## ARTICLE

# Amphetamine maintenance differentially modulates effects of cocaine, methylenedioxypyrovalerone (MDPV), and methamphetamine on intracranial self-stimulation and nucleus accumbens dopamine in rats

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Amphetamine maintenance is effective clinically to reduce the consumption of the monoamine uptake inhibitor cocaine but not of the monoamine releaser methamphetamine, and its effectiveness in treating the abuse of other psychostimulants is not known. The mechanisms for differential amphetamine-maintenance effectiveness to treat different types of psychostimulant abuse are also not known. Accordingly, the present study compared the effects of amphetamine maintenance on abuse-related behavioral and neurochemical effects of cocaine, methamphetamine, and the "bath salts" constituent 3,4-methylenedioxypyrovalerone (MDPV) in rats. In behavioral studies, rats were trained to lever press for electrical brain stimulation in an intracranial self-stimulation (ICSS) procedure. In neurochemical studies, nucleus accumbens (NAc) levels of dopamine (DA) and serotonin (5-HT) were monitored by in vivo microdialysis. Cocaine, methamphetamine, and MDPV each produced dose-dependent ICSS facilitation and increases in NAc DA; cocaine and methamphetamine also increased NAc 5-HT. Amphetamine maintenance ($0.32\,mg/kg/h \times 7$ days) produced (1) sustained increases in basal ICSS and NAc DA with no change in NAc 5-HT, (2) blockade of cocaine but not methamphetamine effects on ICSS and NAc DA, and (3) no blockade of cocaine- or methamphetamine-induced increases in NAc 5-HT. Amphetamine maintenance blocked the increases in NAc DA produced by the selective DA uptake inhibitor MDPV, but it did not block MDPV-induced ICSS facilitation. These results show different effects of amphetamine maintenance on behavioral and neurochemical effects of different psychostimulants. The selective effectiveness of amphetamine maintenance to treat cocaine abuse may reflect attenuation of cocaine-induced increases in NAc DA while preserving cocaine-induced increases in NAc 5-HT.

## INTRODUCTION

There are more than 2 million current (i.e., past month) psychostimulant users in the United States [1], and no pharmacotherapies are currently approved by the Food and Drug Administration for the treatment of psychostimulant abuse. According to the 2016 National Survey on Drug Use and Health [1], cocaine and methamphetamine are the two most commonly abused psychostimulants, and other drugs such as 3,4-methylenedioxypyrovalerone (MDPV) have emerged as problem drugs of abuse during the last decade [2]. Many psychostimulants produce their effects by interacting with transporters for the monoamine neurotransmitters dopamine (DA), serotonin (5-HT), and norepinephrine (NE) (DAT, SERT, and NET, respectively) to increase synaptic monoamine levels in brain areas such as nucleus accumbens (NAc), and drugs that are more potent to increase DA vs. 5-HT tend to produce more robust abuse-related effects [3–6]. Moreover, there are two broad classifications of monoamine transporter ligands based on their transporter interactions. Uptake inhibitors like cocaine and MDPV bind to and block transporters to promote extracellular accumulation of the associated monoamine, whereas releasers like methamphetamine and amphetamine pass through the

transporters and cause a cascade of events leading to monoamine efflux [7, 8].

Although no pharmacotherapies are approved to treat psychostimulant use disorders, amphetamine maintenance decreases cocaine use in double-blind placebo-controlled clinical trials [9–13] and also decreases the choice of cocaine over an alternative reinforcer in laboratory studies in humans, non-human primates, and rats [14–16]. In contrast, amphetamine maintenance is not effective in decreasing methamphetamine use in either clinical trials or preclinical studies [17, 18], and the effects of amphetamine maintenance on abuse-related effects of other psychostimulants like MDPV are unknown. Additionally, the mechanisms that underlie selective amphetamine-maintenance effects on cocaine vs. methamphetamine remain to be determined. Amphetamine maintenance is thought to function as an agonist-type therapy for cocaine abuse because both drugs produce similar behavioral effects and increase NAc DA levels [19, 20]; however, it is not clear why such an agonist-type effect would be selective for cocaine but not methamphetamine.

Intracranial self-stimulation (ICSS) is one preclinical procedure that can be used to evaluate the effects of candidate medications on abuse-related drug effects [21, 22], and we reported previously

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that an amphetamine-maintenance regimen sufficient to reduce cocaine-vs.-food choice in rats [16] also blunted cocaine-induced ICSS facilitation in rats [23]. The present study extended this finding in three ways. First, amphetamine-maintenance effects were compared on ICSS facilitation produced by cocaine, MDPV, and methamphetamine. Second, ICSS facilitation by monoamine transporter ligands correlates with selectivity to increase DA vs. 5-HT levels in NAc [5, 24]. Accordingly, we also used in vivo microdialysis to compare amphetamine-maintenance effects on cocaine-, MDPV-, and methamphetamine-induced changes in NAc DA and 5-HT levels. Given the preclinical and clinical effects of amphetamine maintenance on cocaine and methamphetamine self-administration, we hypothesized that amphetamine maintenance would block the effects of cocaine, but not of methamphetamine, on both ICSS and NAc DA levels, whereas changes in MDPV effects were more difficult to predict. Lastly, effects of amphetamine maintenance on striatal DAT density were also determined, because DAT downregulation is one possible mechanism of amphetamine maintenance-induced decreases in cocaine effects [25, 26].

## METHODS

### Subjects
Studies were conducted in 119 male Sprague–Dawley rats (Envigo, Frederick, MD), weighing 300–350 g at the time of surgery. Rats were individually housed on a 12-h light–dark cycle (lights on 6 a. m.–6 p.m.) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All rats had ad libitum access to food and water in their home cages. Animal maintenance accorded with The National Institutes of Health guidelines on care and use of research animals, and experimental protocols were approved by the Virginia Common-wealth University Institutional Animal Care and Use Committee. Procedures for implantation of microelectrodes, guide cannulae, and minipumps were identical to those published previously [5, 23]. Separate groups of rats were used for studies of ICSS, microdialysis, and receptor binding.

### Intracranial self-stimulation
*Training.* Studies were conducted in operant chambers (Med Associates, St. Albans, VT) using procedures for training, testing, and data analysis identical to those described previously [4, 23, 27, 28]. Daily behavioral sessions consisted of three 10-min components, each consisting of ten 1-min trials. Each trial presented a different frequency of electrical stimulation available for brain stimulation, and the frequency decreased in 0.05 log increments across trials from 158 to 56 Hz. During the first 10 s of each trial, five non-contingent stimulations were delivered. During the remaining 50 s, lever presses produced brain stimulation and illumination of the stimulus lights over the lever under an FR 1 reinforcement schedule. Training continued until stabilization (3 days during which the maximum control rate (MCR) of stimulations per trial and total stimulations per component on each day were within 15% of the running mean across days). This was completed within 12 weeks of surgery, and the final 3 days of training served to establish the "Pre-pump" baseline for ICSS performance.

*Testing.* Once the pre-pump baseline performance was established, drug testing was conducted using an 8-day treatment protocol. On Days 0 and 7, the rats received a series of increasing intraperitoneal (IP) doses of cocaine (1, 3.2, 10 mg/kg), MDPV (0.1, 0.32, 1.0 mg/kg), or methamphetamine (0.1, 0.32, 1.0 mg/kg). Dose ranges of each drug were selected based on previous studies that showed dose-dependent ICSS facilitation by each drug [4, 27, 29]. Dose-effect sessions consisted of three baseline ICSS components followed by three drug injections administered at 30-min intervals.

Two ICSS test components began 10 min after each injection. On Day 1, osmotic minipumps containing either saline, 0.1 or 0.32 mg/kg/h amphetamine were surgically implanted, and three-component ICSS sessions were conducted on Days 2–6 before the second dose-effect session on Day 7. Groups of $N = 6$ rats were used to test each minipump treatment in combination with each test drug, and only cocaine was tested with 0.1 mg/kg/h amphetamine.

*Data analysis.* Data from the first ICSS component on each day were discarded. The principal dependent measure was the number of stimulations per trial. These data were normalized for each rat to a percent maximum control rate (%MCR), with MCR defined as the average of the maximal number of stimulations in any trial of the 2nd and 3rd components of the three pre-pump baseline sessions, and %MCR is calculated using the equation %MCR = (stimulations per trial/MCR) × 100. Data from the three pre-pump baseline sessions were averaged within each rat and then across rats to generate a group pre-pump baseline "frequency–rate" curve. For dose-effect sessions, data from each pair of baseline and test components were averaged within each rat and then across rats to generate group mean frequency-rate curves for baseline and each drug dose. Frequency-rate curves were compared by repeated measures two-way ANOVA, with ICSS frequency and the experimental manipulation as the two factors. A significant interaction was followed by Holm–Sidak post-hoc test. The criterion for significance for this and all other analyses was set at $p < 0.05$. Additionally, the EF50 for each ICSS curve was defined as the "effective frequency" that maintained 50% MCR. EF50 values and 95% confidence limits were interpolated by linear regression from the linear portion of each ICSS curve, and EF50 values were considered to be different if 95% confidence limits did not overlap. In some cases, all points were above 50% MCR; in these cases, interpolation of EF50 values was not possible, and EF50 is shown as "<1.75" because this was the lowest frequency tested.

### Microdialysis
*Procedure.* Procedures for the collection and analysis of NAc microdialysis samples were similar to those described previously [5]. Baseline samples were collected at 10-min intervals until DA and 5-HT levels stabilized (six consecutive baseline samples with <25% variability around the running mean). Subsequently, an injection was administered IP, and samples were collected for another 100 min. Initial studies evaluated the effects of saline and multiple doses of cocaine (1.0, 3.2, 10 mg/kg), MDPV (0.1, 0.32, 1.0 mg/kg), or methamphetamine (0.1, 0.32, 1.0 mg/kg) identical to those tested in ICSS. Each treatment was tested in six rats. Next, to determine the effects of amphetamine maintenance on test-drug effects, new groups were surgically implanted with minipumps containing either saline or 0.32 mg/kg/ h amphetamine and tested between 7 and 13 days after minipump implantation with a dose of cocaine (10 mg/kg), MDPV (0.32 mg/kg), or methamphetamine (0.32 mg/kg) that produced similar 200–250% increases in NAc DA levels during initial dose-effect studies. Each minipump treatment and test-drug group contained six rats.

*Data analysis.* The dependent variables were DA and 5-HT concentrations in each dialysate fraction. For dose-effect studies, data at each time point were expressed as a percentage of the baseline concentration for each neurotransmitter: %Baseline = (test concentration/baseline concentration) × 100. Results were averaged across the rats and analyzed at each drug dose using repeated-measures one-way ANOVA, with time as a fixed effect and subject as a random effect. A significant ANOVA was followed by Dunnett's post-hoc test to compare the monoamine concentrations at each time point with control concentrations

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**Table 1.** EF50 values (95% confidence limits) in log Hz after the administration of cocaine, MDPV, or methamphetamine in rats tested before implantation of minipumps (Pre-pump) or 7 days after implantation of minipumps that delivered either saline or 0.32 mg/kg/h amphetamine

| Drug | Dose (mg/kg) | Pre-pump | Saline | Amphetamine |
|---|---|---|---|---|
| Cocaine | Baseline | 2.02 (1.99–2.05) | 2.02 (2.02–2.03) | 1.90 (1.88–1.92) |
| | 1.0 | 1.93 (1.91–1.94)[a] | 1.96 (1.93–1.99)[a] | 1.87 (1.83–1.90) |
| | 3.2 | 1.89 (1.88–1.90)[a] | 1.88 (1.83–1.92)[a] | 1.91 (1.88–1.93) |
| | 10 | 1.75 (1.49–1.80)[a] | 1.77 (1.57–1.83)[a] | 1.83 (1.82–1.84)[a] |
| MDPV | Baseline | 2.02 (1.97–2.06) | 2.01 (2.01–2.02) | 1.86 (1.80–1.89) |
| | 0.1 | 1.97 (1.96–1.99) | 1.98 (1.94–2.02) | 1.84 (1.76–1.89) |
| | 0.32 | 1.80 (1.78–1.82)[a] | <1.75[b] | <1.75[b] |
| | 1.0 | <1.75[b] | <1.75[b] | <1.75[b] |
| Methamphetamine | Baseline | 2.01 (1.99–2.04) | 2.03 (2.00–2.05) | 1.92 (1.91–1.94) |
| | 0.1 | 1.95 (1.94–1.95)[a] | 1.93 (1.92–1.94)[a] | 1.89 (1.88–1.90)[a] |
| | 0.32 | 1.83 (1.82–1.83)[a] | 1.83 (1.78–1.87)[a] | 1.77 (1.75–1.79)[a] |
| | 1.0 | <1.75[b] | <1.75[b] | <1.75[b] |

[a]Indicates significantly different from "Baseline" as determined by non-overlapping confidence limits
[b]Indicates all points >50% MCR, and EF50 could not be calculated. This outcome was also considered to be significantly different from baseline

determined 10 min after the drug administration. This sample was selected as the control because preliminary experiments indicated a lag time of ~20 min for dialysate to traverse the tubing from the probe to the electrochemical detector at the 1 μL/min flow rate. Accordingly, the 10-min sample was collected prior to drug administration, had advanced into the auto-injector tubing at the time of drug injection, and was evaluated after drug injection.

For minipump studies, baseline DA and 5-HT levels in saline-treated and amphetamine-treated rats were compared by Student's *t*-test with Welch's correction. Because amphetamine maintenance significantly increased the baseline DA levels (see Results), data from minipump studies were expressed as a difference from baseline: Delta baseline = test concentration −baseline concentration. The effects of cocaine, MDPV, and methamphetamine over time during saline or amphetamine maintenance were analyzed by one-way ANOVA as above. Additionally, results of each drug across maintenance treatments were compared by two-way ANOVA with time and treatment as the two factors. A significant interaction was followed by a Holm–Sidak post-hoc test.

[³H]WIN35,428 saturation binding
*Membrane preparation.* The rats were implanted with minipumps containing saline ($N = 6$) or 0.32 mg/kg/h amphetamine ($N = 8$). After 7 days of treatment, the rats were euthanized by rapid decapitation, and whole striatum, including NAc and caudate/putamen, were dissected on ice and frozen at −80 °C until use. On the day of each binding assay, the samples were thawed in cold assay buffer (20 mM sodium phosphate buffer, pH 7.9 with 0.32 M sucrose), homogenized with a Polytron homogenizer, and centrifuged at 50,000×g at 4 °C for 10 min. The supernatant was discarded, the pellet was re-suspended by homogenization in assay buffer, and the protein concentration was determined by the Bradford method.

*Binding assay.* The DA transporter inhibitors WIN35,428 [(−)-2β-carbomethoxy-3β-(4-fluorophenyl)tropane] and RTI-112 [2β-carbomethoxy-3β-(3-methyl-4-chlorophenyl)tropane] were used as the radiolabeled and non-labeled ligands, respectively [30]. Six concentrations of [³H]WIN35,428 (approximately 0.4–30 nM) were incubated in assay buffer containing 40 μg membrane protein for 90 min at 30 °C in a final volume of 0.25 ml. Non-specific binding was determined at each concentration of radioligand in the presence of 30 μM unlabeled RTI-112. The incubation was

terminated by rapid filtration under vacuum through GF/B glass fiber filters using a 48-well Brandel harvester and rinsed three times with 3 ml ice-cold 50 mM Tris-HCl, pH 7.4. Bound radioactivity was determined by liquid scintillation spectrophotometry at 45% efficiency for ³H after overnight equilibration of the filters in Econosafe scintillation fluid (Research Products International, Prospect, IL).

*Data analysis.* Data are reported as specific binding, derived from duplicate determinations. $B_{max}$ and $K_D$ values were determined by iterative curve fitting using single-site saturation analysis ($n_H = 1$) and compared in saline- and amphetamine-treated groups by two-tailed unpaired Student's *t*-test.

Drugs
For behavioral and microdialysis studies, (−)-cocaine HCl, (±)-MDPV HCl, and (+)-amphetamine hemisulfate were obtained from the National Institute for Drug Abuse Drug Supply Program (Bethesda, MD). (+)-Methamphetamine HCl was purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in bacteriostatic saline. Cocaine, MDPV, and methamphetamine were administered IP in a volume of 1 ml/kg. Amphetamine was delivered subcutaneously (SC) via osmotic minipump (2ML2, Alzet, Cupertino, CA) at a rate of 5 μl/h. For receptor binding studies, [³H]WIN35,428 (82.6 Ci/mmol) was purchased from Perkin–Elmer (Waltham, MA). RTI-112 was kindly provided by Dr. F. Ivy Carroll of Research Triangle Institute (Research Triangle Park, NC).

## RESULTS
### Intracranial self-stimulation
*Pre-pump baseline performance.* During pre-pump baseline sessions for all rats in the study, the mean ± SEM MCR was 55.06 ± 1.5 reinforcements per trial and the mean EF50 (95% confidence limits) was 2.02 (2.00–2.03) log Hz. One-way ANOVA indicated no difference in MCRs across treatment groups ($F_{(6,35)} = 1.35$, n.s.), and overlapping confidence limits indicated no difference in pre-pump baseline EF50 values (Table 1).

*Pre-pump effects of cocaine, MDPV, and methamphetamine.* Supplemental Fig. 1 and Table 1 show the effect of cocaine (1.0–10.0 mg/kg), MDPV (0.1–1.0 mg/kg), and methamphetamine (0.1–1.0 mg/kg) on Day 0, before the minipumps were implanted. Data are combined for saline- and amphetamine-treated rats because

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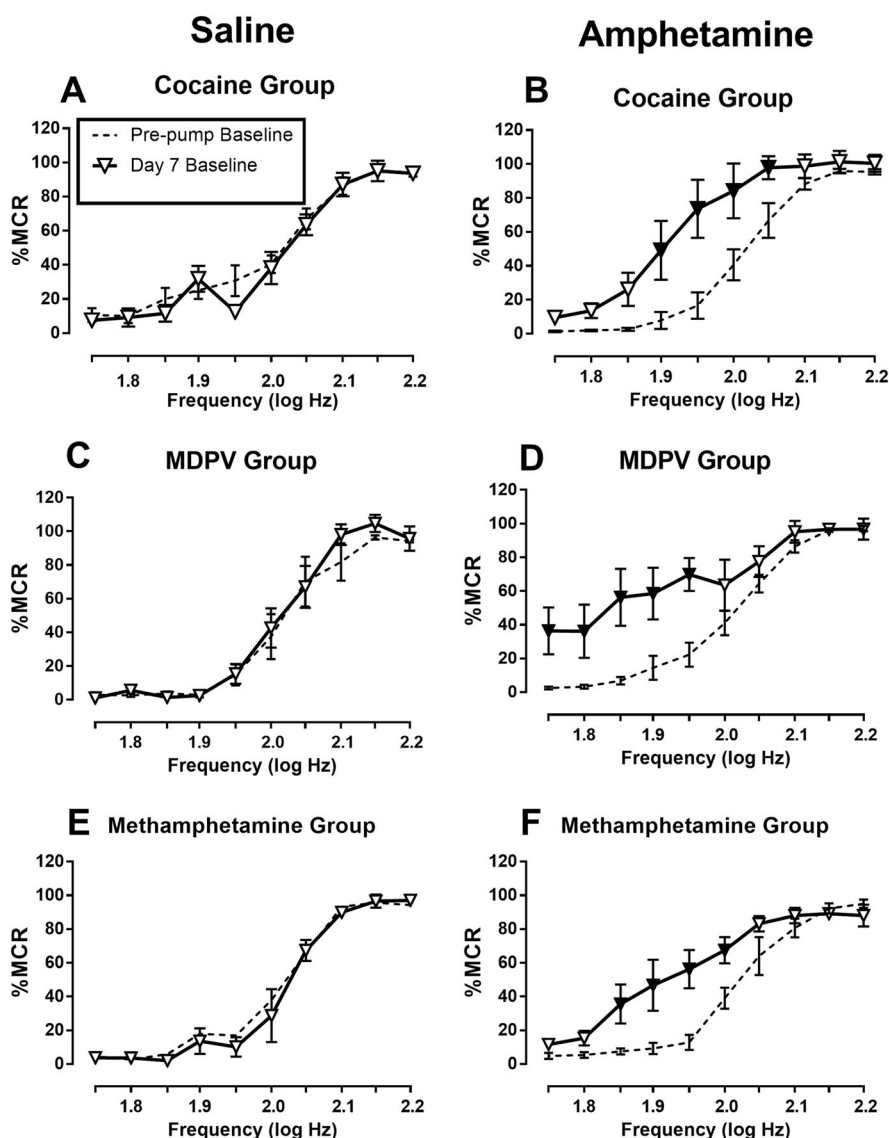
**Fig. 1** Effects of maintenance on saline or 0.32 mg/kg/h amphetamine on baseline ICSS performance in rats subsequently tested with cocaine (**a**, **b**), MDPV (**c**, **d**), or methamphetamine (**e**, **f**). Abscissae: brain stimulation frequency in log Hz. Ordinates: % maximum control rate. All points show mean ± SEM from $N = 6$ rats, and filled points indicate significantly different from "Pre-pump Baseline" as determined by two-way ANOVA followed by the Holm–Sidak post-hoc test ($p < 0.05$)

these data were collected before the minipumps were implanted and before the treatments had started. Brain stimulation maintained a frequency-dependent increase in reinforcement rates under baseline conditions, and all three drugs produced dose-dependent leftward/upward shifts in ICSS frequency–rate curves. Table 1 shows that all three drugs also produced dose-dependent decreases in EF50 values. EF50 values could not be determined for the highest doses of MDPV and methamphetamine, because facilitation was so robust that all points on the frequency–rate curves were above 50% MCR.

*Effects of saline or amphetamine maintenance on baseline ICSS.* Figure 1 shows the effects of saline or 0.32 mg/kg/h amphetamine maintenance on baseline ICSS. In saline-treated rats, the Day 7 baseline frequency–rate curves were not different from the pre-pump baseline in any group (no main effect of day and no significant day × frequency interaction, panels a, c, and e). Conversely, 0.32 mg/kg/h amphetamine maintenance facilitated

ICSS in all three groups [day × frequency interactions: (b) $F_{(9,45)} = 3.45$, $p = 0.0027$, (d) $F_{(9,45)} = 4.18$, $p = 0.0006$, (f) $F_{(9,45)} = 3.24$, $p = 0.0041$]. Additionally, Table 1 shows that EF50 values in saline-treated rats were similar to pre-pump baselines, whereas 0.32 mg/kg/h amphetamine reduced EF50 values. Supplemental Fig. 2A shows that a lower maintenance dose of 0.1 mg/kg/h amphetamine also facilitated ICSS in rats that were subsequently treated with cocaine.

*Effects of cocaine, MDPV, and methamphetamine on ICSS during saline or amphetamine maintenance.* Figure 2 shows the effects of cocaine, MDPV, and methamphetamine in rats treated with saline or 0.32 mg/kg/h amphetamine, and EF50 values are shown in Table 1. Effects of all three drugs during saline maintenance were similar to pre-pump effects. Amphetamine maintenance produced at least a 10-fold decrease in the potency of cocaine to facilitate ICSS. Thus, during saline treatment, cocaine dose-dependently facilitated ICSS at all three doses as indicated both

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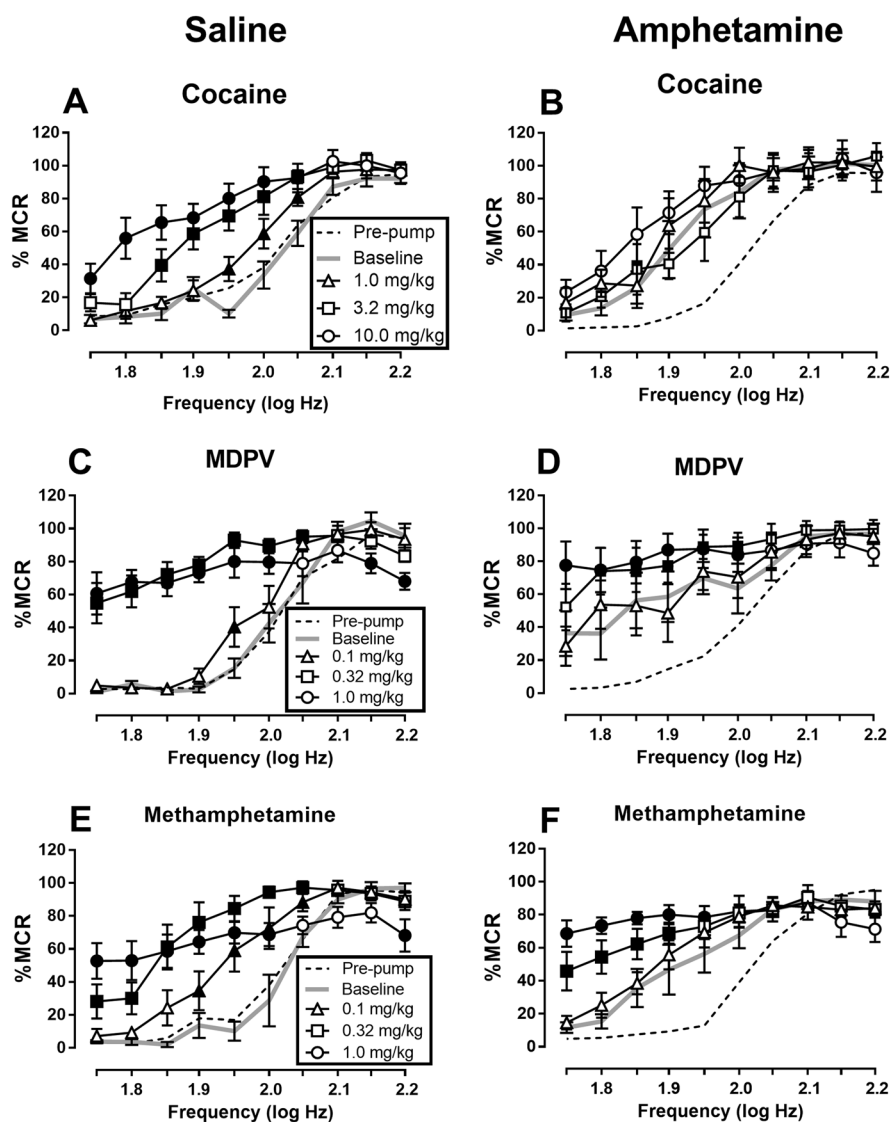
**Fig. 2** Effects of cocaine (**a**, **b**), MDPV (**c**, **d**), and methamphetamine (**e**, **f**) on ICSS on Day 7 of treatment with either saline (**a**, **c**, **e**) or 0.32 mg/kg/h amphetamine (**b**, **d**, **f**). Abscissae: brain stimulation frequency in log Hz. Ordinates: % maximum control rate. All points show mean ± SEM from $N = 6$ rats, and filled points indicate significantly different from "Baseline" as determined by two-way ANOVA followed by the Holm–Sidak post-hoc test ($p < 0.05$)

by two-way ANOVA of frequency–rate data (Fig. 2a: dose × frequency interaction $F_{(27,135)} = 3.93$, $p < 0.0001$) and by reductions in EF50 values (Table 1). However, during maintenance on 0.32 mg/kg/h amphetamine, there was a main effect of cocaine dose on ICSS frequency–rate curves (Fig. 2b: $F_{(3,15)} = 3.92$, $p = 0.030$) but no frequency × dose interaction ($F_{(27,135)} = 1.52$, n.s.). Dunnett's post-hoc test revealed that 10 mg/kg cocaine was different than baseline ($p < 0.05$). Moreover, only 10 mg/kg cocaine significantly reduced EF50 values (Table 1). Supplemental Fig. 2B shows that maintenance on a lower amphetamine dose (0.1 mg/kg/h) failed to blunt cocaine-induced ICSS facilitation.
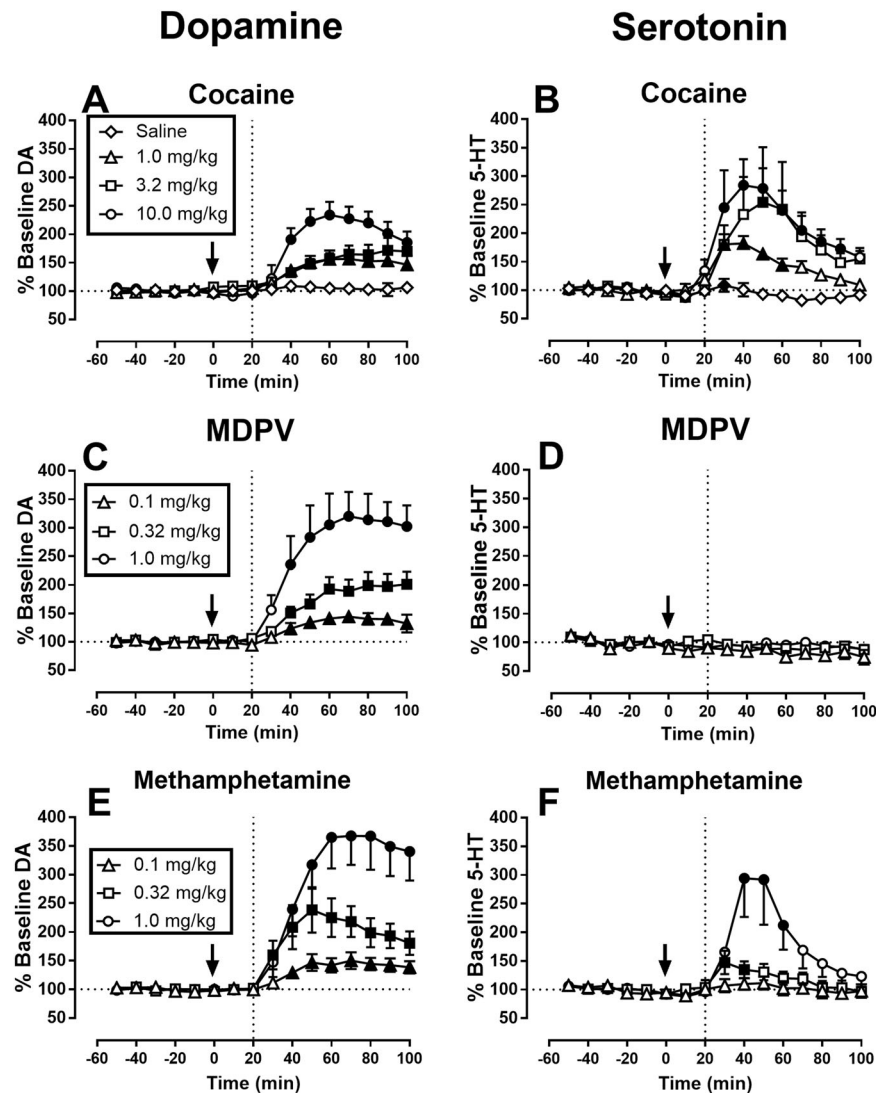
Maintenance on 0.32 mg/kg/h amphetamine had lesser effects on MDPV-induced ICSS facilitation. In saline-treated rats, the lowest dose of 0.1 mg/kg MDPV significantly increased ICSS at only one frequency (1.95 log Hz) and failed to alter the EF50, and higher doses of 0.32 and 1.0 mg/kg MDPV produced robust ICSS facilitation across a broad range of frequencies (Fig. 2c: dose × frequency interaction $F_{(27,135)} = 11.03$, $p < 0.0001$) and also significantly reduced EF50 values (Table 1). In rats treated with 0.32 mg/kg/h amphetamine, 0.1 mg/kg MDPV did not facilitate ICSS at

any frequency or reduce the EF50, but higher doses still facilitated ICSS by both analyses (Fig. 2d: dose × frequency interaction $F_{(27,135)} = 3.32$, $p < 0.0001$; Table 1).

Amphetamine maintenance also had only modest effects on methamphetamine-induced ICSS facilitation. In saline-treated rats, all methamphetamine doses facilitated ICSS both by two-way ANOVA of frequency–rate data (Fig. 2e: dose × frequency interaction $F_{(27,135)} = 9.13$, $p < 0.0001$) and by significant reductions in EF50 values (Table 1). In rats treated with 0.32 mg/kg/h amphetamine, the lowest dose of 0.1 mg/kg methamphetamine no longer facilitated ICSS by analysis of frequency–rate curves, but this dose did still produce a significant, if small, decrease in EF50 value; moreover, higher doses of 0.32 and 1.0 mg/kg methamphetamine produced robust ICSS facilitation by both analyses (Fig. 2f: dose × frequency interaction $F_{(27,135)} = 6.05$, $p < 0.0001$; Table 1).

Microdialysis
*Effects of cocaine, MDPV, and methamphetamine on NAc DA and 5-HT in rats without minipumps.* Supplemental Fig. 3 shows that all microdialysis probe placements were in the NAc. Figure 3 shows

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# Dopamine

## Serotonin



**Fig. 3** Effects of cocaine (**a**, **b**), MDPV (**c**, **d**), and methamphetamine (**e**, **f**) on NAc DA (**a**, **c**, **e**) and 5-HT (**b**, **d**, **f**) levels. Abscissae: time in minutes relative to test-drug injection. Vertical line at 20 min indicates earliest time of drug effect (see Methods). Ordinates: % of baseline DA or 5-HT. All points show mean ± SEM for $N = 6$ rats, and filled points indicate significantly different from the 10-min time point as determined by 1-way ANOVA followed by Dunnett's post-hoc test ($p < 0.05$)

the effects of saline, cocaine, MDPV, and methamphetamine on NAc DA and 5-HT levels in rats without minipumps. Baseline DA and 5-HT levels were $1.46 \pm 0.09$ and $0.27 \pm 0.01$ pg/9 µl, respectively. After saline injection, one-way ANOVA indicated that DA levels did not significantly change (Fig. 3a), but 5-HT levels increased slightly at 30 min to 109% of baseline (Fig. 3b: $F_{(9,45)} = 3.97$, $p = 0.0009$). Cocaine produced a dose- and time-dependent increase in both DA levels (Fig. 3a: 1.0 mg/kg: $F_{(9,45)} = 10.33$, $p < 0.0001$; 3.2 mg/kg: $F_{(9,45)} = 9.28$, $p < 0.0001$; 10.0 mg/kg: $F_{(9,45)} = 16.62$, $p < 0.0001$) and 5-HT levels (Fig. 3b: 1.0 mg/kg: $F_{(9,45)} = 7.74$, $p < 0.0001$; 3.2 mg/kg: $F_{(9,45)} = 2.32$, $p = 0.0305$; 10.0 mg/kg: $F_{(9,45)} = 6.91$, $p < 0.0001$). The 10 mg/kg cocaine dose increased DA levels to a maximum of 233% of baseline after 60 min, and 5-HT levels to a maximum of 284% of baseline after 40 min. MDPV produced a dose- and time-dependent increase in DA (Fig. 3c: 0.1 mg/kg: $F_{(9,45)} = 13.13$, $p < 0.0001$; 0.32 mg/kg: $F_{(9,45)} = 24.64$, $p < 0.0001$; 1.0 mg/kg: $F_{(9,45)} = 10.33$, $p < 0.0001$) but not 5-HT (Fig. 3d). The 0.32 mg/kg MDPV dose used for subsequent studies increased DA levels to a maximum of 201% of baseline after 100 min. Methamphetamine produced a dose- and time-dependent increase in both DA (Fig. 3e: 0.1 mg/kg: $F_{(9,45)} = 9.27$, $p < 0.0001$;

0.32 mg/kg: $F_{(9,45)} = 11.89$, $p < 0.0001$; 1.0 mg/kg: $F_{(9,45)} = 10.26$, $p < 0.0001$) and 5-HT (Fig. 3f: 0.1 mg/kg: n.s.; 0.32 mg/kg: $F_{(9,45)} = 4.44$, $p = 0.0003$; 1.0 mg/kg: $F_{(9,45)} = 7.37$, $p < 0.0001$). The 0.32 mg/kg methamphetamine dose used for subsequent studies increased DA levels to a maximum of 238% of baseline after 50 min, and 5-HT levels to a maximum of 147% of baseline after 30 min.

*Effects of saline or amphetamine maintenance on baseline DA and 5-HT levels.* Mean ± SEM DA levels in the NAc were increased by 7 days of 0.32 mg/kg/h amphetamine treatment ($9.28 \pm 0.75$ pg/9 µl) as compared to 7 days of saline treatment ($1.67 \pm 0.52$ pg/9 µl; $t_{(17.26)} = 7.68$, $p < 0.001$). Conversely, mean ± SEM 5-HT levels in the NAc were similar in amphetamine-treated rats ($0.38 \pm 0.08$ pg/9 µl) as compared to the saline-treated rats ($0.28 \pm 0.06$ pg/9 µl; $t_{(28.04)} = 1.69$, n.s.).

*Effects of cocaine, MDPV, and methamphetamine during saline or amphetamine maintenance.* Figure 4 shows effects of cocaine, MDPV, and methamphetamine on NAc DA and 5-HT levels after 7 treatment days with saline or 0.32 mg/kg/h amphetamine. Data are expressed as change from baseline (delta baseline) rather than

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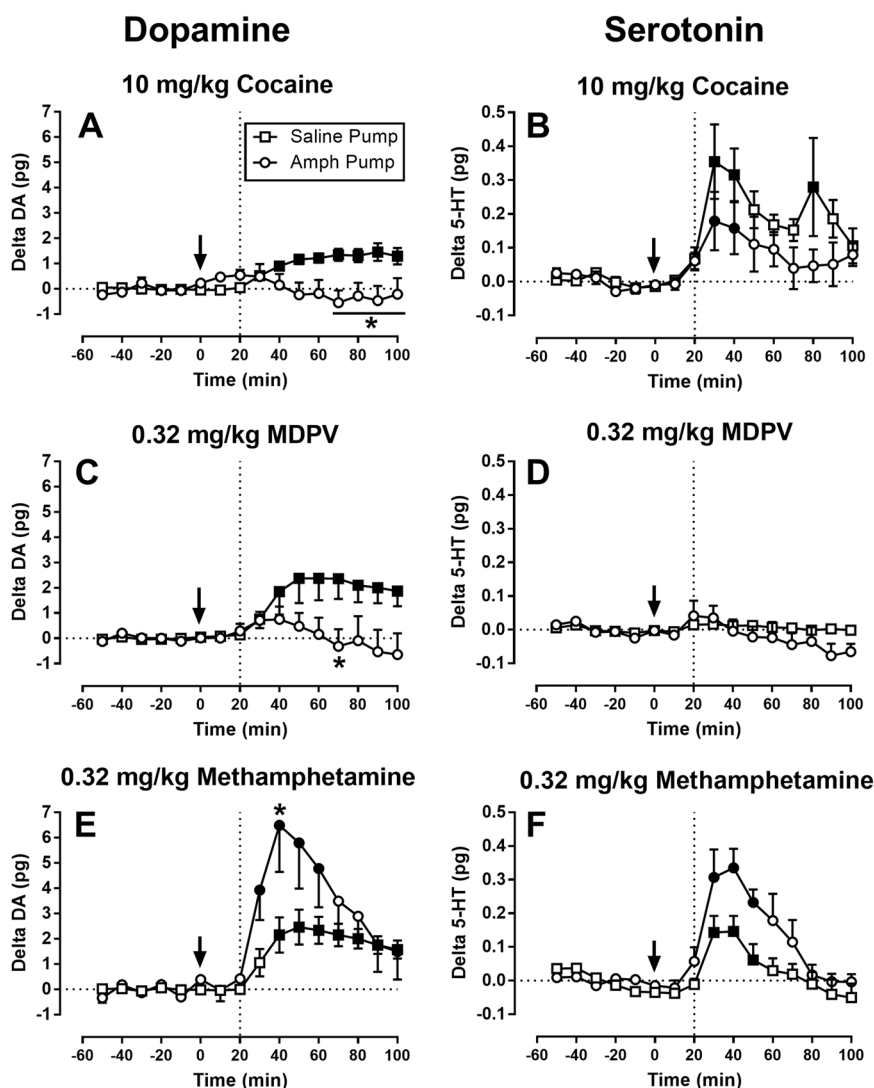
**Fig. 4** Effects of cocaine (**a**, **b**), MDPV (**c**, **d**), and methamphetamine (**e**, **f**) on NAc DA (**a**, **c**, **e**) and 5-HT (**b**, **d**, **f**) levels after saline or amphetamine treatment. Abscissae: time in minutes relative to test-drug injection. Vertical line at 20 min indicates earliest time of drug effect (see Methods). Ordinates: change from baseline DA or 5-HT in pg/9 µl. Note that there is a different range used in DA panels (**a**, **c**, **e**) versus 5-HT panels (**b**, **d**, **f**). All points show mean ± SEM for $N = 6$ rats. Filled points indicate a significant difference from the 10-min time point within a given group as assessed by one-way ANOVA. Asterisks indicate a significant difference between saline and amphetamine-maintenance conditions within a given time point as assessed by two-way ANOVA

% baseline due to the significant difference in DA baselines between the groups. However, for comparison, Supplemental Figs. 4 and 5 show the same data graphed as absolute concentrations or as % baseline, respectively.

One-way ANOVA of data in Fig. 4 indicated significant increases in DA only during saline maintenance for cocaine (Fig. 4a: $F_{(9,45)} = 10.65$, $p < 0.0001$) and MDPV (Fig. 4c: $F_{(9,45)} = 5.13$, $p < 0.0001$), but during both saline and amphetamine maintenance for methamphetamine (Fig. 4e: $F_{(9,45)} = 10.31$, $p < 0.0001$, $F_{(9,45)} = 5.88$, $p < 0.0001$). 5-HT levels were increased during both saline and amphetamine maintenance by both cocaine (Fig. 4b: $F_{(9,45)} = 3.68$, $p = 0.0016$, $F_{(9,45)} = 2.68$, $p = 0.0224$) and methamphetamine (Fig. 4f: $F_{(9,45)} = 13.79$, $p < 0.0001$, $F_{(9,45)} = 6.63$, $p < 0.0001$), whereas MDPV did not alter 5-HT levels during either saline or amphetamine maintenance (Fig. 4d).

Two-way ANOVA of data in each panel of Fig. 4 indicated that amphetamine maintenance significantly attenuated the effects of cocaine on DA (Fig. 4a: treatment × time $F_{(9,90)} = 5.39$, $p < 0.0001$), but it did not significantly alter cocaine effects on 5-HT (Fig. 4b: no main effect of treatment $F_{(1,10)} = 2.36$, n.s. or treatment × time

interaction $F_{(9,90)} = 1.22$, n.s.). Amphetamine maintenance also attenuated effects of MDPV on DA (Fig. 4c: treatment × time $F_{(9,90)} = 4.10$, $p = 0.0002$) without altering the lack of MDPV effect on 5-HT (Fig. 4d: no main effect of treatment: $F_{(1,10)} = 0.78$, n.s.; significant treatment × time interaction $F_{(9,90)} = 2.40$, $p = 0.0174$, but post-hoc test indicated no difference between saline and amphetamine maintenance at any time). In contrast, amphetamine maintenance enhanced the effects of methamphetamine on both DA (Fig. 4e: treatment × time $F_{(9,90)} = 2.76$, $p = 0.0067$) and 5-HT (Fig. 4f: main effect of treatment $F_{(1,10)} = 7.09$, $p = 0.023$).

**[³H]WIN35,428 saturation binding**
Mean ± SEM $B_{max}$ values in rats maintained on saline or 0.32 mg/kg/h amphetamine were $1.53 ± 0.12$ pmol/mg and $1.33 ± 0.07$ pmol/mg of membrane protein, respectively. Mean ± SEM $K_D$ values in rats maintained on saline or 0.32 mg/kg/h amphetamine were $14.7 ± 1.8$ nM and $14.5 ± 1.3$ nM, respectively. Neither $B_{max}$ nor $K_D$ values differed significantly between groups, indicating that amphetamine maintenance at this dose did not significantly alter striatal DAT levels or radioligand binding affinity.

## DISCUSSION

This study compared the effects of amphetamine maintenance on abuse-related behavioral and neurochemical effects of cocaine, MDPV, and methamphetamine in rats. There were three main findings. First, cocaine, MDPV, and methamphetamine all produced dose-dependent increases in ICSS facilitation and NAc DA levels before treatment, and cocaine and methamphetamine also increased NAc 5-HT. Second, amphetamine maintenance for 7 days produced sustained and significant increases in both basal ICSS and NAc DA levels without producing a significant change in basal 5-HT levels or in the density or binding affinity of striatal DAT. Finally, amphetamine maintenance blunted the effects of cocaine on both ICSS and NAc DA levels, it did not blunt methamphetamine-induced increases in ICSS or NAc DA, and it did not block the effects of either cocaine or methamphetamine on NAc 5-HT levels. For MDPV, amphetamine maintenance had little effect on ICSS facilitation, but did block the increases in NAc DA. Taken together, these results are consistent with the conclusion that amphetamine maintenance attenuates abuse-related behavioral effects of cocaine by reducing cocaine effects on NAc DA but conserving cocaine effects on NAc 5-HT. These results also suggest that amphetamine maintenance might be more effective as a pharmacotherapy for cocaine abuse than for MDPV or methamphetamine abuse.

### Effects of cocaine, MDPV, and methamphetamine alone

Effects of cocaine, MDPV, and methamphetamine in the present study are consistent with previously published effects of these compounds on ICSS [4, 23, 27] and in microdialysis studies of NAc DA and 5-HT [31–33]. The effectiveness of these drugs to facilitate ICSS is consistent with other evidence for their abuse-related effects in preclinical studies (e.g., from drug self-administration procedures; [34–36]) and for their abuse by humans. Additionally, the relative potencies of these drugs to facilitate ICSS were similar to their relative potencies to increase NAc DA levels, supporting a role for NAc DA in mediating ICSS facilitation by these compounds. Both cocaine and methamphetamine also dose-dependently increased NAc 5-HT levels, and we and others have reported previously that either increases in 5-HT levels or activation of some 5-HT receptors (e.g., 5-HT2C receptors) can depress baseline ICSS and oppose DA-mediated ICSS facilitation and other stimulant effects [37–40]. However, this type of behavioral depression is usually associated with relatively large increases in NAc 5-HT levels (i.e., >400%; [5, 40]). The cocaine and methamphetamine doses tested here did not increase 5-HT levels to that degree, consistent with the absence of ICSS rate-decreasing effects.

### Amphetamine maintenance facilitates ICSS and increases NAc DA

The sustained facilitation of ICSS during amphetamine maintenance replicated findings from a previous publication [23] and further demonstrated that this sustained behavioral effect was associated with a sustained elevation of NAc DA but not 5-HT. This profile of behavioral and neurochemical effects of 7-day amphetamine maintenance is consistent with both acute amphetamine effectiveness to facilitate ICSS and increase NAc DA but not 5-HT [4, 5] and with in vitro evidence that amphetamine is selective as a substrate for DAT relative to SERT [8]. The similarity in acute and chronic amphetamine effects suggests that little, if any, tolerance developed to the ICSS-facilitating or NAc DA-increasing effects of amphetamine-maintenance doses used here.

### Amphetamine maintenance blocks cocaine effects on ICSS and NAc DA

The effectiveness of 0.32 mg/kg/h amphetamine to blunt ICSS facilitation by 10 mg/kg cocaine replicated findings from a previous study [23] and expanded on this finding in three ways. First, the present study evaluated the effects of 0.32 mg/kg/h

amphetamine maintenance on ICSS-facilitation produced by a broader range of cocaine doses, and ICSS facilitating effects of 1.0 and 3.2 mg/kg cocaine doses were eliminated. This suggests that 0.32 mg/kg/h amphetamine produced at least a 10-fold rightward shift in the cocaine dose–effect curve for ICSS facilitation. Second, the present study also evaluated the effects of a lower amphetamine maintenance dose (0.1 mg/kg/h) on cocaine-induced ICSS facilitation, and this dose was not effective to alter ICSS facilitation by cocaine. The overall potency of amphetamine maintenance to attenuate ICSS facilitation in this study was similar to amphetamine-maintenance potency to reduce cocaine self-administration in rats responding in various drug self-administration procedures, including progressive-ratio, long-access, and cocaine-vs.-food choice procedures [16, 41, 42]. These effects are also consistent with amphetamine-maintenance effectiveness to decrease cocaine self-administration in monkeys and humans and to decrease metrics of cocaine use in clinical trials [9, 43, 44].

In a third extension of previous results, the present study also found that attenuation of cocaine-induced ICSS facilitation by amphetamine maintenance was associated with a selective attenuation of cocaine-induced increases in NAc DA. This correspondence between behavioral and neurochemical results further supports a role for NAc DA in mediating abuse-related effects of cocaine in general and ICSS facilitation in particular. However, two caveats warrant mention. First, although 10 mg/kg cocaine failed to increase NAc DA during 0.32 mg/kg/h amphetamine maintenance, it still produced significant, though blunted, ICSS facilitation. This is consistent with other data to suggest that ICSS may be more sensitive than in vivo microdialysis to small changes in synaptic NAc DA sufficient to alter behavior but insufficient to increase DA levels at extrasynaptic sites sampled by the microdialysis probe [5]. The use of brain stimulation to activate mesolimbic DA neurons during ICSS (Negus and Miller, 2014) [22], but not during in vivo microdialysis, may contribute to higher sensitivity of ICSS by generating higher levels of mesolimbic DA neuronal activity during ICSS than microdialysis. Second, amphetamine maintenance did not block cocaine-induced increases in NAc 5-HT. This is consistent with the lack of amphetamine maintenance effects on baseline 5-HT. Moreover, the retained effectiveness of cocaine to increase NAc 5-HT may also have opposed any residual cocaine effects on NAc DA [40]. Overall, cocaine increased only 5-HT during amphetamine maintenance, and selective SERT inhibitors neither facilitate ICSS [45] nor maintain self-administration [46].

### Amphetamine maintenance blocks MDPV effects on NAc DA but not ICSS

As with cocaine, amphetamine maintenance also blocked increases in NAc DA produced by the other DAT inhibitor MDPV; however, in contrast to results with cocaine, amphetamine maintenance had little effect on MDPV-induced ICSS facilitation. There are at least three possible reasons for this discrepancy. First, as noted above, ICSS may be more sensitive than microdialysis to small changes in synaptic NAc DA levels. Second, consistent with its high selectivity to inhibit DAT over SERT [47, 48], MDPV did not increase NAc 5-HT in the absence or presence of amphetamine maintenance. Consequently, in contrast to cocaine, any residual DA-mediated effects of MDPV during amphetamine maintenance would not be opposed by concurrent increases in 5-HT. Lastly, it is possible that MDPV might facilitate ICSS via some mechanism other than DAT inhibition. At present, no evidence for other plausible mechanisms exists; for example, MDPV also has moderately high affinity for NET, but NET inhibitors do not facilitate ICSS, and MDPV does not have affinity for targets such as D1 DA receptors that can mediate ICSS facilitation [45, 47, 49]. Nonetheless, this possibility warrants consideration. Amphetamine-maintenance effects on MDPV self-administration

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or abuse have not been evaluated, but the present results suggest that amphetamine maintenance would be less effective clinically to reduce MDPV use than cocaine use.

### Amphetamine maintenance fails to block methamphetamine effects

The failure of amphetamine maintenance to block metham-phetamine-induced ICSS facilitation agrees with its failure to block the increases in NAc DA in the present study and with its failure to block methamphetamine self-administration in preclinical studies or methamphetamine abuse in clinical trials [17, 18]. Indeed, the present microdialysis data indicate that amphetamine mainte-nance enhanced rather than reduced methamphetamine effects on NAc DA levels, and also enhanced methamphetamine effects on 5-HT levels. Thus, amphetamine maintenance differentially modulated the neurochemical effects of monoamine uptake inhibitors (cocaine, MDPV) and a monoamine releaser (metham-phetamine) on NAc DA and 5-HT levels.

### Amphetamine maintenance does not reduce DAT density

The mechanisms by which amphetamine maintenance selectively reduces cocaine but not methamphetamine effects on ICSS and NAc DA levels remain to be determined. Although amphetamine-type releasers can reduce DAT density under some circumstances [25], the amphetamine-maintenance regimen used here did not significantly reduce DAT density as measured with the DAT inhibitor ligand WIN35,428, suggesting that decreased cocaine effects cannot be attributed to decreased cocaine binding sites on DAT. An alternative possibility is that amphetamine maintenance produces a sustained inhibition of DAT function due to competi-tion between amphetamine and DA for DAT and/or effectiveness of amphetamine maintenance to promote intracellular-facing conformations of DAT that cannot mediate the uptake of extracellular DA [26, 50]. This might attenuate NAc DA-increasing effects of uptake inhibitors like cocaine and MDPV because transporter function is already inhibited; however, it is currently unknown why transporter substrates like methamphe-tamine would retain their effectiveness to increase DA levels.

## AUTHOR CONTRIBUTIONS

ARJ conducted all the experiments, the initial graphing and analysis of all the data, and drafted the text. MLB supervised the microdialysis studies, SSN supervised the ICSS studies, and DES supervised the receptor binding studies. MLB, SSN, and DES contributed to the experimental design and generation of final data analysis, graphs, and text. All authors approved the final text.

## ADDITIONAL INFORMATION

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