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Stress-Induced Modulation Of Brain Dopamine And Serotonin Receptor Expression In Male And Female Rats

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Abstract

The purpose of this experiment was to study how stress modulates neural mechanisms of inhibitory behavior in male and female rats. Specifically, we examined how stress interacts with sex hormones on brain activity in a region called the striatum, which is known to be involved in the formation of habitual behaviors, a key component in behavioral addiction. In this study, we extracted rats' brain tissue and quantified messenger RNA (mRNA) expression for serotonin 6 (5-HT_6R) and dopamine (D1 and D2) receptors in the dorsolateral striatum (DLS) of male and female rat brains following acute restraint stress exposure using quantitative polymerase chain reaction (qPCR) technique. The 5-HT_6R reduces striatal activity and is known to be involved in the inhibitory control of habitual behaviors. Dopamine, on the other hand, increases striatal brain activity and is a primary neurotransmitter in reward pathways. Our findings suggest that, following acute stress exposure, the expression of 5-HT_6R mRNA is significantly higher in male rats compared to female rats. On the other hand, D1 mRNA expression is greater in female than male rats following the same acute stress exposure. Thus, our findings suggest that stress increases DLS brain activity in female rats but decreases activity in male rats. Such modulations of neural mechanisms would suggest that the presence of stress might lead to increased behavioral addiction in female, but not male, rats. Additionally, we expect that stress effect is dependent on the levels of estrogen present in female rats. Understanding the factors that contribute to sex- specific mechanisms involved in habitual behaviors will help treat drug and other types of addictions.

Keywords: 5-HT_6R, DA1R, estrogen, dorsolateral striatum

1. Introduction

The devastating effects of addiction are quite apparent. In 2017 alone, 19.7 million Americans 12 years and older had a substance abuse disorder. 8.5 million of the 19.7 also had a mental health disorder[1]. In some cases, repeated drug use leads to permanent brain changes that contribute to relapse even after many years of abstinence[2]. Learning to inhibit habitual drug use behaviors is crucial to both quitting and preventing drug relapse. Understanding factors that influence central inhibitory control is vital for developing effective treatments to prevent relapse in addiction sufferers. Some studies suggest that in stressful situations, women have reduced ability to control impulses compared to men[3,4,5]. Thus, identifying sex-specific factors that influence modulation of neurological mechanisms involved in self-control and behavioral inhibition could be relevant for developing sex-specific treatments. This study employed a rodent model to investigate interactive influences of stress on brain changes involved in habit formation across the sexes.

The striatum is a brain region that is central to habit formation and behavioral inhibition, and is relevant to drug abuse vulnerability[6]. The present study investigated the effects of stress on dopamine 1 receptor (DA1R) and serotonin 6 receptor (5-HT_6R) expression in the dorsolateral striatal regions of male and female rats. The striatum contains

ninety percent of all dopamine found in the brain[7]. Levels of dopamine are regulated through multiple receptors, including DA1R. This receptor is important because it is involved in sensorimotor and reward seeking behaviors[8]. The striatum also contains the highest density of 5-HT_6R[9]. 5-HT_6R plays a role in the inhibition of repetitive habitual behaviors[10]. Therefore, both DA1R and 5-HT_6R receptors are likely to influence behavioral addiction, as DA1R regulates responses to increase rewarding stimulation, and 5-HT_6R regulates the inhibition of habit formed behaviors. The goal of this study was to determine how an acute stressor induces changes in the expression of these two receptors in the dorsolateral striatum of male and female rats.

The 5-HT_6R may be of particular importance. Unlike other serotonin receptors, 5-HT_6R is solely expressed in the Central Nervous System (CNS) and there are no other known molecular forms of this receptor[11]. Thus, a pharmacological use of 5-HT_6R modulating drugs is confined to the effects in the CNS[10]. 5-HT_6Rs are expressed presynaptically on inhibitory neurons in the dorsolateral striatum that reduce dopamine signaling[12]. 5-HT_6R stimulation enhances GABA release and inhibits dopamine (DA) release, possibly inhibiting motor responses and/or impulsivity[13]. Consistently, stimulation of dorsolateral striatal 5-HT_6R decreases repetitive, habitual behaviors[9], while inhibition of 5-HT_6R promotes cocaine-seeking relapse in rats[14]. 5-HT_6R is also involved in depression and anxiety, two mental illnesses that can cause those affected to seek and develop behavioral addictions. In 2013 alone, 1.4% of adolescents had a major depressive episode in the same time frame that they had a substance use disorder[15]. In the same year, 3.2% of adults with any type of mental illness had a substance use disorder in the same time frame, and 1.0% of adults had a serious mental illness while also undergoing a substance use disorder[14]. Although it is not yet understood why, both 5-HT_6R agonists, as well as antagonists, have both demonstrated antidepressant and anxiolytic effects[10]. As 5-HT_6R can be utilized to reverse the effects of mental illnesses that can cause those affected to seek and develop behavioral addictions, understanding its role and how it is affected by stress is crucial.

To conduct the experiment, stressed and non-stressed rats were taught behavioral tasks at the outset, then repeated the tasks at a later date. These behavioral tasks were a part of the entire experiment to test the sex differences in the effect of stress on learning. The results of the behavioral portion of this experiment are published in *Sex Differences and the Role of Acute Stress in the Open-Field Tower Maze* (Olga Lipatova et al.)[16]. Prior to euthanization, stressed rats were again exposed to the original acute stressor, and brain tissue was collected from the dorsolateral striatum.

2. Experimental Process

The subjects utilized in this experiment were 23 male and 19 female Sprague Dawley rats. The rats were trained on an Open-Field Tower Maze (OFTM). The subjects learned one of two possible tasks on the OFTM: a place learning task or a response learning task. During place learning (n = 21), subjects were taught to always return to the same tower in the OFTM, regardless of where they were placed within the maze at the beginning of each trial. During response learning (n = 21), the subjects were taught to always choose the tower to either their left or their right when they were placed in the maze, regardless of which towers they were next to. During training, 20 rats were exposed to an acute stressor for thirty minutes prior to entering the maze during the retention test portion of the experiment. The acute stressor consisted of placing a rat in a restraint tube and exposing it to a bright light for thirty minutes. After all training and testing trials were run, the rats were exposed to an identical stressor immediately before they were euthanized. At the time of euthanasia, the blood serum was collected for corticosterone level analysis. The mean serum (±SEM) corticosterone levels obtained following restrained stress was 29.58 ng/ml (±5.55) for males in the control group, 42.66 ng/ml (±19.42) for females in the control group, 181.94 ng/ml (±35.66) for males in the stress group, and 135.91 ng/ml (±71.94) for females in the stress group. The reported levels of corticosterone support that the stressor used induced a moderate physiological stress response. All the behavioral data has been previously published[16].

Harvested micropunches of dorsolateral striatum from all rats were analyzed using quantitative, real-time polymerase chain reaction (qPCR). Brain punches were homogenized in Trizol (Invitrogen) to separate nucleic acid from other organic components. Homogenates were incubated with chloroform to extract RNA in an upper phase of clear liquid that was carefully separated from other layers. The aqueous fluid was then treated with isopropanol to precipitate RNA from solution. Lastly, precipitates were rinsed using a series of ethanol rinses, vortex mixes, and centrifugation to produce a clean pellet. Isolated RNA pellets were eluted in RNA storage solution (Ambion) and stored at -80 °C until used for downstream procedures.

When ready, RNA was used to synthesize first strand complementary DNA (cDNA) to be used for PCR. Briefly, an iscript cDNA synthesis kit (BioRad) was used to complete this step. The enzyme reverse transcriptase and a proprietary blend of raw materials are incubated at 48 °C for 20 minutes per manufacturer recommendations. Aliquots of synthesized cDNA were stored at -20 °C until used for final procedures.

When ready, cDNA samples were plated in triplicate along with iTaq Universal Probes Reaction Supermix (BioRad) and a gene-specific expression assay (Thermofisher) into a 96 well optical plate. These reactions use Taq polymerase to replicate the sample DNA specific to the proprietary gene expression assays designed by the manufacturer. Two probe assays were added to each well. One was a control gene that is constitutively expressed known as Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). The other was a gene of interest that was either the dopamine 1 receptor (DA1R) or the serotonin-6 receptor (5-HT_6R). Control expression allowed for normalization of specific gene of interest expression to the total amount of RNA in the sample. Data were also analyzed using standard curves of expression signal that were generated for GAPDH, DA1R, and 5-HT_6R. Data are expressed as arbitrary units of fluorescence for the gene of interest relative to the expression of the control gene.

3. Results

The results of the behavioral part of the experimental series were previously published in Lipatova et al, 2018. The molecular analysis of the brain tissue indicates that stress induces an increase of DA1R expression in female place learners, while causing a decrease of expression in male place learners (**Figure 1**). A three-way ANOVA (Sex: Male vs Female X Stress: Stressed vs. Non-Stressed X Learning Type: Place vs Response) revealed a significant sex by stress interaction, $F(1, 34) = 4.53$, $p = 0.04$. In addition, stress increased 5-HT_6R expression in male place learners, while decreasing it in female place learners (**Figure 2**). A three-way ANOVA revealed a significant sex by stress interaction, $F(1, 35) = 5.31$, $p = 0.03$.

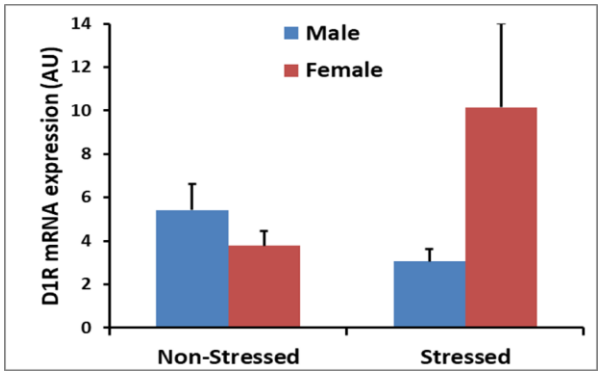

Figure 1: DA1R mRNA Expression in Place Learners. Exposure to stress decreased DA1R expression in male place learners. On the other hand, the same stress exposure increased DA1R expression in female place learners.

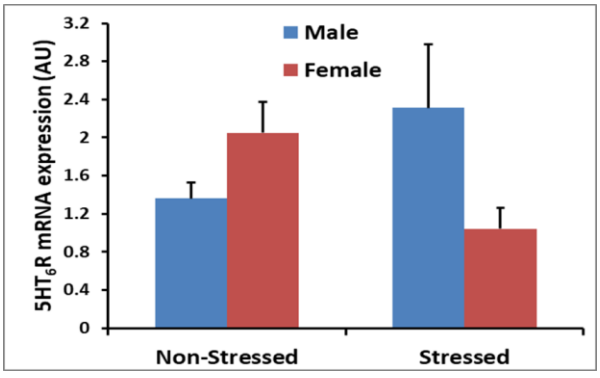

Figure 2: 5-HT_6R Expression in Place Learners. Exposure to stress increased 5-HT_6R expression in male place learners. Exposure to stress decreased 5-HT_6R expression in female place learners.

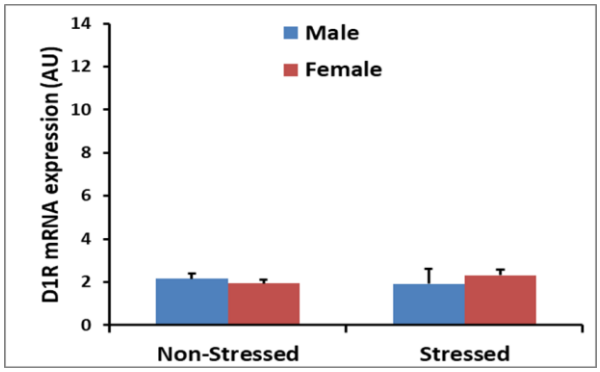

Figure 3: Expression in Response Learners. Exposure to stress did not significantly alter D1R expression in male or female response learners

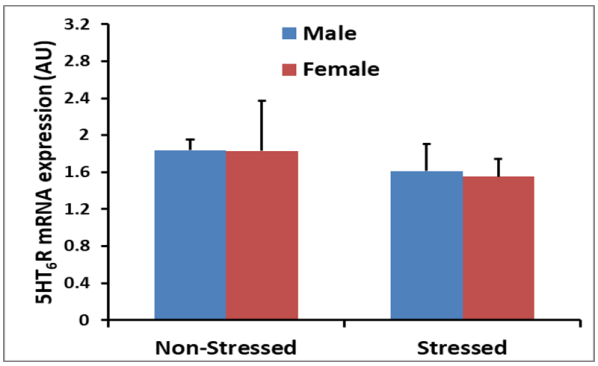

Figure 4: Expression in Response Learners. Exposure to stress did not create a significantly different level of 5-HT_6R expression in male or female response learners.

4. Discussion

Stress induced decreased expression of DA1R in the dorsolateral striatum of male rats, while the expression of DA1R was increased in the dorsolateral striatum of female rats. DA1Rs regulate dopamine activity in the striatal region[6]. Dopamine activity in this brain region is necessary for sensorimotor and exploratory behavior in animals[7] and is known to regulate reward motivated behaviors[17], thus increased dopamine activity likely contributes to addiction related behaviors. Therefore, we hypothesize that, by decreasing the expression of DA1Rs, stress may reduce the addiction related reward seeking behavior in males. On the other hand, stress increased DA1R expression in female rats, thus the increased dopamine activity in the striatum of female rats may escalate reward seeking behaviors related to addiction.

In addition, our findings show that stress increased expression of 5-HT_6Rs in males, and again induced an opposite effect of decreasing this receptor expression in females. Unlike DA1R, 5-HT_6R inhibits neuronal activity in the striatal region through the mechanism described in the introduction above. Increased expression of 5-HT_6Rs in the dorsolateral striatum has been shown to decrease repetitive habitual behaviors in rodents[9], and inhibition of 5-HT_6R promotes cocaine-seeking relapse in rats[14]. Thus, we can infer that stress may increase inhibition in the dorsolateral striatum of male rats, and thus enhance their ability to stop habitual behaviors that are likely to drive addiction. On the other hand, stress appears to do the opposite in female rats. That is, it decreases expression density of 5-HT_6Rs in the dorsolateral striatum, which is likely to increase neural activity in this brain region, thus making them more prone to perpetuate the habitual behaviors present in addiction.

These sex differences were only seen in animals learning the place task and not the response task. We can theorize that the neural plasticity related to learning the place task is more susceptible to stress than plasticity related to response learning. Scientific evidence[18] indicates that stress tends to negatively impact more cognitively demanding forms of learning (such as place learning in the OFTM), rather than repetitive habit-like learning (such as response learning in the OFTM). Thus, it is not surprising to observe cellular changes due to stress in rats that have previously learned place but not response learning.

Future investigations will explore the effect of stress on serotonin and dopamine receptor expression in the dorsomedial striatum, a brain region known to be involved in goal seeking behaviors. In addition, further experiments will address limitations of the present study. One limitation of this study is that it is unknown if learning the place and response tasks in and of itself, influenced expression of the serotonin and dopamine receptors. Nor is it known whether the acute stressor would have had the same sex-specific effect if the behavioral tasks had not been performed. Future studies can be designed to address this question. Another limitation of this study is the high variability in receptor expression within female rats, which is likely due to gonadal hormone fluctuations throughout the estrous cycle. It is possible that the effect of stress on neural plasticity varies across the cyclic variations in hormones seen throughout the female rats' estrous cycle. The expression of dopamine receptors is known to be influenced by estrogen levels. Almey, Filardo, Milner, and Brake[19] found that the estrogen receptors ER-alpha and GPER-1 are located on a small number of acetylcholinergic (ACh) neurons in the dorsal striatum. These neurons are responsible for the release of acetylcholine, which affects dopamine levels in this brain region, implicating one potential route for estrogen's dopamine regulation. One future experiment will explore how stress affects DA1R and 5-HT_6R expression across different phases of the estrous cycle in stressed versus non-stressed female rats. In a follow-up investigation we will utilize ovariectomized female rats while experimentally manipulating levels of estrogen via a slow-releasing subcutaneous implant containing estradiol. It is important to take into account sex when understanding how stress affects individuals, as the stress response is affected by a complex combination of sex chromosome genes as well as hormonal fluctuation[20]. As males enter into adulthood, they are less likely to display stress-induced affective disorders due to an increase in testosterone production, which does not occur in females[20]. Females, on the other hand, have a higher stress sensitivity during aging which is potentially mediated by changes in estrogen levels[20].

5. Conclusions

Stress plays an important role in the regulation of behaviors related to addiction through its influence on DA1R and 5-HT_6R. Our findings show that stress differentially modulates DA1Rs and 5-HT_6Rs in male and female rats, suggesting that stress increases striatal brain activity in females, and decreases striatal activity in males. Such modulation of neural function would suggest that the presence of stress interferes with females' ability to inhibit a striatum-regulated response, which is likely to increase behaviors related to addiction.

6. Endnotes

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