In vivo PK, side effect profile, and efficacy of multiple clinically used compounds in female and male rats

Elizabeth A. Dugan[2] , David Budac[2], Briana Stanfield[2], Mark Urban[2], Vicki E. Brings[1], Sarah A. Woller[1], Smriti Iyengar[1], Taleen Hanania[2], Mark A. Varney[2]

[1]Division of Translational Research, National Institutes of Neurological Disorders and Stroke, National Institutes of Health, Rockville, MD 20852

[2]PsychoGenics Inc., Paramus, NJ, 07652

Background

In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated clinically used compounds, including celecoxib, carbamazepine, and diazepam through the tiered approach established to profile potential novel analgesics. First, pharmacokinetic studies were conducted to guide dosing, select the route of administration, and to determine the time course, supporting subsequent behavioral studies. Next, the modified Irwin and rotarod tests were conducted to evaluate potential neurologic, physiologic, and fine motor effects that may impact outcome measures in the pain models. Following side effect profile assessment, efficacy was evaluated in the plantar incision and L5/L6 spinal nerve ligation (SNL) models. The rat plantar incisional pain model is an established model of acute post-operative pain induced by incision of the skin and the plantaris muscle (Brennan et al. 1996). The model is characterized by transient hind paw tactile allodynia and spontaneous guarding behaviors. SNL is a model of peripheral neuropathic pain resulting from chronic nerve compression in which tactile and cold allodynia are produced (Kim and Chung, 1992).

Methods

Pharmacokinetics: Compounds were administered in male and female SD rats (n=4/group/sex) for serial plasma collections. Separate cohorts of animals were used for evaluation of brain exposures.

Irwin: The modified Irwin test (Irwin 1968, Mathiasen and Moser, 2018) uses a battery of 39 observational assessments to evaluate neurologic and physiologic effects of a test article in male and female rats (n=4/group/sex).

Rotarod test: Compounds were administered in male and female SD rats (n=10/group/sex) 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.

Plantar Incision model: Male and female SD rats received a 1 cm incision in the plantar aspect of the hind paw. Animals (n=10/group/sex) were tested 1-day post-op for hind paw hypersensitivity or guarding score, and effects of compounds were determined following dosing. Paw withdrawal thresholds (PWTs) and guarding scores were assessed in separate cohorts. PWTs were determined with von Frey filaments using the "up-down" method (Chaplan et al. 1994 J. Neurosci Methods. 53(1):55-63). A guarding score was recorded for each animal every 5 minutes for 60 minutes. The scores for each animal were added and a final score was recorded (max 39).

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 compounds were determined following 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). Acetone Evaporation Test on day 21 of SNL surgery: Acetone (~50 µl) was gently applying to the plantar surface of the hind paw and rats are observed for 20 seconds for withdrawal or no withdrawal response.

Rigor

- Sample size were determine based on power analysis.
- Experimenters were blinded to treatments.
- Inclusion/exclusion criteria were applied.
- Animals were randomly assigned to groups.
- Groups balanced by weight and post-injury response.

In vivo Pharmacokinetics

Compound Dose	Plasma Male / Female Drug Levels (µM)						Brain Male / Female Drug Levels (µM)	
Celecoxib	**.5 hour**	**1 hours**	**2 hours**	**4 hours**	**6 hours**	**24 hours**	**4 hours**	**24 hours**
30 mg/kg, PO	0.8 / 1.5	1.4 / 2.6	2.7 / 5.5	3.3 / 5.5	2.9 / 4.2	0.2 / 1.7	9.4 /10.9	.4 / 2.4
Diazepam	**.25 hours**	**.5 hours**	**1 hour**	**2 hours**	**4 hours**	**8 hours**	**.5 hours**	**8 hours**
10 mg/kg, PO	0.6/1.7	0.8/2.0	0.7/1.8	0.4/1.3	0.2/0.9	0.2/0.3	.33 / 1.1	.1 / .1

Carbamazepine levels are maintained through 6 hours post-administration

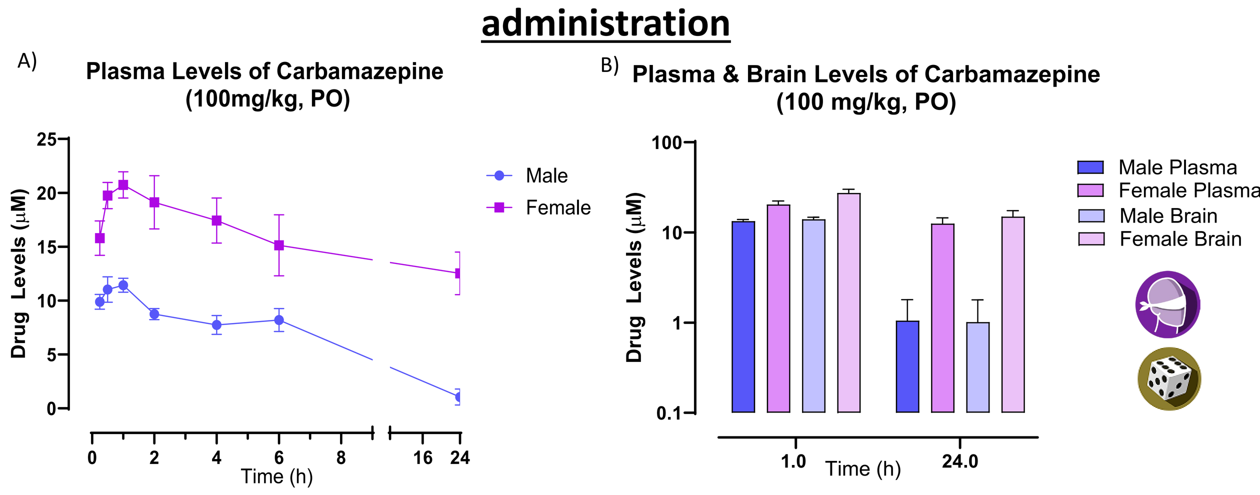


Figure 1: Data points are mean values ± SEM. A) Drug levels in plasma from male and female rats over 24 hours (n=3 for females at 24 hours). B) Drug levels in brain from male and female rats at 1 (n=3 rats per group) and 24-hours (n=4 males per group, n=3 females per group).

Side Effect Profile Assessment

Compound	Doses (mg/kg)	Irwin Observations	Rotarod - Latency to Fall
Celecoxib	3, 10, 30, and 100	No observable behaviors	No reduction in latency to fall
Diazepam	1, 3, 10, and 30	↓ body position, ↓ locomotor activity, sedation, and ↓ pupil size	Reduced at 1 (male) and 2 (male and female) hours

Carbamazepine produced decreased locomotion, ataxia, and increased abdominal tone in the Modified Irwin

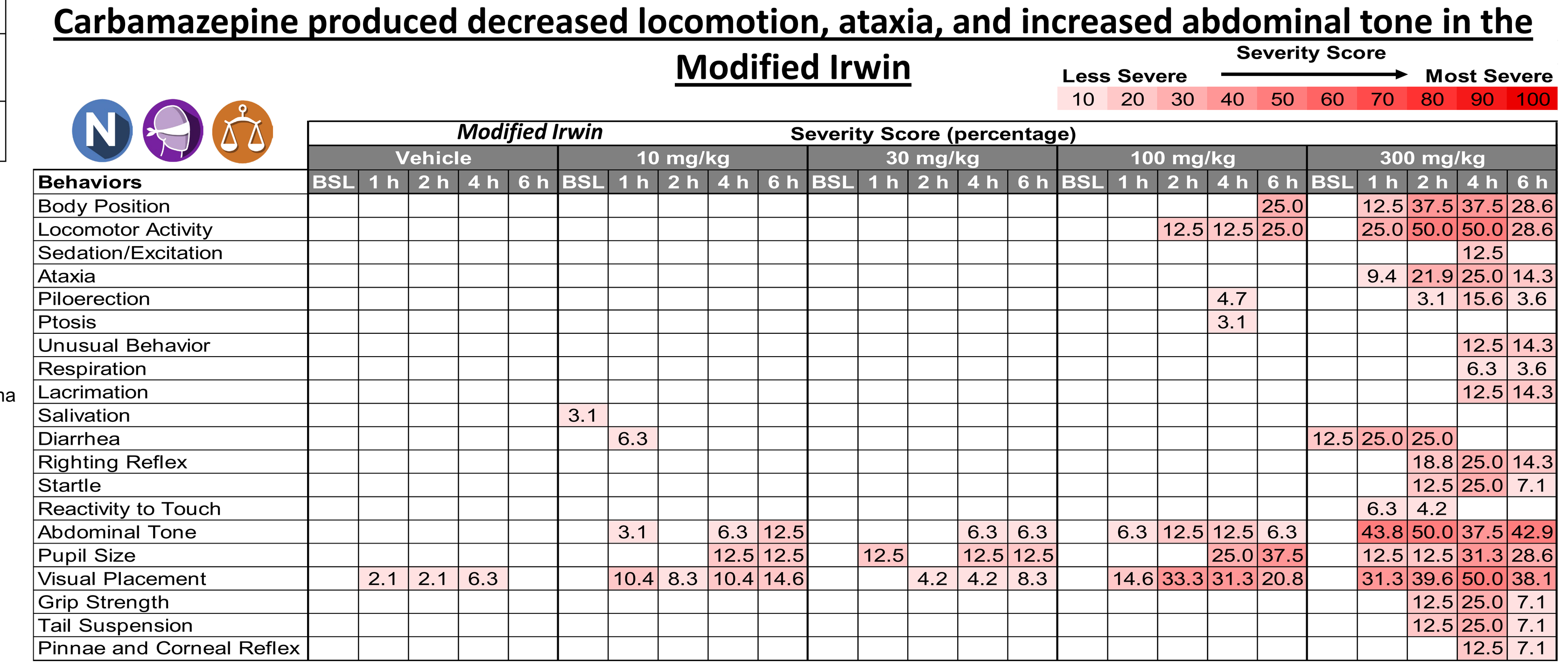


Figure 2: Heat map depicting the severity scores of the observed behaviors. Note: Severity Score = (Sum of Score Across Animals/Maximum Score) *100. Empty cells indicate that a particular behavior was not observed in the 8 animals at the indicated dose and timepoint (thus the severity score would be 0). This table does not indicate the direction of the change (e.g., increase / decrease in a behavior).

Carbamazepine did not affect rotarod performance

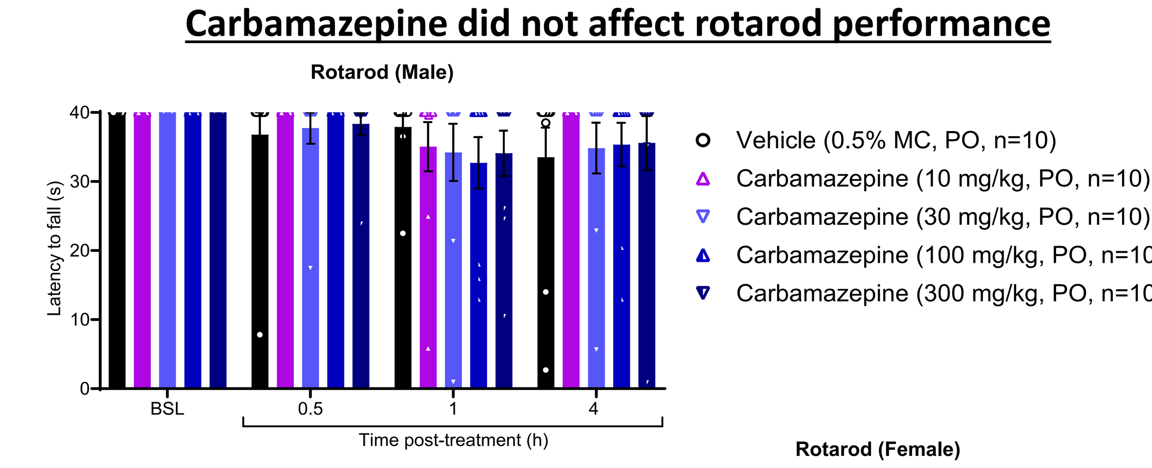
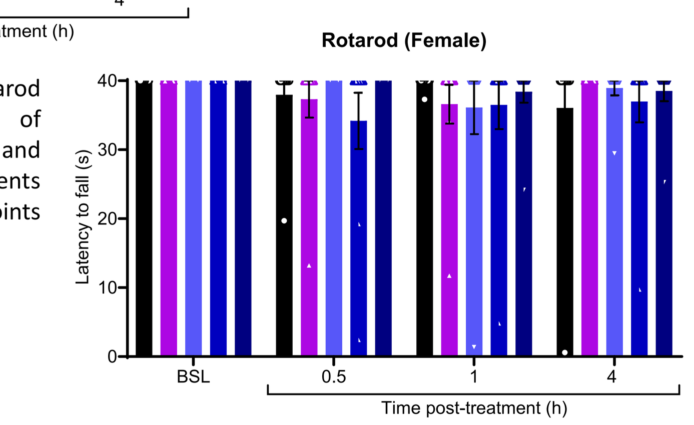


Figure 3. Latencies to fall off the rotarod apparatus following administration of carbamazepine for male (left, n=10) and female (right, n=10) rats. Data represents mean ± SEM, with individual data points superimposed.



Efficacy Assessment

Compound (mg/kg)	Plantar Incision Model								SNL Model					
	Paw Withdrawal Threshold Male/Female				Guarding Score Male/Female				Paw Withdrawal Threshold Male/Female				Acetone Evaporations Male/Female	
Celecoxib	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**3 hours**
3	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/**p<0.001**	p>0.05/**p<0.001**	p>0.05/**p<0.05**	p>0.05/**p>0.0001**	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
10	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/**p<0.01**	p>0.05/**p<0.05**	**p<0.05**/p>0.05	**p<0.05/p<0.05**	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
30	**p<0.01/p<0.05**	**p<0.05/**p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	**p<0.01/p<0.05**	**p<0.01/p<0.01**	**p<0.01/p<0.05**	**p<0.01/p<0.05**	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
Diazepam	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**1 hour**	**2 hours**
1	p>0.05/**p<0.05**	p>0.05/**p<0.05**	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
3	**p<0.05/**p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
10	**p<0.05/p<0.001**	**p<0.05/p<0.001**	**p<0.05/p<0.05**	p>0.05/p>0.05	p>0.05/**p<0.01**	p>0.05/**p<0.01**	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05

Carbamazepine reduced tactile allodynia and guarding behaviors in the plantar incision model

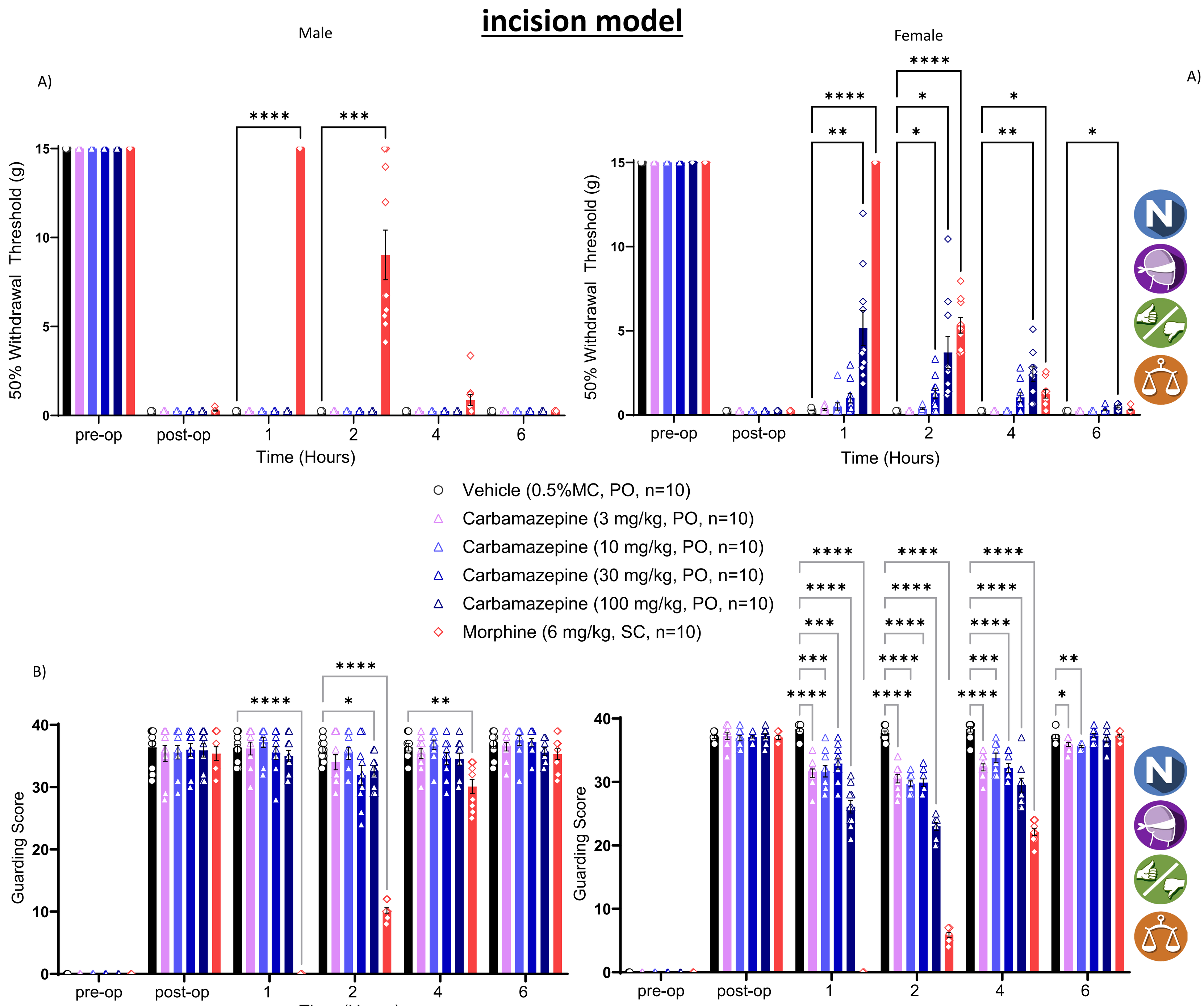


Figure 4: A) Paw withdrawal thresholds (PWTs) and B) Cumulative guarding score for males (left) and females (right) prior to and post-surgery, and post-treatment. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001 .

Carbamazepine did not reduce tactile and cold allodynia in the SNL model

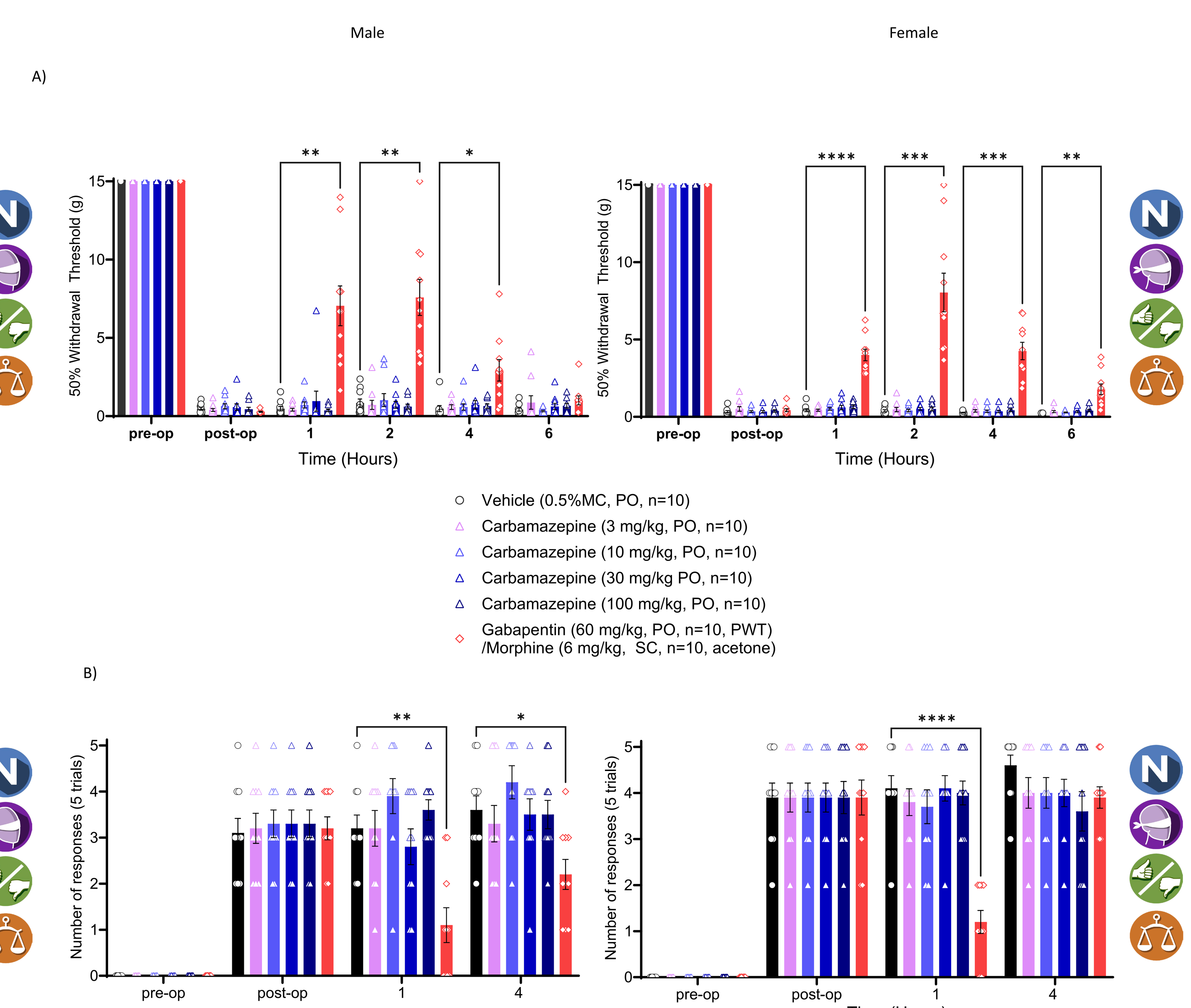


Figure 5.: A) PWT and B) Acetone response in SNL male (left) and female (right). Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

The results of these studies of clinically used standards demonstrate the validation of the models and endpoints within the PSPP program and highlight the goal of providing a robust platform to accelerate the discovery and preclinical development of non-opioid, non-addictive treatments for pain.

PSPP is currently accepting assets for evaluation

For eligibility and participation inquiries, contact:

Smriti Iyengar, Ph.D. smriti.iyengar@nih.gov

NIH National Institute of Neurological Disorders and Stroke

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