Do Medicines that Increase Norepinephrine Release Reduce Meth Seeking Behavior?

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Figure 7: C57BL/6J JAX Mouse

Abstract

Methamphetamine (METH) is a highly addictive central nervous stimulant for which there are no approved pharmacological treatment options and significant problems with relapse years after patients stop using. Conditioned place preference (CPP) is a neurobehavioral test used to study drug dependence in many species. In CPP, mice learn to associate METH experiences with one chamber (striped walls) and placebo experiences with another chamber (gray walls) and then show a preference for the METH chamber by spending more time there when allowed to explore both. The purpose of this study was to test an improved method (more & shorter training sessions) for CPP in mice based on a recently published procedure for rats. This CPP protocol involves habituation, conditioning, extinction, and reinstatement phases to determine if certain medication treatments during extinction can reduce METH-seeking behaviors. Within the phases there is a pre-conditioning test, post-conditioning test, extinction test, and reinstatement test. Previous experiments using a quick-training procedure for CPP did not show strong METH-conditioning (55 to 60% average preference). After conditioning with the improved method, mice spent significantly more time in the METH-paired chamber (70% average preference). During the 21-day extinction phase the average preference decreased, although there was day to day variability (some good and bad preference days). By day 14 the preference decreased to 65% and at day 21 it further decreased to 58%. There was evidence of reinstatement on the test day (65% preference) although it was not to the same extent as the post-conditioning phase. Results for extinction and relapse assessment and the effect of a potential treatment (venlafaxine) will also be presented. There was no evidence of the venlafaxine reducing the reinstatement. Overall, the new protocol significantly improved the conditioning phase although further optimization of the extinction methods is needed.

Introduction/Background

- No current approved pharmacological treatment options for METH addiction/dependence
- Behavioral and cognitive therapies have minimal success
- METH is a highly addictive and very potent central nervous stimulant
- It is also a strong psychomotor stimulant that mimics actions of certain neurotransmitters that affect mood and movement
- METH is a full agonist of trace amine-associated receptor 1 (TAAR 1)
- TAAR 1 regulates catecholamine system which consists of neurotransmitters such as norepinephrine, dopamine, and serotonin
- METH increases level of catecholamines in synapses by stopping reuptake into axon terminal
- Catecholamines are released into bloodstream during physical or emotional stress
- Norepinephrine is responsible for fight-or-flight response and activates the brain and body
- Symptoms from use are drug craving/seeking behavior, paranoia, and irritability
- Short-term effects: increased energy, alertness, increased concentration in fatigued users
- Long-term effects: breakdown of skeletal muscles, psychosis, seizures, persistent drug-stimulus associations (associate certain settings with good feelings from the high)

Materials & Methods

Subjects: Male C57BL/6J mice (Mus musculus obtained from two breeding colonies at Indiana University of Pennsylvania.

Materials:
- 3 transparent acrylic cubes with dimensions of 8” x 8” x 8” and 3/16” thick
- 2 polyvinyl chloride pipes that are 2 ¼” in diameter and 1 ¾” long
- White paper, gray paper, and paper with alternating black and white horizontal bars that are 1” thick. (4 sheets each)
- Plastic mesh mats with 0.1 cm diameter holes to line floor of chamber
- 4 Sony video cameras
- Meth solution: 2 mg/kg and 1 mg/kg
- Venlafaxine solution: 10 mg/kg
- Saline solution: 2 mg/kg

Procedure:

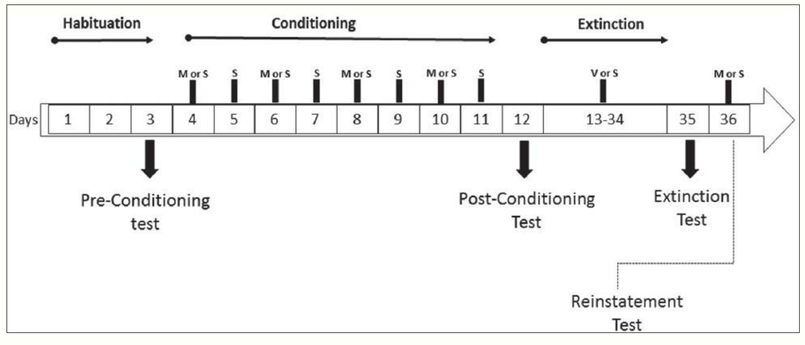

Figure 1: Protocol timeline showing each phase including test days, alternating injections during conditioning and reinstatement (M or S), and different treatment during extinction (V or S)

Hypothesis

Hypothesis: If a main effect of using methamphetamine is increased norepinephrine levels, could using a medication that mimics this effect to a lesser degree reduce drug seeking behavior and prevent relapse?

Results From Individual Test Days Throughout Protocol

Pre-conditioning Results Show No Preference In Chamber

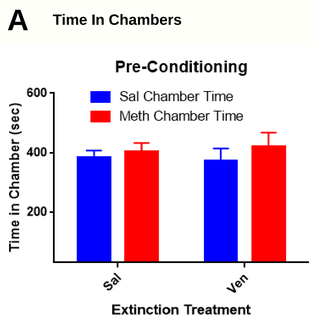

Figure 2: Average time spent in each chamber for the 2 extinction treatment groups during 20 min pre-conditioning test. (N=10 Mice)

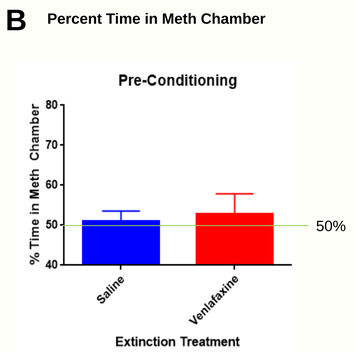

Figure 3: Unitary measure of for preference of chamber expressed as a percentage.

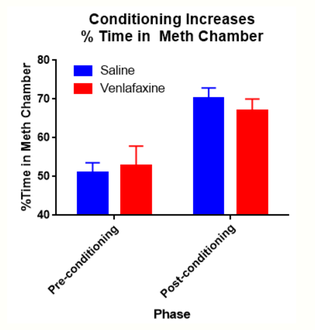

Figure 4: Average percent time spent in meth chamber for Sal. and Ven. groups for pre and post-conditioning phases

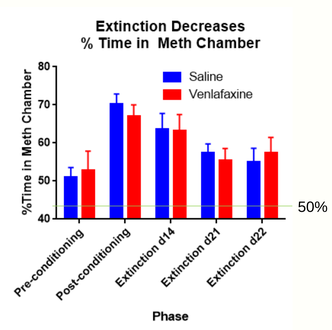

Figure 5: Average percent time spent in meth chamber for both groups during all phases expect reinstatement (extinction shows decreasing trend until last day)

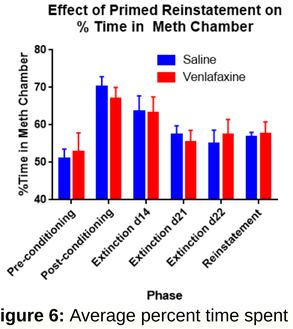

Figure 6: Average percent time spent in METH chamber during all phases and extinction days 14, 21, and 22

Discussion

Major Findings

- Pre-conditioning test results showed no preference to METH or saline chamber
- Increased number of days (8) in conditioning phase led to significantly better METH preference
- 22 days of extinction reduced METH chamber preference but not quite to 50% mark
- Expected an increase in preference to the METH chamber during reinstatement for saline mouse group but did not get those results

Conclusions & Future Projects

- Additional conditioning/training sessions (8 versus 6) resulted in better post-conditioning outcome (greater % time in meth chamber)
- Additional extinction sessions (21 vs 10) led to better reduction in % time in meth chamber.
  -For mice, we may need to have more extinction sessions (e.g., 25 or 28) to get to 50% preference.
- The primed reinstatement (1.0 mg/kg methamphetamine) did not result in a significant increase in preference for the meth chamber.
  -This does not rule out effectiveness of Venlafaxine
  -We may need to use a higher dose of methamphetamine (e.g., 2.0 mg/kg) combined with more extinction sessions to get strong reinstatement for mice.
- The Althobaiti protocol from rats was a good starting point for a mouse protocol, but we need to have more optimization of the procedure to have effective testing of potential therapeutics in mice.
- Future directions include:
  -Investigate the effects of other SNRI’s or SSRI’s
  -Include additional conditioning and extinction days and additional recordings sessions during extinction

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