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Injection of Cocaine-Amphetamine Regulated Transcript (CART) peptide into the nucleus accumbens does not inhibit caffeine induced locomotor activity: implications for CART peptide mechanism

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Abstract

Much evidence suggests that intra-nucleus accumbens (NAc) CART peptide (CART 55-102) injection inhibits locomotor activity (LMA) when there is an increase in the release and activity of dopamine (DA) in the NAc. However, this hypothesis has not been fully tested. One way to examine this is to determine if there is a lack of effect of intra-NAc CART peptide on LMA that does not involve increases in DA release in the NAc. Several studies have suggested that caffeine-induced LMA does not involve extracellular DA release in the NAc core. Therefore, in this study, we have examined the effect of injections of CART peptide (2.5 µg) into the NAc core on the locomotor effects of caffeine in male Sprague-Dawley rats. Several LMA relevant doses of caffeine were used (0, 10, 20 mg/kg i.p.), and an inverted U response curve was found as expected. We determined, in the same animals, that intra-NAc CART peptide had no effect on caffeine induced LMA whereas it blunted cocaine-mediated LMA, as shown by other reports. We also extended a previous observation in mice by showing that at a LMA activating dose of caffeine there is no alteration of CART peptide levels in the NAc of rats. Our study supports the hypothesis that the inhibitory effects of CART peptide in the NAc may be exerted only under conditions of increased extracellular DA release and activity in this region. Our results also suggest that intra-NAc CART 55-102 does not generally inhibit increases in LMA due to all drugs, but has a more specific inhibitory effect on dopaminergic neurotransmission.

Keywords

CART peptide; CART 55-102; caffeine; cocaine; dopamine; locomotion; nucleus accumbens

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Conflicts of Interest
None.

1 Introduction

The mechanism of Cocaine-Amphetamine Regulated Transcript peptide (CART peptide, CART 55-102) effect in the nucleus accumbens (NAc) on dopamine (DA)-mediated activity is not very clear. Injection of CART peptide into the NAc has no effect on spontaneous locomotion. But it inhibits locomotor activity (LMA) when there are increases in extracellular DA levels in the NAc, whether caused by systemic cocaine, intra-NAc amphetamine or intra-NAc DA administration (Jaworski *et al.*, 2003, Job *et al.*, 2014, Job *et al.*, 2013, Kim *et al.*, 2003), for review see (Rogge *et al.*, 2008). Furthermore, intra-NAc CART peptide inhibits LMA due to the co-administration, but not the individual administration, of selective DA D1 and D2 agonists into the NAc (Moffett *et al.*, 2011). Simultaneous activation of DA D1 and D2 receptors in the NAc simulates what occurs after an increase in DA release in this region (Dalia *et al.*, 1998). Thus, the conditions in which intra-NAc CART peptide had a blunting effect on DA-mediated LMA seem to include what occurs after an increase in the extracellular concentration of DA in the NAc.

However, this hypothesis has not been fully tested. One way to test this is to determine the effects of intra-NAc CART peptide on LMA under conditions that do not include an increase in extracellular DA in the NAc. A good model for this is caffeine-induced LMA. The NAc plays a role in systemic caffeine induced LMA: LMA activating doses of systemic caffeine alter NAc activity (Nehlig and Boyet, 2000, Svenningsson *et al.*, 1999), systemic caffeine augments LMA due to intra-NAc DA administration (Anden and Jackson, 1975) and modulation of intra-NAc opioid activity disrupts systemic caffeine-induced LMA (Layer *et al.*, 1991). However, DA release in the NAc is not involved in systemic caffeine induced LMA: destruction of DA terminals in the NAc had no effect on caffeine-mediated LMA (French and Vantini, 1984, Joyce and Koob, 1981, Swerdlow and Koob, 1985) and concomitant DA D1 and D2 activation in the NAc, which simulates what occurs after extracellular DA release in this region, is absent in caffeine-induced LMA (Dalia *et al.*, 1998). Because caffeine does not increase LMA through an increase in the release of DA in the NAc, intra-NAc CART peptide should have no effect on caffeine induced LMA.

While extracellular DA release in the NAc is not required for caffeine-induced LMA (Dalia *et al.*, 1998, French and Vantini, 1984, Joyce and Koob, 1981, Swerdlow and Koob, 1985), there is some evidence that systemic caffeine may increase extracellular DA levels in certain regions of the NAc shell (Borycz *et al.*, 2007, Malave and Broderick, 2014, Quarta *et al.*, 2004, Solinas *et al.*, 2002), though this is controversial (Acquas *et al.*, 2002, De Luca *et al.*, 2007). But what is important to note is that all studies support the observation that caffeine does *not* increase extracellular DA release in the NAc core (Acquas *et al.*, 2002, De Luca *et al.*, 2007, Solinas *et al.*, 2002). Therefore, the NAc core may be a good region to investigate caffeine-induced LMA without extracellular DA release.

In this study, we examined the effect of injections of CART peptide into the NAc core on the locomotor effects of both caffeine and cocaine in male Sprague-Dawley rats. To extend a previous observation in mice (Cho *et al.*, 2015), we also examined the effect of the injection of a LMA activating dose of caffeine on CART peptide levels in the NAc of rats.

2 Methods

2.1 Animals

A total of eleven male Sprague Dawley rats (Charles River Inc., Wilmington, MA), weighing 300-700g during the LMA experiments were used. Five of these animals were used for the investigation of the effects of intra-NAc CART peptide on caffeine-mediated LMA. Six of them were used for the confirmation of a lack of effect of LMA active doses of caffeine on CART peptide levels in the NAc. 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.

2.2 Surgery

The rats for the study of the effects of CART peptide on caffeine-induced LMA were implanted, under isoflurane anesthesia only (no other anesthetics or analgesics were used), with bilateral stainless steel guide cannulae assembly (22 gauge; Plastics One; Roanoke, VA) using the following coordinates: A/P 1.6mm, L/M $\pm$ 1.5mm, D/V -5.7mm (Paxinos and Watson, 1998) to place the guide cannulae 2.0 mm above the core 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 the LMA experiment.

2.3 Locomotor Activity Measurement

LMA was measured using a photocell cage (Omnitech Electronics, Columbus OH) with transparent plexiglass walls (dimensions of 40 × 40 × 30cm) containing 32 photobeams located 5cm above the floor. Each cage was connected to a computer equipped with software (Digipro; Omnitech Electronics) to measure LMA. The cages were placed in locomotor chambers.

On experiment day, rats were placed into the photocell cages in the locomotor chambers for 30 min to habituate to their surroundings before the recording of basal LMA commenced. After 30 min of basal LMA recording, rats were removed from the chambers and exposed to appropriate treatments and placed back into the chambers for additional LMA recording.

2.4 Drug administration

While CART peptide crosses the blood brain barrier and has systemic effects by unknown mechanisms (Job and Kuhar, 2012), we utilized intra-NAc injections to localize the effects of the peptide as mentioned in the Introduction. CART 55-102 was administered via the intra-accumbal route. Caffeine and cocaine were administered via the intraperitoneal (i.p.) route. Intra-accumbal infusions were done using stainless steel bilateral injector cannulae (28 gauge, Plastics One), connected via polyethylene-50 (PE-50) tubing to two 25 μ L microsyringes (Hamilton Co, Reno, NV). The two microsyringes were driven by pumps

connected to Micro4 Microsyringe Pump Controller (World Precision Instruments, Sarasota, FL). To inject, the obturator was removed and the injector cannulae was inserted in its place. Fluid was bilaterally injected (0.5 μ L/side) for 30 seconds. The injected fluid was allowed to diffuse for an additional 30 seconds before removal of the injector cannulae. After removal of the injector cannulae, the obturator was placed back into the guide.

2.5 LMA Experiment design

CART 55-102 (2.5 μ g/ 0.5 μ L) (or saline) was injected into the NAc immediately before an i.p. injection of caffeine (0, 10, 20 mg/kg). For this design, all rats ($n = 5$) received all treatments (counterbalanced design). For the same drug treatment, the CART peptide and control injections were administered at least 3-4 days apart. The different drug treatments were spaced 2-3 weeks apart, and were in the following order: caffeine (20 mg/kg), saline, caffeine (10 mg/kg). After the completion of the caffeine LMA study, the effect of intra-NAc CART peptide on systemic cocaine (10 mg/kg)-mediated LMA was done to confirm that CART peptide had functional activity in these animals (positive control). The experimental design is shown in Figure 1.

2.6 Perfusions and Histological analysis

After the completion of the experiments, the animals received an overdose of Ketamine (70 mg/kg) and dexmedetomidine (0.5 mg/kg), and were then perfused through the cardiovascular system with saline and 4% paraformaldehyde in saline. After removal from the skull, brains were placed in 4% paraformaldehyde overnight, followed by immersion in 30% sucrose for several days. Afterwards, the brains were sliced using a cryostat (Leica CM1900, Leica CM3050S, Leica Microsystems, Germany) set to obtain 60 μ m sections. Nissl staining was performed to further confirm placement of the injector tips. A stereotaxic brain atlas was used to approximate the placement of the injector tips.

2.7 Western blotting Experiment design

This experiment was done to show that at a dose relevant for LMA, caffeine does not alter endogenous CART peptide levels in rats, as shown in mice (Cho *et al.*, 2015). For this, caffeine (30 mg/kg) (or saline) was injected via the intraperitoneal (i.p.) route and 2 h later, animals were sacrificed and the western blotting was used to determine the levels of CART peptide in the NAc. We used this caffeine dose because it is within the range of LMA activating doses in rats. Additionally, this dose has been shown to significantly alter gene expression (NGFI-A and NGFI-B mRNA) in the NAc of Sprague Dawley rats (Svenningsson *et al.*, 1997). For this experiment, 6 rats were used. The rats were divided into saline and caffeine groups ($n = 3$ each).

2.8 Western blots

Details of the western blot procedure are as generally described in Rogge *et al.* (2009). Briefly, animals were given caffeine (30 mg/kg i.p.) and were sacrificed 2 h later by decapitation and the NAc was excised and stored in -80°C till western blot analysis. For western blot analysis, the nuclear proteins from the previously dissected NAc were separated from their cytoplasmic counterparts. 25 μ g of total protein (either nuclear or cytoplasmic),

determined by Bradford assay, were boiled for 5 min in 1:3 diluted 3×SDS sample buffer (187.5 mM Tris [pH 6.8 at 25 °C], 6% [w/v] SDS, 30% glycerol and 0.03% [w/v] bromophenol blue supplemented with 125 mM DTT [Cell signaling technology, Boston, MA]). The mixture was quickly centrifuged and run at 120-V for 2h on a 4–20% Biorad, pre-cast SDS–Tris–Glycine gel (Biorad, Hercules, CA). After overnight transfer at 30-V and 4 °C, the membranes were blocked with 5% non-fat milk in 1× TBS-T (Tris-Buffered Saline, 0.1% Tween-20 [pH 7.6]) for 2 h, and then incubated with the primary antibody overnight. The primary antibody used was CART anti-rabbit polyclonal antibody (produced by the Kuhar lab, Emory University, Atlanta, GA). The signal was detected by using horseradish peroxidase (HRP)-conjugated anti-rabbit antibodies (Cell signaling technology, Boston, MA) and an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL) (Rogge *et al.*, 2009).

2.9 Quantification of CART peptide expression

Western blot raw data were digitally scanned into computer files. Image J software (NIH, Bethesda, MD) was subsequently used to quantify the optical densities of individual Western bands. We used the Kodak #3 calibrated step tablet for grayscale (8-bit) in ImageJ to generate a standard curve to convert raw grayscale values (0-255) into optical density values. The band corresponding to the region of interest was outlined and measured. Thereafter, a geometrically identical area above the band of interest, but separate from other bands in the same lane and separate from the band of interest as well, was measured as ‘background’. The ‘background’ optical density was subtracted from the band of interest's optical density to calculate the optical density of the Western band of interest.

2.10 Statistical Analysis

Data are all expressed as mean ± SEM, and significance was set at $\alpha < 0.05$. Statistical analyses were done using SigmaPlot for Windows v 13.0 (Systat Software Inc, San Jose, CA). For the study regarding the effect of intra-NAc CART peptide on caffeine-mediated LMA, total LMA and time course data analyses were performed. Total LMA data was defined as total distance traveled (in cm) over 90 min and was analyzed using 2-way repeated measures ANOVA. For the positive control study confirming the efficacy of intra-NAc CART peptide in blunting systemic cocaine induced LMA, paired t-tests were used. Time course data were analyzed as total distance traveled (in cm) in 15 min bins and analyzed for each treatment using 2-way repeated measures ANOVA. All data satisfied criteria for equality of variance and normality except the time course data for cocaine-induced LMA which had to be log transformed to satisfy these requirements. The injection sites were analyzed via visual observations of the coordinates with reference to a rat brain atlas (Paxinos and Watson, 1998). For the experiment to confirm a lack of effect of caffeine on CART peptide levels in the NAc, the densitometry data was analyzed using t-tests, with saline and caffeine as independent variables and CART peptide density as the dependent variable.

3 Results

Animals were prepared as described in Methods. Rats were given systemic caffeine at doses of 0, 10, and 20 mg/kg i.p., after saline injections into the NAc. Caffeine-induced LMA displayed an inverted U dose-response result, as expected. Compared to intra-NAc saline administration, when CART peptide (2.5 µg) was administered into the NAc and caffeine was injected i.p., there were no significant changes. Statistical analysis (2-way repeated measures ANOVA) showed no effect of intra-NAc CART peptide ($F_{1,4} = 0.703$, $P = 0.449$). Thus, CART peptide did not appear to affect the LMA induced by systemic caffeine (Fig 2A). In these same animals showing no effect of CART peptide on caffeine induced LMA, CART peptide attenuated the LMA effects of cocaine (paired t-test, $P = 0.003$) (Fig 2B).

An examination of the time course shows that CART peptide altered the cocaine-, but not saline- and caffeine-induced LMA (Fig 3). Statistical analysis (2-way repeated measures ANOVA) showed an effect of intra-NAc CART peptide ($F_{1,4} = 29.805$, $P = 0.005$) only for cocaine (Fig 3D) and not for any other treatments ($P > 0.05$). Thus, intra-NAc CART peptide affected the time course of the LMA induced by cocaine, but not caffeine.

Also, histology examination of the placement of the cannulae for CART peptide injection revealed consistent placement in the core of the NAc (Fig 4).

We also tested if an LMA relevant dose of caffeine changed the CART peptide levels in the NAc. NO effect was found (T-tests, $P = 0.970$) in NAc CART peptide content between saline vs caffeine groups (Fig 5), corroborating a similar study in mice (Cho *et al.*, 2015).

4 Discussion

The results show that intra-NAc core CART peptide has no effect on caffeine-mediated LMA under conditions where it blunted cocaine-induced LMA. Our experiments also confirmed that a motor activating dose of caffeine did not alter endogenous CART peptide levels in the NAc, corroborating a previous report (Cho *et al.*, 2015). Our results replicated other results showing that caffeine exerts a biphasic (an inverted 'U'-shaped) dose-response curve on LMA (Antoniou *et al.*, 1998, Collins *et al.*, 2010, Cook and Beardsley, 2003, El Yacoubi *et al.*, 2000, File *et al.*, 1988, Garrett and Holtzman, 1994, Halldner *et al.*, 2004, Kaplan *et al.*, 1992, Kuzmin *et al.*, 2006, Marin *et al.*, 2011, Powell *et al.*, 2001, Rhoads *et al.*, 2011, Solinas *et al.*, 2002, Svenningsson *et al.*, 1995, Zhang *et al.*, 2011), and buttress other studies that show that intra-NAc CART peptide blunts cocaine-mediated LMA (Jaworski *et al.*, 2003, Job *et al.*, 2012, Job *et al.*, 2014, Job *et al.*, 2013).

The differences observed in the effects of intra-NAc CART peptide on caffeine and cocaine may be related to the effects of these two drugs on extracellular DA release, and its consequences, in the NAc core. Cocaine, but not caffeine, increases extracellular DA release in the NAc core: in male Sprague-Dawley rats, systemic cocaine (10 mg/kg i.p.) increases extracellular DA release in the NAc core (Tanda *et al.*, 2005, Tanda *et al.*, 2007), whereas systemic caffeine (10-30 mg/kg i.p.) does not (Acquas *et al.*, 2002, De Luca *et al.*, 2007, Solinas *et al.*, 2002).

A consequence of extracellular DA release in the NAc a simultaneous activation of DA D1 and D2 receptors in this region (Dalia *et al.*, 1998), which is a necessary condition for CART peptide to exert inhibitory effects on LMA (Moffett *et al.*, 2011). Interestingly, another consequence of extracellular DA release and simultaneous DA D1 and D2 receptor activation is an increase in extracellular glutamate (GLUT) levels in the NAc (Dalia *et al.*, 1998, Reid *et al.*, 1997, Smith *et al.*, 1995). Interestingly, cocaine (Reid *et al.*, 1997), but not caffeine (Dalia *et al.*, 1998, Solinas *et al.*, 2002), increases extracellular DA and GLUT levels in the NAc core. This observation further corroborates the evidence that caffeine does not increase DA levels in the NAc core by showing that it also does not consequentially increase extracellular GLUT in this region (Dalia *et al.*, 1998, Solinas *et al.*, 2002). The distinctions in extracellular GLUT activity in the NAc core between cocaine and caffeine is buttressed by pharmacological and behavioral evidence showing that intra-NAc core injection of a non-selective GLUT antagonist (Pulvirenti *et al.*, 1989) and a selective GLUT NMDA receptor antagonist inhibited cocaine-induced LMA (Pulvirenti *et al.*, 1994), but had no effect on caffeine-induced LMA (Pulvirenti *et al.*, 1991). Intriguingly, these reports are similar to our results showing that intra-NAc core CART peptide attenuates cocaine induced LMA but not caffeine mediated motor activity, suggesting that extracellular DA and/or downstream extracellular DA-GLUT mechanisms may be involved in CART peptide activity in the NAc.

In this study, the cocaine-induced LMA experiment was conducted as a positive control to confirm that CART peptide had functional activity in these animals. However, the cocaine challenge was conducted *after* caffeine treatments, in the same animals. Prior caffeine exposure can alter subsequent DA induced behavioral activity. For example, rats showed potentiation of LMA due to DA D1 agonist (SKF 77434) (Cauli and Morelli, 2002) and acute amphetamine (Simola *et al.*, 2006a, Simola *et al.*, 2006b, Tronci *et al.*, 2006) three days after cessation of a regimen of intermittent systemic caffeine (15 mg/kg i.p.) administration (once every two days for a total of only seven injections). Relatedly, rats showed potentiation of acute methamphetamine induced LMA a week after cessation of prior intermittent systemic adenosine (i.p.) injections (once every three days for a total of only five injections) (Kawashimo *et al.*, 2000). Thus, we cannot exclude the possibility that the prior caffeine administrations led to a sensitization of the cocaine induced LMA such that instead of simply inhibiting the behavioral effects of cocaine, CART peptide administration into the NAc could have prevented caffeine induced potentiating effects on cocaine-induced LMA (Figure 2B).

Caffeine alters LMA at low doses (10-30 mg/kg), and at higher doses (50 mg/kg), it exerts anxiogenic effects (Baldwin and File, 1989, Bhattacharya *et al.*, 1997, Collins *et al.*, 2010, Cook and Beardsley, 2003, El Yacoubi *et al.*, 2000, Garrett and Holtzman, 1994, Halldner *et al.*, 2004, Kaplan *et al.*, 1992, Kuzmin *et al.*, 2006, Patki *et al.*, 2015, Solinas *et al.*, 2002, Svenningsson *et al.*, 1995, Zhang *et al.*, 2011). Higher doses of caffeine alter CART peptide levels in the NAc (Cho *et al.*, 2015). Lower doses of caffeine do not alter CART peptide expression in the NAc in mice (Cho *et al.*, 2015). By showing that caffeine does not alter endogenous CART peptide in the NAc at a LMA relevant dose in our study with rats, we extend the findings of a previous report in mice (Cho *et al.*, 2015).

In conclusion, the inhibitory effects or the lack thereof of intra-NAc core CART peptide on cocaine and caffeine induced LMA, respectively, may depend partly on the differences in how these drugs affect extracellular DA release and activity. Importantly, intra-NAc CART peptide effect is conditional. CART peptide does not nonspecifically inhibit increases in LMA due to all drugs, but has a more specific inhibitory effect on dopaminergic neurotransmission.

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References

- Acquas E, Tanda G, Di Chiara G. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naïve and caffeine-pretreated rats. *Neuropsychopharmacology* : official publication of the American College of Neuropsychopharmacology. 2002; 27:182–93. [PubMed: 12093592]
- Anden NE, Jackson DM. Locomotor activity stimulation in rats produced by dopamine in the nucleus accumbens: potentiation by caffeine. *The Journal of pharmacy and pharmacology*. 1975; 27:666–70. [PubMed: 241810]
- Antoniou K, Kafetzopoulos E, Papadopoulou-Daifoti Z, Hyphantis T, Marselos M. D-amphetamine, cocaine and caffeine: a comparative study of acute effects on locomotor activity and behavioural patterns in rats. *Neuroscience and biobehavioral reviews*. 1998; 23:189–96. [PubMed: 9884112]
- Baldwin HA, File SE. Caffeine-induced anxiogenesis: the role of adenosine, benzodiazepine and noradrenergic receptors. *Pharmacol Biochem Behav*. 1989; 32:181–6. [PubMed: 2543990]
- Bhattacharya SK, Satyan KS, Chakrabarti A. Anxiogenic action of caffeine: an experimental study in rats. *Journal of psychopharmacology*. 1997; 11:219–24. [PubMed: 9305413]
- Borycz J, Pereira MF, Melani A, Rodrigues RJ, Kofalvi A, Panlilio L, et al. Differential glutamate-dependent and glutamate-independent adenosine A1 receptor-mediated modulation of dopamine release in different striatal compartments. *Journal of neurochemistry*. 2007; 101:355–63. [PubMed: 17254024]
- Cauli O, Morelli M. Subchronic caffeine administration sensitizes rats to the motor-activating effects of dopamine D(1) and D(2) receptor agonists. *Psychopharmacology (Berl)*. 2002; 162:246–54. [PubMed: 12122482]
- Cho JH, Cho YH, Kim HY, Cha SH, Ryu H, Jang W, et al. Increase in cocaine- and amphetamine-regulated transcript (CART) in specific areas of the mouse brain by acute caffeine administration. *Neuropeptides*. 2015
- Collins LE, Galtieri DJ, Collins P, Jones SK, Port RG, Paul NE, et al. Interactions between adenosine and dopamine receptor antagonists with different selectivity profiles: Effects on locomotor activity. *Behavioural brain research*. 2010; 211:148–55. [PubMed: 20211657]
- Cook CD, Beardsley PM. The modulatory actions of dopamine D2/3 agonists and antagonists on the locomotor-activating effects of morphine and caffeine in mice. *Pharmacol Biochem Behav*. 2003; 75:363–71. [PubMed: 12873628]
- Dalia A, Uretsky NJ, Wallace LJ. Dopaminergic agonists administered into the nucleus accumbens: effects on extracellular glutamate and on locomotor activity. *Brain Res*. 1998; 788:111–7. [PubMed: 9554973]
- De Luca MA, Bassareo V, Bauer A, Di Chiara G. Caffeine and accumbens shell dopamine. *Journal of neurochemistry*. 2007; 103:157–63. [PubMed: 17655660]

- El Yacoubi M, Ledent C, Menard JF, Parmentier M, Costentin J, Vaugeois JM. The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. *British journal of pharmacology*. 2000; 129:1465–73. [PubMed: 10742303]
- File SE, Baldwin HA, Johnston AL, Wilks LJ. Behavioral effects of acute and chronic administration of caffeine in the rat. *Pharmacol Biochem Behav*. 1988; 30:809–15. [PubMed: 3227034]
- French ED, Vantini G. Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: comparisons to other psychomotor stimulants. *Psychopharmacology (Berl)*. 1984; 82:83–8. [PubMed: 6141586]
- Garrett BE, Holtzman SG. D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. *Pharmacol Biochem Behav*. 1994; 47:89–94. [PubMed: 7906891]
- Halldner L, Aden U, Dahlberg V, Johansson B, Ledent C, Fredholm BB. The adenosine A1 receptor contributes to the stimulatory, but not the inhibitory effect of caffeine on locomotion: a study in mice lacking adenosine A1 and/or A2A receptors. *Neuropharmacology*. 2004; 46:1008–17. [PubMed: 15081797]
- Jaworski JN, Kozel MA, Philpot KB, Kuhar MJ. Intra-accumbal injection of CART (cocaine-amphetamine regulated transcript) peptide reduces cocaine-induced locomotor activity. *J Pharmacol Exp Ther*. 2003; 307:1038–44. [PubMed: 14551286]
- Job MO, Kuhar MJ. Intraperitoneal Administration of CART 55-102 Inhibits Psychostimulant-Induced Locomotion. *J Drug Alcohol Res*. 2012;1.
- 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, Perry J, Shen LL, Kuhar MJ. Cocaine-and-Amphetamine Regulated Transcript (CART) peptide attenuates dopamine- and cocaine-mediated locomotor activity in both male and female rats: lack of sex differences. *Neuropeptides*. 2014; 48:75–81. [PubMed: 24630272]
- 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; 550:179–83. [PubMed: 23819981]
- Joyce EM, Koob GF. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. *Psychopharmacology (Berl)*. 1981; 73:311–3. [PubMed: 6789349]
- Kaplan GB, Greenblatt DJ, Kent MA, Cotreau MM, Arcelin G, Shader RI. Caffeine-induced behavioral stimulation is dose-dependent and associated with A1 adenosine receptor occupancy. *Neuropsychopharmacology*. 1992; 6:145–53. [PubMed: 1599605]
- Kawashimo A, Shimazoe T, Yoshimatsu A, Watanabe S. Repeated adenosine pre-treatment potentiates the acute effect of methamphetamine in rats. *Japanese journal of pharmacology*. 2000; 84:78–81. [PubMed: 11043458]
- Kim JH, Creekmore E, Vezina P. Microinjection of CART peptide 55-102 into the nucleus accumbens blocks amphetamine-induced locomotion. *Neuropeptides*. 2003; 37:369–73. [PubMed: 14698680]
- Kuzmin A, Johansson B, Gimenez L, Ogren SO, Fredholm BB. Combination of adenosine A1 and A2A receptor blocking agents induces caffeine-like locomotor stimulation in mice. *Eur Neuropsychopharmacol*. 2006; 16:129–36. [PubMed: 16054807]
- Layer RT, Uretsky NJ, Wallace LJ. Effects of morphine in the nucleus accumbens on stimulant-induced locomotion. *Pharmacol Biochem Behav*. 1991; 40:21–6. [PubMed: 1685784]
- Malave LB, Broderick PA. Caffeine's Attenuation of Cocaine-Induced Dopamine Release by Inhibition of Adenosine. *Journal of caffeine research*. 2014; 4:35–40. [PubMed: 25054079]
- Marin MT, Zancheta R, Paro AH, Possi AP, Cruz FC, Planeta CS. Comparison of caffeine-induced locomotor activity between adolescent and adult rats. *European journal of pharmacology*. 2011; 660:363–7. [PubMed: 21497160]
- 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]

- Nehlig A, Boyet S. Dose-response study of caffeine effects on cerebral functional activity with a specific focus on dependence. *Brain research*. 2000; 858:71–7. [PubMed: 10700599]
- Patki G, Salvi A, Liu H, Atrooz F, Alkadhi I, Kelly M, et al. Tempol treatment reduces anxiety-like behaviors induced by multiple anxiogenic drugs in rats. *PLoS One*. 2015; 10:e0117498. [PubMed: 25793256]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 4th edition. Academic Press; Burlington, MA: 1998.
- Powell KR, Iuvone PM, Holtzman SG. The role of dopamine in the locomotor stimulant effects and tolerance to these effects of caffeine. *Pharmacol Biochem Behav*. 2001; 69:59–70. [PubMed: 11420069]
- Pulvirenti L, Berrier R, Kreifeldt M, Koob GF. Modulation of locomotor activity by NMDA receptors in the nucleus accumbens core and shell regions of the rat. *Brain Res*. 1994; 664:231–6. [PubMed: 7895034]
- Pulvirenti L, Swerdlow NR, Koob GF. Microinjection of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. *Neuroscience letters*. 1989; 103:213–8. [PubMed: 2771183]
- Pulvirenti L, Swerdlow NR, Koob GF. Nucleus accumbens NMDA antagonist decreases locomotor activity produced by cocaine, heroin or accumbens dopamine, but not caffeine. *Pharmacol Biochem Behav*. 1991; 40:841–5. [PubMed: 1687766]
- Quarta D, Ferre S, Solinas M, You ZB, Hockemeyer J, Popoli P, et al. Opposite modulatory roles for adenosine A1 and A2A receptors on glutamate and dopamine release in the shell of the nucleus accumbens. Effects of chronic caffeine exposure. *Journal of neurochemistry*. 2004; 88:1151–8. [PubMed: 15009670]
- Reid MS, Hsu K Jr, Berger SP. Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. *Synapse*. 1997; 27:95–105. [PubMed: 9266771]
- Rhoads DE, Huggler AL, Rhoads LJ. Acute and adaptive motor responses to caffeine in adolescent and adult rats. *Pharmacol Biochem Behav*. 2011; 99:81–6. [PubMed: 21504758]
- Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ. CART peptides: regulators of body weight, reward and other functions. *Nat Rev Neurosci*. 2008; 9:747–58. [PubMed: 18802445]
- Rogge GA, Jones DC, Green T, Nestler E, Kuhar MJ. Regulation of CART peptide expression by CREB in the rat nucleus accumbens in vivo. *Brain Res*. 2009; 1251:42–52. [PubMed: 19046951]
- Simola N, Cauli O, Morelli M. Sensitization to caffeine and cross-sensitization to amphetamine: influence of individual response to caffeine. *Behavioural brain research*. 2006a; 172:72–9. [PubMed: 16740323]
- Simola N, Tronci E, Pinna A, Morelli M. Subchronic-intermittent caffeine amplifies the motor effects of amphetamine in rats. *Amino acids*. 2006b; 31:359–63. [PubMed: 16874467]
- Smith JA, Mo Q, Guo H, Kunko PM, Robinson SE. Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. *Brain Res*. 1995; 683:264–9. [PubMed: 7552364]
- Solinas M, Ferre S, You ZB, Karcz-Kubicha M, Popoli P, Goldberg SR. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2002; 22:6321–4. [PubMed: 12151508]
- Svenningsson P, Nomikos GG, Fredholm BB. Biphasic changes in locomotor behavior and in expression of mRNA for NGFI-A and NGFI-B in rat striatum following acute caffeine administration. *J Neurosci*. 1995; 15:7612–24. [PubMed: 7472512]
- Svenningsson P, Nomikos GG, Fredholm BB. The stimulatory action and the development of tolerance to caffeine is associated with alterations in gene expression in specific brain regions. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1999; 19:4011–22. [PubMed: 10234030]
- Svenningsson P, Nomikos GG, Ongini E, Fredholm BB. Antagonism of adenosine A2A receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate-putamen and nucleus accumbens. *Neuroscience*. 1997; 79:753–64. [PubMed: 9219939]

- Swerdlow NR, Koob GF. Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat. *Pharmacol Biochem Behav.* 1985; 23:303–7. [PubMed: 3877311]
- Tanda G, Ebbs A, Newman AH, Katz JL. Effects of 4'-chloro-3 alpha-(diphenylmethoxy)-tropane on mesostriatal, mesocortical, and mesolimbic dopamine transmission: comparison with effects of cocaine. *J Pharmacol Exp Ther.* 2005; 313:613–20. [PubMed: 15681658]
- Tanda G, Ebbs AL, Kopajtic TA, Elias LM, Campbell BL, Newman AH, et al. Effects of muscarinic M1 receptor blockade on cocaine-induced elevations of brain dopamine levels and locomotor behavior in rats. *J Pharmacol Exp Ther.* 2007; 321:334–44. [PubMed: 17255465]
- Tronci E, Simola N, Carta AR, De Luca MA, Morelli M. Potentiation of amphetamine-mediated responses in caffeine-sensitized rats involves modifications in A2A receptors and zif-268 mRNAs in striatal neurons. *Journal of neurochemistry.* 2006; 98:1078–89. [PubMed: 16771831]
- Zhang Q, Yu YP, Ye YL, Zhang JT, Zhang WP, Wei EQ. Spatiotemporal properties of locomotor activity after administration of central nervous stimulants and sedatives in mice. *Pharmacol Biochem Behav.* 2011; 97:577–85. [PubMed: 20863845]

Highlights

- Intra-nucleus accumbens (NAc) CART peptide blunts drug-induced locomotion (LMA).
- Intra-NAc CART peptide seems to blunt only LMA involving extracellular DA release.
- Caffeine induced LMA does not require extracellular DA release in the NAc core.
- Intra-NAc core CART peptide does not blunt caffeine induced LMA
- Intra-NAc CART peptide effect on drug-induced LMA is both specific and conditional.

Figure 1. Experiment details

The graph A represents the experiment design. Here rats were given pretreatments of saline and/or CART peptide in counterbalanced design immediately before different doses of systemic caffeine (0, 10, 20 mg/kg i.p.). The graph B represents the order of the caffeine doses and the duration between caffeine treatments. It also includes the time interval between the last caffeine treatment and the cocaine treatment (positive control). For each caffeine or cocaine treatment, animals were pretreated with saline and/or CART peptide, in counterbalanced design (see graph A). The graph C represents the schedule of an experiment (see Methods section for details).

Figure 2. Intra-NAc CART 55-102 has no effect on caffeine-induced LMA: total LMA data
For both graphs, the y-axis shows the total distance traveled in 90 min after intra-NAc injection of CART 55-102 (2.5 μ g/ 0.5 μ L) (or saline) immediately followed by an i.p. injection of different caffeine doses (0, 10, 20 mg/kg i.p.) (Fig 2A) or cocaine (10 mg/kg i.p.) (Fig 2B). The same animals (n = 5) were used for all drug treatments. The asterisk (*) shows significant differences when comparing caffeine doses to saline (Fig 2A). The (#) shows significant differences when comparing saline to CART peptide (Fig 2B). Intra-NAc

CART peptide inhibited cocaine (10 mg/kg i.p.) -mediated LMA but had no effect on caffeine-induced LMA.

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Figure 3. Intra-NAc CART 55-102 has no effect on the time course of caffeine-induced LMA
The graphs represent the time course data of the animals in Fig 2. The y-axis shows the total distance traveled while the x-axis shows time (min). The arrow shows the injection point (intra-NAc CART peptide followed immediately by systemic saline, caffeine or cocaine). The P values are written on the graphs.

Bregma 1.6 mm

Figure 4. Histological analysis of injector tip placements in the NAc core

The closed circles are representative of the locations of the injector tips in the NAc core for the 5 rats used for assessing the effects of CART peptide on both caffeine-induced LMA and cocaine-induced LMA.

A.

B.

Figure 5. Systemic caffeine does not alter CART peptide levels in the NAC

Rats were administered either saline i.p. (n = 3) or caffeine (30 mg/kg i.p., n = 3) and were sacrificed 2 h later. CART peptide levels were analyzed using the western blot technique. Fig 4A shows the western blot data for all the rats including the treatments. Fig 4B shows the average of the optical density measurements in Fig 4A. In Fig 4B, the y-axis shows CART peptide optical density while the x-axis shows the treatment groups (saline, caffeine).