The role of CART peptide in the nucleus accumbens in psychostimulant-induced behavioral sensitization and conditioning

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The role of CART peptide in the nucleus accumbens in psychostimulant-induced behavioral sensitization and conditioning

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ABSTRACT

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Repeated administration of cocaine or amphetamine (AMPH) leads to the development of behavioral sensitization and the nucleus accumbens (NAcc) is known as the neuronal substrate mediating the expression of this behavioral phenomenon. Cocaine and AMPH-regulated transcript (CART) peptide is densely expressed in the NAcc and the recent studies report that microinjections of CART peptide 55-102 (active fragment) into this region significantly attenuated the locomotor effect of acute cocaine and AMPH in the rat. In the present thesis, the role of CART peptide 55-102 was explored in the expression of behavioral sensitization induced by chronic psychomotor stimulants. In the sensitization experiment, rats were divided into four groups: one for saline and the other three for cocaine pre-exposures (15 mg/kg, IP, once daily for 7 days). After 3 weeks of withdrawal, rats were microinjected into the NAcc either saline or CART 55-102 (1.0, or 2.5 μg/0.5 μl/side) followed by cocaine challenge (10 mg/kg, IP). Microinjection into the NAcc of CART peptide 55-102 dose-dependently blocked the expression of chronic cocaine-induced behavioral sensitization. In addition to cocaine, similar results were also obtained in the behavioral sensitization experiment with AMPH.

To investigate which signaling molecules are involved in this effect, the extracellular signal regulated-kinase 1/2 (ERK 1/2) phosphorylation levels, which is known to involve in acute and long-term adaptive processes by drugs of abuse, were further examined after microinjection of CART 55-102 in the NAcc. Additional four groups of rats were either saline or cocaine challenged systemically following microinjection into the NAcc of either saline, CART 55-102 (2.5 μg/0.5 μl/side), or the equivalent mole amount of inactive CART 1-27. The increase of ERK 1/2 phosphorylation levels in the NAcc by cocaine was completely blocked by CART 55-102 microinjection in this site, while it remains unaffected by inactive CART peptide 1-27. These results suggest that CART peptide 55-102 in the NAcc may play a compensatory inhibitory role in the expression of behavioral sensitization by cocaine and these effects may be mediated by its inhibition of ERK 1/2 phosphorylation in this site.

The drug-associated environment, on the other hand, also contributes to elicit craving for drugs and promotes relapse among drug addicts. To understand the role of CART peptide 55-102 in this conditioning effect induced by chronic cocaine, 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 of saline or CART 55-102 (1.0 and 2.5 μg/side). As expected, “Paired” rats showed increase of both 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 of CART peptide 55-102. These results suggest that CART peptide 55-102 in the NAcc plays a significant role in the expression of conditioned locomotion in an environment associated with cocaine, and further extends the notion that CART peptide plays an important regulatory role in

psychostimulant actions in the NAcc.

Key words: CART peptide, cocaine, sensitization, nucleus accumbens, ERK 1/2, conditioning

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1. INTRODUCTION

Drug abuse is defined as a chronic, relapsing brain disease that is characterized by compulsive drug seeking and use[1]. Repetitive use of psychostimulant drugs such as cocaine and amphetamine (AMPH) produces long-term changes in brain and consequently results in long-lasting augmentations of the behavioral and neurochemical responses to these drugs[2]. These enhanced responses are manifested as the behavioral sensitization and it has been proposed as a model for understanding the escalation of drug use that is characteristic of the transition from casual experimentation with drugs to compulsive drug seeking and craving[2-4]. The behavioral sensitization produced by chronic psychostimulant drugs is further strengthened by the association of drug themselves with environmental cues, such as people, sights, and situations[5,6] and it has been well described as a form of Pavlovian conditioning. Such environmental cues significantly contribute to triggering relapse in the abstinent abuser after finishing the rehabilitation medical program[7], that it is needed to understand collectively not only direct pharmacological effects but also conditioning effects of drugs produced by drug-associated environments on the brain[8]. Similar to humans, numerous studies have reported that a distinct environment when exclusively associated with drugs of abuse evokes the hyper-locomotion in the absence of these drugs in rats[9-11]. Several

lines of evidence indicate that the nucleus accumbens (NAcc) is one of the critical sites that mediates locomotor activating and rewarding/reinforcing effects of drug of abuse[12-14]. The NAcc, which receives dopaminergic projections from the ventral tegmental area (VTA), is known as the neuronal substrate mediating the expression of behavioral sensitization and conditioned locomotion induced by psychostimulants[9,15-19].

Cocaine- and AMPH-regulated transcript (CART) peptide is widely and abundantly expressed in the NAcc[12] and colocalize with gamma aminobutyric acid in medium spiny output neurons and receive a dopamine (DA) input in this region[13]. In 1995, Douglass et al[20] demonstrated first that a transcript was increased in the striatum after acute cocaine or AMPH and it was termed cocaine- and AMPH-regulated transcript (CART). This peptide is an endogenous neuropeptide which has various physiological functions including the control of feeding, response to stress, and the rewarding/reinforcing effects of psychomotor stimulants[21]. In the rat, CART peptides are differentially processed in a tissue-specific manner and among them, CART 55-102 peptide is known as a biologically active fragment in regulating numeral physiological processes[22-26]. Among its wide distributions in the brain, CART mRNA and peptides are densely expressed in the NAcc[12-14]. Although the NAcc shows a dense expression for CART peptide[13] and the peptide name deeply implicates the interaction with drug addiction[20], the role of this peptide in drug addiction has not been explored well. Interestingly, it was shown in the rat that microinjection of CART 55-102 peptide into the NAcc significantly attenuated the increase of locomotion by acute AMPH and cocaine, while this peptide alone in this site had no effect on locomotor activity[25,26]. Recently, it has been also shown that microinjection of CART 55-102 into the VTA reduced the locomotor activating effects of systemic cocaine[27]. These results suggest that CART 55-102 may play a negative regulatory role to psychostimulant drugs. However, the effect of this peptide on the psychomotor stimulants-induced locomotor activity after chronic exposure to them has not been explored yet. Thus, it was examined in the present experiments whether this peptide plays a role in the NAcc in the expression of

cocaine or AMPH-induced behavioral sensitization.

There has been a very few studies so far which explore the role of CART peptides in the conditioned behavior such as place preference and taste aversion[24,28]. It has been also shown that the stimuli linked to ethanol availability activate hypothalamic CART expressing neurons[29]. Very recently, microinjection of CART peptide 55-102 into the basolateral amygdala has been shown to block the conditioned place preference induced by AMPH[30]. Taken together, these results suggest that CART 55-102 may have an important role in conditioned behaviors induced by psychostimulants. Thus, it was also examined in the present experiments whether this peptide plays a role in the NAcc in the expression of conditioned locomotion elicited by a cocaine-associated environment.

Extracellular signal-regulated kinase (ERK), a downstream substrate of a mitogen activated protein kinase kinase (MEK), is a protein serine/threonine kinase that plays an important role in various forms of neuronal plasticity[31]. Evidence indicates that ERK pathway importantly involves in both acute and long-term adaptive processes by drugs of abuse[32,33]. For example, ERK1/2 activation is induced by acute cocaine in the NAcc[33], while chronic administration of cocaine or morphine increases its activity in the VTA[32]. Further, recent research shows that the development of behavioral sensitization to cocaine is prevented by systemic pre-treatment of a MEK inhibitor prior to each drug administration[34,35], while its expression is inhibited by blockade of ERK 1/2 phosphorylation directly in the NAcc[36]. Although CART receptors still remain to be identified, experimental evidence indicates that they are G-protein coupled and modulate intra-cellular signaling pathways[37]. Interestingly, it has recently shown that CART 55-102 also activates ERK pathway in primary cultured cortical neurons as well as in a pituitary-derived cell line[38,39]. However, any direct evidence for the effect of CART 55-102 on the regulation of ERK level in the brain tissue including the NAcc has not been obtained yet. Therefore, it was determined whether CART peptide 55-102 in the NAcc has any role in modulation of ERK pathway in the scheme of the expression of cocaine-induced behavioral sensitization.

II. MATERIALS AND METHODS

1. Subjects and surgery

Male Sprague-Dawley rats weighing 220-260 g on arrival were obtained from Samtako (Osan, Korea; for behavioral sensitization experiment) and Orient Bio Inc. (Seongnam-si, Korea; for behavioral conditioning experiment). They were housed three per cage in a 12-h light/dark cycle room with food and water available at all times and all experiments were conducted during the daytime. During the surgical operation, rats were anesthetized with ketamine (100 mg/kg, IP) followed by xylazine (6 mg/kg, IP), 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 (A/P, +3.4; L, ±1.5; D/V, -7.5 mm from bregma and skull)[40] Cannulas were angled at 10° to the vertical, positioned 1mm 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 allowing full recovery for 5 days at least. All procedures involving animals were conducted according to an approved IACUC protocol.

2. Drugs and intracranial microinjections

Cocaine hydrochloride (Belgopia, Belgium), D-amphetamine sulfate (United States Pharmacopeial Convention, Inc., Rockville, MD, USA), and both rat CART peptide 55-102 (American Peptide, Sunnyvale, CA) and CART 1-27 (Takara Biomedicals, Seoul, Korea) 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 1mm below the guide cannula tips. Injections were made in a volume of 0.5 μl per side over 30 s. After 1 min diffusion time, the

injection cannulas were withdrawn and the obturators were replaced. Intraperitoneal (IP) injections were followed and then rats were placed immediately in the activity boxes.

3. Locomotor activity

Locomotor activity was measured in a bank of 6 activity boxes (25 x 35 x 40 cm) (IWOO Scientific Corportation, Seoul, Korea) that were individually kept in larger PVC plastic sound attenuating cubicles. 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 each 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.

4. Western blotting

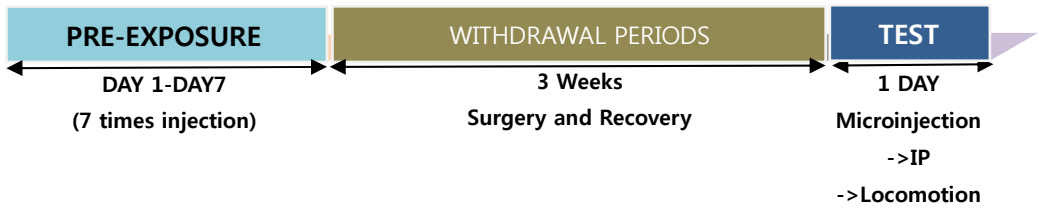
Rats were decapitated 15 min after systemic saline or cocaine injection, the brains were rapidly removed and coronal sections (1.0 mm thick) were obtained with an ice-cold brain slicer. Tissue punches (1.2 mm) were obtained in the NAcc region on an ice-cold plate, immediately frozen on dry ice and stored at -80 °C. Tissues were homogenized in lysis buffer containing 0.32 M sucrose, 2 mM EDTA, 1% SDS, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The concentration of protein was determined by using Pierce BCA protein assay kit (Pierce, Rockford, IL). Samples were then boiled for 10 min and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred electrophoretically to nitrocellulose membranes (Bio-Rad, Hercules, CA), which were then blocked with 5% skim milk in PBS-T buffer [10 mM phosphate-buffered saline plus 0.05% Tween-20]. Specific antibodies against phosphor-ERK 1/2 (1:1000 dilution in PBS-T with 5% bovine serum albumin; Cell Signaling, Beverly,

MA) and total ERK 1/2 (1:1000 dilution in PBS-T with 5% skim milk; Cell Signaling) were used to probe the blots. Primary antibodies were detected with peroxidase-conjugated secondary antibodies (1:2000 dilution in PBS-T with 5% skim milk; KOMA Biotech, Seoul, Korea) followed by enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Arlington Heights, IL) and exposure to X-ray film. Band intensities were quantified based on densitometric values using Fujifilm Science Lab 97 Image Gauge software (version 2.54). Antibodies on the membrane were removed by using Restore™ Western Blot Stripping Buffer (Pierce, Rockford, IL) and re-probed with anti-β-actin antibody (1:10,000 dilution in PBS-T with 5% skim milk; Abcam, Cambridge, UK).

5. Design and Procedures

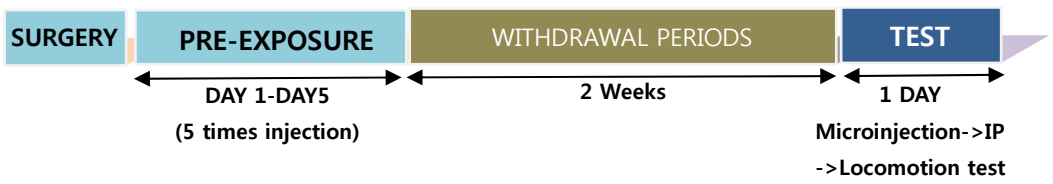
Experiment 1. The role of the NAcc CART peptide 55-102 in the chronic cocaine-induced behavioral sensitization

One week after arrival, two groups of rats were administered once daily with either saline or cocaine (15 mg/kg, IP) for 7 days. Locomotor activities after injection were measured in activity boxes only on Day 1 and 7 during this drug pre-exposure phase. On day 1 and 7, they were first habituated to activity boxes for 1 hour followed by pre-exposure injection, and then stayed in the boxes for an additional 1 hour. On days 2-6, pre-exposure injections were made at home cages. They were home cage-injected for the rest of days during this phase. This procedure is commonly used for the development of sensitization by cocaine[41]. After 3 weeks of drug-free withdrawal period, during which cannula implantations were made described as above, they were all cocaine (10 mg/kg, IP) challenged after microinjection of either saline (for both saline and cocaine pre-exposed groups) or one of two different doses of CART 55-102 (1.0 or 2.5 μg/0.5μl/side) (for cocaine pre-exposed groups). All rats were placed in the activity boxes for a 1-hr habituation period, administered their respective injections and immediately returned to the boxes and their locomotion and rearing measured for an additional 2 h.



Experiment 2. The role of the NAcc CART peptide 55-102 in the chronic AMPH-induced behavioral sensitization

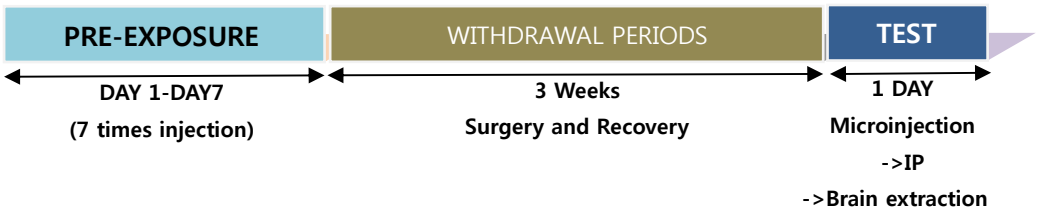
Rats with bilateral guide cannula implanted were pre-exposed 5 times to either saline or AMPH (1 mg/kg, IP) every 2 to 3 days. On day 1 and 5, were first habituated to activity boxes for 1 hour followed by pre-exposure injection, and then stayed in the boxes for an additional 1 hour. On days 2-4, pre-exposure injections were made at home cages. After 2 weeks of drug-free withdrawal, AMPH pre-exposed groups were randomly divided into three and they were microinjected either saline or one of two different doses of CART 55-102 (1.0 or 2.5 μg/side) followed by AMPH (1 mg/kg, IP) challenge, while saline pre-exposed group was tested with saline (microinjection)-AMPH (IP). On the test day, all rats were placed in the activity boxes for a 1-hr habituation period, administered their respective injections and immediately returned to the boxes and their locomotion and rearing measured for an additional 2 h.



Experiment 3. Modulation of the ERK phosphorylation levels by NAcc CART peptide 55-102 in the chronic cocaine-induced behavioral sensitization

Four groups of rats were all administered once daily with cocaine (15

mg/kg, IP) for 7 days. Locomotor activities after cocaine injection were measured only on Day 1 and 7, while they were home cage-injected for the rest of days. On day 1 and 7, they were first habituated to activity boxes for 1 hour followed by pre-exposure injection, and then stayed in the boxes for an additional 1 hour. After 3 weeks of withdrawal including surgery, they were challenged with saline (microinjection) + saline (IP), or one of either saline or CART 55-102 (2.5 μg/0.5 μl/side), CART 1-27 (1.5 μg/0.5 μl/side) microinjection + cocaine (10 mg/kg, IP). The dose of CART 1-27 is approximately equivalent to the mole amount of CART 55-102 used here. Their brains were, then, decapitated 15 min after IP injection for western experiments.



Additional rats were operated for cannular installment. After recovery from surgery, they were microinjected with either saline or one of doses of CART 55-102 (1.0 or 2.5 μg/0.5 μl/side) followed by saline IP injection with 1 min time interval. Their brains were, then, rapidly decapitated 15min after IP injection and coronal sections (1.0 mm thick) were obtained with an ice-cold brain slicer.

Experiment 4. The role of the CART peptide 55-102 in cocaine-induced conditioned locomotion

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/0.5 µl/side). Microinjection was performed only once at this time.

6. Histology

After completion of behavioral experiments, rats were anesthetized and perfused via intra-cardiac 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 were included in the data analysis.

7. Statistics

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 $p<0.05$.

III. RESULTS

Experiment 1. CART 55-102 peptide in the NAcc blocks the expression of behavioral sensitization induced by chronic cocaine

At first, it was examined in the present experiments whether CART peptide 55-102 plays a role in the NAcc in the expression of cocaine-induced behavioral sensitization. Table 1 shows the locomotor activity counts obtained during the pre-exposure day 1 and 7 to either saline or cocaine. As expected, rats exposed to cocaine showed a sensitized locomotor response on day 7 compared to day 1 ($p<0.001$). Further, when they were challenged with a systemic injection of cocaine after 3 weeks of withdrawal, cocaine compared to saline pre-exposed rats showed an enhanced locomotor response (Fig. 1). This effect, however, was dose-dependently blocked by microinjection of CART 55-102 into the NAcc. The ANOVA conducted on these data revealed that they have normal distribution and a significant effect of groups [$F(3,23) =4.32$, $p<0.02$]. Post-hoc Tukey comparisons revealed that CART 55-102 (2.5 μg) significantly decreased ($p<0.05$) the sensitized locomotor activity produced by cocaine for the first hour total of a 2 h test. Time-course analyses showed that the sensitized locomotor activating effects of cocaine persisted for approximately one hour of testing and the ability of the higher dose (2.5 μg) of CART 55-102 to inhibit these effects was apparent throughout this time-course (Fig. 1B). The ANOVA conducted on these data revealed significant effects of groups [$F(3.23)=3.04$, $p<0.05$], time [$F(5,115)] =90.98$, $p<0.001$] and a groups × time interaction [$F(15,115)=2.41$, $p<0.005$]. Post hoc Tukey comparisons revealed that CART 55-102 (2.5 μg) significantly decreased ($p<0.01$-0.001) the sensitized locomotor activity produced by cocaine at the 20 and 40 min time points compared to that observed in animals having received cocaine with saline microinjection. The location of injection cannula tips in the NAcc of rats that were included in this experiment is illustrated in Fig. 1C.

Table 1. Locomotor activity counts during pre-exposures for behavioral sensitization development.

Pre-exposure Group	Day 1	Day 7
Saline (6)	42 ± 12	59 ± 13
Cocaine (21)	289 ±39 ††	487 ± 49 †††,***

All rats were habituated for 1 hr and their locomotor activity measured for an additional 1 hr following their respective injections. Only at day1 and 7, locomotor activity was measured during the pre-exposure injections (once daily total 7 injections). †† $p<0.01$, ††† $p<0.001$, significant differences in cocaine compared to saline pre-exposed animals. *** $p<0.001$, significant difference at day 7 compared to day 1 as revealed by post hoc Tukey comparisons following ANOVA. Numbers in parentheses indicate n/group.

Figure 1

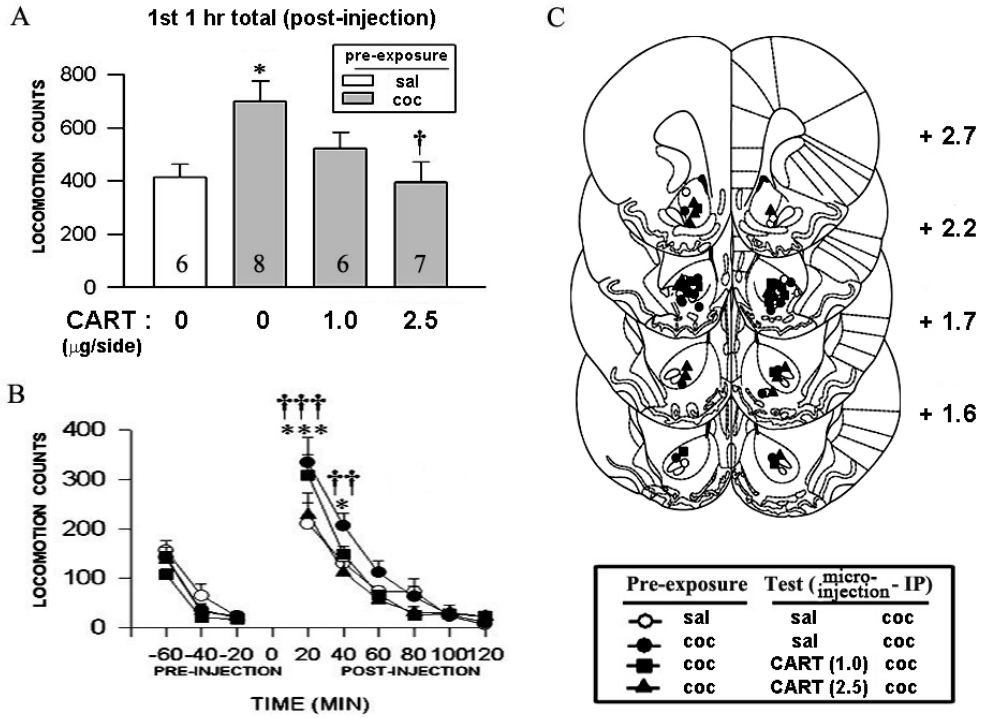

A
1st 1 hr total (post-injection)
pre-exposure
sal
coc
LOCOMOTION COUNTS
800
600
400
200
0
6
8
6
7
CART : 0 0 1.0 2.5
(μg/side)
B
LOCOMOTION COUNTS
400
300
200
100
0
-60 -40 -20 0 20 40 60 80 100 120
PRE-INJECTION
POST-INJECTION
TIME (MIN)
C
+ 2.7
+ 2.2
+ 1.7
+ 1.6
Pre-exposure
Test (micro-injection - IP)
sal sal coc
coc sal coc
coc CART (1.0) coc
coc CART (2.5) coc

Fig. 1. Microinjection into the NAcc of CART 55-102 inhibits the expression of locomotor sensitization by cocaine. All groups were cocaine (10 mg/kg, IP) challenged following different doses of CART 55-102 microinjections as indicated. A, data are shown as group mean (+SEM) locomotor activity counts for the first hour of a 2-h test. Numbers of rats in each group are indicated at the base of the different columns. Symbols indicate significant differences as revealed by post hoc Tukey comparisons following ANOVA. *p<0.05; significantly more counts in cocaine compared to saline pre-exposed animals with saline microinjection. †p<0.05; significant differences in cocaine pre-exposed animals with CART 55-102 (2.5 μg/side) compared to saline microinjection. B, time-course data are shown as group mean (+SEM) locomotor activity counts obtained during the 1 hr preceding (-60 through 0 min) and the 2hr following the CART 55-102 + cocaine injection (0-120 min). Cocaine injections following CART microinjection were administered at time 0. Rats microinjected with saline showed a significantly greater locomotor response to cocaine in cocaine compared to saline pre-exposed groups (*p<0.05, ***p<0.001), but this sensitized response was absent when CART 55-102 was microinjected with cocaine challenge (††p<0.01, †††p<0.001). C, location of the microinjection cannula tips in the NAcc of rats located bilaterally in this site were included[42]. Numbers to the right indicated millimeters from bregma. Symbols represent the different groups: ○, saline (pre-exposure) – saline (intra-NAcc) – saline (IP); ●, cocaine – saline – cocaine; ■, cocaine – CART 55-102 (1.0 μg/side) - cocaine; ▲, cocaine – CART 55-102 (2.5 μg/side) – cocaine.

Experiment 2. CART peptide 55-102 in the NAcc blocks the expression of behavioral sensitization induced by chronic AMPH

Fig. 2 shows the locomotion (A) and rearing (B) counts obtained in rats pre-exposed two weeks earlier to AMPH or saline and tested with a systemic AMPH challenge following NAcc infusion of either saline or CART peptide 55-102. As expect, rats pre-exposed to AMPH compared to saline showed sensitized locomotor activity responses when saline (NAcc)-AMPH (IP) challenged (p<0.05-0.01). These effects, however, were dose-dependently blocked by microinjection of CART 55-102 into the NAcc. The ANOVA conducted on these data revealed a significant effect of groups [F(3,19)=3.87 and 5.47, p<0.03-0.008] for locomotion and rearing, respectively. Post hoc Student-Newman-Keuls comparisons revealed that CART 55-102 (2.5 μg) significantly decreased (p<0.05) the sensitized locomotor activity produced by AMPH pre-exposure. Time-course analyses showed that the sensitized locomotor activating effects of AMPH persisted during the post-injection for approximately 1h of testing (statistical significances are appeared only at 20 and 40 min time bins) and the ability of the higher dose (2.5 μg) of CART 55-102 to inhibit these effects was apparent throughout this time-course. The ANOVA conducted on the post-injection data revealed multiple significant effects of groups [F(3,19)=3.87 and 4.94, p<0.03-0.001] for locomotion and rearing, respectively. Post hoc comparisons revealed that CART 55-102 (2.5 μg) significantly decreased (p<0.01-0.001) the sensitized locomotion and rearing produced by AMPH at the 20 and 40 min time points compared to that observed in animals having received AMPH with saline microinjection. The ANOVA conduced on the pre-injection data revealed no significant effects of groups [F(3.19)=1.08 and 0.58, p<0.4-0.7], but only of time [F(2.38)=103.07 and 73.42, p<0.001] for locomotion and rearing, respectively, indicating that animals were habituated to the level that the activity chamber no longer evokes any differences of locomotor activity by itself. The location of injection cannula tips in the NAcc of rats that were included in this experiment is illustrated in Fig. 2C.

Figure 2

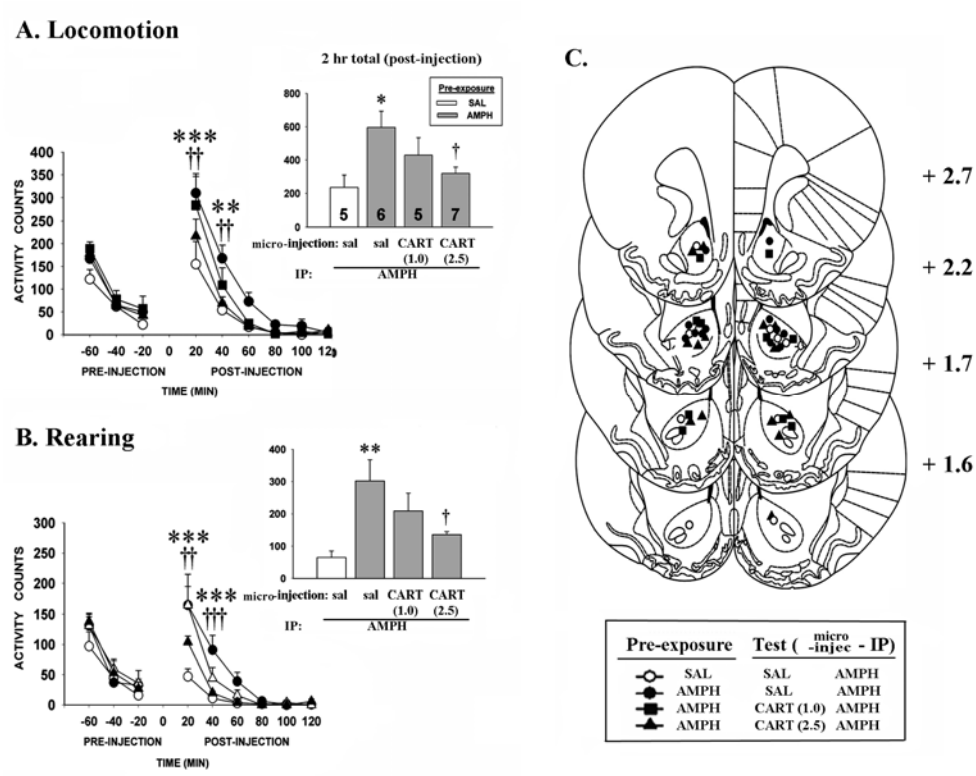

A. Locomotion
2 hr total (post-injection)
Pre-exposure
SAL
AMPH
micro-injection: sal sal CART (1.0) CART (2.5)
IP: AMPH
ACTIVITY COUNTS
PRE-INJECTION
POST-INJECTION
TIME (MIN)
B. Rearing
C.
+ 2.7
+ 2.2
+ 1.7
+ 1.6
Pre-exposure
Test (micro -injec - IP)
SAL SAL AMPH
AMPH SAL AMPH
AMPH CART (1.0) AMPH
AMPH CART (2.5) AMPH

Fig. 2. Microinjection into the NAcc of CART 55-102 inhibits the expression of behavioral sensitization by AMPH. Data are shown as group mean (+SEM) locomotion (A) and rearing (B) counts obtained after sensitization test injection. Insets show total counts obtained in 2 h of testing. Numbers of rats in each group are indicated at the base of the different columns. Time-course data are obtained during the 1 h preceding (−60 through 0 min) and the 2 h following the CART 55-102 (intra NAcc) + AMPH (IP) injection (0 to 120 min), which were administered at time 0. Symbols indicate significant differences as revealed by post-hoc Student–Newman–Keuls comparisons after ANOVA. *$p<0.05$, **$p<0.01$, ***$p<0.001$; AMPH compared with saline pre-exposed rats tested with saline (intra NAcc) – AMPH (IP). †$p<0.05$, ††$p<0.01$, †††$p<0.001$; CART 55-102 (2.5 µg, intra NAcc) – AMPH (IP) compared with saline (intra NAcc) – AMPH (IP). (C) Location of the microinjection cannula tips in the NAcc of rats included in the data analyses. Only rats with injection cannula tips located bilaterally in this site were included[42]. No neuronal damage was observed other than that produced by the insertion of the cannulas. Numbers to the right indicate millimeters from bregma. Symbols represent the different groups: ○, saline (pre-exposure) – saline (intra-NAcc) – saline (IP); ●, AMPH – saline – AMPH; ■, AMPH – CART 55-102 (1.0 µg/side) - AMPH; ▲, AMPH – CART 55-102 (2.5 µg/side) – AMPH.

Experiment 3. CART peptide inhibits cocaine-induced increse of pERK levels in the NAcc

Locomotor activities obtained after cocaine injection were following: 159±33 (Day 1) and 450±58 (Day7) [t-test for day 7 vs. day 1: ***p<0.001, n=14]. Subsequently, the NAcc tissues were punched out and analyzed for both phosphorylated and total ERK 1/2 levels. The 42- and 44 kDa forms of ERK (ERK1/2) were quantified together. A systemic challenge injection of cocaine following microinjection of saline increased the levels of ERK 1/2 phosphorylation in the NAcc about 1.4 times higher than those obtained by saline challenge. These effects, however, were completely blocked by microinjection of CART 55-102 (2.5 μg/side) into this site prior to the challenge injection of cocaine (Fig. 3A, B). The inactive CART 1-27 peptide saves the effect of cocaine on the increase of ERK1/2 phoshorylation levels in this site. The ANOVA conducted on these data showed that they have normal distribution and a significant effect of groups [F(3,10)=15.78, p<0.001]. Post hoc comparisons revealed that the expression levels were lower in CART 55-102 compared to saline microinjected rats (p<0.01). The levels of total ERK1/2 remained unchanged in each group. These effects were specific to the local site where drugs were microinjected because they were not observed in the striatum (data not shown). The region in the NAcc where tissues were punched out for protein analysis is depicted in Fig. 3C.

To investigate whether CART 55-102 peptide itself could change the level of ERK phosphorylation in the NAcc, it was also tested the level of ERK phosphorylation when microinjected CART 55-102 peptide into the NAcc. However, CART 55-102 peptide as compared to saline microinejction produced no changes on the levels of both phosphorylated and total ERK 1/2 in this site (Fig. 4).

Figure 3

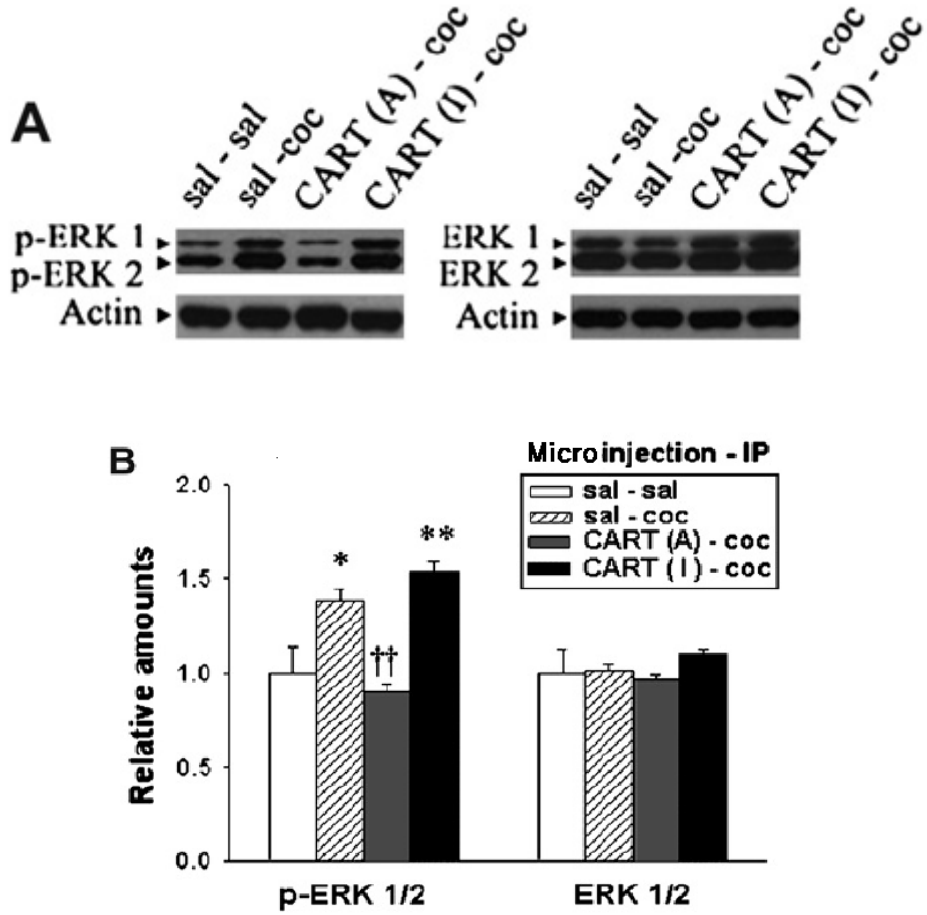

A
sal - sal
sal -coc
CART (A) - coc
CART (I) - coc
p-ERK 1
p-ERK 2
Actin
ERK 1
ERK 2
Actin
B
Microinjection - IP
sal - sal
sal - coc
CART (A) - coc
CART (I) - coc
Relative amounts
2.0
1.5
1.0
0.5
0.0
*
**
††
p-ERK 1/2
ERK 1/2
C

Fig. 3. Microinjection of CART 55-102 into the NAcc blocks the increase of cocaine-induced phosphorylation levels of ERK1/2 in this site. A, representative Western blots labeled with antibodies against phosphorylated and total ERK1/2, and β-actin. Groups indicate microinjection followed by IP injection [CART (A); active CART 55-102, CART (I); inactive 1-27] B, Values are normalized to actin and expressed as mean (+SEM) (n = 3-4 per group) transformed to relative amounts of control values (saline microinjection followed by IP saline as control). Symbols indicate significant differences in the levels of p-ERK1/2 in the NAcc [post-hoc Tukey after ANOVA, *p< 0.05, **p<0.01, saline - saline vs. either saline - cocaine or CART (I) - cocaine, respectively; ††p < 0.01, saline - cocaine vs. CART (A) – cocaine]. Total ERK1/2 levels showed no differences between groups. C, the NAcc region where tissues were punched out is shown (cross-hatched circles). Injection cannula tips were all located bilaterally in this region. Punches (1.2 mm diameter) were prepared bilaterally and pooled for each individual animal's protein isolation [the caudal surface of a coronal section (1.0 mm thick) extending 1.60 - 2.60 mm [42].

Figure 4

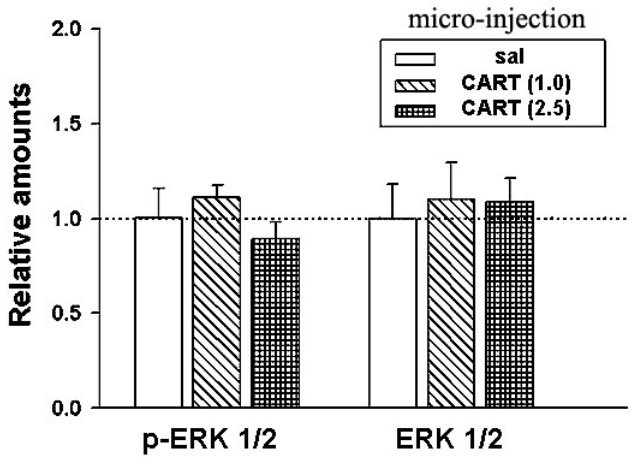


Fig. 4. Microinjection of CART 55-102 alone into the NAcc produces no change on the phosphorylation levels of ERK1/2 in this site. Groups indicate different doses (μg/side) of CART 55-102 microinjection followed by IP injection of saline. Values are normalized to β-actin and expressed as mean (+SEM) (n=3 per group) transformed to relative amounts of control values (saline microinjection followed by IP saline as control). One-way ANOVA showed no differences between groups on both phosphorylated and total ERK1/2 levels (p = 0.44 and 0.90, respectively).

Experiment 4. CART peptide 55-102 blocks the expression of conditioned hyperactivity in a cocaine-associated environment

Table 2 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 1h (Fig. 5). 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. 5A). The location of the injection cannula tips in the NAcc core of the rats that were included in these time-course analyses is illustrated Fig. 5B.

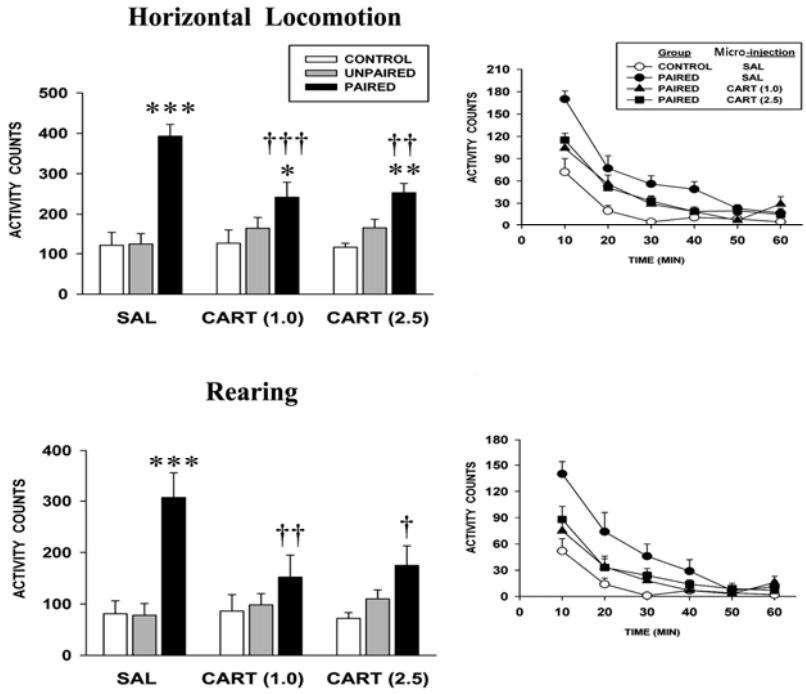
Table 2. Locomotor activity counts on day 1 of each block during conditioning development

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

Figure 5

A.


Horizontal Locomotion
CONTROL
UNPAIRED
PAIRED
ACTIVITY COUNTS
500
400
300
200
100
0
***
†††
*
††
**
SAL
CART (1.0)
CART (2.5)
Group
Micro-injection
CONTROL
SAL
PAIRED
SAL
PAIRED
CART (1.0)
PAIRED
CART (2.5)
210
180
150
120
90
60
30
0
0
10
20
30
40
50
60
TIME (MIN)
Rearing
400
300
200
100
0
***
††
†
SAL
CART (1.0)
CART (2.5)
180
150
120
90
60
30
0
TIME (MIN)

B.

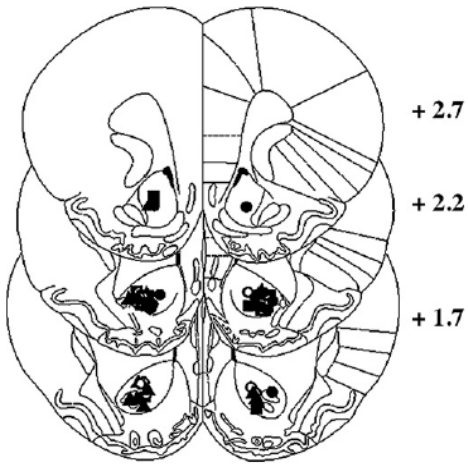

+ 2.7
+ 2.2
+ 1.7

Fig. 5. Microinjection into the NAcc of CART 55–102 inhibits the expression of conditioned hyper-locomotor activity. All rats were tested following an IP saline injection preceded 1-min earlier by NAcc saline or CART 55–102 (1.0 and 2.5 μg/side). The conditioned horizontal locomotion (A) and rearing (B) observed in Paired rats were inhibited by NAcc CART 55-102. Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1-h test. Numbers: Control – saline (8), Control – CART (1.0) (5), Control – CART (2.5) (6), Unpaired –saline (5), Unpaired – CART (1.0) (4), Unpaired – CART (2.5) (5), Paired – saline (8), Paired – CART (1.0) (8), Paired – CART (2.5) (6). Symbols indicate significant differences revealed by post hoc Tukey comparisons following two-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, significantly more counts in Paired rats relative to those in the Control and Unpaired groups. †p<0.05, ††p<0.01, †††p<0.001, significant differences between Paired rats when compared to those microinjected with saline. Time-course data are shown in the insets as group mean (+S.E.M.) locomotor activity counts at 10-min intervals. These are shown for only Paired rats and Control rats tested with NAcc saline for comparison. Location of the injection cannula tips in the NAcc core of the rats illustrated in the insets of Fig. 5. All rats included in the data analyses had injection cannula tips located bilaterally in the NAcc[42]. Numbers to the right indicate millimeters from bregma. Symbols represent different groups: ○, Control – saline; ●, Paired – saline; ▲, Paired – CART (1.0); ■ , Paired – CART (2.5).

IV. DISCUSSION

The present results revealed that microinjection into the NAcc of CART peptide 55-102 inhibited the expression of behavioral sensitization to psychomotor stimulants. The doses of CART peptide 55-102 used here for microinjection experiments have been shown to have no effect, previously on basal locomotor activity[25,26], and in the present experiments on basal levels of total and phosphorylated ERK1/2 in the NAcc [see Fig. 4]. CART peptide has been implicated in the reinforcing and rewarding effects of psychomotor stimulants[23,25,26,43] since its expression was first reported to increase in the NAcc after acute administration of cocaine or AMPH[20]. Besides the anatomical distribution of CART mRNA and peptides in the major brain regions associated with rewarding and reinforcing effects of drugs of abuse[14], a number of behavioral evidence strongly support its role in such effects[12,24-26]. The present results further expand our knowledge of CART peptide and support for its suggestive negative regulatory role in psychomotor stimulants actions in the NAcc.

Repeated pre-exposure to psychomotor stimulants leads to enhanced increase of locomotor response to and self-administration of these drugs in rats[4,44]. This expression of behavioral sensitization has been proposed to model the escalation of drug use and craving that is characteristic of addiction in humans[3]. The NAcc, which receives afferent inputs from dopamine (DA) neurons in the VTA, is known as the neuronal substrate mediating the expression of behavioral sensitization[19,45]. Following previous study showing that CART peptide 55-102 in the NAcc inhibits locomotor activating effects of acute psychomotor stimulants[25,26], the present findings are the first to show that microinjection into the NAcc of this peptide further inhibits the expression of sensitized locomotion produced by chronic psychomotor stimulants. The notion that CART peptide in the NAcc may modulate the effects of psychomotor stimulants by antagonizing rather than mimicking these effects, possibly via homeostatic action, has been previously proposed[25,26,43] based upon the results obtained from the CART effects on acute psychomotor stimulants

and the present results further extend this notion to the scope of behavioral sensitization. Interestingly, microinjection of CART peptide 55-102 into the VTA, a region known as the neuronal substrate for the induction of behavioral sensitization[19,45], has been recently shown to reduce the locomotor activating effects of systemic cocaine when injected together[27], while it increases spontaneous locomotor activity when injected alone[24]. These results add more support to the role of CART peptide as a negative regulator to psychomotor stimulant drugs. However, whether CART peptide 55-102 also shows a similar effect on the induction of behavioral sensitization has not been clarified yet, although it has been previously shown that repeated microinjection of CART peptide 55-102 into the VTA does not produce locomotor sensitization by itself and to a subsequent AMPH or cocaine challege[24]. CART peptide 55-102 in the VTA nonetheless thought to be reinforcing because the repeated injection of this peptide into this site produces conditioned place preference[24], so it can not be totally excluded yet the possibility that CART peptide in the VTA may have other types of modulatory role in mediating the motivational properties of psychomotor stimulants. All together, our present results put more weight on the notion that CART peptide 55-102 in the NAcc may act to oppose the actions of psychomotor stimulants in the expression of behavioral sensitization. It is interesting to know that CART peptide 85-102 has been recently shown to inhibit the expression of morphine-induced locomotor sensitization when administered into the ventricles in the mice [22]. However, because CART peptide 85-102 has not been found yet in natural physiological condition, it is not certain whether this effect actually exists in the brain. However, these results are nonetheless supportive to our present findings that an active fragment of CART peptide inhibits the expression of behavioral sensitization. Our results further indicate that the place in which CART peptide produces its effect is the NAcc, a region known as the neuronal substrate for the expression of sensitization.

The present finding, first time to my knowledge, shows that ERK1/2 activation by cocaine in the NAcc is inhibited by a direct microinjection of CART peptide 55-102 in this site and suggests the opposing effect of CART 55-102 to

cocaine on ERK1/2 activation. In this study, it was also shown that the cocaine-induced increase of ERK1/2 phosphorylation levels in the NAcc was not blocked by inactive CART peptide 1-27, while CART peptide 55-102 alone had no effect on basal levels of both phosphorylated and total ERK1/2 in the NAcc. These results suggest that the inhibitory effect of CART 55-102 in the NAcc on the expression of locomotor sensitization is possibly mediated by its role in the blockade effect on cocaine-induced increase of p-ERK1/2 activation in this site. Whether this blockade effect is mediated by direct interactions of CART peptide 55-102 with DA or glutamate in the NAcc is currently not known. However, evidence shows that CART peptide 55-102 blunted the increased locomotion produced by intra-accumbal dopamine just as it did for the cocaine-induced increase of locomotion[26]. It has been also found that CART expression in the rat NAcc is regulated by DA receptors[46], and these effects are potentiated by cocaine[47]. Considering these results, the opposing role of CART peptide 55-102 in the NAcc in both acute and sensitized locomotor activating effects of cocaine is plausibly mediated by deactivating DA neurotransmission subsequently resulting in the attenuation of p-ERK1/2 activation[47].

Because chronic cocaine induces the increased conditioned locomotor activity, the effect of CART peptide 55-102 on cocaine-induced conditioning was also examined in this study. As a result, intra-accumbal microinjection of CART 55-102 has been found to inhibit 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 “Control” or “Unpaired” groups of rats. The possible involvement of CART peptide in conditioned behavioral responses has been previously shown[24,28]. However, these studies observed different types of conditioned behaviors in different brain areas such as in the ventral tegmental area[24], or the fourth ventricle[28], respectively. Interestingly, our present results first time show that CART 55-102 microinjected into the NAcc inhibits the expression of the conditioned locomotor activity, suggesting that there may exist differential role of CART peptide in the particular type of conditioned behaviors in concert with

different neuronal substrates.

It is possible that similar neuronal substrates in the NAcc likely underlie the expression of both cocaine- and conditioned stimulus-induced locomotion, perhaps reflecting the impartment of cocaine's activating properties to these stimuli by the conditioning process. Interestingly, while only the higher dose (2.5 μg/side) inhibited both acute and sensitized locomotor activity by psychomotor stimulants, the expression of conditioned locomotion was significantly inhibited at the lower dose (1.0 μg/side) and these effects seemed to be saturated at the higher dose (2.5 μg/side) in this study. It is possible that the neuronal substrates in the NAcc previously responding to higher dose of CART 55-102 with the co-presence of cocaine now respond to lower dose of this peptide with the presence of only conditioned stimuli, reflecting relatively weaker signal of conditioned stimuli than cocaine itself.

In the NAcc, dopamine neurotransmission has long been implicated not only in the primary reinforcing and locomotor activating effects of psychomotor stimulants[48], but also in the expression of conditioned behavioral responses[49,50]. Considering the opposing role of the NAcc CART 55-102 in both cocaine- and conditioned stimuli-induced locomotor activity, it is then plausible to suggest that an endogenous negative feedback mechanism (mediated by CART peptide) to attenuate cocaine's effects is similarly activated during the conditioning process to reduce conditioned stimuli's locomotor activating effects. Whether dopamine signaling pathway possibly mediates this mechanism is currently not known. Interestingly, it has been shown that Fos protein expression is enhanced in the NAcc following exposure to a cocaine-associated environment[16,51], while the development of conditioned locomotion with NAcc amphetamine is blocked by co-injecting a protein kinase A inhibitor into this site[10]. More recently, it has also been shown that the stimuli linked to ethanol availability increase Fos-positive CART expressing neurons in the hypothalamus[29]. In hypothalamic synaptosomes, CART peptide 55-102 has been previously shown to dose-dependently inhibit depolarization-induced dopamine release to regulate feeding behavior[52]. Given the findings reviewed above,

it will be important to delineate the particular roles played by CART peptide and its interaction with dopamine signaling in the NAcc in the development and expression of conditioned behaviors.

V. CONCLUSION

In conclusion, the present findings confirm and extend the notion that CART peptide has a negative regulatory role, possibly via homeostatic action, in the expression of behavioral sensitization and conditioned locomotion induced by chronic psychomotor stimulants. It has been further shown that the homeostatic action of CART peptide may be mediated by the modulation of ERK1/2 activation in the NAcc. It will be interesting in the future to further examine how CART peptide in the NAcc interacts with DA signaling pathways resulting in the decrease of ERK1/2 activation in this site (fig. 6).

Figure 6

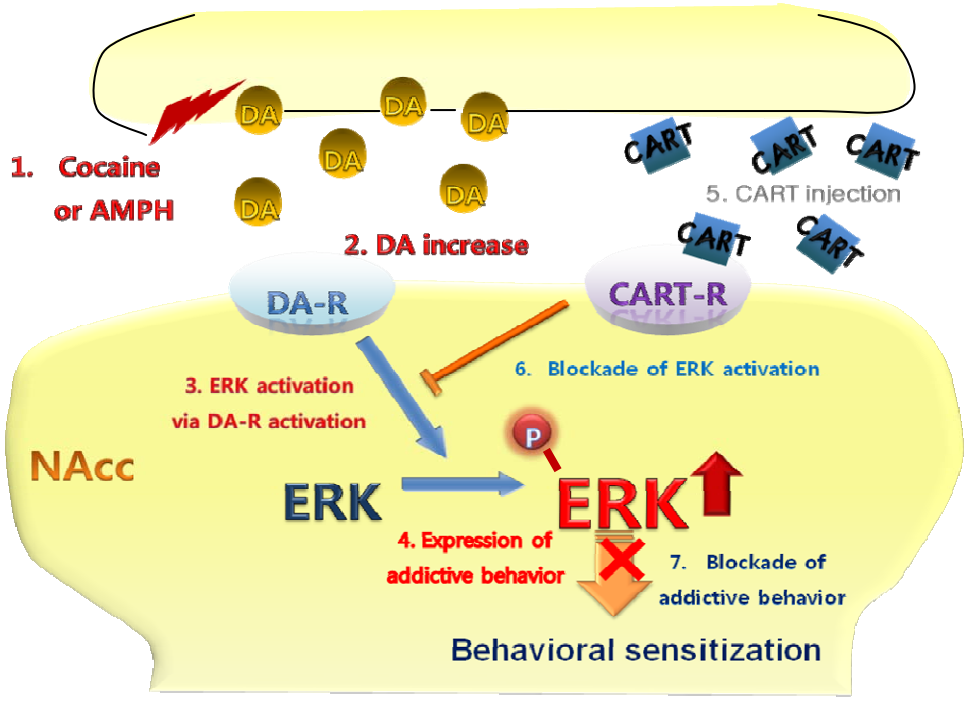


Fig. 6. A hypothetical diagram showing the negative regulatory role of CART peptide 55-102 in the NAcc when chronic psychomotor stimulants are present. Psychomotor stimulant drugs increase the dopamine release in the NAcc and subsequently induce the activation of ERK1/2, eventually leading to the behavioral effects in the animals. The CART peptide 55-102 blocks the behavioral effects of psychomotor stimulants plausibly by blockade of activation of ERK1/2 in the NAcc.

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ABSTRACT (in Korean)

정신자극제에 의한 행동과민반응과 조건화 반응에서 중격측좌핵 내 CART 단백질의 역할

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코카인이나 암페타민과 같은 정신자극제를 반복적으로 주입하면 행동과민반응이 일어나게 되는데, 중격측좌핵(nucleus accumbens)은 이러한 행동과민반응의 발현을 매개하는 곳으로 알려져 있다. CART (cocaine and amphetamine-regulated transcript) 단백질은 이 부위에서 매우 높은 농도로 발현되며, 중격측좌핵에 CART 55-102 (active fragment)를 국소 주입하면, 코카인이나 암페타민을 주었을 때 일어나는 정위행동 (locomotor activity)증가를 억제한다는 것이 보고된 바 있다. 본 연구에서는 중독의 동물모델로 널리 사용되는 행동과민반응에서 CART 55-102의 역할을 알아보고자 하였다. 먼저, 쥐를 4그룹으로 나누어, 한 그룹은 생리식염수를, 나머지 세 그룹은 코카인(15 mg/kg, IP)을 각각 7일간 주입한 다음, 3주간의 약물중지기간(withdrawal period)을 거치게 하였다. 그 후, 쥐에게 생리식염수 또는 CART 55-102 단백질을 중격측좌핵에 국소 주입 한 다음 코카인(10 mg/kg, IP)을 주입하고 행동을 관찰하였다. 그 결과, CART 55-102 단백질은 코카인에 의해서 유도된 행동과민반응을 농도의존적으로 저해함을 알 수 있었다. 이러한 저해 효과는 코카인에서뿐만 아니라, 코카인과 유사한 정신자극제인 암페타민에 의해 유도되는 행동과민반응에서도 동일하게 나타남을 확인하였다. 다음으로 이러한 CART 55-102의 저해 효과가 중격측좌핵 내에서 정신자극제에 의한 행동과민화의 발현에 중요하다고 알려진

extracellular signal regulated-kinase 1/2 (ERK 1/2)의 인산화를 조절함으로서 나타나는 것인지를 알아보고자 하였다. 먼저 모든 쥐에 코카인을 반복적으로 주어 행동과민반응을 유도한 다음 3주의 약물중지기간을 주었다. 생리식염수, CART 55-102 또는 CART 의 비활성 형태인 1-27 단백질을 국소 주입한 다음 코카인 (10mg/kg, i.p.)을 주고 15 분 후에 중격측좌핵 부분에서 ERK 의 인산화를 관찰하였다. 그 결과, 중격측좌핵 내에서 코카인에 의해 증가되는 ERK 의 인산화가 CART 55-102 의 국소 주입에 의해 억제되는 것을 알 수 있었고, 이러한 억제 효과는 비활성 형태인 CART 1-27 단백질에 의해서는 일어나지 않는 것으로 보아 CART 55-102 에 특이적인 효과임을 확인하였다. 이러한 결과를 통해 중격측좌핵 내에서 CART 55-102 단백질은, 중격측좌핵 내의 ERK 의 인산화 수준을 조절 함으로서, 코카인에 의해서 유도되는 행동과민 반응에 대한 보정적 제어 역할을 하는 것을 알 수 있다. 한편, 약물 중독에서, 약물에 의한 직접적인 효과뿐 아니라, 약물과 짝지어진 환경이 약물 중독자에게서 갈망과 약물의 재발을 일으키는데 중요한 역할을 한다는 것이 보고되고 있기 때문에, 본 연구에서는 약물과 짝지어진 환경에 의해 유도되는 조건화 반응에서 CART 55-102의 역할을 알아보고자 하였다. 먼저 세 그룹의 쥐는 다음과 같은 과정으로 약물과 환경을 짝지어주었다. Paired 그룹은 첫째 날에 코카인(15 mg/kg, IP)을 정위행동 측정상자에서 주고 둘째 날에 사육상자에서 생리식염수를 주었으며, Unpaired 그룹은 첫째 날에 정위행동 측정상자에서 생리식염수를, 둘째 날에 사육상자에서 코카인(15 mg/kg, IP)을 주었으며, 마지막으로 Control 그룹은 첫째 날과 둘째 날에 두 환경에서 모두 생리식염수를 준 그룹으로, 앞의 과정을 총 5회 반복하여 실시하였다. 이들 쥐는 1주일간의 약물중지기간을 거친 후, 모든 쥐에 생리식염수 또는 CART 55-102 (1.0, 2.5 μg/side) 단백질을 국소 주입한 다음 생리식염수를 복강 주입하고 정위행동 측정상자에서 1시간 동안 행동을 측정하였다. 그 결과, Paired 그룹은 다른 그룹들과는 달리 조건화된 행동 증가 반응을 보였으며, CART 55-102 단백질을 중격측좌핵 내에 국소 주입하였을 때, 이러한 증가 효과가 억제되는 것을 관찰하였다. 이 결과를 종합하면, CART 55-102 단백질은 중격측좌핵에서 정신자극제에 의한 직접적인 효과를 보여주는 행동과민반응에서뿐 아니라, 약물과 짝지어진 환경에 대한 조건

화 반응을 나타낼 때 중요한 조절작용을 하고 있는 것으로 생각되며, 정신자극제에 대하여 억제효과를 보이는 CART 55-102 단백질의 기전연구는, 약물 중독을 이해하는데 중요한 열쇠가 될 것으로 사료된다.

핵심되는 말: CART 단백질, 코카인, 행동과민반응, 중격측좌핵, ERK 1/2, 조건화 반응

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