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ORIGINAL INVESTIGATION

The effect of chronic amphetamine treatment on cocaine-induced facilitation of intracranial self-stimulation in rats

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Abstract
Rationale Chronic amphetamine treatment reduces cocaine self-administration in pre-clinical and clinical settings, and amphetamine has been proposed as a candidate medication for treatment of cocaine abuse.
Objective The objective of the present study was to investigate whether chronic amphetamine treatment can decrease abuse-related cocaine effects in an assay of intracranial self-stimulation (ICSS).
Methods Thirteen adult male Sprague-Dawley rats were equipped with intracranial electrodes targeting the medial forebrain bundle and trained to lever press for pulses of brain stimulation in a "frequency-rate" ICSS procedure. Cocaine (10 mg/kg) was administered before (day 0), during (days 7 and 14), and after (posttreatment days 1 and 3) 2 weeks of continuous treatment with either amphetamine (0.32 mg/kg/h, n=7) or saline (n=6) via osmotic pump.
Results Prior to treatment, cocaine facilitated ICSS in all rats. Saline treatment had no effect on baseline ICSS or cocaine-induced facilitation of ICSS at any time. Conversely, amphetamine produced a sustained though submaximal facilitation of baseline ICSS, and cocaine produced little additional facilitation of ICSS during amphetamine treatment. Termination of amphetamine treatment produced a depression of baseline ICSS and recovery of cocaine-induced facilitation of ICSS.
Conclusions These data suggest that chronic amphetamine treatment blunts expression of abuse-related cocaine effects on ICSS in rats.

Keywords ICSS · Amphetamine · Cocaine · Withdrawal · Tolerance

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Abbreviations

ICSS	Intracranial self-stimulation
MCR	Maximum control rate
S.C.	Subcutaneous
I.P.	Intraperitoneal

Introduction

There are no current Food and Drug Administration-approved medications to treat cocaine addiction. However, over the past 15 years, studies across a range of species have shown that chronic treatment with amphetamine can decrease cocaine self-administration. In rats, chronic amphetamine decreased responding for cocaine under both a progressive-ratio schedule of reinforcement (Chiodo et al. 2008; Chiodo and Roberts 2009) and a concurrent cocaine-vs.-food choice schedule (Thomsen et al. 2013). Likewise, in rhesus monkeys, chronic amphetamine decreased responding for cocaine under a multiple, second-order schedule of reinforcement (Negus and Mello 2003a), two different progressive-ratio schedules of reinforcement (Czoty et al. 2010; Negus and Mello 2003b), and a concurrent cocaine-vs.-food choice schedule (Negus 2003; Banks et al. 2013). Finally, both human laboratory studies (Greenwald et al. 2010; Rush et al. 2010) and clinical trials (Grabowski et al. 2001, 2004; Mariani et al. 2012; Schmitz et al. 2012) have shown that amphetamine may be efficacious at decreasing cocaine-taking behaviors. The phenomenon of amphetamine-induced reduction in cocaine use has been conceptualized in terms of "agonist therapy," with comparisons drawn to current treatments for nicotine dependence (e.g., nicotine patches) and opioid dependence (e.g., methadone) (Grabowski et al. 2004; Rush and Stoops 2012). However, the molecular mechanisms by which chronic amphetamine treatment might decrease cocaine self-

administration are not clear. There are at least two general possibilities related to the premise that amphetamine and cocaine share common mechanisms as indirect dopamine agonists (Negus and Mello 2003a). First, chronic amphetamine treatment could trigger adaptive neurobiological processes (e.g., downregulation of dopamine transporters) that produce tolerance to its own effects and cross-tolerance to cocaine effects. Second, amphetamine could produce sustained effects that saturate dopamine signaling (e.g., sustained increases in extracellular dopamine levels) and limit the potential of subsequent cocaine doses to promote further dopamine signaling. In this process, brain reward systems could be conceptualized as a stimulus detector, and amphetamine could function as a stimulus that generates "noise" within this system to obscure detection of the cocaine "signal" (analogous to the ability of a loud background noise to obscure detection of other auditory stimuli). The goal of the present study was to distinguish between these two possibilities using an intracranial self-stimulation (ICSS) procedure in rats.

ICSS is one behavioral assay used to assess the abuse liability of drugs (Kornetsky and Esposito 1979; Vlachou and Markou 2011; Wise 1996). In ICSS, subjects are first equipped with chronic electrodes that target brain areas such as the medial forebrain bundle and are then trained to lever press for electrical stimulation delivered via the electrode. Rates of ICSS can then be controlled by altering the frequency or intensity of electrical stimulation, and in the case of medial forebrain bundle stimulation, ICSS is mediated by transsynaptic activation of mesolimbic dopamine neurons that also mediate abuse-related effects of cocaine (Stellar and Rice 1989; Wise 1996). In general, drug-induced facilitation of low ICSS rates maintained by low frequencies or intensities of medial forebrain bundle stimulation is interpreted as an "abuse-related" effect predictive of signals in other preclinical assays of abuse liability (e.g., drug self-administration), whereas attenuation of higher ICSS rates maintained by higher frequencies or intensities of stimulation is interpreted as an "abuse-limiting" effect (Bauer et al. 2013; Carlezon and Chartoff 2007). For example, cocaine reliably facilitates ICSS of the medial forebrain bundle (Esposito et al. 1978; Negus et al. 2012), and this effect is often interpreted as an abuse-related cocaine effect.

The purpose of the present study was to determine cocaine effects on ICSS before, during, and after a regimen of continuous amphetamine treatment similar to those that have been shown previously to decrease cocaine self-administration in rats (Chiodo and Roberts 2009; Thomsen et al. 2013) but that did not produce tolerance to amphetamine-induced ICSS facilitation (Paterson et al. 2000). Specifically, cocaine effects on ICSS were compared in rats treated chronically with saline or amphetamine (0.32 mg/kg/h) delivered via osmotic pump for 14 consecutive days. We hypothesized that chronic amphetamine would attenuate cocaine-induced facilitation of ICSS in a manner similar to its attenuation of cocaine reinforcing effects in assays of self-administration. In addition, we hypothesized that amphetamine treatment itself would produce an initial facilitation of ICSS, that tolerance would fail to develop to this effect during chronic treatment, and that absence of tolerance to amphetamine effects would argue against cross-tolerance as a mechanism to explain attenuation of cocaine effects.

Materials and methods

Subjects

Thirteen adult male Sprague-Dawley rats (Harlan, Frederick, MD, USA) were used. All rats had free access to water and were housed individually on a 12-h light-dark cycle (lights on from 6 a.m. to 6 p.m.) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All rats also had free access to food and weighed between 311 and 406 g at the time of surgery. Animal maintenance accorded with the National Institutes of Health guidelines on the care and use of animal subjects in research (National Research Council 2011). Experimental protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Assay of intracranial self-stimulation

Details regarding the surgery, apparatus, and training regimen have been described previously (Bauer et al. 2013; Bonano et al. 2014; Negus et al. 2012; Altarifi and Negus 2011). In brief, bipolar electrodes were implanted into the left medial forebrain bundle at the level of the lateral hypothalamus (stereotaxic coordinates: 2.8 mm posterior to the bregma, 1.7 mm lateral to the midsagittal line, and 8.8 mm ventral to the skull). Ketoprofen (5 mg/kg) was used as a postoperative analgesic immediately and 24 h after surgery. Animals were allowed to recover for at least 5 days before beginning ICSS training. Operant conditioning chambers consisted of sound-attenuating boxes with a response lever, three stimulus lights centered 7.6 cm above the response lever, a 2-W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Bipolar cables routed through a swivel-commutator connected the stimulator to the electrode (model SL2C, Plastics One, Roanoke, VA, USA). Med-PC IV computer software controlled all programming parameters and data collection (Med Associates, St. Albans, VT, USA).

The house light was illuminated during behavioral sessions, and lever press responding under a fixed-ratio 1 (FR 1) schedule produced delivery of a 0.5-s train of square-wave cathodal pulses (0.1 ms/pulse). During brain stimulation,

stimulus lights over the lever were illuminated, and responding had no scheduled consequences. During initial 60-min training sessions, stimulation intensity was set at 150 μA, and stimulation frequency was set at 126 Hz. Stimulation intensity was then individually manipulated in each rat to identify an intensity that maintained a reinforcement rate >30 stimulations/min. Once an appropriate intensity was identified, changes in frequency were introduced during sessions consisting of three consecutive 10-min components, each of which contained ten 60-s trials. The stimulation frequency was 158 Hz for the first trial of each component, and frequency decreased in 0.05 log unit steps during the subsequent nine trials to a final frequency of 56 Hz. Each trial began with a 10-s time out period, during which responding had no scheduled consequences, and five noncontingent stimulations at the designated frequency were delivered at 1-s intervals during the last 5 s of the time out. During the remaining 50 s of each trial, responding produced both intracranial stimulation at the designated frequency and illumination of the lever lights under an FR 1 schedule as described above. Training continued until frequency-rate curves were not statistically different over 3 days of training as indicated by two-way analysis of variance (ANOVA) and Holm-Sidak post hoc test (see "Data analysis"). Data from these final three training days served as the "pre-pump baseline" for subsequent analysis as discussed below. All training was completed within 5 weeks of surgery.

Treatment Before initiating treatment, animals were split into two groups with statistically similar performance in ICSS as indicated by two-way ANOVA of pre-pump baseline frequency-rate curves. Subsequently, one group was implanted with osmotic pumps filled with saline (n=6), and the other group was implanted with pumps filled with amphetamine (0.32 mg/kg/h; n=7). Pumps were removed after 14 days. The amphetamine dose was selected to match or approximate doses used previously to decrease cocaine self-administration in rats responding under either a concurrent schedule of cocaine and food availability (Thomsen et al. 2013; 0.32 mg/kg/h) or a progressive-ratio schedule (Chiodo and Roberts 2009; 0.21 mg/kg/h). Osmotic pumps (model 2ML2, 5 μl/h flow rate, Alzet, Cupertino, CA, USA) were implanted subcutaneously (S.C.) in the midscapular region, and later removed, while rats were anesthetized with isoflurane. Incision sites were closed with a single running suture. Ketoprofen (5 mg/kg) was used as a postoperative analgesic immediately following surgery. At least 20 h of recovery were allowed before the next training or test session was conducted.

Testing Test sessions were conducted over a period of 18 days, and each test session consisted of six total components, including three consecutive "daily baseline" ICSS components followed by a 10-min time out period and then by three consecutive "test" components. Either saline or cocaine (10 mg/kg, intraperitoneally (I.P.)) was administered at the beginning of the time out, immediately after the daily baseline components, and all subjects remained in the chambers until test components were initiated. A single saline test session was conducted on day -1, the day before pump implantation. Cocaine test sessions were conducted on day 0 (immediately before implantation of osmotic pumps), day 7 of treatment, day 14 of treatment (immediately before removal of osmotic pumps), and days 1 and 3 after pump removal. In addition, training sessions consisting only of three consecutive daily baseline components were conducted on every weekday between cocaine tests.

Fourteen days after pump removal in the amphetamine-treated rats, an additional experiment was performed to examine cocaine effects on responding under conditions of increased stimulation intensity. For this session, stimulation intensity was increased for each animal by 40 % relative to the intensity used during amphetamine treatment. This increase in stimulation intensity was employed to produce an increase in baseline ICSS performance that would be comparable to that produced by amphetamine treatment, and effects of 10 mg/kg cocaine were redetermined.

Data analysis The primary dependent measure was the ICSS reinforcement rate in stimulations/frequency trial. To normalize these raw data, reinforcement rates from each trial in each rat were converted to percent maximum control rate (%MCR) for that rat. The maximum control rate (MCR) was determined for each rat during the three pre-pump baseline sessions at the beginning of the experiment, prior to pump implantation. The first component from these sessions (and from all other sessions) was considered to be an acclimation component, and data were discarded. The MCR was defined as the mean of the maximal rates observed during any frequency trial of the second and third components of the three pre-pump baseline sessions (six total pre-drug baseline components). The MCRs in the saline and amphetamine treatment groups were compared by unpaired, two-tailed t test with Welch's correction. Subsequently, %MCR for each trial was calculated as (reinforcement rate during a frequency trial÷maximum control rate)×100. Graphs show normalized frequency-rate curves, with brain stimulation frequency on the abscissa and ICSS rate expressed as %MCR on the ordinate. Frequency-rate curves were determined and compared across three general conditions: (1) pre-pump baseline curves, showing average results from the pre-pump baseline sessions, (2) daily baseline curves, showing average results collected on a given test day before saline or cocaine administration, and (3) saline/cocaine test curves, showing average results collected on a given test day after saline or cocaine administration. Two-way ANOVAs were used to compare frequency-rate curves, with frequency as one factor and treatment as the second. A Holm-Sidak post hoc test followed all significant ANOVAs, and p-values less than 0.05 were considered significant.

A second dependent measure summarized cocaine effects on ICSS as % daily baseline reinforcers. To calculate this value, the mean number of total stimulations delivered per component across all frequencies was determined before and after cocaine administration on a given test day in each group. The number of total stimulations per component after cocaine was then expressed as a percentage of total daily baseline stimulations per component before cocaine on that day. Data were analyzed by two-way ANOVA with treatment group (saline or amphetamine pump) as a between-subjects factor and time as a within-subjects factor. A significant ANOVA was followed by the Holm-Sidak post hoc test, and the criterion for significance was $p<0.05$.

Treatment effects on body weight were also examined. Baseline body weight was determined in each rat before pump insertion (day 0) and after 14 days of treatment (prior to pump removal). Body weights in the saline- and amphetamine-treated groups were compared before treatment and after treatment by unpaired, two-tailed *t* test with Welch's correction.

Drugs

(−)-Cocaine HCl was provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD, USA). (+)-Amphetamine hemisulfate was purchased from Sigma Aldrich (St. Louis, MO, USA). All compounds were prepared in sterile saline, and all injections were given in volumes of 1 ml/kg. Amphetamine was administered S.C. via chronically implanted osmotic pump, and cocaine was administered I.P. by injection. Doses are expressed in terms of the salt forms above.

Results

The average maximum control rates were not significantly different between treatment groups: 63.64±4.17 and 63.67± 2.68 stimulations per trial (±SEM) for the saline and amphetamine groups, respectively ($p<0.05$). Figure 1 shows effects of I.P. saline or cocaine (10 mg/kg) on ICSS frequency-rate curves before pump implantation in all 13 animals used in the study. Saline had no effect on ICSS, but cocaine produced a leftward shift in the frequency-rate ICSS curve and significantly facilitated ICSS at frequencies of 1.75–2.0 log Hz. Rats were subsequently divided into two groups to evaluate effects of chronic saline or amphetamine treatment on cocaine-induced facilitation of ICSS.

Figure 2 shows ICSS frequency-rate curves determined before (daily baseline) and after (cocaine test) administration of 10 mg/kg cocaine on test days that occurred before (day 0), during (days 7 and 14), and after (posttreatment days 1 and 3, P1 and P3) chronic treatment with saline. The pre-pump baseline ICSS frequency-rate curve is included in each panel for comparison. Daily baseline ICSS curves differed from the pre-pump baseline curve at one or two frequencies on days 7, 14, P1, and P3, indicating some variability in ICSS over time. However, relative to the daily baseline on each day, cocaine facilitated ICSS across a broad range of six to seven frequencies on all test days.

Figure 3 shows ICSS frequency-rate curves determined before (daily baseline) and after (cocaine test) administration of 10 mg/kg cocaine on test days that occurred before (day 0), during (days 7 and 14), and after (P1 and P3) chronic treatment with 0.32 mg/kg/h amphetamine. The pre-pump baseline ICSS frequency-rate curve for this group is also included in each panel for comparison. On day 0, before pump implantation, the daily baseline ICSS curve differed from the pre-pump baseline curve at a single frequency (1.95 log Hz), and cocaine facilitated ICSS across a broad range of six frequencies (1.75–2.0 log Hz) relative to the daily baseline as it did in the saline-treated rats. On days 7 and 14 of amphetamine treatment, the daily baseline ICSS curve was significantly facilitated relative to the pre-pump baseline curve at the four to five frequencies indicated by dollar signs, indicating that amphetamine produced a sustained facilitation of ICSS. On these days, cocaine produced little additional facilitation of ICSS relative to the daily baseline. Specifically, on days 7 and 14, cocaine facilitated ICSS only at the lowest frequency of 1.75 log Hz (day 7) and at frequencies 1.8 and 1.85 log Hz (day 14). On P1, the first day after termination of amphetamine treatment, the daily baseline curve was significantly depressed relative to the pre-pump baseline at the six frequencies indicated by dollar signs (1.95–2.2 log Hz), and cocaine facilitated ICSS relative to this depressed daily baseline across nearly the entire frequency range (1.75–2.15 log Hz). On P3, the daily baseline partially recovered toward pre-pump baseline levels (significantly different at 1.95–2.05 log Hz), and cocaine facilitated ICSS at frequencies of 1.8–2.1 log Hz.

Figure 4 summarizes cocaine effects across time in the saline and amphetamine treatment groups by showing the total number of stimulations per component delivered across all frequencies after cocaine administration expressed as a percentage of the daily baseline number of stimulations per component. Within-group analysis of treatment effects over time indicated that the magnitude of cocaine-induced facilitation did not vary across time in the saline treatment group (black bars); however, relative to day 0 effects, the magnitude of cocaine-induced facilitation decreased during amphetamine treatment and increased on the day after amphetamine pump removal (P1, white bars). Between-group analysis of treatments on each test day indicated that the magnitude of cocaine facilitation was not different between the saline and amphetamine groups on days 0, P1, and P3, but on days 7 and 14, the effect of cocaine was significantly reduced in the amphetamine group relative to the saline group.

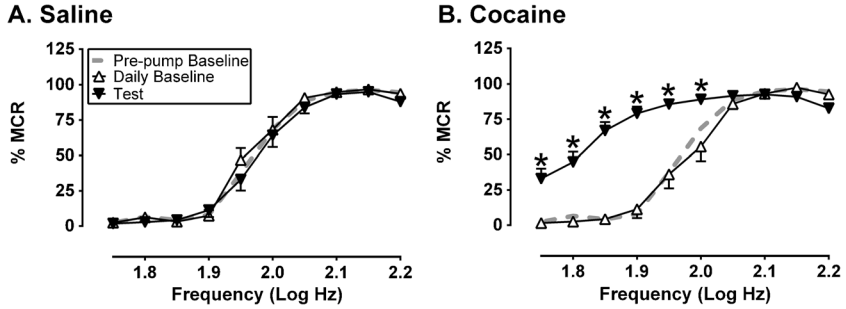

Fig. 1 Saline and cocaine effects on ICSS before pump implantation. *Abscissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: ICSS rate expressed as percent maximum control rate (*%MCR*). *Curves* are shown for (1) the pre-pump baseline, determined during a period of 3 days before implantation of saline or amphetamine osmotic pumps, (2) the daily baseline, determined on a given test day before saline or cocaine administration, and (3) the saline or cocaine test, determined on a given test day after administration of saline or 10 mg/kg cocaine. All points show mean±SEM for 13 rats. *Asterisks* denote points at which cocaine facilitated ICSS relative to the daily baseline, as indicated by a significant two-way ANOVA followed by a Holm-Sidak post hoc test ($p<0.05$): **a** Significant main effect of frequency [$F(9,108)=173.5$, $p<0.0001$] but not treatment [$F(2,24)=1.602$, $p=0.2223$], and the interaction was not significant [$F(18,216)=1.115$, $p=0.3386$]. **b** Significant main effect of frequency [$F(9,108)=172.0$, $p<0.0001$] and treatment [$F(2,24)=77.86$, $p<0.0001$], and a significant interaction [$F(18,216)=23.43$, $p<0.0001$]

The blunted facilitation of ICSS by cocaine during amphetamine treatment may have resulted from low sensitivity of the assay to detect facilitation when ICSS rates were already high across most of the frequency range. To evaluate this possibility, an additional experiment was conducted in the amphetamine-treated rats 14 days after pump removal. On the test day, brain stimulation intensities were increased by 40 %, and effects of cocaine were determined. Figure 5a shows that a 40 % increase in stimulus intensities increased baseline ICSS to levels similar to those observed after 14 days of treatment with amphetamine. Figure 5b shows that, despite producing statistically similar baselines, 10 mg/kg cocaine was able to produce greater facilitation of ICSS under conditions of increased intensity than under conditions of amphetamine treatment.

The saline and amphetamine treatments used in this study had little effect on body weight. Body weights (±SEM) in the saline and amphetamine groups were 428.4±15.93 and 412.2 ±13.54 g, respectively, before treatment (day 0) and 440.6± 11.29 and 444.0±11.23 g, respectively, at the end of the 14-day treatment period (day 14). These values were not significantly different at either time point ($p>0.05$).

Discussion

This study evaluated effects of amphetamine treatment and subsequent cocaine challenges on ICSS in rats. There were two main findings. First, the amphetamine treatment itself produced persistent facilitation of ICSS over the 14 days of treatment, and termination of treatment produced depression of ICSS. Second, cocaine significantly facilitated ICSS before and after treatment with amphetamine, but cocaine-induced facilitation of ICSS was blunted during amphetamine treatment. Taken together, these data suggest that chronic amphetamine treatment produced little tolerance to its own ICSS-facilitating effects and no apparent cross-tolerance to the effects of cocaine. Rather, these results support the hypothesis that amphetamine produced sustained effects on brain reward substrates that obscured expression of abuse-related cocaine effects.

Effect of chronic amphetamine treatment on ICSS Acutely, amphetamine facilitates ICSS (Carey and Goodal 1975; Esposito et al. 1980; Lin et al. 2000; Schmidt et al. 2012; Bauer et al. 2013), and effects of chronic amphetamine in this study are similar to effects described previously using other ICSS procedures (Lin et al. 2000; Paterson et al. 2000; Anderson et al. 1978). For example, in a study that manipulated ICSS stimulation intensity rather than frequency, chronic amphetamine treatment by osmotic pump (0.21 and 0.42 mg/kg/h) produced a sustained facilitation of ICSS as indicated by a sustained reduction in the threshold of stimulation intensity required to maintain responding (Paterson et al. 2000). The present study confirms and expands on these previous findings by demonstrating that amphetamine also produced a sustained increase in low rates of ICSS maintained by low frequencies of stimulation, but high baseline rates of responding maintained by high stimulation frequencies were not significantly affected at any time during treatment. Additionally, the current study examined ICSS over a longer course of amphetamine treatment (14 vs. 6 days) and found that tolerance to the rate-increasing effects of amphetamine did not develop even over this longer treatment period. Tolerance to amphetamine-induced facilitation of ICSS was reported in a procedure that assessed effects of bolus amphetamine doses on response rates maintained by a single stimulation intensity and frequency (Leith and Barrett 1976; Anderson et al. 1978); however, this required an escalating dosage regiment to terminal daily amphetamine doses more than four times higher than those used here, and it was observed only for ICSS maintained by stimulation of brain sites other than the lateral hypothalamus site used here. Also

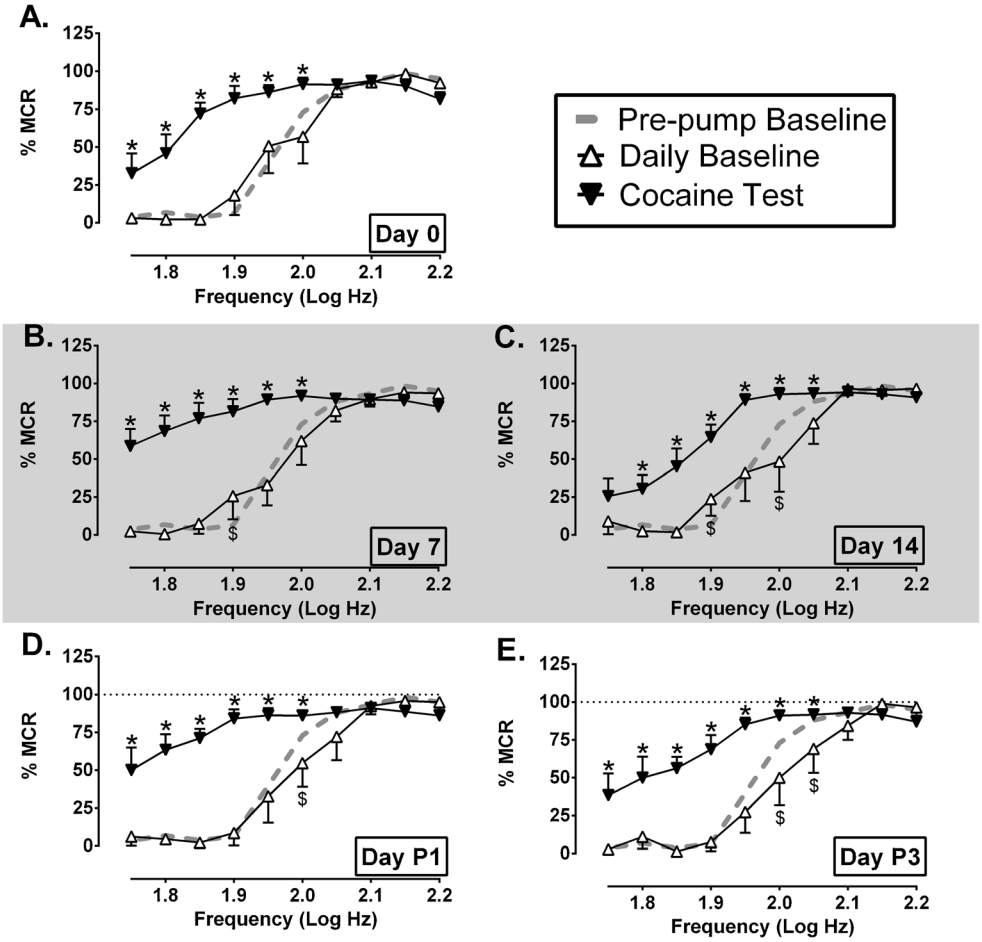

Fig. 2 Cocaine effects on ICSS in saline-treated rats. Panels show ICSS frequency-rate curves determined before cocaine (daily baseline) and after administration of 10 mg/kg cocaine (cocaine test) on test days that occurred before (day 0), during (days 7 and 14), and after (P1 and P3) chronic treatment with saline delivered via osmotic pump. The pre-pump baseline frequency-rate curve is also included in each panel for comparison. *Gray shaded background* indicates period of saline treatment. *Abscissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: ICSS rate expressed as percent maximum control rate (*%MCR*). All points show mean±SEM for six rats. *Dollar signs* denote points of significant difference between daily baseline and pre-pump baseline curves, and *asterisks* denote points at which cocaine facilitated ICSS relative to the daily baseline, as indicated by a significant two-way ANOVA followed by a Holm-Sidak post hoc test ($p<0.05$): **a** Significant main effect of frequency [$F(9,45)=65.67$, $p<0.0001$] and treatment [$F(2,10)=19.59$, $p=0.0003$], and a significant interaction [$F(18,90)=8.822$, $p<0.0001$]. **b** Significant main effect of frequency [$F(9,45)=54.71$, $p<0.0001$] and treatment [$F(2,10)=28.28$, $p<0.0001$], and a significant interaction [$F(18,90)=13.58$, $p<0.0001$]. **c** Significant main effect of frequency [$F(9,45)=50.91$, $p<0.0001$] and treatment [$F(2,10)=9.149$, $p=0.0055$], and a significant interaction [$F(18,90)=5.411$, $p<0.0001$]. **d** Significant main effect of frequency [$F(9,45)=51.90$, $p<0.0001$] and treatment [$F(2,10)=32.16$, $p<0.0001$], and a significant interaction [$F(18,90)=10.74$, $p<0.0001$]. **e** Significant main effect of frequency [$F(9,45)=58.25$, $p<0.0001$] and treatment [$F(2,10)=33.47$, $p<0.0001$], and a significant interaction [$F(18,90)=6.005$, $p<0.0001$]

consistent with previous studies (Paterson et al. 2000; Leith and Barrett 1976), termination of amphetamine treatment produced a withdrawal-associated depression of ICSS that was maximal after 24 h and dissipated over the course of a few days. Taken together, these results suggest that a regimen of continuous amphetamine treatment that reduces cocaine self-administration in rats (Chiodo and Roberts 2009; Thomsen et al. 2013) fails to produce tolerance to amphetamine effects on ICSS; however, the transient depression of ICSS after termination of treatment can be interpreted as a withdrawal sign indicative of amphetamine dependence.

The sustained rate-increasing effects of amphetamine on ICSS contrast with amphetamine effects on operant behaviors maintained by reinforcers other than electrical brain stimulation. For example, chronic amphetamine treatment initially decreases food-maintained responding under various schedules of reinforcement, and these effects dissipate after approximately 7 days (Negus and Mello 2003a, b; Schuster et al. 1966; Demellweek and Goudie 1983; Balster 1985). This dissipation of amphetamine effects on food-maintained responding has been referred to as "behavioral tolerance," because it depends not only on drug exposure but also on opportunities for the subject to perform the target behavior during chronic drug treatment. One hypothesis regarding development of behavioral tolerance to amphetamine effects on operant behavior is that tolerance develops more readily to

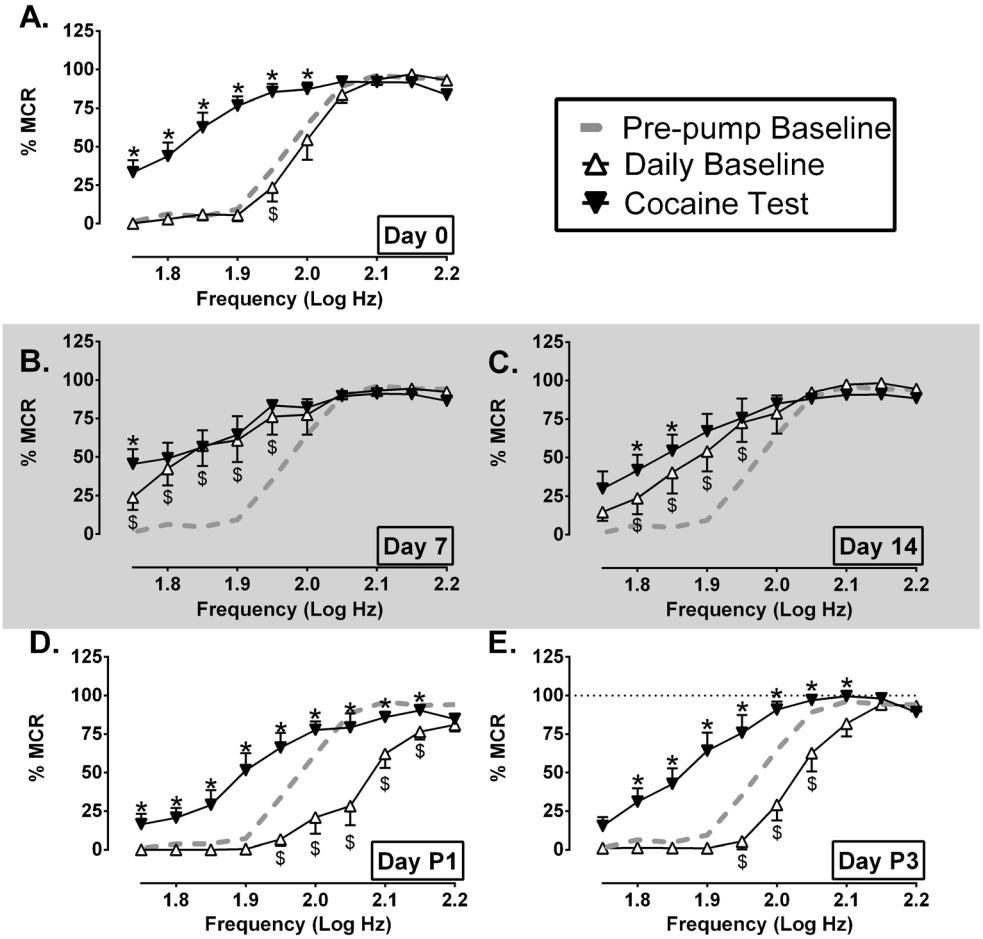

Fig. 3 Cocaine effects on ICSS in amphetamine-treated rats. Panels show ICSS frequency-rate curves determined before cocaine (daily baseline) and after administration of 10 mg/kg cocaine (cocaine test) on test days that occurred before (day 0), during (days 7and 14), and after (P1 and P3) chronic treatment with amphetamine (0.32 mg/kg/h) delivered via osmotic pump. The pre-pump baseline frequency-rate curve is also included in each panel for comparison. *Gray shaded background* indicates period of amphetamine treatment. *Abscissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: ICSS rate expressed as percent maximum control rate (*%MCR*). All points show mean±SEM for seven rats except panel **d**. An equipment malfunction prevented data collection in one rat on P1, so panel **d** shows data for only six rats. *Dollar signs* denote points of significant difference between daily baseline and pre-pump baseline curves, and *asterisks* denote points at which cocaine facilitated ICSS relative to the daily baseline, as indicated by a significant two-way ANOVA followed by a Holm-Sidak post hoc test ($p<0.05$): **a** Significant main effect of frequency [$F(9,54)=101.3$, $p<0.0001$] and treatment [$F(2,12)=87.57$, $p<0.0001$], and a significant interaction [$F(18,108)=16.24$, $p<0.0001$]. **b** Significant main effect of frequency [$F(9,54)=35.09$, $p<0.0001$] and treatment [$F(2,12)=27.02$, $p<0.0001$], and a significant interaction [$F(18,108)=8.347$, $p<0.0001$]. **c** Significant main effect of frequency [$F(9,54)=42.62$, $p<0.0001$] and treatment [$F(2,12)=15.87$, $p=0.0004$], and a significant interaction [$F(18,108)=6.931$, $p<0.0001$]. **d** Significant main effect of frequency [$F(9,45)=98.73$, $p<0.0001$] and treatment [$F(2,10)=41.20$, $p<0.0001$], and a significant interaction [$F(18,90)=8.184$, $p<0.0001$]. **e** Significant main effect of frequency [$F(9,54)=160.3$, $p<0.0001$] and treatment [$F(2,12)=53.27$, $p<0.0001$], and a significant interaction [$F(18,108)=7.471$, $p<0.0001$]

amphetamine effects that decrease rates of reinforcement than to amphetamine effects that do not alter or increase reinforcement rates (Schuster et al. 1966; Demellweek and Goudie 1983; Balster 1985; Rees et al. 1987). According to this hypothesis, amphetamine effects on ICSS might be resistant to tolerance because amphetamine increases rather than decreases rates of reinforcement.

Amphetamine effects on cocaine-induced facilitation of ICSS The current study confirmed previous findings by this lab and others that cocaine facilitates ICSS by increasing low and moderate rates of responding with little impact on high rates of responding (Esposito et al. 1978; Negus et al. 2012). This cocaine-induced facilitation of ICSS was replicable with repeated testing in the saline-treated group in the present study; however, amphetamine treatment blunted cocaine-induced facilitation. Specifically, during amphetamine treatment, cocaine facilitated ICSS to a smaller extent, and significant facilitation was observed at fewer stimulation frequencies, than in saline-treated rats. To the degree that facilitation of ICSS can be interpreted as an abuse-related drug effect (Wise 1996; Carlezon and Chartoff 2007; Bauer et al. 2013), these results can be interpreted to suggest that chronic amphetamine treatment decreased the abuse-related effects of

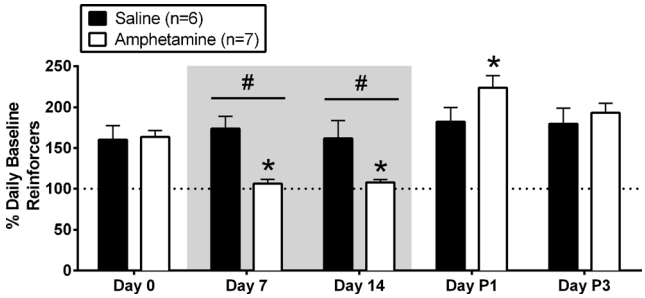

Fig. 4 Summary of cocaine effects across time in each treatment group. *Black and white bars* show cocaine effects on ICSS in saline and amphetamine treatment groups, respectively. *Gray shaded background* indicates period of saline or amphetamine treatment via osmotic pump. *Abscissa*: day of cocaine test before (day 0), during (days 7 and 14), or after (P1 and P3) treatment. *Ordinate*: total number of stimulations per component after cocaine treatment expressed as a percentage of total stimulations per component before cocaine treatment on that day (% daily baseline reinforcers). All points show mean±SEM for six or seven rats. *Asterisks* denote a statistically significant difference in ICSS from day 0 within a given treatment group, and *pound symbols* denote a statistically significant difference in ICSS between treatment groups on a given day, as indicated by a significant two-way ANOVA followed by a Holm-Sidak post hoc test ($p<0.05$): significant effect of time [$F(4,44)=13.77$, $p<0.0001$] but not treatment group [$F(1,11)=0.8450$, $p<0.3777$]; the interaction was significant [$F(4,44)=8.606$, $p<0.0001$]. Note: As noted above, an equipment malfunction prevented data collection in one amphetamine-treated rat on P1; however, this rat completed the other four test sessions and displayed a cocaine effect similar to the group average. To permit inclusion of data for this rat in the overall analysis across days, the cocaine effect for this rat on P1 was assigned the mean effect for the other six amphetamine-treated rats that did complete testing on P1

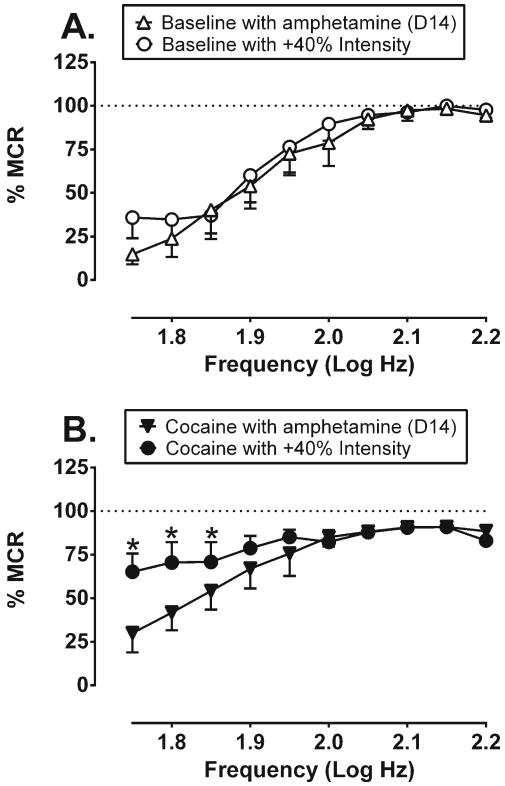

Fig. 5 Cocaine effects on ICSS maintained by high-intensity stimulation. Panel **a** compares daily baseline ICSS frequency-rate curves determined on day 14 of amphetamine treatment (*open triangles*) and after a 40 % increase in stimulation intensity (*open circles*). Panel **b** shows ICSS frequency-rate curves determined after 10 mg/kg cocaine on day 14 of amphetamine (*closed triangles*) and at the increased stimulation intensity (*closed circles*). *Abscissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: ICSS rate expressed as percent maximum control rate (*%MCR*). All points show mean±SEM for seven rats. *Asterisks* denote points at which cocaine facilitated ICSS more in the +40 % intensity condition than in the amphetamine treatment condition, as indicated by a significant two-way ANOVA followed by a Holm-Sidak post hoc test ($p<0.05$): **a** Significant main effect of frequency [$F(9,54)=22.73$, $p<0.0001$], but no main effect of amphetamine vs. +40 % intensity [$F(1,6)=1.224$, $p=0.3110$], and no interaction [$F(9,54)=0.7172$, $p=0.6909$]). **b** Significant main effect of frequency [$F(9,54)=8.074$, $p<0.0001$], significant main effect of amphetamine vs. + 40 % intensity [$F(1,6)=9.954$, $p=0.0197$], and a significant interaction [$F(9,54)=5.638$, $p<0.0001$]

cocaine. This is the first demonstration that chronic amphetamine treatment decreases abuse-related cocaine effects in an ICSS procedure. However, these results from an ICSS procedure agree with previous studies to show that chronic amphetamine treatment decreased abuse-related reinforcing effects of cocaine in assays of self-administration in rats (Chiodo et al. 2008; Chiodo and Roberts 2009; Thomsen et al. 2013), rhesus monkeys (Negus and Mello 2003a, b; Czoty et al. 2010; Negus 2003; Banks et al. 2013), and humans (Greenwald et al. 2010; Rush et al. 2010), and also decreased metrics of cocaine use in double-blind, placebo-controlled clinical trials (Grabowski et al. 2001, 2004; Mariani et al. 2012; Schmitz et al. 2012). Consequently, one implication of the present results is that treatment effects on cocaine-induced facilitation of ICSS may be useful in medication development as a tool to predict treatment effects on both cocaine self-administration in the laboratory and cocaine use by drug-dependent humans.

As discussed in the "Introduction," decreases in abuse-related cocaine effects during chronic amphetamine treatment could reflect one of two general processes: (a) tolerance to amphetamine effects and cross-tolerance to cocaine effects or (b) sustained amphetamine effects that obscured expression of cocaine effects. Results support the latter of these two possibilities. Amphetamine produced a sustained facilitation of ICSS suggesting a sustained effect of amphetamine on underlying brain reward substrates. Two other findings also support the importance of sustained amphetamine effects for attenuation of cocaine effects. First, cocaine-induced facilitation of ICSS recovered completely by 24 h after termination of amphetamine treatment. This suggests that attenuation of cocaine effects depended on the presence of amphetamine and not on amphetamine-induced neuroplasticity (e.g., down-regulation of dopamine transporters; Schmitt and Reith 2010) that might recover only gradually after termination of amphetamine treatment and that might have contributed to the gradual recovery of baseline ICSS. Second, a 40 % increase in stimulation intensity produced a similar increase in ICSS baseline as that produced by amphetamine treatment; however, cocaine produced greater additional ICSS facilitation from

this high-intensity baseline than from the amphetamine baseline. This suggests that attenuation of cocaine-induced facilitation of ICSS depended on the presence of amphetamine and not on any decrease in assay sensitivity associated with the amphetamine-induced change in baseline. The apparent lack of a role for amphetamine tolerance and cocaine cross-tolerance in this ICSS study agrees with previous evidence that pharmacological tolerance does not play a role in amphetamine-induced decreases in cocaine self-administration (Chiodo et al. 2008).

The present study also suggests that cocaine-induced ICSS facilitation recovers more quickly than cocaine self-administration after termination of amphetamine treatment. Thus, in the present study, cocaine-induced facilitation of ICSS recovered on the first day after amphetamine treatment, and within-subjects analysis of data in the amphetamine treatment group even indicated a greater cocaine effect on the first day after termination of treatment than before treatment (although between-groups analysis did not confirm a larger cocaine effect on this day in the amphetamine vs. saline treatment groups). Conversely, cocaine self-administration recovered gradually over the course of 7 days after termination of amphetamine treatment in rats and rhesus monkeys (Negus and Mello 2003a; Chiodo et al. 2008). To the degree that these ICSS data can inform interpretation of self-administration data, these results support the view that slow recovery of cocaine self-administration after amphetamine treatment does not reflect slow recovery of cocaine effects, but rather may reflect learning processes such as those associated with reacquisition of self-administration after a period of extinction (Czoty et al. 2010; Zimmer et al. 2013).

Conclusion

These data provide additional evidence to suggest that chronic amphetamine treatment blunts expression of abuse-related cocaine effects and support a growing body of pre-clinical and clinical literature supporting an agonist therapy approach to cocaine abuse.

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Conflict of interest There are no other conflicts of interest.

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