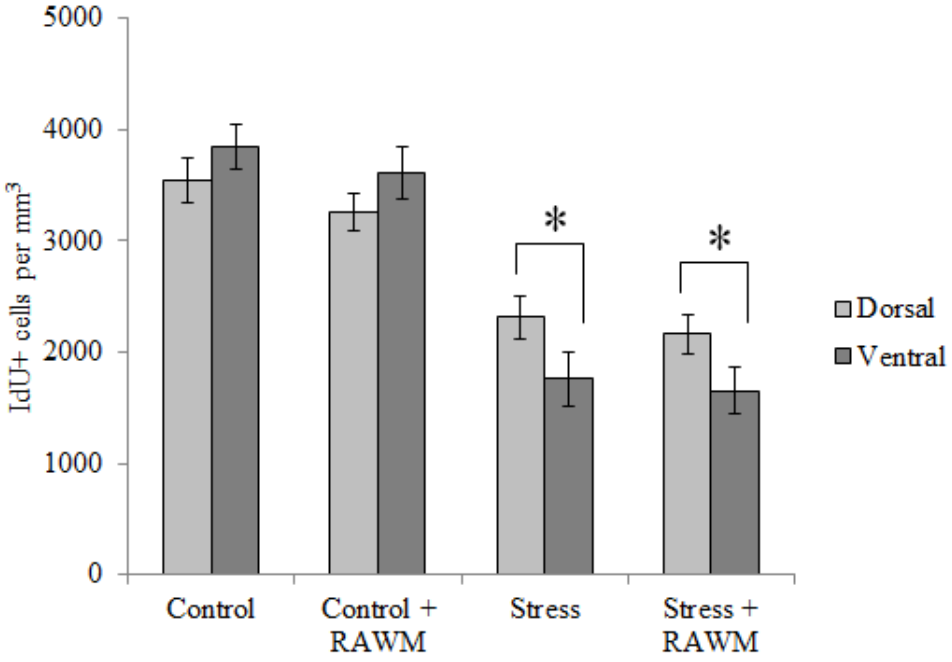

5000
4000
3000
2000
1000
0
IdU+ cells per mm3
*
*
Dorsal
Ventral
Control
Control + RAWM
Stress
Stress + RAWM

Figure 25.

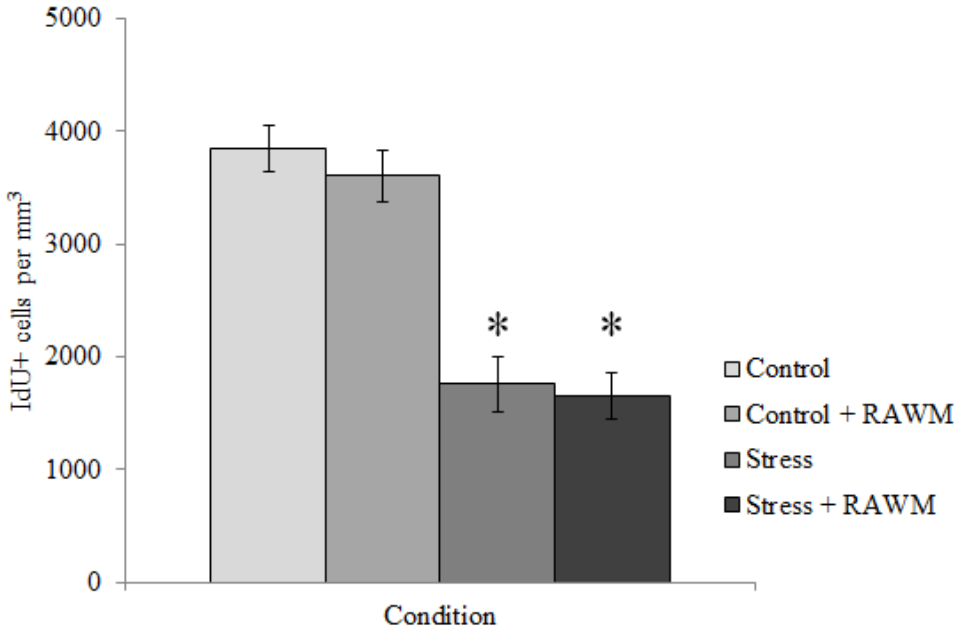

5000
4000
3000
2000
1000
0
IdU+ cells per mm3
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Condition

Figure 26.

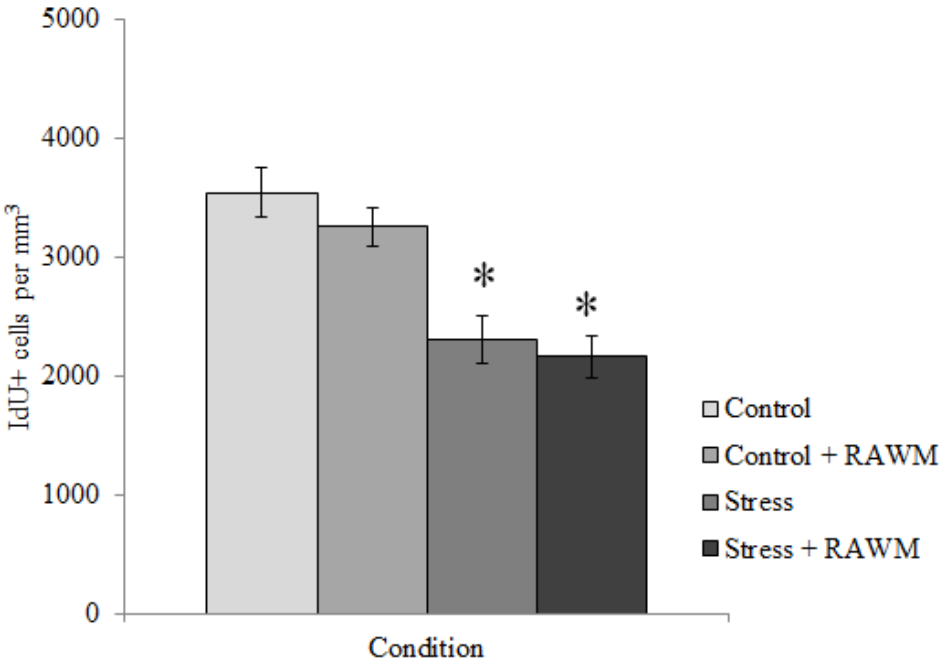

5000
4000
3000
2000
1000
0
IdU+ cells per mm3
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Condition

Figure 27.

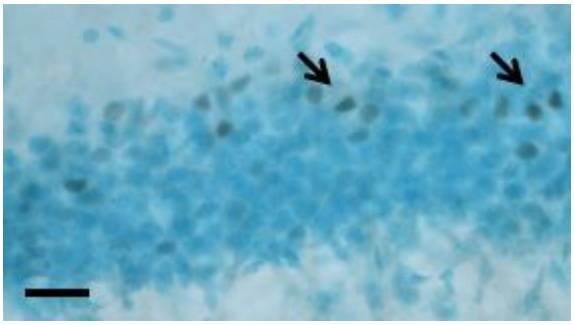


Figure 28.

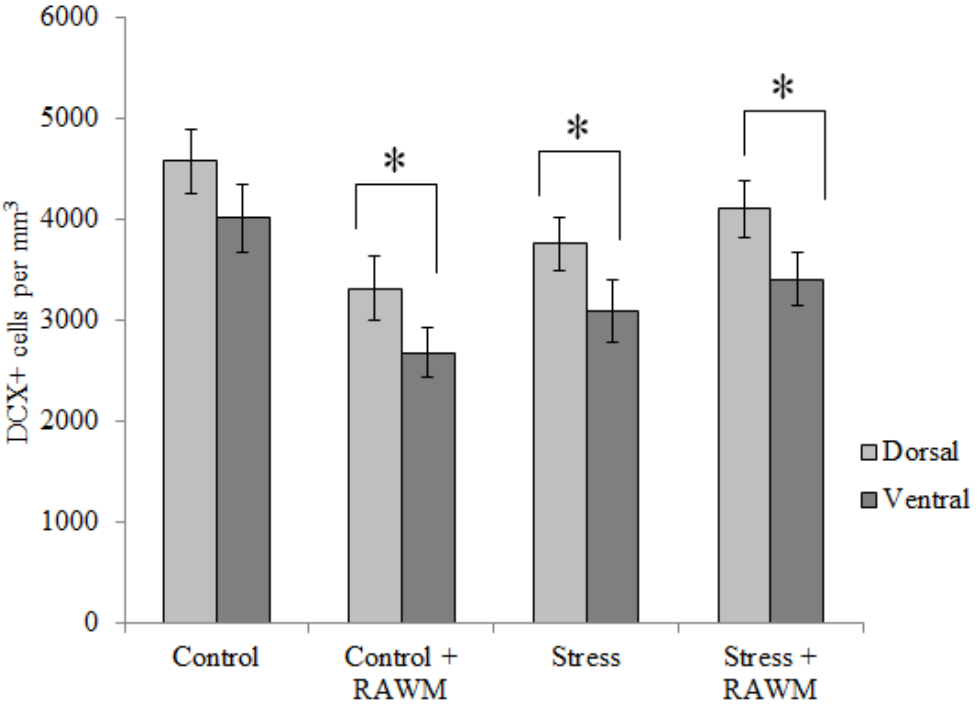

6000
5000
4000
3000
2000
1000
0
DCX+ cells per mm3
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Dorsal
Ventral

Figure 29.

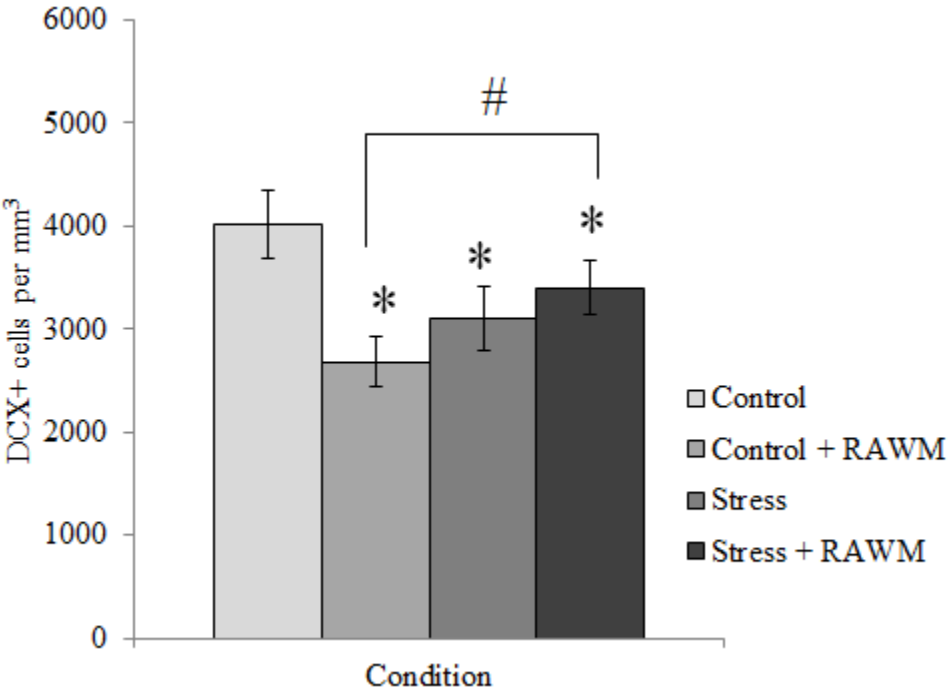

6000
5000
4000
3000
2000
1000
0
DCX+ cells per mm3
#
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Condition

Figure 30.

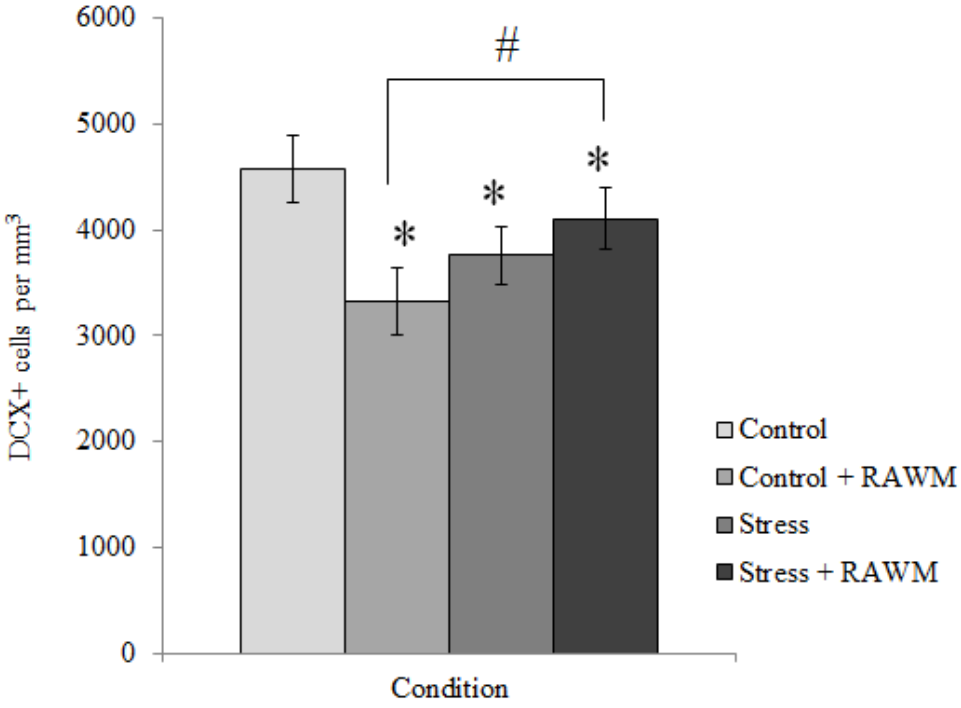

6000
5000
4000
3000
2000
1000
0
DCX+ cells per mm3
#
*
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Condition

Figure 31.

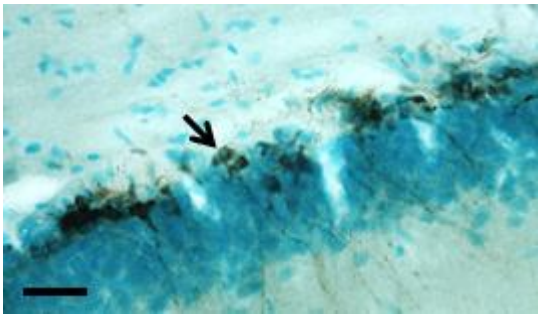


Figure 32.

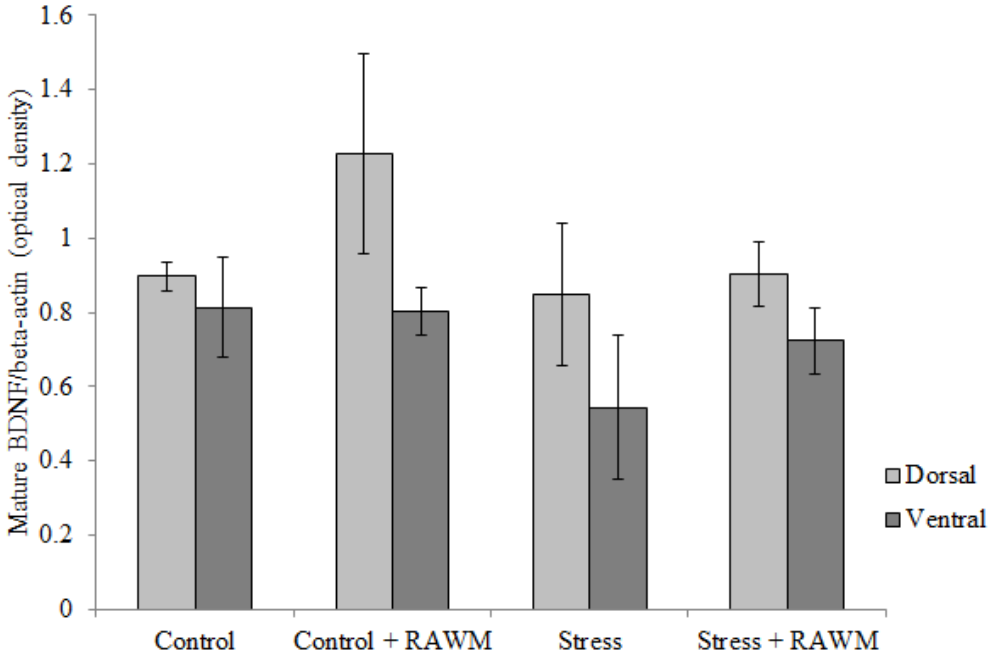
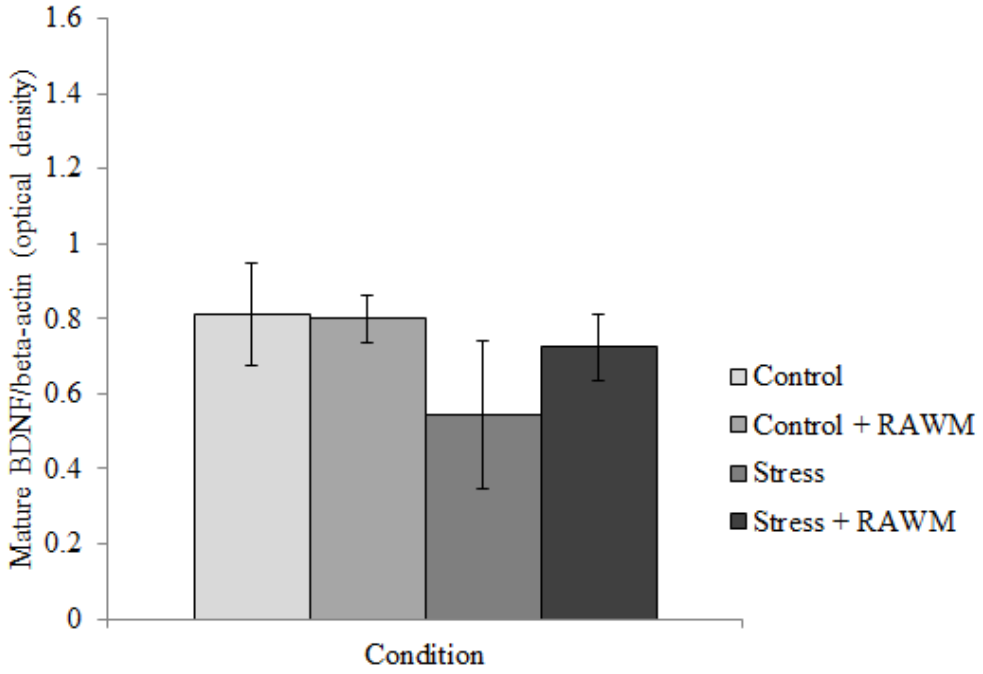

Mature BDNF/beta-actin (optical density)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Control + RAWM
Stress
Stress + RAWM
Dorsal
Ventral

Figure 33.


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mature BDNF/beta-actin (optical density)
Condition
Control
Control + RAWM
Stress
Stress + RAWM

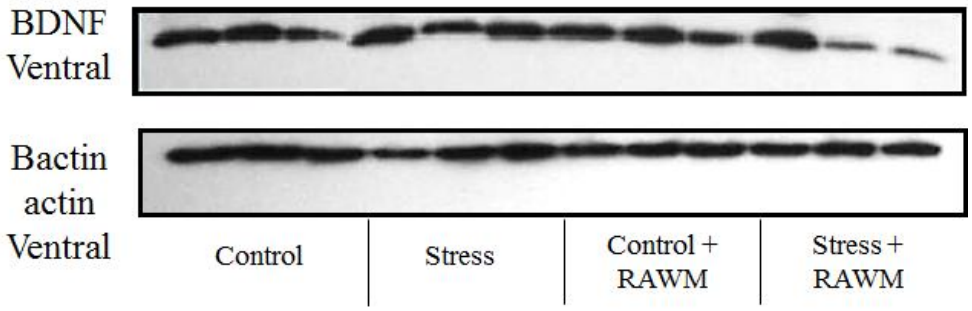
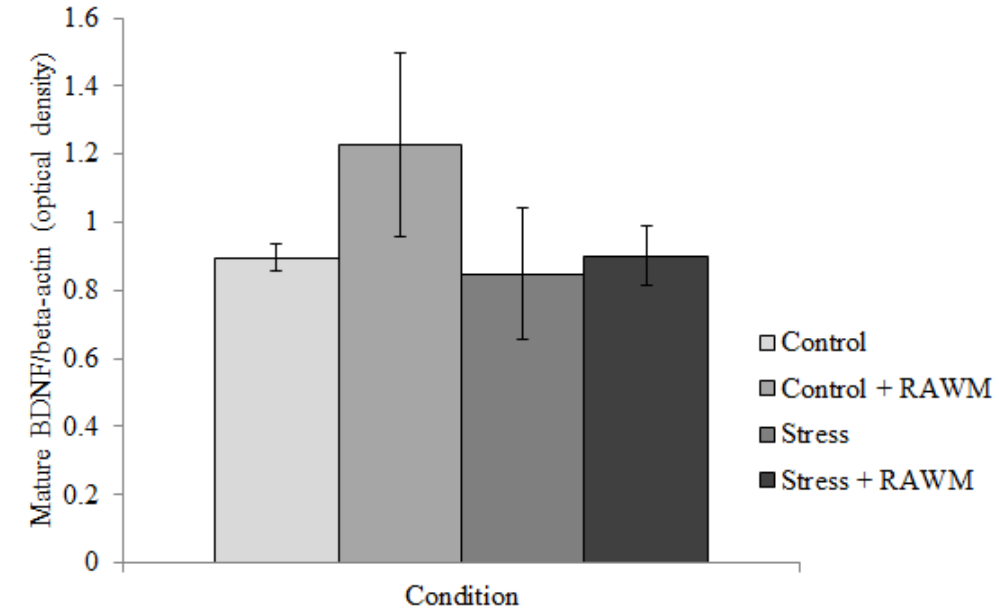

BDNF
Ventral
Bactin
actin
Ventral
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 34.


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mature BDNF/beta-actin (optical density)
Condition
Control
Control + RAWM
Stress
Stress + RAWM

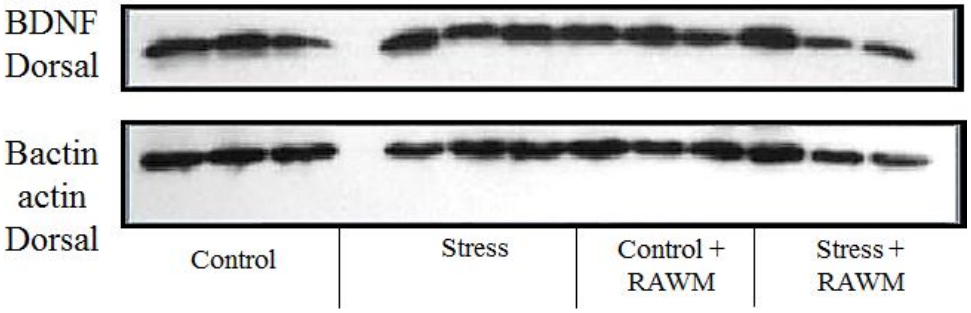

BDNF
Dorsal
Bactin
actin
Dorsal
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 35.

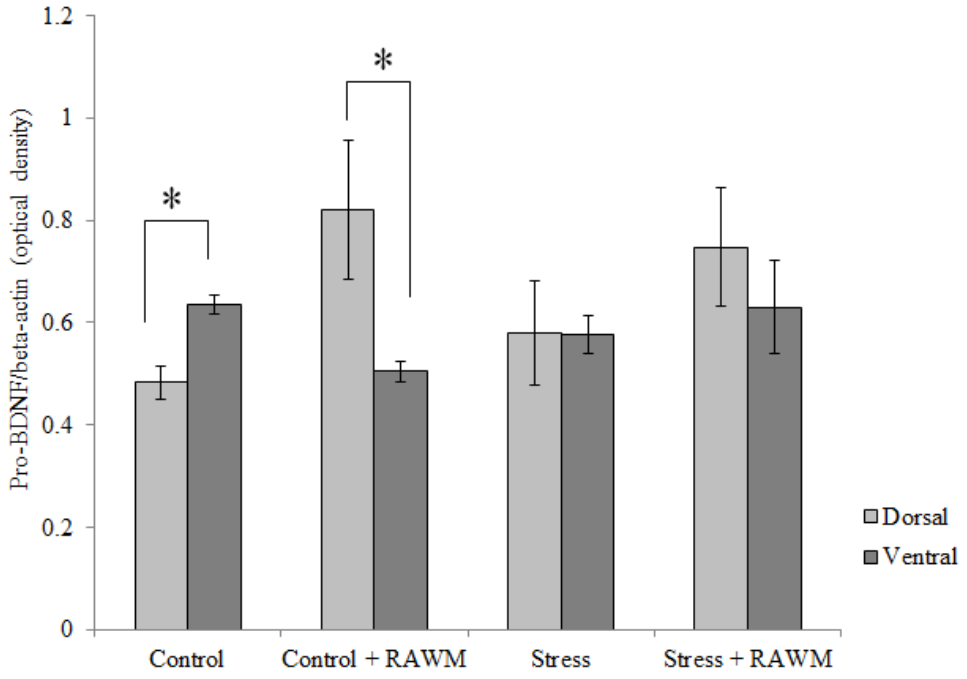

1.2
1
0.8
0.6
0.4
0.2
0
Pro-BDNF/beta-actin (optical density)
*
*
Control
Control + RAWM
Stress
Stress + RAWM
Dorsal
Ventral

Figure 36.

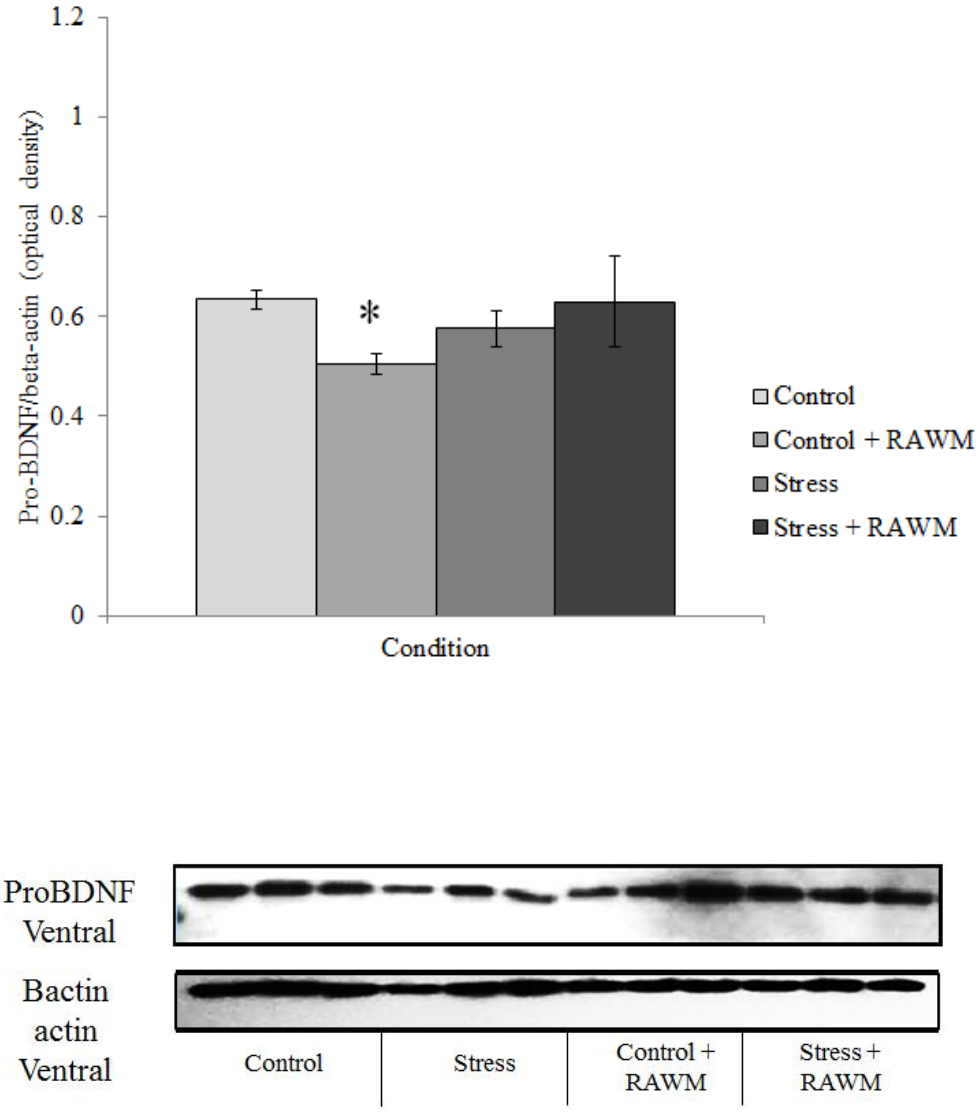
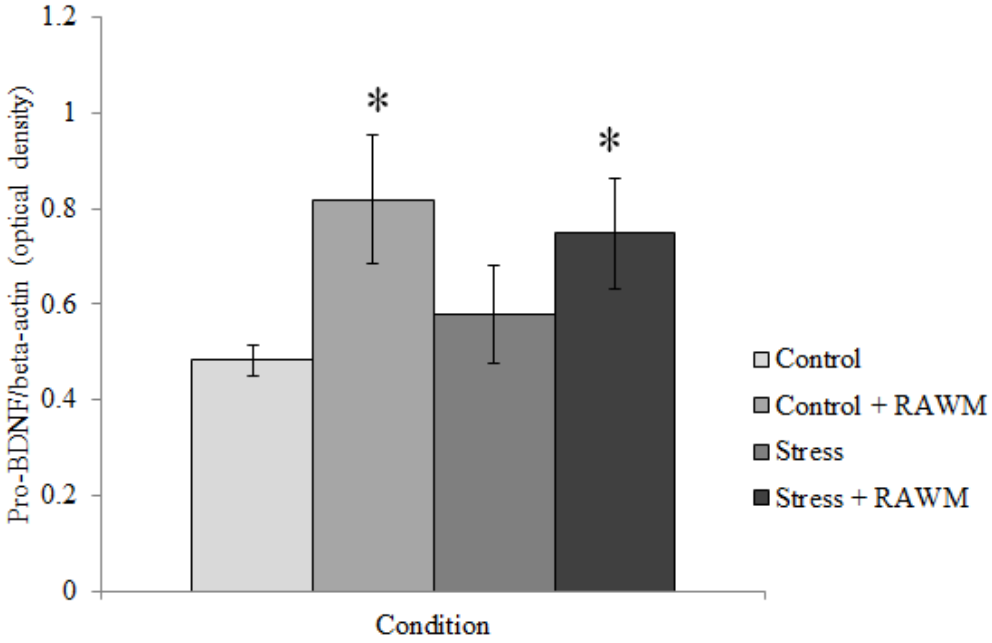

1.2
1
0.8
0.6
0.4
0.2
0
Pro-BDNF/beta-actin (optical density)
*
Condition
Control
Control + RAWM
Stress
Stress + RAWM
ProBDNF
Ventral
Bactin
actin
Ventral
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 37.


1.2
1
0.8
0.6
0.4
0.2
0
Pro-BDNF/beta-actin (optical density)
*
*
Condition
Control
Control + RAWM
Stress
Stress + RAWM

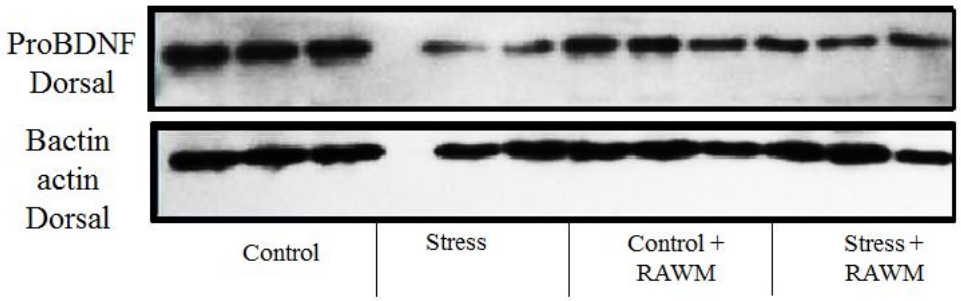

ProBDNF
Dorsal
Bactin
actin
Dorsal
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 38.

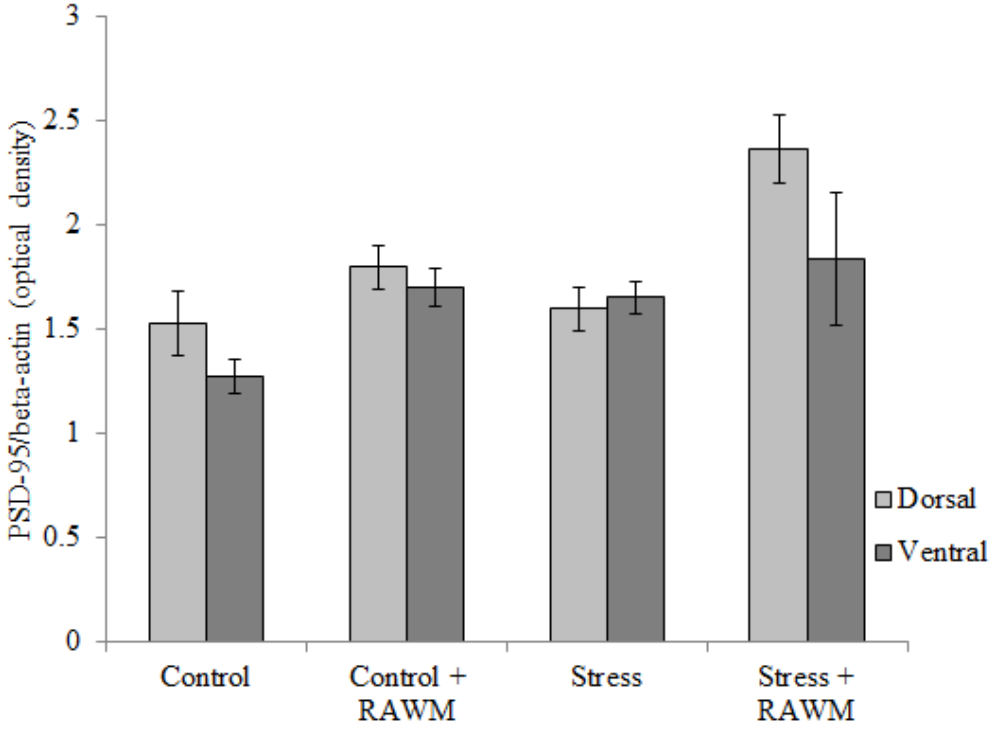

PSD-95/beta-actin (optical density)
3
2.5
2
1.5
1
0.5
0
Control
Control + RAWM
Stress
Stress + RAWM
Dorsal
Ventral

Figure 39.

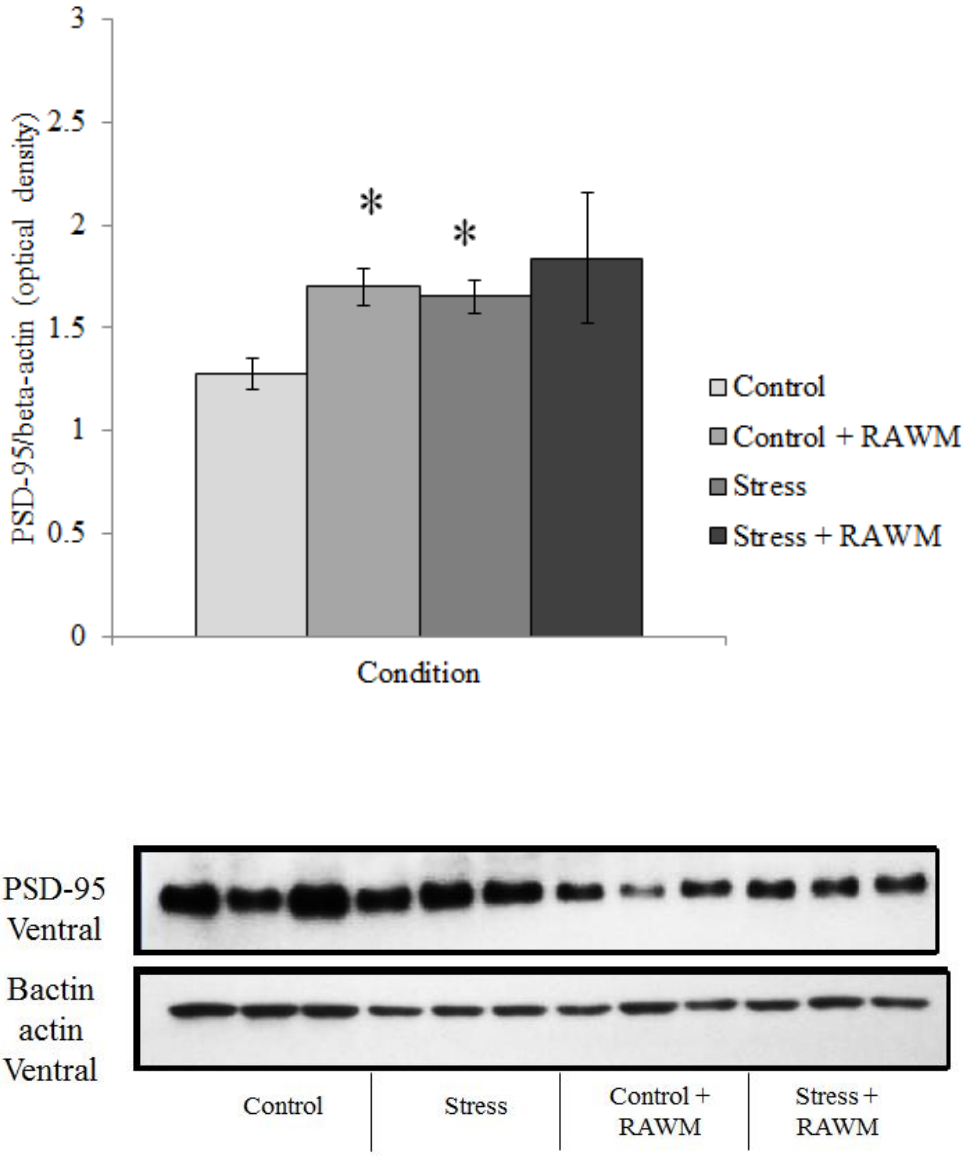

PSD-95/beta-actin (optical density)
3
2.5
2
1.5
1
0.5
0
*
*
Condition
Control
Control + RAWM
Stress
Stress + RAWM
PSD-95
Ventral
Bactin
actin
Ventral
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 40.

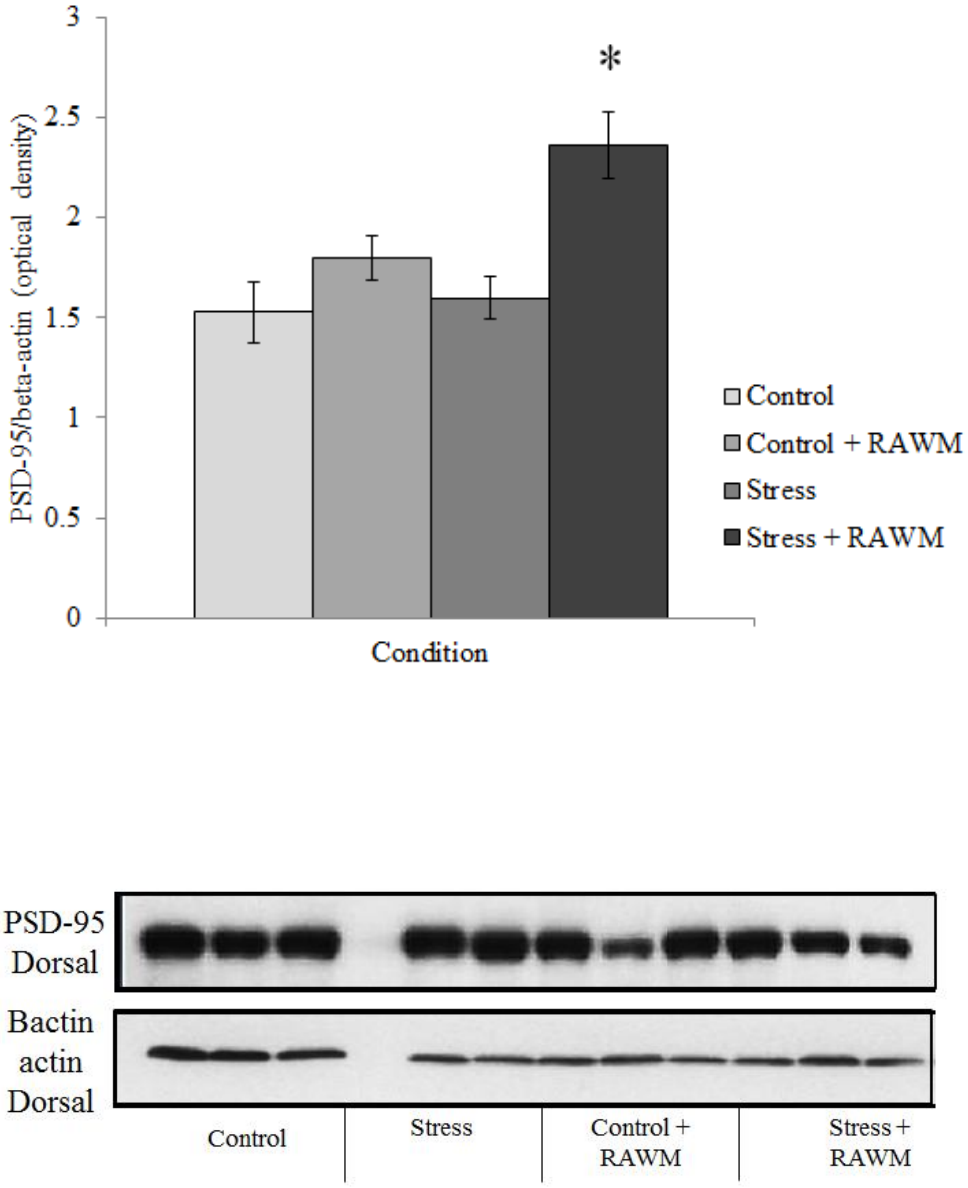
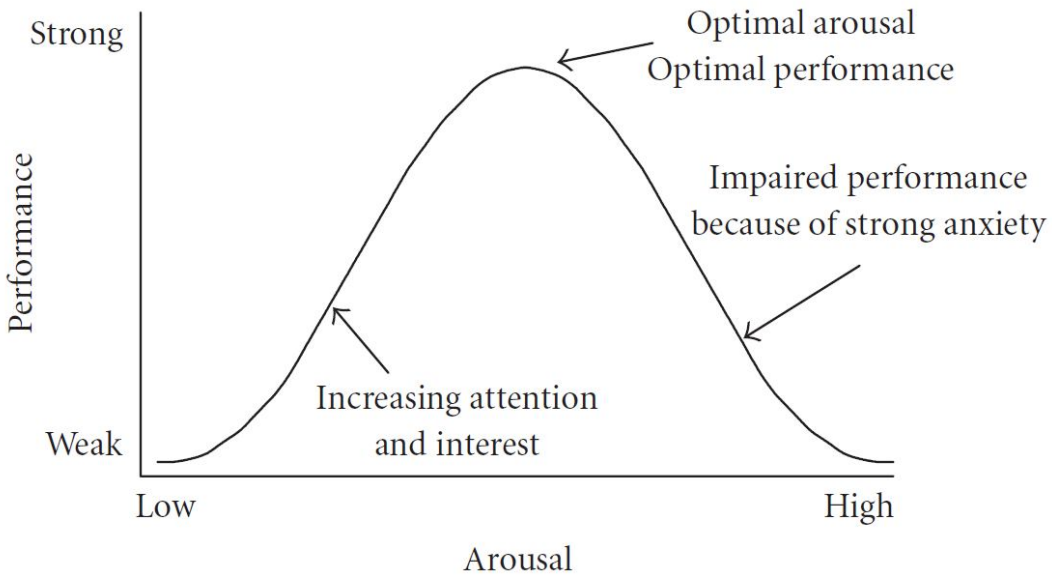

3
2.5
2
1.5
1
0.5
0
PSD-95/beta-actin (optical density)
*
Control
Control + RAWM
Stress
Stress + RAWM
Condition
PSD-95
Dorsal
Bactin
actin
Dorsal
Control
Stress
Control +
RAWM
Stress +
RAWM

Figure 41.


Strong
Performance
Weak
Optimal arousal
Optimal performance
Impaired performance
because of strong anxiety
Increasing attention
and interest
Low
High
Arousal

References

Abidin, I., Yargicoglu, P., Agar, A., Gumuslu, S., Aydin, S., & Ozturk, O. (2004). The effect of chronic restraint stress on spatial learning and memory: Relation to oxidant stress. *International Journal of Neuroscience, 114*(5), 683-699.

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