Repeated Exposure to Methylone: Behavioral Effect in Conditioned Cue Preference Tests

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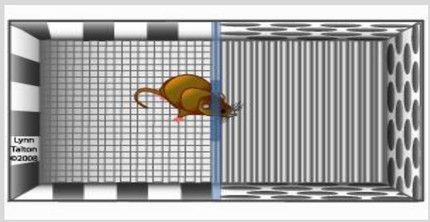
Introduction

The recent media attention surrounding "legal" synthetic drugs has created a swarm of public concern over the possible health risks associated with these chemicals. Specifically labeled as, "not for human consumption," these compounds have managed to skirt existing designer drug laws until recently. Among those commonly available at paraphernalia and adult book stores is methylone (MONE), a triple reuptake inhibitor chemically similar to methylenedioxymethamphetamine (MDMA, or "ecstasy"), marketed as "bath salts" or "room odorizers."

Given its chemical similarities to MDMA, and the current lack of literature concerning the behavioral effects of methylone, this study will assay the abuse potential and long-term toxicological effects. In particular, exposure to methylone may ultimately lead to incentive sensitization and a susceptibility to addiction or addictive behavioral tendencies.

To assess MONE as a putative reinforcing agent, this study utilizes the conditioned cue preference (CCP) task. This project addresses repeated methylone exposure in CCP tests. Swiss Webster mice were randomly assigned to two groups and given intraperitoneal injections over the course of a week. The conditioned cue preference task was employed to determine whether mice in the MONE group would show a significant shift in floor style preference from pre to post-test measurements.



Conditioned Cue Preference

Conditioned cue preference is a technique designed to measure preferences for environmental stimuli that have been paired with a positive or negative reward. Typically, the environment is a box with distinct environmental cues such as tactile, visual, or olfactory variations. Subjects are pretested to assess initial environment preference, then operantly conditioned to prefer one particular type of environmental cue. Later tested in a normal state, the preference for environmental cues are assessed. Reward learning is indicated by a shift in preference to the conditioned environment.

For the purpose of this study, a box containing tactile floor cues (holed or grated flooring) was implemented. Subjects were habituated to the CCP box, pretested for bias, conditioned, and post-tested to observe potential changes as a result of ethanol and triadimefon administration through reward learning.

Methods

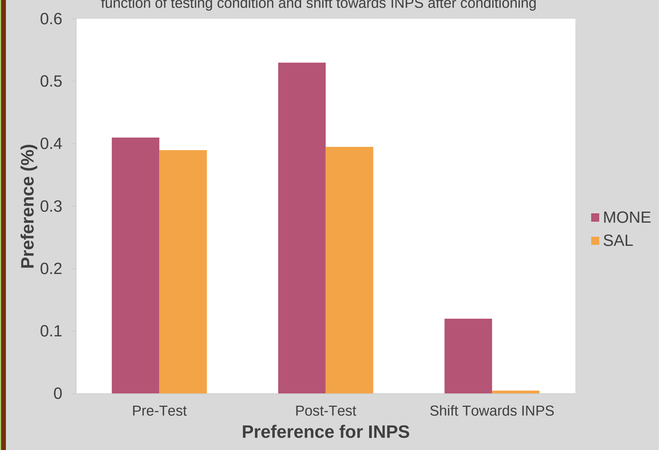
Subjects were Swiss Webster mouse males (N=23, approximately 12 weeks old, weights ranging from 28-36 g) obtained from Charles River Laboratory (Wilmington, MA) maintained on a reversed 12:12 light schedule with unrestricted access to food and water. All animal care and protocols were in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. Public Health Service and approved by the Institutional Animal Care and Use Committee of Winona State University. All subjects were randomly assigned to condition.

Subjects were habituated to the CCP box for a period of 5 minutes prior to injection schedules and initial preference testing. Subjects were then pretested for cue preference in a 20 minute session. Following the initial preference testing, subjects were administered 8 injections over a 4 day period with either saline, or methylone hydrochloride 15 mg/kg in 0.9% saline vehicle.

The conditioning phase occurred over an 8-day period, in which subjects in both the MONE and control groups were administered a single injection every other day. Subjects receiving the MONE injection were administered a 15g/kg dosage in a saline solution. Subjects in the non-MONE group received saline alone during this period. The conditioning phase subjected the mice to 10 minute conditioning in either holed or grated flooring to satisfy the necessary tactile cue conditioning.

Subjects were then post-tested for 20 minutes to ascertain whether mice in the MONE group spent a significantly higher amount of time in the conditioned environment than control group.

Figure 1. Preference (in percentage) for initially no preferred side (INPS) as a function of testing condition and shift towards INPS after conditioning



Results

Figure 1 shows preference for the initially non-preferred/drug-paired side pre- and post-test using a number of different measures. It should be noted that subjects in both groups demonstrated an initial overall preference for the grid floor (60%) as opposed to the hole floors, contradicting some previous reports that suggest subjects tend to spend half of their time on both floor types (Cunningham, Ferree, & Howard, 2003) and replicating the particular preference shown in a previous study from our laboratory (Holden, Fitzgerald, Bussell, & Ehlers, 2010). Thus, this apparatus was confirmed as a "biased" apparatus. Preference after the conditioning phase, recorded during the post-test, was 52% and 39% for the MONE and SAL groups ,respectively. Percent shift from original (pretest) to final (posttest) preference was also compared for both groups, with that shift averaging 12% and 0% for MONE and SAL groups respectively, $t(21) = 2.33$, $p<.05$. Dependent sample t-test of posttest-pretest difference scores showed a significant shift in preference toward the initially non-preferred side for the MONE group, $t(7)=3.87$, $p<.01$, but not the SAL group, $t(14)=0.43$, *ns*.

Discussion

In the current study, the overall preference in the MONE group for the drug-paired side was just over 50%, which represented a significant *shift* in preference but not a strong overall preference for the drug-paired side. The reinforcing properties of MONE, therefore, seem to be rather less (at least at this dosage) than some other kinds of reinforcing events examined with this approach (see Holden et al., 2010). Also of concern is the possibility that if the subject shows a shift in preference, that might reflect an anxiety-reduction effect of the drug on fear of a less preferred cue (accompanied by increased exploration of the side where that cue is present) rather than a reinforcing effect per se. While this possibility cannot entirely be discounted, it seems unlikely due to the fact that psychostimulants typically produce an anxiogenic effect, both in published studies (e.g., Maldonado & Navarro, 2000) and in unpublished data from our laboratory.

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