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Research report

Microinjection of CART peptide 55–102 into the nucleus accumbens core inhibits the expression of conditioned hyperactivity in a cocaine-associated environment

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ABSTRACT

A distinct environment associated with psychomotor stimulants like cocaine and amphetamine can elicit conditioned locomotion in rats. This study examined the contribution of CART 55–102 peptide in the NAcc core to the expression of conditioned locomotion in a cocaine-associated environment. Rats in different groups were administered injections in five 2-day blocks: Paired, cocaine (15 mg/kg, IP) in locomotor activity boxes on day 1 and saline in their home cages on day 2; Unpaired, saline in the activity boxes on day 1 and cocaine in their home cages on day 2; or Control, saline in both environments. One week after the last conditioning block, all rats were tested for their conditioned locomotor response in the activity boxes for 1 h following an IP saline injection, which was preceded by a bilateral microinjection into the NAcc core of saline or CART 55–102 (1.0 and 2.5 μg/side). As expected, Paired rats showed both increased locomotor activity and rearing compared to rats in either the Unpaired or Control groups. However, the expression of this conditioned hyper-locomotion was inhibited by microinjection into the NAcc core of CART 55–102. These results suggest that CART 55–102 peptide in the NAcc plays a role in the expression of conditioned locomotion in an environment associated with cocaine, and further extends the notion that CART 55–102 plays an important regulatory role in psychomotor stimulants actions in the NAcc.

1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) is an endogenous neuropeptide which has various physiological functions including a role in feeding, stress, and drug addiction [29]. It is widely and abundantly expressed in the brain including the nucleus accumbens (NAcc) [7,28,37], a region importantly mediating locomotor activating and rewarding/reinforcing effects of drugs of abuse [4,36,38]. It has been previously shown in the rat that microinjections into the NAcc of CART 55–102 peptide, a biologically active fragment, significantly attenuated the locomotor effects of acute amphetamine [25] and cocaine [21]. Further, it has been recently shown that CART 55–102 in the NAcc also blocks the expression of behavioral sensitization by amphetamine and cocaine [26,44] and reduces cocaine self-administration in rats [23], suggesting its role as a homeostatic negative modulator in the site of psychomotor stimulant action [19,22,44].

Pavlovian conditioning has been well described with the effects of various drugs of abuse [11] and suggested to contribute to craving and promote relapse in drug addicts [6,32]. Similarly in rats, numerous studies have reported that a distinct environment when exclusively associated with drugs of abuse evokes conditioned behavioral effects including hyper-locomotion in the absence of these drugs [2,10,13,41]. Evidence also indicates that the expression of conditioned responses to stimuli associated with psychomotor stimulants is mediated in the NAcc. For example, dopamine overflow and activation of Fos-related proteins have been reported to increase in the NAcc by psychomotor stimulant associated cues [12,14], while activation of GABA neurotransmission or 6-hydroxydopamine lesions in this site disrupts conditioned locomotion induced by psychomotor stimulant [13,16]. Several lines of evidence reflecting the anatomically and functionally heterogeneous organization of the NAcc [45] further indicate that the core, but not the shell, of this nucleus is more likely to be involved in the expression of conditioned behavioral responses to the stimuli associated not only with psychomotor stimulant [15,17,33,40] but also more recently with other types of reinforcements [5,43].

Although the NAcc shows a dense expression for CART peptide [28,37], the role of the NAcc CART in the expression of conditioned locomotion in the presence of psychomotor stimulant associated stimuli has not yet been explored. Interestingly, only a very few studies so far have implicated CART peptides in the conditioned behavior such as place preference and taste aversion [1,27]. More recently, it has also been shown that the stimuli linked to ethanol availability activate hypothalamic CART expressing neurons [8]. These results suggest that CART peptides are involved in condi-

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tioned behaviors but probably with differential role depending on the site. Thus, we examined in the present experiments the role this peptide plays in the NAcc core in the expression of conditioned locomotion elicited in a cocaine-associated environment.

2. Methods

2.1. Animals and surgery

Male Sprague–Dawley rats weighing 220–250 g on arrival were obtained from Orient Bio Inc. (Seongnam-si, Korea). They were housed three per cage in a 12-h light/dark cycle room (lights out at 8:00 pm) and all experiments were conducted during the daytime. Rats had access to food and water *ad libitum* at all times. All animal use procedures were conducted according to an approved Institutional Animal Care and Use Committee protocol. Rats were anesthetized with IP ketamine (100 mg/kg) and xylazine (6 mg/kg), placed in a stereotaxic instrument with the incisor bar at 5.0 mm above the interaural line and implanted with chronic bilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA) aimed at the NAcc core (A/P, +3.4; L, ±1.5; D/V, −7.5; mm from bregma and skull) [35]. Cannulas were angled at 10° to the vertical, positioned 1 mm above the final injection site, and secured with dental acrylic cement anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge obturators were placed in the guide cannulas, and rats were returned to their home cages for a 7-day recovery period.

2.2. Drugs and intracranial microinjections

Cocaine hydrochloride (Belgopia, Belgium) and rat CART peptide 55–102 (American Peptide, Sunnyvale, CA) were dissolved in sterile 0.9% saline. Bilateral intracranial microinjections into the NAcc were made in the freely moving rat. Injection cannulas (28 gauge) connected to 1 μl syringes (Hamilton, Reno, NV) via PE-20 tubing were inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μl per side over 30 s. After 1 min, the injection cannulas were withdrawn and the obturators were replaced.

2.3. Locomotor activity

Locomotion and rearing were measured with a bank of six activity boxes (35 cm × 25 cm × 40 cm) (IWOO Scientific Corporation, Seoul, Korea) made of translucent Plexiglas. Each box was individually housed in a PVC plastic sound attenuating cubicle. The floor of each box consisted of 21 stainless steel rods (5 mm diameter) spaced 1.2 cm apart center-to-center. Two infrared light photo beams (Med Associates, St. Albans, VT, USA) positioned 4.5 cm above the floor and spaced evenly along the longitudinal axis of the box estimated horizontal locomotion. Two additional photo beams, positioned on the sidewall 14.5 cm above the floor and 7.5 cm from the front and back walls, estimated rearing.

2.4. Design and procedure

Rats were randomly assigned to three groups: Paired, Unpaired and Control. During conditioning, rats in these three groups were administered injections in five 2-day blocks. Rats received cocaine (15 mg/kg, IP) in locomotor activity boxes on day 1 and saline in their home cages on day 2 (Paired), saline in the activity boxes on day 1 and cocaine in their home cages on day 2 (Unpaired), or saline in both environments (Control). On the test, one week after the last conditioning block, all rats were tested for their conditioned locomotor response in the activity boxes for 1 h following an IP saline injection preceded 1 min earlier by a bilateral microinjection into the NAcc of saline or CART 55–102 (1.0 and 2.5 μg/side). Microinjection was performed only once at this time.

The doses of CART 55–102 were chosen based on our previous findings [25,44] showing that they produced no effects on basal locomotor activity when microinjected into the NAcc.

After completion of the behavioral experiments, rats were anesthetized and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and further post-fixed in 10% formalin. Coronal sections (40 μm) were subsequently stained with cresyl violet for verification of cannula tip placements. Only rats with injection cannula tips located bilaterally in the NAcc core were included in the data analyses. Of the 65 rats surgically prepared for testing, 10 (1 Control, 5 Unpaired and 4 Paired) were dropped for failing to meet this criterion. The resulting *n*/group was 19 (Control), 14 (Unpaired) and 22 (Paired).

2.5. Statistical analyses

The data were analyzed with two-way ANOVA (analysis of variance) followed by post hoc Tukey comparisons. Differences between experimental conditions were considered statistically significant when $p < 0.05$.

Table 1
Locomotor activity counts on day 1 of each block during conditioning.

Group	Horizontal locomotion		Rearing	
	Block 1	Block 5	Block 1	Block 5
Paired (22)	592 ± 56***	908 ± 77***,†††	287 ± 40***	329 ± 50***
Unpaired (14)	179 ± 24	123 ± 17	120 ± 21	91 ± 17
Control (19)	171 ± 14	121 ± 13	110 ± 8	74 ± 10

Rats were administered cocaine (15 mg/kg, IP) in locomotor boxes on day 1 and saline in their home cages on day 2 (Paired), saline in the activity boxes on day 1 and cocaine in their home cages on day 2 (Unpaired), or saline in both environments. This procedure was repeated five times. Data are shown as group mean (±S.E.M.) 1-h total locomotor activity counts obtained on day 1 of each block during conditioning.
*** $p < 0.001$, significant differences compared to both Control and Unpaired.
††† $p < 0.001$, significant increase at block 5 compared to block 1 in Paired rats; revealed by post hoc Tukey tests following two-way ANOVA. Numbers in parentheses indicate *n*/group.

3. Results

Table 1 shows the locomotor activity counts obtained on day 1 of the first and the fifth conditioning blocks in response to IP injections of either cocaine (Paired) or saline (Unpaired and Control). Two-way between–within ANOVA with groups as the between and blocks as the within factors revealed significant effects of groups for both horizontal locomotion and rearing [$F_{2,52} = 110.58$, $p < 0.001$, and 29.45, $p < 0.001$, respectively]. It also revealed significant effect of groups × blocks interactions [$F_{2,52} = 10.35$, $p < 0.001$] for horizontal locomotion. Only Paired rats showed a significant increase in horizontal locomotion on the fifth compared to the first block of conditioning ($p < 0.001$; as revealed by post hoc Tukey comparisons).

One week after the last conditioning block, all rats were tested for their conditioned locomotor response in the activity boxes for 1 h (Fig. 1). The two-way between–within ANOVA with groups as the between and doses as the within factors conducted on the 1-h total locomotor activity counts revealed multiple significant effects of groups [$F_{2,46} = 31.26$, $p < 0.001$] and groups × doses interactions [$F_{4,92} = 3.81$, $p < 0.01$] for horizontal locomotion and significant effects of groups [$F_{2,46} = 13.12$, $p < 0.001$] for rearing, respectively. As expected, in the NAcc saline condition, Paired rats showed increased locomotor activity (horizontal and rearing) compared to rats in either Unpaired or Control groups ($p < 0.001$; post hoc Tukey comparisons). These effects, however, were either diminished (horizontal) or lost (rearing) in Paired rats with the NAcc CART 55–102 microinjection. Post hoc Tukey comparisons revealed that CART 55–102 significantly inhibits the expression of conditioned hyper-locomotion in Paired rats ($p < 0.001$–0.05), while it has no effects in either Unpaired or Control groups. Time-course analyses of these findings showed that the conditioned horizontal locomotion and rearing observed in Paired rats persisted for 40 min of 1-h testing and that the ability of NAcc CART 55–102 to inhibit these effects was apparent throughout this time course (Fig. 1, insets). The location of the injection cannula tips in the NAcc core of the rats that were included in these time-course analyses is illustrated in Fig. 2.

4. Discussion

The present results revealed that microinjection into the NAcc core of CART 55–102 inhibits the expression of the conditioned locomotor activity normally evoked by the presence of environmental stimuli associated with cocaine. This effect was not observed in either saline Control or cocaine injected but Unpaired group of rats. This is the first demonstration, to our knowledge, that CART 55–102 in the NAcc plays a role in the expression of conditioned locomotion.