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Short communication

NMDA receptor antagonists prevent conditioned activation of intracranial self-stimulation in rats

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Abstract

Rats with bipolar electrodes implanted unilaterally into the ventral tegmental area were trained to lever-press for response contingent electrical stimulation (continuous reinforcement). After preliminary lever-press training, two types of daily sessions were held on 10 consecutive days: type T^+, during which current intensity was set at the *T*hreshold level and each response was accompanied by the visual signal (stimulus lights above the lever briefly went off); and type ST^-, during which current was set at the *S*ub*T*hreshold level and there were no visual stimuli. On day 11, combination of the subthreshold current intensities and stimulus lights previously associated with the threshold stimulation (session type ST^+) resulted in significantly elevated response rates compared to the performance under the subthreshold current without visual stimuli (session type ST^-). This effect was dose dependently blocked by competitive NMDA receptor antagonist (±)-CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) and CGS 19755 (*cis*-4-(phosphonomethyl) piperidine-2-carboxylic acid). The present findings suggest that the activation of intracranial self-stimulation induced by a conditioned visual stimulus is dependent on the NMDA receptor functioning.

Keywords: NMDA receptor antagonist; Electrical brain stimulation; Conditioning; Reward; (Rat)

1. Introduction

There is an increasing body of evidence that glutamate receptors are implicated in the mechanisms underlying the development and expression of conditioned behaviors. Recent studies have demonstrated that glutamate receptor antagonists affect both acquisition and expression of drug-conditioned place preference in rats (Bespalov et al., 1994b; Cervo and Samanin, 1995; Kaddis et al., 1995; Tzschentke and Schmidt, 1995). Previously, systemic administration of the non-competitive NMDA receptor antagonist MK-801 (dizocilpine; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]-cyclohepten-5,10-imine maleate) has been shown to block the development of amphetamine- and apomorphine-conditioned hyperactivity (Druhan et al., 1993; Stewart and Druhan, 1993; Segal et al., 1995). Experimental evidence suggests the involvement of glutamate receptors in the development of conditioned tolerance to the analgesic effects of morphine (Bespalov et al., 1994a) and in the expression of the conditioned component of sensitization to psychostimulant-induced locomotion (Kalivas et al., 1995). NMDA receptor blockade in the nucleus accumbens septi prevents locomotor stimulation conditioned by morphine and amphetamine (Bespalov and Zvartau, 1996b), as well as amphetamine-potentiated responding with conditioned food reinforcement (Burns et al., 1994). Conditioned dopamine release was shown to be dependent upon NMDA receptors (Saulskaya and Marsden, 1995b), and glutamatergic transmission was found to be involved in both expression and acquisition of conditioned responses (Saulskaya and Marsden, 1995a; Shors et al., 1995).

It was earlier suggested that NMDA receptors are not implicated in the behaviors elicited by the stimuli previously associated with the drug action, i.e., expression of drug-conditioned place preference (Cervo and Samanin, 1995). The present study sought to test whether NMDA receptor antagonists affect the conditioned activation of intracranial self-stimulation. The design of the present experiments was based on the model of intracranial self-

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stimulation facilitated by a brief visual stimulus previously associated with the rewarding stimulation (Bespalov and Zvartau, 1996a).

2. Materials and methods

2.1. Animals

Forty adult male Wistar rats (250–300 g; State Breeding Farm 'Rappolovo', St.-Petersburg, Russian Federation) were used. Animals were housed individually with food and water available ad libitum. All experiments were conducted during the light period of a 12/12-h day-night cycle (08:00–20:00 h). All rats were drug-naive, except for 7 rats that had previously been given a single injection of the opiate antagonist naloxone (0.3 mg/kg, s.c.).

2.2. Drugs

(±)-CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) and CGS 19755 (*cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid) (both from Research Biochemicals International, Natick, MA, USA) were dissolved in equimolar NaOH to form stock solutions (pH = 7.1 ± 0.2). Further dilutions were made with 0.9% sterile saline. Drugs and saline were injected intraperitoneally in a volume of 1 ml/kg.

2.3. Apparatus

Two standard operant conditioning chambers equipped with two side by side levers, 18 cm apart, were used. White stimulus lights located 2 cm above each lever illuminated the chamber throughout the experimental session. By pressing one of the levers ('rewarding' lever) rats were able to initiate brain stimulation (300 ms/response; rectangular cathodal wave with a pulse frequency of 100 Hz, pulse delay of 500 ms and 0.1 ms pulse duration) delivered through Kvant stimulus isolation units ('Pharmacolog', St.-Petersburg, Russian Federation) coupled to ES-51 stimulators. Responses on the other lever were recorded but did not have any programmed consequences.

2.4. Procedure

Each animal was anesthetized with Nembutal (55 mg/kg, i.p.), and bipolar stainless steel electrodes of 0.2 mm thickness, insulated except at the cross-section at the tip, were stereotaxically implanted, using a David Kopf Micromanipulator. Electrodes were lowered into the left ventral tegmental area (coordinates AP: −4.3 mm from bregma, L: 1.1 mm from the midline, V: 8.1 mm from a flat skull, angle: 0, incisor bar: 0). Four stainless-steel jeweler's screws and dental cement were used to anchor the electrode assembly to the skull.

Seven days postoperatively the rats were trained to press a lever for response-contingent intracranial electrical stimulation (continuous reinforcement). During this initial training the minimal current intensity inducing approximately 40–60 responses per minute was determined for each experimental subject and was called 'threshold' current intensity. Threshold intensities used in present study ranged from 6 μA to 30 μA.

After subjects successfully acquired lever pressing, training sessions of 12 min duration were introduced. Each training and later test sessions consisted of three 4-min periods. During the second 4-min period of each training/test session the current intensity was set at zero level and the stimulus lights above the 'rewarding' lever were on irrespective of the subject's behavior.

Initially, during the first and third periods current intensity was set at the 'threshold' level. During the first and third but not second periods of these sessions stimulus lights above the 'rewarding' lever went off simultaneously with each depression of the lever and went on again 500 ms later (session T^+, threshold stimulation with light stimuli). All subjects had training sessions in both operant chambers, in a random order.

Once stable rates of responding (less than 15% variation across three consecutive tests) were established (6–10 days), rats were randomly divided into experimental groups and the second daily training session was introduced. During these sessions the current intensities (set for the first and third 4-min periods) were decreased so that the response rates were 7–15 responses per minute (20–25% of the rates during the T^+ sessions) and stimulus lights above the 'rewarding' lever were on irrespective of the responding on the lever (session ST^-, subthreshold stimulation without light stimuli). A randomly alternating sequence was designed to provide two daily training sessions (T^+ and ST^-) for 10 consecutive days (days 1–10 of the experiment). There was an interval of at least 2 h between daily sessions. Performance during the ST^- session on day 10 served as a baseline for the subsequent analyses.

On day 11 of the experiment, a single test session was held. For the experimental groups, current intensity was set as for the ST^- sessions but the stimulus lights were as for the T^+ sessions (ST^+ session). Control group ($n = 7$) had a ST^- instead of a ST^+ session. Thirty minutes prior to the test session, rats from the control group were injected with saline while rats from the experimental groups were injected with (±)-CPP (5, 10, 15 mg/kg), CGS 19755 (1, 3 mg/kg) or saline.

During the next 5 days rats from experimental groups underwent one T^+ session per day. Performance during the T^+ session on day 15 served as a baseline for the subsequent analyses. Thirty minutes prior to the last T^+ session (day 16), these rats were treated with (±)-CPP, CGS 19755, or saline as described above.

There were six experimental groups treated as follows: (1) saline ($n = 8$); (2) (±)-CPP (5 mg/kg, $n = 7$); (3)

(±)-CPP (10 mg/kg, $n = 7$); (4) (±)-CPP (15 mg/kg, $n = 6$); (5) CGS 19755 (1 mg/kg, $n = 5$); (6) CGS 19755 (3 mg/kg, $n = 7$).

2.5. Statistics

Total numbers of responses on the 'rewarding' lever during the last 3 min of the first and third test periods were summed for each subject. The rate of lever-pressing during the first minute of either the first ('warm-up') or the third (first minute that followed the second 4-min period with no rewarding stimulation) period was much lower than during each of the remaining 3 min. Because of this difference, data for the first minute were excluded from further analysis.

Data were subjected to two-way analysis of variance (ANOVA) with repeated measures on one factor (Session). Factors were Session (baseline vs. test) and Treatment (saline and various doses of NMDA receptor antagonists). Analysis was performed using the General Linear Models (GLM) procedure of SAS-STAT software (version 6.11, SAS Institute, Cary, NC, USA). Group comparisons were performed using a post-hoc Duncan's multiple range test (only where ANOVA revealed significant effects).

2.6. Histology

After completion of the experiments the rats were decapitated, their brains were quickly removed and stored in 4% formalin. The stimulation site at the end of the electrode tract was examined under a microscope in cresyl violet-stained sections of 50-μm thickness. The slices were analyzed by an independent trained observer who was blind to the treatment conditions. Data for six rats with electrode tip placements outside the ventral tegmental area were excluded from the statistical analysis.

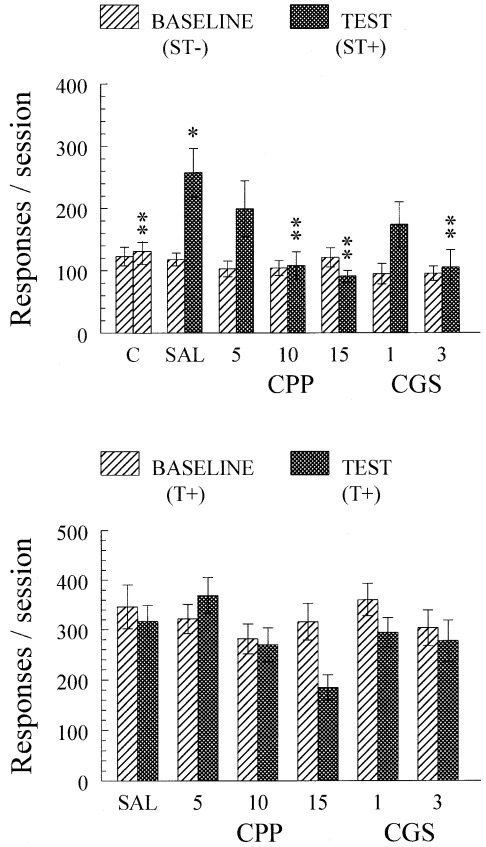

Fig. 1. Effects of (±)-CPP (5–15 mg/kg), CGS 19755 (1–3 mg/kg) and saline ('SAL') on rates of responding for subthreshold (upper panel, ST^- and ST^+ sessions) and threshold (lower panel, T^+ sessions) ventral tegmental area stimulation. Baseline recordings were made in a drug-free state. T^+ session, threshold stimulation paired with visual stimuli; ST^+, subthreshold stimulation accompanied by visual stimuli paired previously with threshold stimulation; ST^-, subthreshold stimulation with no visual stimuli. Data points above 'C' represent responding in control group exposed to two consecutive daily ST^- sessions. * $P < 0.05$, compared to the performance in the 'C' group during the second ST^- session, ** $P < 0.05$, compared to the 'SAL' group's performance during the ST^+ session (Duncan's test). $n = 6$ ($n = 7$ for 'C' and 'SAL' groups, $n = 5$ for (±)-CPP (5 mg/kg), $n = 4$ for CGS 19755 (1 mg/kg)).

3. Results

Analysis of the behavior of rats, performing under the conditions of subthreshold stimulation (ST^- and ST^+ sessions, Fig. 1, upper panel), revealed that both Session (baseline vs. test) and Treatment (doses of NMDA receptor antagonists) were significant determinants of the lever-pressing rate ($F(1,41) = 9.93$, $P = 0.0033$, $F(6,41) = 5.13$, $P = 0.0007$, respectively). As displayed in Fig. 1 (upper panel), introduction of the ST^+ sessions (day 11) for the saline-treated experimental group ('SAL' group) resulted in response rates that were significantly higher than baseline rates (Duncan's test, $P < 0.05$). This increase was observed in 6 out of 7 rats from the saline-treated experimental group. Response rates in these subjects ranged from 163% to 210% of the level of responding in the preceding baseline ST^- session (Fig. 1, upper panel). Post-hoc comparisons revealed that: (1) the response rate in the experimental group treated with saline was significantly higher than in saline-treated controls, and (2) the increased response rates of experimental groups exposed to ST^+ conditions were not observed in groups pretreated with 10 and 15 mg/kg of (±)-CPP or 3 mg/kg of CGS 19755 (Fig. 1, upper panel).

The experiment conducted on day 16 under T^+ conditions (Fig. 1, lower panel) revealed that at the doses used in the present study, neither (±)-CPP nor CGS 19755 significantly decreased responding on the 'rewarding' lever ($F(5,33) = 2.21$, $P = 0.081$).

Responding on the 'rewarding' lever during the second test period (current intensity set at zero level) was very low throughout the study (mean ± S.E.M. = 2.1 ± 1.8 per minute) and did not significantly differ between groups or across days of experiment. Also, there were no significant differences between overall response rates during the last 3 min of the first and third periods.

4. Discussion

In this study the NMDA receptor antagonists (±)-CPP and CGS 19755 prevented the expression of conditioned activation of intracranial self-stimulation behavior. This effect was demonstrated to be dose dependent for both drugs and was observed at dose levels that had little or no effect on response rates by themselves.

There is experimental evidence for the conditioned nature of the visual stimuli-induced facilitation of self-stimulation behavior that was reported here, as this effect was shown to extinguish with repeated presentations of conditioned stimuli and can be reinstated after additional pairings between unconditioned and conditioned stimuli (Bespalov and Zvartau, 1996a). Since classical conditioning involves associative learning, it should be dependent on memory-related processes of neural plasticity. NMDA receptors have been implicated in mechanisms underlying neural plasticity (Danysz et al., 1995). Therefore, it is tempting to speculate that the memory-disrupting properties of NMDA receptor antagonists are responsible for the effects seen in the present experiments.

However, it is possible that (±)-CPP and CGS 19755 prevented conditioned facilitation of self-stimulation responding by interfering with the processing of exteroceptive stimuli. For instance, non-competitive antagonists of NMDA receptors impair attention to exteroceptive stimuli in the modified open field (with a stimulus object) (Dai and Carey, 1994) and prepulse inhibition in acoustic startle paradigms (Mansbach, 1991; Wedzony et al., 1994). However, systemic administration of competitive antagonists of NMDA receptors failed to disrupt prepulse inhibition (Mansbach, 1991; Wedzony et al., 1994), suggesting differential involvement of various classes of NMDA receptor antagonists in sensorimotor gating of acoustic startle and, perhaps, in processing of relevant sensory information.

Since the procedure used in these experiments does not discriminate between reward and performance functions, it is possible that as a result of repeated pairings, visual stimuli could acquire both rewarding and motor-stimulating properties. Therefore, we cannot speculate any further on the mechanisms of the effects of NMDA receptor antagonists observed in our study. The only conclusion that can be drawn from the present results is that NMDA receptor antagonists prevent facilitation of intracranial self-stimulation responding induced by visual stimuli previously associated with rewarding stimulation of the ventral tegmental area.

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