



Redefining Drug Discovery Through Innovation

Preclinical Models of Abuse Liability

Introduction

Drug abuse liability refers to the likelihood a drug to be used in non-therapeutic situations, due to underlying psychoactive effects it produces (such as euphoria, sedation, or mood changes)

The assessment of the abuse potential of a drug product under development is generally conducted as a component of its safety evaluation. Therefore the ability to detect/refute drug abuse liability is critical in drug development, licensing, and for post-marketing surveillance.

PsychoGenics has been validating and applying rodent models of drug abuse liability for both internal studies and for clients for more than 10 years. Our models and tests include many protocols in self-administration, drug discrimination, conditioned place preference, withdrawal analysis, etc.



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Intravenous Self Administration



Self Administration

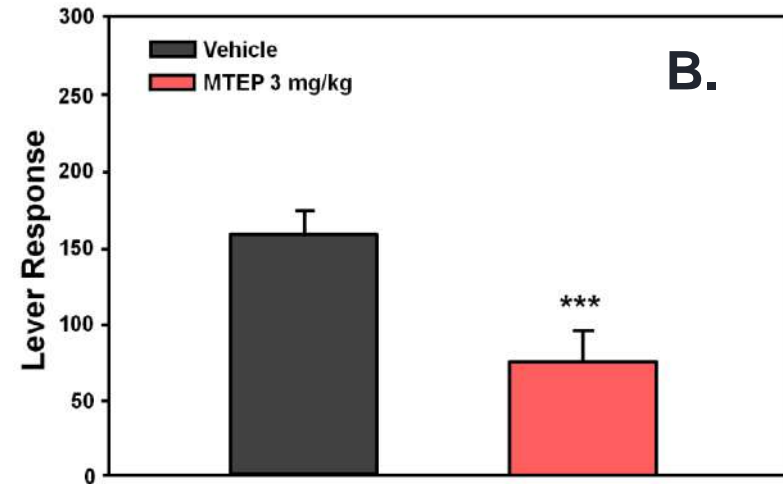
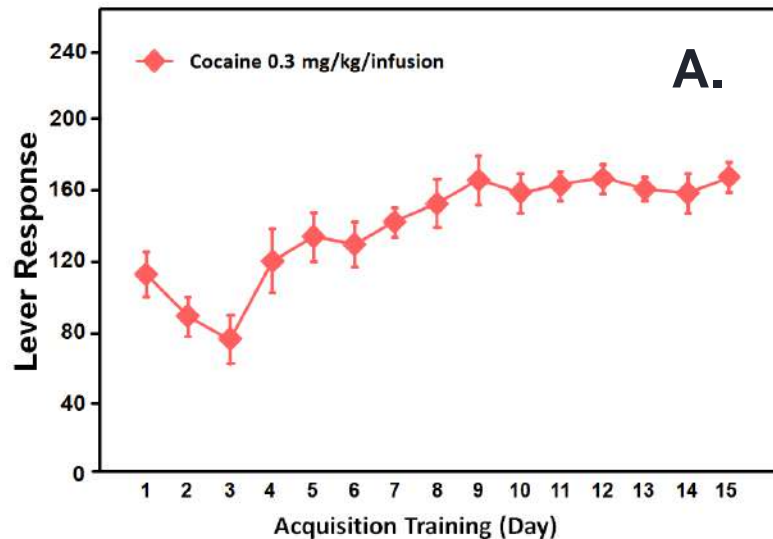
- The intravenous self-administration (SA) model is regarded as a “gold standard” for studying the reinforcing properties of novel compounds.
- SA model possesses high reliability and predictive validity. Lever press results in delivery of an IV infusion of drug. Most abused drugs differentially increase the rate of responding on the drug-delivery lever.
- Applications of SA model are mostly in three areas:
 - *Compound's effects on inhibition drug abuse*
 - *Compound's effects on inhibition of reinstatement of drug abuse*
 - *Evaluation of novel compound's abuse liability*



Methods

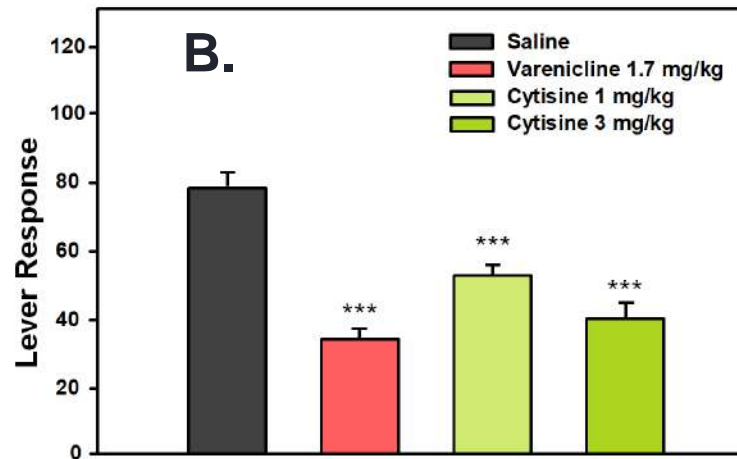
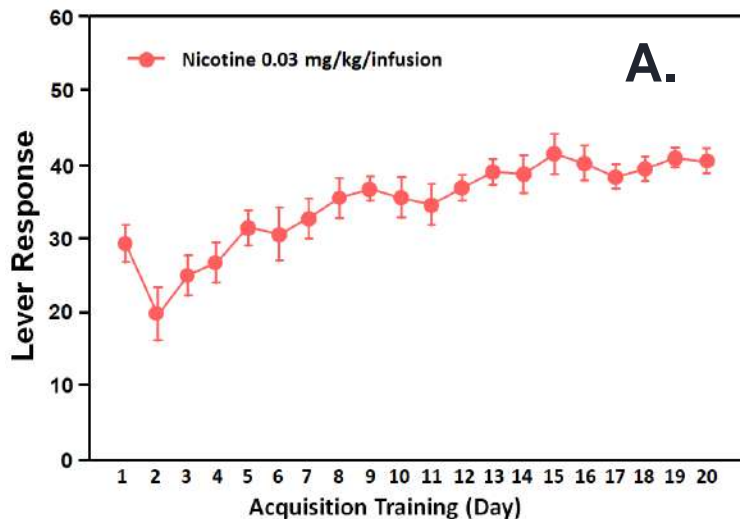
- Self-administration takes place in operant chambers (Med Associates, VT). Each chamber contained two levers and one of them is active.
- A stimulus light was located above each lever. A house light (providing illumination) is located at the top. An infusion pump is mounted above each chamber and it delivers drug solution via tubing connected to a fluid swivel.
- Prior to intravenous catheterization surgery, rats are trained to press the active lever to obtain food reward. Food training starts after the rats are food-restricted and reach ~ 85% of the free-feeding body weight.
- After acquiring the lever-press response in food training, rats are implanted with a jugular vein catheter.
- One week after the surgery, rats are trained to self-administer compound (such as cocaine, nicotine, morphine, etc.) in a fixed-ratio (FR) schedule of reinforcement. Depending on compound, a FR2, FR3 or FR5 schedule is used, i.e. 2, 3 or 5 lever presses for one drug delivery.
- Usually 0.1ml solution is infused in 1 sec for each drug delivery.

MTEP Inhibits Cocaine Self-Administration

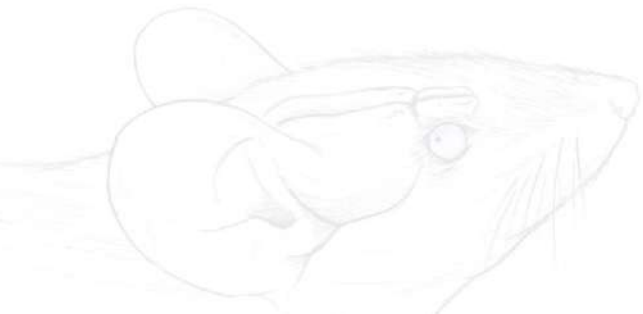


(A) Cocaine self-administration can be acquired through about 15 days of training
(B) MTEP (3-((2-Methyl-4-thiazolyl)ethynyl) pyridine, a selective allosteric antagonist of mGluR5) decreases cocaine intake in trained rats.

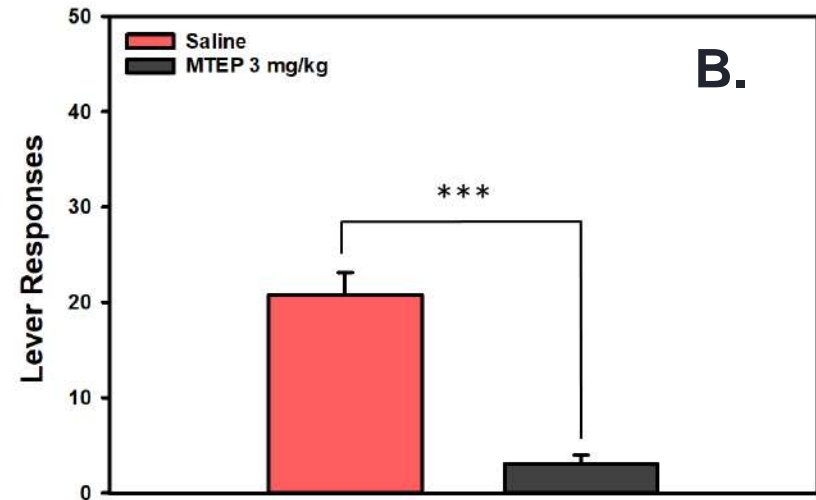
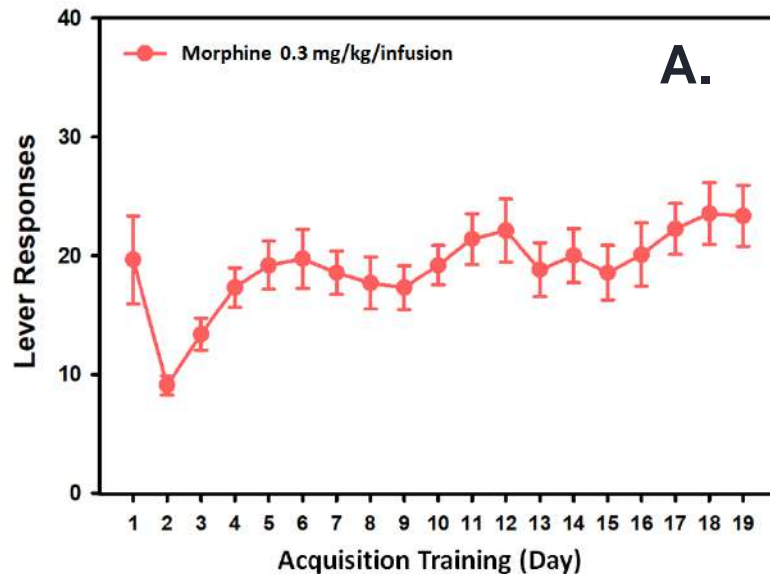
Nicotine Self-Administration



(A) Nicotine self-administration can be acquired in ~20 days of training. (B) Varenicline (Chantix; a partial agonist for the $\alpha 4\beta 2$ receptor) and cytisine (an acetylcholine agonist) decrease nicotine intake in trained rats.

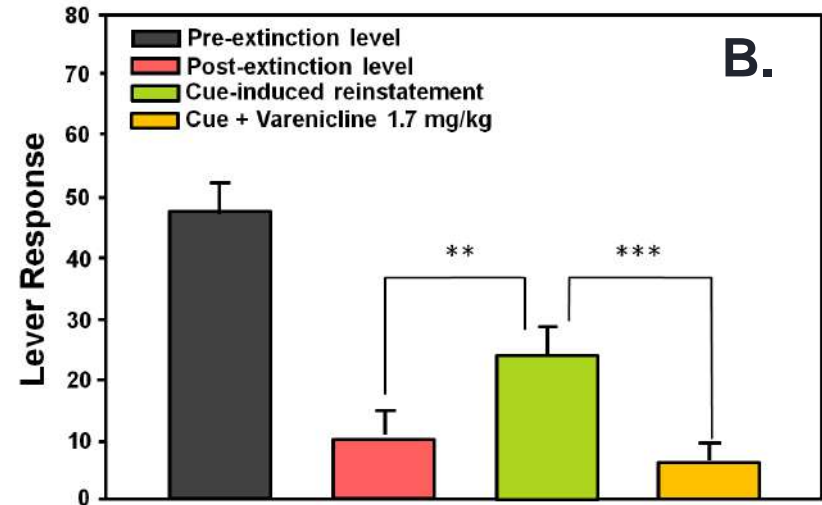
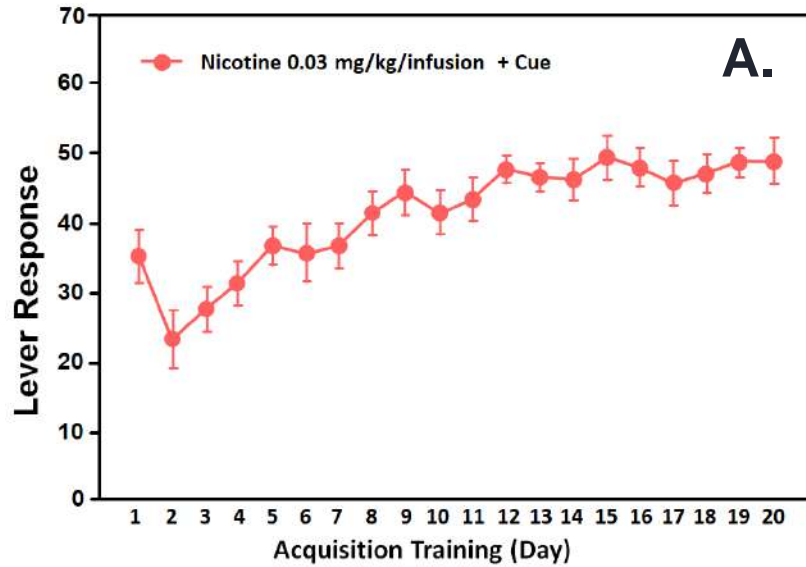


Morphine Self-Administration



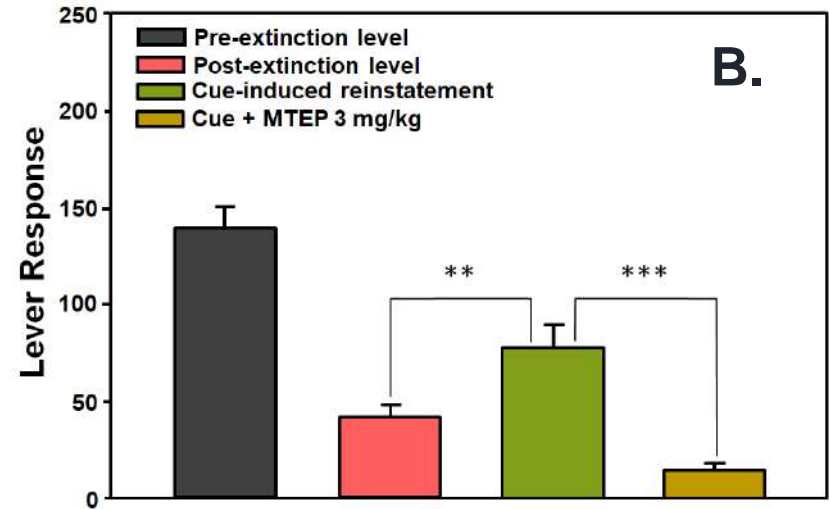
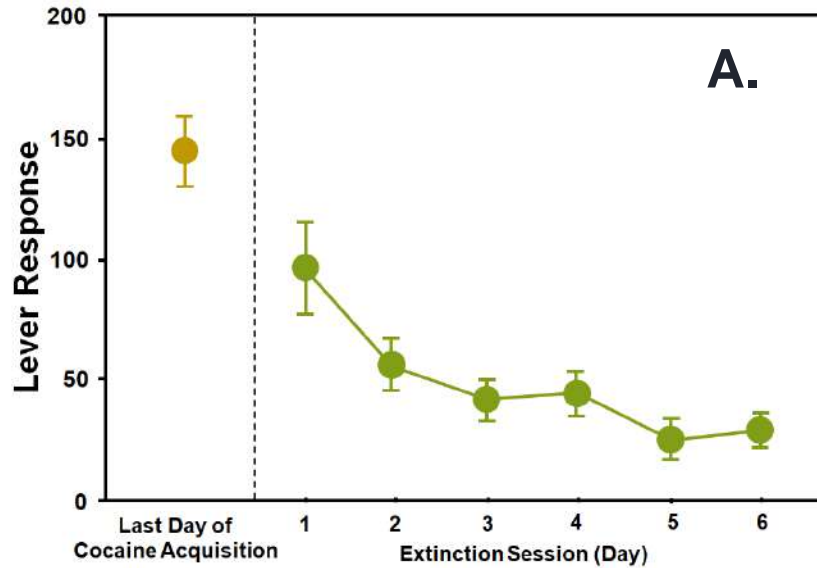
(A) Morphine acquisition can be achieved with self-administration training in about 20 days. (B) MTEP (3 mg/kg; ip) decreased morphine intake in trained rats.

Reinstatement of Nicotine Abuse



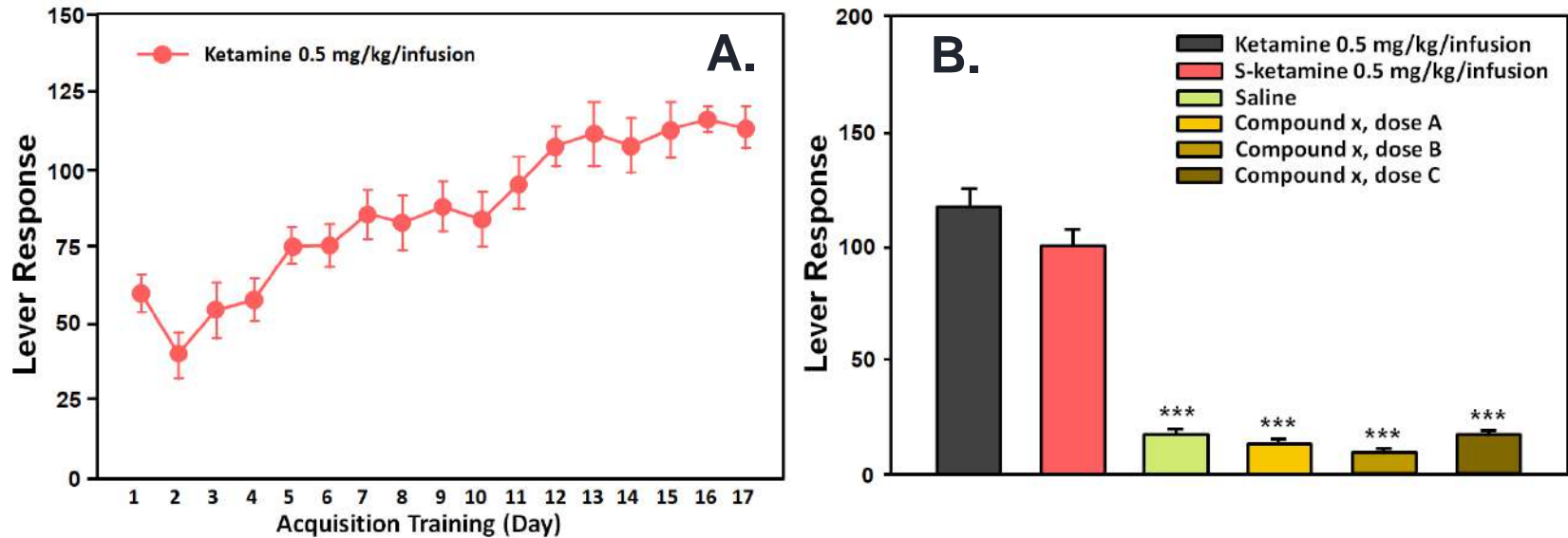
(A) Rats are trained in a similar way when studying reinstatement, but a cue (tone + light flash) is associated with drug infusion during training. (B) Varenicline 1.7 mg/kg entirely prevented cue-induced reinstatement.

Reinstatement of Cocaine Abuse



(A) Extinction session before the test of cocaine reinstatement (Acquisition curve before this session is not shown) (B). After lever response extinguished, cue only induced significant reinstatement, and MTEP 3 mg/kg treatment entirely prevented reinstatement of cocaine self-administration.

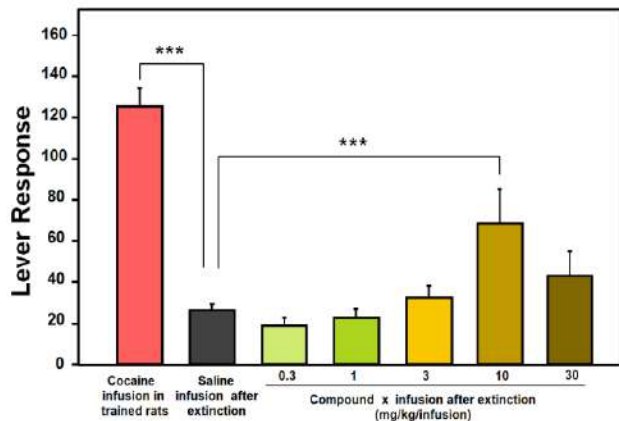
Substitution of Ketamine Self-Administration



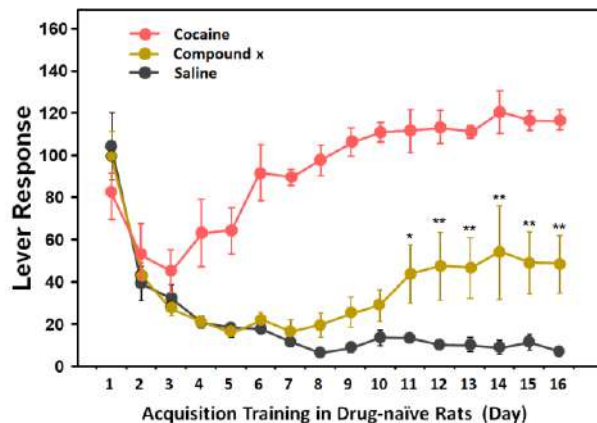
Substitution is usually a first step of assessing a novel compound's abuse liability. (A) Acquisition curve of ketamine. (B) S-ketamine showed full substitution to ketamine, meanwhile, compound x did not show substitution at either dose, suggesting this compound is not in the same category of ketamine or it unlikely possesses abuse liability.

SA Study III b: A thorough procedure to assess abuse liability

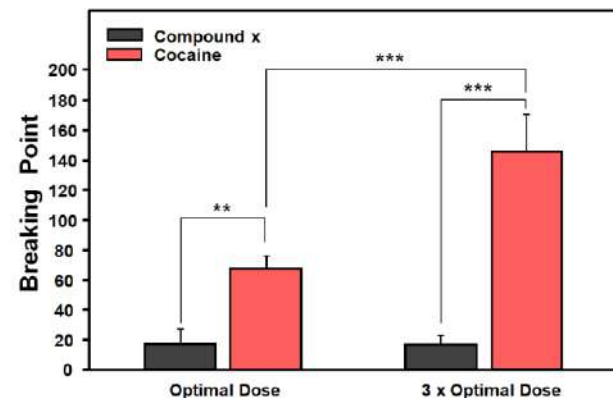
A. Stage I



B. Stage II



C. Stage III



A three-stage study was used to provide full assessment of a novel compound's abuse liability. (A) Substitution test (B) Self administration of test compound in drug-naïve rats. (C) Progressive ratio (PR) schedule showing that compound x's abuse liability is much weaker than that of cocaine.



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Drug Discrimination



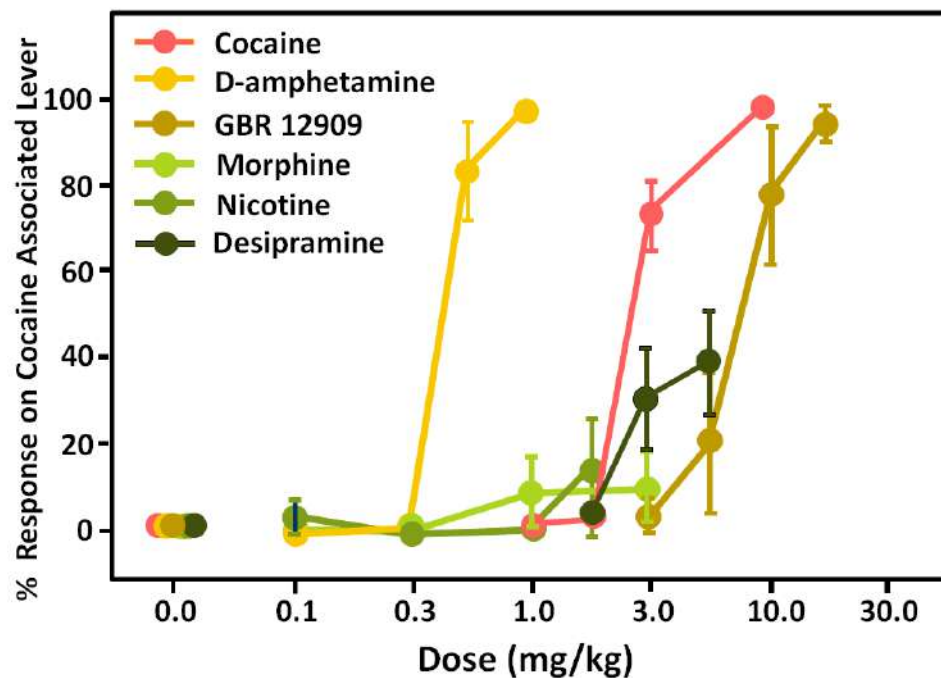
Introduction

- Drug discrimination (DD) is an important behavioral assay used to assess the degree of overlap of interoceptive stimulus effects with relevant drugs of abuse..
- In DD training, animals are trained to make a specific response (such as to one lever) after administration of a known drug of abuse, and a different response after administration of vehicle (such as to the other lever).
- When a trained subject receives a novel compound, the degree of drug-appropriate responding indicates the degree of similarity of the subjective effects of the novel compound compared to the drug of abuse.
- In studies of abuse liability, the assessment of novel compounds via combination of drug self-administration and drug discrimination models provide critical data for the development of medications with maximized therapeutic benefit and minimized risk for abuse.

Methods

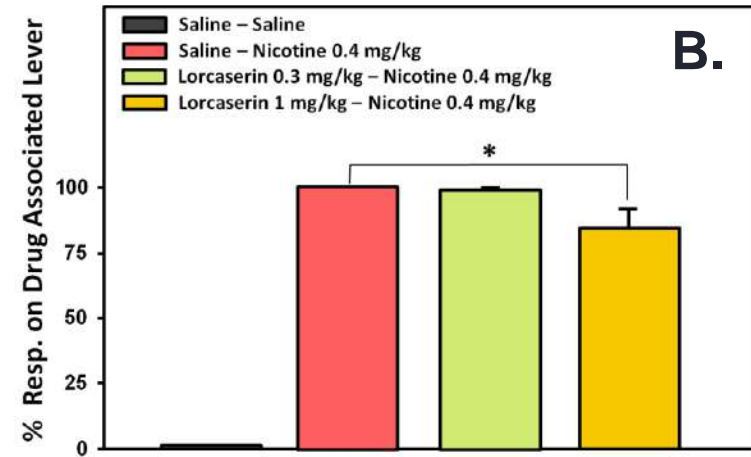
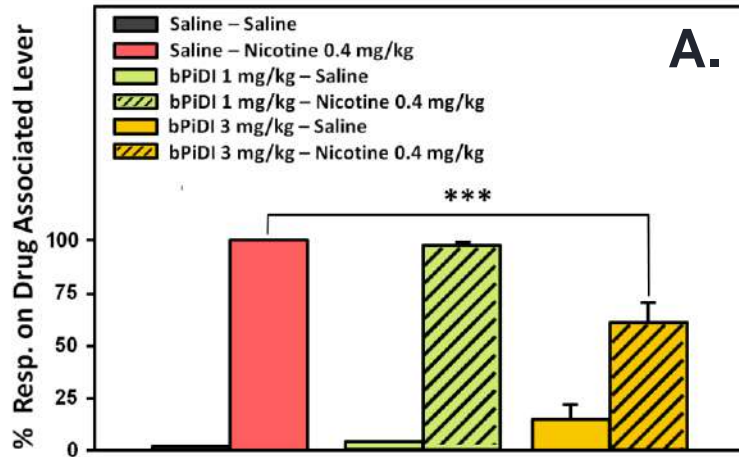
- DD takes place in operant chambers (Med Associates, VT). Each chamber contained two levers, one of them is assigned for drug and the other for vehicle.
- Rats are trained to press the lever to obtain food reward. Food training starts after the rats are food-restricted and reach ~ 85% of the free-feeding body weight.
- Rats are trained / tested according under a double-alternation two-week schedule [Drug (D), Vehicle (V), D, D, V; V, D, V, V, D].
- During training sessions, rats are administered either drug (optimal dose and pretreatment) or vehicle and are placed in the operant chambers. After drug administration, responding on one lever is reinforced by delivery of a 45 mg food pellet will gradually increase to a fixed-ratio 10 schedule; responses on the inappropriate lever resets the FR response requirement. After vehicle administration, responding on the opposite lever is reinforced. Lever assignment is counter-balanced across subjects.
- After consistent responding on the appropriate drug- or vehicle-associated lever for at least 80% of total lever presses across the whole session and during the first FR10 of the session, acquisition is achieved and compound testing can be initiated.

Cocaine Substitution



d-amphetamine and dopamine reuptake inhibitor GBR 12909 achieved full substitution; tricyclic antidepressant desipramine showed partial substitution; morphine and nicotine showed no substitution.

Nicotine



(A) The $\alpha 6\beta 2$ nACh receptor antagonist bPiDI show weak substitution to nicotine when administered alone. When co-administered with nicotine it significantly disrupts nicotine's discrimination from vehicle. (B). The 5-HT_{2C} receptor agonist lorcaserin, when co-administered with nicotine, significantly disrupts nicotine's discrimination



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Conditioned Place Preference and Conditioned Place Aversion

Introduction

- Conditioned Place Preference (CPP) is a behavioral test based on Pavlovian conditioning. It is commonly used to evaluate preferences for environmental stimuli that have been associated with a positive stimulus (e.g., food, a reinforcing drug or a relief of pain) paired with placement in a distinct environment containing various cues.
- Usually CPP tests animals in a drug-free state and it is sensitive to both reward and relief from aversion. After acquisition, the animal spends more time in the compartments previously associated with the positive stimulus. To date, CPP has been confirmed as a valid behavioral assay to evaluate may abused compound in both primates and rodents.
- Open field chambers with two or three compartments are used for rodent CPP test. Perceptive cues are applied to create a distinctive texture for the two compartments.
- On the other hand, if the animals spend significantly more time in the vehicle-paired compartment versus the drug-paired compartment, then this is considered a conditioned place aversion (CPA). Typically, drugs that elicit aversive effects produce CPA. Many drugs of abuse produce both CPP and CPA, depending on the dose administered. In drug-dependent animals, withdrawal effects generally produce CPA.

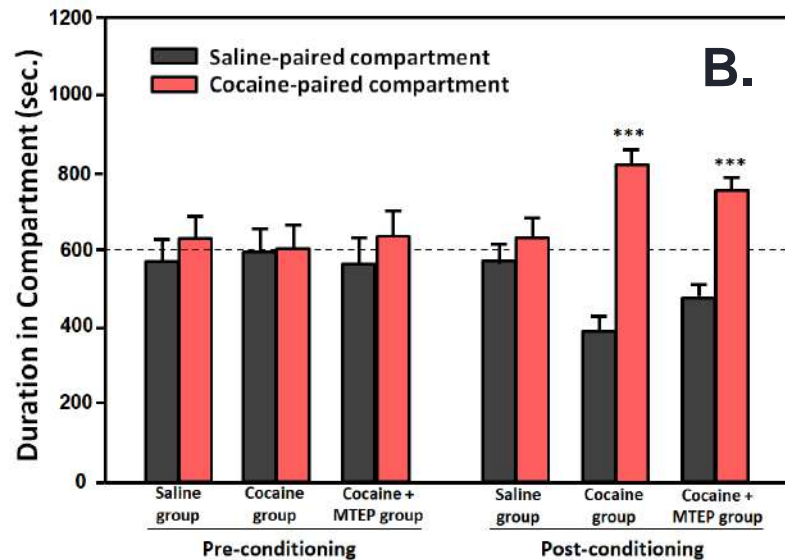
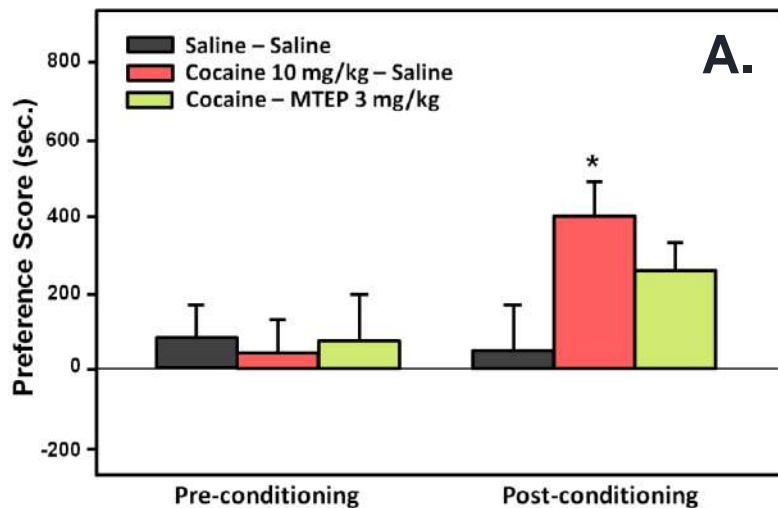
Methods

Typically there are three stages in a CPP study:

- *Habituation*: On day 1 the subjects are allowed to explore the testing arena twice (20 min each time with ~5 hr in between).
- *Conditioning*: During day 2-9, differentiation between “drug compartment ” and “vehicle compartment” is achieved through 8-day conditioning as shown in the table below. The rats in drug group receive drug on days 1, 3, 5 and 7, and vehicle on days 2, 4, 6, 8. After injection, they are confined in “drug compartment” or “vehicle compartment” for 30 min.
- *Test*: Post-conditioning bias test is conducted on day 10. The animals are allowed to explore the testing arena freely for 20 min. No treatment is given.

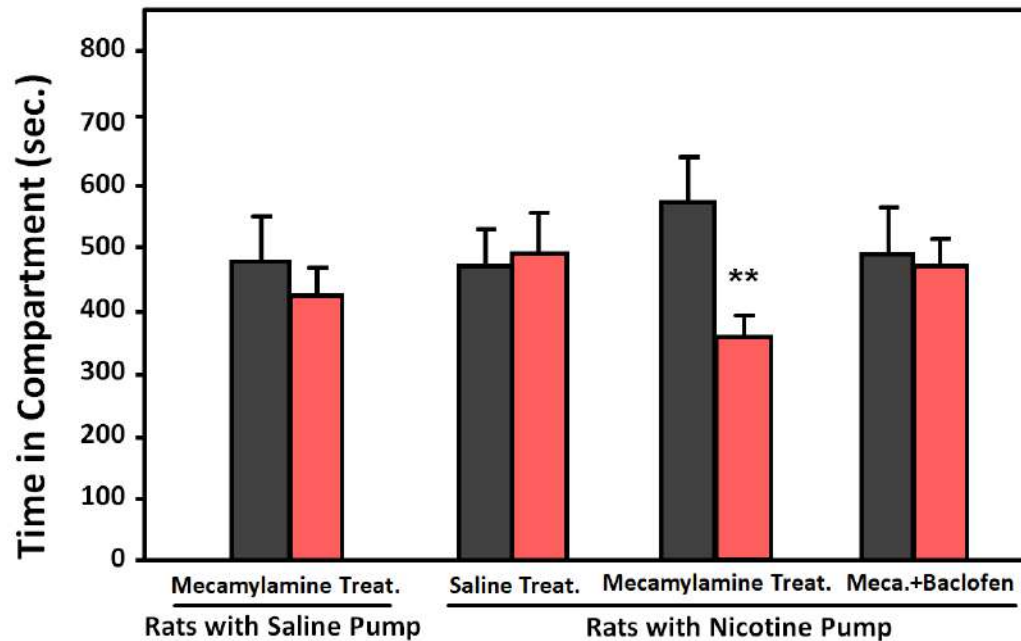
		Mon	Tue	Wed	Thu	Fri	Sun	Mon	Tue	Wed
Cocaine group	In CPP box	Habi x 2	Coc	Veh	Coc	Veh	Veh	Coc	Veh	Test
Vehicle group	In CPP box	Habi x 2	Veh	Veh	Veh	Veh	Veh	Veh	Veh	Test

Cocaine-induced CPP



(A) Using Preference Score, cocaine showed significant bias compared to saline. MTEP's bias did not significantly differ from vehicle. (B) Duration in paired compartment: rats treated with MTEP spent significantly more time in cocaine-paired compartment compared to vehicle, suggesting that MTEP only partly blocked the effects of cocaine.

Mecamylamine-Induced CPA in Nicotine Dependent Rats



Mecamylamine 2 mg/kg caused significant CPA as seen by the decreased time rats spent in the mecamylamine compartment. The GABAB agonist baclofen (3 mg/kg) prevented mecamylamine-induced CPA.




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Withdrawal

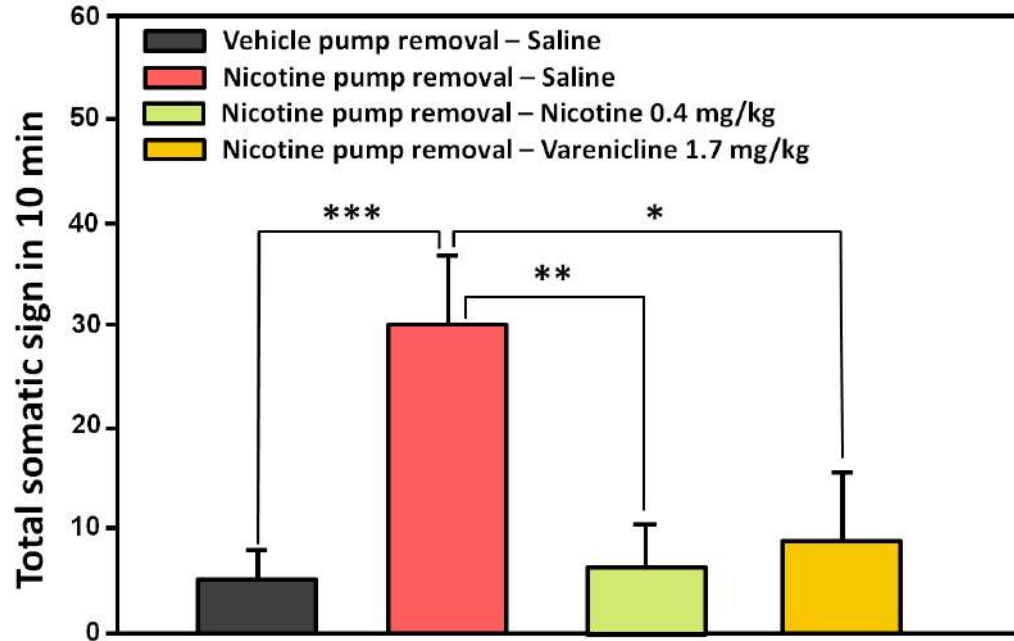


Introduction

- Withdrawal of a dependent drug has been studied extensively in animal models. A typically method is to assess withdrawal syndrome, which is defined as a set of symptoms occurring in discontinuation or dosage reduction of abused drugs.
- The most common way to study withdrawal syndrome is to observe and record somatic signs in a structured survey-like behavioral test.
- To assess withdrawal syndrome, physical dependence is generated first by administering a drug repeatedly or continuously for 1- 3 weeks. Osmotic mini-pumps are often used to continuously deliver drugs in our studies.
- Observation of somatic signs is conducted with an open-field. The scoring lasts 10-20 min (depend on study design), with both on-site scoring and video-taping. The following list of symptoms are scored :

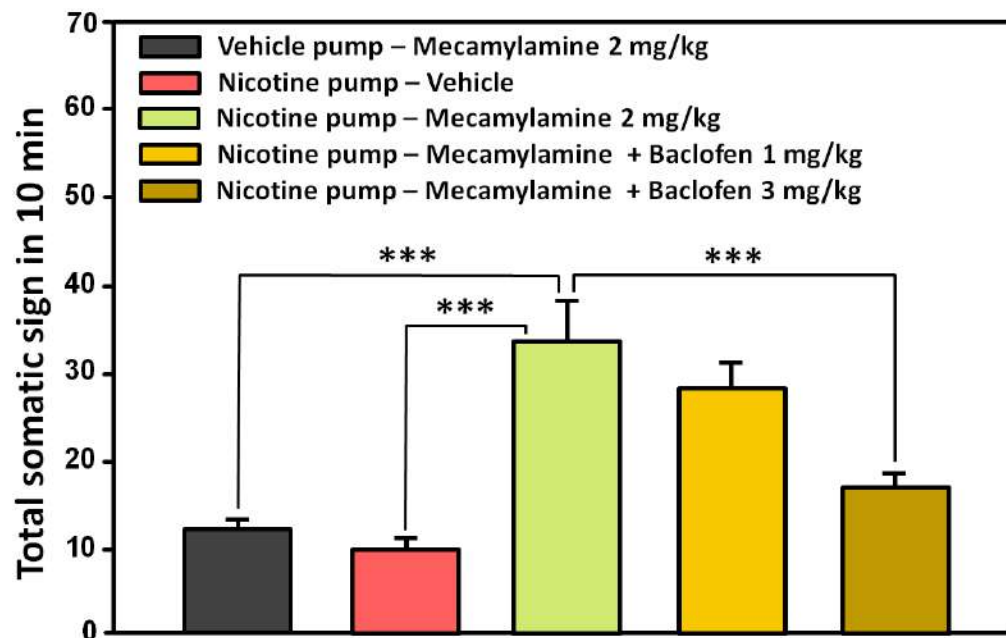
- 
- **Gasps**
 - **Writhes**
 - **Head-shakes**
 - **Body-shakes**
 - **Scratching**
 - **Yawning**
 - **Ptosis**
 - **Escape attempts**
 - **Chewing**
 - **Teeth chattering**
 - **Genital licks**
 - **Paw-lift**
 - **Diarrhea**
 - **Eye-blinks**

Spontaneous Withdrawal



24 hr after removal of nicotine mini-pump,, somatic signs significantly increased. Both nicotine 0.4 mg /kg and varenicline 1.7 mg/kg treatment decreased these symptoms.

Precipitated Withdrawal



Precipitated withdrawal usually happens when an antagonist is administered when the abused drug is still on board. Mecamylamine increased withdrawal symptoms in nicotine-treated rats. Baclofen significantly decreased mecamylamine's effects.