

Video Article

Assessment of Cocaine-induced Behavioral Sensitization and Conditioned Place Preference in Mice

Laura N. Smith^{*1}, Rachel D. Penrod^{*1}, Makoto Taniguchi¹, Christopher W. Cowan¹

¹Department of Psychiatry, Harvard Medical School, McLean Hospital

^{*}These authors contributed equally

Correspondence to: Christopher W. Cowan at cwcowan@mclean.harvard.edu

URL: <https://www.jove.com/video/53107>

DOI: [doi:10.3791/53107](https://doi.org/10.3791/53107)

Keywords: Behavior, Issue 108, cocaine, locomotor sensitization, conditioned place preference, mice, psychostimulant

Date Published: 2/18/2016

Citation: Smith, L.N., Penrod, R.D., Taniguchi, M., Cowan, C.W. Assessment of Cocaine-induced Behavioral Sensitization and Conditioned Place Preference in Mice. *J. Vis. Exp.* (108), e53107, doi:10.3791/53107 (2016).

Abstract

It is thought that rewarding experiences with drugs create strong contextual associations and encourage repeated intake. In turn, repeated exposures to drugs of abuse make lasting alterations in the brain function of vulnerable individuals, and these persistent alterations likely serve to maintain the maladaptive drug seeking and taking behaviors characteristic of addiction/dependence². In rodents, reward experience and contextual associations are frequently measured using the conditioned place preference assay, or CPP, wherein preference for a previously drug-paired context is measured. Behavioral sensitization, on the other hand, is an increase in a drug-induced behavior that develops progressively over repeated exposures. Since sensitized behaviors can often be measured after several months of drug abstinence, depending on the dose and length of initial exposure, they are considered observable correlates of lasting drug-induced plasticity. Researchers have found these assays useful in determining the neurobiological substrates mediating aspects of addiction as well as assessing the potential of different interventions in disrupting these behaviors. This manuscript describes basic, effective protocols for mouse CPP and locomotor behavioral sensitization to cocaine.

Video Link

The video component of this article can be found at <https://www.jove.com/video/53107/>

Introduction

Research aimed at understanding drug addiction using animal models must take a variety of approaches to address each of the assorted components that obstruct treatment success, including reward/reinforcement/motivation and withdrawal and relapse, as well as the general persistence that further complicates these issues in addiction. Since rewarding experiences associated with taking a drug of abuse are thought to motivate subsequent use, studies focusing on drug-context associations may be particularly useful for understanding brain mechanisms that contribute to drug taking and seeking. One such assay, conditioned place preference (CPP) is a high-throughput method for comparing group differences in reward sensitivity. The traditional interpretation of the task involves classical, or Pavlovian, conditioning, where a conditioned stimulus (CS) is paired with an unconditioned stimulus (UCS), and after multiple pairings, the CS elicits the same behavior as the UCS (however, see^{39,40}). Theoretically, animals learn to associate an interoceptive state (reward or aversion) with contextual cues. The relative aversive or appetitive intensity of the interoceptive state is then assessed by then determining the animal's preference for the contextual cues. The use of place conditioning to measure drug-reward associations dates back to at least 1957, to a study using morphine on rats in a Y-maze^{3,4}. Over the past several decades, variations on this method have been widely used to study place preference and aversion in rodents to various stimuli, and it remains particularly useful in the study of associations induced by drugs of abuse. In drug-addiction research, the assay has been used to assess the rewarding properties of a number of drugs and the contribution of different brain systems and proteins to drug reward (for reviews, see^{5-7,44}). While there are superior methods of assessing factors that contribute to drug addiction, namely drug self-administration, CPP is a simple and much more accessible approach to measuring reward function.

Most current protocols for conditioned place preference and aversion (CPA) use an apparatus that allows rodents to have access to two distinct chambers, either via a doorway or smaller connecting chamber. Distinctions between the two chambers are often based, at a minimum, on visual and tactile cues, including wall color and floor texture, but sometimes include other elements, such as olfactory cues. "Biased" designs typically attempt to reverse a pre-existing, innate preference for one chamber over the other, such as the one that rodents generally show for a black chamber over white. "Unbiased" designs aim to create a preference to one of two chambers that were initially equally appealing by randomly counterbalancing assignment to either chamber within a group. A "balanced" design is used when animals show small preferences, but do not, as a group, favor the same chamber. Goals of this latter design are to produce 1) pre-test preference scores for the (eventual) cocaine-paired chamber that are not significantly different between experimental groups and 2) negligible preference for the cocaine-paired chamber at pre-test, either positive or negative⁸. The balanced design is ideal for use with the described chambers, which utilize contradicting biases for wall color (black over white) and flooring (wire over bar), resulting in a roughly equal distribution of small preferences for both the black and white sides in different animals. Balancing calculations are described in further detail below.

During conditioning, animals are exposed to a drug and quickly placed into one of these two environments for a limited time period. Exposure is typically via intraperitoneal (i.p.) or sometimes subcutaneous (s.c.) injection, although paradigms for intravenous (i.v.) self-administration⁹, and intracranial infusions³⁸ in a place preference apparatus have also been developed. These pairings are complemented by non-drug (vehicle) pairings of the same length conducted in the opposite chamber, which can take place on the same day as drug pairings or on separate days. In general, when allowed to explore the apparatus after conditioning, animals will spend more time where they received a rewarding drug (*i.e.*, one that humans and animals will voluntarily self-administer), while they will avoid a place where they were given a drug that induced illness (*e.g.*, lithium chloride). Several studies have been dedicated to optimizing the conditions for place preference to different drugs of abuse (for review, see⁷). Cocaine doses (i.p.) for mice generally range from 1 to 20 mg/kg, with doses less than 5 mg/kg often used to parse high sensitivity in one group. Two or more drug pairings are typically required for adult mice¹⁰, and the length of these pairings is an important consideration. Very low doses of cocaine require an immediate and brief conditioning, likely because this method captures the most rewarding period of the exposure. Delayed or very long conditioning periods can result in no preference, or may even induce aversion^{11,12}. Here is presented a basic method for obtaining conditioned place preference to cocaine in adult mice.

While the CPP assay is an ideal method for assessing reward-related learning and memory of drug-context associations, behavioral sensitization is arguably easier to perform and allows the assessment of changes that develop over repeated treatment. Also known as reverse-tolerance, behaviors undergoing sensitization are incrementally enhanced over repeated exposures to a particular drug of abuse, especially psychostimulants, and cross-sensitization is known to occur between some, but not all, of these drugs. One of the first assessments of cocaine-induced locomotor sensitization, in particular, in rodents was published in 1976¹³. A number of labs have shown that sensitized locomotion is detectable long after drug cessation, depending on the original length, location and dose of exposure¹⁴⁻¹⁷, and the current protocol has been used to detect sensitization as long as 10 months following seven days (30 mg/kg) of cocaine treatment in mice¹⁸. The test can be performed using either photobeam or video-tracking technologies, in apparatuses of differing sizes and shapes, making it simple for many labs to perform. The robust nature, simplicity and persistence of locomotor sensitization makes its assessment an ideal part of examining basic mechanisms of long-lasting changes in drug-induced behavior.

As is expanded upon in the discussion, an important consideration when performing the locomotor sensitization assay is whether drug is given in the home- or test-cage environment. To take advantage of the robust sensitization that occurs when drug administration occurs outside of the home cage, this protocol employs this method. However, it has been observed that when animals are not adequately habituated to a new environment before drug exposure, a novelty-induced ceiling effect occurs on Day 1, which can partially or fully mask the progressive nature of sensitization. It is likely that this represents synergistic locomotor-activating effects of the drug together with novelty, and while the mechanisms underlying such effects may be interesting, the method described is designed to reduce the role of novelty and allow the effects of the drug to be measured more independently. While it is expected this method will be useful in the assessment of other locomotor-sensitizing drugs, it has primarily evaluated its effectiveness with cocaine in C57BL/6 mice.

Protocol

All experimental procedures have been approved by the McLean Hospital Institutional Animal Care and Use Committee. NOTE: The following protocol describes a single approach to CPP and locomotor sensitization, many details of which differ from other successful protocols (*e.g.*, light- vs. dark-phase testing, consecutive vs. intermittent dosing, etc.). Novices may wish to begin with these protocols, or simply use them as guides, adapting alterations from the literature based on the experimental question(s) at hand. Automated measurement methods are described; however, it is possible to use non-automated means for each assay (*i.e.*, video recording, hand-scoring).

1. Conditioned Place Preference

1. Equipment and Room Set-Up:

1. Handle experimental mice for 1-3 min each day, for at least 3-5 days prior to testing.
NOTE: Never-handled mice may find removal from the chamber stressful, which can interfere with or alter conditioning.
2. Obtain four or more three-chambered CPP apparatuses, preferably equipped with photobeams for automated data collection¹⁸. Ensure that each chamber has two larger, visually- and tactilely-distinct chambers connecting to a smaller, neutral chamber via doors that can be raised/lowered to control access. Lids on each chamber should open for insertion/removal of mice and be mounted with small, individually controllable (dimnable) lights (one per chamber).
NOTE: CPP chamber designs vary and can be purchased commercially or constructed by researchers. For the "balanced" design, a recommended scheme is one larger chamber with white walls and wire grid flooring and the other with black walls and bar flooring. The middle chamber should have gray walls and solid gray Plexiglass flooring. For explanation purposes, these chambers will be referred to as "white," "black" and "middle." Lids should be clear Plexiglass. Alternate compartment configurations (one- or two-chamber) are also possible and discussed elsewhere (see Discussion).
3. Setup the room as it will be during testing: turn off or set overhead lights to the dimmest setting and close the door. Use a light meter inside each chamber and set the lid lights so that middle chambers are slightly brighter (15-20 lux) than black and white chambers (6-10 lux) in order to discourage mice from spending time there.
NOTE: If the average time spent in the middle is as much or more than in the black or white chambers, further increase the lighting contrast described in Step 1.1.3. Alternatively, use less appealing flooring for the middle. (*e.g.*, ~220 0.5 cm diameter holes, evenly spaced in 6 x 3.5 x 1/4" Plexiglass), but avoid making this chamber aversive. Pilot any alterations to ensure that decreases in middle time are not due to decreases in total explorations (crossings).
4. Program automated data collection ("procedure," **Figure 1A**) or manually collect data according to the following parameters. Set trials to start upon the first beam break in either conditioning chamber. Set "test" sessions to be 20 min in length and to track time spent and beam breaks in each chamber. Set "conditioning" sessions to be 30 min long and (optionally) to measure beam breaks in each chamber. Set all chamber lights to illuminate during trial.

5. Prepare full volume of cocaine solution required for the experiment at hand. Dissolve cocaine HCl in 0.9% NaCl (saline), basing the final concentration on an injection volume of 0.1 ml/10 g body weight (e.g., for a dose of 5 mg/kg, solution concentration is 0.5 mg/ml). Vortex mixture for 30-45 sec, sterile filter (0.2 µm syringe filters) and store at RT.

2. Testing:

NOTE: The general timeline for CPP is PRE-TEST, CONDITIONING, and then POST-TEST. The pre-test may be separated from conditioning by 1-3 days; however, conditioning and the post-test day should take place on consecutive days (**Figure 2A**). This timing should be kept the same for all cohorts in the same experiment.

1. Test during the animals' light phase using the same apparatus for each animal across days.
2. Each trial day (i.e., tests and conditioning days), move mice to the behavioral anteroom and allow them to sit undisturbed in their home cages for 1-1.5 hrs before the trial. Choose a location where mice can be moved swiftly from their cage to the apparatus, with minimal disruption, when the trial begins. Turn on all equipment so that any noises associated with the test (e.g., equipment fans) are present.
3. Thoroughly clean each apparatus (inner walls, flooring and trays) with mild, alcohol-, glycol-ether- or ammonia-based disinfecting wipes prior to and after each mouse (use the same type of cleaner throughout the experiment). Do not neglect flooring undersides.
4. Check that room lights are set appropriately (off or dimmed) at the beginning of each day.
5. Proceed with the following according to whether it is a "test" or "conditioning" day:
 1. On Trial Days 1 and 6 (i.e., the pre- and post-tests), place the inter-chamber doors in the open position (**Figure 2B**).
 2. Load the "test" computer program and enter animal IDs (**Figure 1A**), then issue start command (**Figure 1B**), if applicable.
 3. Gently lower each mouse into the middle chamber of its assigned apparatus, facing the back wall, and softly close the lid. Once all chambers are loaded, leave the testing room and minimize noise.
 4. Leave mice inside each apparatus until trials for all mice have closed/ended (**Figure 1C**). Export trial data.
6. Just prior to or following the pre-test, weigh the animals. Use these weights for dose calculations during conditioning.
7. In order to prepare for conditioning trials, calculate each mouse's pre-test "preference" for the black and white chambers by subtracting time spent in each of these from the other (i.e., "black minus white" & "white minus black"; see **Figure 3**, Columns 9 & 10).
8. Since mice with strong initial preferences make balancing difficult, establish an acceptable limit for preference scores (e.g., <33% of total trial time) and exclude mice that exceed it from calculations. Use a liberal limit (<66% of total trial time) to maximize inclusion when attrition is likely after testing is complete (e.g., due to off-target surgical manipulations).
NOTE: Mice exceeding the limit can still be tested, with an attempt to keep their pre-test preferences balanced. Later, these mice can be excluded from analysis, if necessary, to balance pre-test scores. If extreme pre-test biases (i.e., >800 sec) disproportionately affect one group, consider altering the conditioning environments and/or using other assays.
9. Choose the black or white chamber as the drug-pairing chamber for each mouse, meanwhile summing the corresponding pre-test preference scores within each group with the following priorities in mind:
NOTE: Take care to balance scores between groups *within* each cohort as well as *across* any previous cohorts.
 1. Make the sums for all groups as equivalent as possible.
 2. Make the sums as close to zero as possible (i.e., choose the preferred side for some mice and the non-preferred side for others). If a near-zero sum is impossible for any given group, re-adjust all others to closely match the best obtainable score for the limiting group, favoring slightly negative group sums over positive.
 3. As much as feasible, keep assignments of black versus white and preferred versus non-preferred chambers for drug pairings even within each group.
 4. As it is not always possible to meet the above goals, correct any deviations by making opposing balancing considerations in later cohorts; however, try to avoid producing cohorts with wildly different average pre-test preferences.
10. On Conditioning Days 2-5, place inter-chamber doors in the closed position (**Figure 2C**).
11. Prepare individual syringes with cocaine (Days 2 & 4) or saline (Days 3 & 5) solution, as described in Step 1.1.5 and based on body weights measured at pre-test.
NOTE: Dose should be chosen with regard to experimental expectations, considerations mentioned in the Introduction, and potential floor and ceiling effects. It is often best to conduct independent experiments using at least two different doses.
12. Load "conditioning" computer program and enter animal IDs (**Figure 1A**), then issue start command (**Figure 1B**), if applicable.
13. Scruff and inject each mouse (i.p.), immediately lowering them into the appropriate black or white chamber of their assigned apparatus facing the back wall, then softly close the lid. Once all chambers are loaded, leave the testing room and minimize noise.
14. Remove mice from their chamber as close to exactly 30 min as feasible (i.e., the first mice are removed from their chambers while other mice are still conditioning). Remove animals as quietly as possible, without introducing noise.

3. Statistical Analysis:

1. Choose a method of analysis. Either subtract time spent on the saline-paired side during the post-test from time spent on the cocaine-paired side during the post-test (cocaine - saline, sec) or use the time spent in the post-test drug-paired chamber minus the pre-test time spent on the drug-paired chamber.
NOTE: if using the first method, also plot line graphs of average time spent in the middle, saline- and drug-paired chambers during the pre- and post-tests for each group. Compared to the pre-test, the post-test should show increased time in the drug-paired side and decreased time spent in the saline-paired side (see **Figure 6**, bottom, and Discussion for explanation).
2. Depending upon the nature and number of groups being compared, use a t-test, One- or Two-Way ANOVA, as appropriate, possibly with post-hoc analysis, to analyze either of the above-presented subtraction scores.
NOTE: Cocaine preference scores tend to be variable, and furthermore, can be negative (i.e., indicate aversion to the drug-paired side). Mice that show aversion should not be removed (unless they are statistical outliers), since this result is normal and likely important to determining differences between groups. Expect to need sample sizes of 12 to 30 animals per group, depending on the effect size of the treatment.

2. Locomotor Sensitization

1. Equipment and Room Set-Up

1. Obtain a 4 x 8 (X x Y) photobeam array (outside dimensions 11.5" x 20"). Construct an open-topped chamber made of black Plexiglas (inner dimensions 22 1/6" x 13 3/4" x 9 3/8") to house the array (**Figure 4A**).
2. Prepare the testing room so that a red light (ceiling- or wall-mounted) can be used during testing.
3. Prepare separate program sessions for daily habituation and injection trials.
 1. Set habituation trials to be between 30-60 min and injection trials to be between 60-120 min (**Figure 5B**). The recommended length for each is 60 min. Keep trial length consistent across multiple cohorts within the same experiment.
 2. Set trials to start recording upon the first beam break that occurs after the start signal has been initiated (**Figure 5B**). For the injection trial, set the start signal so that boxes can be started individually (not in unison), if possible.
 3. Set beam breaks to be recorded in user-defined "bins," preferably 5 min each (**Figure 5B**).
4. Prepare full volume of cocaine solution required for the experiment at hand. Dissolve cocaine HCl in 0.9% NaCl (saline), basing the final concentration on an injection volume of 0.1 ml/10 g body weight (e.g., for a dose of 5 mg/kg, solution concentration is 0.5 mg/ml). Vortex mixture for 30-45 sec, sterile filter (0.2 µm syringe filters) and store at RT.

2. Testing

NOTE: The initial phase of testing runs for 10-11 consecutive days. Mice receive two daily trials: habituation (injection-free) and injection. Administer saline for the first three to four days (see Discussion for importance of saline habituation), and cocaine for the next seven using the same dose (e.g., 15 mg/kg/day).

1. Each day, acclimate mice in their home cages to the behavioral anteroom for 30 min to 1 hr.
2. Prepare clean standard mouse-sized clear acrylic housing cages with an extremely thin layer of fresh bedding (e.g., pine chips), so as to not obscure photobeams, if applicable (**Figure 4C & D**).
3. Place cages against the Y-axis of the photobeam array near one end, so that five beams are spaced evenly along its length. X-axis beams are not used in this test (**Figure 4C & D**).
4. Take mice from their home cage, in random order, scruff and weigh them. Remove each mouse from the weigh boat by the base of their tail (with support) and place directly into their assigned locomotor cage. Cover each cage with a standard filter-top lid.
5. Prepare individual syringes with saline or cocaine solution, as described in Step 2.1.7, based on the current day's body weights. Begin with a dose of 15 or 20 mg/kg, and consider a second experiment using a higher or lower dose (see Discussion for relevant factors).
6. Once habituation trials have ended for all cages, load the injection program.
7. One at a time, remove mice from their test cage, scruff and give their injection (i.p.). Before returning the mouse to its test cage, quickly initiate the start signal for that cage (**Figure 5C**, bottom).
8. After all injection trials have ended, return mice to their home cages and their housing room.
9. If desired, allow mice to undergo a series of withdrawal periods and drug challenges, such as the following: same dose as original, half-dose, double-dose, then saline, allowing seven days before the same-dose challenge and three to seven days before each additional challenge. Whatever the choice of challenges, maintain similar withdrawal periods across cohorts within an experiment.

NOTE: If the original dose is high (e.g., 30 mg/kg or above), the double-dose should be skipped or replaced with a lower dose. The saline challenge reveals any conditioned locomotor activation by the cocaine-paired environment alone, and in the process, the amount of sensitized locomotion that is cocaine-dependent (see Discussion).

3. Statistical Analysis

1. Choose the portion(s) of each trial that will be used for analysis. Most cocaine-induced locomotion in rodents occurs within the first ~15-30 min after drug injection. Depending on involved variables, consider analyzing cumulative locomotion for multiple time windows (e.g., the first 15, 30, 60 and/or 120 min) or focus on independent segments of the trial.
2. Using the time frame chosen, sum the beam breaks for each animal by day for habituation and injection trials separately and then average the sums for each group. Plot means and standard errors of the mean (S.E.M.) in a line graph over all days. As treatments may alter how mice respond to the drug early and/or late in each daily trial, also plot average group beam breaks by 5-min bin over the course of each daily trial.

NOTE: Plotting daily activity averages for habituation and injection trials (e.g., first 15 min) makes it artificially appear that locomotion is dampened by saline injection, but remember that activity has (most likely) declined to this level by the end of habituation each day.

3. Analyze consecutive saline and drug treatment days separately using repeated measures (RM) ANOVAs having the within-subjects factor of day/time and including any between-subjects factors in the design, as appropriate. Use a t-test or One-Way ANOVA to investigate group differences on Day 1 of cocaine (acute exposure), and a Multivariate ANOVA for the challenges. When appropriate, follow significance with post-hoc tests or Univariate ANOVAs.

Representative Results

Representative results from the CPP assay are shown in **Figure 6** using wild-type C57BL/6N mice at approximately nine weeks of age. The study design was a 2 x 3 mixed factorial, with a within-subjects variable of Test (pre and post) and a between-subjects variable of Treatment (saline and cocaine 5 and 10 mg/kg). A RM ANOVA showed a significant interaction between Test and Treatment ($F_{2,20}=3.68$, $p<0.05$), which was interpreted *in lieu* of significant main effects observed for both Test ($F_{1,20}=9.86$, $p<0.01$) and Treatment ($F_{1,20}=4.37$, $p<0.05$). Post hoc comparisons showed that none of the groups differed from one another during the pre-test. At the post-test, the 5 mg/kg and 10 mg/kg did not differ significantly from each other; however, both groups showed significantly greater preference for the cocaine-paired chamber than the saline-only control group (Tukey's: 5 mg/kg, $p<0.5$; 10 mg/kg, $p<0.01$). In addition, Bonferroni post hoc tests showed that both the 5 ($p<0.05$) and 10 ($p<0.05$) mg/kg groups showed greater preference for the cocaine-paired side at post-test compared to pre-test, while the saline-saline group showed no change from pre- to post-test.

Representative results from the locomotor assay are shown in **Figure 7A & B** using wild-type C57BL/6N littermate control mice from a series of separate experiments that have been previously published¹⁸. After acclimation to saline injections for 3-4 days, mice received one cocaine injection (i.p.) at 5, 10, 15 or 30 mg/kg per day for seven days. Following a seven- or 14-day withdrawal period, each group received a cocaine challenge at the original dose, then after further (variable) withdrawal periods, some received additional cocaine challenges at other doses, as shown. For the purpose of demonstrating the effect of cocaine dose on locomotor sensitization in the current publication, the four different dose groups were combined into a 4 x 7 mixed-factorial design, with a within-subjects variable of Day (first 7 cocaine injections) and a between-subjects factor of Dose. A RM ANOVA showed a significant interaction between Dose and Day ($F_{18,276}=12.53$, $p<0.0001$), which was interpreted instead of significant main effects that were also observed for both Day ($F_{6,276}=18.25$, $p<0.0001$) and Dose ($F_{3,46}=22.63$, $p<0.0001$). Tukey's post hoc analyses showed significant overall differences between 5 and 15 mg/kg ($p<0.0001$), 5 and 30 mg/kg ($p<0.0001$), and 10 and 15 mg/kg ($p<0.01$). A number of significant differences were observed when groups were compared each day. Noted here are differences for Day 1 Cocaine (5 vs. 30 mg/kg, $p<0.01$; 10 vs. 30 mg/kg, $p<0.05$; 15 vs. 30 mg/kg, $p<0.01$) and Day 7 Cocaine (5 vs. 10 mg/kg, $p<0.05$; 5 vs. 15 mg/kg, $p<0.0001$; 10 vs. 15 mg/kg, $p<0.01$; 15 vs. 30 mg/kg, $p<0.0001$). **Figure 7C** shows mock figures illustrating the major aspects of locomotor sensitization that may differ amount treatment groups. While these features are illustrated independently here, it is also possible to observe differences in combinations thereof.

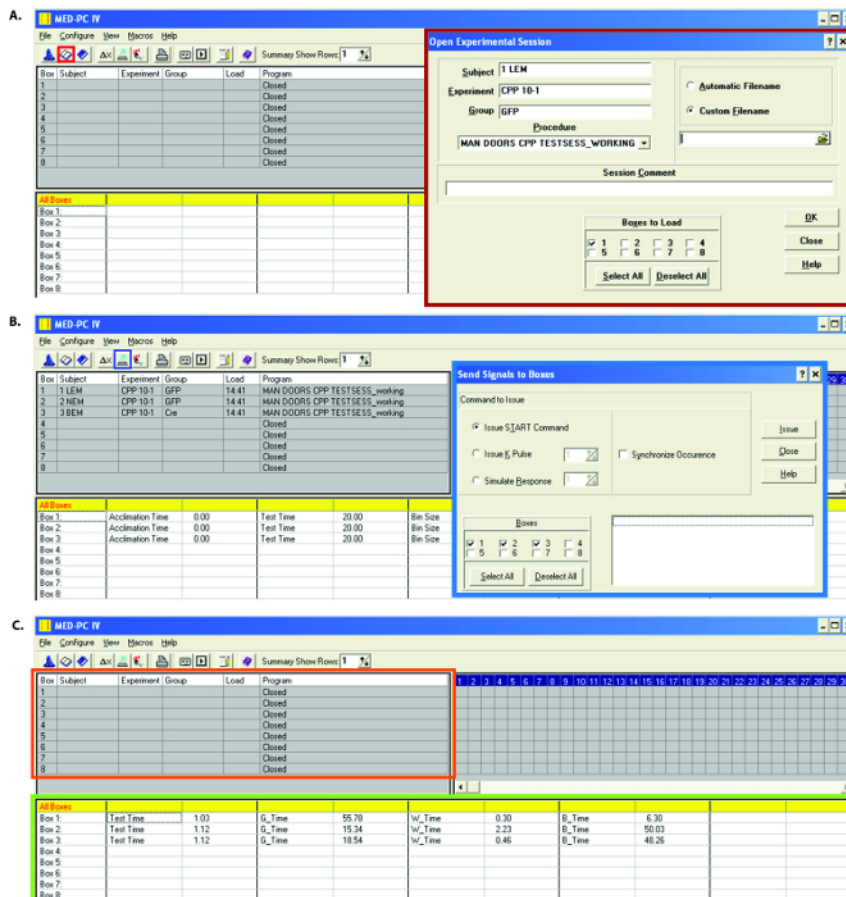


Figure 1. Med PC CPP basic session control. (A) Default display, open session icon (red box), and open session dialog box (inset). Select "custom filename" and use folder window to navigate to data folder and name the session. Select appropriate program from the "procedure" drop down list. For each box in use, check box number and enter pertinent information into subject, experiment, and group boxes. (B) Load chamber display, start signal icon (blue box), and send signal dialog box (inset). Once all chamber data has been entered, select chambers to be loaded and issue start signal. Chambers will now be triggered by beam-break to start the session timer and collect data. (C) Ended session display. As each chamber completes the designated program, the data collection area (green box) will become static and the chamber information area (orange box) will show chambers as "closed." [Please click here to view a larger version of this figure.](#)

A.

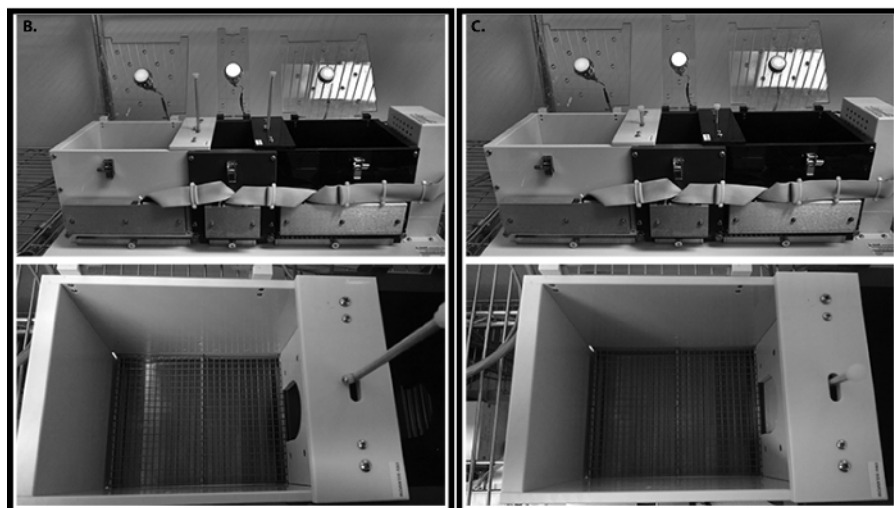


Figure 2. CPP chamber configuration. (A) Sample experimental time line. Pre-test is followed by daily alternating cocaine (gray) and saline (white) conditioning sessions. Post-test is conducted 24hrs after the last conditioning session. (B) Open chamber configuration for use in pre- and post-test sessions. Note that inter-chamber doors are in the raised/open position. (C) Closed chamber configuration for use in conditioning sessions. Note that manual doors are lowered, with no access between chambers. [Please click here to view a larger version of this figure.](#)

Pre-Test											
Group	Mouse ID	Run	Chamber	Black-Side Time (s)	White-Side Time (s)	Middle Time (s)	Total Time in Chamber (s)	Black - White	White - Black	Side Chosen	Pre-Test Preference Score
Treatment 1	Mouse 1A	1	1	401.46	544.15	254.39	1200	-142.69	142.69	white	142.69
	Mouse 1B	1	3	357.71	577.8	264.49	1200	-220.09	220.09	white	220.09
	Mouse 1C	1	7	293.63	630.58	275.79	1200	-336.95	336.95	black	-336.95
	Mouse 1D	2	4	380.96	407.08	411.96	1200	-26.12	26.12	black	-26.12
	Mouse 1E	2	2	512.09	82.62	605.29	1200	429.47	429.47	white	429.47
Treatment Group 1: SUM of Pre-Test Preferences											-0.29
Treatment 2	Mouse 2A	1	2	601.07	295.36	303.57	1200	305.71	305.71	black	305.71
	Mouse 2B	1	6	878.52	281.41	40.07	1200	597.11	597.11	white	-597.11
	Mouse 2C	2	7	591.07	371.41	237.52	1200	219.66	219.66	white	-219.66
	Mouse 2D	2	5	276.72	363.36	558.92	1200	-86.64	86.64	black	-86.64
	Mouse 2E	2	8	225.89	758.34	215.77	1200	-532.45	532.45	white	532.45
Treatment Group 2: SUM of Pre-Test Preferences											-0.59

animals exceeding pre-test preference limit (shown in red) are omitted from balancing calculations, but are still paired toward & away from their preferred chamber, within each group, as much as possible.

try to keep the sum of each group's preference similar

if all of the animals assigned to the same chamber during different runs can be drug paired in opposite chamber sides without negatively affecting the balancing of group sums, conditioning runs can be consolidated (see light blue boxes)

Figure 3. Balanced design calculations for CPP. Example showing calculations and goals when balancing pre-test scores in CPP. [Please click here to view a larger version of this figure.](#)

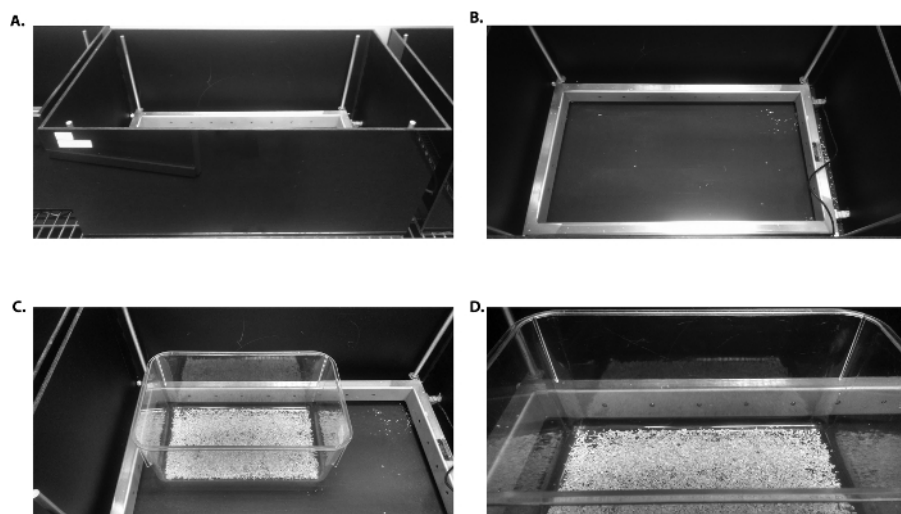


Figure 4. Locomotor Chamber Configuration. (A) Outer chamber consists of black opaque box inside which the beam array fits snugly (B). Housing/shoe box sized chambers should be filled with a thin layer of bedding (C) and aligned so that beams are evenly distributed along the long axis (D). [Please click here to view a larger version of this figure.](#)

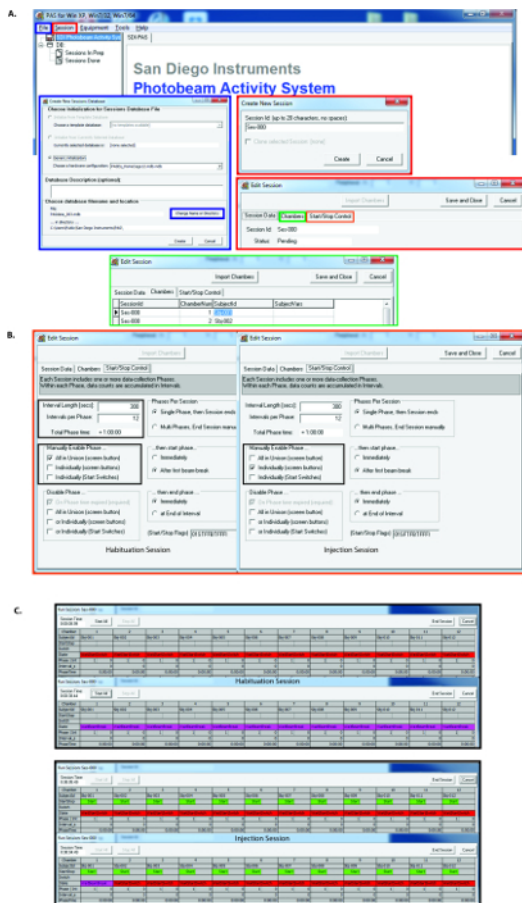


Figure 5. Locomotor Chamber Computer Operation. (A) Default Computer Program. Start new session database (blue box and inset). Rename file and directory for each experiment (blue sub-inset). Create new session (red box and inset) with unique identifier. Edit Session (red inset) and enter animal identifier information in the chamber tab (green box and inset) and set up start/stop control for each session type (orange box). (B) Select appropriate start stop control for each session type. Set interval length to 300 seconds (for 5 min bin) and 12 intervals per phase (for 1 hr session). For habituation sessions (left), chambers will be triggered to start in unison (Manually Enable Phase... All in Unison) and will begin monitoring for activity immediately after the first beam break. For injection sessions (right), chambers will be triggered individually (Manually Enable Phase... Individually Screen Buttons) and will begin monitoring for activity immediately after the first beam break. Both session types will end when the total phase time has expired for each chamber individually. (C) Once the session has been started, habituation sessions can be started by selecting the "start all" button (top). Note that after triggering, all chambers will be waiting for first beam break to begin session. For injection sessions, each chamber can be started independently (bottom). Note that after triggering individual chambers (while animal is out of chamber for injection), chambers will be waiting for the first beam break to begin session. [Please click here to view a larger version of this figure.](#)

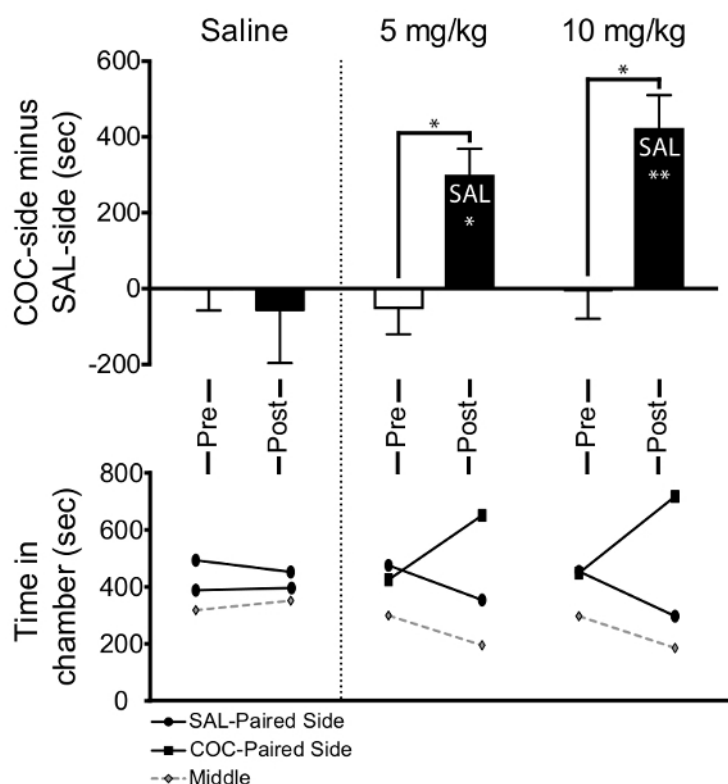


Figure 6. Representative CPP results. (Top) In CPP, mice showed significant preference for a chamber previously paired with cocaine (either 5 or 10 mg/kg), while mice that received saline pairings in both chambers did not develop any preference. Column abbreviations denote significant between-group differences from the labeled bar compared to the noted group at the same testing time point. (Bottom) By plotting time spent in cocaine- versus saline-paired chambers during the pre- and post-tests, increases in the cocaine-paired side can be seen in the 5 and 10 mg/kg groups that are at the expense of time spent in both the saline-paired and middle chambers. In contrast, there were no striking differences in time spent in any chamber between pre- and post-tests in the saline control group. Data presented as mean $\pm$ SEM. [Please click here to view a larger version of this figure.](#)

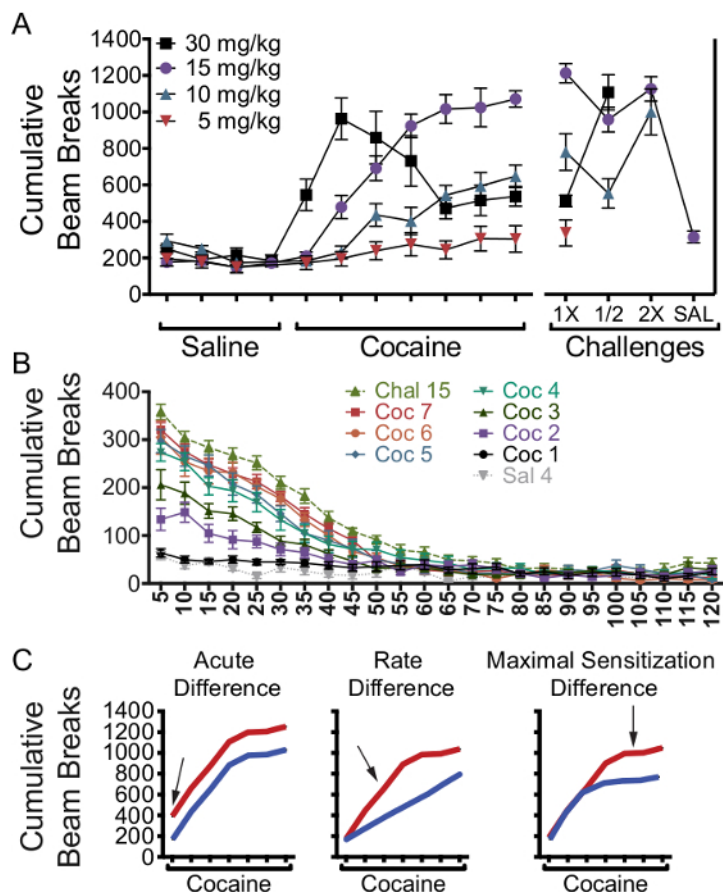


Figure 7. Representative locomotor sensitization results. (A) Cumulative beam breaks for the first 20 min of each daily trial for four separate doses: 5, 10, 15 and 30 mg/kg in wild-type mice followed by same-, half-, double-dose and saline challenges, modified from (18). All groups received same-dose challenges either one (10, 15, 30 mg/kg) or two (5 mg/kg) weeks following the last cocaine exposure. Some groups received further challenges, which were performed at variable withdrawal periods. (B) Plot of locomotor data (beam breaks) summed by 5-min bin for daily trials during the 15 mg/kg cocaine sensitization experiment. (C) Illustrations showing differences in locomotor sensitization for two treatment groups that are primarily driven by (left) acute cocaine response differences, (middle) a sensitization rate difference or (right) differences in maximal sensitization. Data presented as mean $\pm$ SEM. [Please click here to view a larger version of this figure.](#)

Discussion

This protocol demonstrates methods for conditioned place preference and locomotor sensitization, each of which can be used by the average lab to assess aspects of drug-induced behavioral plasticity. As with most behavioral tests, there are additional worthy considerations beyond the basic protocol. First, each of these techniques can be conceived as having two phases, induction and expression. "Induction" covers the development of the behavior-for CPP it occurs during conditioning, and for sensitization it is the initial period of (typically consecutive) drug exposures. "Expression" for CPP is the post-test, while for sensitization it can be defined as a drug challenge given either after withdrawal or simply as the last consecutive exposure.

It is worthwhile to consider limiting manipulations to one of these phases versus the other to better parse their potential effects. Viruses with temporally limited effectiveness (e.g., HSV) or drug co-administrations/pretreatments (e.g., agonists/antagonists) are useful in such efforts. When taking this approach, it may be further necessary to use compressed protocols so that a particular phase will better coincide with viral expression. For CPP, it is possible to conduct a two-day conditioning method, as we have described previously³⁷. Especially for locomotor sensitization, changes in the withdrawal period between induction and expression combined with these methods, may uncover processes involved in the maintenance or stability of the behavior. In addition, such approaches can be used to study the phenomenon of cross-sensitization, where a sensitized behavior is induced using one drug but can be expressed by exposure to a different drug. Since cross-sensitization does not occur between all sensitizing drugs of abuse, may elicit a sensitized behavior that differs somewhat from the original, and is not necessarily bidirectional for any given set of drugs, its examination may offer unique opportunities to understand where and how different drugs affect brain plasticity and function.

Proper interpretation of CPP, in particular, depends upon ruling out alternative explanations of the findings. Data generated in a three-chambered apparatus should be further scrutinized before defining subtraction of time spent in the saline-paired side from the cocaine-paired side at post-test as preference, since an increase in a preference for the drug-paired side that results solely from a decrease in time spent in the saline-paired chamber is likely unfit for such an interpretation. The middle chamber allows for this result since the mouse may alter time spent there instead of the drug-paired chamber. It is also possible to observe increased time spent in the drug-paired side at the expense of the middle chamber instead of the saline-paired side; arguably, this outcome may still be acceptably interpreted as an increase in drug preference. The inclusion

of third compartment provides a neutral chamber that allows unbiased placement of the animal during pre- and post-test sessions⁴¹. Although useful for addressing initial placement biases contributing to test-day scores, the third chamber is not required for conditioning. Alternative CPP designs that feature two distinct compartments or a single compartment with varied stimulus configurations are discussed elsewhere⁴¹⁻⁴².

Any deficit in place preference for a drug should also be accompanied by assessments of both ability to learn contextual associations and general reward function. There are a number of adjustments that can be made to the CPP paradigm that can aid in the interpretation of altered preference, including modifying the salience of the UCS (drug) by increasing (or decreasing) the number of pairings or using higher or lower drug dose. CPP can be performed using palatable food (e.g., high fat, sucrose) or social interactions to assess whether the observed change is specific to drug or is relevant to natural rewards; non-CPP approaches useful for this purpose include intracranial self-stimulation, sucrose preference, and/or appetitive approach tasks. However, all of these options vary in their ability to adequately address the desired question. Food-based CPP may be particularly beneficial since normal responses demonstrate an ability to learn and form appropriate contextual associations with a natural reward. Additional controls for assessing learning ability include tasks that rely on contextual learning/memory (contextual fear conditioning and CPA). CPA has the advantage of being run similarly to CPP, often in the same chambers, replacing the appetitive drug with an aversive experience (e.g., lithium chloride injection). Animals that show deficits in CPP, but normal CPA, demonstrate an ability to form appropriate contextual associations, which indicates that impairments in drug CPP most likely relate to reward (drug-specific or otherwise). One caution for CPA using lithium chloride to consider is whether this drug is a known treatment for any condition that may be modeled by the experimental animals. For example, lithium treatment has been shown to counteract learning deficits in the mouse model of Fragile X⁴³, which would confound this control method.

In addition to the classic CPP paradigm, there are potential extensions users may find useful, such as testing extinction of the learned drug-context association and its reinstatement after CPP. Conditioned responses (CRs), once established, can be maintained for extended periods^{19,20} when animals are left undisturbed. Despite the relative persistence of the CR, it can be effectively extinguished by repeated presentations of the CS (context) in the absence of the UCS (drug). Two CPP extinction methods appear in the literature: repeated test exposure without injection²¹ or re-pairings of the previously drug-paired side with vehicle²². Extinction processes reflect new learning, as opposed to "unlearning" of the original conditioning, an idea effectively demonstrated through "reinstatement." Reinstatement is classically triggered by re-exposure to the UCS, which produces recovery of the CR. In the context of CPP, a single injection of the training drug will cause animals to show place preference. Interestingly many non-training drug cues can also produce reinstatement of CPP, including priming with alternative drugs²² and a variety of stressors²³⁻²⁶. Extinction and reinstatement experiments are of particular interest to the drug addiction field as models of drug treatment and relapse. Interventions that improve the rate of extinction and/or reduce the magnitude of reinstatement could be valuable targets for human pharmacotherapies.

Compared to CPP, locomotor sensitization is considered to be much less dependent upon learning, and therefore, may be a preferred method for assessing drug-induced plasticity in rodent models with known cognitive difficulties. That said, there is certainly evidence that at least in some sensitization paradigms, a learned drug-context association develops and contributes to the sensitized response. Supporting evidence includes greater sensitization observed when drug is administered in a test environment outside of the home-cage, and sometimes, a lack of sensitization altogether when dosing occurs in the home-cage (e.g.,²⁷⁻²⁹). Notably however, context-independent sensitization has clearly been demonstrated in other studies (e.g.,³⁰⁻³²). Experimental details that may contribute to whether a context-dependent increase in sensitization is observable include drug dose, length of exposure, whether any group must be transported for the test of sensitization and certain aspects of pre-exposure to the testing chamber; however, these details remain somewhat unclear. One method of determining the contribution of contextual sensitization is to give a saline challenge following drug sensitization³³. The use of the described paradigm minimizes the contribution of context-dependent sensitization as evidenced by very little locomotor activation in previous studies upon saline challenge. The contextual contribution to sensitization has been reviewed in a number of papers, often including discussions of behaviors other than locomotor activity³⁴.

To limit contamination of drug-induced activation and sensitization with the locomotor stimulating effects induced by a novel environment, mice are acclimated to saline injections for three to four days at the beginning of each experiment. As can be seen in **Figure 7**, mice typically show reduced locomotion between the first and last saline acclimation day. In addition, the mice are noticeably calmer and easier to handle and inject by the last saline exposure. Previous works have tested multiple strains and genotypes of mice using this paradigm and do not see much variance in saline acclimation activity across them; however, it is possible to occasionally observe very strong hyperactivity phenotypes associated with particular genotypes that are not overcome using this method. In these cases, it may be desirable to perform several saline acclimation days (until locomotion plateaus in the hyperactive group), and then normalize the hyperactive group to the control group using the last saline injection day data. While not ideal, it allows for a more reasonable comparison of the effects of a drug on locomotion between these groups. Other options that may allay extreme hyperactivity are to extend the habituation trial length prior to injection each day by one to 5 hr, and/or use longer injection trials (2 - 4 hr) each day.

There are additional considerations that are important to the interpretation of locomotor behavior. As illustrated in **Figure 7C**, observed group differences may be driven primarily by disparities in the acute locomotor response, the rate of sensitization over days, the maximal limit of sensitization, or some combination of these factors. Parsing the contributions of these factors individually can be helpful. For this purpose, the acute locomotor response can be statistically analyzed alone. Then the rate of sensitization can be assessed using a program capable of curve fitting and a rate normalization process that discounts any acute locomotor differences. Altered maximal sensitization is usually revealed in a RM ANOVA over the consecutive drug exposure days, where follow-up day-by-day post-hoc comparisons are significant on days after group responses have plateaued. One should be aware that maximal sensitization cannot be determined for any group that maintains a linear locomotor response over drug exposure days, such as portrayed in **Figure 7C** (middle; blue line). In such a case, drug exposure may be extended to try and determine maximal sensitization.

Lastly, it is typically best to compare sensitization between groups using at least two primary drug doses performed in separate cohorts of animals. Differences in any of the above aspects of locomotor activation, at one dose or both, should be used to guide further testing to rule out other explanations, including alterations in the development of stereotypy. Repeated exposure to some drugs, such as cocaine and amphetamine, not only produces sensitized locomotion in a dose-dependent manner, but also sensitizes competing stereotypical behaviors. These stereotypies become particularly overt at high doses, such that locomotor sensitization is often partially or completely obscured, but revealed again upon administration of a lower dose. For this reason, a "deficit" in locomotor sensitization in one experimental group may actually reflect heightened sensitivity to the drug-induced development of stereotypies. The assessment of stereotypy can be challenging, but there

are a number of published scales³⁵ and other approaches³⁶. Using both a general stereotypy scale and an assessment of specific behaviors is recommended, as published previously¹⁸.

In conclusion, a number of behavioral tests have been developed in animal models in an attempt to parse the complexity of human addiction. Conditioned place preference and locomotor sensitization are two basic tests widely used in rodents and, respectively, they may be particularly useful in the assessment of early drug-associated reward and the persistent plasticity induced by repeated use. There are a number of considerations for the design and interpretation of each type of study, making it worthwhile to carefully consider the experimental goals and previous literature when planning these assessments.

Disclosures

The authors declare that they have no competing financial interests.

Acknowledgements

The authors thank Karen Dietz and Shari Birnbaum for previous input on behavioral design considerations and Lauren Peca for help with behavioral testing. The authors also acknowledge the generous support of the Simons Foundation (Simons Foundation Autism Research Initiative grant to C.W.C.), NIDA (DA008277, DA027664, and DA030590 to C.W.C., F32DA027265 to L.N.S. and F32DA036319 to R.D.P.), the FRAXA Research Foundation and Eleanor and Miles Shore Fellowship Program (fellowship support to L.N.S.), and the John Kaneb Fellowship Program (fellowship support to M.T.).

References

1. National Institute on Drug Abuse. *Drug Facts: Understanding Drug Abuse and Addiction*. <<http://www.drugabuse.gov/publications/drugfacts/understanding-drug-abuse-addiction>> www.drugabuse.gov: (2012).
2. Kasanetz, F., *et al.* Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science*. **328** (5986), 1709-1712 (2010).
3. Beach, H. D. Morphine addiction in rats. *Can J Psychol.* **11** (2), 104-112 (1957).
4. van der Kooy, D. Place Conditioning: A simple and effective method for assessing the motivational properties of drugs. In: *Methods of Assessing the Reinforcing Properties of Abused Drugs*. Bozarth M.A. ed., **Chap 13**, 229-240 (2012).
5. Carlezon, W. A. Place conditioning to study drug reward and aversion. *Methods Mol Med.* **84**, 243-249 (2003).
6. Prus, A. J., James, J. R., & Rosecrans, J. A. Conditioned Place Preference. In: *Methods of Behavior Analysis in Neuroscience*. Buccafusco J.J. ed., **Chap 4**, (2009).
7. Tzschentke, T. M. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol.* **12** (3-4), 227-462 (2007).
8. Aguilar, M. A., Rodriguez-Arias, M., & Miñarro, J. Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev.* **59** (2), 253-277 (2009).
9. Feduccia, A. A., & Duvauchelle, C. L. Novel apparatus and method for drug reinforcement. *JoVE.* (42) (2010).
10. Brabant, C., Quertemont, E., & Tirelli, E. Influence of the dose and the number of drug-context pairings on the magnitude and the long-lasting retention of cocaine-induced conditioned place preference in C57BL/6J mice. *Psychopharmacology.* **180** (1), 33-40 (2005).
11. Pliakas, A. M., Carlson, R. R., Neve, R. L., Konradi, C., Nestler, E. J., & Carlezon, W. A. Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J Neurosci.* **21** (18), 7397-7403 (2001).
12. Knackstedt, L. A., Samimi, M. M., & Ettenberg, A. Evidence for opponent-process actions of intravenous cocaine and cocaethylene. *Pharmacol Biochem Behav.* **72** (4), 931-936 (2002).
13. Post, R. M., & Rose, H. Increasing effects of repetitive cocaine administration in the rat. *Nature.* **260** (5553), 731-732 (1976).
14. Marin, M. T., Cruz, F. C., & Planeta, C. S. Cocaine-induced behavioral sensitization in adolescent rats endures until adulthood: lack of association with GluR1 and NR1 glutamate receptor subunits and tyrosine hydroxylase. *Pharmacol Biochem Behav.* **91** (1), 109-114 (2008).
15. Henry, D. J., & White, F. J. The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. *J Neurosci.* **15** (9), 6287-6299, (1995).
16. Hope, B. T., Simmons, D. E., Mitchell, T. B., Kreuter, J. D., & Mattson, B. J. Cocaine-induced locomotor activity and Fos expression in nucleus accumbens are sensitized for 6 months after repeated cocaine administration outside the home cage. *Eur J Neurosci.* **24** (3), 867-875 (2006).
17. Shuster, L., Yu, G., & Bates, A. Sensitization to cocaine stimulation in mice. *Psychopharmacology.* **52** (2), 185-190 (1977).
18. Smith, L. N., Jedynak, J.P., Fontenot, M.R., Hale, C.R., Dietz, K.C., Taniguchi, M., Thomas, F.S., Zirlin, B.C., Birnbaum, S.G., Huber, K.M., Thomas M.J., & Cowan, C.W. Fragile X mental retardation protein regulates synaptic and behavioral plasticity to repeated cocaine administration. *Neuron.* **82** (3), 645-658 (2014).
19. Mueller, D., & Stewart, J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behav Brain Res.* **115** (1), 39-47 (2000).
20. Sakoori, K., & Murphy, N. P. Maintenance of conditioned place preferences and aversion in C57BL6 mice: effects of repeated and drug state testing. *Behav Brain Res.* **160** (1), 34-43 (2005).
21. Bardo, M. T., Neisewander, J. L., & Miller, J. S. Repeated testing attenuates conditioned place preference with cocaine. *Psychopharmacologia.* **89** (2), 239-243 (1986).
22. Itzhak, Y., & Martin, J. L. Cocaine-induced conditioned place preference in mice: induction, extinction and reinstatement by related psychostimulants. *Neuropsychopharmacology.* **26** (1), 130-134 (2002).
23. Kreibich, A. S., & Blendy, J. A. cAMP response element-binding protein is required for stress but not cocaine-induced reinstatement. *J Neurosci.* **24** (30), 6686-6692 (2004).

24. Briand, L. A., & Blendy, J. A. Not all stress is equal: CREB is not necessary for restraint stress reinstatement of cocaine-conditioned reward. *Behav Brain Res.* **246**, 63-68 (2013).
25. Redila, V. A., & Chavkin, C. Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology.* **200** (1), 59-70 (2008).
26. Ribeiro Do Couto, B., Aguilar, M. A., Manzanedo, C., Rodriguez-Arias, M., Armario, A., & Minarro, J. Social stress is as effective as physical stress in reinstating morphine-induced place preference in mice. *Psychopharmacology.* **185** (4), 459-470 (2006).
27. Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R. Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization. *Life sciences.* **28** (7), 755-760 (1981).
28. Badiani, A., Browman, K. E., & Robinson, T. E. Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of cocaine and amphetamine. *Brain Res.* **674** (2), 291-298 (1995).
29. Li, Y., Acerbo, M. J., & Robinson, T. E. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. *Eur J Neurosci.* **20** (6), 1647-1654 (2004).
30. Partridge, B., & Schenk, S. Context-independent sensitization to the locomotor-activating effects of cocaine. *Pharmacol Biochem Behav.* **63** (4), 543-548 (1999).
31. Le Foll, B., Diaz, J., & Sokoloff, P. Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. *Synapse.* **47** (3), 176-183 (2003).
32. Heidbreder, C. A., Babovic-Vuksanovic, D., Shoaib, M., & Shippenberg, T. S. Development of behavioral sensitization to cocaine: influence of kappa opioid receptor agonists. *J Pharmacol Exp Ther.* **275** (1), 150-163, (1995).
33. Tirelli, E., Michel, A., & Brabant, C. Cocaine-conditioned activity persists for a longer time than cocaine-sensitized activity in mice: implications for the theories using Pavlovian excitatory conditioning to explain the context-specificity of sensitization. *Behav Brain Res.* **165** (1), 18-25 (2005).
34. Anagnostaras, S. G., Schallert, T., & Robinson, T. E. Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology.* **26** (6), 703-715 (2002).
35. Spangler, R., Zhou, Y., Schlussman, S. D., Ho, A., & Kreek, M. J. Behavioral stereotypies induced by 'binge' cocaine administration are independent of drug-induced increases in corticosterone levels. *Behav Brain Res.* **86** (2), 201-204 (1997).
36. Kelley, A. E. Measurement of rodent stereotyped behavior. *Curr Protoc Neurosci.* **Chap. 8**, 8.8. 1-8.8. 13 (1998).
37. Taniguchi, M., Carreira, M.B., Smith, L.N., Zirlin, B.C., Neve, R.L., & Cowan, C.W. Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. *Neuron.* **73** (1), 108-120 (2012).
38. Zangen, A., Solinas, M., Ikemoto, S., Goldberg, S.R., & Wise, R.A. Two brain sites for cannabinoid reward. *J Neurosci.* **26** (18), 4901-4907 (2006).
39. Huston, J.P., de Souza Silva, M.A., Topic, B., & Müller, C.P. What's conditioned in conditioned place preference? *Trends Pharmacol Sci.* **34** (3), 162-166 (2013).
40. Schechter, M.D., & Calcagnetti, D.J. Trends in place preference conditioning with a cross-indexed bibliography; 1957-1991. *Neurosci Biobehav Rev.* **17**, 21-41 (1993).
41. Bevins, R.A., & Cunningham, C.L. *Place Conditioning: A Methodological Analysis.* In M.Anderson (Ed.), *Tasks and Techniques: A sampling of methodologies for the investigation of animal learning, behavior, and cognition*; **Chap 9**, 99-110 (2006).
42. Hitchcock L.N., Cunningham C.L., & Lattal, K.M. Cue configuration effects in the acquisition of a cocaine-induced place preference. *Behav Neurosci.* **128** (2), 217-227 (2014).
43. Liu, Z-H, Chuang, D.M., & Smith C.B. Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. *Int J Neuropsychopharmacol.* **14** (5), 618-30 (2011).
44. Bardo, M.T., Rowlett, J.K., & Harris, M.J. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. *Neurosci Biobehav Rev.* **19** (1), 39-51 (1995).