Neurobiology of Stress 3 (2016) 68–73

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: http://www.journals.elsevier.com/neurobiology-of-stress/

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

Alex G. Lee [a], Jordan M. Nechvatal [a], Bin Shen [b], Christine L. Buckmaster [a], Michael J. Levy [a], Frederick T. Chin [b], Alan F. Schatzberg [a], David M. Lyons [a, *]

[a] Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA, USA
[b] Department of Radiology, Stanford University School of Medicine, Stanford, CA, USA

ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form
1 February 2016
Accepted 2 February 2016
Available online 8 February 2016

Keywords:
Cognitive control
Emotion regulation
Dopamine
DRD2/3
Striatum

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

* Corresponding author. Department of Psychiatry and Behavioral Sciences, 1201 Welch Rd., MSLS Room P104, Stanford University School of Medicine, Stanford, CA 94305-5485, USA.
E-mail address: dmlyons@stanford.edu (D.M. Lyons).

http://dx.doi.org/10.1016/j.ynstr.2016.02.001

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

saturation were continuously monitored. An intravenous catheter was placed using aseptic technique in either the cephalic or saphenous vein. The radiotracer was produced to provide ~2.0 mCi of [^{11}C]raclopride in a volume no greater than 0.75 mL injected at 0.2 mL per 30 s at ~10:00 h. The mass (0.17 ± 0.02 μg; mean ± SEM) and specific activity (4.3 ± 0.3 Ci/μmol) of [^{11}C]raclopride administered to each monkey did not differ significantly across the three treatment conditions. After administration, the catheter was flushed with an amount of heparinized saline equivalent to the volume of the catheter.

Fully three-dimensional dynamic emission data were then collected for 60 min on a microPET R4 (Siemens Preclinical Solutions, Knoxville, TN) using an energy window of 250–750 keV and 137 successive time frames of increasing duration (14 at 5 s, 29 at 10 s, 45 at 20 s, 30 at 40 s, 19 at 60 s). The microPET scanner has 96 scintillation detector modules compactly arranged in a 14.8 cm diameter ring with an axial length of 7.8 cm. The tomograph has a spatial resolution of 1.85 mm full-width at half-maximum (FWHM) in the axial direction and 1.66 mm FWHM in the transaxial direction, which provides linear resolution of ~2.5 mm in all three dimensions. Resulting emission data were used to compute [^{11}C] raclopride binding for each voxel with a simplified kinetic model that uses the cerebellum as a reference brain region devoid of DRD2/3 (Gunn et al., 1997).

Whole brain anatomical data were acquired with MRI in separate sessions on a Signa 3T scanner (General Electric, Milwaukee, WI) at 9.5 years of age (range 8.4–10.3 years). Squirrel monkeys were sedated as described above and MRI was conducted with the following three-dimensional inversion recovery prepared fast spoiled gradient pulse sequence: TR = 12 ms, TE = 3 ms, TI = 300 ms, flip angle = 15, NEX = 4, matrix = 160 × 160, FOV = 8 cm, voxel size = 0.5 × 0.5 × 0.5 mm, slice thickness = 0.5 mm, gap = 0 mm, total scan time = 18 min. PET data were spatially co-registered to MRI data (Fig. 1) using SPM8 software (www.fil.ion.ucl.ac.uk/spm/). [^{11}C]raclopride binding measures (BP_{ND}) were determined for dorsal caudate nucleus, dorsal putamen, and ventral striatum as defined elsewhere (Mawlawi et al., 2001).

2.4. Data analysis

Mixed factor analysis of variance (ANOVA) was used to assess BP_{ND} measures. Stress inoculation (NS, SI, SI + Mom) was considered a between-subjects factor with striatal region (caudate, putamen, ventral striatum) and brain side (left, right) considered within-subjects factors. Body weight was included as a statistical covariate in keeping with suggestions for small primate PET neuroimaging (Morris et al., 1998).

Post hoc pairwise comparisons with the NS control treatment condition were conducted with Dunnett tests. Post hoc comparisons between striatal regions were conducted with Bonferroni corrections and comparisons between SI and SI + Mom treatment conditions were conducted with t-tests. Effect sizes were determined using Cohen's *d* and associations between DRD2/3 BP_{ND} measures and previously reported cognitive control errors (Parker et al., 2012) were assessed with Pearson correlation coefficients. All test statistics were evaluated with two-tailed probabilities at $P < 0.05$.

3. Results

Significant stress inoculation ($F(2,20) = 4.01$, $P = 0.034$) and striatal region ($F(2,40) = 37.61$, $P < 0.001$) main effects were discerned in the 3-way ANOVA. As expected, DRD2/3 BP_{ND} was greater in caudate and putamen compared to ventral striatum ($P < 0.001$). Across striatal regions, DRD2/3 BP_{ND} was respectively 9.3% and 10.7% greater in SI and SI + Mom squirrel monkeys compared to NS controls. Laterality main effects and all of the interactions were not statistically significant.

The 3-way ANOVA was decomposed to separately examine each striatal region with left and right brain sides combined (Fig. 2). Significant stress inoculation effects were discerned for ventral

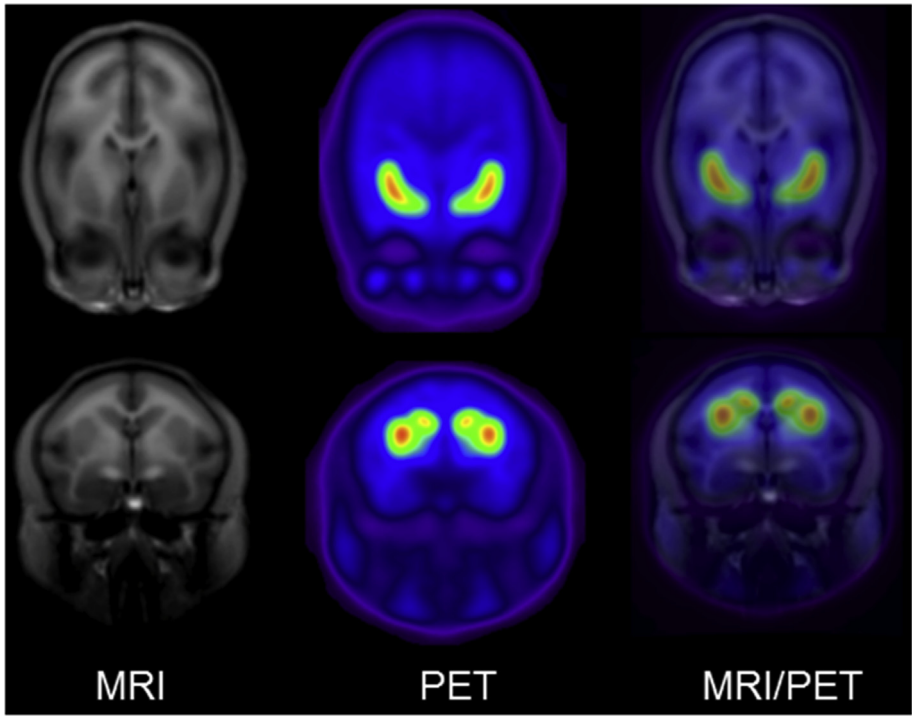

Fig. 1. Axial (top) and coronal (bottom) images of neuroanatomy (MRI) and DRD2/3 availability (PET) detected *in vivo* by [^{11}C]raclopride binding in squirrel monkey brain.

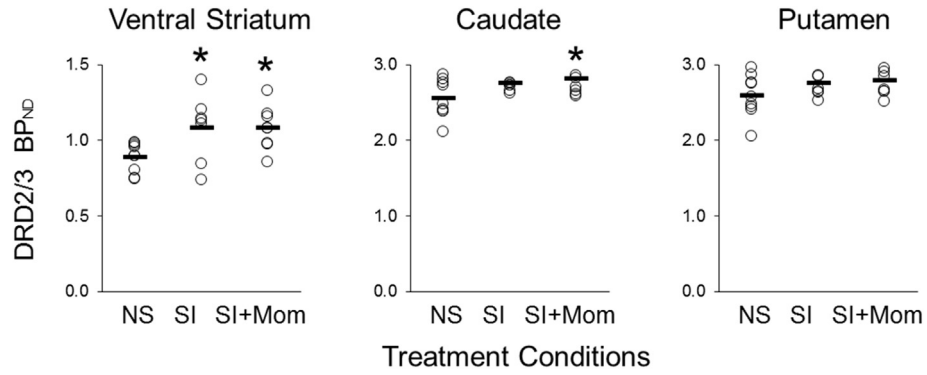

Fig. 2. Stress inoculation effects on DRD2/3 availability in ventral striatum, caudate, and putamen. Data from individual squirrel monkeys are plotted for the no-stress control (NS), stress inoculated alone (SI), and stress inoculated with mother (SI + Mom) treatment conditions. Horizontal bars signify treatment condition means and asterisks represent significant Dunnett test comparisons with the NS control condition ($P < 0.05$). DRD2/3 BP_{ND} measures reflect left and right brain sides combined. Note different y-axis measurement scales for ventral striatum compared to caudate and putamen.

striatum ($F(2,20) = 4.73$, $P = 0.021$) and caudate ($F(2,20) = 3.56$, $P = 0.048$) but not putamen. Squirrel monkeys from the SI condition did not differ from SI + Mom squirrel monkeys in any of the striatal regions. Post hoc Dunnett tests established that DRD2/3 BP_{ND} was greater in ventral striatum for both SI ($P = 0.041$) and SI + Mom ($P = 0.037$) squirrel monkeys compared to NS controls. Dunnett tests likewise established that DRD2/3 BP_{ND} was greater for SI + Mom squirrel monkeys compared to NS controls in caudate ($P = 0.038$) but not putamen (Fig. 2).

Effect size estimates confirmed that DRD2/3 regulation by stress inoculation varied systematically across striatal regions. For the SI and SI + Mom conditions combined, treatment effects relative to the NS condition were greatest in ventral striatum ($d = 1.41$) followed by caudate ($d = 1.07$) and putamen ($d = 0.78$). Differences between regions diminished the overall increase described above for stress inoculation main effects across striatal regions. Effects in ventral striatum were the most robust with DRD2/3 BP_{ND} measures respectively 22.1% and 21.8% greater in SI and SI + Mom squirrel monkeys compared to NS controls.

Behavioral assessments of cognitive control from an earlier study (Parker et al., 2012) were completed for 6 of 7 SI squirrel monkeys and 7 of 9 NS squirrel monkeys. Cognitive control tests were not conducted with any of the SI + Mom squirrel monkeys. Similar DRD2/3 BP_{ND} measures in SI and SI + Mom squirrel monkeys suggest that increased DRD2/3 availability relative to NS controls (Fig. 2) reflects stress inoculation and not subsequent testing effects.

Cognitive control errors for SI and NS squirrel monkeys analyzed together were inversely correlated with bilateral measures of DRD2/3 availability in ventral striatum ($r = -0.62$, df 11, $P = 0.02$) but not caudate ($r = -0.45$, df 11, $P = 0.12$) nor putamen ($r = -0.32$, df 11, $P = 0.29$). Higher DRD2/3 availability in ventral striatum correlated with fewer cognitive control errors (Fig. 3) and the correlation largely reflects group differences between SI and NS squirrel monkeys. The correlation was nearly significant, however, for NS squirrel monkeys analyzed alone ($r = -0.71$, df 5, $P = 0.076$) but not for SI squirrel monkeys with higher overall levels of DRD2/3 availability and fewer cognitive control errors.

4. Discussion

Significant stress inoculation and striatal region main effects for DRD2/3 availability were detected *in vivo* by neuroimaging. DRD2/3 availability was greater in caudate and putamen compared to ventral striatum. 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.

In natural and semi-natural conditions squirrel monkey mothers and other group members intermittently leave newly weaned offspring beginning at 3–6 months of age to forage for food on their own (Boinski & Fragaszy, 1989; Lyons et al., 1998). Initially, brief intermittent separations studied in controlled experimental conditions elicit distress peep-calls and increase plasma levels of the stress hormone cortisol with partial habituation of these

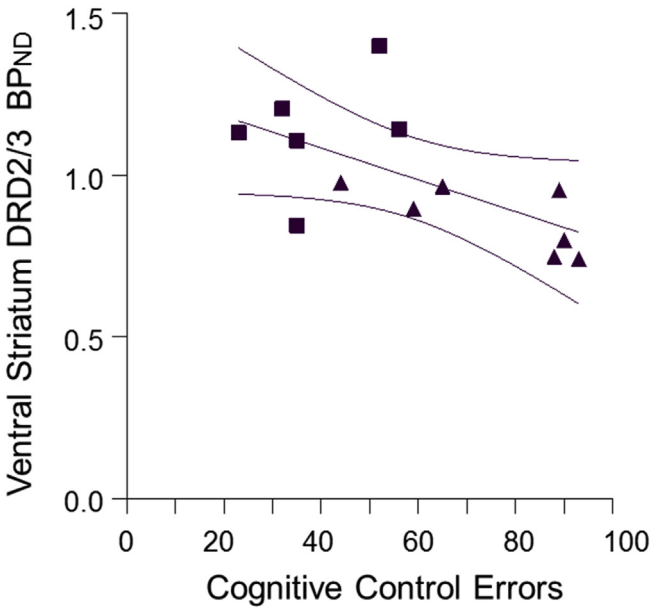

Fig. 3. Correlation between DRD2/3 availability in ventral striatum and cognitive control errors ($r = -0.62$, df 11, $P = 0.02$) for squirrel monkeys from the no-stress control (NS, triangles) and stress inoculated alone (SI, squares) treatment conditions. Cognitive control errors were assessed as described elsewhere (Parker et al., 2012) and represent the number of straight reaches on test trials with the box opening oriented left or right. DRD2/3 BP_{ND} for ventral striatum reflects left and right brain sides combined. Linear regression and 95% confidence intervals are depicted.

measures observed over repeated separations (Coe et al., 1983; Hennessy, 1986). Later in life, squirrel monkeys exposed to intermittent separations show fewer behavioral indications of anxiety, diminished stress levels of cortisol, and enhanced sensitivity to glucocorticoid feedback regulation of the hypothalamic–pituitary–adrenal (HPA) axis compared to squirrel monkeys not exposed to prior separations (Lyons et al., 2009; Lyons et al., 2010). Whereas severe forms of chronic stress impair HPA axis regulation and induce anxiety in macaque monkeys (O'Connor & Cameron, 2006; Sanchez, 2006), intermittent separations that simulate naturally occurring but stressful conditions mimic the effects of stress inoculation in humans.

In an earlier study of cognitive control conducted at 1.5 years of age, stress inoculated squirrel monkeys exposed to prior intermittent separations performed significantly better than control squirrel monkeys not exposed to prior separations (Parker et al., 2005). Performance on the same cognitive test in marmoset monkeys and macaque monkeys is impaired by lesions of prefrontal cortex (Diamond, 1990; Dias et al., 1996; Wallis et al., 2001) but not lesions of hippocampus (Diamond et al., 1989). In a different sample of squirrel monkeys from those examined here, stress inoculation early in life increased ventromedial prefrontal cortical volumes compared to controls (Lyons et al., 2002). Larger volumes do not reflect increased cortical thickness but instead represent surface area expansion of ventromedial prefrontal cortex due to growth and maturation of underlying white matter (Katz et al., 2009).

To determine whether improvements in cognitive control of impulsivity continue on into adulthood, we tested a subset of the same squirrel monkeys as those examined here using different procedures at 3.5 years of age to assess inhibition of a motivated pre-potent response (Parker et al., 2012). Stress inoculated squirrel monkeys exposed to prior intermittent separations again performed better than control squirrel monkeys not exposed to prior separations (Parker et al., 2012). Moreover, performance at 3.5 years correlated with cognitive control measures collected at 1.5 years of age (Parker et al., 2012). These findings suggest that stress inoculation early in life induces enduring trait-like transformations in cognitive control of impulsivity.

Cognitive control of impulsive behavior is a complex function mediated by multiple neural mechanisms. Larger ventromedial prefrontal size in humans predicts diminished impulsivity (Matsuo et al., 2009) and prefrontal cortical regions interact with striatal dopamine systems to regulate cognitive control (Jentsch & Pennington, 2014). Studies of rats, monkeys, and humans have found that impulsive motivation for psychostimulant drugs is associated with low striatal DRD2/3 measures (Dalley et al., 2011; Nader et al., 2006; Trifilieff & Martinez, 2014; Volkow et al., 2011) and high striatal DRD2/3 availability in humans has been linked to resilience against addictions (Volkow et al., 2002; Volkow et al., 2006). Pharmacological DRD2/3 agonists decrease impulsive behavior in rats (Fernando et al., 2012) and improve reversal learning as an index of cognitive control in humans dependent on psychostimulant drugs (Ersche et al., 2011).

We found that DRD2/3 availability was greater in squirrel monkey caudate and putamen compared to ventral striatum as observed in PET studies of humans (Mawlawi et al., 2001) and other non-human primates (Groman et al., 2011). DRD2/3 availability was also greater in stress inoculated squirrel monkeys compared to no-stress controls. Stress inoculation effects were greatest in ventral striatum followed by caudate and putamen. Differences between striatal regions in neuroanatomical connections and functions (Haber & Knutson, 2010; Choi et al., 2012) support our finding that enhanced cognitive control correlated with higher DRD2/3 availability in ventral striatum. Pharmacological mimicry of increased DRD2/3 availability by stress inoculation may therefore provide a novel target for new treatments of psychiatric disorders in humans that involve deficits in cognitive control.

Our results should be interpreted in the context of potential limitations. Findings from females may or may not hold true for males. Behavioral measures of cognitive control were not collected from all monkeys which limited the size of samples used for correlations but allowed assessment of stress inoculation versus subsequent testing effects. The [^{11}C]raclopride PET radiotracer primarily binds dopamine D2 receptors (Narendran et al., 2006) but also D3 receptors in ventral striatum (Gurevich & Joyce, 1999) and does not distinguish pre-versus post-synaptic locations. DRD2/3 BP_{ND} measures also reflect receptor availability and not receptor densities because *in vivo* dopamine levels affect [^{11}C]raclopride binding (Laruelle, 2000). Nevertheless, *in vivo* PET measures of DRD2/3 availability correlate with *in vitro* estimates of striatal DRD2/3 densities in vervet monkeys (Groman et al., 2014). Moreover, cerebrospinal fluid levels of the dopamine metabolite homovanillic acid do not differ significantly in stress inoculated squirrel monkeys compared to controls (Parker et al., 2007). Lastly, the search for drugs that mimic increased DRD2/3 signaling as a target for new clinical interventions must contend with unwanted side effects (Jentsch & Pennington, 2014).

5. Conclusions

We found stress inoculation and striatal region main effects for DRD2/3 availability in squirrel monkeys. DRD2/3 availability was greater in caudate and putamen compared to ventral striatum. 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.

Conflict of interest

Dr. Schatzberg reports equity in Merck, Pfizer, Neurocrine, XHale, and Corcept Therapeutics (co-founder). Dr. Schatzberg has received lecture fees from Merck and consulted to Takeda/Lundbeck, Pfizer, Depomed, and Neuronetics. Drs. Lee, Nechvatal, Shen, Buckmaster, Levy, Chin, and Lyons report no biomedical financial interests or potential conflicts of interest.

Acknowledgments

Supported by the National Institutes of Health DA35503 (Lyons) and NCI ICMIC P50 CA114747 (Gambhir). Funding agencies did not design the study or influence the writing of this report. We thank G. Nelson and K. Newburn for technical veterinary assistance, C. Pacharinsak for veterinary anesthesia consultation, and S-H. Kim for assistance with the radiochemistry.

References

Altemus, M., Sarvaiya, N., Neill Epperson, C., 2014. Sex differences in anxiety and depression clinical perspectives. Front. Neuroendocrinol. 35, 320–330.
Boinski, S., Fragaszy, D.M., 1989. The ontogeny of foraging in squirrel monkeys, Saimiri oerstedi. Anim. Behav. 37, 415–428.
Brady, A.G., 2000. Research techniques for the squirrel monkey (*Saimiri*). Ilar J. 41, 10–18.
Brockhurst, J., Cheleuitte-Nieves, C., Buckmaster, C.L., Schatzberg, A.F., Lyons, D.M., 2015. Stress inoculation modeled in mice. Transl. Psychiatry 5, e537.
Choi, E.Y., Yeo, B.T.T., Buckner, R.L., 2012. The organization of the human striatum

estimated by intrinsic functional connectivity. J. Neurophysiol. 108, 2242–2263.
Coe, C.L., Glass, J.C., Wiener, S.G., Levine, S., 1983. Behavioral, but not physiological, adaptation to repeated separation in mother and infant primates. Psychoneuroendocrinology 8, 401–409.
Compton, R.J., Arnstein, D., Freedman, G., Dainer-Best, J., Liss, A., Robinson, M.D., 2011. Neural and behavioral measures of error-related cognitive control predict daily coping with stress. Emotion 11, 379–390.
Craske, M.G., Kircanski, K., Zelikowsky, M., Mystkowski, J., Chowdhury, N., Baker, A., 2008. Optimizing inhibitory learning during exposure therapy. Behav. Res. Ther. 46, 5–27.
Dalley, J.W., Everitt, B.J., Robbins, T.W., 2011. Impulsivity, compulsivity, and top-down cognitive control. Neuron 69, 680–694.
Diamond, A., Zola-Morgan, S., Squire, L.R., 1989. Successful performance by monkeys with lesions of the hippocampal formation on AB and object retrieval, two tasks that mark developmental changes in human infants. Behav. Neurosci. 103, 526–537.
Diamond, A., 1990. Developmental time course in human infants and infant monkeys, and the neural bases of, inhibitory control of reaching. Ann. N. Y. Acad. Sci. 608, 637–676.
Dias, R., Robbins, T.W., Roberts, A.C., 1996. Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. Behav. Neurosci. 110, 872–886.
Ersche, K.D., Roiser, J.P., Abbott, S., Craig, K.J., Muller, U., Suckling, J., Ooi, C., Shabbir, S.S., Clark, L., Sahakian, B.J., Fineberg, N.A., Merlo-Pich, E.V., Robbins, T.W., Bullmore, E.T., 2011. Response perseveration in stimulant dependence is associated with striatal dysfunction and can be ameliorated by a D(2/3) receptor agonist. Biol. Psychiatry 70, 754–762.
Fernando, A.B., Economidou, D., Theobald, D.E., Zou, M.F., Newman, A.H., Spoelder, M., Caprioli, D., Moreno, M., Hipolito, L., Aspinall, A.T., Robbins, T.W., Dalley, J.W., 2012. Modulation of high impulsivity and attentional performance in rats by selective direct and indirect dopaminergic and noradrenergic receptor agonists. Psychopharmacol. Berl. 219, 341–352.
Groman, S.M., Jentsch, J.D., 2012. Cognitive control and the dopamine D_2-like receptor: a dimensional understanding of addiction. Depress. Anxiety 29, 295–306.
Groman, S.M., Lee, B., London, E.D., Mandelkern, M.A., James, A.S., Feiler, K., Rivera, R., Dahlbom, M., Sossi, V., Vandervoort, E., Jentsch, J.D., 2011. Dorsal striatal D2-like receptor availability covaries with sensitivity to positive reinforcement during discrimination learning. J. Neurosci. 31, 7291–7299.
Groman, S.M., James, A.S., Seu, E., Tran, S., Clark, T.A., Harpster, S.N., Crawford, M., Burtner, J.L., Feiler, K., Roth, R.H., Elsworth, J.D., London, E.D., Jentsch, J.D., 2014. In the blink of an eye: relating positive-feedback sensitivity to striatal dopamine D2-like receptors through blink rate. J. Neurosci. 34, 14443–14454.
Gunn, R.N., Lammertsma, A.A., Hume, S.P., Cunningham, V.J., 1997. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 6, 279–287.
Gurevich, E.V., Joyce, J.N., 1999. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacology 20, 60–80.
Haber, S.N., Knutson, B., 2010. The reward circuit: linking primate anatomy and human imaging. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 35, 4–26.
Hennessy, M.B., 1986. Multiple, brief maternal separations in the squirrel monkey: changes in hormonal and behavioral responsiveness. Physiol. Behav. 36, 245–250.
Jentsch, J.D., Pennington, Z.T., 2014. Reward, interrupted: inhibitory control and its relevance to addictions. Neuropharmacology 76 (Pt B), 479–486.
Katz, M., Liu, C., Schaer, M., Parker, K.J., Ottet, M.C., Epps, A., Buckmaster, C.L., Bammer, R., Moseley, M.E., Schatzberg, A.F., Eliez, S., Lyons, D.M., 2009. Prefrontal plasticity and stress inoculation-induced resilience. Dev. Neurosci. 31, 293–299.
Langer, O., Nagren, K., Dolle, F., Lundkvist, C., Sandell, J., Swahn, C.G., Vaufrey, F., Crouzel, C., Maziere, B., Halldin, C., 1999. Precursor synthesis and radiolabelling of the dopamine D-2 receptor ligand C-11 raclopride from C-11 methyl triflate. J. Label. Compd. Radiopharm. 42, 1183–1193.
Laruelle, M., 2000. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 20, 423–451.
Lyons, D.M., Kim, S., Schatzberg, A.F., Levine, S., 1998. Postnatal foraging demands alter adrenocortical activity and psychosocial development. Dev. Psychobiol. 32, 285–291.
Lyons, D.M., Afarian, H., Schatzberg, A.F., Sawyer-Glover, A., Moseley, M.E., 2002. Experience-dependent asymmetric variation in primate prefrontal morphology. Behav. Brain Res. 136, 51–59.
Lyons, D.M., Parker, K.J., Katz, M., Schatzberg, A.F., 2009. Developmental cascades linking stress inoculation, arousal regulation, and resilience. Front. Behav. Neurosci. 3, 32.
Lyons, D.M., Parker, K.J., Schatzberg, A.F., 2010. Animal models of early life stress: implications for understanding resilience. Dev. Psychobiol. 52, 616–624.
Matsuo, K., Nicoletti, M., Nemoto, K., Hatch, J.P., Peluso, M.A., Nery, F.G., Soares, J.C., 2009. A voxel-based morphometry study of frontal gray matter correlates of impulsivity. Hum. Brain Mapp. 30, 1188–1195.
Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D.R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M., 2001. Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 21, 1034–1057.
McNally, R.J., 2007. Mechanisms of exposure therapy: how neuroscience can improve psychological treatments for anxiety disorders. Clin. Psychol. Rev. 27, 750–759.
Meichenbaum, D., 1993. Stress inoculation training: a twenty year update. In: Woolfolk, R.L., Lehrer, P.M. (Eds.), Principals and Practices of Stress Management. Guilford Press, New York, pp. 373–406.
Morris, E.D., Chefer, S.I., London, E.D., 1998. Limitations of binding potential as a measure of receptor function; a two-point correction for mass. In: Carson, R.E., et al. (Eds.), Quantitative Functional Brain Imaging with Positron Emission Tomography. Academic Press, New York, pp. 407–413.
Nader, M.A., Morgan, D., Gage, H.D., Nader, S.H., Calhoun, T.L., Buchheimer, N., Ehrenkaufer, R., Mach, R.H., 2006. PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nat. Neurosci. 9, 1050–1056.
Narendran, R., Slifstein, M., Guillin, O., Hwang, Y., Hwang, D.R., Scher, E., Reeder, S., Rabiner, E., Laruelle, M., 2006. Dopamine (D2/3) receptor agonist positron emission tomography radiotracer [11C]-(+)-PHNO is a D3 receptor preferring agonist in vivo. Synapse 60, 485–495.
O'Connor, T.G., Cameron, J.L., 2006. Translating research findings on early experience to prevention: animal and human evidence on early attachment relationships. Am. J. Prev. Med. 31, S175–S181.
Parker, K.J., Buckmaster, C.L., Schatzberg, A.F., Lyons, D.M., 2004. Prospective investigation of stress inoculation in young monkeys. Arch. Gen. Psychiatry 61, 933–941.
Parker, K.J., Buckmaster, C.L., Justus, K.R., Schatzberg, A.F., Lyons, D.M., 2005. Mild early life stress enhances prefrontal-dependent response inhibition in monkeys. Biol. Psychiatry 57, 848–855.
Parker, K.J., Buckmaster, C.L., Sundlass, K., Schatzberg, A.F., Lyons, D.M., 2006. Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. Proc. Natl. Acad. Sci. U. S. A. 103, 3000–3005.
Parker, K.J., Rainwater, K.L., Buckmaster, C.L., Schatzberg, A.F., Lindley, S.E., Lyons, D.M., 2007. Early life stress and novelty seeking behavior in adolescent monkeys. Psychoneuroendocrinology 32, 785–792.
Parker, K.J., Buckmaster, C.L., Lindley, S.E., Schatzberg, A.F., Lyons, D.M., 2012. Hypothalamic-pituitary-adrenal axis physiology and cognitive control of behavior in stress inoculated monkeys. Int. J. Behav. Dev. 36.
Russo, S.J., Murrough, J.W., Han, M.H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nat. Neurosci. 15, 1475–1484.
Sanchez, M.M., 2006. The impact of early adverse care on HPA axis development: nonhuman primate models. Horm. Behav. 50, 623–631.
Sapolsky, R.M., 2015. Stress and the brain: individual variability and the inverted-U. Nat. Neurosci. 18, 1344–1346.
Saunders, T., Driskell, J.E., Johnston, J.H., Salas, E., 1996. The effect of stress inoculation training on anxiety and performance. J. Occup. Health Psychol. 1, 170–186.
Schiml, P.A., Mendoza, S.P., Saltzman, W., Lyons, D.M., Mason, W.A., 1999. Annual physiological changes in individually housed squirrel monkeys (*Saimiri sciureus*). Am. J. Primatol. 47, 93–103.
Seery, M.D., Holman, E.A., Silver, R.C., 2010. Whatever does not kill us: cumulative lifetime adversity, vulnerability, and resilience. J. Pers. Soc. Psychol. 99, 1025–1041.
Serino, S., Triberti, S., Villani, D., Cipresso, P., Gaggioli, A., Riva, G., 2014. Toward a validation of cyber-interventions for stress disorders based on stress inoculation training: a systematic review. Virtual Real. 18, 73–87.
Stetz, M.C., Thomas, M.L., Russo, M.B., Stetz, T.A., Wildzunas, R.M., McDonald, J.J., Wiederhold, B.K., Romano Jr., J.A., 2007. Stress, mental health, and cognition: a brief review of relationships and countermeasures. Aviat. Space Environ. Med. 78, B252–B260.
Trifilieff, P., Martinez, D., 2014. Blunted dopamine release as a biomarker for vulnerability for substance use disorders. Biol. Psychiatry 76, 4–5.
Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R.D., Taylor, K.M., Martinez, D., Moore, H., Balsam, P.D., Simpson, E.H., Javitch, J.A., 2013. Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. Mol. Psychiatry 18, 1025–1033.
Volkow, N.D., Wang, G.J., Fowler, J.S., Thanos, P.P., Logan, J., Gatley, S.J., Gifford, A., Ding, Y.S., Wong, C., Pappas, N., 2002. Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synapse 46, 79–82.
Volkow, N.D., Wang, G.J., Begleiter, H., Porjesz, B., Fowler, J.S., Telang, F., Wong, C., Ma, Y., Logan, J., Goldstein, R., Alexoff, D., Thanos, P.K., 2006. High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. Arch. Gen. Psychiatry 63, 999–1008.
Volkow, N.D., Wang, G.J., Fowler, J.S., Tomasi, D., Telang, F., 2011. Addiction: beyond dopamine reward circuitry. Proc. Natl. Acad. Sci. U. S. A. 108, 15037–15042.
Wallis, J.D., Dias, R., Robbins, T.W., Roberts, A.C., 2001. Dissociable contributions of the orbitofrontal and lateral prefrontal cortex of the marmoset to performance on a detour reaching task. Eur. J. Neurosci. 13, 1797–1808.