# Evaluation of novel non-opioid, non-addictive pain therapeutics within the NIH HEAL Initiative PSPP program: a case study

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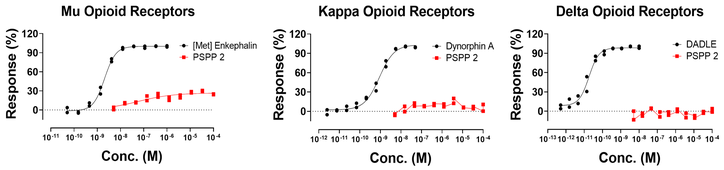
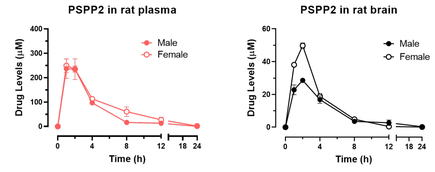
## Abstract

The National Institute of Neurologic Disorders and Stroke (NINDS) Preclinical Screening Platform for Pain (PSPP), a program within the NIH Helping to End Addiction Long-term℠, or NIH HEAL Initiative℠, aims to accelerate the development of novel non-opioid, non-addictive therapeutics for pain. To support the PSPP goals, PsychoGenics Inc. was awarded a contract to screen and profile these novel therapeutics and to validate new endpoints and models. PSPP employs a tiered approach to evaluation of assets. In Tier 1, assets are screened in cell-based functional assays to assess activity at opioid receptors and other receptors associated with abuse liability. Also, in Tier 1, the pharmacokinetic (PK) profile of the asset in both plasma and brain is determined. In Tier 2, a side effect profile is assessed using an accelerating rotarod and modified Irwin test. Subsequently, assets are evaluated using evoked and non-evoked pain endpoints in two pain models: 1) the plantar incision model, representative of acute to sub-chronic pain mechanisms and 2) the L5/L6 spinal nerve ligation (SNL) model, representative of persistent pain mechanisms. Finally, in Tier 3, assets are evaluated *in vivo* for abuse liability and in disease specific pain models. This tiered approach to evaluation of assets will be illustrated using a representative example that has been screened in Tier 1 in the *in vitro* assays and PK, and has been profiled in Tier 2 on rotarod performance and in plantar incision and L5/L6 SNL models as well as in the intravenous self-administration model in Tier 3, enabling further evaluation in disease specific pain models within Tier 3. Together, these data demonstrate the merits of evaluating promising pain assets rigorously in a tiered approach and highlight efforts to enhance novelty and reproducibility within the NINDS PSPP program to support the goal of identifying novel non-opioid, non-addictive pain therapeutics.

## *In vitro screens* and pharmacokinetic study

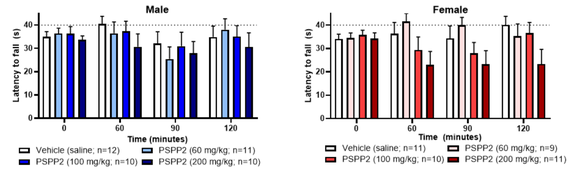



PSPP 2 was tested at concentrations between 5nM and 100 µM and did not show appreciable agonist, antagonist, or positive allosteric modulatory activity across the range of targets tested. Of interest, PSPP 2 did not show agonist activity at mu, kappa, or delta opioid receptors.



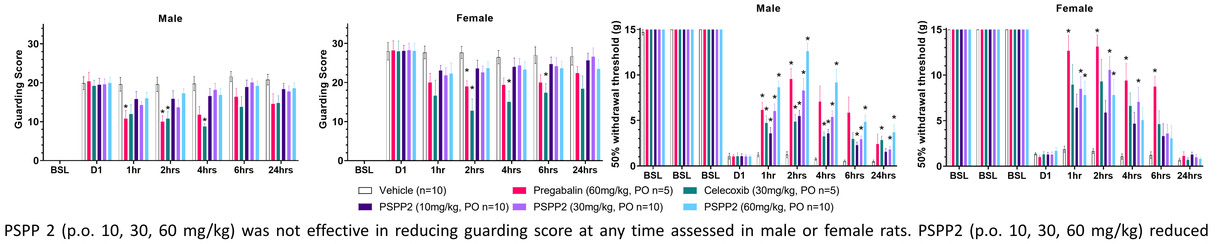

PSPP 2 (p.o., 60 mg/kg) showed high levels of exposure in plasma and brain with maximal levels achieved at 2 hours in both male (n=3) and female (n=3) rats. The plasma half-life was around 2.5-3 hours and brain half-life ranged from 2.4-4 hours.

## Rotarod test



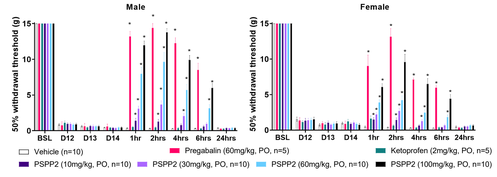

Male and female SD rats (n=9-12/group) were examined on the rotarod after p.o. dosing of PSPP 2 (60, 100, or 200 mg/kg). In female animals, there was a significant main effect of dose (2-way RM ANOVA).

## Plantar incision




PSPP 2 (p.o. 10, 30, 60 mg/kg) was not effective in reducing guarding score at any time assessed in male or female rats. PSPP2 (p.o. 10, 30, 60 mg/kg) reduced mechanical allodynia at 1, 2, 4, and 6 hours after administration in male animals. In female rats, PSPP 2 (p.o. 30 and 60 mg/kg) reduced mechanical allodynia at 1 and 2 hours. The 30 mg/kg dose was also effective at 4hrs. * $p$<0.05 relative to vehicle at each time by 2-way RM ANOVA with Dunnett's multiple comparison

## L5/L6 spinal nerve ligation (SNL)




PSPP 2 was evaluated for efficacy in the spinal nerve ligation model of chronic pain following p.o. dosing in male and female SD rats. PSPP2 and the positive comparator pregabalin reversed tactile hypersensitivity, while the negative comparator ketoprofen did not produce effects. * $p$<0.05 relative to vehicle at each time point by 2-way RM ANOVA with Dunnett's multiple comparison.

## Methods

### *In vitro* and *in vivo* profiling of PSPP 2

***In vitro* screen:** PSPP 2 was evaluated across a range of concentrations in functional assays to detect activity at opioid receptors and targets associated with abuse liability (Eurofins DiscoverX and Eurofins Panlabs).

**Pharmacokinetics:** PSPP 2 was dosed orally (p.o.) in male and female SD rats for serial plasma collections. Separate cohorts of animals were used for evaluation of brain exposures.

**Rotarod test:** PSPP 2 was dosed orally (p.o.) in male and female SD rats and animals were evaluated on an accelerating rotarod. The rotarod accelerated from 0-17 RPM over 5 seconds and was then maintained at 17 RPM for an additional 40 seconds. Latency to fall (seconds) was recorded.

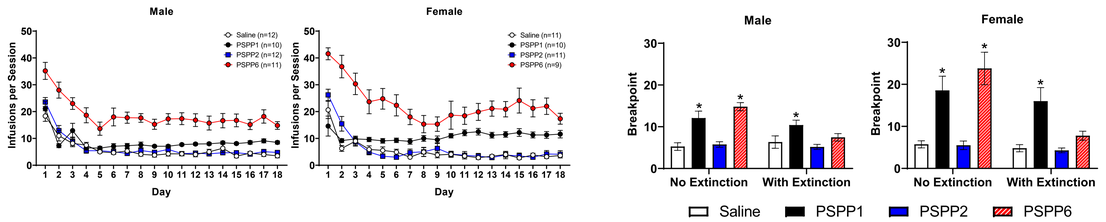
**Spinal nerve ligation (L5/L6) model:** Male and female SD rats received tight ligation of the L5 and L6 spinal nerves. Animals were tested 14 days post-op for hind paw hypersensitivity, and effects of PSPP2 were determined following oral (p.o.) dosing. Paw withdrawal thresholds were determined with von Frey filaments using the "up-down" method (Chaplan et al. 1994 J. Neurosci Methods. 53(1):55-63).

**Plantar Incision model:** Male and female SD rats received a 1 cm incision in the plantar aspect of the hind paw. Animals were tested 1-day post-op for hind paw hypersensitivity and guarding score, and effects of PSPP2 were determined following oral (p.o.) dosing. Paw withdrawal thresholds were determined with von Frey filaments using the "up-down" method (Chaplan et al. 1994 J. Neurosci Methods. 53(1):55-63).

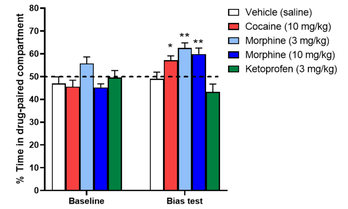
**Self-Administration:** Male and female SD rats were trained to lever press for food. Once they achieved stable responding, animals were switched to self-administer one of 3 different compounds. After acquisition, break point was assessed prior to and following extinction.

**Conditioned place preference (CPP):** Conditioned place preference consisted of habituating male SD rats to an open field chamber with two compartments (Day 1), treating rats with either saline or drug on alternating days (Day 2-9), and placing them in the saline or drug compartment for 20 minutes. Bias test was performed on Day 10 in which the animals explored the open field chamber for 20 minutes and % Time in the drug-paired compartment was determined.

## Self-administration and conditioned place preference (CPP)




Male and female SD rat did not acquire self-administration of PSPP 2 (3.3 mg/kg/infusion). The PSPP 2 curve overlaps with the saline acquisition curve. In contrast, PSPP 1 (0.5 mg/kg/infusion) and PSPP 6 (0.012 mg/kg/infusion) were readily self-administered. Male and female rats were also assessed on a progressive ratio schedule without extinction (i.e. the day after acquisition ended) and with extinction (i.e. 4 days of receiving saline only for a lever press). PSPP 2 breakpoints were not different from the saline control group. * $p$ < .05 relative to vehicle by 2-way ANOVA with Dunnett multiple comparison test.




A conditioned place preference (CPP) paradigm has been developed as an alternative abuse liability test for compounds that cannot be administered intravenously. Increased time in the drug-paired compartment was observed for male rats treated with morphine and cocaine, but not with the NSAID ketoprofen. * $p$<0.05, **p<0.01 vs vehicle, Dunnett's test

## Summary

- Within PSPP assets are evaluated in a tiered manner, as illustrated by the example of PSPP 2.
  - PSPP 2 was tested at concentrations between 5nM and 100 µM and did not show appreciable agonist, antagonist, or positive allosteric modulatory activity across the range of targets tested.
  - Based on PK, for behavioral assays, a pretreatment time of 1 hour is recommended.
  - Higher doses of PSPP 2 (100 and 200 mg/kg) produce deficits on rotarod performance.
  - PSPP 2 transiently reduced mechanical allodynia in the plantar incision model but did not affect guarding score.
  - In the L5/L6 SNL model, PSPP 2 dose-dependently reduced mechanical allodynia when administered 14 days after injury.
  - Rats did not acquire intravenous self-administration of PSPP 2, indicating a low abuse potential at the concentration assessed.
  - For assets that cannot be formulated for IV delivery, abuse liability can be assessed using the CPP paradigm.
- These data are illustrative of the multi-pronged approach in evaluating assets within the PSPP program at PsychoGenics, Inc.

## Conclusion

Under the NIH HEAL Initiative, PSPP aims to accelerate the development of non-opioid, non-addictive pain therapeutics including small molecules, biologics, natural products, and devices.

## PSPP Participation and Eligibility

PSPP is currently accepting assets for evaluation continuously, on an ongoing basis.

For eligibility and participation inquiries, contact:

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Dr. Sarah Woller, Scientific Project Manager,
sarah.woller@nih.gov

For more information about PSPP, visit:
https://heal.nih.gov/research/preclinical-translational/screening-platform

Or scan here: