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Locomotor conditioning by amphetamine requires cyclin-dependent kinase 5 signaling in the nucleus accumbens

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ABSTRACT

Intermittent systemic exposure to psychostimulants leads to several forms of long-lasting behavioral plasticity including nonassociative sensitization and associative conditioning. In the nucleus accumbens (NAcc), the protein serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) and its phosphorylation target, the guanine–nucleotide exchange factor kalirin-7 (Kal7), may contribute to the neuroadaptations underlying the formation of conditioned associations. Pharmacological inhibition of Cdk5 in the NAcc prevents the increases in dendritic spine density normally observed in this site following repeated cocaine. Mice lacking the *Kal7* gene display similar effects. As increases in spine density may relate to the formation of associative memories and both Cdk5 and Kal7 regulate the generation of spines following repeated drug exposure, we hypothesized that either inhibiting Cdk5 or preventing its phosphorylation of Kal7 in the NAcc may prevent the induction of drug conditioning. In the present experiments, blockade in rats of NAcc Cdk5 activity with roscovitine (40 nmol/0.5 μl/side) prior to each of 4 injections of amphetamine (1.5 mg/kg; i.p.) prevented the accrual of contextual locomotor conditioning but spared the induction of locomotor sensitization as revealed on tests conducted one week later. Similarly, transient viral expression in the NAcc exclusively during amphetamine exposure of a threonine–alanine mutant form of Kal7 [mKal7(T1590A)] that is not phosphorylated by Cdk5 also prevented the accrual of contextual conditioning and spared the induction of sensitization. These results indicate that signaling via Cdk5 and Kal7 in the NAcc is necessary for the formation of context-drug associations, potentially through the modulation of dendritic spine dynamics in this site.

1. Introduction

Repeated intermittent amphetamine exposure can lead to several forms of behavioral plasticity including associative conditioning and nonassociative sensitization. The formation of associative memories can link contextual stimuli to unconditioned drug effects allowing these cues to elicit drug-like excitatory conditioned responses as demonstrated by the locomotor activating effects of amphetamine and contexts previously paired with amphetamine (Stewart, 1992; Stewart and Vezina, 1988). In the case of sensitization, drug-evoked behavioral and neurochemical responses become exaggerated with successive infusions of the drug (Vezina, 2004). This form of plasticity accrues independent of association formation as demonstrated by the ability of amphetamine infusions into the ventral tegmental area (VTA) to produce sensitization in the absence of drug conditioning (Singer et al., 2009; Vezina and Stewart, 1990). Although associative conditioning and nonassociative sensitization reflect distinct processes, drug-paired and drug-unpaired environments can come to control the expression of sensitized responding (Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Stewart and Vezina, 1988, 1991; Wang and Hsiao, 2003). As both of these forms of plasticity are known to regulate drug-related behaviors and have been linked, separately and together, to addiction vulnerability in humans and animal models (Vezina and Leyton, 2009; Leyton and Vezina, 2013), it is important to elucidate their underlying neuronal mechanisms.

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Drugs of abuse are typically administered in the presence of a large number of salient environmental stimuli, providing ample opportunity for the formation of drug-stimulus associations and the possibility for these associations to subsequently influence responding. Morphological changes in dendritic spines have long been thought to underlie aspects of this type of memory storage and have been observed following learning, the induction of long-term potentiation, and behavioral enrichment (Geinisman et al., 2001; Lamprecht and LeDoux, 2004; Leuner et al., 2003). In the nucleus accumbens (NAcc), exposure to sensitizing regimens of systemic amphetamine injections produces long lasting increases in dendritic spine density (Robinson and Kolb, 1997, 1999). Considering that these are not observed following repeated infusions of amphetamine into the VTA, it is likely that they reflect associative drug conditioning rather than nonassociative sensitization (Singer et al., 2009). Consistent with this possibility, Marie et al. (2012) showed that the development of cocaine CPP correlates with increased dendritic spine density in the NAcc. Thus, preventing these increases in NAcc dendritic spine density normally observed in rats exposed to systemic amphetamine would be predicted to inhibit the development of conditioning while preserving the induction of sensitization. This reasoning provided the rationale for the present experiments to investigate the contribution to the induction of conditioning of proteins known to regulate dendritic spine dynamics.

Two such proteins, the proline-directed serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) and its phosphorylation target, the guanine–nucleotide exchange factor kalirin-7 (Kal7), are known amongst other actions to regulate cytoskeletal stability related to dendritic spine formation and retraction (Penzes and Jones, 2008; Xie et al., 2007) and have been implicated in drug-induced spine proliferation in the NAcc. Preventing Cdk5 phosphorylation of Kal7 at its threonine 1590 (T1590) residue, for example, reduces spine maturity (Xin et al., 2008). As predicted, pharmacological inhibition of Cdk5 in the NAcc blocks cocaine-induced increases in dendritic spine density in this site (Norrholm et al., 2003) but enhances the induction of locomotor sensitization (Bibb et al., 2001; Taylor et al., 2007). Similarly, interfering with Kal7 function spares (Wang et al., 2013) or even enhances (Kiraly et al., 2010) locomotor sensitization while preventing the increases in dendritic spine density normally observed in the NAcc following cocaine exposure. However, the link between the actions of Cdk5 and Kal7 in the NAcc and the development of drug conditioning is not clear.

Pharmacological inhibition of Cdk5 in the lateral septum and hippocampus has been reported to block the acquisition of fear conditioning (Fischer et al., 2002) and when applied to the basolateral amygdala, to prevent the acquisition of cocaine CPP (Li et al., 2010). However, its effect in the NAcc on the development of drug conditioning has yet to be assessed and no experiments have yet been conducted with amphetamine. Strategies using transgenic mice or long-lasting viral-mediated gene transfer to target the NAcc have yielded a number of different results regarding the functions of Cdk5 and Kal7 in Pavlovian and instrumental conditioning. For example, mice with either Cdk5 knocked out (Hawasli et al., 2007) or subjected to transient p25 expression and elevated Cdk5 activity (Fischer et al., 2005) both show enhanced contextual fear conditioning with the latter effects possibly involving a Cdk5 substrate shift (Meyer et al., 2008). Selective Cdk5 knock-out in the NAcc has been reported to lower the threshold dose required for acquisition of cocaine CPP (Benavides et al., 2007) while decreased acquisition of cocaine CPP has been shown in Kal7 knock-out mice (Kiraly et al., 2010). Reducing Kal7 in the NAcc with lentiviral delivery of Kal7 shRNA decreased incentive motivation but had no effect on the acquisition of cocaine self-administration (Wang et al., 2013) while Cdk5 knock-out mice show similarly unaffected acquisition of instrumental responding but enhanced incentive motivation (Benavides et al., 2007). However, regardless of the outcome, the results obtained in these latter experiments are difficult to interpret because, unlike in pharmacology studies, the Cdk5 and Kal7 manipulation strategies used did not distinguish between acquisition and expression of conditioning, making it difficult to ascertain whether the results obtained were due to effects on one, the other, or a combination of the two. As with sensitization (Vezina, 2004), different neuronal mechanisms underlie the acquisition and expression of excitatory conditioning (Aujla and Beninger, 2004; Banasikowski et al., 2010; Cervo and Samanin, 1995) and these may be differentially affected by changes in Cdk5 and Kal7. Thus, the roles played by Cdk5 and Kal7 in the NAcc specifically in the development of drug conditioning remain unclear.

The present experiments assessed the contribution of Cdk5 and Kal7 in the NAcc to the induction of amphetamine-induced locomotor conditioning. Locomotor sensitization was also assessed to control for potential contributions to non-associative plasticity. The approach used targeted induction specifically by pharmacologically inhibiting Cdk5 or using a transient viral infection system to express exclusively during amphetamine exposure a threonine–alanine mutant form of Kal7 (mKal7) that is not phosphorylated by Cdk5. Our results indicate that Cdk5 and Kal7 signaling in the NAcc is necessary for the induction of excitatory contextual drug conditioning, possibly through a pathway involving Cdk5 phosphorylation of Kal7.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Madison, WI) weighing 250–275 g on arrival were used. Rats were individually housed in a reverse cycle room (12-h light/12-h dark; lights on at 2000 h) with food and water available *ad libitum*. All procedures were performed during the dark phase of the light cycle. Following a 4–5 day acclimation period, all rats were anesthetized with a mix of ketamine (100 mg/kg, IP) and xylazine (10 mg/kg, IP), placed in a stereotaxic instrument with the incisor bar positioned 5.0 mm above the interaural line, and implanted with 22 gauge chronic bilateral guide cannulae angled at 10° to the vertical and aimed at the NAcc shell (A/P, +3.4; M/L, ±0.8; DV, −7.5 mm from bregma and skull; as per the angled brain atlas of Pellegrino et al., 1979) with tips positioned 1 mm (for the roscovitine experiment) or 4 mm (for the HSV-mKal7 experiment) above the final injection site. The NAcc shell was targeted because previous studies of the effects of NAcc roscovitine examined this subnucleus (Norrholm et al., 2003; Taylor et al., 2007) and it is uniquely innervated by the ventral hippocampus, a structure known to process contextual information (Moses et al., 2002). The cannulae (Plastics One, Roanoke, VA) were imbedded in a dental cement cap secured by six screws fastened to the skull. After surgery, 28 gauge obturators were placed into the guide cannulae (either flush for the HSV-mKal7 experiment or protruding 1 mm beyond the guide cannula tips for the roscovitine experiment) and rats were returned to their home cage for 10–14 days of recovery. All surgical procedures were conducted using aseptic techniques according to an approved Institutional Animal Care and Use Committee protocol.

2.2. Locomotor testing chambers

A bank of 8 open field activity boxes (Med Associates, St. Albans, VT) was used to measure locomotor responding to saline and amphetamine. Each open field (43.2 × 43.2 × 30.5 cm) was constructed of acrylic walls, a wire mesh floor, a removable Plexiglas top, and was fitted with a 16 × 16 horizontal grid of infrared sensors positioned 3.5 cm above the floor. Separate interruptions of photocell beams were detected as ambulatory counts and recorded via an electrical interface by a computer situated in an adjacent room using Med Associates Open Field Activity Software (SOF-811).

2.3. Effect of inhibiting Cdk5 in the NAcc on the induction of locomotor conditioning and sensitization by amphetamine

2.3.1. Behavioral procedures

In this experiment, rats were subjected to three phases: drug exposure, withdrawal, and testing.

The exposure phase used a discrimination learning paradigm that consisted of four 3-day conditioning blocks (Table 1). Injections were given on the first 2 days of each block (the first in the open field and the second in the home cage);

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