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Mechanisms by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats

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ABSTRACT

Neonatal testosterone, either acting directly or through its conversion to estradiol, can exert organizational effects on the brain and behavior. The goal of the current study was to examine sex differences and determine the role of neonatal testosterone on prefrontal cortex-dependent impulsive choice behavior in prepubertal rats. Male and female prepubertal rats were tested on the delay-based impulsive choice task. Impulsive choice was defined as choosing an immediate small food reward over a delayed large reward. In a first experiment to examine sex differences, males made significantly more impulsive choices than did females. In a second experiment to examine the organizational effects of testosterone, females treated with neonatal testosterone made significantly more impulsive choices than did control females and their performance was indistinguishable from that of control males. In a third experiment to determine if the effect of testosterone on performance is due to the actions of androgens or estrogens through its conversion to estradiol, males treated neonatally with the aromatase inhibitor formestane, which blocks the conversion of testosterone to estradiol, females treated neonatally with the non-aromatizable androgen dihydrotestosterone, and females treated neonatally with estradiol made significantly more impulsive choices than did control females and their performance was indistinguishable from that of control males. Results indicate that male pubertal rats display increased impulsive choice behavior as compared to females, that this sex difference results from organizing actions of testosterone during the neonatal period, and that this effect can result from both androgenic and estrogenic actions.

Introduction

The ability to inhibit a behavior or action is essential for performing simple everyday tasks. Inhibitory control deficits contribute to many psychological disorders, such as drug addiction (Moeller et al., 2001), pathological gambling (Steel and Blaszczynski, 1998), and attention deficit-hyperactivity disorder (ADHD) (Breedlove et al., 2007). The prevalence rates of ADHD (Breedlove et al., 2007) and pathological gambling (Blanco et al., 2006) are higher for men than they are for women. In addition, different levels of inhibitory control are reported in men and women. Prepubertal girls display higher levels of inhibitory control than do prepubertal boys (Li-Grining, 2007), and girls are rated by their parents as having higher levels of inhibitory control than are boys (Moilanen et al., 2009). A similar sex difference in inhibitory control levels has been reported in college-aged men and women (Kirby and Marakovic, 1996).

Impulsivity can be defined as action without forethought and classified into at least two distinct processes: impulsive action and impulsive choice (Evenden, 1999). Impulsive actions arise from a lack of behavioral inhibition resulting in an inability to control or suppress premature or inappropriate actions (Eagle and Baunez, 2010). Impulsive action is commonly measured in humans and rodents using stop-signal tasks and serial reaction time tasks (Eagle and Baunez, 2010; Harrison et al., 1999). On the other hand, impulsive choices stem from making decisions or choices without appropriate deliberation of the alternative options (Eagle and Baunez, 2010). An impulsive choice is often demonstrated as an aversion to a delayed reward (Dalley et al., 2008). It is commonly measured in humans and rodents using delay-discounting paradigms in which impulsive choice is defined as the selection of a small immediate reward over a larger but delayed reward (Cardinal et al., 2004).

Studies investigating impulsive behavior in rodent models have primarily used male subjects. The results of the studies examining sex differences in impulsivity in rodents have yielded mixed results. Results from our laboratory (Bayless et al., 2012) and others (Jentsch and Taylor, 2003) indicate that adult male rats exhibit more impulsive action or poorer inhibitory control than do female rats on tasks of spatial divided attention. Additionally, sex differences were reported in novelty-seeking behavior, such that male rats display increased impulsive behavior compared to female rats at mid-adolescence (pnd 40) but not at early-adolescence (pnd 28) or early adulthood (pnd 80) (Cyrenne and Brown, 2011). However, no difference in impulsive

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action between adult male and female rats has also been reported (Burton and Fletcher, 2012). Sex differences in impulsive choice behavior in mice have been reported during an operant delay-discounting task. Under strong food deprivation male mice made more impulsive choices than did female mice, yet under mild food deprivation female mice made more impulsive choices than did male mice (Koot et al., 2009). Together, these studies suggest that factors such as age, motivation, and task demands may play a role in sex differences in impulsivity levels.

The developmental levels of gonadal hormones affect the expression of behavior later in life and could contribute to differences in impulsive behavior among males and females. Phoenix et al. (1959) were the first to demonstrate that prenatal testosterone masculinizes and defeminizes behavior of guinea pigs resulting in an increase in male-like behaviors and a decrease in female-like behaviors. Unless androgens, such as testosterone, are present during a critical period of development, sexual differentiation proceeds in an inherently female direction (Nelson, 2005b). In the hypothalamus, the effects of neonatal testosterone are dependent upon its conversion into estradiol by the enzyme aromatase once inside the cell (Gorski, 1993). Because testosterone can be aromatized into estradiol or converted into the androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase, the organizational effect of testosterone in other brain areas, such as the prefrontal cortex (PFC), could result from the actions of either androgens or estrogens. Since the publication of the Phoenix et al. (1959) paper, various behaviors, ranging from hypothalamic-mediated sex behaviors (Quadagno and Rockwell, 1972; Quadagno et al., 1973; Thomas et al., 1983) to hippocampal spatial memory (Roof, 1993; Williams et al., 1990) have been shown to be influenced by the organizing effects of neonatal gonadal hormone levels. However, no studies to date have investigated the organizational effects of gonadal hormones on the PFC or associated behaviors.

The goal of the current study was to investigate the effects of biological sex and the role of neonatal testosterone exposure on impulsive choice, an aspect of impulsivity that is dependent upon the PFC (Eagle and Baunez, 2010). Impulsive choice was measured using the delay-based impulsive choice task. In this task, rats must choose between an immediate small food reward and a delayed large food reward. Impulsive choice is defined as selection of the immediate small food reward over the delayed large food reward. Animals were tested prior to puberty to isolate early life organizational effects of testosterone by avoiding adult levels of hormones. In an initial experiment, sex differences in performance on the delay-based impulsive choice task were determined. In a second experiment, the organizational effects of testosterone on impulsive choice were determined by assessing the impact of neonatal administration of testosterone on performance in female rats. In a third experiment, the mechanisms by which neonatal testosterone affects impulsive choice responding were determined by treating rats during the neonatal period with hormones or drugs that resulted in either androgenic or estrogenic actions.

Materials and methods

Experiment 1: Sex differences in impulsive choice

Subjects

Ten female and ten male Long–Evans hooded rats, received at 24 days of age, were purchased from Harlan Sprague–Dawley (Indianapolis, IN). 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 group housed with three to four same sex rats per cage in a temperature controlled vivarium under a 12-h light/dark cycle (lights on at 7:00 a.m.). In order to facilitate behavioral testing, the experiment was conducted in two replicates consisting of five males and five females each. Rats were placed on food-restricted diets and weighed daily throughout the experiment to maintain their body weights at approximately 90% of the average free feeding weight for aged-matched Long–Evans rats according to a standard growth chart (Harlan).

Apparatus

Behavioral testing on the delay-based impulsive choice task was conducted in a plastic T-maze (arms: 10 cm wide × 40 cm long × 20 cm high). The maze consisted of a solid black plastic floor and clear plastic walls. The start arm (north) led to two goal arms (east and west, respectively). A clear plastic 25-cm-high sliding door, placed 5 cm into the entrance of each goal arm, confined the rat into a goal arm upon entrance. A second clear plastic 25-cm-high sliding door, placed 5 cm from the end wall of each goal arm, controlled access to the food reward.

Habituation

At 25 days of age, cage mates were placed into the maze for two separate 15-min acclimation periods during which an equal amount of Froot Loops were placed at the end of each goal arm. All four sliding doors were removed, and the rats were allowed to travel freely throughout the maze. Several Froot Loops were placed in the home cages each day.

No-Delay trials

Beginning the day after habituation, rats were trained to choose between a low-reward (LR) arm that contained one piece of Froot Loop and a high-reward (HR) arm that contained five pieces of Froot Loop. Rats were trained and tested in assigned male/female pairs in which rats alternated trials each session. The location of the HR arm was counterbalanced across pairs but always in the same location for any given rat. The maze was cleaned with ethanol between trials. To ensure the completion of all behavioral testing before the onset of puberty, rats were given up to two sessions on a single day. Each session started with a forced trial into each of the LR and HR arms during which a black plastic sliding door blocked access to the opposite arm. The order of these forced trials alternated each session. Following the forced trials, rats were given five choice trials in which they were free to choose either the LR or HR arm. When a rat entered an arm, the first sliding door was lowered to confine the rat in the arm. The second sliding door was then lifted to give the rat immediate access to the food reward. Sessions of this No-Delay condition continued until rats were choosing the HR arm on at least 80% of the trials for two consecutive sessions. All rats achieved criterion performance within 3 days of training.

Delay trials

After all rats reached criterion on the No-Delay sessions, rats were given three 15-s Delay sessions. The goal of the first session at each delay was to habituate and expose the rats to the delay conditions. Performance during the final two sessions was used for analyses. During these sessions, a 15-s delay was imposed when rats entered the HR arm. The 15-s delay on the HR arm was imposed during the two forced trials and all five choice trials. When a rat entered the HR arm the first sliding door was lowered to confine the rat in the arm. The rat then had to wait 15 s before the second sliding door was lifted to provide access to the larger food reward. When a rat entered the LR arm the first sliding door was lowered to confine the rat in the arm and the second sliding door was lifted to provide immediate access to the smaller food reward. After the three 15-s Delay sessions, rats were given three 30-s Delay sessions. The procedure during these sessions was the same as in the 15-s Delay sessions except that the delay before accessing the reward on the HR arm was increased to 30 s. All testing was completed before rats reached 35 days of age and before the onset of puberty. The onset of puberty is typically around 35 days of age for female rats and 45 days of age for male rats (Kennedy and Mitra, 1963).