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Apparatus bias and the use of light and texture in place conditioning

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Abstract

In a typical conditioned place preference (CPP) preparation, animals alternately experience drug and vehicle effects in distinct chambers of an apparatus, spending more time in the drug-paired chamber post-conditioning. However, if all animals prefer the same chamber before conditioning, data interpretation may be compromised. Unbiased apparatus has been systematically validated with ethanol in mice ([Cunningham, C.L., Feree, N.K., Howard, M.A. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology (Berl) 2003;170:409–422]); the present study sought to identify and eliminate bias in a standard black-and-white apparatus and validate that apparatus for CPP with morphine and cocaine in rats. Apparatus bias was assessed in 24 adult female Sprague–Dawley rats. Subjects preferred the black chamber under bright lighting conditions, with no preference in the dark. Subjects then underwent a counterbalanced CPP regimen to 5 mg/kg SC morphine ($n=12$) or 20 mg/kg IP cocaine ($n=12$) using only tactile conditioned stimuli. Significant absolute preferences for the drug-paired chamber were produced by both drugs, with no effect of drug-paired chamber assignment on CPP expression; vehicle-treated controls ($n=12$) showed no preferences. Bias-free CPP to morphine and cocaine using standard apparatus in rats is possible. Implications for place conditioning are discussed, including the potential value of systematically exploiting apparatus bias in addition to eliminating it.

Keywords: Conditioned place preference; Place conditioning; Apparatus bias; Ambient lighting; Texture; Morphine; Cocaine

1. Introduction

Many laboratories that specialize in animal models of drug abuse do so by assessing the rewarding effects of abused substances, and one of the most widely used methods for assaying such drug effects is the conditioned place preference (CPP) technique (van der Kooy, 1987; Schechter and Calcagnetti, 1993; Tzschentke, 1998; Carboni and Vacca, 2003). Cardinal among the reasons for CPP's popularity is its relative ease, quickness and economy. Given these characteristics, it comes as no surprise that virtually every conceivable combination of shape, size and sensory modality have been utilized for conditioned stimuli in CPP, with a wide array of parametric variations in routes of drug administration, number of conditioning trials and temporal manipulations. Cataloging the many possible configurations of CPP apparatus and protocol is beyond the scope of this paper, but a clear dichotomy that does emerge from the procedural variations is one that has been demanding increased attention in recent years—that between the "biased" and the "unbiased." The terms "biased" and "unbiased" typically refer to two different place conditioning procedures, whereby individuals are either conditioned with drug against their naturally non-preferred chamber (biased design) or drug-paired chamber assignments are randomly and equally distributed between alternatives (unbiased, or "counterbalanced," design). However, attention has recently been called to the issue of biased and unbiased place conditioning *apparatus*, that is, when all subjects exhibit a significant preference for the same chamber prior to conditioning.

Cunningham et al. (2003) directly assessed the effects of apparatus bias on ethanol (2 g/kg) CPP in mice. In their study, both biased and unbiased apparatuses were used in

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separate, but otherwise identical, unbiased CPP procedures that also included dedicated vehicle-treated control groups. All animals were randomly assigned to either the biased or unbiased apparatus and subjected to a pre-test session; drug-paired chamber assignments were then counterbalanced within both experiments. Data from each experiment's test session were analyzed in a variety of forms common to the CPP literature, including percent time in the drug-paired chamber, drug minus vehicle time "difference score" and post-conditioning minus pre-conditioning time spent in the drug-paired chamber. For each apparatus (unbiased and biased), Analyses of Variance (ANOVA) were used to compare the alcohol-treated groups to the vehicle-treated groups while determining what effect if any the drug-paired chamber had on the expression of CPP. Significant, uniform place preferences were evident only in the unbiased apparatus, while place conditioning was consistently seen in the biased apparatus only when drug was paired with the non-preferred chamber, clearly demonstrating the impact of apparatus bias on the conditioning of place preferences. Perhaps most interestingly, the results of the experiments with both the biased and unbiased apparatuses appeared identical when data across conditioning subgroups were collapsed (see their Fig. 8).

The results of Cunningham et al. (2003) support a CPP strategy that incorporates a pre-test for assessing apparatus bias and warrant their recommendation of always including drug-paired chamber as a factor in the statistical analysis to account for potential bias effects. Furthermore, the similarity in the collapsed results of each experiment demonstrate how the mean of the bimodal response to a biased apparatus can be identical to the homogeneous response to an unbiased apparatus, providing an estimate of drug reward that may be paradoxically both accurate and misleading when described through measures of central tendency. From these observations, another conclusion that may be drawn is that an effective way to manage bias is to eliminate it altogether through the design of unbiased apparatus, and it was in that context that the present study was conducted. The following report echoes Cunningham et al. (2003) in its focus on bias, but extends to the use of a conventionally designed CPP apparatus, using rats as the model species, and with morphine and cocaine as the experimental compounds. Specifically, the purpose of the following set of experiments was to address apparatus bias by identifying a common source of bias, systematically eliminating that bias and further validating the use of unbiased apparatus for place conditioning with drugs of abuse.

2. General method

2.1. Subjects

Adult female Sprague–Dawley rats served as subjects (mean weight = 292 ± 29 g). All animals were individually housed in hanging wire mesh cages (24 × 19 × 18 cm) and had ad libitum access to food and water in the home cage. Animal housing rooms operated on a 12-h light/dark schedule (lights on at 0800 h) and were maintained at an ambient temperature of 23 °C. All procedures were conducted between 1000 and 1530 h. All procedures were in compliance with National Research Council guidelines and conformed to regulations established by the Animal Care and Use Committee at American University (National Research Council, 1996; NRC, 2003).

2.2. Place conditioning apparatus

The apparatus was constructed of wood and consisted of two main conditioning chambers (30 × 30 × 39 cm each) joined by a smaller middle chamber (10 × 30 × 39 cm). One of the conditioning chambers was painted flat black on all sides with a smooth, black Plexiglas floor; the other conditioning chamber was painted flat white on all sides with a textured clear plastic floor with black stripes applied beneath the surface. The smaller middle chamber was painted gray on all sides with heavy steel mesh attached directly to the floor. Vertically sliding wood panels separated the chambers, and each side of each panel was painted to match the chamber it faced. Six identical apparatuses were utilized for running multiple animals simultaneously. A white noise generator was used in the CPP room throughout all procedures. All test sessions were digitally recorded by a ceiling-mounted night-vision equipped camera (Sony DVR201) for subsequent behavioral scoring. An animal was operationally defined as "in a chamber" once both forepaws had crossed the threshold into the same chamber.

2.3. Data analysis

Unless otherwise noted, time spent in the two main chambers was transformed into a percentage of the combined time spent in only those two chambers. For example, an individual animal's datum for time spent in the black chamber = seconds in black / (seconds in black + seconds in white) × 100. Tests for preference between the two main chambers were performed on the transformed percentages described above via repeated-measures ANOVA. Each ANOVA contained at least one repeated-measures factor of Chamber (two levels), with additional factors of interest added based on the experiment (see below). Bonferroni-corrected *t*-tests were employed for warranted post hoc comparisons. Statistical significance for all analyses was set at $\alpha = .05$.

3. Assessment of apparatus bias

The purpose of the first experiment was to test explicitly whether or not rats had a significant natural

preference for the black chamber over the white chamber, and if so whether or not that bias could be neutralized by manipulating the ambient lighting within the CPP procedure room.

3.1. Subjects

Twenty-four adult female Sprague–Dawley rats served as subjects.

3.2. Apparatus

The aforementioned place conditioning apparatus was used.

3.3. Procedure

Testing took place over two consecutive days. Each subject was transported from its home cage and immediately placed in the middle holding chamber of the CPP apparatus. The barriers between the chambers were then removed for the animal to freely explore the entire apparatus for 15 min. On Day 1 of testing, 12 of the animals were tested in the CPP room while it was lit by overhead fluorescent house lights augmented by four 60-W flood lights ceiling-mounted above the CPP chambers; all lighting was directed down towards the apparatuses. The other 12 animals were identically tested, but in total darkness. On Day 2, the animals were tested identically to Day 1, but in the opposite lighting conditions.

3.4. Results and discussion

A 2 × 2 repeated measures ANOVA on percent time with factors of Chamber (Black vs. White) and Lighting (Lights On vs. Lights Off) was performed. No main effects of Chamber or Lighting were found (F's $(1,23)<3.115$, p's $>.091$); however, there was a significant Chamber x Lighting interaction ($F(1,23)=37.651$, $p<.0009$). As seen in Fig. 1, Bonferroni-corrected paired-samples t-tests revealed a significant preference for the Black chamber over the White chamber in the Lights On condition ($t(23)=4.303$, $p<.0009$), a significant decrease in time spent in the Black chamber from Lights On to Lights Off ($t(23)=6.138$, $p<.0009$) and an equally significant increase in time spent in the White chamber from Lights On to Lights Off ($t(23)=-6.138$, $p<.0009$). Percent time spent in the Black and White chambers did not differ significantly in the Lights Off condition ($t(23)=-1.219$, $p=.235$), indicating a lack of preference when tested in the dark.

These data suggest that rats prefer dark colored chambers over light colored chambers, i.e., that the apparatus was biased. This finding is not unprecedented (Garcia et al., 1957; Rossi and Reid, 1976; Mucha and Iversen, 1984; Campbell and Spear, 1999), yet the use of the black-and-white CPP apparatus is still common in many laboratories, and is a standard feature of many commercially available automated place conditioning systems (e.g., MED Associates, Columbus Instruments). What differentiates the above experiment from other demonstrations of black-over-white preference is the identification of bright ambient white light as a major facilitating factor for this bias. A number of place conditioning studies have reported correcting apparatus bias through lighting manipulations (Haile et al., 2001; Mucha and Iversen, 1984), but the parameters employed are often unspecified. The present study specifically offers complete removal of white lighting as a possible solution to black-over-white apparatus bias.

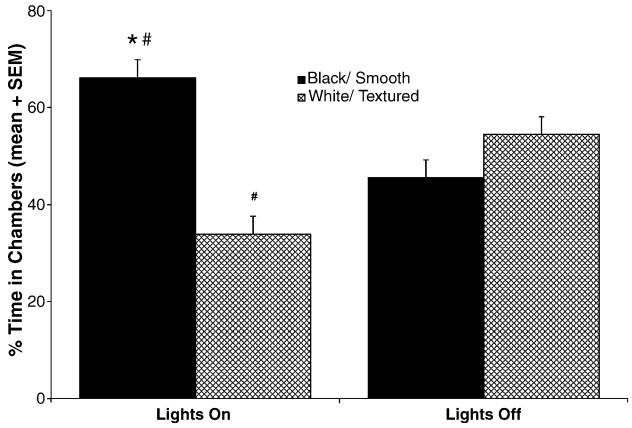

Fig. 1. Percent session time spent in each chamber in the Lights On and Lights Off conditions (mean+SEM). All subjects were tested in both lighting conditions, one condition per day for two consecutive days, in a counterbalanced within-subjects design. Eliminating white lighting from the test room eliminated the black-over-white bias. Significant chamber preference within lighting condition is indicated by *, significant within-chamber difference across lighting conditions is indicated by # (all p's $<.0009$).

In the above experiment, complete removal of white lighting rendered the apparatus unbiased for unconditioned preferences; however, the question remained whether or not this unbiased apparatus could support drug-induced place preference conditioning. Without lighting to enable discrimination between the black and white chambers, the only systematically manipulated sensory cue remaining was the texture of the floors, specifically the smooth Plexiglas of the Black chamber versus the textured plastic of the White chamber. Many published reports have revealed tactile stimuli as sufficient for place conditioning either explicitly (Vezina and Stewart, 1987a) or by default (e.g., Bozarth, 1987; Cunningham et al., 2003), but effective conditioning via texture without white light using the conventionally designed apparatuses employed herein had yet to be demonstrated. The following series of experiments was performed to validate the newly unbiased apparatus for CPP with morphine and cocaine, respectively. These two compounds are pharmacologically distinct (Nestler, 2004), but both have reliably produced CPP in our laboratory at the doses tested (5 mg/kg, Simpson and Riley, 2005; and 20 mg/kg cocaine, Busse et al., 2005) and are among the most

popular drugs used in place conditioning (Bardo et al., 1995; Tzschentke, 1998).

4. Validation of unbiased apparatus

4.1. Subjects

The 24 adult female Sprague–Dawley rats from the apparatus bias experiment above served as subjects. These rats were assigned to either the Morphine group ($n=12$) or Cocaine group ($n=12$). An additional 12 adult female Sprague–Dawley rats were included as a Vehicle Control group.

4.2. Apparatus

The aforementioned unbiased place conditioning apparatus was used.

4.3. Drugs and solutions

Morphine sulfate (generously supplied by NIDA) was mixed at a concentration of 5 mg/ml in saline and administered via subcutaneous (SC) injection at a dose of 5 mg/kg. All within-group non-drug saline injections were also administered SC and were equivolume to morphine. Cocaine hydrochloride (also generously supplied by NIDA) was mixed at a concentration of 10 mg/ml in saline and administered via intraperitoneal (IP) injection at a dose of 20 mg/kg. All within-group non-drug saline injections were also administered IP and were equivolume to cocaine. Half of the Vehicle Control animals ($n=6$) received SC injections of saline equivolume to morphine while the other half ($n=6$) received IP injections of saline equivolume to cocaine.

4.4. CPP Regimen

All subjects were weighed and briefly handled daily beginning 14 days prior to the CPP procedures. The actual CPP regimen itself took place over 9 consecutive days. On Day 1, half of the subjects received a morphine ($n=6$), cocaine ($n=6$) or saline ($n=6$) injection and were confined to either the smooth or textured chamber for 30 min; the remaining animals ($n=6$ per group) were injected with saline and also placed in a chamber for 30 min. The following day, animals from the drug groups received the opposite injection of the previous day and were confined to the opposite chamber; vehicle control animals received another saline injection and were confined to the opposite chamber of the previous day. This 2-day sequence constituted one cycle, and the CPP procedure consisted of four consecutive cycles over the course of 8 days. Day 9 was test day, where no injections were administered and all animals were given access to the entire apparatus for 15 min. Although the Lights Off condition of the apparatus bias experiment was conducted in total darkness, all subsequent conditioning and testing procedures were conducted under an 85-W red light to ensure experimenter safety while conducting the procedures (rats cannot perceive red; Jacobs et al., 2001). All CPP procedures were counterbalanced for which chamber was paired with the drug, that is, 12 animals (six from the morphine group and six from cocaine) were randomly assigned the smooth chamber to be drug-paired while the remaining 12 experienced their respective drug's effects in the textured chamber. The vehicle control animals received saline injections alternately in both conditioning chambers.

4.5. Results and discussion

4.5.1. Morphine

A 2×2 mixed ANOVA on percent time with a repeated-measures factor of Chamber (Drug vs. Vehicle) and between-groups factor of drug-paired stimulus (CS+; Smooth floor vs. Textured) yielded a significant main effect of Chamber ($F\ (1,10)=12.470$, $p=.005$) with no Chamber × CS+ interaction ($F\ (1,10)=.038$, $p=.849$). These data revealed a significant preference for the morphine-paired chamber over the vehicle-paired chamber that was not influenced by the unique tactile cues associated with the drug.

Using the raw time data (seconds), a 2×2 mixed ANOVA with a repeated-measures factor of Phase (pre- vs. post-conditioning) and a between-groups factor of CS+ yielded a significant main effect of Phase ($F\ (1,10)=15.276$, $p=.003$) with no main or interaction effects involving CS+ (F's $(1,10)<.179$, p's $>.682$). These data indicated a significant increase in time spent in the drug-paired chamber from the pre-conditioning test (the Lights Off condition from the apparatus bias experiment) to the final post-conditioning CPP test that was not influenced by the unique tactile cues associated with the drug.

4.5.2. Cocaine

A 2×2 mixed ANOVA on percent time with a repeated-measures factor of Chamber (Drug vs. Vehicle) and between-groups factor of drug-paired stimulus (CS+; Smooth floor vs. Textured) yielded a significant main effect of Chamber ($F\ (1,10)=5.899$, $p=.036$) with no Chamber × CS+ interaction ($F\ (1,10)=.097$, $p=.762$). As with morphine, these data revealed a significant absolute preference for the cocaine-paired chamber over the vehicle-paired chamber that was not influenced by the unique tactile cues associated with the drug chamber.

Turning again to the raw time data, a 2×2 mixed ANOVA with a repeated-measures factor of Phase and a between-groups factor of CS+ yielded a significant main effect of Phase ($F\ (1,10)=7.931$, $p=.018$) with no main or interaction effects involving CS+ (F's $(1,10)<1.609$, p's $>.233$). These data also indicated a significant increase in time spent in the drug-paired chamber from the pre-conditioning test to the

final post-conditioning CPP test that was not influenced by the unique tactile cues associated with the drug.

4.5.3. Vehicle control group

A 2 × 2 mixed ANOVA on percent time with a repeated-measures factor of Chamber ("drug-paired" vs. "vehicle-paired") and between-groups factor of counterbalanced CS+ assignment (smooth vs. textured) yielded no significant main or interaction effects (F's $(1, 10) < .011$, p's $> .918$), indicating a lack of preference for either chamber. An additional paired-samples t-test comparing percent session time spent in the smooth vs. textured chambers (regardless of CS+ assignment) confirmed that there was no preference for a specific tactile cue (t (11) = −.097, p = .924). CPP test data for the Morphine, Cocaine, and Vehicle Control groups are presented in Fig. 2.

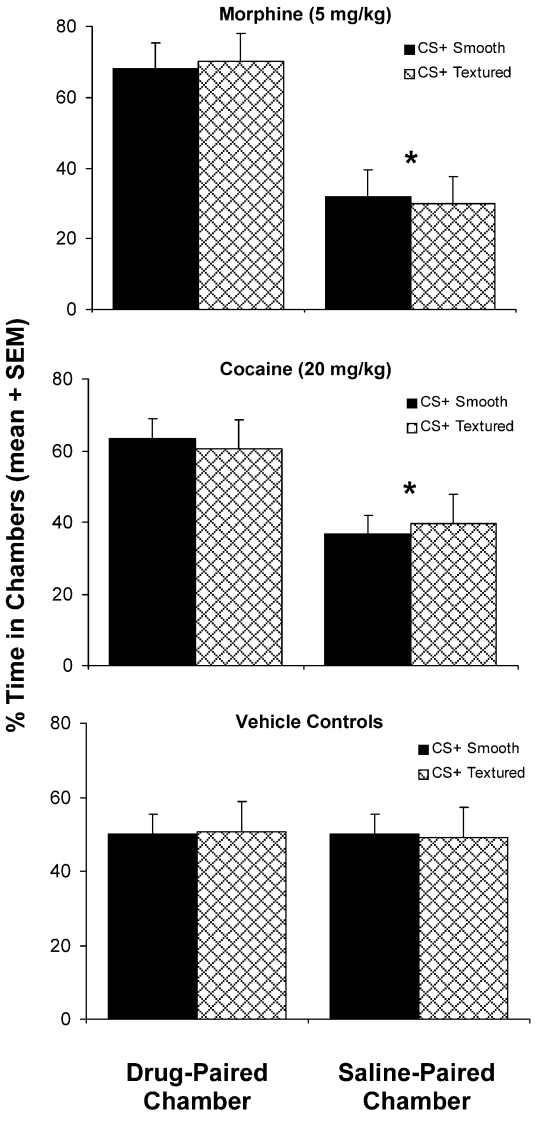

Fig. 2. Percent test session time spent in the drug-paired and vehicle-paired chambers (mean+SEM) after a fully unbiased CPP regimen. Animals conditioned with morphine (top panel) and cocaine (middle panel) showed significant preferences for the drug-paired chamber over the saline-paired chamber (p's < .036, indicated by *) regardless of which chamber (smooth or textured) was paired with drug. Control animals that had saline paired with both chambers (bottom panel) showed no preference (p = .918).

Morphine and cocaine reliably conditioned significant absolute place preferences using only tactile cues in the unbiased apparatus. The data from these experiments reveal the utility of a conventional apparatus rendered unbiased by removal of white light and further support the use of tactile stimuli as sufficient for place conditioning with rats. In their demonstration of texture's adequacy for place conditioning, Vezina and Stewart (1987a) argued for the sole use of tactile cues because animals cannot engage those cues without providing what has been operationally defined as the dependent variable. That is, if an animal has been conditioned to associate a drug's rewarding effects with multiple sensory cues, when tested for CPP it may spend considerable time in the vehicle-paired chamber engaging the distal cues from the drug-paired chamber (e.g., looking at or smelling) without actually providing the approach and contact behavior with the proximal tactile cues of the drug-paired chamber that defines place preference. Their data coupled with this compelling argument suggest that texture alone is not only sufficient for drug-induced CPP, but may actually be preferable. The position of Vezina and Stewart (1987a) was formulated based on morphine CPP data collected using a unique open field apparatus that was divided into quadrants and outfitted with modular floor panels of contrasting textures (also see Vezina and Stewart, 1987b). The present data corroborate their demonstration of tactile cues as sufficient for CPP and extend this principle to the use of conventionally designed apparatus with both morphine and cocaine.

5. General discussion

The results of the present study suggest that rats may prefer black chambers over white chambers, that black-over-white apparatus bias may be corrected by eliminating white light and that tactile cues alone are sufficient for place conditioning with morphine and cocaine in rats using a conventionally designed apparatus. Most importantly, the data generated by the unbiased CPP apparatus were essentially free from the interpretive complications associated with a biased procedure, a biased apparatus, or an unbiased procedure with an apparatus of unknown bias potential. Rats conditioned with either morphine or cocaine showed significant preferences for the drug-paired chamber over the saline-paired chamber (≈65% of test session time) and significant increases in raw time spent in the drug chamber from pre- to post-conditioning (≈+40% shift), whereas the vehicle-treated control rats showed no preference. Of course, the ultimate test of apparatus bias is whether or not stimulus assignment affects the expression of place conditioning effects, and none of the present data

suggests that the magnitude of CPP was dependent upon the tactile cues associated with the drug.

In addition to the empirical and interpretational benefits of a fully unbiased place conditioning protocol exemplified above, important theoretical issues also add to the appeal of unbiased apparatus. Paramount among them is controlling animals' natural tendency to explore. A number of reports have shown that vehicle-treated rats with strong initial preferences for a chamber may have those preferences attenuated by repeated exposures to the apparatus, moving closer to an even distribution of time between chambers over trials (Garcia et al., 1957; Rossi and Reid, 1976; Scoles and Siegel, 1986; Reid et al., 1989). The less apparatus bias there is, the less room there is for such a shift, and thus the more confident investigators can be in concluding that significant pre- to post-conditioning shifts are drug-induced rather than the consequence of habituation. Another conceptual advantage to using unbiased apparatus is its potential for assessing bidirectional effects within the same preparation, such as drug-induced reward or aversion (Cunningham et al., 2002; Ettenberg et al., 1999; Haile et al., 2001). Unbiased apparatus is also essential for making within-group assessments of preference for multiple rewarding stimuli, such as comparing two doses of a drug, two different drugs, or drugs of abuse vs. natural rewards (e.g., Mattson et al., 2003).

The value of a fully unbiased place conditioning protocol may be apparent, but it is worth noting that eliminating apparatus bias is not the only way of approaching the issue. In fact, the use of a biased procedure in a strongly biased apparatus may have its own advantages. Comparisons between several groups may demand that responses be scaled over a wider range; if so, then, for example, pairing drug with the chamber that animals spent 20% of pre-test session time in may leave more room for detecting differences than starting with the 50/50 distribution of pre-test time in a perfectly unbiased apparatus. Such an approach may still yield a conservative within-group estimate of drug reward, but provides flexibility for detecting differences between several groups by curtailing potential "ceiling" effects. A complementary argument may also apply to curtailing "floor" effects in assessing place aversions to drugs paired with a strongly preferred chamber (cf. Heinrichs and Martinez, 1986; Le Foll and Goldberg, 2005).

For investigators interested in utilizing unbiased apparatus, the case presented in the present report revealed a relatively simple solution for a common source of bias in a black-and-white CPP apparatus. The simplicity of removing white light from the CPP room to eliminate bias, however, may not be universally effective. For example, recent CPP pre-test data collected from our laboratory using the very same apparatuses revealed a strong preference for the textured chamber among male Fischer and Lewis rats (≈75% of transformed session time, no strain difference). Thus, apparatus bias, like drug reward itself, may be as much a function of the individual's or group's "properties" as of the apparatus' or drug's properties (Kabbaj et al., 2004; Kalivas, 2003). Indeed, controlling all possible sources of experimental variance is virtually impossible (e.g., Crabbe et al., 1999); however, the present data further illustrate that apparatus bias-free place conditioning is at least possible, and given the value of the place conditioning paradigm (Bardo and Bevins, 2000), may be worth pursuing. A corollary endorsement of systematically controlling and exploiting apparatus bias may also be made, depending on the goals and complexity of the investigation. At the very least, the present study supports Cunningham et al.'s (2003) call for full consideration of apparatus bias when designing, analyzing and reporting place conditioning experiments.

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