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# Microinjection of cocaine- and amphetamine-regulated transcript 55–102 peptide into the nucleus accumbens could modulate anxiety-related behavior in rats

Hyung Shin Yoon, Naoki Adachi, Hiroshi Kunugi *

*Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo, 187-8502, Japan*

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ABSTRACT

Cocaine- and amphetamine-regulated transcript (CART) peptide is abundantly expressed in the nucleus accumbens (NAcc) and is involved in stress, anxiety and reward responses. To examine the role of CART peptide in anxiety-related behavior, naïve rats were bilaterally injected with CART 55–102 peptide (0.5, 1.0 or 2.5 μg/0.5 μl/side) or vehicle into the NAcc. Following this, their anxiety-related behavior was assessed using the elevated plus maze and the open field tests with a one-week interval between the tests. There was no difference in the time spent in open arms, or number of entries into open arms on the elevated plus maze in the CART-treated animals at any dose, when compared with the vehicle-treated group. However, there was a significant increase in the time spent in the center of the open field with administration of the low dose of CART peptide (0.5 μg/0.5 μl/side), although this effect disappeared at the high dose (2.5 μg/0.5 μl/side). None of the doses of CART peptide altered total locomotion in these tests. To further determine the possible anxiety-modulating effect of CART peptide at low dosages, the light and dark test was performed. Additional groups of rats given doses of 0.01 μg/0.5 μl/side or 0.5 μg/0.5 μl/side of CART peptide showed increased exploration time in the light side. These results suggest that accumbal-CART peptide reduces anxiety-like behavior in a dose-dependent manner.

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## 1. Introduction

Cocaine- and amphetamine-regulated transcript (CART) peptide is a neuropeptide involved in a variety of physiological actions, such as natural and psychostimulant reward processes, feeding and body weight, sleep, and stress response (Douglass et al., 1995; Hunter et al., 2004, 2005; Jaworski et al., 2003b; Keating et al., 2010; Lambert et al., 1998). Diverse lengths of CART peptides are produced by intracellular processing of two different forms, short and long, in a tissue-specific manner (Thim et al., 1999). The CART 55–102 peptide is produced by processing the long form and is one of the main biologically active forms (Dylag et al., 2006). It is known to have a stronger anorexic effect when compared to any other forms (Thim et al., 1998). It has been shown to modulate anxiety-related behavior when microinjected intracerebroventricularly (i.c.v.), whereas CART 62–102, a different main active form, did not (Chaki et al., 2003), implicating diverse effects of different CART peptides.

CART peptide is highly expressed in the brain, including the nucleus accumbens (NAcc) (Couceyro et al., 1997; Koylu et al., 1998; Philpot and Smith, 2006). This brain region is the center of the reward pathway and is involved in stress response. Anatomically, the NAcc receives afferents from the emotion-related region such as the amygdala (Zorrilla and Koob, 2013) as well as from the reward-related region such as the ventral tegmental area, implicating that the NAcc might be involved in emotional behaviors as well as in reward-related behaviors. However, most previous studies of the accumbal-CART peptide have focused on the role of CART 55–102 peptide in drug-related reward, feeding behaviors (Jaworski et al., 2003a; Kim et al., 2003, 2007; Yang et al., 2005; Yoon et al., 2007, 2010) or stress response (Hunter et al., 2005; Kang et al., 2010). To our knowledge, there is little information on the possible role of CART 55–102 peptide in emotion-related behaviors, especially anxiety, when it is injected into the NAcc region. In other brain regions, a few reports imply the possibilities that CART 55–102 peptide could modulate anxiety behaviors but their interpretations seem inconsistent; some studies report that CART peptide produced anxiety-like behaviors (Chaki et al., 2003; Dandekar et al., 2008) and the effects of CART peptide related to anxiety and/or stress-related behaviors

First Author: Hyung Shin Yoon, PhD, E-mail: hsyoon@ncnp.go.jp.
Second Author: Naoki Adachi, PhD, E-mail: adachi@ncnp.go.jp.
* Corresponding Author. Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo, 187-8502, Japan.
*E-mail address:* hkunugi@ncnp.go.jp (H. Kunugi).

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(see review, Stanek, 2006). Other reports have suggested that CART peptide might be a neurotransmitter with antidepressant-like or anxiolytic effects (Job et al., 2011; Mao, 2011; Pae et al., 2007). Therefore, we examined whether CART 55–102 peptide could modulate anxiety-related behaviors in naïve rats when microinjected directly into the NAcc.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats aged 8 weeks (260–280 g) were obtained from Charles River Laboratories Japan (Yokohama, Japan). They were housed two per cage with a 12-h light/dark cycle (lights on at 8:00 am and off at 8:00 pm). They were habituated and handled for 1 week before all experiments began. Rats had access to food and water *ad libitum*. All experiments were conducted during the light cycle. All animal experiments were approved by the ethics review committee for animal experimentation at the National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan.

### 2.2. Surgery

Rats were anesthetized with Avertin (Trimoethanol 140 mg/kg, i.p., Sigma, MO) and placed in a stereotaxic instrument with the incisor bar at 5.0 mm above the interaural line. Bilateral guide cannulas were implanted (22 gauge; Plastics One, Roanoke, VA) toward the NAcc core (A/P: +3.4; L: ±1.5; D/V: −7.5 mm from bregma and skull) (Pellegrino et al., 1979). Cannulas were angled at 10° to the vertical, positioned 1 mm above the final injection site, and secured with dental acrylic cement (Shofu Inc., Japan) anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge obturators were placed in the guide cannula and rats were caged one per cage to allow for a recovery period of more than 7 days.

### 2.3. Intra-accumbal microinjection of CART 55–102 peptide

Rat CART 55–102 peptide (American peptide, Sunnyvale, CA) was dissolved in sterile saline (0.9%). Bilateral intracranial microinjections into the NAcc were made in freely moving rats. The doses of CART 55–102 peptide in the present study were chosen based on previous studies that show an inhibitory effect on cocaine-induced sensitization at 2.5 μg/0.5 μl/side and on cocaine-induced conditioned hyperactivity at 1.0 and 2.5 μg/0.5 μl/side when microinjected into the NAcc without alteration of basal locomotor activity (Yoon et al., 2007, 2010). As described previously (Yoon et al., 2007, 2010), 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. Vehicle (0.9% saline) or CART 55–102 peptide at a volume of 0.5 μl per side was injected over 30 seconds. Following a diffusion time of 1 minute, the injection cannulas were withdrawn and the obturators were replaced.

### 2.4. Behavioral tests

All behavior experiments were performed between 11:00 and 15:00 to avoid the effect of circadian fluctuation in endogenous CART peptide levels (Vicentic et al., 2005), and were started within 5 minutes of microinjection. The starting time of behavior measurement from the CART 55–102 peptide microinjection was determined based on previous report (Yoon et al., 2010) indicating that the maximum effect of the CART 55–102 peptide microinjected into the NAcc core was observed within 10 minutes. Therefore, the elevated plus maze (EP) and the open field (OF) tests were started immediately after the microinjection, and the light and dark (LD) test was started 5 minutes after the microinjection, because of its shorter observation time than that of the EP and OF tests. In order to avoid any prior test effects, there was a one week interval between the EP test and the OF test. This allows sufficient time for the prior drug effect to be washed out (Jaworski et al., 2003a). The EP and OF tests were carried out based on the procedure as described in our previous report (Chiba et al., 2010). For the EP test, immediately following vehicle or CART peptide microinjection into the NAcc, rats were placed into a closed arm and allowed to move freely for 10 minutes (closed arms: $10 \times 50 \times 40$ cm; open arms: $10 \times 50 \times 0.5$ cm; floors for the maze: black acrylic plastics). For the OF test, following another microinjection, rats were introduced into an open-field apparatus ($100 \times 100 \times 40$ cm), consisting of a black rubber floor and black walls, and allowed to explore freely for 15 minutes. Their behaviors were monitored using a charge-coupled device (CCD) camera and calculated automatically, with the exception of rearing and grooming, with Image-EP and Image-OF software (O'Hara & Co., Japan), respectively. The central square in OF was set up as an area enclosed by the peripheral zone 20 cm from each wall. Rearing and grooming were counted manually by an observer.

Additional groups of animals were used for the LD test. After recovery from surgery, animals were microinjected with CART 55–102 peptide (0.01, 0.5, 1.0 or 2.5 μg/0.5 μl/side) or vehicle (0.5 μl/side). Due to a shorter LD test time, rats were allowed to remain in their home cage for 5 minutes following microinjection. To start the test, rats were placed in the dark compartment of the box for 1 minute to habituate. Following this, the center door was opened and rats were allowed to move freely between light and dark compartments for 5 minutes. Their behaviors in the light compartment were monitored by a CCD camera. The time spent and the numbers of rearing in the light compartment were measured manually by an observer.

### 2.5. Histology

Following completion of the behavioral experiments, rats were anesthetized and perfused via intracardiac infusion with saline (0.9%) and formalin (10%). Brains were removed and further post-fixed in formalin (10%). Bilateral cannula positions were verified in coronal sections (40 μm) and only data obtained from animals with the verification of correct insertion into the NAcc core were included in the analysis.

### 2.6. Statistical analysis

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

## 3. Results

Experiments in which cannula tips were successfully located in the NAcc cores bilaterally were included in the analysis (Fig. 1). Data were excluded if tips were located in the NAcc shell or a tip was inserted only unilaterally in the NAcc core. CART 55–102 peptide had no significant effect on total distance moved ($F(3,22) = 1.2$, $p = 0.32$, Fig. 2A) and total entry number ($F(3,22) = 0.99$, $p = 0.42$, Fig. 2B) at any dose in the EP test, as expected. We found no significant difference in the time spent in open arms ($F(3,22) = 0.93$, $p = 0.45$, Fig. 2C) or the number of entries into open arms ($F(3,22) = 1.1$, $p = 0.37$, Fig. 2D) at any dose of CART 55–102 peptide, when compared with vehicle-treated rats.

In the OF test, there was a significant increase in the percentage of time spent in the center area ([time spent in the center area/total experimental time] × 100) when a low dose of CART 55–102 peptide (0.5 μg/side) was microinjected into the NAcc, compared

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