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Striatal dopamine D2/3 receptor regulation by stress inoculation in squirrel monkeys

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ABSTRACT

Intermittent mildly stressful situations provide opportunities to learn, practice, and improve coping in a process called stress inoculation. Stress inoculation also enhances cognitive control and response inhibition of impulsive motivated behavior. Cognitive control and motivation have been linked to striatal dopamine D2 and/or D3 receptors (DRD2/3) in rodents, monkeys, and humans. Here, we study squirrel monkeys randomized early in life to stress inoculation with or without maternal companionship and a no-stress control treatment condition. Striatal DRD2/3 availability in adulthood was measured *in vivo* by [^{11}C]raclopride binding using positron emission tomography (PET). DRD2/3 availability was greater in caudate and putamen compared to ventral striatum as reported in PET studies of humans and other non-human primates. DRD2/3 availability in ventral striatum was also consistently greater in stress inoculated squirrel monkeys compared to no-stress controls. Squirrel monkeys exposed to stress inoculation in the presence of their mother did not differ from squirrel monkeys exposed to stress inoculation without maternal companionship. Similar effects in different social contexts extend the generality of our findings and together suggest that stress inoculation increases striatal DRD2/3 availability as a correlate of cognitive control in squirrel monkeys.

1. Introduction

Intermittent exposure to mildly stressful situations is a feature of stress inoculation training for people who work in conditions where performance in the face of adversity is required, e.g., medical and military personnel, police, firefighters, and rescue workers (Meichenbaum, 1993; Saunders et al., 1996; Stetz et al., 2007). Stress inoculation is further supported by evidence that mild but not minimal nor severe stress exposure promotes subsequent coping and emotion regulation as described by U-shaped functions (Russo et al., 2012; Sapolsky, 2015; Seery et al., 2010). Exposure psychotherapies likewise teach patients to imagine a graded series of stressful situations and encourage interaction with stressors *in vivo* (McNally, 2007). These procedures promote learning (Craske et al., 2008) and provide opportunities to practice acquired coping skills (Serino et al., 2014).

Previously, we showed that stress inoculation early in life enhances subsequent coping and emotion regulation modeled in mice (Brockhurst et al., 2015) and squirrel monkeys (Lyons et al., 2009; Lyons et al., 2010). In keeping with suggestions that emotion regulation is an aspect of cognitive control (Compton et al., 2011), we also found that stress inoculation enhances cognitive control of impulsive motivated behavior (Parker et al., 2005; Parker et al., 2012). Impaired cognitive control and dysregulation of motivation have been linked to low striatal dopamine D2 and/or D3 receptor (DRD2/3) levels in rodents, monkeys, and humans (Dalley et al., 2011; Groman et al., 2011; Nader et al., 2006; Trifilieff & Martinez, 2014; Volkow et al., 2011). Conversely, increased experimental expression of dopamine D2 receptors in ventral striatum enhances motivation in mice (Trifilieff et al., 2013). High striatal DRD2/3 availability in humans is associated with resilience against the development of addictions (Volkow et al., 2002; Volkow et al., 2006) and pharmacological DRD2/3 agonists decrease impulsive behavior in rats (Fernando et al., 2012). DRD2/3 agonists similarly improve reversal learning as an index of cognitive control in

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humans dependent on psychostimulant drugs (Ersche et al., 2011).

Here, we examine striatal DRD2/3 regulation by stress inoculation in female squirrel monkeys. Females are studied because stress-related mental health disorders are 2–3 times more common in women than men (Altemus et al., 2014). Impaired cognitive control of thoughts, feelings, and behavior is a key dimension of various psychiatric disorders in humans (Groman & Jentsch, 2012) and neural mechanisms that mediate this dimension may provide novel targets for new treatment interventions. Based on our findings that cognitive control of impulsivity is improved in squirrel monkeys exposed early in life to stress inoculation (Parker et al., 2005; Parker et al., 2012), we test for increased striatal DRD2/3 availability in stress inoculated squirrel monkeys studied as adults by measuring [^{11}C]raclopride binding *in vivo* with positron emission tomography (PET).

2. Materials and methods

Female squirrel monkeys (*Samiri sciureus*) that were born and raised at the Stanford University Research Animal Facility served as subjects. All squirrel monkeys were initially housed in undisturbed mixed-sex natal groups through 16 weeks of age. Group composition was determined by birth dates to minimize developmental differences between natal groups. Seasonal synchronous breeding facilitated the generation of age-matched groups.

Groups were housed indoors in 1.8 × 1.2 × 1.8 m species appropriate cages that were cleaned daily. Housing and testing occurred in climate controlled rooms with an ambient temperature of 26 °C. Light/dark cycles were 12:12 h with lights on at 0700 h. All squirrel monkeys were provided unrestricted access to fresh drinking water and monkey chow with fruit and vegetable supplements. Various toys, swinging perches, and simulated foraging activities were provided for environmental enrichment. To facilitate husbandry-related activities and experimental manipulations, squirrel monkeys were trained to leave the home cage through a small sliding door connected to a transport box used for capture and transportation. All procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1. Experimental design

Squirrel monkeys were randomized to one of three 10-week treatment conditions that occurred between 17 and 27 week of age as described elsewhere in detail (Parker et al., 2004; Parker et al., 2006). In the no-stress (NS) control condition, squirrel monkeys were continuously maintained in undisturbed natal groups. In the stress inoculation (SI) condition, squirrel monkeys were individually removed from natal groups once each week for 10 total 1-hr separation sessions (Parker et al., 2004). In the SI + Mom condition, squirrel monkeys were treated identically except that each monkey along with its mother was removed together from natal groups once each week for 10 total 1-hr sessions (Parker et al., 2006). After each social separation in the SI and SI + Mom conditions, squirrel monkeys were returned to the home cage and reunited with the natal group. All separations occurred between 1200 and 1800 h and no more than one squirrel monkey from each natal group was separated on a given day.

After completion of the treatment conditions at 27 week of age, behavioral measures of coping and emotion regulation were collected from all squirrel monkeys in the presence of mothers during involuntary exposure to a novel environment at 9 months of age (Parker et al., 2004; Parker et al., 2006). Mothers were then permanently removed and their offspring were housed with peers. Cognitive control of impulsive behavior was examined in NS and SI squirrel monkeys at 1.5 and 3.5 years of age (Parker et al., 2005; Parker et al., 2012). Novelty seeking was also assessed in NS and SI squirrel monkeys at 2.5 years of age (Parker et al., 2007). Magnetic resonance imaging (MRI) of brain was conducted with NS and SI squirrel monkeys at 3.3 years of age (Katz et al., 2009) and place preference conditioning tests of NS and SI squirrel monkeys were conducted at 4.4 years of age (unpublished observations). Cognitive control, novelty seeking, place preference conditioning, and previous neuroimaging procedures were not conducted with SI + Mom squirrel monkeys and provided the opportunity to assess stress inoculation versus subsequent testing effects. After 4.4 years of age, no tests were conducted with any of the squirrel monkeys except for procedures described below.

All females were housed separately from males in same-sex social groups beginning at puberty around 2.5 years of age to prevent breeding. Striatal DRD2/3 availability was assessed *in vivo* with PET using [^{11}C]raclopride in 24 randomly selected females from the 3 rearing conditions: 9 NS, 7 SI, and 8 SI + Mom females at 11.2 years of age (range 10.5–11.7 years). Lifespan in captivity is ~20 years (Brady, 2000). All neuroimaging was conducted during non-breeding seasons when sex hormone levels remain stable at non-detectable levels in these seasonally breeding primates (Schiml et al., 1999).

2.2. [^{11}C]raclopride radiosynthesis

[^{11}C]raclopride was prepared in a GE TRACERlab FX C Pro module (GE Healthcare) using previously published methods (Langer et al., 1999) with the following modifications. Briefly, [^{11}C] carbon dioxide was delivered from a GE PETtrace cyclotron (GE Healthcare) into the synthetic module, where the methylating agent [^{11}C]methyl triflate, was formed from [^{11}C]carbon dioxide via reduction, halogenation, and triflation. [^{11}C]Methyl triflate was bubbled with a flow rate of 20 mL/min into solution containing acetone (300 μl), *O*-Desmethyl free base precursor (1 mg, 3.3 μmol, ABX), and NaOH (3 μL, 1 N) at −20 °C. The reaction mixture was warmed to room temperature within 1 min, diluted with 1 mL water, and loaded on a semi-preparative HPLC column for purification (Phenomenex Luna C18 5 micro, 250 × 10 mm, 30% acetonitrile, 70% 0.1 M NH_4HCO_2 with 0.5% AcOH, 7 mL/min). The fraction corresponding to [^{11}C]raclopride (Rt = 9.2 min) was collected in a round flask preloaded with 20 mL water. Mobile phase was removed using a solid phase extraction (SPE) process, then [^{11}C]raclopride was eluted from the SPE cartridge with ethanol and subsequent dilution with saline (ethanol < 10% *v*/*v*). Final [^{11}C] raclopride for imaging was sterilized by passing it through a 0.22 μm Millex MP (33 mm) sterile filter. Overall synthesis time was 45 min and radiochemical yield of [^{11}C]raclopride was 2.0 ± 0.6% (n = 40). Analytical HPLC (Phenomenex Gemini C18 5 micro, 250 × 4.6 mm, 60% acetonitrile, 40% 0.1 M NH_4HCO_2 with 0.5% AcOH, 1 mL/min) showed the final product (Rt = 7.8 min) to have >99% radiochemical and chemical purities and high specific activity (10.6 ± 3.9 Ci/μmol; n = 40). Both radiochemical yield and specific activity were decay-corrected to the end of synthesis.

2.3. Neuroimaging

Whole brain anatomical and [^{11}C]raclopride data were acquired with established methods. Each squirrel monkey was fasted overnight and then sedated with an intramuscular injection of 10 mg/kg ketamine hydrochloride. Atropine sulfate was subcutaneously administered at 0.04 mg/kg. Ophthalmic ointment was placed in both eyes and anesthesia was induced with ~1% isoflurane gas. Heart rate, respiration, body temperature, and blood oxygen