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Synaptic Adaptations in the Nucleus Accumbens Caused by Experiences Linked to Relapse

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Abstract

Background—Excitatory synaptic transmission in the nucleus accumbens (NAc) regulates the reinstatement of drug-seeking, an animal model of relapse in human drug addicts. However, the functional adaptations at NAc synapses that mediate reinstatement are not clearly understood.

Methods—We assessed the behavioral responses of mice to cocaine administration by measuring locomotor stimulation and the acquisition, extinction, and reinstatement of conditioned place preference. Synaptic function was then examined by preparing acute brain slices and performing whole cell voltage-clamp recordings from individual medium spiny neurons in the NAc shell.

Results—We find that reduced excitatory synaptic strength in the NAc shell is a common functional adaptation induced by multiple experiences known to cause reinstatement, including stress and drug re-exposure. The same synaptic adaptation is observed shortly after reinstatement of conditioned place preference by a cocaine priming injection.

Conclusions—This common synaptic modification associated with stress, drug re-exposure, and reinstatement defines a potential synaptic gateway to relapse.

Keywords

Addiction; Stress; Nucleus Accumbens; Synaptic Plasticity; Relapse; Reinstatement

Introduction

Drugs of abuse share a common capacity to commandeer synaptic plasticity in the mesolimbic dopamine system (1). In the nucleus accumbens (NAc) – a central component of the mesolimbic dopamine system – adaptations in glutamate transmission are specifically implicated in the reinstatement of cocaine-seeking (2), a prominent animal model of relapse (3). Manipulations of NAc glutamate signaling have profound effects on reinstatement by drug priming injections (2, 4, 5), stress (6), and drug-associated cues (7). However, few

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studies have directly examined changes in NAc excitatory synaptic function that occur following experiences that cause reinstatement and relapse.

Repeated cocaine exposure leads to an enhancement of NAc excitatory synaptic transmission that develops following the termination of drug exposure (7-11) and is reversed by re-exposure to cocaine (8, 10, 12) – one experience associated with relapse. Stressful events also provoke relapse in human drug addicts and reinstatement in animal models (3). While acute stress can modify synaptic function in many regions of the brain, including NAc (13), the effect of stress on NAc synaptic transmission has not previously been examined in animals with a history of cocaine exposure. Here, we find that cocaine treatment reverses the impact of stress on NAc excitatory synapses, leading to a loss of excitatory synaptic strength. This synaptic adaptation is also observed shortly after the reinstatement of cocaine-conditioned place preference, defining a potential synaptic gateway to reinstatement and relapse.

Methods and Materials

Male C57Bl/6J mice (Jackson Lab) were given at least one week to acclimate to housing conditions and were at least 4 weeks old at the beginning of each experiment. All procedures conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Procedures for cocaine treatment and synaptic electrophysiology have been previously described (8, 12); details are provided in the Supplement. For forced swim stress, mice were placed in a 1L beaker containing ~800 mL of water at room temperature (~23°C). After swimming for 6 minutes, mice were lightly dried with a towel and returned to the home cage. This stress procedure elevates circulating levels of corticosterone and reinstates conditioned place preference in mice (14, 15). The place conditioning apparatus and procedure are described in the Supplement, along with details concerning statistical analysis.

Results

The locomotor responses following the first (Day 1) and last (Day 5) cocaine injection are presented in Figure 1B. Cocaine-treated mice were classified as sensitized if their locomotor response to cocaine increased more than 45% from the first to the last injection (see Supplement). Out of 19 cocaine-treated mice, 13 (68%) met this criterion and were classified as sensitized, while the remaining six mice (32%) were classified as non-sensitized. These proportions, as well as the tendency for a larger initial response to cocaine in non-sensitized animals, are similar to previous studies of cocaine sensitization in rats (10, 16). Ten to 14 days after the last injection, some mice from each treatment group were exposed to a single session of forced swim stress, and acute brain slices were prepared 24 hours later (Figure 1A).

We first measured the relative contributions of AMPA receptors (AMPA) and NMDA receptors (NMDARs) to the synaptic current recorded at a holding potential of +40 mV. The AMPAR/NMDAR ratio is a measure of excitatory synaptic strength that has proven to be a sensitive assay for detecting synaptic adaptations caused by drug exposure (1). Analysis of the AMPAR/NMDAR ratio from all three treatment groups (Figure 1C) revealed a significant Group $\times$ Stress interaction [$F(2,68) = 4.08$, $p = .021$]. In the absence of stress, the AMPAR/NMDAR ratio was specifically increased in sensitized mice [$p = .025$ compared to saline]. Parallel changes in cell-surface expression of AMPARs have been reported in rats that develop cocaine sensitization (10, 16).

The increase in synaptic strength associated with cocaine sensitization was reversed 24 hours after a single session of forced swim stress (Figure 1C) [$F(1,25) = 5.09$, $p = .033$] – a

manipulation that provokes reinstatement at this same time point (17). In the saline-treated control group, there was a non-significant tendency for stress to increase the AMPAR/NMDAR ratio [$F(1,32) = 1.82, p = .19$]. This increase in synaptic strength is more robust following a more intense stress protocol (13). Stress had no discernible effect in mice that did not develop cocaine sensitization [$F(1,11) < 1$], consistent with other evidence for correlated individual differences in sensitivity to stress and psychostimulant exposure (18).

The synaptic incorporation of AMPARs lacking the GluA2 subunit is increased in NAc following drug-free periods longer than 10-14 days (7, 9). However, we found no changes in the current-voltage relationship of AMPAR EPSCs (Figure S1 in the Supplement), suggesting alterations in the subunit composition of AMPARs did not lead to changes in the AMPAR/NMDAR ratio. This is consistent with our previous report that the current-voltage relationship of AMPAR EPSCs is not changed 10-14 days after repeated cocaine exposure (8). There were also no differences in the paired-pulse ratio that would indicate a change in the probability of glutamate release under any treatment condition (Figure S2 in the Supplement).

One striking aspect of these results is that the synaptic effect of stress in cocaine-treated animals mirrors the effect of cocaine re-exposure (8), indicating that this synaptic adaptation represents a common consequence of experiences that cause relapse in humans and reinstatement in animals models (3). To more directly assess this possibility, we examined the synaptic changes that accompany the reinstatement of cocaine-conditioned place preference (CPP) (Figure 2A). CPP was established by repeatedly injecting cocaine in the presence of distinct contextual cues, leading to a significant increase in time spent on the side of the apparatus associated with cocaine (Figure 2B, "CPP") [$F(1,25) = 81.58, p < .001$]. CPP was then extinguished by repeatedly exposing animals to these cues in the absence of cocaine over 8-10 sessions, leading to a significant decrease in time spent on the drug side (Figure 2B, "Ext") [$F(1,25) = 232.4, p < .001$] (Figure 2B). A priming injection of saline (Sal') had no effect on extinguished cocaine CPP, but a priming injection of cocaine (Coc') restored preference for the drug-related cues (Figure 2B, "Prime") [Session $\times$ Prime interaction: $F(1,25) = 12.67, p = .002$].

We prepared acute brain slices *two hours* after the priming injection, to allow cocaine to clear the system but still assess synaptic changes in close temporal proximity to the reinstatement event. Analysis of the AMPAR/NMDAR ratio from all three treatment groups revealed a significant main effect [$F(2,39) = 4.51, p = .017$]. Compared to an experimentally naïve control group, there was a net increase in the AMPAR/NMDAR ratio caused by CPP conditioning, extinction, and a priming injection of saline (Figure 2C) [$p = .017$]. This increase likely reflects the combined effects of cocaine exposure (8) and extinction training (6). However, a priming injection of cocaine reversed this increase in the AMPAR/NMDAR ratio (Figure 2C) [$p = .037$] – precisely the same synaptic adaptation observed 24 hours after cocaine re-exposure (8) or stress (Figure 1).

Conclusions

Our results indicate that stress and drug re-exposure, two experiences linked to relapse in human drug addicts, both cause a reduction of excitatory synaptic strength in the NAc shell of mice exposed to cocaine. This modification of synaptic function was observed 24 hours after forced swim stress, coincident with enhanced cocaine-seeking (17). The effect of stress in cocaine-treated mice contrasts with the effect of acute stress in drug-naïve mice, as the latter causes an increase in excitatory synaptic strength (13). Cocaine exposure thus reverses the impact of stress on NAc excitatory synapses, providing another example of cocaine-

induced metaplasticity (8, 11). This altered sensitivity to stress may be one factor that leaves recovering drug addicts vulnerable to relapse.

The reinstatement of cocaine CPP was also accompanied by a reduction of excitatory synaptic strength in the NAc shell. This synaptic adaptation appeared to develop rapidly, as it was present only two hours after a priming injection of cocaine. These results are the first to demonstrate a common form of plasticity at NAc excitatory synapses triggered by different experiences associated with relapse. Cocaine-associated cues have also been shown to cause internalization of cell surface AMPARs in the NAc (10) – the biochemical parallel of a decrease in the AMPAR/NMDAR ratio. Although stress- and drug-induced reinstatement involve distinct upstream mechanisms (3), synaptic changes in NAc may be part of a convergent downstream pathway mediating reinstatement by both experiences. In our experimental preparation, stimulation at the rostral border of NAc in a parasagittal brain slice should prominently activate synaptic input from the prefrontal cortex (12). As prefrontal input to NAc shell arises primarily from infralimbic cortex (19), we speculate that the decrease in AMPAR/NMDAR ratio reflects a specific loss of excitatory drive from infralimbic cortex to medium spiny neurons in NAc shell, which promotes the reinstatement of extinguished drug-seeking behavior (20). This shared synaptic consequence of multiple experiences linked to relapse should inform the development of targeted therapeutic interventions to diminish the likelihood that abstinent drug addicts return to compulsive drug use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Stress after cocaine sensitization decreases NAc synaptic strength. (A) Experimental time line. (B) Locomotor activity following the first (Day 1) and last (Day 5) exposure to saline or cocaine. (C) *Top*: representative AMPAR and NMDAR EPSCs from saline alone, cocaine (sensitized) alone, and cocaine (sensitized) + stress. Calibration: 100 ms, 20 pA. *Bottom*: mean AMPAR/NMDAR ratio 24 hours after saline alone ($n = 16/7$); saline + stress ($n = 18/7$); cocaine (sensitized) alone ($n = 13/5$); cocaine (sensitized) + stress ($n = 14/4$); cocaine (non-sensitized) alone ($n = 6/3$); or cocaine (non-sensitized) + stress ($n = 7/2$). *Significant increase from saline alone; #significant decrease from cocaine (sensitized) alone.

Figure 2. Reduced NAc synaptic strength following reinstatement of cocaine CPP. (A) Experimental time line. (B) Acquisition, extinction, and reinstatement of cocaine CPP following a priming injection of saline (Sal', n = 13) or cocaine (Coc', n = 14). *Significant increase compared to Initial; #significant decrease compared to CPP; \$significant difference between Sal' and Coc'. (C) Mean AMPAR/NMDAR ratio in NAc shell of naïve mice (n = 13/4) or two hours after priming injection with saline (n = 14/9) or cocaine (n = 15/8). *Significant increase compared to Naïve; #significant decrease compared to Sal'.