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Methamphetamine blocks exercise effects on *Bdnf* and *Drd2* gene expression in frontal cortex and striatum

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ABSTRACT

Exposure to drugs of abuse can produce many neurobiological changes which may lead to increased valuation of rewards and decreased sensitivity to their costs. Many of these behavioral alterations are associated with activity of D2-expressing medium spiny neurons in the striatum. Additionally, *Bdnf* in the striatum has been shown to play a role in flexible reward-seeking behavior. Given that voluntary aerobic exercise can affect the expression of these proteins in healthy subjects, and that exercise has shown promise as an anti-addictive therapy, we set out to quantify changes in D2 and *Bdnf* expression in methamphetamine-exposed rats given access to running wheels. Sixty-four rats were treated for two weeks with an escalating dose of methamphetamine or saline, then either sacrificed, housed in standard cages, or given free access to a running wheel for 6 weeks prior to sacrifice. Rats treated with methamphetamine ran significantly greater distances than saline-treated rats, suggesting an augmentation in the reinforcement value of voluntary wheel running. Transcription of *Drd2* and *Bdnf* was assessed via RT-qPCR. Protein expression levels of D2 and phosphorylation of the TrkB receptor were measured via western blot. *Drd2* and *Bdnf* mRNA levels were impacted independently by exercise and methamphetamine, but exposure to methamphetamine prior to the initiation of exercise blocked the exercise-induced changes seen in rats treated with saline. Expression levels of both proteins were elevated immediately after methamphetamine, but returned to baseline after six weeks, regardless of exercise status.

1. Introduction

In order to engage in adaptive decision making, an animal must compare previously experienced reward with currently available reward sources to determine optimal effort expenditure. Flexible responses to reward require maintenance of reward history that incorporates both frequency of reward and overall reward availability in an environment. Substance abuse can be conceptualized, in part, as a consequence of this adaptive response to a distorted percept of reward history and availability brought about by exposure to a drug (Nesse, 1994). Many drugs of abuse, including methamphetamine, exert their reinforcing effects by dramatically increasing dopamine (DA) signaling in the striatum, mimicking the effects of many natural rewards. Neuroplastic changes, especially adaptations in DA receptors and transporters, have been found to contribute to many of the cognitive and behavioral changes that are associated with compulsive drug seeking and addiction (Groman et al., 2013; Kosheleff et al., 2012; Izquierdo et al., 2010). Experience with methamphetamine, therefore, may recalibrate striatal reward circuitry to change reward valuation of and responses to future rewards. In support of this idea, we recently reported that repeated, escalating methamphetamine pretreatment increased the ability to learn from positive feedback in reversal learning during protracted methamphetamine withdrawal, when reward history and current reward experiences are most opposed (Stolyarova et al., 2014a). Responses to positive feedback have been associated with DA transporter binding (Stolyarova et al., 2014a) and variation in D2 receptor availability (Groman et al., 2011). Additionally, we found increased willingness to work for large-over-small food rewards after methamphetamine in an effortful decision-making task in rats

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(Stolyarova et al., 2014b), indicative of increased reward sensitivity to natural reinforcement following drug exposure. Indeed, genetic knockout of D2 receptors in the ventral striatum impairs motivation (Tran et al., 2002), while viral overexpression of postsynaptic D2 increases motivation to expend effort for rewards (Trifilieff et al., 2012), strongly implicating dysregulated D2 signaling in altered reward learning following methamphetamine.

Voluntary wheel running has also been shown to induce plastic changes in the mesolimbic reward pathways, including D2 transcription (Greenwood et al., 2011). Aerobic exercise has been proposed as a reinforcing behavior that can potentially normalize DA and glutamatergic signaling in addiction (Lynch et al., 2013; O'Dell et al., 2012; Sobieraj et al., 2014) and lead to improvements in various facets of cognition (Gomez-Pinilla and Hillman, 2013; Creer et al., 2010), including those abilities of particular relevance to cognitive flexibility, that depend on striatal DA (Eddy et al., 2014). Additionally, exercise has been shown to be protective against addiction at all stages of its progression. Six weeks of voluntary running attenuates acquisition and maintenance of methamphetamine self-administration behavior (Engelmann et al., 2014), and blocks drug- and cue-primed reinstatement to cocaine self-administration (Smith and Witte, 2012).

Altered reward learning following methamphetamine also suggests a role for proteins involved in cognition such as brain-derived neurotrophic factor (BDNF), which is critical for cell survival and synaptic signaling. Infusion of exogenous BDNF into the striatum enhances cognitive flexibility in a strategy set-shifting task (D'Amore et al., 2013), whereas methamphetamine exposure results in impairments (Groman et al., 2013; Parsegian et al., 2011). Voluntary aerobic exercise (wheel running) increases *Bdnf* exon IV transcription by affecting the activity of epigenetic regulatory proteins (Gomez-Pinilla et al., 2011). Given the foregoing, we hypothesized that treatment with methamphetamine would reduce the expression of *Bdnf* mRNA in the striatum, and that this would be normalized by voluntary aerobic exercise. We further hypothesized that exercise would also normalize methamphetamine induced alterations in *Drd2* transcription. To test this we treated rats with methamphetamine or saline, and either allowed or did not allow them access to an exercise wheel. Following this treatment, we assessed transcription of *Drd2* and *Bdnf* in the striatum, as well as protein expression levels of D2 and activation of the TrkB receptor in the striatum. Additionally, we measured *Bdnf* mRNA in the frontal cortex due to the region's role as a major source of striatal *Bdnf*.

2. Methods and materials

2.1. Subjects

Sixty-four male Long-Evans rats (Charles River Laboratories, Raleigh, NC) weighing between 250 and 300 g at the beginning of the study were maintained under a 12-hr light/12-hr dark cycle (lights on 6:00–18:00) under temperature- and humidity-controlled conditions. Food and water were available *ad libitum*. After arrival in the facility, animals were left undisturbed for 3 days to acclimate to the vivarium, then individually handled over the next 5 days for a minimum of 10 min per day. During acclimation, handling, and methamphetamine pre-exposure, rats were pair-housed; each methamphetamine-treated rat was housed with a saline-treated rat to minimize aggression. During exercise periods, all rats were singly-housed. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of California at Los Angeles, Chancellor's Animal Research Committee.

2.2. Drug treatment

Rats were treated with methamphetamine using a subchronic escalating regimen adapted from Segal et al. (2003), which was shown to be protective against DA neurotoxicity following subsequent binge exposure. In short, rats were given three daily injections of D-methamphetamine ("mAMPH", n = 32; Sigma, St. Louis, MO; 0.1–6.0 mg free base/kg, s.c., escalating in 0.1 mg/kg increments up to 2.1 mg/kg, then in 0.2 mg/kg increments from 2.1 mg/kg to 6.0 mg/kg) or physiological saline solution ("Sal", n = 32; 1 ml/kg, s.c.) for two weeks. Injections took place during the light cycle, at 10:00, 13:15, and 16:30. One methamphetamine-treated animal succumbed to cerebral ischemia following the second-highest dose, and was excluded from the dataset.

2.3. Voluntary wheel running

Following methamphetamine treatment, a subset of rats were individually housed either in standard shoebox cages ("Sed", n = 23) or in cages equipped with running wheels with radius = 0.175 m ("Ex", n = 24) for 6 weeks. The remaining 16 rats were euthanized the day after cessation of drug treatment (Fig. 1). Hourly wheel revolutions and running distance were recorded for exercising rats. The running intensity data from 8 rats were unanalyzable due to a malfunction in the magnetic switches used to record these data. These rats were excluded from any analysis involving running intensity, but included for analyses in which presence or absence of a running wheel was an independent variable. Subsequently, data were recorded digitally using an optical counter and Lafayette Activity Wheel Monitor (AWM) Software.

2.4. Real-time quantitative PCR

As outlined above, rats were euthanized either 6 weeks (n = 47) or one day ("Immed", n = 16) after the final methamphetamine or Sal treatment with an overdose of sodium pentobarbital (250 mg/kg, i.p.) and decapitated. Bilateral frontal cortex and striatum were rapidly dissected over a cold plate at 4 °C and flash frozen by immersion in isopentane over dry ice before being stored at −80 °C. Frontocortical dissections included ventral (orbital) and medial sectors of the frontal cortex, but excluded most lateral, posterior regions (agranular insular). Striatal dissections included both

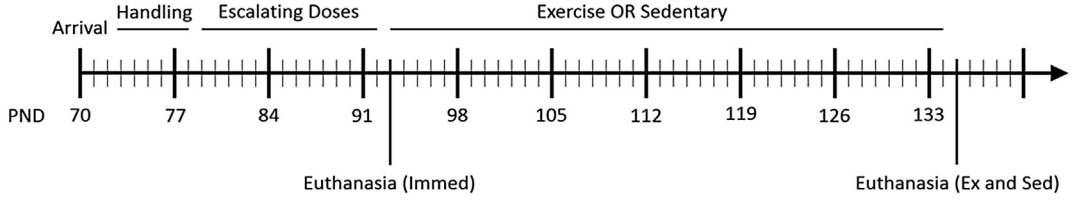

Fig. 1. Experimental timeline. Subjects arrived at our facilities on PND70, and were allowed to acclimate to environmental conditions and experimenter handling. Escalating doses of methamphetamine or saline were given for two weeks from PND 78–92, then animals were divided into three groups. One group was sacrificed immediately (n = 16), the second was placed in standard shoebox cages for six weeks (n = 23), and the third was placed in cages equipped with running wheels for six weeks (n = 24). At the conclusion of the exercise or sedentary period, all animals were sacrificed.