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Amphetamine sensitization in mice is sufficient to produce both manic- and depressive-related behaviors as well as changes in the functional connectivity of corticolimbic structures

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ABSTRACT

It has been suggested that amphetamine abuse and withdrawal mimics the diverse nature of bipolar disorder symptomatology in humans. Here, we determined if a single paradigm of amphetamine sensitization would be sufficient to produce both manic- and depressive-related behaviors in mice. CD-1 mice were subcutaneously dosed for 5 days with 1.8 mg/kg D-amphetamine or vehicle. On days 6–31 of withdrawal, amphetamine-sensitized (AS) mice were compared to vehicle-treated (VT) mice on a range of behavioral and biochemical endpoints. AS mice demonstrated reliable mania- and depression-related behaviors from day 7 to day 28 of withdrawal. Relative to VT mice, AS mice exhibited long-lasting mania-like hyperactivity following either an acute 30-min restraint stress or a low-dose 1 mg/kg D-amphetamine challenge, which was attenuated by the mood-stabilizers lithium and quetiapine. In absence of any challenge, AS mice showed anhedonia-like decreases in sucrose preference and depression-like impairments in the off-line consolidation of motor memory, as reflected by the lack of spontaneous improvement across days of training on the rotarod. AS mice also demonstrated a functional impairment in nest building, an ethologically-relevant activity of daily living. Western blot analyses revealed a significant increase in methylation of histone 3 at lysine 9 (H3K9), but not lysine 4 (H3K4), in hippocampus of AS mice relative to VT mice. In situ hybridization for the immediate-early gene activity-regulated cytoskeleton-associated protein (Arc) further revealed heightened activation of corticolimbic structures, decreased functional connectivity between frontal cortex and striatum, and increased functional connectivity between the amygdala and hippocampus of AS mice. The effects of amphetamine sensitization were blunted in C57BL/6J mice relative to CD-1 mice. These results show that a single amphetamine sensitization protocol is sufficient to produce behavioral, functional, and biochemical phenotypes in mice that are relevant to bipolar disorder.

1. Introduction

Patients with bipolar disorder suffer from a diverse array of symptoms, including manic behaviors, depressive behaviors, and cognitive deficits, that ultimately impair their ability to function in day to day life (Oswald et al., 2007; Balanza-Martinez et al., 2010; Bowie et al., 2010; Rosa et al., 2014). Despite the devastating impact of bipolar disorder and its widespread prevalence, very little is understood of the pathophysiology underlying the disease. Heritability studies clearly demonstrate a genetic component in the etiology of bipolar disorder (McGuffin et al., 2003). That said, genome wide association studies have been largely inconsistent, with only a few replicated targets (e.g., *ANK3*, *CACNA1C*, and *DAT*) (Greenwood et al., 2001; Horschitz et al., 2005; Greenwood et al., 2006; Pinsonneault et al., 2011; Bhat et al., 2012; Maletic and Raison, 2014). Inconsistencies in the genomics literature may be related to the fact that epigenetic dysregulation has also been implicated in the pathophysiology of bipolar disorder and symptomatically-related psychiatric illnesses, particularly

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alterations in DNA and histone methylation (Abdolmaleky et al., 2004; Veldic et al., 2005; Abdolmaleky et al., 2006; Kuratomi et al., 2008; Rao et al., 2012; Xiao et al., 2014). Environmental stressors also clearly impact susceptibility, onset, and progression of bipolar disorder (McGuffin et al., 2003; Post and Leverich, 2006). Thus, any complete understanding of the pathophysiology of bipolar disorder is likely to include multiple genetic hits that, ultimately, impair stress resiliency by compromising the function of corticolimbic circuitry (Post and Leverich, 2006; Maletic and Raison, 2014).

One pathway in the brain for which multiple genetic hits have been identified in patients with bipolar disorder is the dopamine system, and it has long been hypothesized that sensitization of dopaminergic signaling contributes to both the manic and depressive phases of bipolar disorder (Mamelak, 1978; Berk et al., 2007; Cousins et al., 2009; Post and Kalivas, 2013). As noted above, one of the few genetic targets found to be reproducibly associated with bipolar disorder is the dopamine transporter (DAT) (Greenwood et al., 2001; Horschitz et al., 2005; Greenwood et al., 2006; Bertolino et al., 2009). Phenotypes relevant to bipolar disorder have been observed in mice with genetic knockdown or deletion of DAT, including locomotor hyperactivity, impaired response inhibition, perseverative motor behaviors, heightened reactivity to novelty, heightened aggression, altered social behaviors, and somewhat riskier choices in a mouse Iowa Gambling Task (Ralph et al., 2001; Zhuang et al., 2001; Ralph-Williams et al., 2003; Rodriguiz et al., 2004; Young et al., 2011; van Enkhuizen et al., 2014). DAT clears dopamine from the synaptic cleft, so when genetic mutations or psychostimulants inhibit DAT function, synaptic dopamine levels increase. Consistent with a proposed role for altered DAT function in the pathophysiology of bipolar disorder, abuse and subsequent withdrawal from the psychostimulant amphetamine by humans was noted long ago to produce manic and depressive symptoms, respectively, associated with bipolar disorder (Mamelak, 1978).

Studies in rodents have used various schedules of amphetamine use and withdrawal to produce either manic-related behaviors (Antelman et al., 1980; Hamamura and Fibiger, 1993; Diaz-Otanez et al., 1997; Barr et al., 2002; Pacchioni et al., 2002; Conversi et al., 2008; Cruz et al., 2012), depressive-related behaviors (Barr et al., 1999; Barr and Phillips, 1999; Cryan et al., 2003; Che et al., 2013), or cognitive deficits observed in patients with bipolar disorder (Kozak et al., 2007). That said, few have determined whether a singular sensitization paradigm is capable of producing both manic- and depressive-related behaviors (e.g., (Dencker and Husum, 2010)). Further, it has yet to be assessed whether amphetamine sensitization is sufficient to reproduce deficits in activities of daily living, changes in epigenetic markers, or alterations in neural circuit activation/functional connectivity that have been reported in patients with bipolar disorder. As such, we seek here to test the hypothesis that a singular amphetamine sensitization regimen is sufficient to recapitulate in mice the many varied symptoms associated with bipolar disorder, including manic-related behaviors (hyperactivity in response to a dopaminergic or stress challenge), depressive-related behaviors (reduced sucrose preference and impaired consolidation of procedural memory), and impaired function in an ethologically-relevant activity of daily living (nest building), in addition to increased activation/altered functional connectivity of corticolimbic structures (as measured by mRNA expression of the activity-regulated immediate-early gene Arc) and changes in histone methylation.

2. Methods

2.1. Subjects

Male CD-1 mice (Charles River) weighing 14–16 g and C57BL/6J (Jackson) aged 6–7 weeks were group-housed upon arrival 4/cage on ventilated racks under standard laboratory conditions. These strains were selected based on our previous experience with amphetamine-related animal model development (Kanes et al., 2007; Kelly et al., 2007, 2009). Each experiment/time point utilized separate cohorts of mice (see figure legends for group's n). Housing and testing rooms were maintained on a L:D/600 h:1800 h lighting cycle, and food and water were available *ad libitum* in the home cage. Animals habituated to the facility for 1 week prior to the initiation of experimentation and all testing was conducted during the light phase. All procedures were conducted in concordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (Pub 85-23, revised 1996) and were fully approved by Institutional Animal Care and Use Committee of Pfizer Research and University of South Carolina. All efforts were made to minimize animal suffering and to reduce the number of animals used. There are no animal alternatives for the vivo techniques used herein.

2.2. Drugs and amphetamine sensitization procedure

All compounds were dissolved in sterile saline and were administered in a volume of 10 mL/kg subcutaneously (s.c.). All drug weights were corrected for salts. D-amphetamine and lithium chloride were obtained from Sigma Aldrich and quetiapine was synthesized in house. The amphetamine sensitization procedure was based on that of Dencker and Husum (Dencker and Husum, 2010). Briefly, 1 week following arrival, mice were given daily injections of 1.8 mg/kg D-amphetamine (amphetamine sensitized, AS) or vehicle (vehicle treated, VT) for 5 days. To inject mice, the home cage was transferred from the ventilated rack to a fume hood located in the colony room and mice were removed from the home cage, injected, and immediately returned to the home cage 1 by 1. Once all mice in the cage had received their injection, the home cage was returned to the ventilated rack. Mice were then withdrawn from drug 4–31 days before being tested (Fig. 1).

2.3. Locomotor activity and restraint stress

Locomotor activity (total distance and total number of horizontal beam breaks) was recorded using the Accuscan Versamax system, as previously reported (Kelly et al., 2009). Briefly, mice were placed in 20.3 cm × 20.3 cm plexiglass chambers and infrared beam activity was monitored and translated into total distance traveled using the Versadat software. For studies employing a D-amphetamine challenge, mice were first injected with vehicle or a mood stabilizer and then were habituated to the locomotor chamber for 30 min. Following the habituation period, mice received either vehicle or a low-dose D-amphetamine challenge and locomotor activity was recorded for 30 min. For studies employing a restraint stress, mice were habituated to the chamber for 30 min and then restrained for 30 min by being placed in a 50 mL conical tube (with the tip cut off to allow for adequate air circulation), which was set in the chamber. Unrestrained mice remained free in the chamber during this period. Following the 30 min restraint period, locomotor activity of both the restrained and unrestrained mice was measured for 30 min.

2.4. Sucrose preference

One day before testing began, animals were single housed and remained so for the duration of the study. When the test began, animals were assigned 1 bottle of water and 1 bottle of 1% sucrose. Both bottles were weighed prior to being placed in the cage on a Friday. The following Monday, bottles were removed from the cages and again weighed. The total mLs of fluid consumed was then inferred from the weight differential based on the fact that 1 mL of water weighs 1 g.

2.5. Rotarod

Mice were tested on an accelerating rotarod using a regimen similar to our previously published work (Kelly et al., 2010). Mice were trained for 5 trials/day with a 1-h interval between trials. Trials lasted until the mouse fell off the rotating rod, with a 5-min maximum. Latencies to fall off the rod were recorded.

2.6. Nesting

To assess nest-building behavior, mice were individually placed into a clean home cage with fresh bedding. After 5 min, a nestlette was introduced into the box and animals were allowed 2.5 h to build a nest using the nestlette material. At the end of test, an experimenter blind to treatment scored the state of the nestlette/nest (0 = nestlette untouched, 1 = nestlette slightly chewed, 2 = nestlette >50% chewed, 3 = nestlette completely chewed and some pieces moved, 4 = nestlette no longer identifiable/shredded into fluffy bits, 5 = a clearly defined, circular, fluffy nest evident, 6 = fluffy nest clearly defined with mouse inside).

2.7. Molecular and biochemical analyses

For molecular and biochemical analyses, animals were sacrificed by cervical dislocation. Brains were collected fresh and hemisected on an ice-cold tray. For *in situ* hybridization, the left hemisphere was first flash frozen in isopentane sitting on dry ice and then placed on dry ice to allow for evaporation of isopentane. Flash frozen hemispheres were then placed in tubes sitting on dry ice. For Western blotting, whole hippocampi were freshly dissected and then immediately placed in tubes sitting on dry ice. Frozen tissue was stored at −80 °C until processing.

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