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Cocaine-and-Amphetamine-Regulated-Transcript (CART) peptide attenuates dopamine- and cocaine-mediated locomotor activity in both male and female rats: lack of sex differences

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Abstract

Cocaine-and-Amphetamine Regulated Transcript peptide (CART peptide) is known for having an inhibitory effect on dopamine (DA)- and cocaine-mediated actions and is postulated to be a homeostatic, regulatory factor in the nucleus accumbens (NAc). Some sex differences in cocaine-mediated LMA and in the expression and function of CART peptide have been reported. However, it is not known if the inhibitory effect of CART peptide on cocaine-mediated locomotor activity (LMA) is sexually dimorphic. In this study, the effect of CART 55-102 on LMA due to intra-NAc DA and i.p. cocaine were determined in male and female Sprague-Dawley rats. The results show that CART 55-102 blunted or reduced both the DA- and cocaine-induced LMA in both males and females. In conclusion, CART peptide is effective in blunting DA- and cocaine-mediated LMA in both males and females.

Key words/Phrases

Cocaine-and-Amphetamine Regulated Transcript peptide; CART55-102; cocaine; dopamine; locomotion; nucleus accumbens; sex differences

1. Introduction

Cocaine-and-Amphetamine Regulated Transcript (CART) peptides (usually CART 55-102) are involved in a variety of physiological systems and effects (Rogge et al., 2008, Zhang et

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Contributors
Michael J Kuhar is the PI on the DA15162 and DA15040, and supervised the research and the preparation of this manuscript. Martin O Job carried out all of the laboratory work, statistical analysis, literature review, and prepared the manuscript. Ms Li-ling Shen assisted with the estrous cycle phase determinations and brain histology. JoAnna Perry assisted in the laboratory work and brain histology. All of the authors approved the final version of this paper.

Conflict of Interest
The authors have no conflicts of interest.

al., 2012). The mesolimbic dopaminergic system which includes projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is important in cocaine-mediated locomotion (LMA) and reward (Wise, 1984). Dopamine (DA)-containing nerve terminals originating in the VTA synapse on CART peptide-containing neurons in the NAc (Smith et al., 1997, Koylu et al., 1998, Smith et al., 1999), suggesting that CART peptide modulates mesolimbic DA function. Incidentally, these CART-peptide-containing neurons are activated by cocaine (Douglass et al., 1995, Hunter et al., 2005, Hubert and Kuhar, 2008), suggesting that CART peptide is important in cocaine-mediated effects. In human cocaine abusers, CART expression levels were increased and decreased in the NAc (Albertson et al., 2004, Bannon et al., 2005) and VTA (Tang et al., 2003), respectively. In some studies, cocaine increased the expression of CART mRNA in the NAc (Douglass et al., 1995, Hunter et al., 2005).

The administration of CART 55-102 into the NAc blunted the effects of DA and cocaine (Jaworski et al., 2003, Job et al., 2013), whereas depletion of CART peptides in the NAc (using shRNAs) led to a potentiation of cocaine-mediated LMA (Job et al., 2012). The mechanisms for these effects are not completely clear; however this blunting action of CART peptide seems to involve simultaneous activation of both D1 and D2 DA receptors in the NAc (Moffett et al., 2011). Also, this blunting action of CART peptide is lost after repeated cocaine administration (Job et al., 2013). For these reasons, CART peptide is thought to be a homeostatic regulator of DA- and cocaine-induced actions in the NAc (Jaworski et al., 2003, Jaworski et al., 2008, Rogge et al., 2008, Job et al., 2012).

There are sex differences in the effects of cocaine (Quinones-Jenab et al., 2001, Walker et al., 2001, Lynch et al., 2002, Hu et al., 2004, Fuchs et al., 2005, Jackson et al., 2006, Quinones-Jenab, 2006, Becker and Hu, 2008, Fattore et al., 2008, Anker and Carroll, 2010, Carroll and Anker, 2010, Cotto et al., 2010, Anker and Carroll, 2011, Andersen et al., 2012, Quinones-Jenab and Jenab, 2012). Furthermore, there are sex differences in the effects of cocaine on DA in the NAc (Lynch et al., 2002, Festa et al., 2006, Lynch et al., 2007, Festa et al., 2010). With all these sex differences in cocaine-mediated DA function, it is important to determine if there are differences also in CART peptide-mediated modulation of cocaine and DA function.

Interestingly, there are sex differences in CART peptide expression and function (Fagergren and Hurd, 1999, Rondini et al., 2004, Balkan et al., 2006, Gozen et al., 2007, Xu et al., 2010, Battistoni et al., 2011, Gerrits et al., 2011, Hostetler et al., 2011, Rodrigues et al., 2011, Bloem et al., 2012). Because of the findings that there are sex differences in (a) the effects of cocaine on DA in the NAc and (b) in CART peptide expression and function, we explored if there are sex differences in the effects of CART peptide on cocaine-induced LMA in rats. Even though the effects of CART peptide have been previously explored in males, males were included in many of these experiments to provide a rigorous comparison to females. Also, because cocaine produces its effects through DA systems, we explored the effects of CART peptide on DA-induced LMA.

2. Methods

2.1 Animals and Surgery

A total of 87 Sprague Dawley rats (47 males and 40 females), weighing between 225–275g at the time of purchase (Charles River Inc., Wilmington, MA), were used for the behavioral studies. The rats were pair-housed according to sex prior to surgery and individually housed following surgery. They were provided rat chow and water *ad libitum*, and maintained on a 12 hour light: dark cycle (lights on at 7am). Experiments and animal care were in accordance with the Institute of Animal Care and Use Committee of Emory University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Using stereotaxic surgical procedures under isoflurane inhalation anesthesia, or with a cocktail of Ketaset (Ketamine HCl, Fort Dodge Animal Health, Iowa, USA) and Dexdomitor (dexmedetomidine HCl, Orion Corporation, Espoo, Finland) injection anesthesia, rats were implanted with a bilateral stainless steel guide cannulae assembly (22 gauge; Plastics One; Roanoke, VA) using the following coordinates (from Bregma) for the NAc: A/P 1.6 mm, L/M ± 1.5 mm, D/V −5.7 mm (Paxinos and Watson, 1998) to place the guide cannulae 2.0 mm above the shell-core boundary of the NAc. The assembly was secured to the skull with acrylic dental cement and 2–4 stainless steel screws. A bilateral obturator extending 0.5mm past the tip of the cannulae was inserted into the guide cannulae to prevent blockage. All rats were allowed to recover for at least one week before LMA experiments. Following recovery from surgery, the animals were prepared for infusions and LMA measurement. One to two days before testing, animals were habituated to the testing chambers for approximately 30 minutes after being briefly handled. The testing days were separated by at least 2 days of no testing. Male and female rats were used on the same day under the same experimental conditions. For the cocaine study, estrous cycle status in females was determined retrospectively. Rats were only used for either DA or cocaine administration, but not both.

2.2 Experimental Design

The following experiments were done in males and females: (1) a determination of the effect of CART peptide on the cocaine dose-response curve, (2) a determination of the dose-response effect of CART peptide on cocaine (10 mg/kg) mediated LMA, and (3) a determination of the effect of CART peptide on DA-mediated LMA. Estrous cycle was determined for females used in cocaine (10 mg/kg ip)-mediated LMA (data not shown). For the cocaine experiments, the females ranged in age from 4–6 months and the males ranged in age from 3.4–7.8 months. For the DA-related experiments, the females ranged in age from 3.6–8.7 months and the males ranged in age from 3.4–8.3 months. The details of the animal use are summarized in Table 1.

A total of 25 male rats and 20 female rats were used for the cocaine study (Table 1). For the cocaine dose-response experiment, both male and female rats were used in a crossover design such that for a given dose of cocaine, the rats received both saline + cocaine and CART55-102 + cocaine. In these experiments, rats received bilateral intra-accumbal infusions of either CART 55-102 (2.5 μg in 0.5 μL) per side or an equivalent volume of

saline (0.5 μL/side) followed immediately by an injection (ip.) of different doses of cocaine (0.0, 5.0, 7.5, 10.0, and 15.0 mg/kg all administered in 1 mL/kg) and LMA was measured. The cocaine 0 mg/kg was the same as an equivalent volume of saline (1 mL/kg). Animals were used for 2 treatments (with and without CART peptide, counterbalanced design) for only one cocaine dose. The number of animals used per cocaine dose are given in Table 1.

Some animals were re-used for CART 55-102 dose-response experiment. In this experiment, *some* of the animals that had been used in previous experiment (not used for cocaine (10 mg/kg ip) dose in the cocaine dose response study), were assigned to 2 groups (CART1 μg + cocaine, CART2.5 μg + cocaine) and analyzed with the saline + cocaine (10 mg/kg ip, data from previous experiment). The reproducibility of our re-used data was tested by comparing the CART2.5 μg + cocaine group with the CART2.5 μg + cocaine results from previous experiment using t-tests. In this experiment, 17 males and 14 females from the previous experiment were re-used. In this design, both male and female rats were used only once.

A total of 22 male rats and 20 female rats were used for the DA study (Table 1). In this design, males and females were assigned to one of 6 groups (saline + saline, CART1-27 + saline, CART 55-102 + saline, saline + DA, CART1-27 + DA, CART 55-102 + DA). For this study, both male and female rats were used only once. The details of the animal use are summarized in Table 1.

2.3 Intra-NA infusions and LMA Experiments

The microinfusion assembly included stainless steel bilateral injector cannulae (28 gauge; Plastics One), polyethylene-50 (PE-50) tubing, two 25 μL microsyringes (Hamilton Co, Reno, NV), two microsyringe pumps and Micro4 Microsyringe Pump Controller (World Precision Instruments, Sarasota, FL). The two 25 μL syringes were filled with sterile water with an air bubble introduced to enable a reading on the syringe scale of amount infused. The microsyringes were then placed in the microsyringe pumps which were in turn connected to the microsyringe pump controller. Two PE-50 tubes, cut to the same length, were filled with sterile water and connected to the two 25 μL syringes on one end and to the stainless steel bilateral injector cannulae on the other end. Air was withdrawn into the assembly through the bilateral microinjector cannulae before the solutions for infusion were drawn. In other words, an air bubble was introduced between the sterile water and the solution to be infused, also to enable a visual confirmation of infusion. For each infusion, the rats were gently restrained by the handler. The bilateral injector cannulae were placed into the bilateral guide cannulae such that it extended 2.0 mm past the guide cannulae. Fluid was bilaterally injected (0.5 μL/side) for 30 seconds through the bilateral injector cannulae. The injected fluid was allowed to diffuse for an additional 30 seconds before gentle removal of the injector cannluae.

LMA was measured in 10 minute intervals using a photocell cage (Omnitech Electronics, Columbus OH) with the dimensions of 40 × 40 × 30cm. The cages were made of transparent plexiglass walls and contained 32 photobeams located 5cm above the floor to record LMA. Each cage was placed in a stainless steel box and connected to a computer equipped with software (Digipro; Omnitech Electronics) to measure LMA. On testing days, animals were habituated to the chambers for 30 minutes, followed by 30 minutes of basal LMA

measurement. Next, animals were given an accumbal infusion of the designated treatment and returned to the chamber for 60 or 90 minutes of LMA measurement, depending on the treatment (DA or cocaine, respectively). Time courses were obtained in each experiment as we have in the past, but the data shown represent total locomotor activity added over 90 min and are shown as one bar value.

2.4 Estrous cycle determinations and brain histology

After the LMA experiments, female rats were lightly anesthetized with isoflurane and vaginal samples were obtained to determine estrous phase. Vaginal secretion was collected with a plastic pipette containing 50 μL of distilled water. The pipette tip was inserted gently into the vagina and fluid withdrawn and placed on glass slides. The slides were allowed to dry at room temperature and stored at −4°C until staining. Staining of vaginal samples was done with cresyl violet. The images were scanned at 20X in the Aperio, and downloaded from Spectrum. The images were not changed or altered in anyway except the scale was adjusted in Adobe Illustrator Encapsulated PostScript (EPS) to fit into a Word document page. The estrous phase is defined by the preponderant cellular types in the vaginal samples. A proestrus sample predominantly contains nucleated epithelial cells whereas an estrus sample predominantly contains anucleated cornified cells.

After completion of the all experiments, animals received an overdose of Ketamine (70 mg/kg) and Dexdomitor (0.5 mg/kg), and were then perfused intracardially with saline and 4% paraformaldehyde. After removal from the skull, brains were placed in 4% paraformaldehyde overnight, followed by immersion in 30%w/v sucrose paraformaldehyde solution for several days. The brains were sliced (60 μm thickness) using a cryostat (Leica CM1900, Leica CM3050S, Leica Microsystems, Germany) and processed for Nissl staining. A stereotaxic brain atlas (Paxinos, 1998.) was used to approximate the placement of the injector tip (see Fig 5).

2.5 Statistical Analyses

LMA data were acquired in 10-minute bins and total locomotor activity data over 90 min (for cocaine studies) and 60 min (for DA studies) were analyzed. Data are all expressed as mean ± SEM, and significance was assumed to be $p < 0.05$. All analyses were performed using IBM SPSS Statistics 20.0. When required, data were transformed to satisfy equality of variance test.

For the cocaine dose-response study, rats were used for both saline + cocaine and CART + cocaine in counterbalanced design (repeated measures) as described in Methods. The analysis for the effect of CART peptide on LMA due to different doses of cocaine in male and female rats (Fig 1) employed a 2 × 5 × 2 (sex x cocaine dose x treatment) mixed factorial repeated measures. The factors were 2 (sex: male, female) × 5 (cocaine dose: 0, 5, 7.5, 10, 15 mg/kg i.p.) × 2 (treatment: without- and with- CART peptide).

For the analysis of the effect of different doses of CART peptide on cocaine-10 mg/kg-mediated LMA in male and female rats (CART peptide dose-response, Fig 2), a 2-way ANOVA was used. The factors were sex (2 levels: males and females) and CART dose (3 levels: CART0 + coc10, CART1 + coc10 and CART2.5 + coc10).

For the analysis of the effect of estrous phase on the effect of CART peptide (CART 2.5 μg) on cocaine-10 mg/kg-mediated LMA (Fig 3), a 2-way ANOVA was used (data not shown). The factors were estrous phase (2 levels: estrus and proestrus) and treatment (2 levels: saline and CART).

For the effect of CART peptide on DA-mediated LMA in males and females (Fig 4), a 2 × 3 × 2 mixed factorial ANOVA was used with factors sex (2 levels: males and females), treatment (3 levels: saline, CART 1-27 and CART 55-102) and DA dose (2 levels: 0 and 15 μg). Significant main effects or interactions were followed up with simple effects tests. The significant differences after Bonferroni *post hoc* analysis are shown on the graphs.

3. Results

Animals were prepared as described in Methods, and the effect of CART peptide on cocaine-mediated LMA were examined. Different doses of cocaine were injected into male and female rats immediately after intra-NAc infusion of saline or CART 55-102, as described in methods. A dose-dependent effect of cocaine alone on LMA in males and females was observed. When the injection of cocaine was preceded by an intra-NAc infusion of CART 55-102 there was an inhibitory effect of CART peptide on LMA (Fig 1). When various doses of CART 55-102 were utilized in both males and females, CART 55-102 was effective in suppressing LMA in both sexes due to cocaine (10 mg/kg ip) at the 2.5 μg dose but not at the 1.0 μg dose (Fig 2). Generally, female rats in estrus were more sensitive to cocaine relative to females in proestrus and CART peptide was effective in attenuating cocaine (10 mg/kg ip) -mediated LMA generally, regardless of estrous phase (data not shown).

As with cocaine, CART peptide suppressed DA-mediated LMA in both males and females (Fig 3). In these experiments CART 1-27 was used as an additional control, as it has never found to produce any effects: the effect of CART 1-27 was identical to that of saline in these experiments. As expected it had no effect on DA-induced LMA.

The locations of the injections were determined as described in methods, and as expected they were found throughout the extent of the NAc, but there was no systematic difference in the locations in males compared to that in the females (Fig 4).

4. Discussion

The new finding of this study is that intra-accumbal CART 55-102 inhibits cocaine- and DA-induced LMA in females, and that there are no sex differences in this effect. We determined that the effect of cocaine on LMA is greater in females in the estrus phase than in the proestrus phase, and this result agrees with similar evidence that shows that the effect of cocaine is greater in the follicular phase than the luteal phase in humans (Sofuoglu et al., 1999, Evans et al., 2002, Terner and de Wit, 2006, Evans, 2007, Evans and Foltin, 2010). Additionally, in agreement with others, our results confirm that LMA induced by intra-accumbal DA and ip cocaine is greater in females than in males (Sell et al., 2000, Walker et al., 2001, Fuchs et al., 2005, Kippin et al., 2005, Quinones-Jenab, 2006, Becker and Hu, 2008, Parylak et al., 2008).

Additional observations were confirmed and support previous data. Notably, CART 55-102 had no effect on basal LMA but blunted DA- and cocaine-induced LMA in males (Jaworski et al., 2003). A previous study by showing that the non-active CART peptide CART 1-27 does not affect systemic cocaine-mediated LMA (Jaworski et al., 2003). This study complements the previous study by showing that in addition, CART 1-27 does not alter intra-NAc DA-mediated LMA. It appears that the regulatory action of CART55-102 only comes into play when there is stimulation of the DA pathway. These blunting actions in males, and now found in females, buttress the suggestion that endogenous CART peptide regulates the actions of cocaine in the NAc. This regulation is such that CART peptide tends to oppose the effect of large DA increases in the NAc, homeostatically restoring basal activity (Jaworski et al., 2003, Jaworski et al., 2008, Rogge et al., 2008, Job et al., 2012).

While sex differences in cocaine-mediated LMA have been observed, there are numerous reports that indicate that there are no sex differences in basal DA receptor mRNA (DA D1, D2), dopamine transporter binding levels and tyrosine hydroxylase profile in the NAc of adult rats (Festa et al., 2006, Wissman et al., 2012). Also, there are no sex differences in DA (D1 and D2) receptor density in the NAc in adult rats (Andersen et al., 1997) though there are sex differences during development (Andersen et al., 1997, Andersen and Teicher, 2000). Furthermore, acute cocaine administration did not change DA receptor binding in the NAc of both males and female rats (Festa et al., 2006). Incidentally, there are no sex differences in the effects of systemic DA D2 antagonist blockade of cocaine-mediated LMA (Schindler and Carmona, 2002), giving some credence to the observations that there are no sex differences in DA D2 receptors in the NAc. Since CART peptide attenuates LMA due to combined intra-accumbal DA D1 and D2 activation (Moffett et al., 2011), the lack of sex differences in DA D1 and D2 basal expression and binding properties after acute cocaine administration may explain why there are no sex differences in CART peptide-mediated suppression of cocaine-mediated LMA.

Like others, we have shown that there are sex differences in cocaine-mediated LMA, with females showing a higher response than males. This observation may be partly due to higher basal levels of DA in the NAc of females compared to males (Duchesne et al., 2009). It may also be partly due to sex differences in cocaine-induced enhancement of structural changes in spine density in the medium spiny neurons of the NAc, with greater female vs male changes correlating with greater female vs male behavioral responses to cocaine (Wissman et al., 2011, Wissman et al., 2012). But, as discussed in the previous paragraph, one can infer that these sex differences may not even be due to DA because there is ample evidence that DA systems in the NAc in males and females are not much different. This gives rise to the idea that the sex differences in cocaine-mediated LMA may be due to DA system differences between males and females in other regions of the brain apart from the NAc or sex differences may be due to other neurochemicals in the NAc apart from DA. Interestingly, even though CART peptide in the NAc is actively involved in regulating cocaine-mediated LMA, it may not play a role in the mechanism of sex differences in cocaine-mediated LMA in the NAc.

There are studies showing sex differences in CART peptide expression and function (Fagergren and Hurd, 1999, Rondini et al., 2004, Balkan et al., 2006, Gozen et al., 2007, Xu

et al., 2010, Battistoni et al., 2011, Gerrits et al., 2011, Hostetler et al., 2011, Rodrigues et al., 2011, Bloem et al., 2012). These studies focused on CART mRNA. However, without data on actual CART peptide protein levels, it is difficult to affirmatively conclude that CART peptide content in brain is sexually dimorphic. Furthermore, even if CART peptide levels are different between males and females, without data on actual CART peptide receptor levels, it is hard interprete the significance of such a difference. Unfortunately, the CART peptide receptor has not yet been cloned. In the absence of CART peptide protein and receptor levels, one way to interprete the possible sex differences in CART peptide function in the NAc is to determine if there are any differences in the downstream targets of CART peptide in this region of the brain. Dopamine receptor-mediated actions in NAc output neurons are thought to be such downstream targets of CART peptide modulation of dopamine function (Hubert et al., 2008, Hubert et al., 2010). Again, as discussed in the previous paragraph, the lack of sex differences in the downstream targets of CART peptide (DA D1 and D2 receptor) may explain why we do not observe any sex differences in the effect of CART peptide on cocaine-mediated LMA in the NAc.

Based on some preliminary experiments, we had reported in an abstract (Perry et al., 2012) that there were possible sex differences in CART peptide's effects on DA-mediated. But in this more extensive study, we found no sex differences in the effects of CART peptide on DA-induced LMA. The implication of our study is that CART peptide may be clinically effective in attenuating the effects of cocaine in both males and females.

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References

Albertson DN, Pruetz B, Schmidt CJ, Kuhn DM, Kapatos G, Bannon MJ. Gene expression profile of the nucleus accumbens of human cocaine abusers: evidence for dysregulation of myelin. J Neurochem. 2004; 88:1211–1219. [PubMed: 15009677]

Andersen ML, Sawyer EK, Howell LL. Contributions of neuroimaging to understanding sex differences in cocaine abuse. Exp Clin Psychopharmacol. 2012; 20:2–15. [PubMed: 21875225]

Andersen SL, Rutstein M, Benzo JM, Hostetter JC, Teicher MH. Sex differences in dopamine receptor overproduction and elimination. Neuroreport. 1997; 8:1495–1498. [PubMed: 9172161]

Andersen SL, Teicher MH. Sex differences in dopamine receptors and their relevance to ADHD. Neurosci Biobehav Rev. 2000; 24:137–141. [PubMed: 10654670]

Anker JJ, Carroll ME. The role of progestins in the behavioral effects of cocaine and other drugs of abuse: human and animal research. Neurosci Biobehav Rev. 2010; 35:315–333. [PubMed: 20398693]

Anker JJ, Carroll ME. Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones. Curr Top Behav Neurosci. 2011; 8:73–96. [PubMed: 21769724]

Balkan B, Gozen O, Yararbas G, Koylu EO, Akinturk S, Kuhar MJ, Pogun S. CART expression in limbic regions of rat brain following forced swim stress: sex differences. Neuropeptides. 2006; 40:185–193. [PubMed: 16644010]

Bannon M, Kapatos G, Albertson D. Gene expression profiling in the brains of human cocaine abusers. Addict Biol. 2005; 10:119–126. [PubMed: 15849025]

Battistoni S, Kloting I, Cifani C, Massi M, Polidori C. Gender differences in Nociceptin/Orphanin FQ-induced food intake in strains derived from rats prone (WOKW) and resistant (Dark Agouti) to metabolic syndrome: a possible involvement of the cocaine- and amphetamine-regulated transcript system. Genes Nutr. 2011; 6:197–202. [PubMed: 21484154]

Becker JB, Hu M. Sex differences in drug abuse. Front Neuroendocrinol. 2008; 29:36–47. [PubMed: 17904621]

Bloem B, Xu L, Morava E, Faludi G, Palkovits M, Roubos EW, Kozicz T. Sex-specific differences in the dynamics of cocaine- and amphetamine-regulated transcript and nesfatin-1 expressions in the midbrain of depressed suicide victims vs. controls. Neuropharmacology. 2012; 62:297–303. [PubMed: 21803054]

Carroll ME, Anker JJ. Sex differences and ovarian hormones in animal models of drug dependence. Horm Behav. 2010; 58:44–56. [PubMed: 19818789]

Cotto JH, Davis E, Dowling GJ, Elcano JC, Staton AB, Weiss SR. Gender effects on drug use, abuse, and dependence: a special analysis of results from the National Survey on Drug Use and Health. Gend Med. 2010; 7:402–413. [PubMed: 21056867]

Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci. 1995; 15:2471–2481. [PubMed: 7891182]

Duchesne A, Dufresne MM, Sullivan RM. Sex differences in corticolimbic dopamine and serotonin systems in the rat and the effect of postnatal handling. Progress in neuro-psychopharmacology & biological psychiatry. 2009; 33:251–261. [PubMed: 19100810]

Evans SM. The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. Exp Clin Psychopharmacol. 2007; 15:418–426. [PubMed: 17924776]

Evans SM, Foltin RW. Does the response to cocaine differ as a function of sex or hormonal status in human and non-human primates? Horm Behav. 2010; 58:13–21. [PubMed: 19733571]

Evans SM, Haney M, Foltin RW. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology (Berl). 2002; 159:397–406. [PubMed: 11823892]

Fagergren P, Hurd YL. Mesolimbic gender differences in peptide CART mRNA expression: effects of cocaine. Neuroreport. 1999; 10:3449–3452. [PubMed: 10599860]

Fattore L, Altea S, Fratta W. Sex differences in drug addiction: a review of animal and human studies. Womens Health (Lond Engl). 2008; 4:51–65. [PubMed: 19072451]

Festa ED, Jenab S, Weiner J, Nazarian A, Niyomchai T, Russo SJ, Kemen LM, Akhavan A, Wu HB, Quinones-Jenab V. Cocaine-induced sex differences in D1 receptor activation and binding levels after acute cocaine administration. Brain research bulletin. 2006; 68:277–284. [PubMed: 16377433]

Festa ED, Shivers KY, Jenab S, Quinones-Jenab V. Cocaine-induced sex differences in D1 dopamine receptor mRNA levels after acute cocaine administration. Ethn Dis. 2010; 20:S1-24–27.

Fuchs RA, Evans KA, Mehta RH, Case JM, See RE. Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats. Psychopharmacology (Berl). 2005; 179:662–672. [PubMed: 15682307]

Gerrits H, Bakker NE, van de Ven-de Laat CJ, Bourgondien FG, Peddemors C, Litjens RH, Kok HJ, Vogel GM, Krajnc-Franken MA, Gossen JA. Gender-specific increase of bone mass by CART peptide treatment is ovary-dependent. J Bone Miner Res. 2011; 26:2886–2898. [PubMed: 21887702]

Gozen O, Balkan B, Yararbas G, Koylu EO, Kuhar MJ, Pogun S. Sex differences in the regulation of cocaine and amphetamine-regulated transcript expression in hypothalamic nuclei of rats by forced swim stress. Synapse. 2007; 61:561–568. [PubMed: 17447258]

Hostetler CM, Kowalczyk AS, Griffin LL, Bales KL. CART peptide following social novelty in the prairie vole (Microtus ochrogaster). Brain research. 2011; 1414:32–40. [PubMed: 21871610]

Hu M, Crombag HS, Robinson TE, Becker JB. Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology. 2004; 29:81–85. [PubMed: 12955098]

Hubert GW, Jones DC, Moffett MC, Rogge G, Kuhar MJ. CART peptides as modulators of dopamine and psychostimulants and interactions with the mesolimbic dopaminergic system. Biochemical pharmacology. 2008; 75:57–62. [PubMed: 17854774]

Hubert GW, Kuhar MJ. Cocaine administration increases the fraction of CART cells in the rat nucleus accumbens that co-immunostain for c-Fos. Neuropeptides. 2008; 42:339–343. [PubMed: 18314190]

Hubert GW, Manvich DF, Kuhar MJ. Cocaine and amphetamine-regulated transcript-containing neurons in the nucleus accumbens project to the ventral pallidum in the rat and may inhibit cocaine-induced locomotion. Neuroscience. 2010; 165:179–187. [PubMed: 19825396]

Hunter RG, Vicentic A, Rogge G, Kuhar MJ. The effects of cocaine on CART expression in the rat nucleus accumbens: a possible role for corticosterone. European journal of pharmacology. 2005; 517:45–50. [PubMed: 15972209]

Jackson LR, Robinson TE, Becker JB. Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology. 2006; 31:129–138. [PubMed: 15920500]

Jaworski JN, Hansen ST, Kuhar MJ, Mark GP. Injection of CART (cocaine- and amphetamine-regulated transcript) peptide into the nucleus accumbens reduces cocaine self-administration in rats. Behav Brain Res. 2008; 191:266–271. [PubMed: 18485497]

Jaworski JN, Kozel MA, Philpot KB, Kuhar MJ. Intra-accumbal injection of CART (cocaine-amphetamine regulated transcript) peptide reduces cocaine-induced locomotor activity. The Journal of pharmacology and experimental therapeutics. 2003; 307:1038–1044. [PubMed: 14551286]

Job MO, Licata J, Hubert GW, Kuhar MJ. Intra-accumbal administration of shRNAs against CART peptides cause increases in body weight and cocaine-induced locomotor activity in rats. Brain Res. 2012; 1482:47–54. [PubMed: 22975438]

Job MO, Shen LL, Kuhar MJ. The inhibition of cocaine-induced locomotor activity by CART 55-102 is lost after repeated cocaine administration. Neurosci Lett. 2013; 550:179–183. [PubMed: 23819981]

Kippin TE, Fuchs RA, Mehta RH, Case JM, Parker MP, Bimonte-Nelson HA, See RE. Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. Psychopharmacology (Berl). 2005; 182:245–252. [PubMed: 16001116]

Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. J Comp Neurol. 1998; 391:115–132. [PubMed: 9527537]

Lynch WJ, Kiraly DD, Caldarone BJ, Picciotto MR, Taylor JR. Effect of cocaine self-administration on striatal PKA-regulated signaling in male and female rats. Psychopharmacology (Berl). 2007; 191:263–271. [PubMed: 17180335]

Lynch WJ, Roth ME, Carroll ME. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology (Berl). 2002; 164:121–137. [PubMed: 12404074]

Moffett MC, Song J, Kuhar MJ. CART peptide inhibits locomotor activity induced by simultaneous stimulation of D1 and D2 receptors, but not by stimulation of individual dopamine receptors. Synapse. 2011; 65:1–7. [PubMed: 20506412]

Parylak SL, Caster JM, Walker QD, Kuhn CM. Gonadal steroids mediate the opposite changes in cocaine-induced locomotion across adolescence in male and female rats. Pharmacology, biochemistry, and behavior. 2008; 89:314–323.

Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. Burlington MA: Academic Press; 1998.

Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. Burlington MA: Academic Press; 1998.

Perry, J.; Job, M.; McNamara, I.; Kuhar, MJ. Sex differences in CART peptide's effects on dopamine-induced locomotor activity in rats: A hypothesis as to why females are more sensitive to cocaine than males. College on Problems of Drug Dependence (CPDD) 74th Annual Meeting; Palm Springs, CA. 2012.

Quinones-Jenab V. Why are women from Venus and men from Mars when they abuse cocaine? Brain research. 2006; 1126:200–203. [PubMed: 17010952]

Quinones-Jenab V, Jenab S. Influence of sex differences and gonadal hormones on cocaine addiction. ILAR J. 2012; 53:14–22. [PubMed: 23520596]

Quinones-Jenab V, Perrotti LI, Fabian SJ, Chin J, Russo SJ, Jenab S. Endocrinological basis of sex differences in cocaine-induced behavioral responses. Ann N Y Acad Sci. 2001; 937:140–171. [PubMed: 11458535]

Rodrigues BC, Cavalcante JC, Elias CF. Expression of cocaine- and amphetamine-regulated transcript in the rat forebrain during postnatal development. Neuroscience. 2011; 195:201–214. [PubMed: 21903152]

Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ. CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci. 2008; 9:747–758. [PubMed: 18802445]

Rondini TA, Baddini SP, Sousa LF, Bittencourt JC, Elias CF. Hypothalamic cocaine- and amphetamine-regulated transcript neurons project to areas expressing gonadotropin releasing hormone immunoreactivity and to the anteroventral periventricular nucleus in male and female rats. Neuroscience. 2004; 125:735–748. [PubMed: 15099687]

Sell SL, Scalzitti JM, Thomas ML, Cunningham KA. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. The Journal of pharmacology and experimental therapeutics. 2000; 293:879–886. [PubMed: 10869388]

Smith Y, Kieval J, Couceyro PR, Kuhar MJ. CART peptide-immunoreactive neurones in the nucleus accumbens in monkeys: ultrastructural analysis, colocalization studies, and synaptic interactions with dopaminergic afferents. J Comp Neurol. 1999; 407:491–511. [PubMed: 10235641]

Smith Y, Koylu EO, Couceyro P, Kuhar MJ. Ultrastructural localization of CART (cocaine- and amphetamine-regulated transcript) peptides in the nucleus accumbens of monkeys. Synapse. 1997; 27:90–94. [PubMed: 9268069]

Sofuoglu M, Dudish-Poulsen S, Nelson D, Pentel PR, Hatsukami DK. Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. Exp Clin Psychopharmacol. 1999; 7:274–283. [PubMed: 10472516]

Tang WX, Fasulo WH, Mash DC, Hemby SE. Molecular profiling of midbrain dopamine regions in cocaine overdose victims. J Neurochem. 2003; 85:911–924. [PubMed: 12716423]

Terner JM, de Wit H. Menstrual cycle phase and responses to drugs of abuse in humans. Drug Alcohol Depend. 2006; 84:1–13. [PubMed: 16413143]

Walker QD, Cabassa J, Kaplan KA, Li ST, Haroon J, Spohr HA, Kuhn CM. Sex differences in cocaine-stimulated motor behavior: disparate effects of gonadectomy. Neuropsychopharmacology. 2001; 25:118–130. [PubMed: 11377925]

Wise RA. Neural mechanisms of the reinforcing action of cocaine. NIDA research monograph. 1984; 50:15–33. [PubMed: 6440023]

Wissman AM, May RM, Woolley CS. Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity. Brain Struct Funct. 2012; 217:181–190. [PubMed: 21987050]

Wissman AM, McCollum AF, Huang GZ, Nikrodhanond AA, Woolley CS. Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. Neuropharmacology. 2011; 61:217–227. [PubMed: 21510962]

Xu L, Bloem B, Gaszner B, Roubos EW, Kozicz T. Stress-related changes in the activity of cocaine- and amphetamine-regulated transcript and nesfatin neurons in the midbrain non-preganglionic Edinger-Westphal nucleus in the rat. Neuroscience. 2010; 170:478–488. [PubMed: 20638450]

Zhang M, Han L, Xu Y. Roles of cocaine- and amphetamine-regulated transcript in the central nervous system. Clin Exp Pharmacol Physiol. 2012; 39:586–592. [PubMed: 22077697]

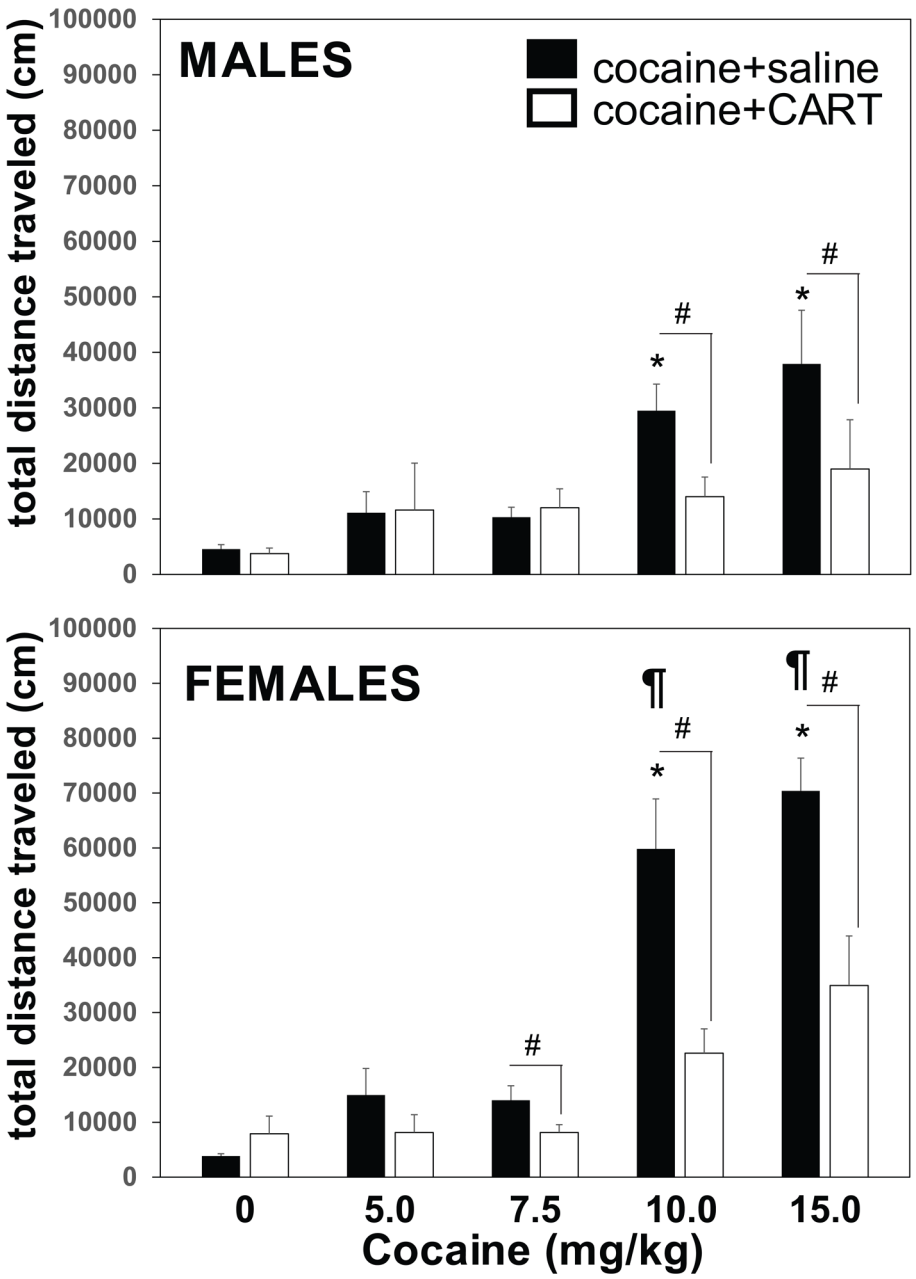

Fig. 1. The effect of CART peptide on the LMA due to different doses of cocaine in male and female rats (cocaine dose-response)

CART55-102 (2.5 ug) or saline was administered directly into the NAc of male and female rats immediately before an ip injection of different doses of cocaine (0, 5, 7.5, 10 and 15 mg/kg ip) and LMA was measured. The groups and number of animals/group are shown in Table 1. The y-axis represents total distance traveled in 90 mins and the detailed time courses of LMA after treatment are not shown. The experiments were done in counterbalanced design such that for each cocaine dose, all animals had *both* saline and CART peptide treatments. The statistical analysis procedure was done as described in the Methods section. For within subject effects (repeated measures design), there was a

significant CART effect ($F_{1, 35}$ = 32.509, ***P<0.0001), a significant CART x cocaine dose effect ($F_{4, 35}$ = 7.822, ***P<0.0001), no significant CART x sex interaction ($F_{1, 35}$ = 1.941, P = 0.171) and no significant CART x sex x cocaine dose interaction ($F_{4, 35}$ = 1.073, P = 0.385). Between subjects, ANOVA showed a significant difference in sex ($F_{1, 35}$ = 8.988, ***P = 0.005), cocaine dose ($F_{4, 35}$ = 27.369, ***P<0.0001), but no sex x cocaine dose interaction ($F_{4, 35}$ = 2.197, P = 0.09). Bonferroni *post hoc* tests showed that for both sexes, cocaine 10 mg/kg ip and cocaine 15 mg/kg ip increased LMA compared to saline and CART blunted cocaine 10- and cocaine 15-mediated LMA. Bonferroni *post hoc* tests showed that females are more responsive to cocaine 10 mg/kg ip and cocaine 15 mg/kg ip than males. The significant differences after *post hoc* analysis are shown on the graph. The p-values * show the differences when comparing saline + cocaine effects and saline + saline (controls) between groups. The p-values # show the difference when comparing CART + cocaine and saline + cocaine to each other within a cocaine dose group. The p-values ¶ show a comparison between males and females within each cocaine-dose and CART treatment group.

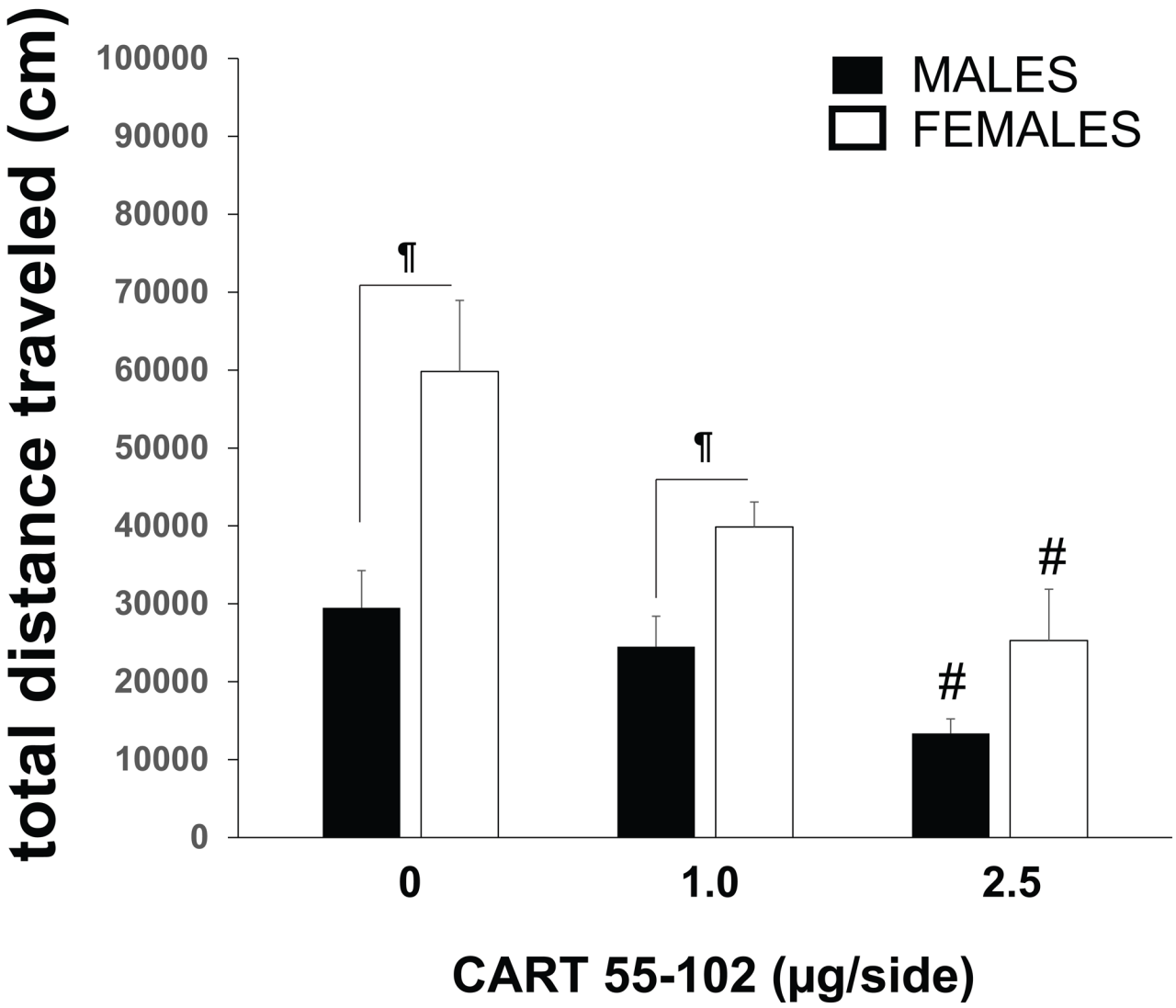

Fig. 2. The effect of different doses of CART peptide on cocaine-10 mg/kg-mediated LMA in male and female rats (CART peptide dose-response)

CART 55-102 (0.0, 1.0 and 2.5 µg) was administered directly into the NAc of male and female rats immediately before an ip injection of saline or cocaine-10 mg/kg and LMA was measured. The y-axis represents the total distance covered in 90 mins. For males and females, the CART 0 + cocaine group is the same as in Fig 1, with other animals re-used for the other groups. 2- way ANOVA determined a significant difference in sex ($F_{1, 25}$ = 20.633, ***P < 0.0001), a significant difference in the effect of CART ($F_{2, 25}$ = 12.034, ***P < 0.0001) and no CART dose x sex interaction ($F_{2, 25}$ = 0.777, P = 0.470). Bonferroni *post hoc* tests showed that, for both sexes, CART2.5 blunted cocaine-mediated LMA. Bonferroni *post hoc* tests showed that females are more responsive to cocaine 10 mg/kg ip than males. The significant differences after *post hoc* analysis are shown on the graph. The p-values * show difference when comparing CART doses control (CART0 + cocaine) within sex and p-values ¶ show a comparison between males and females within CART dose treatment groups.

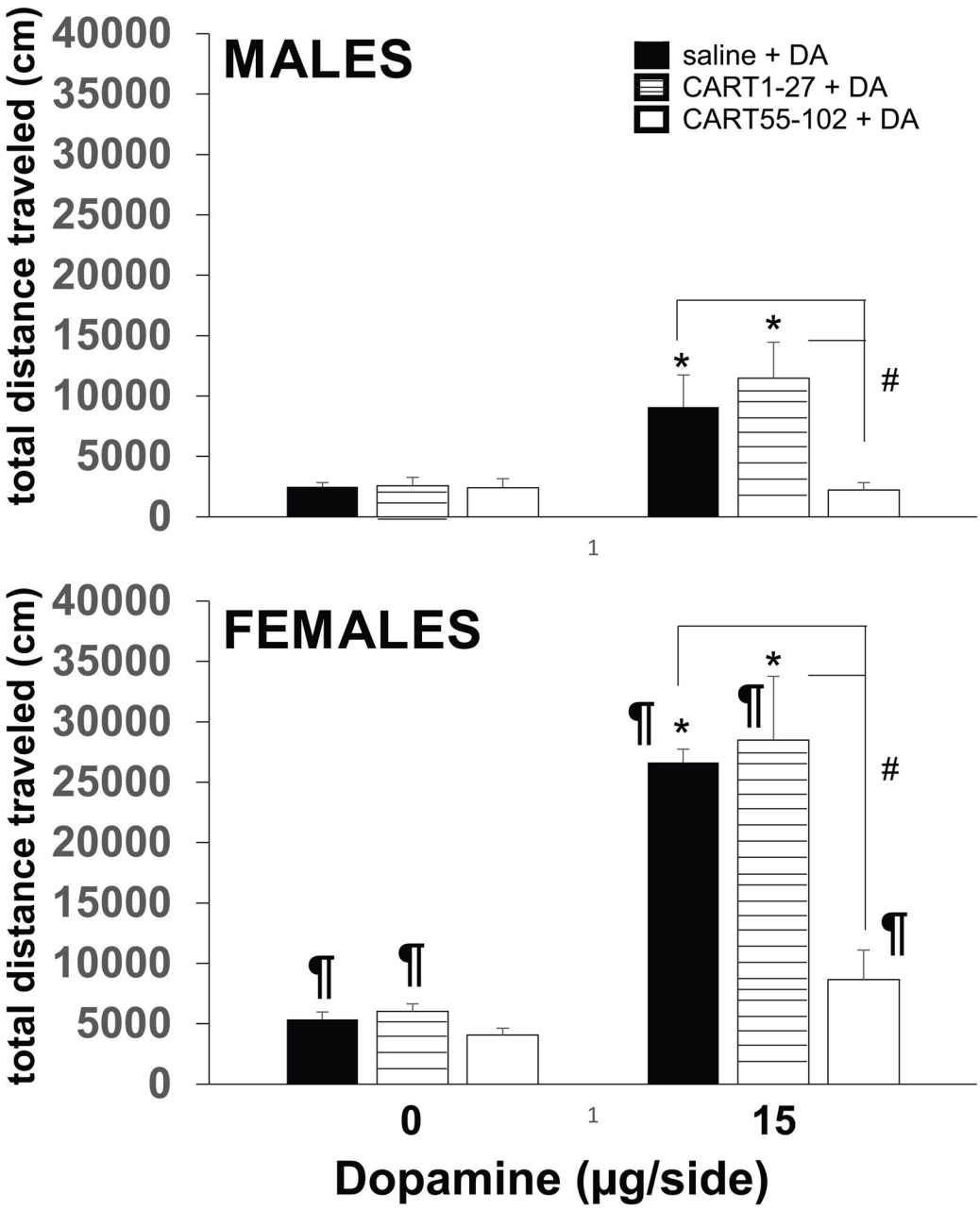

Fig. 3. The effect of CART peptide on DA-mediated LMA in males and females

CART55-102 (2.5 ug), CART 1-27 (2.5 µg) or saline was co-administered alone or with dopamine (DA)-15 µg (DA15) directly into the NAc of male and female rats and LMA was measured. The groups and number of animals/group are shown in Table 1. The y-axis represents total distance covered in 60 mins after infusion. The statistical analysis procedure was done as described in the Methods section. 3-way ANOVA showed a difference in sex ($F_{1, 30}$ = 44.363, ***P < 0.0001), CART ($F_{2, 30}$ = 13.307, ***P<0.0001), DA ($F_{1, 30}$ = 51.251, ***P < 0.0001), CART x DA ($F_{2, 30}$ = 7.043, ***P = 0.003) but no differences in sex x CART ($F_{2, 30}$ = 0.000429, P = 1.000), sex x DA ($F_{1, 30}$ = 1.871, P = 0.182) and sex x CART x DA ($F_{2, 30}$ = 0.409, P = 0.668). Bonferroni *post hoc* tests showed that in both sexes

(a) DA15 increased LMA compared to DA0, (b) CART 1-27 did not blunt DA15-mediated LMA (c) CART 55-102 suppressed DA mediated LMA. Bonferroni *post hoc* tests showed that (a) basal and DA15-mediated LMA was higher in females than in males. The significant differences after *post hoc* analysis are shown on the graph. The p-values * show the differences when comparing DA0 to DA15 (controls) between CART treatment groups, and the p-values # show the difference when comparing saline, CART 1-27 and CART 55-102 within each DA-dose group. The p-values ¶ show a comparison between males and females within each DA-dose and CART treatment group.

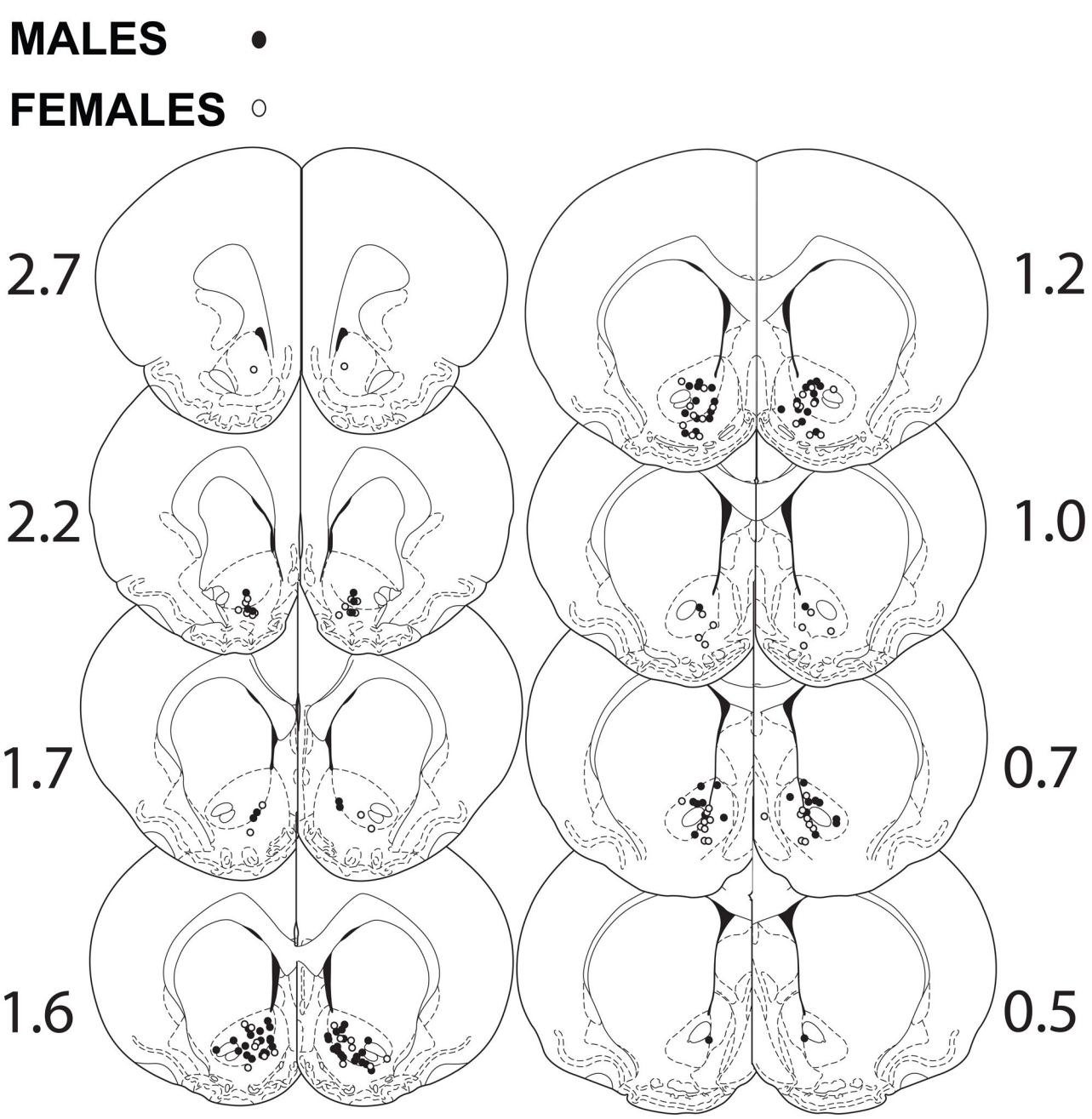

Fig. 4. Histological analysis of injector tip placements in the NAc

The numbers in the left and right columns of the histological diagram show the distances from Bregma as described in Methods. Males: filled circles. Females: open circles.

Table 1

Summary of animal use

Details of the experimental designs, treatment paradigms and the number of animals used in the different experiments. * Some animals used for the cocaine dose-response were re-used for the CART dose-response study.

Cocaine dose-response experiment

Treatment Saline and CART (counterbalanced design)	Cocaine (mg/kg ip)	Number of males used	Number of females used
	0	7	3
	5	3	4
	7.5	6	4
	10	6	4
	15	3	5
Total		25	20

Dopamine experiments

Treatment	Dopamine (μg/side)	Number of males used	Number of females used
Saline	0	4	3
	15	3	4
CART 1-27 (2.5 μg/side)	0	3	3
	15	5	3
CART 55-102 (2.5 μg/side)	0	3	3
	15	4	4
Total		22	20