SENSITIZATION OF LOCOMOTOR ACTIVITY IN THE MOUSE AS A POTENTIAL INDICATOR OF ABUSE LIABILITY

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Introduction

- Repeated exposure to psychostimulants, such as cocaine, produces behavioral sensitization which is characterized by augmented locomotor response to repeated stimulant challenge.
- Sensitization has been proposed to play a critical role in the development and maintenance of drug addiction (Robinson and Berridge, 1993).
- The present study determined whether locomotor sensitization in the mouse was a good predictor of abuse liability in humans.

Hypothesis

- Drugs that are abused in humans will show locomotor sensitization in the mouse model.

Sensitization Definition

- Increase in locomotor activity following successive injections of the drug and/or increased sensitivity to a challenge dose following a washout period.

General Methods

Animals

- Male C57BL/6J or Balb/cJ mice from Jackson Laboratory (Bar Harbor, ME) were housed in groups of four and maintained on a 12hr/12hr light/dark cycle. Room temperature was maintained between 20 and 23ºC with a relative humidity between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. All procedures were approved by PsychoGenics' Institutional Animal Care and Use Committee.

Locomotor Activity

- Locomotor activity was measured in Plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St. Albans, VT) surrounded by infrared photobeam sources. Distance traveled (cm) was measured as the index for activity. Mice were injected with vehicle or the appropriate test compound and placed in the chambers for a 30 min session.

Forced Swim Test

- Balb/cJ male mice were individually placed into clear glass cylinders (15 cm tall x 10 cm wide, 1 L beakers) containing 23±1ºC water 12 cm deep (approximately 800 mL). The time the animal spent immobile was recorded over a 6 min trial. Immobility was defined as the absence of all movement except those required by the mouse to keep its head above the water.

Statistical Analysis

- Locomotor activity data were analyzed using analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test when appropriate. An effect was considered significant if $p<0.05$.

Table 1. Compounds Tested

Compound	Strain	Chronic Dose (mg/kg)	Challenge Dose (mg/kg)	Route of Administration	Vehicle	Ki (nM) DAT	NET	SERT	Reference
Cocaine	C57BL/6J	15	10	i.p.	water	275	129	177	Kozikowski et al., 2003
	Balb/cJ	15	25	i.p.	water				
Bupropion	C57BL/6J	10&20	10	i.p.	water	570	1,400	19,000	Sanchez and Hyttel, 1999
Modafinil	C57BL/6J	75&150	75	p.o.	0.5% arabic gum	3,800	>10,000	-	Nishino et al., 1998
DOV 216,303	C57BL/6J	30&75	50	i.p.	water	78	20	14	Skolnick et al., 2003
JZAD-IV-22	C57BL/6J	30&60	30	i.p.	water	14	11	13	In house data

Table 2. Locomotor Sensitization Design

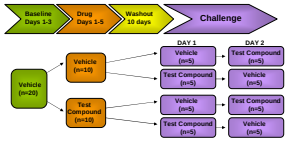
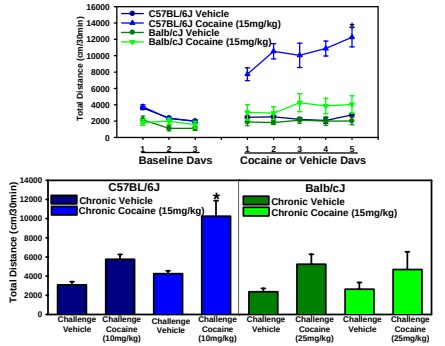
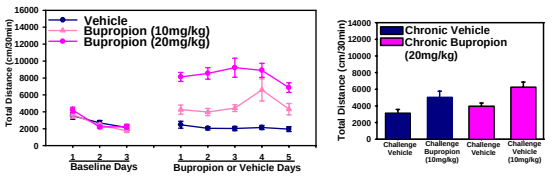


Figure 1: Cocaine produced sensitization in C57BL/6J but not Balb/cJ mice



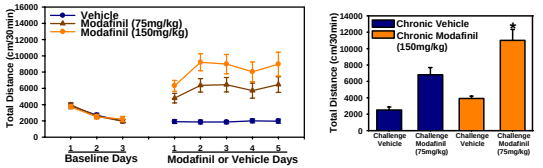
C57BL/6J or Balb/cJ mice were administered vehicle for baseline days 1-3 and either vehicle or cocaine (15mg/kg) for test days 1-5 and activity was measured for 30 min (top figure). Following a 10 day washout, mice that received chronic vehicle or cocaine were challenged with either vehicle or cocaine (10mg/kg for C57BL/6J and 25 mg/kg for Balb/cJ, i.p.) and locomotor activity was measured for 30 min (bottom figure).
Data are mean ±SEM (n=10/group).
**p<0.05 vs. Day 1 Cocaine (top figure)*
**p<0.05 vs. chronic vehicle, challenge cocaine (bottom figure)*

Figure 2: Bupropion, an antidepressant DAT/NET inhibitor, did not produce sensitization



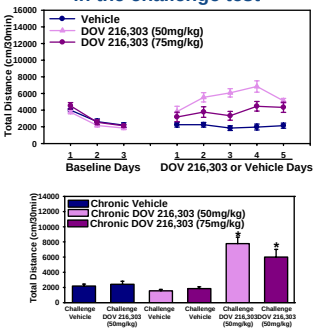
C57BL/6J mice were administered vehicle for baseline days 1-3 and either vehicle or bupropion (10 or 20mg/kg, i.p.) for test days 1-5 and activity was measured for 30 min (left figure). Following a 10 day washout, mice that received chronic vehicle or 20mg/kg of bupropion were challenged with either vehicle or 10mg/kg of bupropion and locomotor activity was measured for 30 min (right figure).
Data are mean ±SEM (n=7-9/group).

Figure 3: Modafinil, a wake-promoting DAT inhibitor, produced sensitization in the challenge test



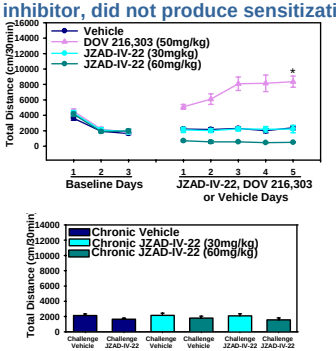
C57BL/6J mice were administered vehicle for baseline days 1-3 and either vehicle or modafinil (75 or 150mg/kg, p.o.) for test days 1-5 and activity was measured for 30 min (left figure). Following a 10 day washout, mice that received chronic vehicle or 150 mg/kg of modafinil were challenged with either vehicle or 75mg/kg of modafinil and locomotor activity was measured for 30 min (right figure).
Data are mean ±SEM (n=12-14/group).
**p<0.05 vs. chronic vehicle, challenge modafinil*

Figure 4: DOV 216,303, the antidepressant DAT/NET/SERT inhibitor, produced sensitization in the challenge test



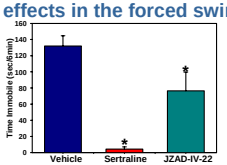
C57BL/6J mice were administered vehicle for baseline days 1-3 and either vehicle or DOV 216,303 (50 or 75mg/kg, i.p.) for test days 1-5 and activity was measured for 30 min (top figure). Following a 10 day washout, mice that received chronic vehicle or DOV 216,303 (50 or 75mg/kg) were challenged with either vehicle or 50mg/kg of DOV 216,303 and locomotor activity was measured for 30 min (bottom figure).
Data are mean ±SEM (n=9-10/group).
**p<0.05 vs. chronic vehicle, challenge DOV 216,303*

Figure 5: JZAD-IV-22, a DAT/NET/SERT inhibitor, did not produce sensitization



C57BL/6J mice were administered vehicle for baseline days 1-3 and either vehicle, DOV 216,303 (50mg/kg) or JZAD-IV-22 (30 or 60mg/kg, i.p.) for test days 1-5 and activity was measured for 30 min (top figure). Following a 10 day washout, mice that received chronic vehicle or JZAD-IV-22 (30 or 60mg/kg) were challenged with either vehicle or 30mg/kg of JZAD-IV-22 and locomotor activity was measured for 30 min (bottom figure).
Data are mean ±SEM (n=9-10/group).
**p<0.05 vs. Day 1 DOV 216,303*

Figure 6: JZAD-IV-22 produced antidepressant-like effects in the forced swim test



Mice received a 30 min pretreatment with either 10% DMSO vehicle, sertraline (20mg/kg) or JZAD-IV-22 (30mg/kg) and time immobile was measured in forced swim for 6 min.
Data are mean ±SEM. (n=10/group).
**p<0.05 vs. Vehicle*

Summary

- C57BL/6J, but not Balb/cJ mice, showed locomotor sensitization to cocaine (15mg/kg).
- Bupropion, an antidepressant DAT/NET inhibitor, produced locomotor hyperactivity, but not locomotor sensitization in mice. Bupropion shows no evidence of abuse liability in humans.
- Modafinil, a wake-promoting weak DAT inhibitor, showed locomotor sensitization in the challenge test. Although modafinil is a schedule IV medication under the Controlled Substances Act, post-marketing evidence suggests that modafinil has limited potential for large-scale abuse. Modafinil's limited abuse liability, however, may be related to its poor chemical properties (eg. poor solubility and degradation when heated).
- DOV 216,303, a DAT/NET/SERT inhibitor that showed efficacy in a Phase II clinical trial for depression, produced locomotor sensitization.
- JZAD-IV-22, a DAT/NET/SERT inhibitor, that was efficacious in the mouse forced swim, did not produce acute locomotor activation or locomotor sensitization.

Conclusions and Future Directions

The locomotor sensitization assay:

- shows some predicative validity in assessing abuse liability in humans.
- can be utilized to screen therapeutic compounds with a dopaminergic component for abuse liability.
- suggests that PsychoGenics' "triple" reuptake inhibitors such as JZAD-IV-22 may have less potential for abuse in humans than DOV 216,303.
- Additional compounds, both with and without abuse liability in humans, will be screened to further validate the mouse locomotor sensitization assay.

References

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