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When administered into the nucleus accumbens core or shell, the NMDA receptor antagonist AP-5 reinstates cocaine-seeking behavior in the rat

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Abstract

Nucleus accumbens glutamate transmission plays a critical role in cocaine priming-induced reinstatement of drug seeking. Previous studies have demonstrated that systemic or intra-accumbens shell administration of an NMDA receptor antagonist reinstates cocaine-seeking behavior. However, it is unclear if antagonizing NMDA receptors in the nucleus accumbens core or shell subregions will differentially affect cocaine reinstatement. To investigate this possibility, we microinjected the competitive NMDA receptor antagonist AP-5 (0, 3 or 30 μg) into either the nucleus accumbens core or shell and assessed the reinstatement of cocaine-seeking behavior. When microinjected into the shell, both doses of AP-5 produced reinstatement of cocaine seeking. In contrast, when administered into the core, only the highest dose of AP-5 reinstated cocaine-seeking behavior; moreover, the magnitude of this effect was substantially less than when AP-5 was administered into the shell. This study provides evidence that pharmacological antagonism of NMDA receptors in the nucleus accumbens core or shell promotes the reinstatement of cocaine seeking.

Keywords: Relapse; Addiction; Psychostimulant; Glutamate

Cocaine craving in human addicts can be elicited by three major factors: environmental cues associated with drug taking, a stressful life-event or re-exposure to cocaine [26]. Drug craving is modeled in rodents and non-human primates using the reinstatement model of cocaine seeking [13,19,39]. The nucleus accumbens plays a major role in mediating the reinstatement of cocaine seeking [27,36]. The nucleus accumbens receives a major dopaminergic projection from the ventral tegmental area (VTA) and glutamatergic projections from the prefrontal cortex, hippocampus and amygdala [5,34,43]. The accumbens is composed of two major subregions, the core and the shell, which have differential afferent and efferent anatomical projections [7,20,21,44]. Functionally, the accumbens shell is considered the more limbic subregion, mediating the acute effects of drug reward [8,35]. In contrast, the core is more involved in the compulsivity of drug addiction [16] and the effects of drug-associated cues [15,18,25]. In terms of cocaine priming-induced reinstatement of drug seeking, dopamine receptors play differential roles in the shell versus the core [2–4]. For example, infusion of dopamine receptor antagonists into the accumbens shell, but not the core, attenuates the reinstatement of cocaine-seeking behavior [2–4]. Similarly, dopamine receptor agonists promote the reinstatement of cocaine seeking when microinjected into the shell, but not core [4,37]. Collectively, these studies demonstrate that increased dopamine transmission in the nucleus accumbens shell, but not the core, critically mediates the reinstatement of cocaine-seeking behavior.

The glutamatergic projection from the medial prefrontal cortex (mPFC) to the nucleus accumbens is one of the key anatomical substrates underlying cocaine priming-induced reinstatement of drug seeking [27,37]. There are two major classes of ionotropic glutamate receptors: AMPA/kainate and NMDA. While AMPA receptors in the nucleus accumbens are critically involved in the reinstatement of cocaine-seeking behavior [9,10,30,32,40], there is evidence that NMDA receptors may also play a role. For example, systemic administration of MK-801, an NMDA receptor channel blocker, robustly reinstated cocaine seeking, without an increase in non-specific operant responding ([12], but see also [6]). In a subsequent study, it was found that intra-accumbal administration of the competitive NMDA receptor antagonist, CPP, had no effect on the

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reinstatement of cocaine seeking when administered either alone or prior to a systemic priming injection of cocaine [9]. In contrast, intra-accumbal administration of AP-5, a competitive NMDA receptor antagonist, reinstated cocaine-seeking behavior when microinjected into the nucleus accumbens shell subregion [32]. Notably, these studies either did not differentiate between the core and shell subregions of the nucleus accumbens [9] or examined the shell but not the core [32]. Therefore, in the present study we systematically examined the ability of an NMDA receptor antagonist to reinstate cocaine-seeking behavior when administered into either the nucleus accumbens core or shell.

Male Sprague–Dawley rats (*Rattus norvegicus*) weighing 250–300 g were purchased from Taconic Laboratories (Germantown, NY). Animals were individually housed with food and water available ad libitum. The animal facilities have a 12-h light/12-h dark cycle, with the lights on at 7:00 a.m. All experimental procedures were performed during the light cycle. Behavioral experiments used Med-Associates (East Fairfield, VT) equipment enclosed within ventilated, sound-attenuating chambers. The operant chambers are equipped with two response levers (active and inactive), house light and injection pumps for intravenous drug administration.

Prior to surgery, rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine (Sigma/RBI, St. Louis, MO). An indwelling silastic catheter (CamCaths, Cambridge, UK) was inserted into the right jugular vein (side opposite the heart) and sutured into place. The catheter was connected to a back-mount, which was sealed with a plastic obturator when not in use. To maintain patency and prevent infection, catheters were flushed daily with 0.3 ml of the antibiotic Timentin (ticarcillin disodium/potassium clavulanate, 0.93 mg/ml) dissolved in heparinized saline. In all animals, guide cannulae (14 mm, 24 gauge; Small Parts Inc., Roanoke, VA) for microinjection experiments were bilaterally implanted 2 mm dorsal to the nucleus accumbens core and shell and were cemented into place by affixing dental acrylic to stainless steel screws secured in the skull. The coordinates for the placement of guide cannulae, relative to bregma [33], were as follows: nucleus accumbens shell: +1.0 mm A/P, ±1.0 mm M/L, −5.0 mm D/V; nucleus accumbens core: +1.0 mm A/P, ±2.5 mm M/L, −5.0 mm D/V. Obturators (14 mm, 33 gauge) were placed into the guide cannulae to prevent occlusion.

Following a 7-day recovery period from surgery, the rats were placed in operant chambers and trained to lever press for intravenous cocaine infusions (0.25 mg cocaine/59 μl saline/infusion over 5 s). A 20-s timeout period during which responses have no scheduled consequences followed each cocaine infusion. Rats initially were trained using a fixed ratio (FR)1 schedule of reinforcement with each daily 2 h self-administration session initiated by an intravenous injection of cocaine. This injection was performed in order to fill the catheter (i.e. little or none of this non-contingent injection reached the systemic circulation). When the animals achieved stable responding under the FR1 schedule (i.e. less than 15% variation in response rates over two consecutive days and >15 infusions each day) they were switched to an FR5 schedule. During all phases of the experiment, inactive lever responding was also recorded. To avoid overdose, rats were limited to a maximum of 30 cocaine infusions per self-administration session. Following 21 days of self-administration (FR1 + FR5), lever pressing was extinguished by replacing cocaine with saline. Daily 2-h extinction sessions were conducted until responding was less than 15% of the responses maintained by cocaine self-administration. During the reinstatement phase of the experiment, 3 or 30 μg DL-2-Amino-5-phosphonovaleric acid lithium salt (AP-5) or its saline vehicle was administered into the core or shell of the nucleus accumbens immediately prior to a 2-h reinstatement session. Doses were chosen based upon a previous reinstatement study from our laboratory, as well studies that assessed locomotor activity and behavioral sensitization [29,31,32]. Lower doses, used extensively in learning and memory studies, have not generally been successful in disrupting cocaine-associated behaviors [11,17,22,38,42]. For the reinstatement sessions, the FR5 schedule was used and satisfaction of the response requirement resulted in a saline infusion. Each reinstatement session was followed by extinction sessions until responding was again less than 15% of the response rate maintained by cocaine self-administration. At various points during the extinction phase, reinstatement of cocaine-seeking behavior was assessed by administering a systemic injection of cocaine (10 mg/kg, i.p.). If a rat failed to reinstate to cocaine (operationally defined as fewer than 40 active lever responses per session), no additional data was collected using that subject.

Obturators were removed from the microinjection guide cannulae and replaced by injection needles (33 gauge; Small Parts Inc.), which extend 2 mm below the end of the guide cannulae into the nucleus accumbens core or shell. Bilateral infusions were made over 120 s in a volume of 0.5 μl/side. To allow for the compound to diffuse away from the tips of the microinjectors, the microinjectors were left in place for 60 s after the infusion and then removed. Whenever possible, each animal served as its own control. That is, each animal received vehicle as well as both doses of AP-5 (up to a maximum of 3 microinjections per brain region per animal). Deviation from this design occurred only when it was unavoidable (i.e. when technical difficulties, such as clogged microinjection cannulae, loss of intravenous catheter patency or an animal no longer reinstated to cocaine, made it impossible to test all doses of a test compound on a specific animal). Treatments were counterbalanced across sessions to avoid any rank-order effects. Similar microinjection procedures (specifically, the use of a 0.5 μl microinjection volume) have been used to identify functional differences between the nucleus accumbens core and shell [2,3,14,23,28,37]. DL-2-Amino-5-phosphonovaleric acid lithium salt (AP-5) was purchased from Sigma–Aldrich. Cocaine was a gift from the National Institute on Drug Abuse (NIDA).

After the reinstatement experiments were completed, rats were overdosed with pentobarbital (100 mg/kg, i.p.) and perfused intracardially with 60 ml of 0.9% saline followed by 60 ml of 10% formalin. The brains were removed and stored in 10% formalin. Subsequently, 100 μm coronal sections were taken at the level of the nucleus accumbens with a Vibratome (Technical Products Int., St. Louis, MO, USA). Coronal sections were mounted on gel-coated slides and stained with cresyl