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SEX DIFFERENCES IN MYELIN-ASSOCIATED PROTEIN LEVELS WITHIN AND DENSITY OF PROJECTIONS BETWEEN THE ORBITAL FRONTAL CORTEX AND DORSAL STRIATUM OF ADULT RATS: IMPLICATIONS FOR INHIBITORY CONTROL

D. W. BAYLESS [a*] **AND J. M. DANIEL** [a,b]

[a] *Department of Psychology, Tulane University, New Orleans, LA 70118, USA*

[b] *Neuroscience Program, Tulane University, New Orleans, LA 70118, USA*

Abstract—Impulsive actions and decisions often lead to undesirable outcomes. Lesion and neuroimaging studies have revealed that the orbital frontal cortex (OFC) and dorsal striatum (dSTR) play key roles in inhibitory control. It has been proposed that greater OFC input into the dSTR reflects enhanced top-down cognitive control and less impulsive responding. We previously reported a sex difference in inhibitory control, such that female rats make fewer impulsive errors than do male rats. The goal of the present study was to investigate differences in the OFC and dSTR of young adult male and female rats. In Experiment 1, we measured levels of two myelin-associated proteins, myelin basic protein (MBP) and myelin proteolipid protein (PLP), in the OFC and dSTR. Western blot data revealed that females had significantly higher levels of both MBP and PLP in the OFC but similar levels in the dSTR as compared to males. In Experiment 2, we infused the anterograde tracer, biotinylated dextran amine (BDA), into the OFC and measured the density of BDA in the dSTR. BDA was visualized using histochemistry followed by light microscopy imaging and densitometry analysis. Density of BDA in the dSTR was significantly greater in females as compared to males indicating that the projections from the OFC to dSTR may be greater in females as compared to males. Our results suggest a potential neuroanatomical sex difference that may contribute to the reported differences in inhibitory control levels of male and female rats.

Key words: sex difference, orbital frontal cortex, striatum, myelin, anterograde tracer, inhibitory control.

INTRODUCTION

Inhibitory control is vital for normal everyday functioning, and deficits in inhibitory control contribute to many neuropsychiatric disorders (Steel and Blaszczynski, 1998; Breedlove et al., 2007). Studies investigating the neuroanatomical circuitry of impulsivity have demonstrated the important role that the prefrontal cortex (PFC) plays in inhibitory control in humans and animals (Dove et al., 2000; Brass and von Cramon, 2002; Cardinal, 2006; Eagle and Baunez, 2010). Human subjects with damage to the PFC display impulsive actions during stop-signal tasks (Aron et al., 2003) and impulsive choices during gambling tasks (Bechara et al., 1994). It has been proposed that the PFC influences impulsivity by modulating the operations of lower brain areas involved in reward-based behaviors, such as the striatum (Galvan et al., 2006; Perry et al., 2011; Peper et al., 2012). The striatum is involved in reward associated stimulus–response behaviors (Eichenbaum, 2012). In this view, the PFC acts as a top-down modulator of these lower brain areas, integrating behaviorally relevant information and preventing the over-reliance on fixed action patterns (Perry et al., 2011; Peters and Buchel, 2011). Using functional magnetic resonance imaging (fMRI), the striatum has been shown to be hyper-responsive when individuals choose immediate small rewards over delayed large rewards (McClure et al., 2004), and the magnitude of striatal activation correlates with the amount of impulsive choices individuals make during a delay-discounting task (Hariri et al., 2006). In addition, using transcranial magnetic stimulation (TMS) to disrupt the PFC leads to in an increase in preference for immediate small rewards over delayed large rewards (Figner et al., 2010), suggesting that blocking the ability of the PFC to modulate the operations of lower brain areas results in increased impulsivity. Furthermore, results using tract-based diffusion tensor imaging have revealed that lower integrity within the frontostriatal white matter tract predicts a greater increase in impulsivity as the delay for a large reward over an immediate small reward increases (Peper et al., 2012).

Studies investigating sex differences in inhibitory control and the symptomology of attention deficit

*Corresponding author. Address: Department of Anatomy, University of California San Francisco, San Francisco, CA 94158, USA. Tel: +1-415-514-4383; fax: +1-415-514-4360.
E-mail addresses: daniel.bayless@ucsf.edu (D. W. Bayless), jmdaniel@tulane.edu (J. M. Daniel).

Abbreviations: ABC, avidin–biotin peroxidase complex; ADHD, attention deficit hyperactivity disorder; ANOVA, analysis of variance; BDA, biotinylated dextran amine; dlSTR, dorsolateral striatum; dmSTR, dorsomedial striatum; dSTR, dorsal striatum; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; MBP, myelin basic protein; OFC, orbital frontal cortex; PBS, phosphate-buffered saline; PFC, prefrontal cortex; PLP, myelin proteolipid protein; TTBS, 0.1% Tween/1 X Tris-buffered saline; vSTR, ventral striatum.

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hyperactivity disorder (ADHD) indicate that males display greater inhibitory control deficits and are more frequently diagnosed with the hyperactive-impulsive subtype of ADHD than are females (Gershon, 2002; Rucklidge, 2010; Davies, 2014). In addition, many but not all studies examining inhibitory control in the general population indicate that males are more impulsive than are females (Kirby and Marakovic, 1996; Rosenblitt et al., 2001; Whiteside and Lynam, 2003; Fillmore and Weafer, 2004; Skinner et al., 2004; Heyman and Gibb, 2006; Reynolds et al., 2006; Li-Grining, 2007; Van et al., 2008; Moilanen et al., 2009). Our laboratory and others have reported that adult male rats make more impulsive actions than do females when a delayed response is required during the 5-choice serial reaction time task and other tests of spatial divided attention (Jentsch and Taylor, 2003; Bayless et al., 2012). In addition, our laboratory has demonstrated that prepubertal male rats make more impulsive choices for an immediate small food reward over a delayed large food reward than do females and that this sex difference is organized by neonatal exposure to estrogens and androgens (Bayless et al., 2013).

One possible neuroanatomical mechanism that could contribute to a sex difference in inhibitory control would be a difference in myelination and density of projections from the orbital frontal cortex (OFC) to dorsal striatum (dSTR), such that females as compared to males have increased OFC input into the dSTR leading to enhanced inhibitory control. The levels of myelination or white matter vary across brain regions, and sex differences in brain connectivity are reported (for review see, Gong et al., 2011). To our knowledge no study to date has examined sex differences in myelination and projections from the OFC to dSTR. Projections from the dSTR diverge into two routes: the direct pathway leading to excitation of thalamic projections to motor cortex and the indirect pathway leading to inhibition of thalamic projections to motor cortex (Mink, 1996; Graybiel, 2000). The balance between these two pathways allows for the release of desired behavioral patterns while inhibiting undesired behavioral patterns (Miller and Buschman, 2007). The excitatory glutamatergic inputs of the OFC into the dSTR are hypothesized to modulate the activity of the direct and indirect pathways of the dSTR (for review see, Eagle and Baunez, 2010). A neuroanatomical sex difference in the density of projections from the OFC to dSTR in female rats as compared to males could provide females with increased OFC input into the dSTR leading to increased inhibition of thalamic projections to motor cortex via the indirect pathway, thereby dampening motor output and enhancing the ability of females to inhibit undesirable behaviors.

The goal of the present study was to test the hypothesis that there is a sex difference in male and female rats such that myelination and density of projections from the OFC to dSTR are greater in females as compared to males. In Experiment 1, we examined sex differences in the levels of myelin-associated proteins in the OFC and dSTR via western blotting for myelin basic protein (MBP) and myelin proteolipid protein (PLP). MBP is responsible for the adhesion of multilayered compact myelin to axons and to itself and is the second most abundant protein in central nervous system (CNS) myelin (Boggs, 2006). PLP is a hydrophobic integral membrane protein and the most abundant protein in CNS myelin (Greer and Lees, 2002). Increased levels of MBP and PLP are indicative of increased levels of myelination. In Experiment 2, in order to gain insights into a possible sex difference in the density of the projections from the OFC to dSTR, we infused an anterograde tracer into the OFC of male and female rats and measured the density of the tracer in the dSTR.

EXPERIMENTAL PROCEDURES

Experiment 1: analysis of myelin-associated protein levels in the OFC and dSTR

Animals. Nine female and eight male Long-Evans hooded rats, approximately 60 days old, were purchased from Harlan Sprague–Dawley. Animal care was in accordance with the guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all procedures were approved by the Institutional Animal Care and Use Committee of Tulane University. Rats were housed in same-sex pairs in a temperature-controlled vivarium under a 12-h light/dark cycle (lights on at 7:00 a.m.). Prior to use in the present experiment, rats were used to pilot behavioral tasks for future use in the laboratory.

Vaginal cytology. To control for effects of fluctuating ovarian hormones, vaginal smears of female rats were collected by lavage each morning and analyzed daily beginning 2 weeks prior to brain dissection. To control for handling effects, males underwent sham smears during which a small amount of water was placed on the genitals using a medicine dropper. Females were sacrificed at the proestrous stage of the estrous cycle, at which time circulating estradiol levels are at their peak and vaginal cytology is characterized by large nucleated epithelial cells (Becker et al., 2005). Each male was paired with a particular female and was sacrificed on the same day as that female.

Tissue dissection and processing. At approximately 85 days old, male and female rats were deeply anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (7 mg/kg) and killed by decapitation. Whole brains were removed and quick-frozen on dry ice. In a cryostat at −20 °C, coronal sections containing the OFC and dSTR were cut using coordinates from Paxinos and Watson (1998) (OFC: AP +4.2 mm to +2.7 mm; dSTR: AP +1.7 mm to −0.4 mm). Using a scalpel and visual cues from natural boundaries such as the corpus callosum, the OFC and dSTR were dissected (as described in Spijker, 2011). Tissue samples from both hemispheres were pooled for each animal and stored at −80 °C until processing. Tissue was homogenized in 20 µl/mg lysis buffer