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NMDA receptors on striatal neurons are essential for cocaine cue reactivity in mice

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Abstract

Background—Environmental cues associated with cocaine evoke craving and seeking. This process, termed cue reactivity, is a critical element of cocaine addiction. While glutamatergic neurotransmission has been implicated in this effect of cocaine, the precise subtype and localization in the brain of the glutamatergic receptor critical for cocaine cue reactivity is not well understood.

Methods—We used a conditional NMDA receptor (NMDAR) knockout mouse whose NMDAR gene was deleted by Cre expression restricted to striatal neurons. To evaluate the role of NMDAR in cocaine cue reactivity, conditional knockout mice and control mice (n=5–8 per group) were conditioned for place preference (CPP) with cocaine (5 and 10 mg/kg, s.c.) for 3 days. Their subsequent place preference was examined in a drug-free state.

Results—While control mice developed cocaine CPP, mice deficient for NMDAR on striatal neurons failed to develop CPP.

Conclusions—NMDAR on striatal neurons is essential for the development of cocaine cue reactivity in the place conditioning paradigm. Our finding identifies a brain region whose constitutive NMDAR level serves as a determinant for susceptibility to this aspect of cocaine addiction.

Keywords

Cocaine; Addiction; Conditioned Place Preference; Striatum; Conditional Knockout Mouse; Cue Reactivity

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Introduction

A prominent element of cocaine addiction is the ability of environmental cues, associated with the effects of cocaine, to elicit craving and sustained drug-taking (1). This phenomenon is termed cue reactivity. Cue reactivity can be assessed in rodents using a Pavlovian place conditioning paradigm, in which environmental cues are paired with cocaine injections. After repeated pairings, rodents approach the environmental cues, even when the cues are no longer paired with cocaine. This preference for cues previously paired with a drug is known as conditioned place preference (CPP).

Because cue reactivity involves a conditioning process, neuronal elements relevant to memory formation are likely to be involved in this aspect of cocaine addiction. The N-methyl-D-aspartic acid receptor (NMDAR) has been implicated in many forms of learning and memory, including Pavlovian conditioning (2). The role of NMDAR in cocaine CPP has been investigated through the use of the non-competitive NMDAR antagonist MK801. However, this antagonist also increases glutamate release (3) and binds to the dopamine D2 receptor (4).

A genetically engineered mouse provides a specific molecular ablation to reveal the contribution of constitutionally-altered molecular function to conferring susceptibility to addiction (5,6). NMDAR “knockdown” in the whole brain eliminates cocaine CPP in mice (7). However, the specific location of this effect in the brain is still not clear. Selective deletion of NMDAR in dopaminergic neurons in the mouse midbrain abolishes (8) or has no effect on (9) the development and expression of cocaine CPP.

The NMDAR gene is also expressed in striatal projection neurons and interneurons (10). The striatum is divided into parallel sub-structures, based on their anatomical similarities. The caudate-putamen and nucleus accumbens/olfactory tubercle are referred to as the dorsal and ventral striatum, respectively (11). Because the caudate-putamen and nucleus accumbens are implicated in cue reactivity in cocaine addiction (12), we examined the role of striatal NMDAR in cocaine CPP, using mice in which NMDAR was selectively abolished in striatal neurons (13).

Methods and Materials

Animals

Adult male conditional NMDAR KO (RGS9-cre/NR1^{flox/flox}) and control (NR1^{flox/flox}) mice were used in this study. These strains of mice have been used in a previous study (13). Mice were maintained under a 14-h light:10-h dark cycle with food and water available *ad libitum* unless otherwise specified. Animal handling and use procedures followed a protocol approved by the Animal Care and Use Committee of Albert Einstein College of Medicine, in accordance with NIH guidelines.

Drugs

Cocaine hydrochloride (HCl) (Sigma Chemical Co., St Louis, MO, USA) was dissolved in 0.9% saline and was injected subcutaneously (s.c.) in a volume of 2 ml/kg.

CPP

The apparatus used was a rectangular Plexiglas box composed of three distinct compartments. One of the two large compartments (24.5 cm × 18 cm × 33 cm) had black-and-white striped walls and a wire mesh floor with 2.1 mm × 2.1 mm openings. It was lit at 5.6 lux. The other large compartment had gray walls and a wire mesh floor with 3.7 mm × 3.7 mm openings. It

was lit at 3.66 lux. A third, central compartment (13 cm × 18 cm × 33 cm) separated these two large compartments.

The CPP experiment was carried out over 5 days. On Day 1, the guillotine doors were opened 5 cm above the floor and the mice were allowed to explore the three compartments freely for 15 min. Mice, on a group basis, did not show any bias toward one compartment of the apparatus. Conditioning was given on Days 2–4. Mice received two conditioning sessions 5 h apart. Mice received a single injection of cocaine HCl (5 or 10 mg/kg, s.c.) and a single injection of saline 5 h apart on each of Days 2–4, with the order of cocaine injections and the compartment paired with cocaine counterbalanced. During conditioning, guillotine doors were closed and mice were confined for 30 min to one of the two large compartments. Approximately equal numbers of animals received pairings with cocaine in each compartment. On Day 5, animals were placed in the center compartment with no injections and allowed to move freely for 15 min between the two large compartments through open guillotine doors. An observer blinded to experimental conditions recorded the amounts of time the mice spent in the two large compartments.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA). Exploratory ANOVAs with Bonferroni correction were used to determine the nature of an interaction effect. Type II error was set at 5%.

Results

Neither control nor KO mice showed a pre-conditioning bias to either compartment (Fig. 1A; Genotype, $F_{1,25} = 1.79$, n.s.; Compartment, $F_{1,25} = 1.65$, n.s.; Genotype × Compartment, $F_{1,25} = 0.04$, n.s.). The CPP scores on the post-conditioning test day showed a significant interaction between Genotype and Compartment ($F_{1,23} = 5.89$, $P = 0.024$). Exploratory ANOVAs showed that CPP was abolished in KO mice (Dose, $F_{1,14} = 1.39$, n.s.; Compartment, $F_{1,14} = 0.002$, n.s.; Dose × Compartment, $F_{1,14} = 0.004$, n.s.), while control mice showed equally robust CPP at both cocaine doses (Dose, $F_{1,9} = 0.08$, n.s.; Compartment, $F_{1,9} = 15.30$, $P = 0.0036$; Dose × Compartment, $F_{1,9} = 0.08$, n.s.).

Discussion

We assessed the role of NMDAR on striatal neurons in cue reactivity, an element of cocaine addiction, in the place conditioning paradigm. Our results show that mice deficient in NMDAR on striatal neurons failed to show cocaine CPP.

In the conditional KO mice, the excision of NMDAR was restricted by RGS9-dependent Cre expression (13). RGS9 mRNA is expressed in striatal cholinergic interneurons, the striatal indirect pathway projecting to the external segment of the pallidum, and the striatal direct pathway to the internal segment of the pallidum and the midbrain (14–16). RGS9-dependent Cre expression reduced the amount of NMDAR produced in cell bodies of striatal neurons and expressed in their dendrites and terminals (13). By contrast, NMDAR expressed on terminals of corticostriatal projection neurons and striatal glial cells were not significantly affected (13). Thus, the effects of NMDAR deletion on behavior in our conditional mouse model is potentially attributable to NMDAR expressed on terminals of striatal neurons in the globus pallidus/ventral pallidum and the midbrain, as well as the dendrites and cell bodies of striatal neurons within the striatum.

Although RGS9 is highly enriched in striatal neurons, low levels of RGS9 exist elsewhere (17,18). It is possible that NMDAR may be reduced in neurons other than striatal neurons.

However, in our conditional NMDAR KO mouse, RGS9-cre activity is confined to striatal neurons and is not found in the cortex, cerebellum or midbrain (13). NMDAR reduction outside the striatum is likely to be minimal. Our mouse model complements other conditional NMDAR deletion mouse lines whose NMDAR levels are reduced in dopaminergic neurons (8,9).

The place conditioning paradigm does not simply evaluate the rewarding effects of addictive substances. Rather, the CPP evaluates the detection of sensory cues and the approach-inducing, incentive effects of cocaine. The CPP also examines the acquisition of an association between the representations of the two events, the retrieval and expression of such an association, and the expression of an approach behavior. A defect in any of these processes would disrupt a CPP.

The general ability to detect sensory stimuli and guide behavior through classical conditioning does not seem to be impaired in conditional NMDAR KO mice. Passive avoidance is normal in these mice (13). Moreover, the motor ability to express a conditioned behavior is apparently normal, since conditional KO mice exhibit normal motor activity in an open field (13). Although our experiment was not designed to identify the exact process through which a basal NMDAR tone influences CPP, indirect evidence suggests that NMDAR might be required for some acquisition, but not for expression processes. When infused into the accumbens, a subregion of the striatum, during acquisition, the NMDAR antagonist AP-5 prevents the establishment of conditioned approach to cues associated with food. However, AP-5 did not impair expression of the once-acquired approach behavior (19). Thus, a basal, constitutive NMDAR tone is likely to alter the susceptibility to cocaine cue reactivity through its impact on the incentive effects of cocaine, their association with sensory cues, or both.

Within the striatum, NMDAR levels in the nucleus accumbens are likely to be a determinant of cocaine CPP. In this subregion of the striatum, repeated cocaine injections cause alterations in NMDAR-dependent long-term depression (LTD) in mice (20). Consistent with this finding, our conditional KO mice show impairments in LTD in the nucleus accumbens (13). It is unclear whether reduced LTD in the nucleus accumbens plays a functional role in cocaine CPP. Therefore, a future challenge is to determine a causal association between altered synaptic plasticity in the accumbens and elements of cocaine addiction.

Pre-existing traits accentuate or reduce susceptibility to addiction (5,6). The capacity to form cue reactivity may be associated with pre-existing traits. Basal, constitutive NMDAR tone may contribute to addiction susceptibility through its effects on cue reactivity. Our conditional KO mouse provides a novel means to explore the role of striatal NMDAR in this aspect of addiction to other substances.

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Figure 1.

Cocaine CPP in conditional NMDAR KO mice. A) Pre-conditioning test. B) Post-conditioning test. * $P < 0.0125$ and ** $P < 0.0025$ cocaine- versus saline-paired compartments, as determined by one-way ANOVAs with Bonferroni correction. C, Control; KO, knockout littermates. WT, 5 mg/kg: $n = 6$, 10 mg/kg: $n = 5$; KO, 5 mg/kg: $n = 8$, 10 mg/kg: $n = 8$.