Cocaine- and Amphetamine-Regulated Transcript (CART) Peptides Modulate the Locomotor and Motivational Properties of Psychostimulants

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ABSTRACT

Drug addiction results from a subversion of neural circuits that control motivation. Although the hedonic and addictive properties of psychostimulants and drugs of abuse are predominantly attributed to dopamine and glutamate, it is appreciated that other signaling molecules in the brain are important. This study suggests that cocaine- and amphetamine-regulated transcript (CART) peptides modulate the locomotor and motivational properties of psychostimulants. The behavioral effects of cocaine and amphetamine were examined in Carttm1Amgen knockout (Cart KO) and wild-type (WT) mice. Acute amphetamine administration increased in locomotor activity in WT mice, but this response was attenuated in Cart KO mice. Repeated amphetamine produced locomotor sensitization in WT mice, but this response was attenuated in Cart KO mice. Repeated amphetamine produced locomotor sensitization in WT mice but hardly any in Cart KO mice. Amphetamine elicited conditioned place preference in both genotypes, but amphetamine’s potency was reduced in the Cart KO mice. Intravenous cocaine self-administration was observed in both genotypes, but Cart KO mice consumed less cocaine and responded less for cocaine than WT mice. The behavioral effects of psychostimulants were reduced in the mutant Cart KO mice. By contrast, open field activity and sucrose preference of drug-naive mice WT and Cart KO mice were not significantly different. The attenuated effects of amphetamine and cocaine in Cart KO mice suggest a positive neuromodulatory role for CART peptides in the locomotor and motivational properties of psychostimulants and implicate CART peptides in psychostimulant addiction.
lar to that of the rodent but more extensive in the neocortex (Douglass et al., 1995; Hurd and Fagergren, 2000). The neu-renchymal distribution of CART peptides suggests that they participate in locomotor activity, the appraisals of re-warding/reinforcing stimuli, and in learning associated with habitation that is critical in the transition from drug use to drug addiction (Kelley, 2004).

Behavioral and neurochemical studies support a role for CART peptides in the locomotor activity and motivation. Exogenous and endogenous CART peptides suppress food intake much like psychostimulants (Kristensen et al., 1998; Lambert et al., 1998). This anorectic action coupled with the psychostimulant regulation of CART expression in the striatum, which occurs specifically within the nAcc (Hurd et al., 1999), first suggested that CART peptides are endogenous stimulant-like compounds. Subsequent studies showed that injection of CART peptide 55-102 into the VTA increased locomotor activity and produced conditioned place preference in rats (Kimmel et al., 2000); other CART peptides have similar effects on locomotion (Bannon et al., 2001; Kimmel et al., 2002). Intracerebroventricular injection of CART peptide 55-102 increased dopamine turnover in dopaminergic terminal fields, including the nAcc and dorsal striatum (Yang et al., 2004). Thus, CART peptides affect locomotion and motivation on their own or in concert with other transmitter systems. CART peptides are also important in anxiety, pain, arousal, startle response, regulation of calcium channels, and neuroendocrine hormone secretion (Kask et al., 2000; Ban-Non et al., 2001; Yermolaieva et al., 2001; Chaki et al., 2003; Smith et al., 2004). Thus, the molecular, anatomical, and behavioral data show a role for CART peptides in motivation and implicate them in the behavioral properties of psycho-stimulants.

To explore the role of CART peptides in actions of psycho-stimulants, we compared the behavioral effects of psycho-stimulants in adult, male wild-type (WT) and Carttm1Amgen knockout (Cart KO) mice. The lack of pharmacological agents, particularly antagonists, to block CART peptide activ-ity prompted the use of mutant mice lacking CART pep-tides. These studies show that psychostimulant-mediated in-creases in locomotor activity, locomotor sensitization, conditioned place preference, and intravenous drug self-ad-ministration are attenuated in Cart KO mice as compared with WT mice. They suggest that the behavioral effects of psychostimulants are modulated by CART peptides. These data are the first to show a causal link between CART pep-tides and the locomotor and motivational effects of psycho-stimulants.

**Materials and Methods**

All animal procedures were done in accordance with the rules established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approval of the Insti-tutional Animal Care and Use Committee of the Rosalind Franklin University of Medicine and Science/Chicago Medical School.

**Materials.** Oligonucleotides were purchase from Sigma-Genosys (Woodlands, TX). d-Amphetamine, cocaine hydrochloride, heparin, and sucrose were purchased from Sigma-Aldrich (St. Louis, MO). Ketamine and xylazine were purchased from Burns Veterinary Sup-ply, Inc. (Elk Grove, IL). Gentamicin was purchased from ICN Bio-medicals (Aurora, OH). Bullet cameras and video splitters were purchased from Security Depot (Miami, FL). The digital video device was a Philips Progressive Scan DVD75 (Chicago, IL). The intrave-nous silastic catheter was purchased (Fisher Scientific, Pittsburgh, PA). Operant activity boxes were purchased from MED Associates (St. Albans, VT).

**Nomenclature.** CART refers to the mRNA or gene, whereas the encoded protein/peptide products are referred to as CART peptide. Cart is used to denote the mouse mRNA or gene. The CART peptide numbering system is based on the 102-amino acid propeptide or the long variant in rat as originally described by Douglass et al. (1994).

**Drug-induced Locomotion Studies.** Amphetamine was selected for the locomotor studies because of the extensive literature and history on its effects, especially in mice, where there is often variability among the strains (Shuster et al., 1977; Phillips et al., 1994). Mice were given amphetamine in a novel environment because psychostimulants and drugs of abuse produce more robust locomotor responses in novel environment rather than familiar ones such as the home cage (Badiani et al., 1995).

Mice were handled (i.e., held) for 1 to 2 min daily for the 3 days prior to testing. On the test day, mice were transferred to the test room and immediately placed in the center of an open field (60 × 60 × 40 cm, LWH) made of an aluminum floor, black Plexiglas walls, and an open top. Their behavior over 60 min was videotaped. One mouse was tested at a time, and the order of testing alternated between genotypes. The floor and walls of the test box were cleaned with hot water and dried in between tests. The test was conducted between 9:00 AM and 3:00 PM.

Open field activity was measured by counting the number of entries or crosses made into different areas or quadrants of the field, the number of rears, and the time spent in along the wall (i.e., thigmotaxis) in the center of the field. The open field was divided into nine equal quadrants. Thigmotaxis was counted only when the mouse was within one-body width of the wall. Locomotor activity over the first 10 min and then for the first 2 min of every 10-min bin was assessed and is reported. Two independent investigators blind to genotype analyzed the videotapes.
activity system with two photobeams 3 cm from the floor from which ambulation was calculated and six beams located 6 cm from the floor from which vertical activity score was calculated (San Diego Instruments, San Diego, CA). Mouse activity was monitored daily with this photobeam system for the 30 min of habituation or time before receiving an injection and for 2 h after an i.p. injection of saline or d-amphetamine dissolved in saline (1 ml/kg). Mouse activity was digitally recorded with cameras every other day onto DVDs with a digital recorder device. Locomotor activity was defined as ambulation, vertical activity, stereotypic grooming, and stereotyped head-bobs. Ambulation and vertical activity measurements were derived from a photobeam activity system. Stereotypies quantified from visual inspection of the video records. Stereotypies were defined as continuous, repeated movements produced in one location. The duration, and not the number of bouts, of each stereotypy were scored for the 1st min of every 10 min over 2 h after each injection. Three independent investigators blind to genotype scored the stereotypies. The scores represent the mean of three independent investigators’ scores. There was over a 90% concordance between investigator-reported scores.

Conditioned Place Preference (CPP). Amphetamine was chosen for this study, in part, because it reliably elicits CPP, even in mice where strain variability is prevalent (Belzung and Barreau, 2000). CPP was conducted with the biased method for drug conditioning (i.e., drug was paired with least preferred preconditioning environment). A three-chambered Plexiglas box (22 × 14 × 15 cm LWH, per chamber) with different texture floors was used. The textured floors were made of rods (4-mm diameter stainless steel rods spaced 8 mm apart) or mesh (1-mm diameter stainless steel thread-weave) in 4 mm × 4 mm squares). The textured floor chambers were positioned on either side of a smooth, white Plexiglas floored chamber, and sliding doors separated each. Three of the four walls of the textured floor chambers were black, and one was transparent, through which the mice were videotaped. The top of the chamber had a transparent Plexiglas ceiling. Two separate CPP boxes were placed in an open test room with low ambient lighting (1.5- to 2.5-ft candles); the room did not house any animals when these experiments were conducted. Mice were first habituated to handling and i.p. saline injections as follows. Mice were held daily for 1 to 2 min for 3 days between 9:00 and 10:00 AM. On the 2nd and 3rd day of handling, mice were given saline i.p. 4 to 6 h after being handled. On day 4, an initial assessment of chamber preference was conducted. Mice were given access to all three chambers for 20 min. Their activity was videotaped, and the time spent in each of the chamber was measured and reported as the preconditioning chamber preference. Mice that spent greater than 15% more time in any one chamber compared with the other two were eliminated from the study. Of the remaining mice, those with the strongest preconditioning chamber bias were used in the saline control group. The chamber walls were cleaned with hot water, and the removable floors and underlying waste bin were cleaned with soap and water after each session. On day 5, mice were exposed to all three chambers as one the previous day, but the activity was not monitored. On days 6 to 9, drug conditioning was performed. On each day between 9:00 AM to 12:00 PM, mice were injected with saline i.p. and confined for 45 min in the most preferred textured floor chambers, and access to the other chambers was blocked. Between 12:00 to 3:00 PM, mice were injected with d-amphetamine i.p. and confined for 45 min to the least preferred textured floor chamber, and access to the other chambers was blocked. A minimum of 3 h was allowed between saline and drug conditioning. On day 11, place preference was determined between 12:00 to 3:00 PM by giving mice access to all three chambers for 20 min and videotaping the activity. No injections were given prior to this test. Place preference was determined by subtracting the amount of time spent in the drug-paired compartment before drug conditioning (on day 4) from the time spent there after drug conditioning (on day 11).

In this study, CPP was produced with the biased method (i.e., drug conditioning is performed in the least preferred preconditioning compartment) to enhance the possibility of observing place preference. This biased protocol is questioned because it may measure the removal of aversion to an environment or reveal an anxiolytic effect of the drug instead of measuring a positive hedonic effect (Carr et al., 1989); however, this criticism is now being questioned (Cunningham et al., 2003). Interestingly, the unbiased CPP method must contend with this issue when an animal is assigned, albeit randomly, to the least preferred environment for drug conditioning. A ceiling effect is also a problem for the unbiased method when animals are randomly assigned to the most preferred preconditioning chamber for drug conditioning. Although neither method is ideal, these problems are resolved by using a CPP box with minimal chamber bias (Bardo and Bevins, 2000). The three-chambered CPP box used in these experiments produced a small preconditioning chamber bias (Fig. 1). The middle chamber with the smooth floor (31.3 ± 0.9% preference) was preferred less than the mesh floor (34.0 ± 0.9% preference) or rod floor (34.7 ± 0.9% preference) [two-factor analysis of variance (ANOVA): floor preference, F$_{2,129}$ = 14.6, p < 0.05; floor preference X genotype, F$_{2,129}$ = 10.31, p < 0.001] during the preconditioning test (Fig. 1A). The bias against the smooth floor chamber resided in the WT mice and not the Cart KO mice, which showed no statistically significant preconditioning chamber bias (Fig. 1B). Thus, the problems associated with the biased CPP method were minimized by using a CPP box with a small preconditioning chamber bias, eliminating mice with a strong preconditioning chamber bias and parceling the remaining mice with the strongest preconditioning chamber bias into saline control groups.

Intravenous Cocaine Self-Administration. Cocaine was chosen because it is a reliable reinforcer, and its use would allow us to generalize these results to psychostimulants. Mice were housed in a reverse 12-h light/12-h dark cycle with lights on at 8:00 PM. After 7 days, mice were implanted with an indwelling silastic catheter (2 French) in the external jugular under ketamine (100 mg/kg) and xylazine (20 mg/kg) anesthesia. A 1.2-cm segment of the catheter was inserted into the vein, and the distal end of the catheter exited the midcapsular region in between the shoulder blade. Mice were allowed 4 to 5 days to recover. Catheters were flushed daily with 25 to 50 μl each of saline, gentamicin (0.8 mg/ml), and heparin (70 U/ml). Gentamicin was given only 4 days postoperatively. Mice were allowed to self-administer cocaine in sound-attenuated operant boxes 4 to 5 days after surgery. Unlimited cocaine was available daily for 2 h between 10:00 AM and 2:00 PM. Mice could nose-poke for cocaine from either of two holes on one wall under a fixed ratio-1 schedule of reinforcement. A nose-poke in one hole assigned as the “active hole” resulted in one intravenous (i.v.) infusion of cocaine (1 mg/kg).
mg/kg/injection, 120 μl infused over 4 s), activation of a light inside the nose-poke hole for 20 s, and no scheduled consequences of further nose-pokes during this 20-s time period. The number of injections and nose-pokes in the active and inactive hole were recorded throughout the entire session. A cocaine dose-response curve was generated for each mouse by serially lowering the dose of cocaine, once stable operant responding was observed at each cocaine dose. Stable responding was defined as 3 consecutive days or sessions with cocaine intake that varied <10%, active hole nose-pokes that varied <20%, and nose-poke accuracy or the active/inactive nose-poke ratio that varied <20%. Catheter patency was checked every 5 to 6 days by infusing 20 to 25 μl of ketamine/xylazine anesthetic i.v. at least 3 h after a self-administration session. Mice were removed from the study if loss of righting reflex and consciousness was not observed within 5 and 10 to 15 s, respectively.

**Sucrose Preference.** A two-bottle, unlimited access preference test was used to measure sucrose preference in the home cages. Upon arriving in the university animal care facility, mice were given access to two identical drinking bottles of tap water for several days to establish a baseline. Fluid intake was measured daily between 10:00 AM and 12:00 PM. After drinking preferences for water stabilized, 0.5, 1, or 2% sucrose dissolved in tap water was placed in one of the two drinking bottles for each mouse. Fluid intake was measured daily for 4 days, and the position of the bottles was switched after 2 days. The values reported represent an average of the last 2 days of intake.

**Statistical Analysis.** All data are reported as the mean ± standard error. The open field data were analyzed with a Student’s t test (unpaired, two-tailed). All other data were analyzed using ANOVA. Statistically significant effects in ANOVAs were followed with post hoc tests. Within-group drug-to-saline control comparisons were performed with Dunnett’s test. Within-group comparisons with repeated measures were made with Bonferroni/Dunn’s test. Between-group comparisons were made with Tukey’s test. Acute amphetamine locomotor activity was analyzed with a two-factor ANOVA, with genotype and dose as between factors. Repeated amphetamine data were analyzed with a mixed-factor ANOVA, with genotype and dose as between factors and days as the within factor. Significant interactions were further examined with two- and one-factor ANOVAs. Conditioned place preference and sucrose preference data were analyzed with a two-factor ANOVA, with genotype and dose as between factors. Self-administration data were analyzed with a two-factor ANOVA, with genotype as the between factor and dose as the within factor. Data were analyzed with StatView (version 5.0.1; SAS Institute, Cary, NC). The ED50 and p values for the cocaine self-administration dose-intake curve were determined with GraphPad Prism (version 4; GraphPad Software Inc. San Diego, CA). Statistical significance was set at p < 0.05.

**Results**

**Cart KO and WT Mice.** Adult male mice (7–9 weeks old at the start of the study) with a deletion of the entire coding region of *Cart* gene (*Cart1m1Ang*) or Cart KO) were used in these studies. This eliminated the possibility that any bioactive CART peptides could be generated from the *Cart* locus (Wierup et al., 2005). Body weight, longevity, and fecundity did not differ between WT and Cart KO mice (data not shown). The breeding background had little effect on body weight in young adult male mice, like those used here that are on a Black Swiss background, or whether the *Cart* gene deletion is on a C57BL/6 background (Asnicar et al., 2001; Wierup et al., 2005); only later in life does body weight differ between genotypes.

**Exploratory Activity in a Novel Open Field.** Locomotor activity in a novel, open field was similar between Cart KO and WT mice (Table 1). Crosses into different quadrants of the field or ambulation and rears did not differ between genotypes over the first 10 min or the entire 60-min test period (p = 0.47–0.97, t test, two-tailed). The number of entries into the center of the field, the time spent in the center of the field, and the time along the walls (i.e., thigmotaxis) did not differ between genotypes over the first 10 min or the entire 60-min test period (p = 0.14–0.84, t test, two-tailed). Thus, the anxiety produced by the center of the open field (Britton and Britton, 1981) did not differ between Cart KO and WT mice. This finding contrasts with an anxiogenic effect of CART peptides in rats (Kask et al., 2000; Chaki et al., 2003). Failure to observe reduced anxiety in the Cart KO mice may reflect compensatory changes that obscured this phenotype, or it may suggest that CART peptides are relevant in selective states of anxiety. The open field data suggest that exploratory behavior and motor function of WT and Cart KO mice were similar.

**Reduced Amphetamine-Induced Locomotor Activity in Cart KO Mice.** Four distinct psychostimulant-induced behaviors, including hyperlocomotion, locomotor sensitization, conditioned place preference, and drug self-administration, were examined in WT and Cart KO mice. Psychostimulants and many drugs of abuse increase locomotor activity (Wise and Bozarth, 1987). In this study, locomotor activity was defined as ambulation, vertical activity, stereotypic grooming, and stereotypic head bobs.

Acute amphetamine administration increased locomotor activity in WT and Cart KO mice (Fig. 2A). Amphetamine produced a dose-dependent increase in ambulation in both genotypes (two-factor ANOVA: dose, F3,50 = 13.72, p < 0.001). The two highest amphetamine doses were significantly greater than saline and 1 mg/kg amphetamine in both genotypes (p < 0.05). Cart KO mice exhibited more ambulation than in WT mice at 6 mg/kg amphetamine (p < 0.05). However, WT mice were engaged in greater vertical activity at this dose instead (see below). Amphetamine produced a dose-dependent increase in vertical activity in WT mice only (two-factor ANOVA: genotype, F1,50 = 4.29, p < 0.05; dose, F3,50 = 3.22, p < 0.05; genotype × dose, F3,50 = 4.45, p < 0.01) and stereotypic grooming (two-factor ANOVA: genotype, F1,50 = 8.04, p < 0.01; dose, F3,50 = 10.99, p < 0.0001; genotype × dose, F3,50 = 6.37, p < 0.001) at 6 mg/kg amphetamine (p < 0.05). Thus, vertical activity and stereotypic grooming were greater in WT mice than in Cart KO mice (p < 0.05). Acute amphetamine induced stereotypic head bobs in WT mice in a dose-dependent trend that never reached statistical significance; these were rarely seen in Cart KO mice after acute amphetamine administration. Ambulation and vertical activity during the 30-min habituation period prior

**Table 1**

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<tr>
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<th>WT KO</th>
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<tr>
<td><strong>Crossovers</strong></td>
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<tr>
<td>10 min</td>
<td>174 ± 14 159 ± 28 120 ± 15 122 ± 21</td>
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<td>60 min</td>
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<tr>
<td><strong>Crosses (middle)</strong></td>
<td>19 ± 2 21 ± 5 15 ± 2 14 ± 4</td>
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<tr>
<td><strong>Rears</strong></td>
<td>30 ± 5 39 ± 11 34 ± 8 34 ± 9</td>
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<tr>
<td><strong>Time in middle (s)</strong></td>
<td>27 ± 5 25 ± 2 36 ± 5 25 ± 5</td>
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<tr>
<td><strong>Time along walls (s)</strong></td>
<td>408 ± 30 449 ± 14 523 ± 30 581 ± 21</td>
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Exploratory behavior of WT and Cart KO mice in a novel open field. Mice were exposed to a large open field for 1 h in low ambient lighting. Their activity over the entire first 10 min and the total 60 min (i.e., first 2 min of every 10 min) is reported. No statistically significant differences were found between genotypes (p < 0.8–0.9, t test, two-tailed) (n = 8 genotype).
to receiving amphetamine did not differ between WT and Cart KO mice (data not shown). In summary, acute amphetamine increased ambulation, vertical activity, and stereotypic grooming in WT mice but only ambulation in Cart KO mice. The effects of acute amphetamine on locomotor activity were reduced in Cart KO mice compared with WT mice.

Repeated amphetamine administration produced different effects on locomotor activity in WT and Cart KO mice (Fig. 2B). Locomotor sensitization was observed in WT mice but rarely in Cart KO mice. Repeated amphetamine sensitized ambulation in dose-dependent manner in WT mice only (mixed-factor ANOVA, day as the repeated measure: dose, $F_{3,49} = 22.03, p < 0.0001$; genotype X dose, $F_{3,49} = 2.75, p < 0.05$; day, $F_{13,637} = 4.76, p < 0.0001$; day X genotype, $F_{13,637} = 4.24, p < 0.0001$; day X dose, $F_{39,637} = 2.25, p < 0.0001$; day X genotype X dose, $F_{39,637} = 1.85, p < 0.005$) and at the two highest amphetamine doses ($p < 0.05$). WT mice exhibited greater ambulation than Cart KO mice on days 8 to 14 at 6 mg/kg amphetamine ($p < 0.05$). Repeated amphetamine sensitized vertical activity in dose-dependent manner in WT mice only (mixed-factor ANOVA, day as the repeated measure: dose, $F_{3,49} = 9.26, p < 0.0001$) at 3 mg/kg amphetamine
WT mice exhibited more vertical activity than Cart KO mice between days 11 to 14 at 3 mg/kg amphetamine and on days 1, 4, and 14 at 6 mg/kg amphetamine (p < 0.05). In summary, repeated amphetamine elicited robust sensitization of ambulation and vertical activity in WT mice but not in Cart KO mice.

Stereotypic grooming and head bobs failed to sensitize with repeated amphetamine administration except in one case (Fig. 2B). Stereotypic grooming increased in a dose-dependent manner in Cart KO mice only (mixed-factor ANOVA, day as the repeated measure: genotype effect, F(1,44) = 6.74, p < 0.01; dose effect, F(3,44) = 24.33, p < 0.0001; genotype X dose interaction, F(3,44) = 8.67, p < 0.0001) and at 3 mg/kg amphetamine (p < 0.05). In Cart KO mice, the 6-mg/kg amphetamine dose showed a trend toward sensitization but never reached statistical significance. Repeated amphetamine was unable to sensitize head bobs in either genotype. Although stereotypic behaviors generally failed to sensitize with repeated amphetamine administration, they were greater in WT mice than in Cart KO mice. Stereotypic grooming was significantly greater in WT mice than Cart KO mice at 6 mg/kg amphetamine on all days measured except day 5 (p < 0.05). Stereotypic head bobs were greater in WT mice than Cart KO mice at 6 mg/kg amphetamine on day 5 (p < 0.05); stereotypic head bobs were rare in Cart KO mice. In summary, repeated amphetamine administration sensitized stereotypic grooming in Cart KO mice only, but stereotypies were more pronounced in WT mice than Cart KO mice. Taken together, the ability of amphetamine to increase locomotor activity and produce locomotor sensitization was considerably attenuated in Cart KO mice by comparison with WT mice.

Reduced Amphetamine-Conditioned Place Preference in Cart KO Mice. The CPP paradigm was used to measure the ability of amphetamine to produce a positive association with an environment. This effect is characteristic of rewarding/reinforcing stimuli, such as drugs of abuse (Carr et al., 1989; Bardo and Bevins, 2000). Five mice from each genotype were not included in the study because they exhibited a strong preconditioning chamber bias. One Cart KO mouse died after an i.p. injection of saline. WT mice, but not Cart KO mice, exhibited an aversion to the smooth floor of the CPP apparatus during preconditioning test (Fig. 1). The smooth floor was white, a color that may produce aversion in rodents. The aversion of WT mice to the smooth floor should have a small impact on the conditioning properties of amphetamine because this chamber was not used for drug conditioning. Amphetamine produced CPP in dose-dependent manner (two-factor ANOVA: dose, F(1,43) = 18.05, p < 0.0001) (Fig. 3). Both amphetamine doses elicited CPP in WT mice, but only the higher dose was effective in Cart KO mice (p < 0.01). In Cart KO mice, 0.3 mg/kg amphetamine was as ineffective as saline in producing CPP and was significantly less than the CPP produced in WT mice (p < 0.05). It is noteworthy that equal amounts of CPP were produced by 1 mg/kg amphetamine in both Cart KO and WT mice. Amphetamine never elicited place aversion in any mouse. In conclusion, amphetamine produced place preference in Cart KO and WT mice, but its conditioning effects were attenuated in Cart KO mice.

Reduced Cocaine Self-Administration in Cart KO Mice. One of the main characteristics of drugs of abuse is that they are voluntary consumed or self-administered (Schuster and Thompson, 1989). Drug self-administration is one of the most powerful measures of a drug’s reinforcing effect (Shippenberg and Koob, 2002). Intravenous cocaine self-administration was used to examine the reinforcing effects of cocaine in Cart KO and WT mice. Mice were trained to respond for cocaine at 1 mg/kg/infusion under a fixed ratio-1 schedule of reinforcement. WT and Cart KO mice acquired cocaine self-administration at similar rates. Stable responding (see Materials and Methods for definition) at 1 mg/kg cocaine was attained at similar rates, by day 6.3 ± 0.5 for WT mice and by day 7.8 ± 0.9 for Cart KO mice (p = 0.15, t test, two-tailed). Thereafter, a dose-response curve was generated by serially decreasing the cocaine dose. Cocaine self-administration behavior diverged between the genotypes once the cocaine dose was lowered (Fig. 4). Cocaine intake (two-factor ANOVA, dose as repeated measure: genotype, F(1,20) = 5.58, p < 0.05; dose, F(4,80) = 75.90, p < 0.0001; genotype X dose, F(4,80) = 2.53, p < 0.05) and responding for cocaine (two-factor ANOVA, dose as repeated measure: genotype, F(1,20) = 8.70, p < 0.01; dose, F(4,80) = 6.56, p < 0.0001; genotype X dose, F(4,80) = 2.82, p < 0.05) was greater in WT mice than in Cart KO mice. WT mice exhibited greater cocaine intake at 0.125, 0.25, and 0.5 mg/kg cocaine (p < 0.05) and greater response rates at 0.125 and 0.25 mg/kg cocaine than Cart KO mice (p < 0.05). The right shift in the cocaine intake curve of Cart KO mice compared with the WT mice curve (ED50 = 0.20 and 0.38 mg/kg, WT and KO, respectively; p < 0.005) represented a 2-fold reduction in the potency of amphetamine without a change in its efficacy (Fig. 4A). By contrast, the dose-response curve of Cart KO mice shifted down compared with that of the WT mice (Fig. 4B). The efficacy of cocaine as a reinforcer in Cart KO mice appeared reduced, while its potency remained unaltered.
dose, and genotype (mixed-factor ANOVA, dose as repeated measure: genotype, $F_{1,40} = 4.05, p < 0.05$; hole, $F_{1,40} = 136.26, p < 0.0001$; dose, $F_{4,200} = 7.25, p < 0.0001$; genotype X dose, $F_{4,200} = 3.15, p < 0.05$; hole X dose, $F_{4,200} = 4.93, p < 0.001$). Simplified analysis of the nose-poke activity in the active and inactive holes showed differential responding based on hole and/or genotype at 0.5 mg/kg cocaine (two-factor ANOVA: hole, $F_{1,38} = 91.33, p < 0.0001$), at 0.25 mg/kg cocaine (two-factor ANOVA: genotype, $F_{1,40} = 7.67, p < 0.01$; hole, $F_{1,38} = 67.13, p < 0.0001$; genotype X hole, $F_{1,40} = 7.40, p < 0.01$) and at 0.125 mg/kg cocaine (two-factor ANOVA: genotype, $F_{1,40} = 7.59, p < 0.01$; hole, $F_{1,40} = 50.66, p < 0.0001$; genotype X hole, $F_{1,40} = 6.05, p < 0.05$). At all cocaine doses, WT and Cart KO mice showed significantly greater activity in the active than in the inactive hole ($p < 0.01$) and significant differences in the active holes between genotypes at 0.125 and 0.25 mg/kg cocaine ($p < 0.01$). In the course of this study, one mouse from each genotype was eliminated from this study because of a blocked i.v. catheter. In summary, WT and Cart KO self-administered cocaine, but the amount of cocaine consumed and responding for cocaine was attenuated in Cart KO mice.

To evaluate the possibility of a biased assessment of the reinforcing effects of cocaine from the serial dilution dose-response data, separate groups of mice were trained with a single, modest cocaine dose (i.e., 0.5 mg/kg/infusion). Cocaine self-administration was acquired at similar rates between Cart KO and WT mice. Stable responding for cocaine was observed at 5.4 ± 0.2 days for WT mice ($n = 8$) and 5.3 ± 0.2 days for Cart KO mice ($n = 7$) ($p = 0.99, t$ test, two-tailed). However, cocaine intake (12.3 ± 1.6 mg/kg, WT; 7.6 ± 1.0 mg/kg, Cart KO; $p < 0.05$, $t$ test, two-tailed) and responding for cocaine (active and inactive hole nose-pokes: 27.3 ± 4.4 and 1.8 ± 0.2, WT; 18.7 ± 3.2 and 7.9 ± 4.0, Cart KO; $p = 0.15, t$ test (active hole responses, WT versus KO), two-tailed) differed between genotypes and was 68% in Cart KO mice compared with WT mice. In summary, a modest dose of cocaine supported self-administration in WT and Cart KO mice but again there was an attenuation in the amount of cocaine consumed and responding for cocaine in the Cart KO mice.

**Sucrose Preference Is Similar between Cart KO and WT Mice.** To determine whether the diminished motivational response in Cart KO mice could result from a wide-ranging alteration in motivation, the motivational value of a natural reward was assessed. A sucrose preference test was conducted to address this possibility in WT and Cart KO mice (Catalanotto and Lacy, 1977). In a two-bottle, 24-h unlimited access test, a sucrose solution was preferentially consumed over water in a dose-dependent manner by WT and Cart KO mice (two-factor ANOVA: concentration, $F_{1,36} = 8.29, p = 0.001$; genotype, $F_{1,36} = 0.005, p = 0.94$) (Fig. 5). The 2% sucrose solution was preferred over water by both genotypes ($p < 0.01$), but there was no difference between Cart KO and WT mice sucrose preferences at any sucrose concentration ($p > 0.05$). The amount of sucrose solution, water, and total fluid consumed did not differ between genotypes (data not shown). In summary, sucrose preference did not differ between Cart KO and WT mice.

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**Fig. 4.** Intravenous cocaine self-administration in WT and Cart KO mice. Both WT and Cart KO mice were given daily unrestricted access to cocaine for 2 h on a fixed ratio-1 schedule of reinforcement. A and B, a dose-intake and dose-response curve was generated for each animal by training at 1 mg/kg/infusion and serially lowering the dose once stable responding was established at each dose. Group means reflect averages of 3 days of stable intake for each animal at each dose. Stable responding was defined as cocaine intake that varied <10% and active hole nose-pokes that varied <20%. Cocaine intake, cocaine responding, and active hole nose-poke behavior were significantly reduced at several cocaine doses in the KO mice compared with WT mice ($\dagger, p < 0.05$; Tukey's test). C, nose-poke behavior in the active and inactive hole at each cocaine dose. At all cocaine doses, activity in the active hole was significantly greater than in the inactive hole in both genotypes ($\star, p < 0.05$; Bonferroni-Dunn). A significant difference in active hole nose-pokes between genotypes was observed at two amphetamine doses ($\dagger, p < 0.01$; Tukey's test). Means ± S.E.M. are reported; $n = 11$/genotype.

The difference in cocaine intake between WT and Cart KO mice was reflected by the activity in the nose-poke holes (Fig. 4C). Responding for cocaine depended on the hole, cocaine intake, cocaine dose, and genotype (mixed-factor ANOVA, dose as repeated measure: genotype, $F_{1,40} = 4.05, p < 0.05$; hole, $F_{1,40} = 136.26, p < 0.0001$; dose, $F_{4,200} = 7.25, p < 0.0001$; genotype X dose, $F_{4,200} = 3.15, p < 0.05$; hole X dose, $F_{4,200} = 4.93, p < 0.001$). Simplified analysis of the nose-poke activity in the active and inactive holes showed differential responding based on hole and/or genotype at 0.5 mg/kg cocaine (two-factor ANOVA: hole, $F_{1,38} = 91.33, p < 0.0001$), at 0.25 mg/kg cocaine (two-factor ANOVA: genotype, $F_{1,40} = 7.67, p < 0.01$; hole, $F_{1,38} = 67.13, p < 0.0001$; genotype X hole, $F_{1,40} = 7.40, p < 0.01$) and at 0.125 mg/kg cocaine (two-factor ANOVA: genotype, $F_{1,40} = 7.59, p < 0.01$; hole, $F_{1,40} = 50.66, p < 0.0001$; genotype X hole, $F_{1,40} = 6.05, p < 0.05$). At all cocaine doses, WT and Cart KO mice showed significantly greater activity in the active than in the inactive hole ($p < 0.01$) and significant differences in the active holes between genotypes at 0.125 and 0.25 mg/kg cocaine ($p < 0.01$). In the course of this study, one mouse from each genotype was eliminated from this study because of a blocked i.v. catheter. In summary, WT and Cart KO self-administered cocaine, but the amount of cocaine consumed and responding for cocaine was attenuated in Cart KO mice.

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Discussion

We show that CART peptides are important for psychostimulant induced locomotor activity, locomotor sensitization, conditioned place preference, and self-administration. These psychostimulant-mediated behaviors were attenuated in Cart KO mice by comparison with WT mice. The data suggest a causal link between CART peptides and psychostimulant effects.

Studies with knockout mice must be interpreted with caution because deletion of the gene and breeding strategies may inadvertently alter the phenotype under investigation. The reduced responses to psychostimulants of Cart KO mice were not due to sensorimotor or cognitive impairments. First, locomotor activity in a novel open field was indistinguishable between WT and Cart KO mice. Second, Cart KO mice developed similar levels of CPP as WT mice at 1 mg/kg amphetamine, thereby suggesting similar capacities for forming contextual associations. Moreover, cocaine self-administration acquisition rates and discrimination between active and inactive nose-poke holes did not differ between genotypes. Third, fecundity, which requires partner recognition and receptivity, was similar between genotypes. These data suggest there were no overt sensorimotor or cognitive impairments in Cart KO mice compared with WT mice. A potential limitation of these studies is variation in the background of the WT and Cart KO mice because each genotype was maintained as a separate colony without backcrossing onto the Black Swiss background. However, there was no drift in body weight or litter size within genotype throughout the course of these studies. Moreover, the amphetamine locomotor studies took over 10 months to perform, but variation in the responses to amphetamine within genotypes never emerged. Differences between WT and Cart KO mice in open field activity and sucrose preference might be expected if their backgrounds differed, but this was not observed. Therefore, the attenuated psychostimulant responses of Cart KO mice were more likely to result from changes in the properties of these drugs in these mutant mice.

Locomotor responses to acute amphetamine were attenuated in Cart KO mice. Ambulation was the only locomotor response enhanced by acute amphetamine in Cart KO mice. By contrast, WT mice exhibited increases in ambulation, vertical activity, and stereotypic grooming. Perhaps higher amphetamine doses would have elicited greater locomotor responses in Cart KO mice, but the similarity in the responses to two high amphetamine doses is one argument against this possibility. It is possible that CART peptides may be important for vertical activity and stereotypes and less so for ambulation. The reduced potency and possible efficacy of amphetamine in Cart KO mice may reflect a decreased ability to engage central motor systems. The inability of psychostimulants to stimulate CART peptide release in Cart KO mice may be important because CART peptides stimulate locomotor activity on their own. Injection of CART peptide 55-102 into the VTA was shown to increase locomotor activity in rats (Kimmel et al., 2000). Systemic haloperidol blocked this effect, thus suggesting dopaminergic regulation of CART peptide effects. Moreover, CART peptides modulate dopaminergic activity. Injection of CART peptide 55-102 into the lateral ventricles was shown to increase dopamine turnover in the nAcc and dorsal striatum (Yang et al., 2004). CART peptide modulation of psychostimulant locomotor activity is more complex, however. Injection of CART peptide 55-102 into the nAcc blocked cocaine and amphetamine induced locomotor activity (Jaworski et al., 2003; Kim et al., 2003). This inhibitory action of CART peptides may reflect a local negative feedback within the nAcc. Therefore, psychostimulants may induce increases in extracellular dopamine within the nAcc that stimulates CART peptide release at distal sites such as the VTA, medial substantia nigra, and ventral pallidum as well as locally in the nAcc. CART peptides released in the ventral midbrain probably modulate feedback to the nAcc perhaps by further increasing dopamine release as well as transfer of information from the limbic to motor pathways in the striatum, or translate motivation into action. The data suggest that CART peptides modulate psychostimulant induced locomotor activity presumably by regulating dopaminergic activity within mesolimbic and mesostriatal pathways.

Repeated amphetamine produced little locomotor sensitization in Cart KO mice compared with WT mice. The development of locomotor sensitization was delayed and the magnitude of the response in Cart KO mice was a fraction of that observed in WT mice. These data contrast with a lack of locomotor sensitization produced by repeated injections of CART peptide 55-102 into the VTA of rats (Kimmel et al., 2000). Whether this disparity arises from methodological issues, or whether CART peptides alone are insufficient for sensitization to develop requires further research. CART peptides in the VTA are probably important for psychostimulant sensitization because this brain area is central to the development amphetamine locomotor sensitization (Dougherty and Ellinwood, 1981; Vezina and Stewart, 1990). Sensitization is considered important for understanding drug addiction because it reflects a long-lasting behavioral alteration that persists even after drug intake ceases in animals (Robinson and Berridge, 1993). The data suggest a role for CART peptides in the initiation, and perhaps in the expression of psychostimulant locomotor sensitization, and subsequently implicates them in psychostimulant addiction.

Amphetamine’s ability to produce conditioned place preference
Changes in motivation. Body weight and sucrose preference susceptibility to psychostimulant self-administration. Reinforcing properties of psychostimulants as well as the reinforcing effects of psychostimulants. In summary, that control motivation support a role for CART peptides in reinforcing in Cart KO mice. CART peptides in brain areas responded less for cocaine than WT mice; cocaine was less drug-naive mice had access to a modest dose of cocaine (i.e., Cart KO dose-intake curve is incongruent with this vulnerability to drugs of abuse (Piazza et al., 2000). The horizontal shift of the dose-response curves as those observed for Cart KO mice reveals a change in the reinforcing property of cocaine.

Cocaine self-administration was attenuated in Cart KO mice compared with WT mice. The amount of cocaine consumed as well as responding for cocaine was reduced in Cart KO mice compared with WT mice. Yet, Cart KO mice learned to respond for cocaine at the same rate as WT mice. The Cart KO mice cocaine dose-response curve shifted down compared with that of WT mice. This vertical shift in the dose-response curve is consistent with a change in the efficacy of cocaine for maintaining response rates. By contrast, the cocaine dose-intake curve of Cart KO mice shifted to the right relative to the WT mice curve. This horizontal shift represented a change in the potency of cocaine to support intake. The decrease in cocaine intake and responding for cocaine in Cart KO mice most likely reflected a change in the reinforcing property of cocaine.

The cocaine self-administration data also suggest a possible role of CART peptides in the susceptibility to psychostimulant self-administration. Downward vertical shifts in the dose-response curves as those observed for Cart KO mice is associated with decreased vulnerability to self-administer drugs of abuse (Piazza et al., 2000). The horizontal shift of Cart KO dose-intake curve is congruent with this vulnerability model, but it does not preclude reduced vulnerability.

These data predicted that cocaine would be less reinforcing in Cart deficient mice at low to moderate doses. In fact, when drug-naive mice had access to a modest dose of cocaine (i.e., 0.5 mg/kg/infusion), the Cart KO consumed less and responded less for cocaine than WT mice; cocaine was less reinforcing in Cart KO mice. CART peptides in brain areas that control motivation support a role for CART peptides in the reinforcing effects of psychostimulants. In summary, these data suggest that CART peptides are important for the reinforcing properties of psychostimulants as well as the susceptibility to psychostimulant self-administration.

Deletion of the Cart gene failed to elicit generalized changes in motivation. Body weight and sucrose preference was similar between Cart KO and WT mice. Differences in body weight between WT and Cart KO mice appear in older (i.e., 40-week old) male mice and in heterozygous female mice fed a high fat diet (Asnicar et al., 2001; Wierup et al., 2005), but not in 7–9 week old male mice used in these studies. Either CART peptides do not modulate food preference or intake in young, sated mice, or compensatory changes have obscured this phenotype. The similarity in sucrose preference between WT and Cart KO mice suggest that CART peptides may modulate stimuli with relatively high values, or in conditions where a stimulus assumes a high rewarding/reinforcing value. Whether CART peptides help to discriminate the value of the stimuli requires further investigation.

This study demonstrates a causal link between CART peptides and the behavioral effects of psychostimulants. In the absence of CART peptides, psychostimulant-mediated hyperlocomotion, locomotor sensitization, conditioned place preference, and drug self-administration are considerably attenuated. The data suggest that CART peptides are neuromodulators of the behavioral properties of psychostimulants, and are important for psychostimulant addiction. Unless CART peptides are selective modulators of psychostimulants, they may be important for drug addiction in general, but this warrants further research.

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References


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