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EARLY LIFE STRESS, DRUG ABUSE, EXERCISE EFFECTS ON BDNF AND SEX-
INFLUENCED EXERCISE DIFFERENCES

By

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Honors Thesis submitted in partial fulfillment for the designation of Department Honors

Psychology

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Abstract

In 2011, the U.S. reported 3 million child maltreatment cases, an uncomfortably high but recurring figure each year. Research shows exposure to early life stress (ELS) increases an individual's susceptibility to substance abuse, specifically of nicotine, alcohol, and cocaine. Increased susceptibility may result from dysregulation of the HPA axis sustaining activation into adulthood after ELS. Hyperactivation of the HPA axis significantly reduces hippocampal BDNF, a neurotrophin involved in neuronal growth and plasticity. Reduced hippocampal BDNF may be a factor in substance abuse vulnerability. Additionally, research shows exercise protects hippocampal BDNF from stress induced down-regulation. To explore these relationships, this study used maternal separation (MS) to model ELS in rats. Following MS, rats voluntarily exercised for three weeks, or were sedentary, followed by cocaine conditioned place preference. We quantified hippocampal BDNF from these groups and predicted MS would down-regulate BDNF and exercise would ameliorate this effect. Finally, we predicted BDNF levels would correlate with total running activity. We found no significant effect of MS or exercise, and total running activity weakly correlated with BDNF expression. Our results parallel the behavioral results of this experiment, in which there also were no significant effects of exercise on sensitivity to the locomotor or rewarding effects of cocaine. Thus, although no significance was found in this study, it may provide further insight into the relationships between ELS, exercise, and substance abuse and provide footing for improvement in the development of designs exploring them.

Introduction

Millions of child maltreatment reports are made each year in the United States, and over half a million of those reports can be confirmed (U.S. Department of Health and Human Services, 2012). Early life stress of this kind has been shown to be predictive of certain behavioral characteristics and of the presentation of certain diseases and psychological disorders in later life (Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Heim et al., 2000). More specifically, chronic early life stress has been linked to increased abuse of nicotine, alcohol and cocaine (Maddahian, Newcomb, & Bentle, 1988). Vreugdenhil, Kloet, Schaaf, & Datson (2001) define stress as a physical or psychological challenge to normal homeostasis that results in a cascade of events that includes the activation of the hypothalamic-pituitary-adrenal (HPA)-axis. The mechanism by which early life stress exerts its effects is thought to be related to the dysregulation of the HPA axis during development (Gutteling, de Weerth, & Buitelaar, 2005; O'Connor et al., 2005). Normal functioning of the HPA axis works to regulate the stress response through a negative feedback loop involving the release of stress hormones. In adults who have experienced chronic stress during their early development, their HPA axis appears to be hyperactive, failing to appropriately regulate its release of stress hormones (Heim et al., 2000; Plotsky et al., 2005).

Both animals and humans exposed to early life stress exhibit inappropriate decreases or impairment in neurogenesis and synaptic plasticity into adulthood (Herpfer et al., 2012; Liu, Diorio, Day, Francis, & Meaney, 2000; Mirescu, Peters, & Gould, 2004), likely as a result of stress and its effect on the HPA axis. However, it is now known that brain-derived neurotrophic factor (BDNF) plays a critical role in the stress response as well and also interacts with HPA axis functioning (Lippmann, Bress, Nermeroff, Plotsky, & Monteggia,

2007; Taliaz et al., 2011). It is BDNF's role as a neurotrophin that implicates it as the primary agent facilitating neurogenesis and synaptic plasticity in crucial brain areas involved in the stress response. Neurotrophins are a family of growth factors that mediate the growth, survival, and development of neurons, playing a developmental and neuroprotective role in the brain. During times of stress, glucocorticoids secreted by the HPA axis rise and subsequently cause levels of BDNF to fall (Smith, Makino, Kvetnansky, & Post, 1995). Chronic activation of the HPA axis causes a long-term decrease in BDNF and, thus, its neurotrophic functions become weakened (Nibuya, Takahashi, Russell, & Duman, 1999; Smith, Makino, Kventnansky, & Post, 1995). It may be that this decreased neurotrophic activity contributes to the increased susceptibility to substance abuse and other diseases seen in adults exposed to early life stress (Brake, Zhang, Diorio, Meaney, & Gratton, 2004).

However, although much research suggests adverse effects of early life stress, evidence is emerging to suggest some of those effects may be attenuated, or even reversed, by behavioral interventions. Interestingly, the incidence of substance abuse among adolescents with a history of early life stress appears to be lower if physical activity and fitness were prominent during their development (Field, Diego, & Sanders, 2001; Kirkcaldy, Shephard, & Siefen, 2002). Thus, exercise may play a protective role against drug-seeking behavior in adulthood when it is performed during development. Furthermore, exercise has previously been shown to increase levels of BDNF expression in brain areas involved in the stress response, such as the ventral midbrain, prefrontal cortex, and hippocampus (Maniam & Morris, 2010; Neeper et al., 1996). Thus, exercise should be studied as an important mechanism that may be capable of ameliorating the adverse effects of early life stress during

development on the behavioral and physiological responses to stressful later life events and vulnerability to substance abuse.

BDNF

Neurotrophins are growth factors that mediate the growth, survival, and maintenance of neurons. These include neurotrophin 3 (NT-3), neurotrophin 4 (NT-4), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) (Dechant & Neumann, 2002).

Neurotrophic mechanisms work by activating signaling pathways that lead to the transcription of proteins involved in neuronal growth and plasticity. Specifically, three signaling pathways are activated upon specific binding of a neurotrophin to its receptor: the phospholipase C- γ pathway, the phosphatidylinositol-3-kinase (PI-3-K) pathway, and the mitogen-activated protein kinase (MAP-kinase) pathway, also known as the extracellular signal-regulated protein kinase (ERK) pathway (Chao, 2003; Kaplan & Miller, 2000; Rhee, 2001; Russel, 1995; Segal & Greenberg, 1996). These pathways lead to increased proliferation and differentiation of neurons and to anti-apoptotic features.

BDNF specifically binds to the tyrosine-kinase B (TrkB) receptor (Barbacid, 1995; Barde, 1989), activating transcription in the hippocampus through the cyclic-AMP response element binding (CREB) protein and leading to transcription of protective factors, through the ERK and PI-3-K pathways (Xheng, Zhou, Moon, & Wang, 2012; Barnabe-Heider & Miller, 2003). The protective functions of BDNF and its ability to modulate synaptic plasticity in the hippocampus may be attributed to its ability to modulate pre- and postsynaptic transmission and to distinguish among GABAergic and glutamatergic cell types (Gottschalk, Pozzo-Miller, Figueroa, & Lu, 1998). Previous studies of neurotrophin-induced synaptic modulation involving BDNF found BDNF to affect presynaptic release of glutamate

to glutamatergic, but not GABAergic postsynaptic neurons, emphasizing its role at excitatory synapses (Schinder et al., 2000). It has more recently been found that BDNF can modulate presynaptic transmitter release to GABAergic postsynaptic neurons as well, and that this modulation produces inhibitory action at glutamatergic synapses (Poo & Wardle, 2003), which explains why BDNF was found to reduce efficacy of inhibitory transmission in the hippocampus and not to increase excitatory transmission (Tanaka, Saito, & Matsuki, 1997).

In the course of early development, BDNF has been shown to modulate the development and maintenance of the serotonergic system (Djalali et al., 2005; Mamounas et al., 2000; Rumajogee et al., 2002), promote long-term potentiation (LTP) (Korte, Staiger, Griesbeck, Thoenen, & Bonkoeffer, 1996; Kovalchuk, Hanse, Kafitz, & Konnerth, 2002), and play a role in the development of resilience to stress (Taliaz et. al., 2011). Further, BDNF knockout mice do not survive more than a few weeks, indicating BDNF to be of critical developmental importance (Ernfors, Lee, & Jaenisch, 1994). BDNF appears to particularly mediate the growth and survival of hippocampal neurons, especially in the context of stress; having high concentrations of glucocorticoid receptors, hippocampal neurons are more susceptible to apoptosis and damage following stress (Kawata et al., 1996). Finally, midbrain dopamine neurons self-express BDNF, which may be responsible for survival of those neurons (Ceccattelli, Ernfors, Villar, Persson, & Hokfelt, 1991; Numan, & Seroogy, 1999), and will be shown later to play a critical role in drug sensitization following the stress-activated release of glucocorticoids.

Maternal Separation

Both laboratory and clinical studies in animal models and humans have consistently shown a link between early life stress and the concomitant change in the stress response

patterns of adults (Kalinichev, Easterling, Plotsky, & Holtzman, 2002). Specifically, early life stress has been shown to alter HPA axis responsiveness to later stressors (Essex et al., 2011), which cause the HPA axis to become hyper-activated and to secrete increased amounts of glucocorticoid stress hormones (Heim et al., 2000; Rinne et al., 2002). For clear ethical reasons, early life stress cannot be directly manipulated for study in human populations. However, rodent models of maternal separation have proven to be effective in producing similar changes to the HPA axis, as well as similar behavioral responses to stress as are seen in humans who experience early life stress (Lippmann, Nemeroff, Plotsky, Monteggia, 2007; Marais, van Rensburg, van Zyl, Stein, & Daniels, 2008). Thus, rodent maternal separation is a useful procedure for mimicking the effects of early life stress on humans and for studying the possible mechanisms and treatments for associated disorders that result from HPA axis dysregulation, such as anxiety, depression, and substance abuse.

Maternal separation also produces similar changes in BDNF expression as are seen in stressed humans as homeostasis is challenged and the HPA axis is dysregulated (Vreugdenhil, Kloet, Schaaf, & Datson, 2001). As stated, the increased secretion of glucocorticoids by the HPA axis has an adverse effect on the survival of neurons in several brain regions, particularly in the hippocampus. The stress imposed by prolonged maternal separation has been shown to cause a prolonged rise in circulating glucocorticoids, even after the stress is removed, which results in significantly reduced BDNF expression in the hippocampus (Smith, Makino, Kvetnansky, & Post, 1995). Additionally, apoptosis and decreased proliferation of hippocampal neurons has been observed in maternally separated rodents (Mirescu, Peters, & Gould, 2004). When BDNF is looked at in adult rodents that have been maternally separated, BDNF remains downregulated, and the HPA axis continues

to be hyper-responsive to environmental stressors (Nishi, Horii-Hayashi, Sasagawa, & Matsunaga, 2013; Smith, Makino, Kvetnansky, & Post, 1995; Ueyama et al., 1997).

Exercise

The benefits of exercise are numerous and wide-ranging. Of importance to this study is the impact exercise has on the functioning of the HPA axis, especially during childhood and adolescence. The HPA axis responds to stress by releasing glucocorticoids that go on to do harm to the brain and to produce adverse behavioral pathologies if not appropriately regulated (Nibuya, Takahashi, Russell, & Duman, 1999; Smith, Makino, Kventnansky, & Post, 1995). Exercise, however, has been shown to regulate the HPA axis through a curious mechanism. Exercise presents a voluntary stress to the organism that activates the HPA axis and actually increases the amount of circulating glucocorticoids (Stranahan et al., 2006). It would be expected, then, that increased HPA axis activation would have an adverse effect on the brain similar to stress. However, in the hippocampus, exercise works to reduce the affinity of glucocorticoids and mineralocorticoids for their receptors and thus protects the hippocampus from stress-induced activation and damage (Adlard & Cotman, 2004). The hippocampus of exercised animals also shows increased dendritic branching and density, further ameliorating damage by involuntary stress and augmenting hippocampal functions (Redila & Christie, 2006; Stranahan, Khalil, & Gould, 2010). Thus, exercise represents a protective mechanism regulating HPA axis sensitivity by superimposing its affinity regulation on the HPA axis negative feedback system; normally this system facilitates damage when hyperactive by decreasing the number of glucocorticoid receptors being expressed and increasing the amount of circulating hormones to toxic levels.

Furthermore, exercise has been shown to increase hippocampal BDNF expression (Maniam & Morris, 2010; Neeper et al., 1996). Given the protective effects known of BDNF, increasing its expression would potentially protect against stress-induced damage. It is our proposal that although stress in the form of maternal separation causes an increase in HPA axis activation and a subsequent decrease in BDNF expression, the positive effects of exercise may overwhelm the adversity posed by maternal separation. In fact, stressed animals that engage in voluntary exercise show an increase in BDNF expression following prolonged exercise despite significantly increased glucocorticoid secretion by the HPA axis (Adlard & Cotman, 2004; Johnson, Rhodes, Jeffrey, Garland, & Mitchell, 2003; Neeper et al., 1996; Oliff, Berchtold, Isakson, & Cotman, 1998). Given that early life stress causes reductions in BDNF and has been linked to increased substance abuse following early life stress (Cleck & Blendy, 2008), it is possible that exercise could preventatively function to reduce the consequences of early life stress.

Cocaine

Many addictive drugs such as cocaine produce their positive-reinforcing effects by increasing dopamine transmission in mesolimbic and mesocortical pathways (Goeders & Smith, 1983). Cocaine acts by blocking transporters for the reuptake of dopamine, serotonin, and norepinephrine, and dopamine has been shown to play a stimulatory role in the regulation of the HPA axis (Borowsky & Kuhn, 1991). Additionally, stress and cocaine administration cause similar hyper-responsiveness of the HPA axis and of the mesolimbic dopamine pathway, increasing dopamine release (Saal, Dong, Bonci, & Malenka, 2003). Thus, both cocaine addicts and stressed individuals exhibit HPA axis dysregulation and maintenance through similar mechanisms. BDNF and its receptor, TrkB, are up-regulated in

response to cocaine (Fumagalli, Pasquale, Caffino, Racagni, & Riva, 2007), and it is possible that BDNF may differentially regulate long-term neuroadaptations in response to cocaine.

Increasing BDNF levels in the midbrain by infusion has the effect of suppressing cocaine seeking in rats (Berglind et al., 2007), indicating that BDNF may help regulate responses to cocaine as it regulates responses to stress. Further, in stressed animals, BDNF and its receptor, TrkB, fail to be upregulated by acute cocaine challenge (Fumagalli, Pasquale, Caffino, Racagni, & Riva, 2009), suggesting BDNF as a factor in the cross-sensitization between stress and cocaine, and identifying it as a possible target for understanding vulnerability to drugs of abuse.

The purpose of the present study was to evaluate the effects of early life stress, exercise, and cocaine exposure on hippocampal BDNF expression, as the hippocampus expresses high levels of BDNF and is particularly active in neuronal growth, neurogenesis, and neuronal connectivity (Conner, Lauterborn, Yan, Gall, & Varon, 1997; Jacobi, Soriano, Sega, & Moses, 2009). In both rats and humans, BDNF has been repeatedly implicated in the regulation of the HPA axis and the HPA axis has been directly linked to hippocampal BDNF expression. By elucidating the many factors that contribute to changes in the HPA axis and BDNF expression, predictions about the likelihood of behaviors such as drug abuse might be made. Our experimental design inspected the effects of rearing condition, exercise, cocaine, and sex on BDNF expression. We anticipated main effects for rearing condition and exercise, as well as a condition by exercise interaction, such that maternal separation significantly decreases BDNF expression relative to control conditions and exercise rescues this effect.

It is not common that sex differences exist in exercising rats. However, in a recent experiment, we documented a dramatic decrease in wheel running for MS females relative to

control females, but the same disparity was not observed among MS and control males. The group difference in wheel running emerged in the third and final week of wheel access, which, in this experiment, coincided with the onset of puberty. Female rats are known to increase wheel running during puberty. Thus, it is not clear whether decreased wheel running in maternally separated females is a stable behavioral effect, or if it reflects a delay in puberty onset. It may be possible that glucocorticoid alteration by MS plays a role in female running motivation, though this has not been observed (Deroche, Marinelli, le Moal, & Piazza, 1997). To address this issue, we allowed a smaller group of female rats two additional weeks of wheel access. We evaluated the female rats' BDNF expression by comparing female rats from our experiment with female rats from this smaller group. We expected a main effect of exercise such that the rats allowed to run longer would show increased BDNF expression. We also expected an interaction between rearing condition and exercise duration such that maternally separated rats would show a decreased level of BDNF expression relative to controls while longer-exercised rats would show higher levels of BDNF expression relative to controls.

Methods

Subjects

Four litters (n=46) of male and female Long-Evan rats were used. Rats were assigned randomly to maternally separated (MS) or control conditions. MS rats were weighed and isolated for 3hr/day in individual plastic containers on a heating pad to maintain nest temperature (30-33°C). Control rats were weighed and gently handled daily. MS or controlled conditions lasted from post natal day (PND) 2-14. Refer to Figure 1 for the full experimental design.

Exercise

From PND 21-52, pups in each rearing condition were randomly assigned to exercise or sedentary conditions. Pups in the exercise condition were housed in cages with accessible running wheels. Each running wheel was equipped with a magnetic counter. Total wheel counts (one count = $\frac{1}{4}$ turn) were recorded each morning. Pups in the control condition were housed in standard cages. All rats had *ad libitum* access to standard chow and tap water.

Drug Administration and Conditioned-Place-Preference (CPP)

From PND 42-52 rats from each group were subjected to cocaine conditioned place preference testing. Rats from each of the four groups were assigned to cocaine or saline conditions before testing. Cocaine was dissolved in 0.9% saline solution at a concentration of 20 mg/ml, expressed as an HCl salt. The rats were given cocaine by subcutaneous injections. Cocaine CPP was carried out in a 3-compartment CPP chamber (Lafayette instruments) with differing floor textures (cross hatched or random) and wall colors (black or white) in the end compartments. An 8 x 19 photobeam array attached to a PC monitored time spent in each chamber and distance traveled.

Rats were habituated to the chamber on Day 1 of CPP testing. Free access to both compartments for 30 minutes was allowed, and single-sample t-tests were used to determine any baseline preferences for either chamber. On days 2, 4, 6, and 8 of testing, rats were given saline injections and confined to the compartment initially preferred. On days 3, 5, 7, and 9 of testing, rats were given injections of cocaine (20mg/kg) and placed in the non-preferred compartment, or given saline if in the control group. Rats were kept in the compartment for 30 minutes after injection.

On day 10 of CPP testing, rats were given free access to both chambers for 30 minutes, and the difference in preference from day 1 was calculated using the following equation:

$$\frac{(\text{Time in drug paired chamber D10}) - (\text{Time in drug paired chamber D1})}{\text{Time in drug paired chamber D1}}$$

Tissue Collection

24 hours after the final CPP test day, rats were sacrificed, and tissues from the hippocampus, ventral midbrain, and prefrontal cortex were obtained and frozen at -80°C for later analysis. Only hippocampal tissue was analyzed for BDNF content.

Protein Analysis

BDNF quantification followed the sandwich ELISA technique. BDNF protein levels were quantified using the Promega Enzyme Immuno Assay (EIA) kit according to manufacturer recommendations. Tissue from the hippocampus was homogenized in lysis buffer. The solution was centrifuged, and the supernatant was collected. Samples were stored at -20°C.

Prior to performing the ELISA, a total protein concentration assay (PCA) was performed with two 96-well plates and Pierce® BCA Protein Assay Kit by Thermo Scientific according to manufacturer recommendations. Samples were diluted in deionized water in a sample to water ratio of 1:8 for a total volume of 25µl. A standard curve was prepared using albumin standard (BSA) and deionized water to achieve a final range of 0-2,000 µg/mL. Following preparation of the standard curve, 200 µl of the working reagent (WR) was prepared and added to each well containing sample. The WR was composed of 25 ml of

bicinchoninic acid (BCA) reagent A and 500µl of BCA reagent B for colorimetric detection. The plates were incubated for 30 minutes at 37°C. The plate was allowed to cool to room temperature before reading the absorbance with an automated plate reader at 562 nm.

Quantification was performed using two 96-well plates. Plates were coated with Anti-BDNF mono-clonal antibody (10µl) and carbonate coating buffer (9.99ml) thoroughly mixed and pipetted with a multichannel pipettor. Wells were sealed with a plate sealer and incubated over night at 4°C without shaking. The plate was blocked using Block and Sample (B&S) 1X Buffer. All washes were performed using Tris Buffered Saline with Tween (TBST) 1X wash buffer.

A standard curve for BDNF was constructed in duplicate on each plate and plotted on a logarithmic scale. A final BDNF concentration range of 0-500pg/ml was achieved with 1:2 serial dilutions of a solution containing BDNF Standard and B&S 1X Buffer. A Protein Concentration Assay (PCA) was performed to achieve equal protein loading during the ELISA. A final quantity of 20µg of each sample was converted to µl using the standard curve regression from the PCA and was loaded into each well with enough Dulbecco's Phosphate-Buffered Saline (DPBS) to achieve 100µl per well. The plate was incubated for 2h at room temperature with shaking.

Following washing, 30 µl of Anti-Human BDNF polyclonal antibody was added to 14.97 ml B&S 1X buffer to create enough solution for all samples. One hundred (100) µl of this solution was added to each well and the plate was sealed and incubated for 2h at room temperature with shaking. Following washing, 75µl of Anti-IgY HRP Conjugate was added to 14.925 ml B&S 1X buffer to create enough solution for all samples. One hundred (100) µl

of this solution was added to each well and the plates were incubated at room temperature for 1h with shaking. Room temperature Tetramethylbenzidine (TMB) One Solution was added to each well (100µl) and incubated at room temperature for 10 minutes for color development. The reaction was stopped using 100µl of 1N HCl added to each well. Absorbance was read using an automated plate reader at 450nm absorbance immediately following HCl addition.

Statistical Analysis

Final BDNF concentrations (pg/ml) were calculated using the line of best fit equation for the BDNF standard curve. Results were analyzed using factorial ANOVA with IBM SPSS Statistics software version 21. Four-way ANOVA analyzed rearing condition x exercise x drug x sex effects on BDNF expression. Pearson correlations were performed for total wheel running activity and BDNF expression.

Results

A sandwich ELISA was performed using 20µg of hippocampal protein from each sample, loaded with DPBS for a final volume of 100µg per well. Following absorbance readings for two plates, the BDNF standard curve was plotted using a logarithmic scale (Figures 2 and 3). We used the linear regression lines from each plate ($y=1.3467x + .0283$, $R^2= .9841$, $y=1.1907x + .0142$, $R^2= .9985$) to calculate sample BDNF concentrations in pg/ml ($M = 33.13$, $SD = 8.53$). The mean concentration of duplicates from each sample was used for statistical analyses.

We predicted a significant upregulation of hippocampal BDNF in response to exercise (Figure 4) and a significant effect of maternal separation decreasing hippocampal

BDNF expression relative to controls. A potential interaction between rearing condition and exercise was also postulated (Figure 5). A four-way ANOVA was performed and yielded no main effects or interactions for condition, $F(1, 45) = .05, p=.82$, exercise, $F(1, 45) = 1.50, p=.23$, drug, $F(1, 45) = 1.88, p=.12$, or sex, $F(1, 45) = .06, p = .81$.

We also predicted a positive correlation between total running activity and hippocampal BDNF expression. A Pearson's correlation found no such relationship, $r(44) = .27, p > .05$. Finally, we predicted that female rats allowed to run for additional time would show increased BDNF expression compared to female rats who were not allowed to run longer. A t-test did not support this prediction. Females running longer ($M=36.06, SD=14.39$) and who ran the standard 3 weeks ($M = 34.61, SD= 8.55$) did not show significant differences in BDNF expression, $t(9) = -.197, p=.85$.

Discussion

Our study sought to identify whether ELS, exercise, substance abuse, or sex, had significant effects or interactions on hippocampal BDNF expression in rats. Although differences in BDNF are noted, no significant effects or interactions were found. Although we did not predict a main effect of cocaine administration increasing BDNF expression, we did note a drug effect that approached significance. BDNF expression also increased in an approaching significant, drug x exercise interaction. Cocaine upregulated BDNF expression relative to saline controls, and exercise upregulated BDNF in a potential ceiling effect, discussed later. Additionally, our study sought to identify whether or not total running activity correlated with BDNF expression, but we did not find a strong correlation.

Maternal separation models of early life stress have been repeatedly shown to mimic the developmental dysregulation of the HPA axis and subsequent downregulation of BDNF in the hippocampus of humans and rodents (Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007; Liu, Diorio, Day, Francis, & Meaney, 2000; Mirescu, Peters, & Gould, 2004; Nishi, Horii-Hayashi, Sasagaw, & Matsunaga, 2013). We thus anticipated a main effect of rearing condition such that MS rats would display reduced levels of BDNF expression relative to control rats. Our results did not confirm this prediction. However, relative to control rats (n=22), MS (n=24) did appear to downregulate BDNF expression. Due to the complex nature of the design, however, a majority of the rats were subjected to later factors, either exercise or cocaine administration, or both, that are known to influence BDNF expression. Relatively few (MS, n=7, C, n=5) rats were subjected only to a manipulation in rearing condition. Thus, a small sample size limits the interpretation of our results, but is still in support of previous literature on ELS and BDNF expression (Smith, Makino, Kventnansky, & Post, 1995; Taliaz et al., 2011).

Although several studies cite a significant upregulation of BDNF expression in the hippocampus with exercise (Adlard & Cotman, 2004; Oliff, Berchtold, Isakson, & Cotman, 1998; Redila & Christie, 2006), we did not confirm this effect in our study. However, as was the case with MS, rats who exercised had, in general, higher levels of BDNF expression than rats who did not exercise. It may be the case that a small sample size played a role in our results. Additionally, studies that looked at the effect of voluntary exercise on BDNF expression typically employed an average of 6 weeks of exercise in their designs (Daniels, Marais, Stein, & Russell, 2012; Maniam & Morris, 2010). Our study design employed only 3 weeks of voluntary exercise. Other studies have also shown BDNF to be sensitive to output

(amount of exercise) (Johnson, Rhodes, Jeffrey, Garland, & Mitchell, 2003) and time (duration of exercise) (Ploughman et al., 2005) such that BDNF increases correlate with total exercise activity.

When we looked at overall running activity in terms of total wheel revolutions, our data did not support a correlation between total running activity and BDNF expression. However, wheel-running data for some individuals was unavailable due to technical malfunction during the recording stages of the experiment, limiting the interpretation of our data to even fewer individuals and possibly masking a correlation. Furthermore, wheel-running data varied significantly in the third week for MS females, who ran significantly less than control females, and may be a complicating factor in understanding the relationship between BDNF expression and exercise. Thus, we sought to explore the possibility of an MS effect on exercise activity and ultimately on BDNF expression in females. Comparison of BDNF expression in female rats subjected to the standard running time of 3 weeks and female rats allowed to run for additional time were not significant, however. In fact, females allowed to run longer had lower BDNF expression compared to females who ran for 3 weeks.. Although the group of females who ran longer was 3 weeks older at the time of sacrifice, BDNF expression does not appear to change significantly throughout development (Webster, Herman, Kleinman, & Weickert, 2006). A possible explanation for this is the difference in total females in each group and the standard deviations being large

Although we did not support a main effect of exercise or rearing condition in our data, it may be possible that a dysregulating factor such as MS is necessary for the compensatory effect of exercise to emerge. Our results showed an increase in BDNF expression for MS, exercised rats such that MS rats that exercised showed higher BDNF expression than non-

MS, exercised controls, comparable to non-MS, sedentary controls. This evidence would suggest that MS acted against BDNF expression and perhaps altered those rats' baseline BDNF levels as predicted by past research (Adlard & Cotman, 2004). Exercise, then, might have a greater opportunity to exert an effect on the compromised BDNF expression and dysregulated HPA axis of MS rats than it would for a normally functioning HPA axis.

The effect of cocaine administration was not anticipated to be significant by our main hypotheses. However, cocaine administration increased BDNF expression in an approaching significant manner (Figure 6). Previous literature has shown BDNF to be upregulated by cocaine administration (Fumagalli et al., 2009), an effect that is likely involved in addiction and reward pathways which sustain cocaine abuse in humans and in rodents allowed to self-administer cocaine (Deroche, Marinelli, le Moal, & Piazza, 1997; Goeders & Smith, 1983; Grimm et al., 2003).

Wheel-running has been shown to attenuate cocaine self-administration in rats, however, which is consistent with the results we obtained. Cocaine administration and exercise in our study interacted in the anticipated direction such that exercise diminished the difference in BDNF expression between cocaine- and saline-administered rats relative to sedentary controls (Figure 7). It may be possible that exercise produces a ceiling effect such that cocaine administration cannot significantly increase BDNF expression further from exercising controls. Figure 7 suggests this possibility as BDNF expression appeared to independently maximize during exercise and with cocaine administration. Also consistent with previous studies, female rats appeared to be more sensitive to the effects of exercise on BDNF expression when confronted with cocaine administration challenge (Figure 8) (Cosgrove et al., 2002; Larson & Carroll, 2005). Finally, our results are consistent with

behavioral data previously obtained in this experiment (Pritchard et al., 2012). Cocaine CPP was measured over a period of 10 days and a final preference score was indicated. Exercise did not have a main effect on cocaine CPP scores, suggesting that any BDNF expression differences we found in our study may have been insufficient to produce significant behavioral results.

Behavioral data from Pritchard et al. (2012) did show a main effect of rearing condition, however, such that MS rats showed a preference for the cocaine-paired CPP chamber, but controls did not. Additionally, rearing condition showed a main effect on cocaine-induced locomotor activity, such that MS rats showed a decreased locomotor response to cocaine relative to control rats. Finally, the exercised groups showed smaller MS effects than sedentary controls in measures of cocaine-induced locomotor activity, similar to the effect of exercise on BDNF expression during cocaine-administration, suggesting that behavior might be accurately predicted by exercise and hippocampal BDNF expression (Figure 9).

Our study failed to show significant effects of ELS, exercise, drug administration, or sex on BDNF expression. However, our results consistently support the general trends we anticipated based on our literature review and also support the overall trend in behavioral data obtained in the previous experiment on these rats. Thus, it may be true that the confines of our experimental design did not allow for the sensitivity to detect significant differences. Additionally, although the ELISA method is a highly sensitive method for protein detection, it also has limitations that could influence our results. The potential for non-specific binding and cross-reactivity is increased for the sandwich ELISA, which uses multiple antibodies, and thus the signal could be false or confounded. Results also rely on the quality of the kit

and materials. A western blot, in comparison, has less risk for cross-reactivity and can be more specific for the target protein because of the separation of protein components by SDS-page. In this way, we may have been able to target specific forms of BDNF protein for comparison. Western blots are limited by their cost and relative difficulty to perform, however.

Meaningful expectations may be garnered from our data. Importantly, despite the complexity of our design and the possibility of individual differences in the regulation of BDNF and the HPA axis, our data point to a protective effect of exercise for each variable under consideration. In summary, although no significant effects of exercise were found in our study, some data indicate a potential protective effect of exercise on BDNF expression. MS rats who exercised appear to have higher overall BDNF expression than either MS rats who did not exercise or control rats who did not exercise. Further, exercising appeared to increase BDNF only in MS rats while control rats who exercised and who did not exercise showed stable BDNF expression.

Future studies on the protective effect of exercise and the attenuation of the adverse effects of ELS on BDNF and the HPA axis should consider a design that would be more sensitive to detecting differences by means of a greater sample size in every condition. Furthermore, this study used a relatively high dose of cocaine in the cocaine CPP portion that could have confounded both our BDNF results and the behavioral results previously obtained. Also, a longer period of exercise might be considered in future designs such that BDNF regulation might require a more generous period of time to manifest itself behaviorally and in terms of quantifiable BDNF. Sex differences may be further elucidated

with a longer period of exercise, as the timing of the onset of puberty and estrous cycling may be cause for changes in running activity that influence BDNF expression.

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Figure 1: Experimental Design; MS = maternally separated; C = control; Ex = exercise; Sed = sedentary; Co = cocaine administration; Sal = saline

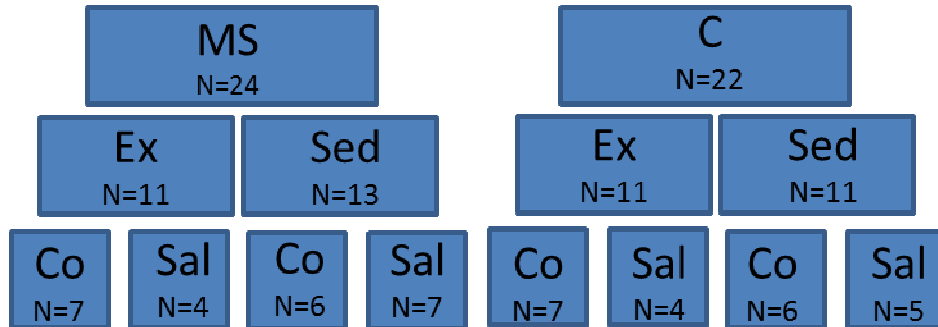


Figure 2: BDNF Standard curve, plate 1

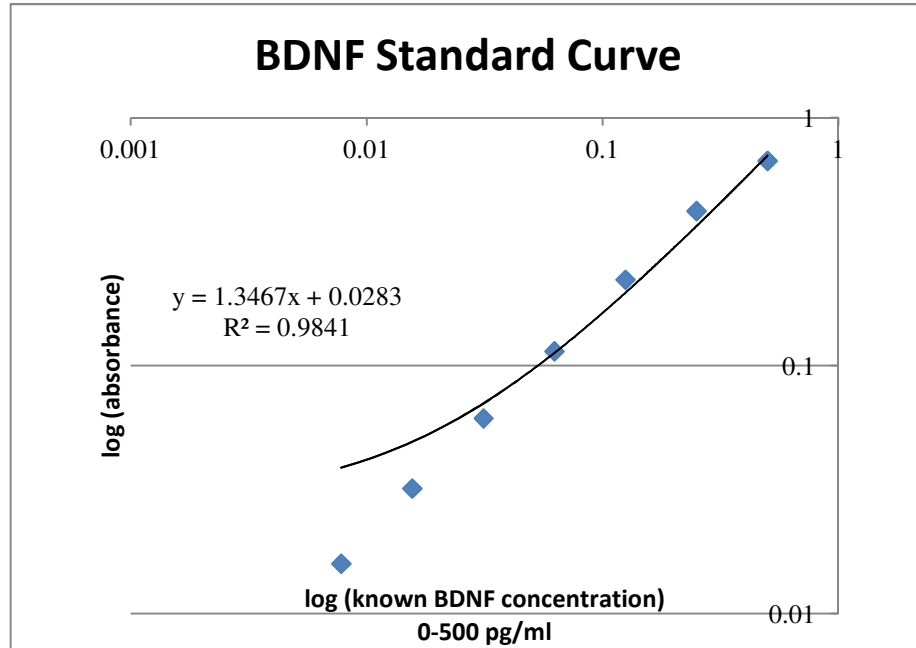


Figure 3: BDNF Standard curve, plate 2

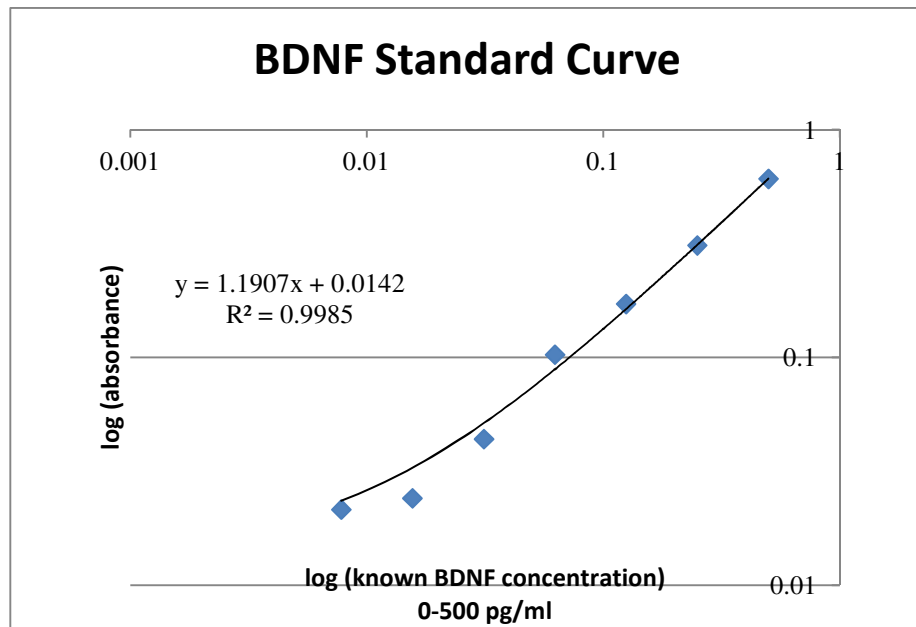
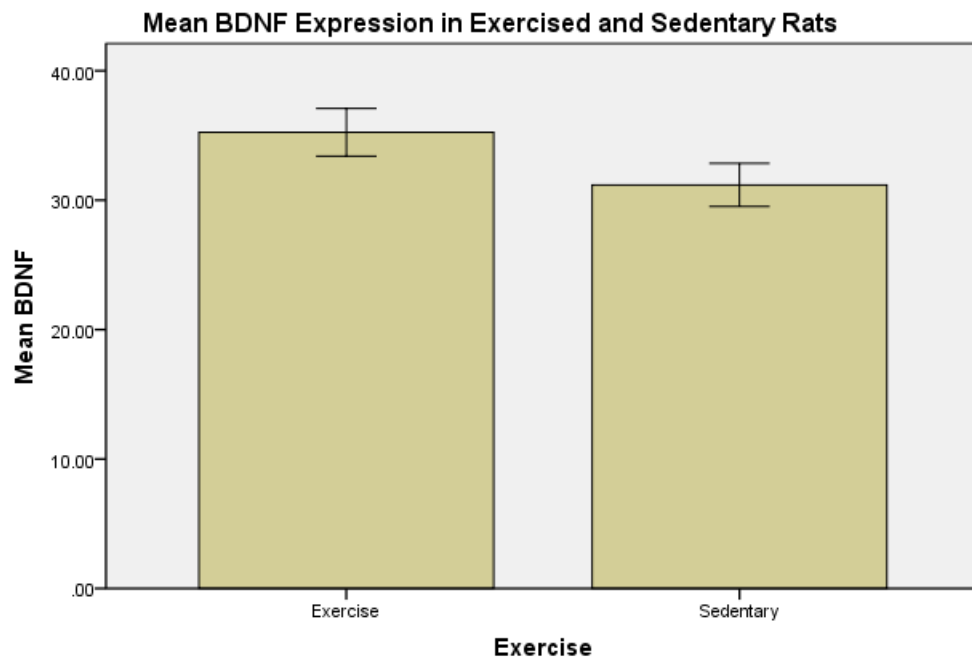


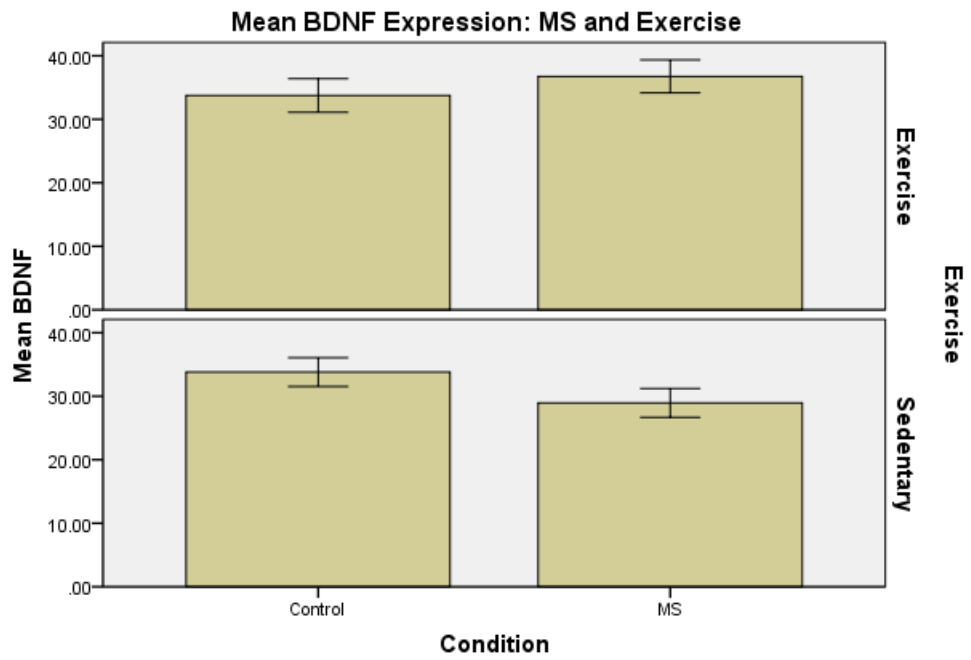
Figure 4



Mean hippocampal BDNF expression in rats who voluntarily exercised and in rats who were sedentary.
Error bars represent 1 standard error.

Error bars: ± 1 SE

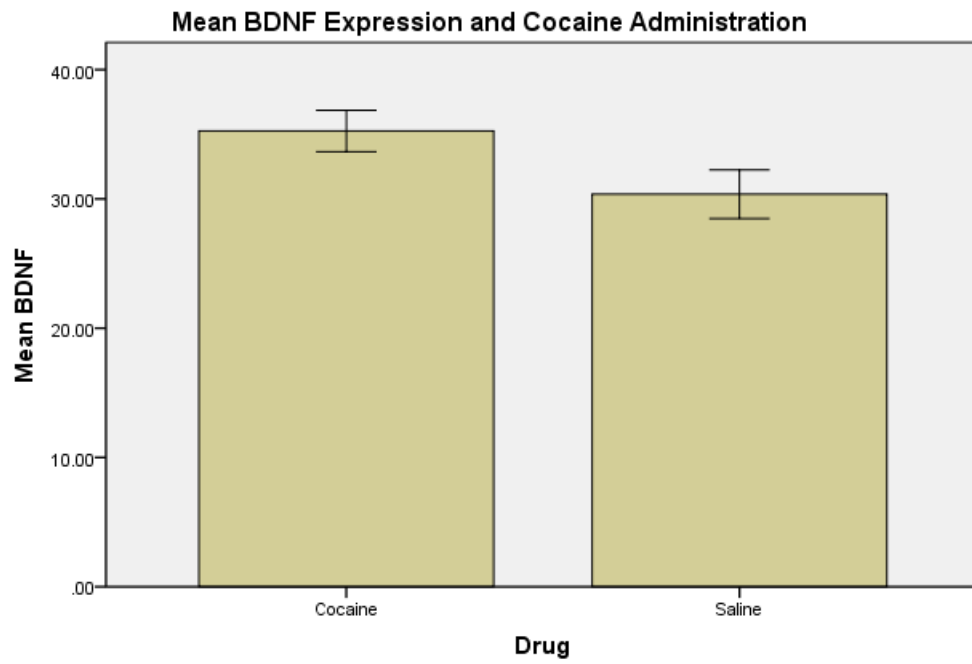
Figure 5



Mean hippocampal BDNF expression in rats who were maternally separated and in control rats relative to the exercised or sedentary condition. Error bars represent 1 standard error.

Error bars: +/- 1 SE

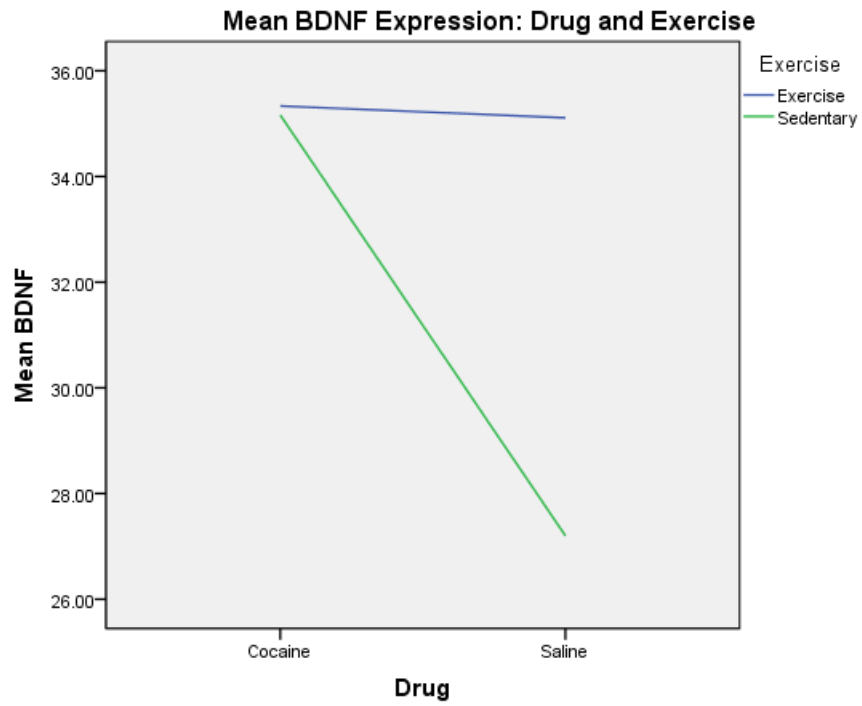
Figure 6



Mean hippocampal BDNF expression in rats who were administered cocaine and in rats who were administered saline. Error bars represent 1 standard error.

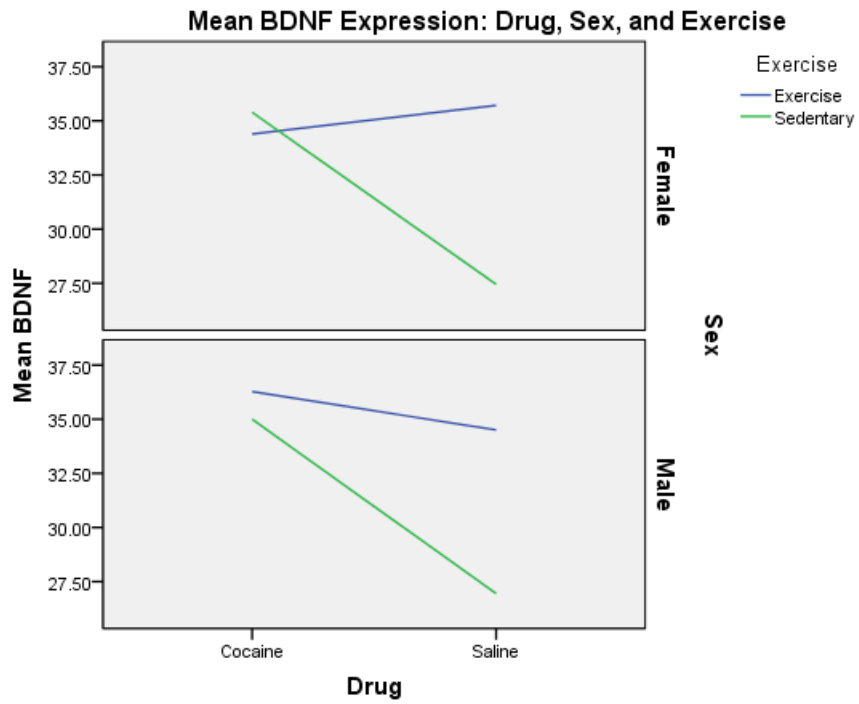
Error bars: +/- 1 SE

Figure 7



Mean hippocampal BDNF expression in cocaine- and saline-administered rats who exercised and who were sedentary.

Figure 8



Mean hippocampal BDNF expression in cocaine- and saline-administered male and female rats who exercised and who were sedentary.

Figure 9: Mean Cocaine-induced locomotor activity in maternally separated and control rats who exercised and who were sedentary (Pritchard et al., 2012).

