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SHORT COMMUNICATION

Stimuli associated with the presence or absence of amphetamine regulate cytoskeletal signaling and behavior

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Abstract
Drug-paired stimuli rapidly enlarge dendritic spines in the nucleus accumbens (NAcc). While increases in spine size and shape are supported by rearrangement of the actin cytoskeleton and facilitate the synaptic expression of AMPA-type glutamate receptors, it remains unclear whether drug-related stimuli can influence signaling pathways known to regulate these changes in spine morphology. These pathways were studied in rats trained on a discrimination learning paradigm using subcellular fractionation and protein immunoblotting to isolate proteins within dendritic spine compartments in the NAcc shell. An open field chamber was repeatedly associated with amphetamine in one group (Paired) and explicitly unpaired with amphetamine in another (Unpaired). Rats in a third group were exposed to the open field but never administered amphetamine (Control). When administered saline and returned to the open field one week later, Paired rats as expected displayed a conditioned locomotor response relative to rats in the other two groups. NAcc shell tissues were harvested immediately after this 30-minute test. Re-exposing Paired rats to the drug-paired excitatory context significantly decreased p-GluA2(S880), an effect consistent with reduced internalization of this subunit and increased spine proliferation in these rats. In contrast, re-exposing Unpaired rats to the drug-unpaired context, capable of inhibiting conditioned responding in these animals,

Abbreviations: AMPAR, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor; Arp2/3, actin-related protein 2/3; CS, conditioned stimulus; DA, dopamine; IP, intraperitoneal; LIMK, LIM-domain-containing protein kinase; MSN, medium spiny neuron; NAcc, nucleus accumbens; PKC, protein kinase C; PSD, post-synaptic density

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significantly decreased levels of both actin binding protein Arp2/3 and p-cofilin, consistent with spine volatility, shrinkage, and inhibition of spine proliferation in these rats. These findings show that contextual stimuli previously associated with either the presence or absence of amphetamine differentially regulate cytoskeletal signaling pathways in the NAcc.

1. Introduction

Rapid increases in dendritic spine size and shape are elicited by drug-paired stimuli in nucleus accumbens (NAcc) medium spiny neurons (MSNs), suggesting a role for these morphological effects in the expression of conditioned excitatory behavioral responding (Gipson et al., 2013; Singer et al., 2016). Recent studies have reported that rearrangement of the actin cytoskeleton regulates spine dynamics following psychostimulant administration (Shen et al., 2009), but it remains largely unknown whether conditioned stimuli can initiate actin cycling in the absence of drug. Conversely, stimuli explicitly unpaired with drug can inhibit the expression of conditioned responding (Vezina and Leyton, 2009), but again, it remains unknown what effects if any these stimuli may have on cytoskeletal signaling.

With the help of various binding proteins, actin cycles between monomeric (globular, "G") and polymerized (filamentous, "F") states. In the spine, a complex involving actin-related protein 2/3 (Arp2/3), cofilin, and cortactin is necessary to promote F-actin branching (Hotulainen and Hoogenraad, 2010; Ichetovkin et al., 2002). Inhibiting Arp2/3 reduces actin polymerization and results in smaller spines (Nakamura et al., 2011). Cofilin also binds and removes G-actin to disassemble F-actin, again resulting in smaller spines. Phosphorylation of cofilin by LIM-domain-containing protein kinase (LIMK) prevents this effect, thereby stabilizing F-actin to maintain spine length (Van Troys et al., 2008).

The lengthening and branching of F-actin in the post-synaptic density (PSD) provides structural support for changes in synaptic plasticity (Hotulainen and Hoogenraad, 2010; Matsuo et al., 2008; Shen et al., 2009), including psychostimulant-induced increases in the surface expression and function of glutamatergic α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPARs) in the NAcc (Boudreau et al., 2007; Loweth et al., 2010). Interestingly, the AMPAR GluA2 subunit also contributes to spine formation (Passafaro et al., 2003) via interactions between its extracellular N-terminal domain and presynaptic processes (Saglietti et al., 2007). Phosphorylation by protein kinase C (PKC) of membrane-bound GluA2 at S880 results in the rapid internalization of this subunit (Chung et al., 2000), an event that, in addition to inducing long-term depression (Chung et al., 2003), would significantly curtail the contribution of GluA2 to spine proliferation (Passafaro et al., 2003).

Here we show that contextual stimuli previously associated with either the presence or absence of amphetamine differentially regulate these signaling pathways in the NAcc, implicating them in the conditioned regulation of spine dynamics and the elicitation of conditioned locomotor responding by amphetamine-associated cues. Protein expression was examined specifically in the NAcc shell because this site is known to process contextual, as opposed to discrete, stimulus information (Bossert et al., 2013, 2007; Everitt and Robbins, 2005; Singer et al., 2014a, 2014b). Contextual cues also elicit rapid changes in dendritic spine morphology in the NAcc shell (Singer et al., 2016).

2. Experimental procedures

2.1. Subjects

Male Sprague–Dawley rats (Harlan Sprague–Dawley; Madison, WI) weighing 250–275 g on arrival were individually housed with food and water available *ad libitum* in a reverse cycle room (12- h light/12-h dark, lights on at 8 pm). Rats were given 4–5 days to acclimate to housing conditions. All procedures were performed during the dark phase of the lighting cycle and conducted according to an approved Institution of Animal Care and Use Committee (IACUC) protocol.

2.2. Behavioral conditioning

Behavioral training and testing were conducted as previously described (Singer et al., 2016, 2014a, 2014b). Briefly, rats were conditioned using a discrimination learning paradigm over the course of five consecutive 3-day blocks. On the first day of each block, rats were transported to an experimental room, administered either amphetamine (Paired rats; 1 mg/kg, IP) or saline (Unpaired and Control rats; 1 ml/kg, IP), and placed in Med Associates open fields for 2-hours. On the second day, rats were transported to another room in which Unpaired rats were administered amphetamine (1 mg/kg, IP) and Paired and Control rats administered saline. Following these injections, rats were immediately returned to their home cages and transported back to the colony room. Thus, the open field contextual stimuli were associated with the presence of amphetamine and the development of excitatory conditioning in Paired rats. Conversely, these contextual stimuli were associated with the absence of amphetamine and the development of conditioned inhibition in Unpaired rats. This discrimination learning paradigm is known to establish stimuli explicitly unpaired with the unconditioned stimulus as conditioned inhibitors. When used in a summation procedure, these stimuli reduce responding to a drug unconditioned stimulus (Vezina and Leyton, 2009). Importantly, procedures known to extinguish conditioned inhibition in this paradigm selectively disinhibit the expression of locomotor sensitization by amphetamine to reveal sensitized responding in Unpaired rats (Stewart and Vezina, 1991). No procedures took place on the third day. After the fifth 3-day block, rats were left undisturbed for 1-week. On the subsequent test for conditioning, all rats were administered saline and their locomotor responding in the open fields was recorded for 30-minutes. Rats used for subcellular fractionation and immunoblotting were then immediately sacrificed without anesthesia, their brains rapidly

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