

Discovery Research

Efficacy and Safety Pharmacology



Discovery Research
Suven Life Sciences Ltd
Serene Chambers, Road-5, Avenue-7, Banjara Hills,
Hyderabad-500034, India.
Contacts: info@suven.com, nvsrk@suven.com

Overview



S. No	Title	Page No
1	Cognition	3 - 23
2	Psychosis	24 - 38
3	Anxiety	39 - 48
4	Depression	49 - 63
5	Pain	64 - 118
6	Parkinson	119 - 120
7	Others	121 - 122
8	Safety Pharmacology	123 - 124
9	Models under validation	125
10	Quality Assurance	126
11	Contacts	127

Rodent Models of cognition



Object recognition task

Morris water maze

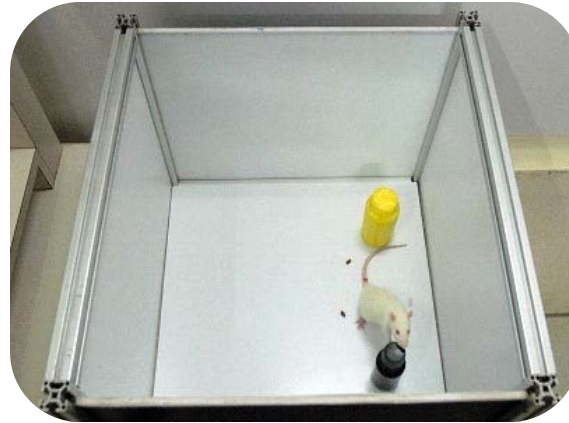
Radial arm maze

T-Maze

Social recognition task

Contextual fear conditioning

Cerebral blood flow



Novel object recognition task (Time induced memory deficit) –



Study Outline

Species: Rats

Strain: Wistar

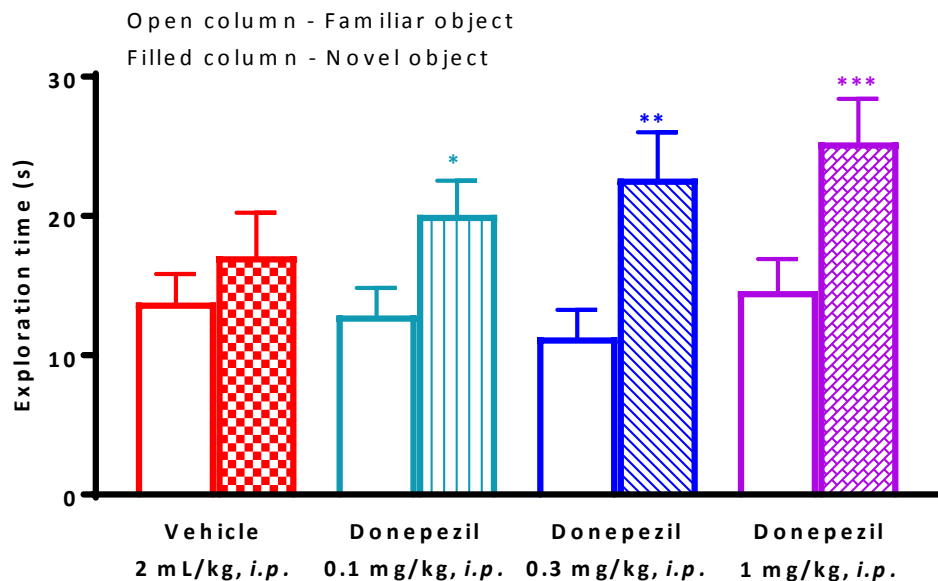
Experimental Procedure:

On day 1 the animals were habituated in the arena for 20 minutes. On day 2, animals were administered vehicle or test drug. Trial-1 is familiarization task, where the animals are allowed to explore arena containing 2 yellow bottles of same dimensions for a period of 3 minutes. On day 3, animals were administered vehicle or test drug. After an intertrial interval (ITI) of 24 hrs animals were subjected to Trial II. Trial-II is recognition task, where the animals are allowed to explore arena containing one yellow and one black bottle for a period of 3 minutes.

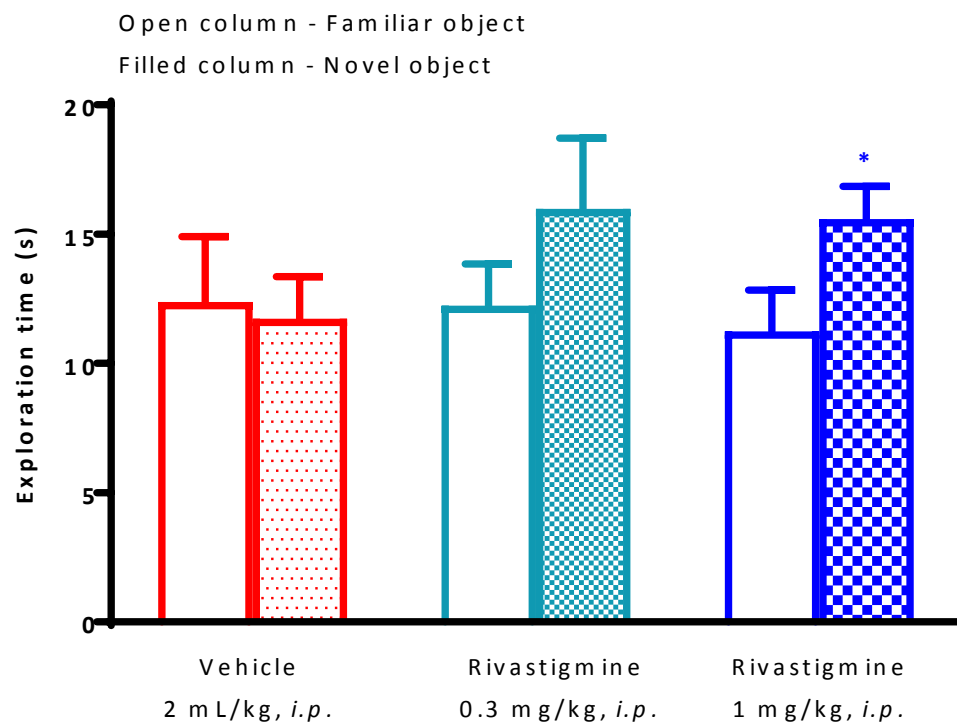
Novel object recognition task (Time induced memory deficit) –



Validation data



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs familiar object



Novel object recognition task (Scopolamine induced memory deficit) – Study Outline



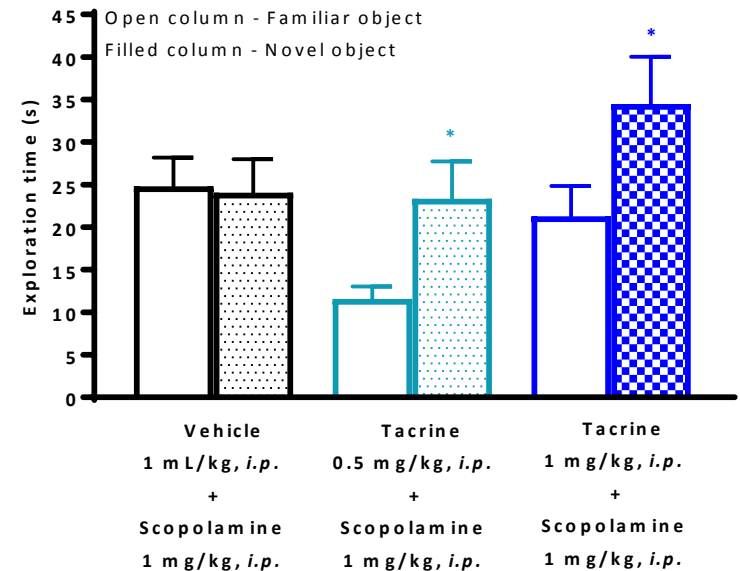
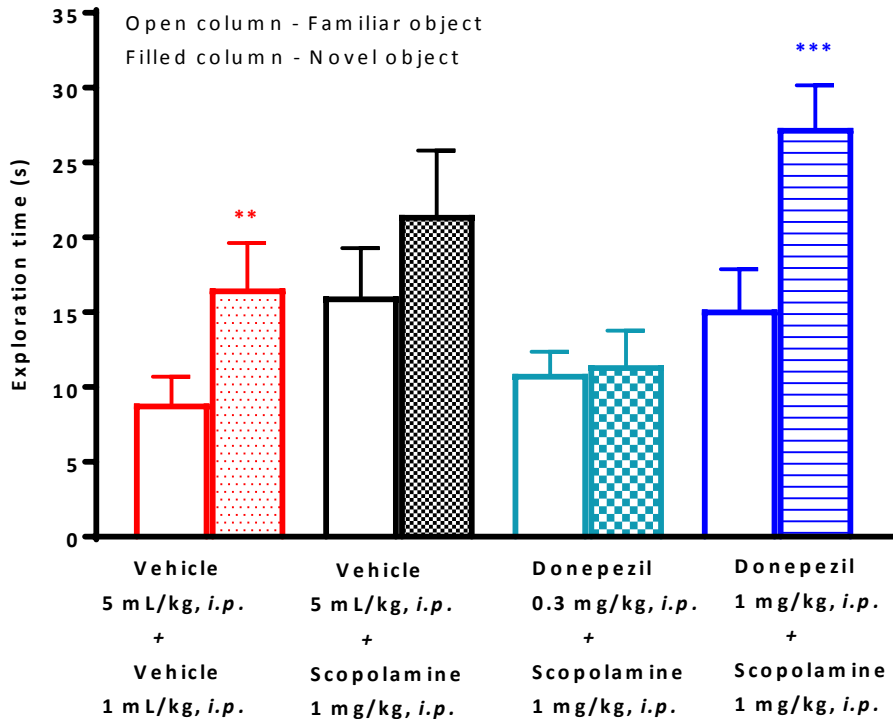
Species: Rats

Strain: Wistar

Experimental Procedure:

On day 1, the animals were habituated in the arena for 20 minutes. On day 2, animals were administered either vehicle or test compound. Scopolamine (1mg/kg, *i.p.*) was administered 20 min prior to the trial 1. Trial-1 is a familiarization task, where the animals are allowed to explore arena containing 2 yellow bottles of same dimensions for a period of 3 minutes. After an intertrial interval (ITI) of 3 min, animals were subjected to Trial II. Trial-II is recognition task, where the animals are allowed to explore arena containing one yellow and one black bottle for a period of 3 minutes.

Novel object recognition task (Scopolamine induced memory deficit) – Validation data



Data represent Mean \pm SEM. *** $p < 0.01$, ** $p < 0.01$, * $p < 0.05$ Vs familiar object

Novel object recognition task (Time induced memory deficit) –



Study Outline

Species: Mice

Strain: CD1

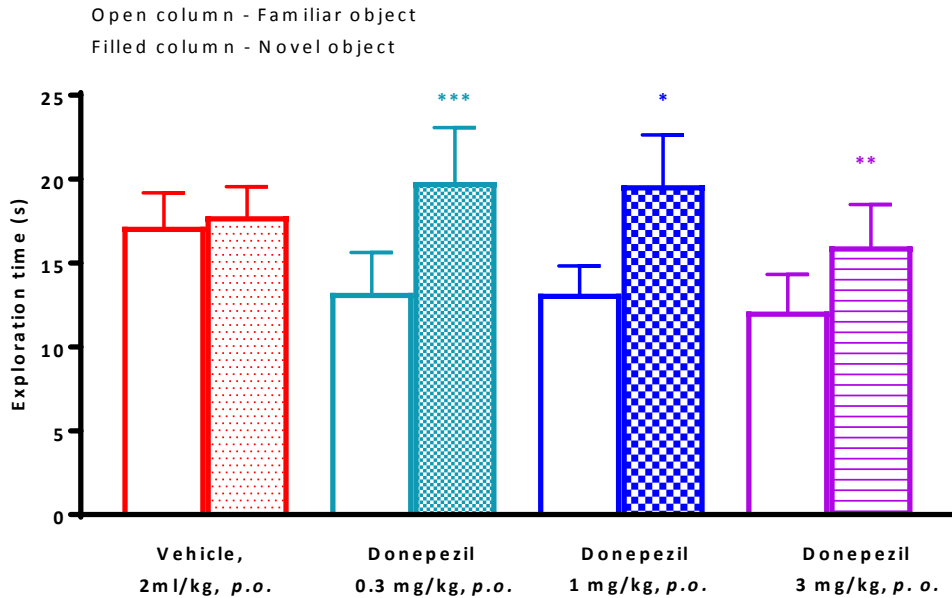
Experimental Procedure:

On day 1, animals were habituated to the arena for a period of 45 minutes. On day 2, animals were again habituated to the arena for a period of 10 minutes and returned to home cages. 60 min prior to trial-1, the animals were administered with vehicle or donepezil. Trial-1 is the familiarization task in the arena with 2 stainless steel flasks of same dimensions for a period of 3 minutes. On day 3, 60 min prior to trial-2, the animals were administered with vehicle or donepezil at dose levels of 0.3, 1 and 3 mg/kg, *p.o.* Trial-2 is the recognition task in the arena with one colorless steel flask and one red color steel flask for a period of 3 minutes.

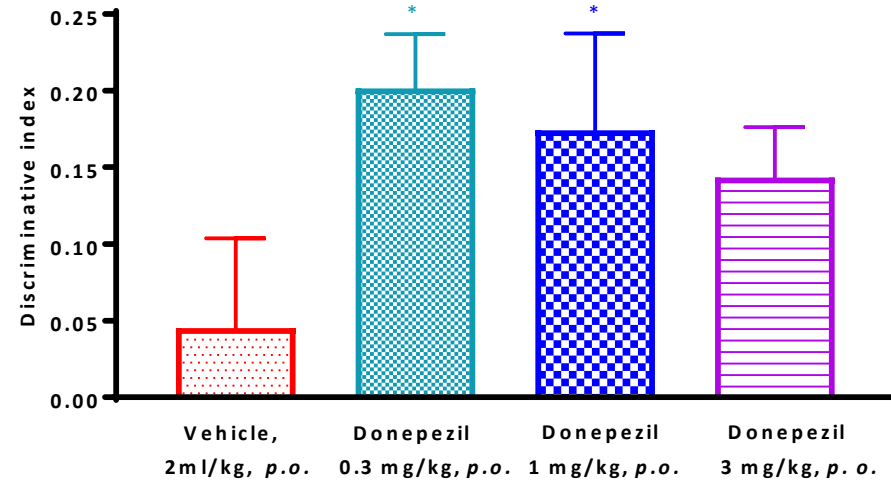
Novel object recognition task (Time induced memory deficit) –



Validation data



Data represents Mean \pm SEM. * p <0.05, ** p <0.01, *** p <0.001 Vs familiar object



Data represents Mean \pm SEM. * p <0.05 Vs vehicle

Morris water maze task (Scopolamine induced memory deficit) –



Study Outline

Species: Rats

Strain: Wistar

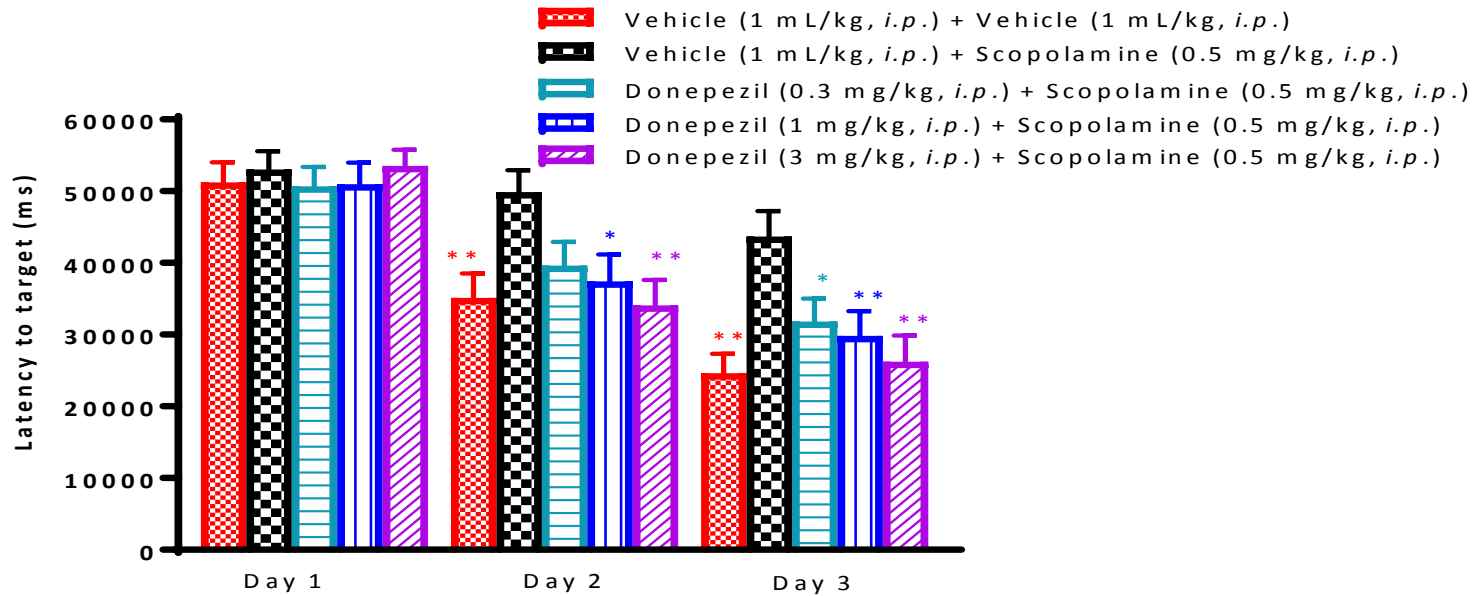
Experimental Procedure:

Water maze consists of a 1.8 m diameter; 0.6 m high circular water maze filled with water $24 \pm 2^\circ\text{C}$. A platform 16 cm diameter will be placed 1.0 cm below the water surface in the center of one of the four imaginary quadrants, which remain constant for all the rats. Rats were administered vehicle or test compound 60 min before acquisition training and half hour after administration of vehicle or test compounds, scopolamine was administered at a dose of 0.5 mg/kg. Rats were lowered gently, feet first into water. A rat was allowed to swim for 60 s to find the platform. If the platform was found during this time the trial was stopped and rat was allowed to stay on platform for 30 s before being removed from the maze. If the platform was not found during 60 s trial, then the rat was manually placed on the platform and allowed to stay on platform for 10 s before being removed from the maze. Each rat received 4 trials per day.

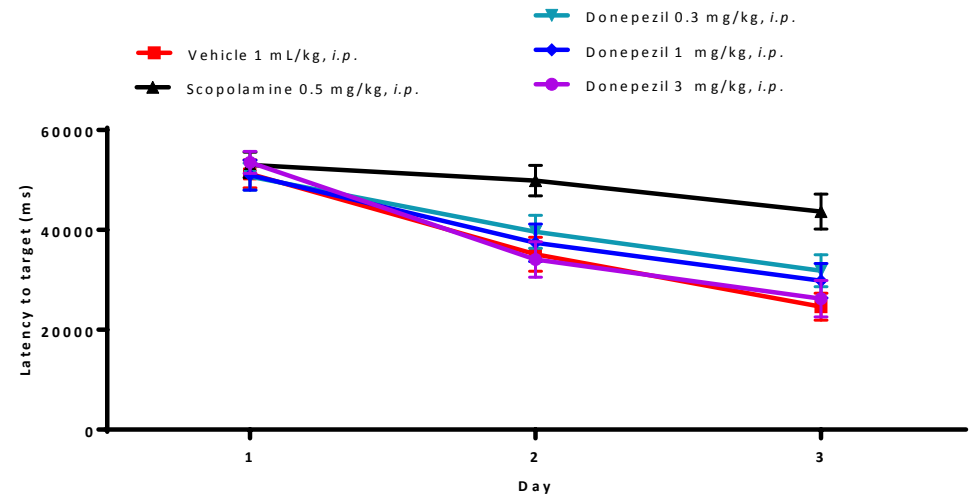
Morris water maze task (Scopolamine induced memory deficit) –



Validation data



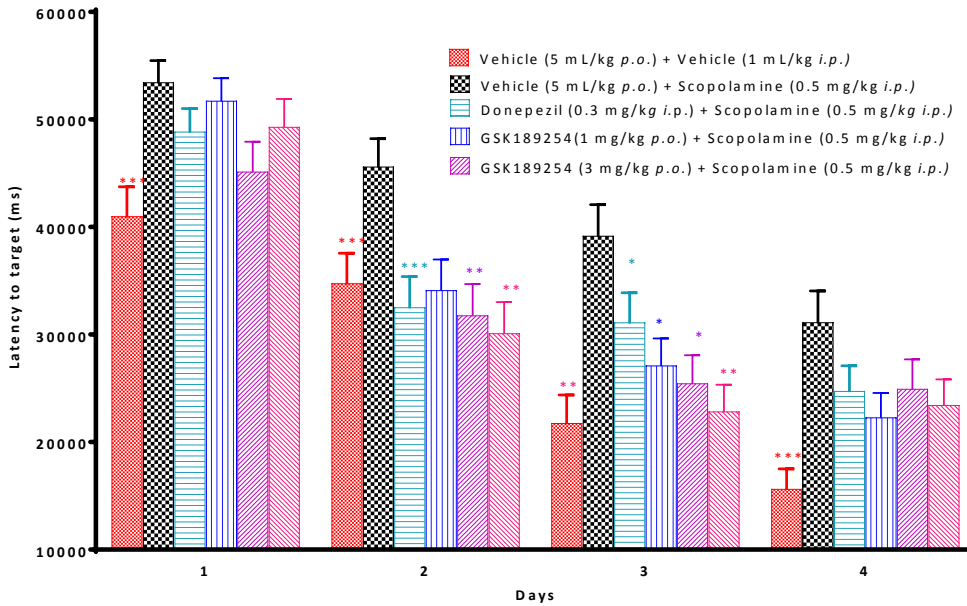
Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Scopolamine



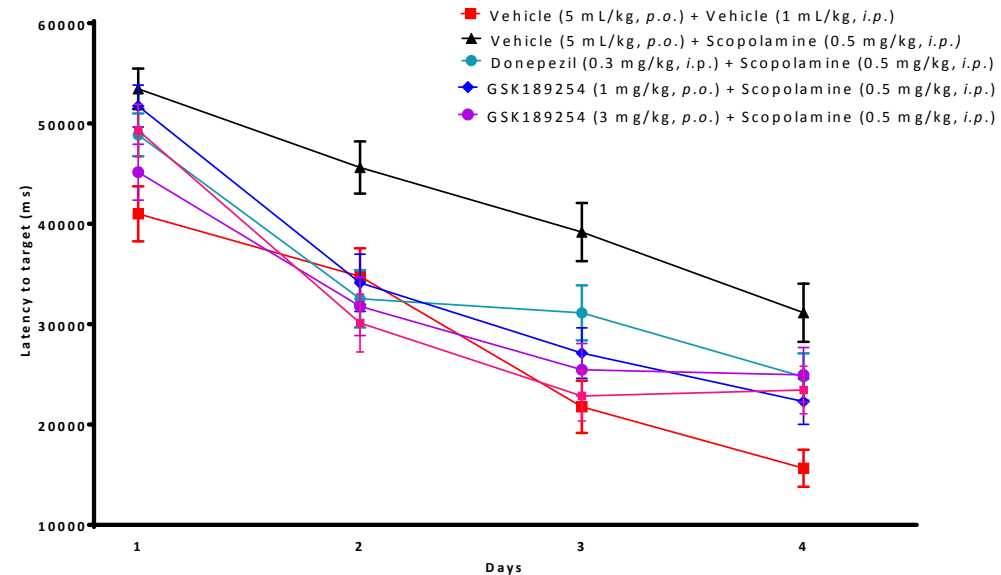
Morris water maze task (Scopolamine induced memory deficit) –



Validation data



Data represent Mean \pm SEM. *** p <0.001, ** p <0.01, * p <0.05 Vs scopolamine



Radial arm maze (Scopolamine induced memory deficit) – Study



Outline

Species: Rats

Strain: Wistar

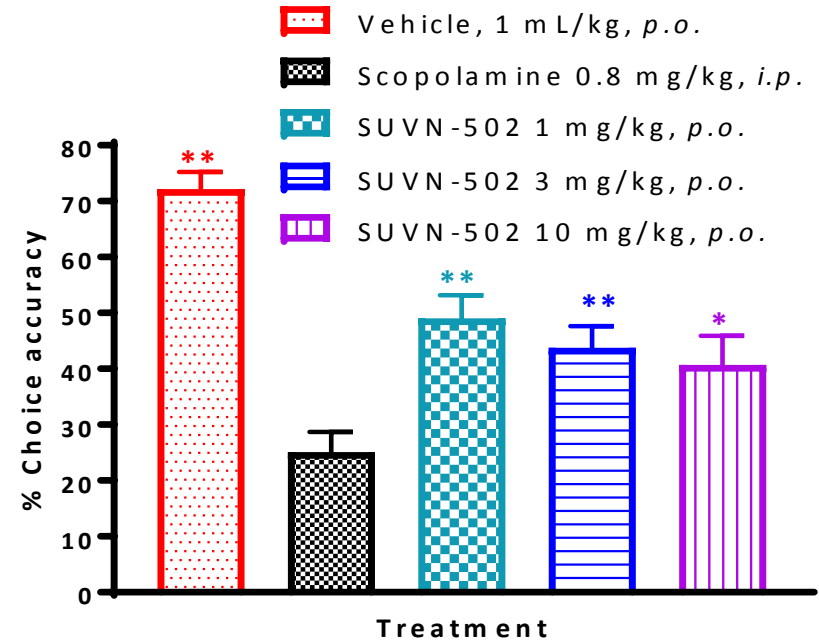
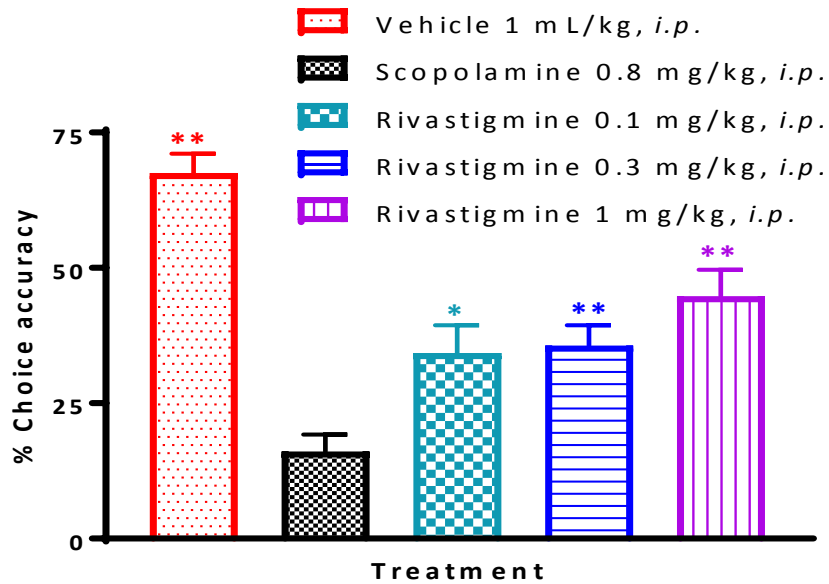
Experimental Procedure:

Radial arm maze test was conducted in an octagonal maze with eight radiating arms elevated to a height of 80 cm. Experiment was carried out for a period of 6 days. On day 1 the pellets was spread through out the maze and rats were habituated to the maze. On day 2 the rats were grouped and habituated to the radial arm maze for a period of 10 mins, with food placed near the food cups. From day 3 to day 6 the food was placed in the food cups only and each of the arms was baited only once at the beginning of the trial. Forty min after administration of test compound, scopolamine (0.8 mg/kg, *i.p*) was injected and after a period of 20 min the rats were subjected to the trial in the radial arm maze. The rat were individually introduced into the radial arm maze for duration of 10 mins or 16 arm entries or till all the pellets have been eaten.

Radial arm maze (Scopolamine induced memory deficit) – Validation



data



Data represent Mean ± SEM. **p<0.01, *p<0.05 Vs Scopolamine

SUVN-502 is a 5-HT₆ antagonist

T-maze (Scopolamine induced memory deficit) – Study Outline



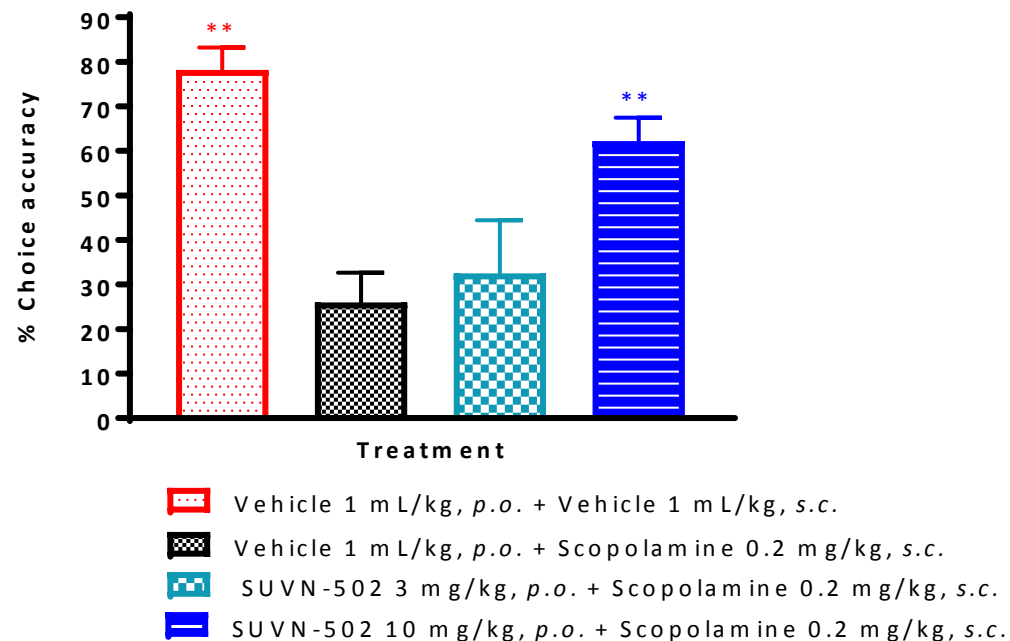
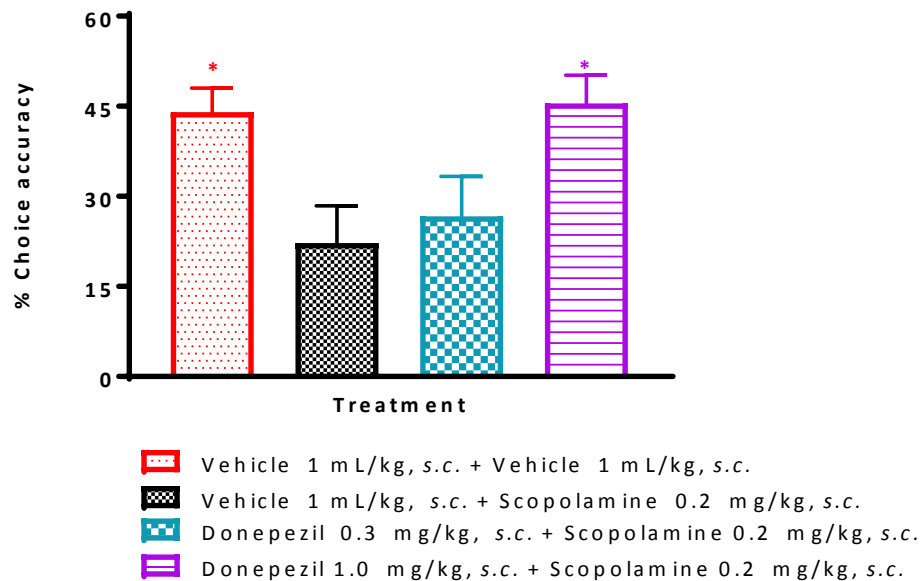
Species: Rats

Strain: Wistar

Experimental Procedure:

On day 1 the animals were habituated to the maze for 10 minutes by keeping experimental pellets in both arms of the T-Maze, except start arm. On day 2, the animals were treated with Vehicle or test compound and Scopolamine 0.2 mg/kg, s.c. was administered 30 min before the trial. Each animal was subjected to 6 trials initially one forced trial into baited arm. This was followed by 5 continuous choice trials. During the choice trial the pellet was placed in the arm which was not baited previously.

T-maze (Scopolamine induced memory deficit) – Validation data



Data represent Mean ± SEM. **p<0.01, *p<0.05 Vs Scopolamine

Social recognition task (Time induced memory deficit) –



Study Outline

Species: Rats

Strain: Wistar

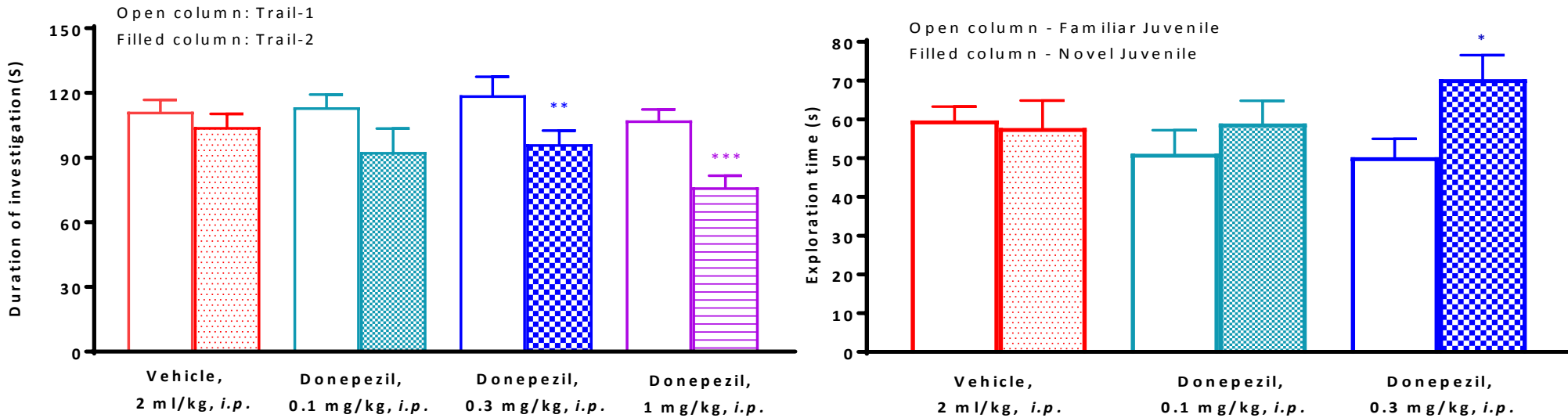
Experimental Procedure:

On experiment day 1 , all groups of animals were treated with vehicle or donepezil and habituated to their respective test arenas for a period of 30 min prior to trial-1. In trial-1, adult rats were presented with juvenile rats in their respective test arenas for a period of 5 minutes. Time spent by the adult rat socially investigating the juvenile was noted. On day 2 , after 24 h ITI, animals were treated with vehicle or donepezil and habituated to their respective test arenas for a period of 30 min prior to trial-2. In trial-2, adult rats were presented with respective juvenile (or familiar and novel juvenile) rats and in their respective test arenas for a period of 5 minutes. Time spent by the adult rats socially investigating the juvenile in trial -2 was noted. Ratio of investigation duration (RID) was calculated and compared among various groups.

Social recognition task (Time induced memory deficit) –



Validation data



Data represents Mean \pm SEM. *** p <0.001, ** p <0.01 vs. Trial 1 or Familiar juvenile

Contextual fear conditioning – Study Outline



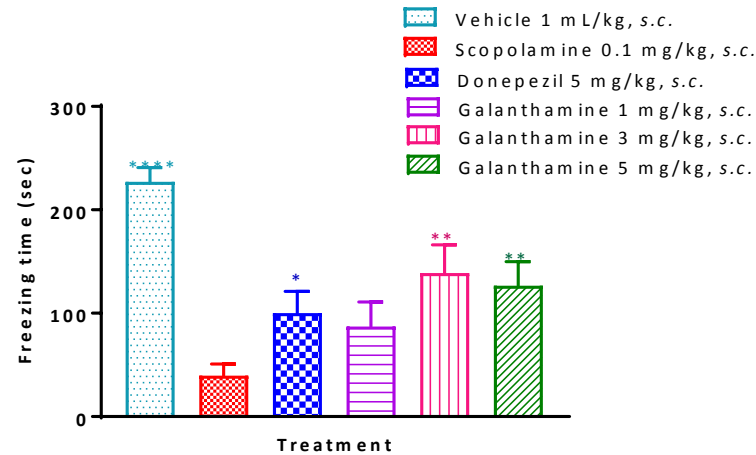
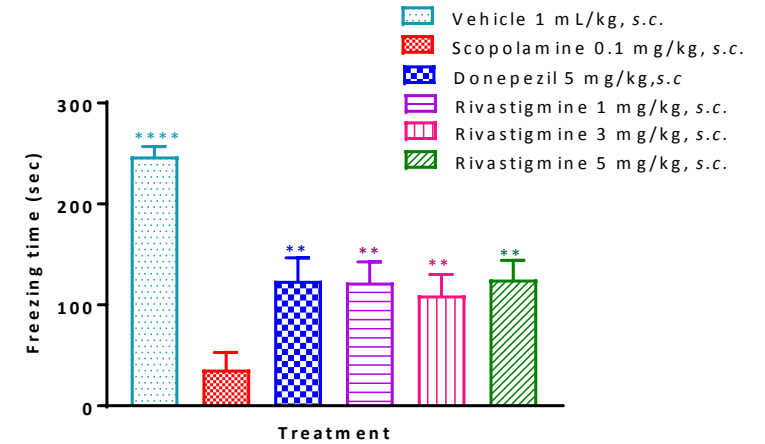
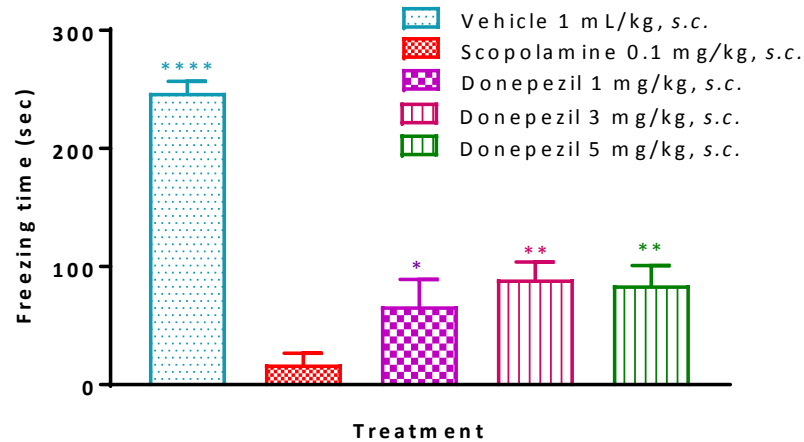
Species: Rats

Strain: Wistar

Experimental Procedure:

On day 1, rats were placed in the operant behavior chamber and allowed to acclimatize for 1 min. Rats received a conditioned stimulus (CS) (tone for 10 sec) followed by an unavoidable foot shock (unconditioned stimulus (US): electric shock of 0.7 mA for 1 sec). Following a 40 secs interval between each administration, tone and shock were repeated to deliver a total of six CS-US pairings. On day 2, animals were treated with Vehicle/Scopolamine and Vehicle/test drug. After post dose interval, animals were scored for the duration of freezing without shock stimulus. Shocking and scoring was controlled through freeze frame software.

Contextual fear conditioning – Validation data



Data represent Mean \pm SEM. ****P<0.0001, **P<0.01, *P<0.05 Vs Scopolamine

Cerebral blood flow – Study Outline



Species: Rats

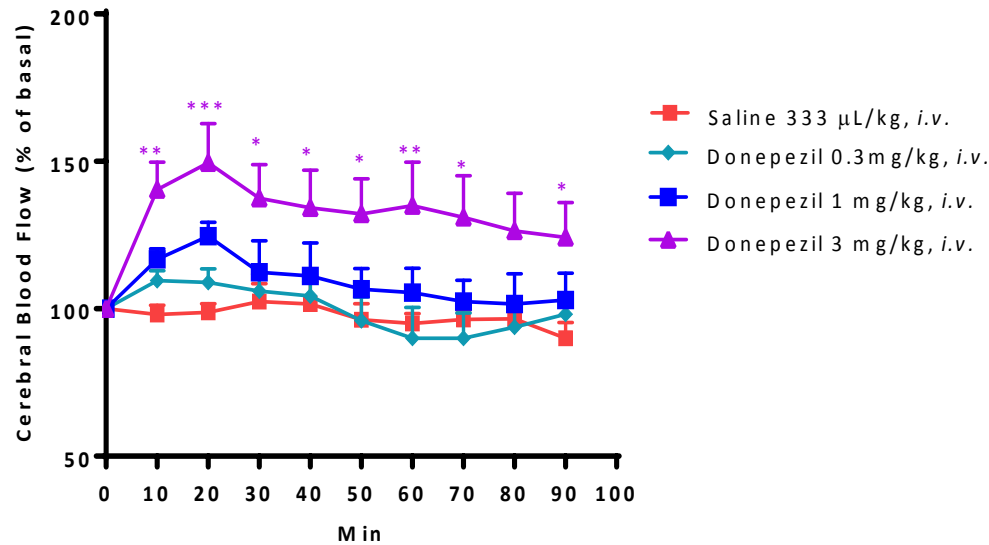
Strain: Wistar

Experimental Procedure:

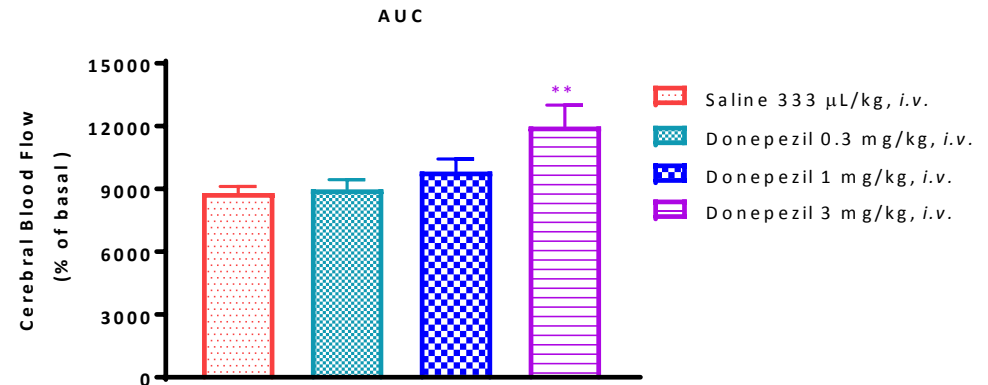
Cerebral blood flow (CBF) studies were conducted on wistar rats of 300-350gm. Rats were anesthetized with 12 % urethane. The animal's body core temperature was maintained at 37 °C via a heating pad and rectal temperature probe. The femoral vein on one side was cannulated with PE10 tubing for drug application. Then animals were placed into a stereotaxic frame and a midline incision was made to expose the skull. A burr hole was drilled over the frontal cortex (stereotaxic coordinates 1 mm anterior and 4 mm lateral to bregma) leaving the underlying dura intact. A Laser Doppler Probe (ADInstruments Inc.) was placed over the hole to monitor CBF. The Laser Doppler probe connected to a computerized data acquisition system (PowerLab, ADInstruments Inc.) for data acquisition. Recordings were started after CBF was stable for 30 min. Data is shown as percent increase relative to resting basal blood flow level.



Cerebral blood flow – Validation data



Data represent Mean \pm SEM. * $p < 0.05$ ** $p < 0.01$ Vs vehicle





Summary:

- **Donepezil and Rivastigmine** reversed the time induced episodic memory deficit in the novel object recognition task.
- **Donepezil and Tacrine** reversed the scopolamine induced episodic memory deficit in the novel object recognition task.
- **Donepezil and GSK189254** reversed the scopolamine induced spatial memory deficit in the water maze task.
- **Rivastigmine and SUVN-502** reversed the scopolamine induced working memory deficit in the radial arm maze task.
- **Donepezil and SUVN-502** reversed the scopolamine induced working memory deficit in the T-Maze task.
- **Donepezil** reversed time induced social recognition memory deficit in social recognition task.
- **Donepezil** increased the cerebral blood flow.
- **Donepezil, Rivastigmine and Galantamine** reversed the scopolamine induced memory deficit in the fear conditioning task.

Rodent Models of Psychosis



Prepulse inhibition

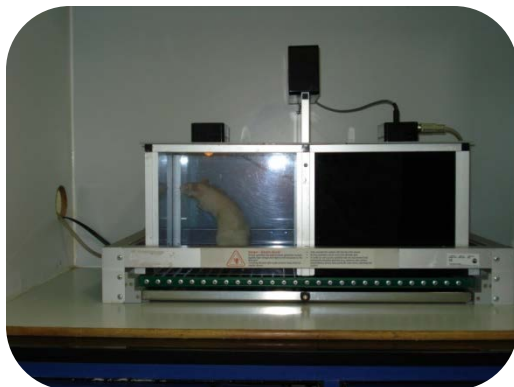
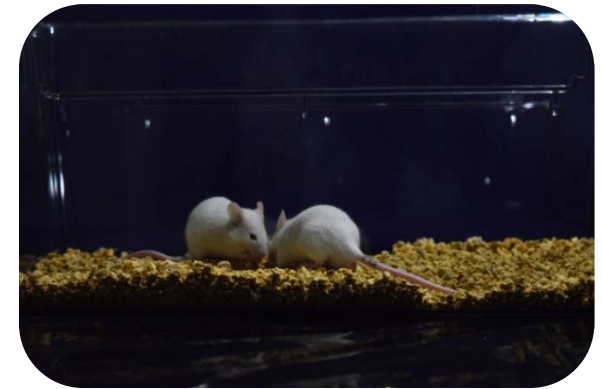
MK-801 induced hyperlocomotion & stereotypy

Amphetamine induced hyperlocomotion & stereotypy

Condition avoidance response

Dominant submissive assay (Mania)

Resident intruder task





MK-801 induced PPI deficits in rats - Study Outline

Species: Rats

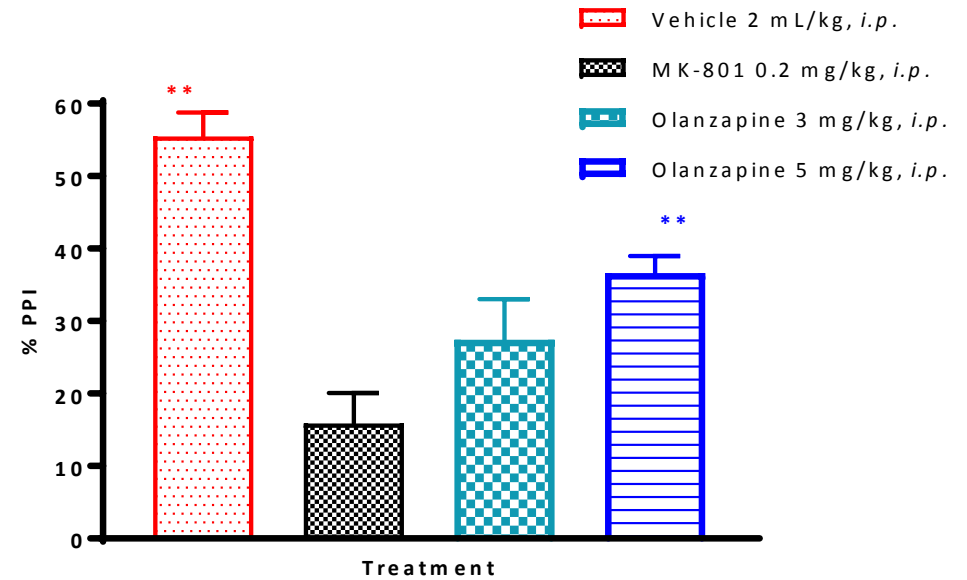
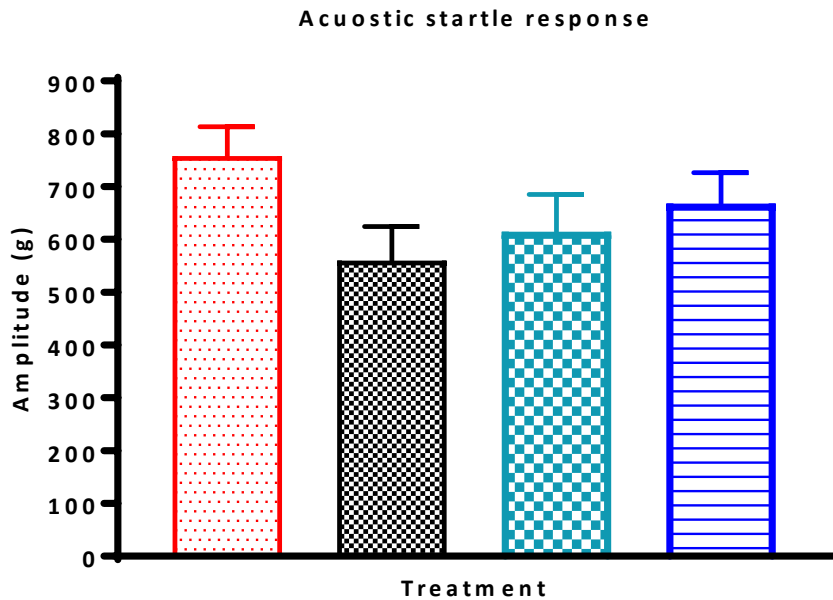
Strain: Wistar

Experimental Procedure:

Animals were randomized based on the basal startle response (98 dB). Olanzapine and MK-801 was administered 50 and 30 min before the habituation respectively. Rats were habituated to background noise (60 dB) for 5 min. Then the rats were subjected to trials (5 trials of 63 dB pp+ 98 dB p, 5 trials of 66 dB pp+ 98 dB p, 5 trials of 72 dB pp+ 98 dB p, 5 trials of No pulse, 15 trials of 98 dB pulse only). Activity of animal was recorded during the stimuli by the transducer and stored by the software. Maximum amplitude of response was taken as a measure of startle response and used as parameter for analysis.



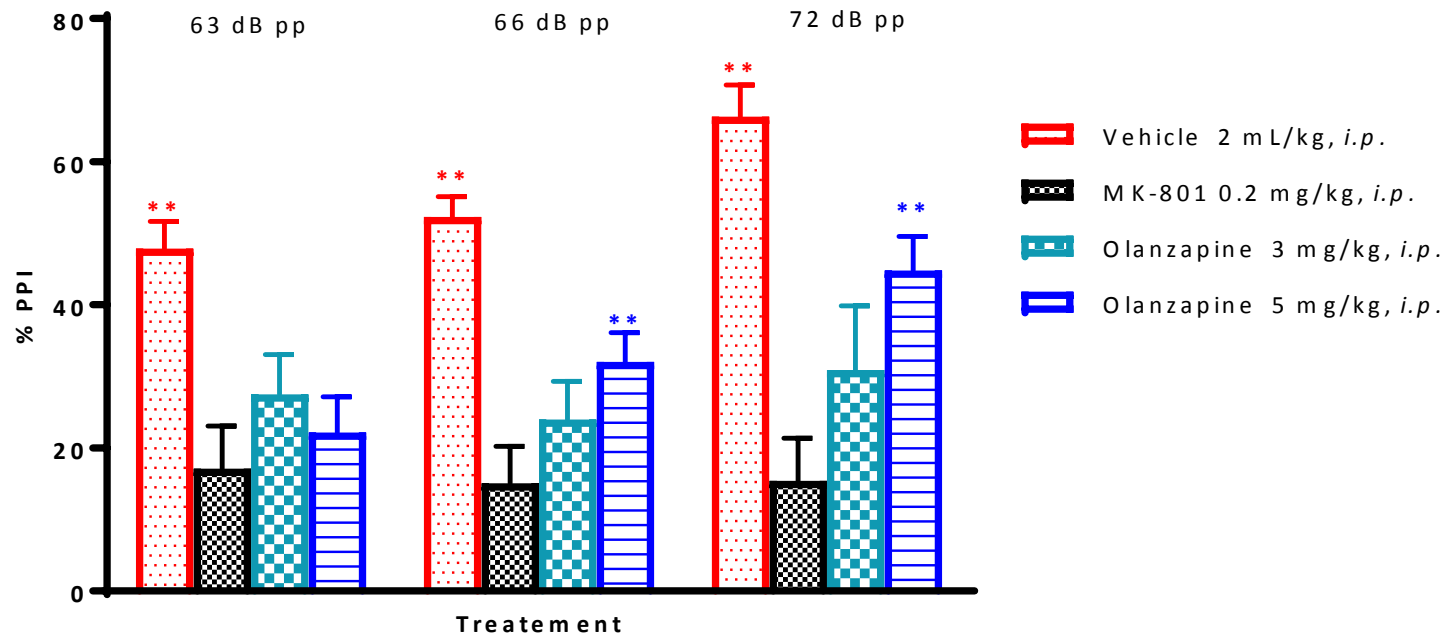
MK-801 induced PPI deficits – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$ vs MK-801



MK-801 induced PPI deficits – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$ Vs MK-801

MK-801 induced hyperlocomotion - Study Outline



Species: Rats

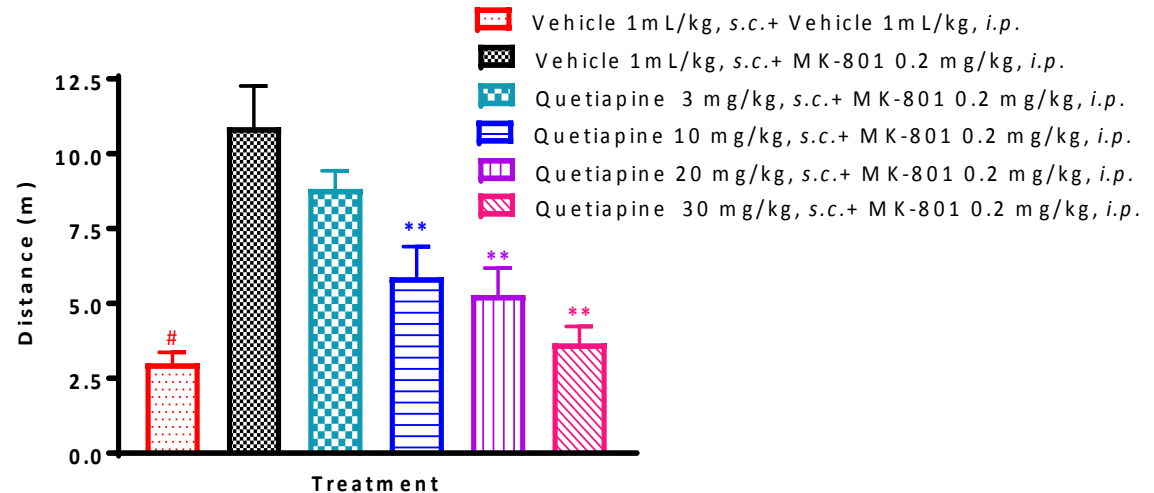
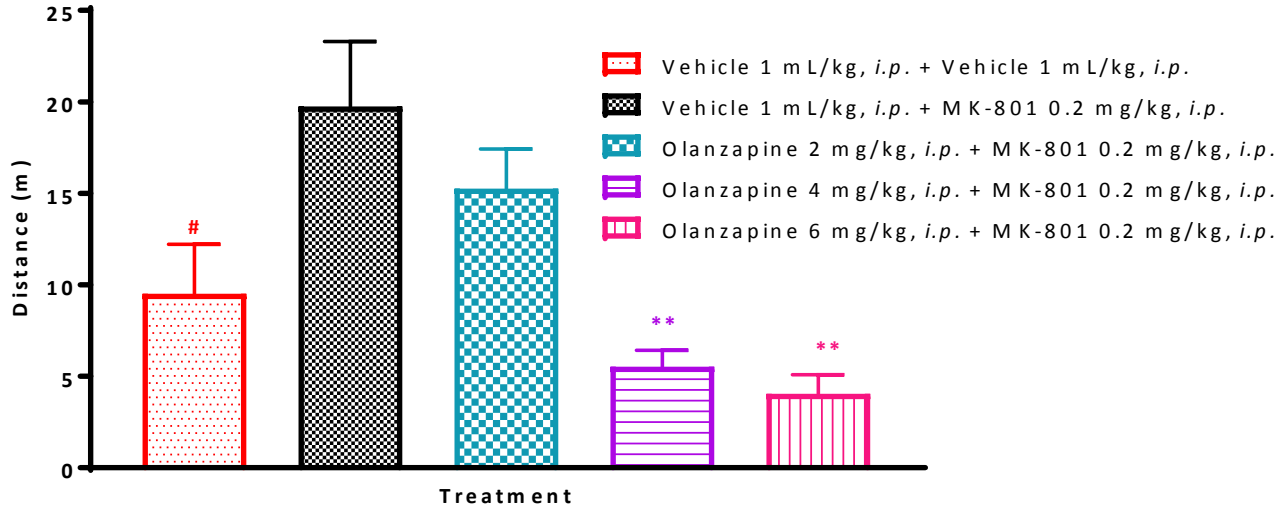
Strain: Wistar

Experimental Procedure:

Male Wistar rats were used. Rats were weighed and randomized according to body weight. The rats were brought to the laboratory 1 hr prior to test and handled. Thirty minutes prior to the trial vehicle or test drug was administered. After a post dosing interval MK-801 was injected. Then rat was placed in the open field and locomotion recorded for a period of 15 minutes.



MK-801 induced hyperlocomotion – Validation data



Data represent Mean \pm SEM. # p <0.0001, ** p <0.01 Vs MK-801

Amphetamine induced hyperlocomotion - Study Outline



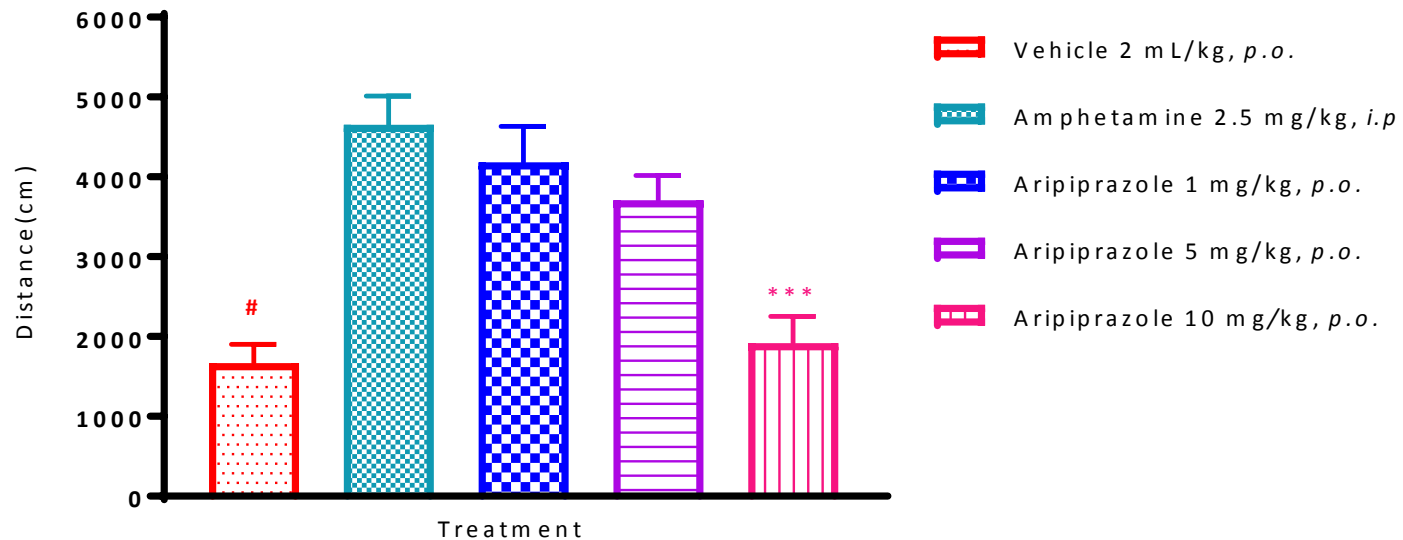
Species: Rats

Strain: Wistar

Experimental Procedure:

Male Wistar rats of were used. Rats were weighed and randomized according to their body weights. Following day, the rats were brought to the laboratory 1 hr prior to test. Animals were habituated for a period of 15 min. to the open field areas. Vehicle or Aripiprazole were administered 120 minutes prior to the trial. Vehicle or Amphetamine were administered 30 minutes prior to the trial. After the post dose interval, rats were placed in the open field and locomotion was recorded for a period of 15 minutes.

Amphetamine induced hyperlocomotion - Validation data



Data represent Mean \pm SEM. # p <0.0001, ** p <0.01 Vs Amphetamine

Condition avoidance response - Study Outline



Species: Rats

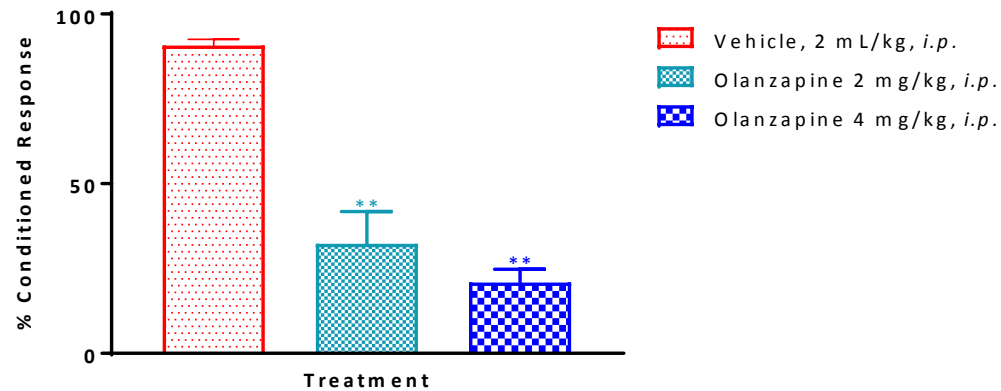
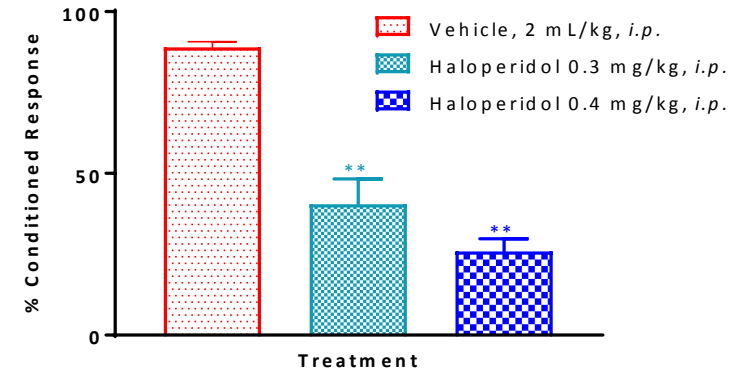
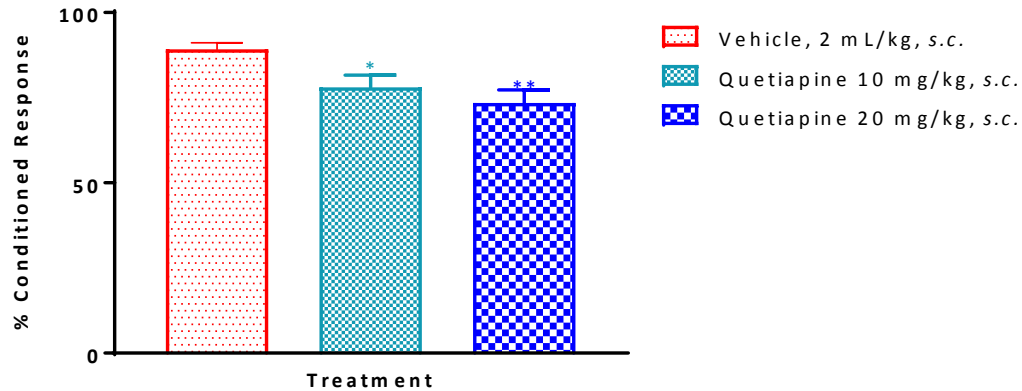
Strain: Wistar

Experimental Procedure:

Rats were trained for one week before drug testing. Training consisted of 20 trials per day with an inter trial interval of 30s. Each trial consisted conditioned stimulus (CS), white noise and unconditioned stimulus (UCS). The rat had 5 s to move into the opposite compartment in order to avoid the a shock of 0.6 mA through the grid floor which increased after 10 s by 0.2 mA.



Condition avoidance response – Validation data



Data represent Mean ± SEM. *p<0.05, **p<0.01 Vs Vehicle

Dominant submissive assay– Study Outline



Species: Rats

Strain: Wistar

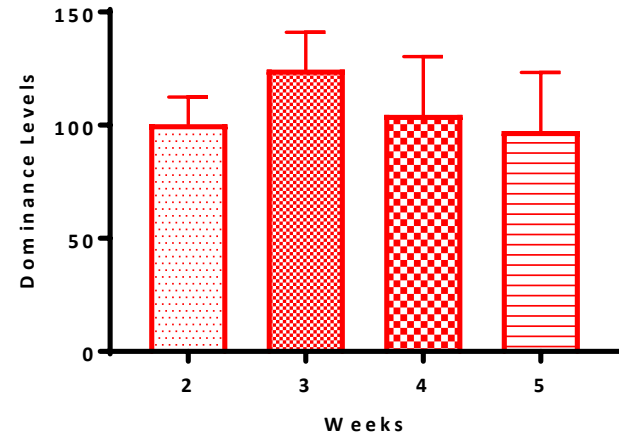
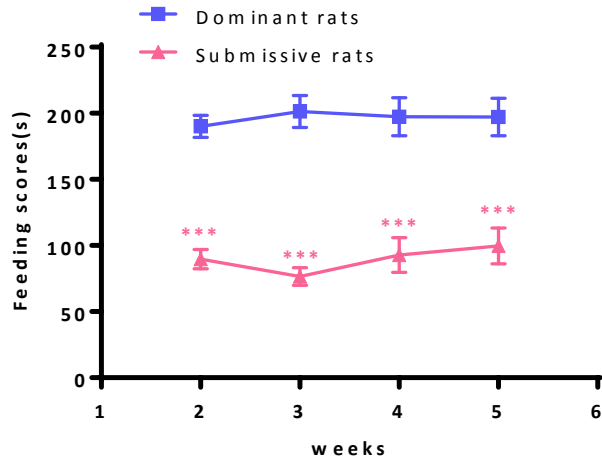
Experimental Procedure:

The testing apparatus was constructed from transparent plastic and consisted of two identical chambers connected by a tunnel. A beaker of sweetened milk was placed in an opening in the floor at the mid point of the tunnel. Habituation of the animal to the apparatus and milk (one week). Selection of dominant and submissive pair (one week). Animals were food-deprived overnight prior to testing. At the end of the testing animals were returned to their home cages and given free access to food for 1hour. The animals were also given free access to food from Friday afternoon to Sunday morning. The time spent drinking during the second week (selection period) was scored for each pair of animals and the pairs were selected using the selection criteria. The difference between the average daily drinking scores of the two animals was significant in the two-tailed t-test, $p < 0.05$. The dominant animal's score was at least 25% greater than the submissive animal's score. The selected animals were subjected to treatment and time spent drinking was recorded for each animal of the pair for a period of 5 min (three weeks). Dominant animals were administered test drug.

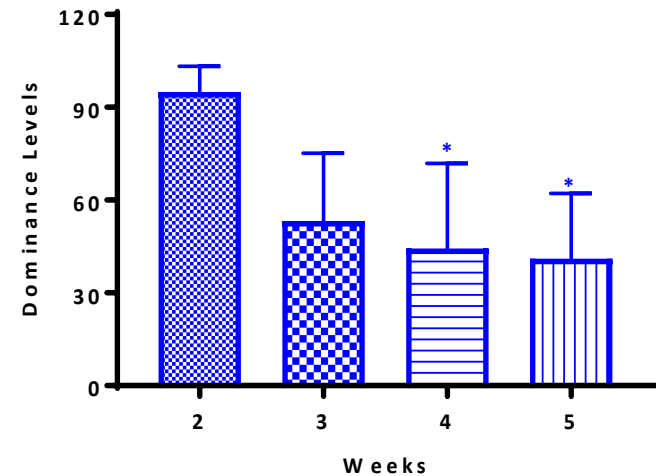
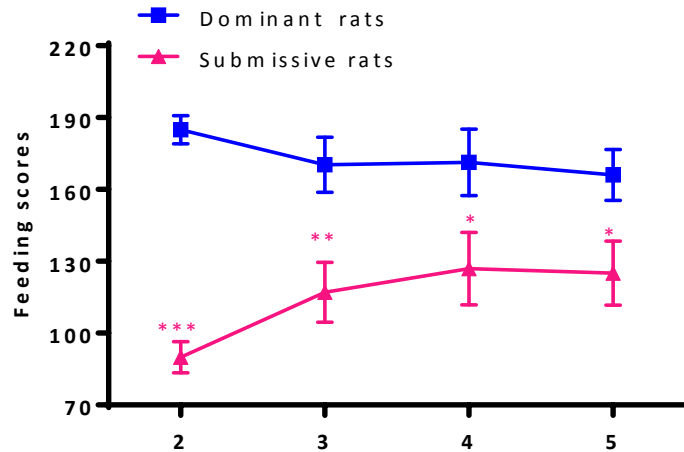


Dominant submissive assay – Validation data

Vehicle



Sodium Valproate 10 mg/kg



Data represent Mean \pm SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ Vs Dominant animals or week 2

Resident intruder task– Study Outline



Species: Mice

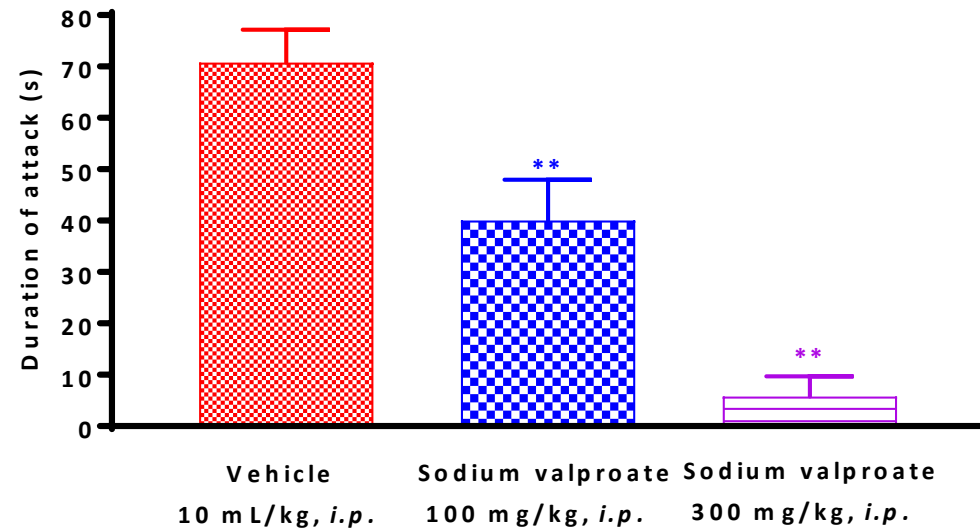
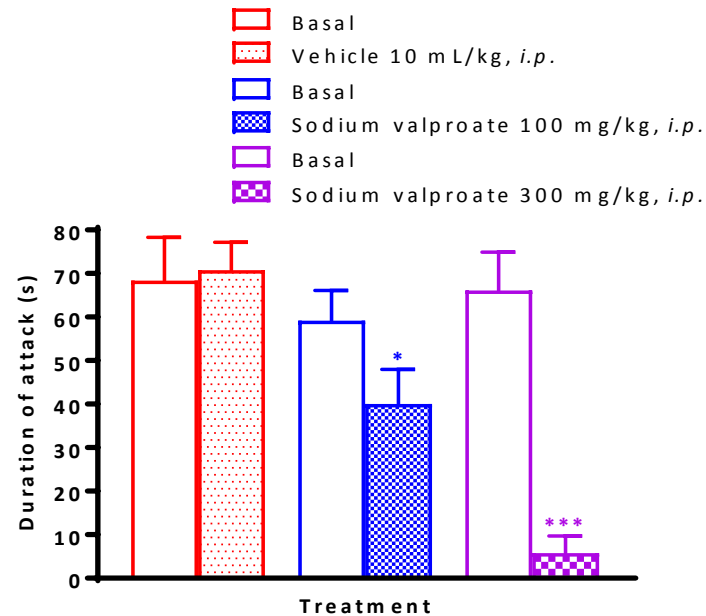
Strain: CD1

Experimental Procedure:

Male CD1 mice of weight 30-40g (Resident) and 25-35g (Intruder) were used. Resident mice were habituated individually with a ovariectomized female mouse in each cage and intruders were habituated socially for 1 week. β -estradiol at a dose of 0.2 mg/kg, s.c. was administered to female mice during habituation. On day 1 and day 2, intruder was exposed to resident mice for a period of 10 min and duration of attack was measured. On day 4 from initial exposure, animals were randomized based on their duration of attacking and administered respective treatments. Sodium valproate (100 and 300 mg/kg, i.p.) and vehicle was administered 30 min prior to the trial. After post dose interval resident mice were exposed to same intruder for 10 min and duration of attack was recorded.



Resident intruder task– Validation data



Data represents Mean \pm SEM of duration of attack, * $p < 0.05$, *** $p < 0.001$ vs basal or Vehicle.



Summary:

- **Olanzapine** reversed the sensory motor gating deficits induced by MK-801.
- **Olanzapine and Quetiapine** reversed the MK-801 induced hyperlocomotion
- **Aripiprazole** reversed the amphetamine induced hyperlocomotion
- **Olanzapine, Quetiapine and haloperidol** showed antipsychotic like property in the condition avoidance response task.
- **Sodium valproate** significantly reduced the dominance behavior in the dominant submissive assay.
- **Sodium valproate** significantly reduced the duration of attack in the resident intruder task.

Rodent models of anxiety



Elevated Plus Maze

Vogel conflict test

Hole board

Novelty induced hypophagia



Elevated plus maze – Study Outline



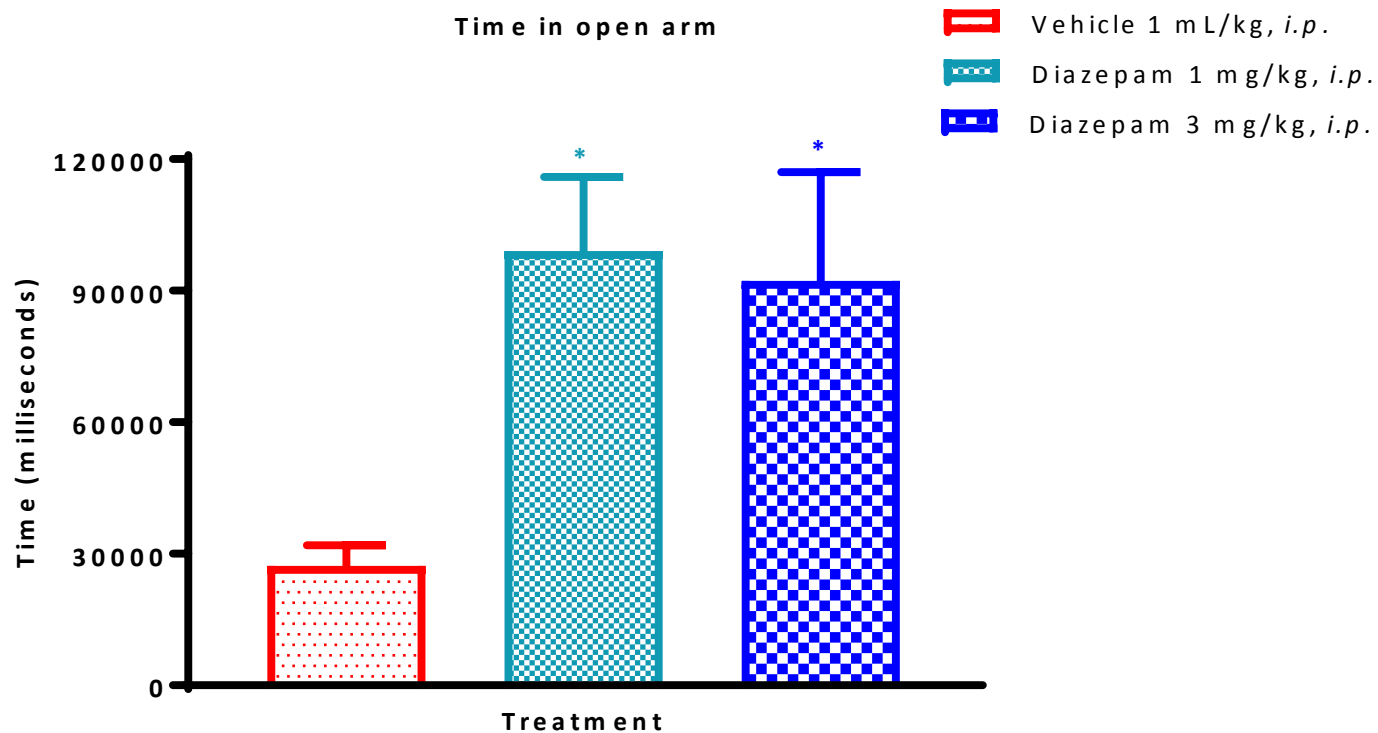
Species: Rats

Strain: Wistar

Experimental Procedure:

The elevated plus maze is black colored wooden arena in the form of a plus. It has two open arms 50 x 11 cm and 2 closed arms 50 x 11 cm partially enclosed on 3 sides by walls of 39 cm height the arms connected by central 10 x 10 cm square. The experiment was done under constant lighting conditions (50 lux in the open arms). One hour prior to the experiment the rats were treated either with vehicle or test compound and placed in the central square facing the enclosed arm. Tracking was done for a period of 5 minutes with the help of TSE Videomot 2.

Elevated plus maze – Validation data



Data represent Mean \pm SEM. * $p < 0.05$ Vs Vehicle

Vogel conflict – Study Outline



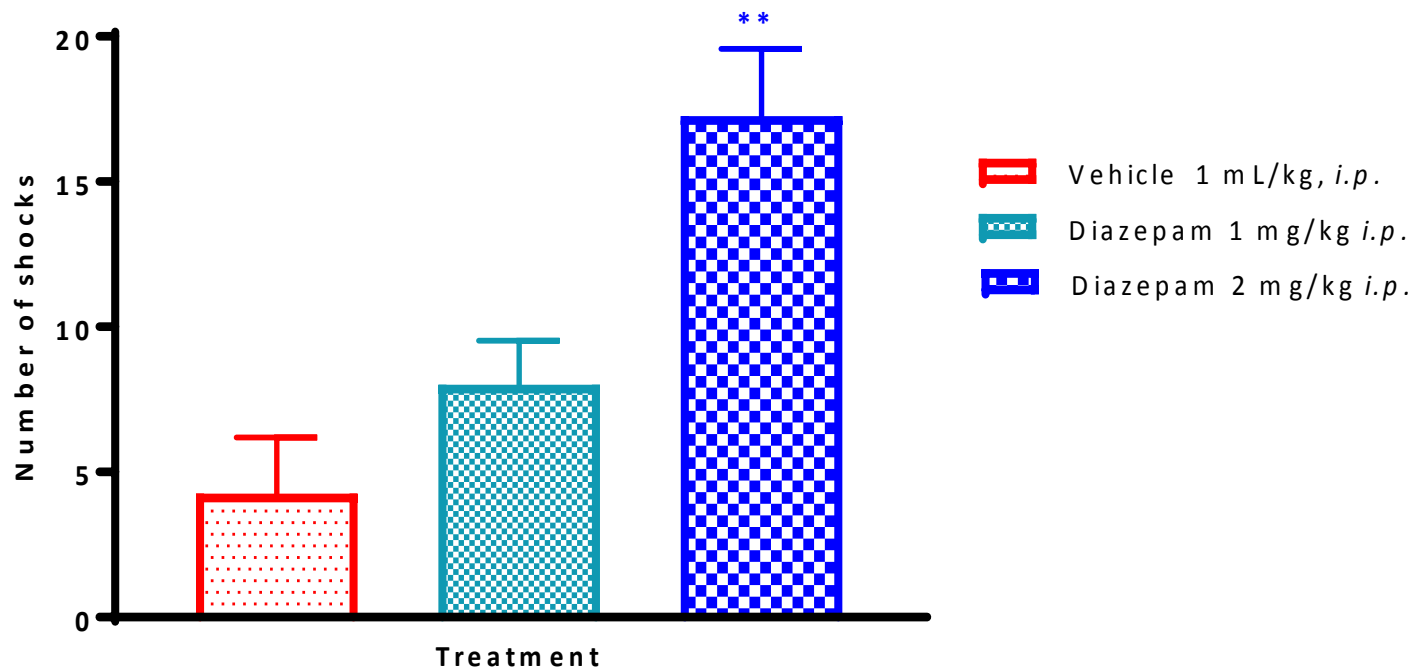
Species: Rats

Strain: Wistar

Experimental Procedure:

The experiment was carried out over a period of 3 days. The rats were water deprived for 24 hours. Then they were subjected to basal response in the test cage, during which the instrument counts the number of licks over a period of five minutes. After the basal response the rats were returned to their home cages, where they were given access to drinking water for a period of thirty minutes. Again the rats were water deprived for 24 hours. After 24 hours, the rats were administered with the test drugs. Following the post dose interval the rats were subjected to test response for a period of ten minutes, during which the instrument delivers a shock 1.9 mA for every 10 licks.

Vogel conflict – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$ Vs Vehicle

Hole board – Study Outline



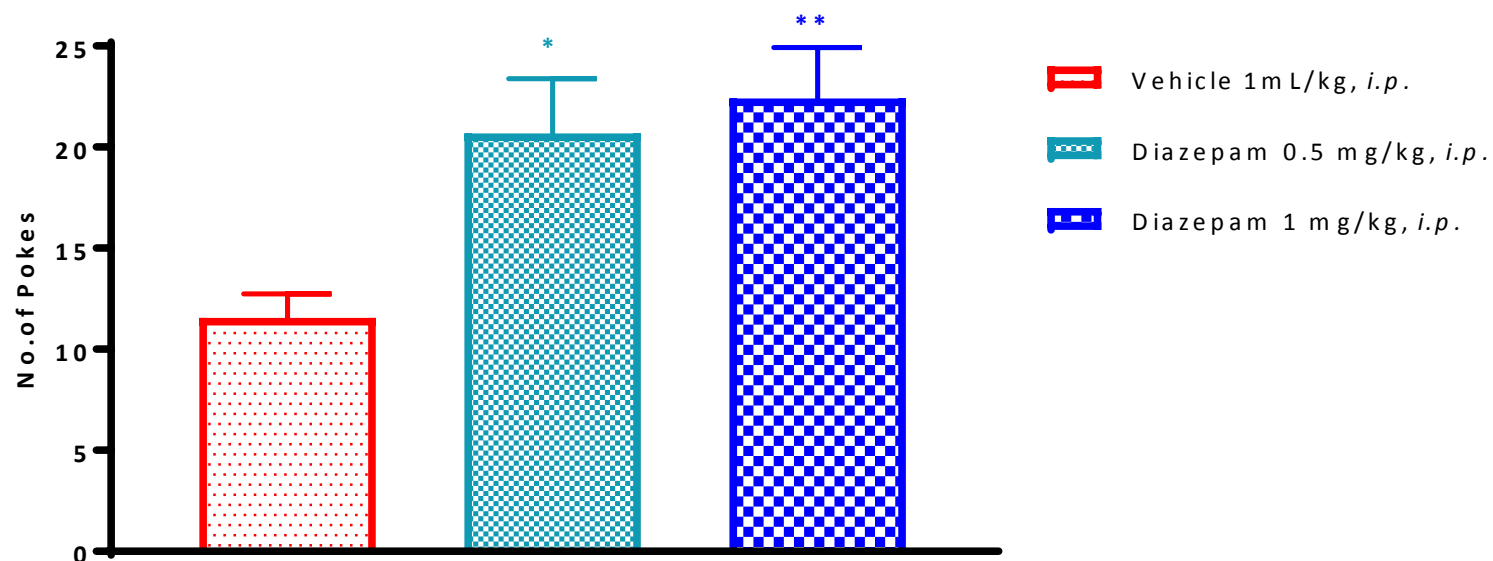
Species: Rats

Strain: Wistar

Experimental Procedure:

The hole board consists of a perforated base of dimensions 57 x 57 cm with 16 holes (3 cm diameter). Black plastic walls enclose the board. One hour prior to the experiment the rats were administered with vehicle or test compound and placed in the center of the hole board facing away from the observer. The latency of head dips, number of head dips and cumulative time of head dips was calculated individually for each animal for a period of 5 minutes.

Hole board – Validation data



Data represent Mean \pm SEM. **p<0.01, * p<0.05, Vs Vehicle

Novelty induced hypophagia – Study Outline



Species: Rats

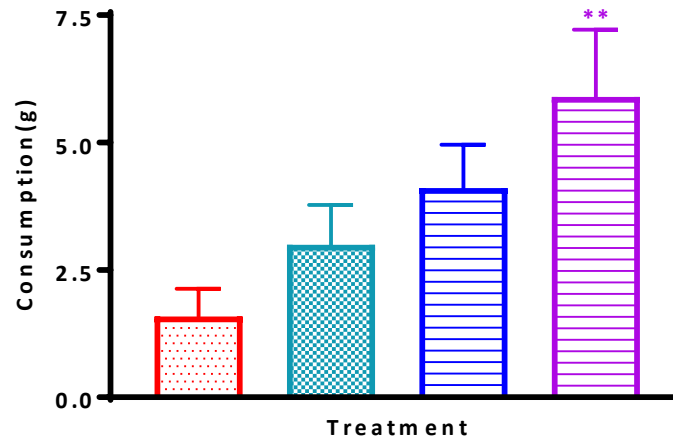
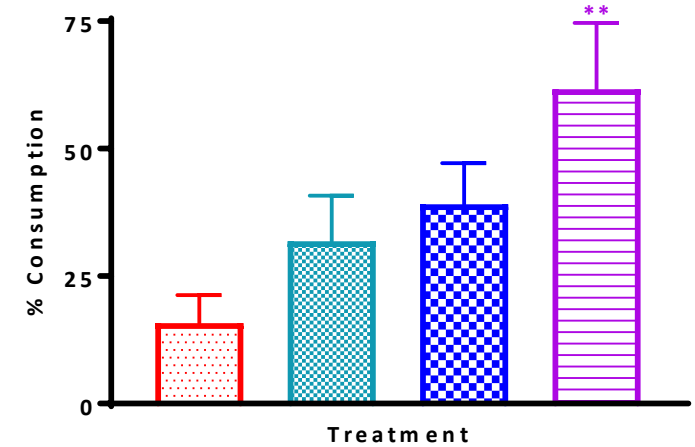
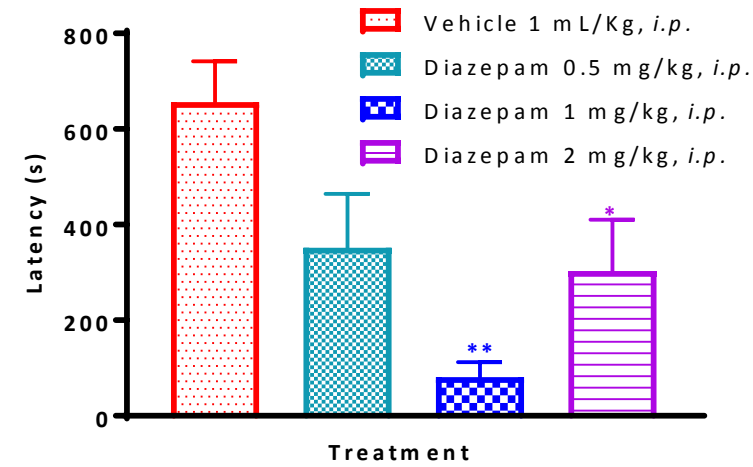
Strain: Wistar

Experimental Procedure:

Animals were trained to consume milkmaid (sweetened condensed milk) in their home cage without husk for 7 days for a period of 15 mins every day. Average latency and total consumption of 5th, 6th & 7th day were taken as home cage readings. On the 8th day the rats were introduced into novel cages of dimension 49x49x49 of white acrylic walls for a period of 15 mins. Latency and total consumption was recorded. On the 9th day home cage testing was performed. Latency and total consumption was recorded.



Novelty induced hypophagia – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle



Summary:

- **Diazepam** increased the time spent in the open arm in the elevated plus maze task.
- **Diazepam** increased the number of shock accepted in the Vogel conflict assay.
- **Diazepam** increased the number of nose pokes in the hole board assay.
- **Diazepam** decreased the latency for food consumption in a novel environment.

Rodent models of depression



Forced swim test (Rat & Mouse)

Tail suspension test

Dominant submissive assay

DRL-72s



Mouse forced swim test (mFST) – Study Outline



Species: Mice

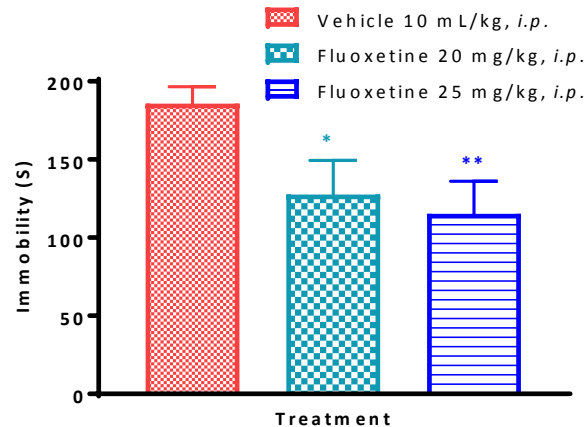
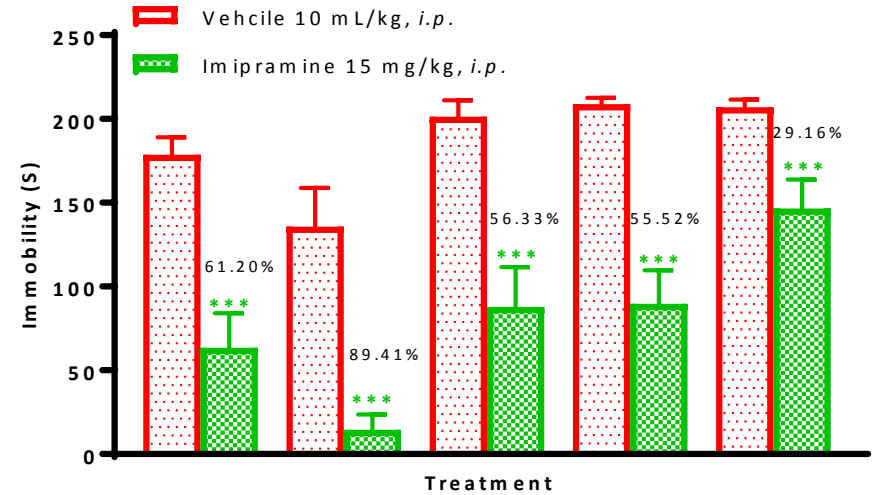
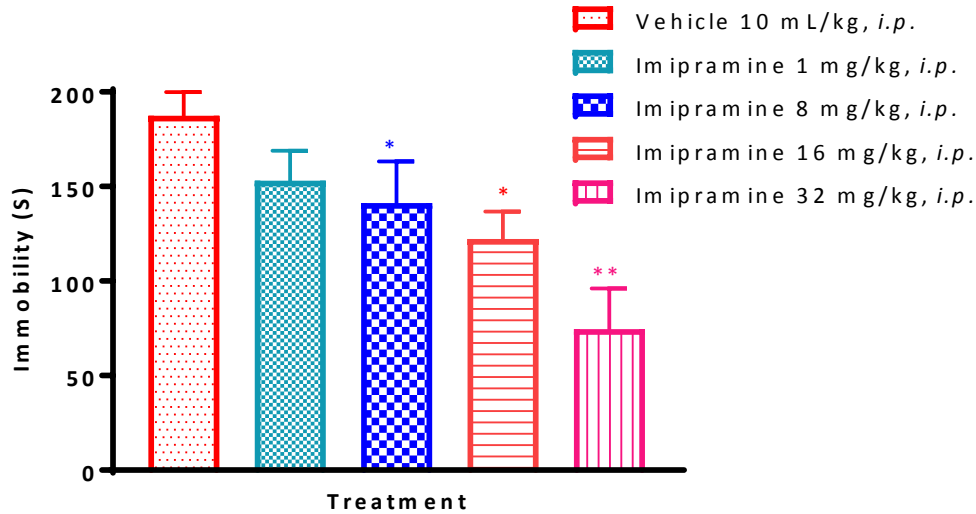
Strain: Swiss

Experimental Procedure:

Male Swiss albino mice of 25-30g were used. Mice were weighed and randomized according to body weight. The mice were placed in transparent cylinder (18 cm diameter) containing water (12 cm high) for a period of 6 minutes. The immobility of the mice in the last 4 minutes was recorded.



Mouse forced swim test (mFST) – Validation data



Data represent Mean \pm SEM. *** p <0.001, ** p <0.01; * p <0.05 Vs Vehicle

Rat forced swim test (rFST) – Study Outline



Species: Rats

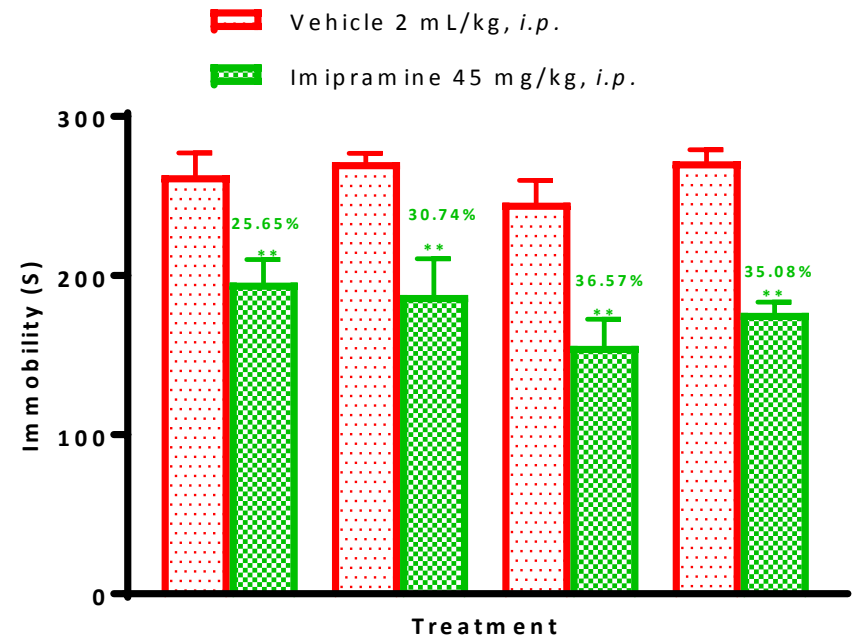
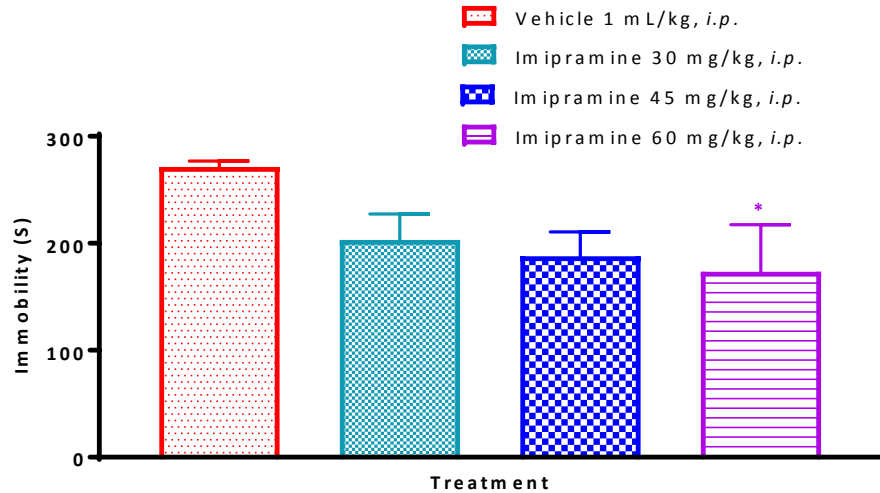
Strain: Wistar

Experimental Procedure:

Male Wistar rats of 180-230g were used. Rat were weighed and randomized according to body weight. The rat were placed in transparent cylinder (50cm height & 30 cm diameter) containing water (30 cm high) for a period of 15 minutes. On the next day rats were administered respective treatments and placed in the cylinder for 6 min. The immobility of the rat during the last 5 minutes was recorded.



Rat forced swim test (rFST) – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$; * $p < 0.05$ Vs Vehicle

Tail suspension test– Study Outline



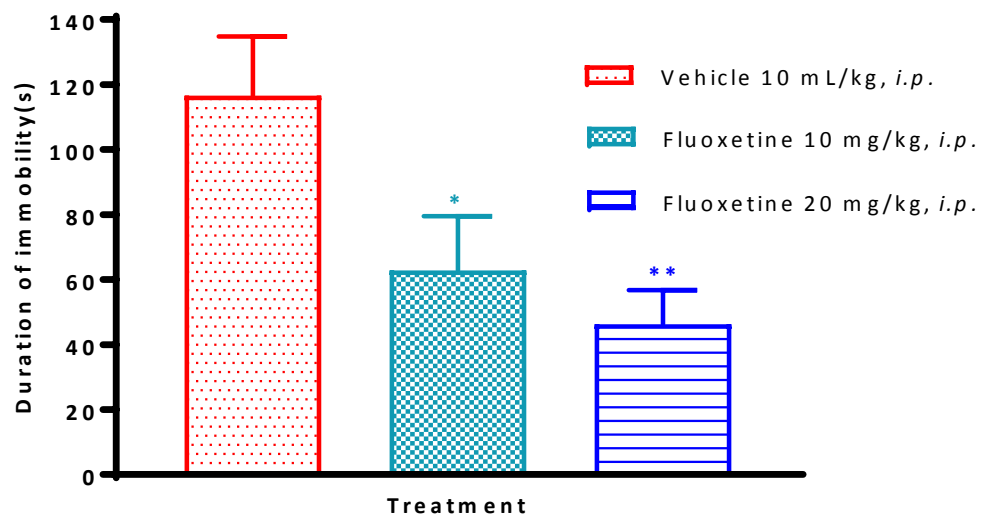
Species: Mice

Strain: Swiss

Experimental Procedure:

Male mice of 30-40g were used. Mice were weighed and randomized according to their body weight. The experiment was carried out in a wooden compartment. Mice were hanged at a height of 50 cm from the base of a wooden compartment using adhesive tapes. The mice were hanged 1cm from the tip of the tail. The immobility of the mice in the last 5 minutes were recorded in the total 6min trial.

Tail suspension test– Validation data



Data represent Mean \pm SEM. **P<0.01, *P<0.05 Vs Vehicle

Dominant submissive assay– Study Outline



Species: Rats

Strain: Wistar

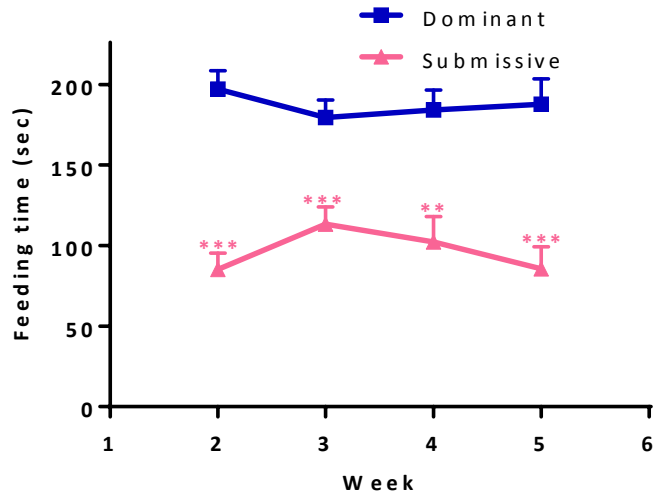
Experimental Procedure:

The testing apparatus was constructed from transparent plastic and consisted of two identical chambers connected by a tunnel. A beaker of sweetened milk was placed in an opening in the floor at the mid point of the tunnel. Habituation of the animal to the apparatus and milk (one week). Selection of dominant and submissive pair (one week). Animals were food-deprived overnight prior to testing. At the end of the testing animals were returned to their home cages and given free access to food for 1hour. The animals were also given free access to food from Friday afternoon to Sunday morning. The time spent drinking during the second week (selection period) was scored for each pair of animals and the pairs were selected using the selection criteria. The difference between the average daily drinking scores of the two animals was significant in the two-tailed t-test, $p < 0.05$. The dominant animal's score was at least 25% greater than the submissive animal's score. The selected animals were subjected to treatment and time spent drinking was recorded for each animal of the pair for a period of 5 min (three weeks). Submissive animals were administered test drug.

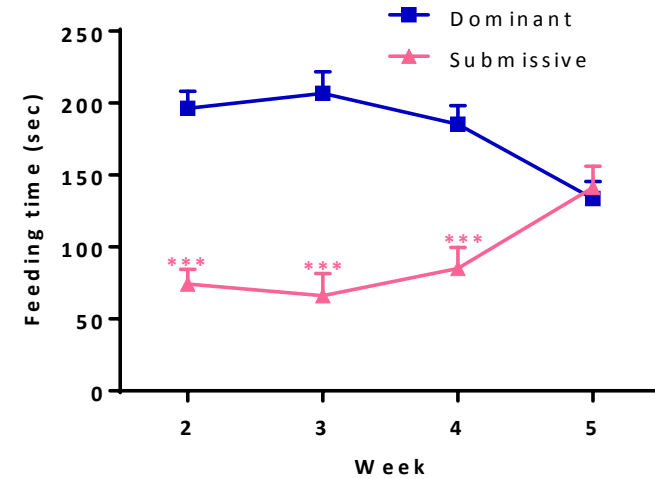


Dominant submissive assay (Depression) – Validation data

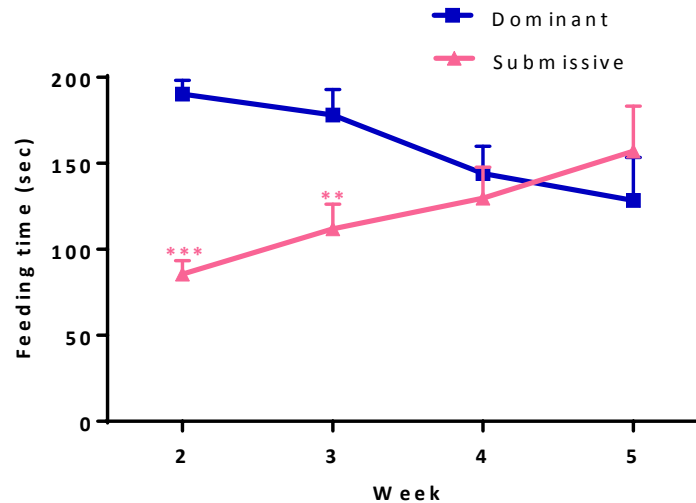
Vehicle 2 mL/kg, i.p.



Fluoxetine 10 mg/kg, i.p.



Imipramine 10 mg/kg, i.p.



Data represent Mean \pm SEM.

*** p <0.001, ** p <0.01 Vs Dominant animals

Differential reinforcement at low rates-72s– Study Outline



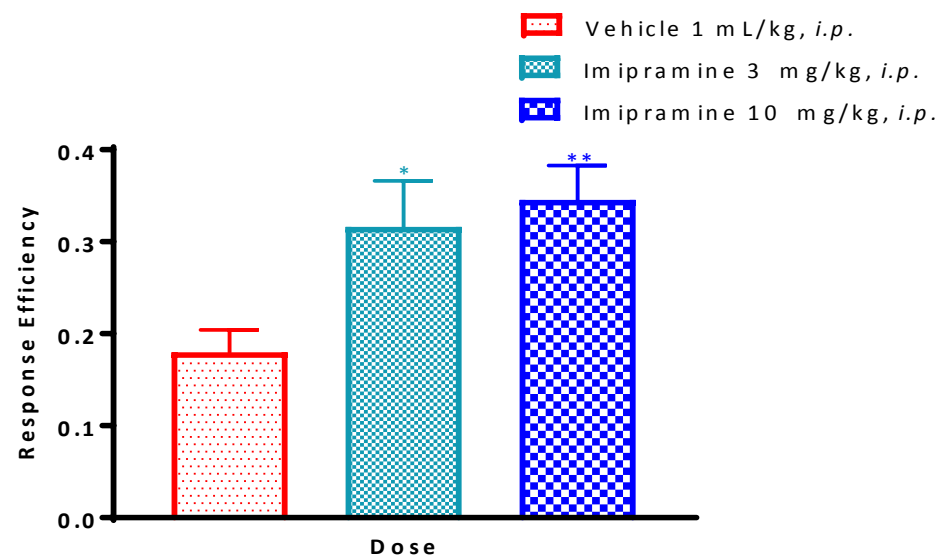
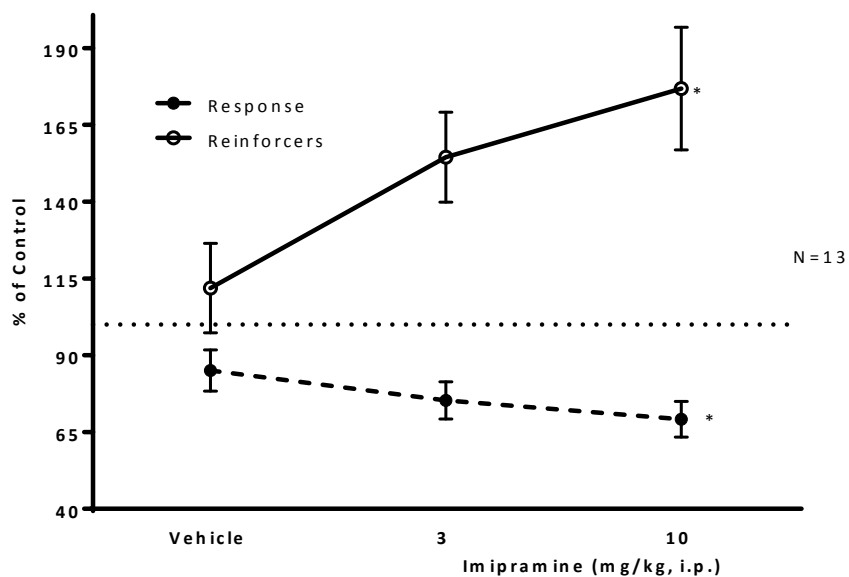
Species: Rats

Strain: Wistar

Experimental Procedure:

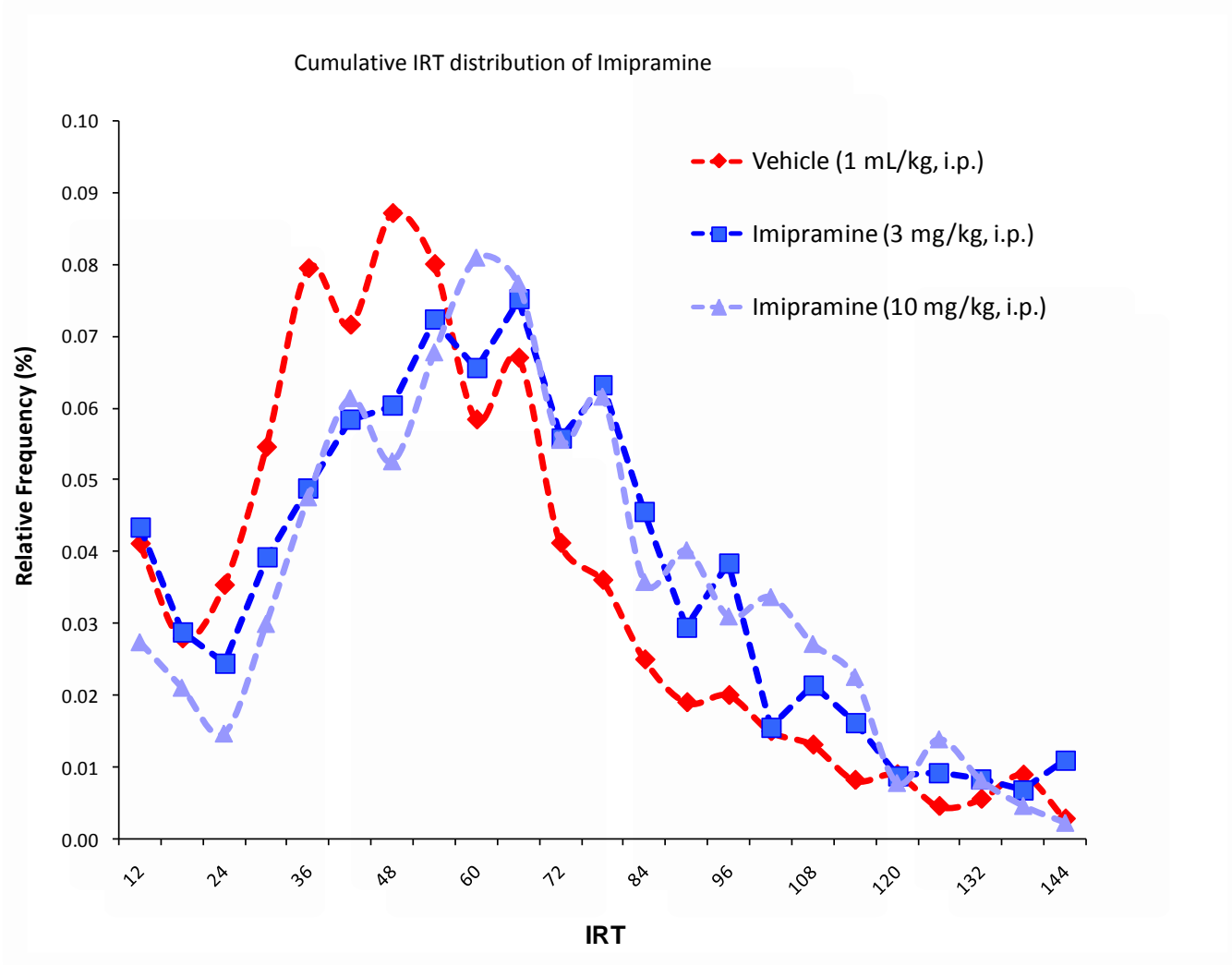
Rats are trained to lever press for a 4" access to .025 ml of water for each correct response during daily 60 minute sessions. All testing takes place on weekdays only. At the beginning of each session, the house light is illuminated and remains lit until the session ends. No other stimuli are presented during testing. After successful lever press training, rats are then required to respond under a DRL-24 second schedule, where only lever presses that are separated by 24 seconds are reinforced. Upon stable responding on a DRL-24 second schedule (5-10 sessions), rats are trained on a DRL-72 second schedule until responding stabilizes at approximately 15% efficiency (approx 25-35 sessions). Specifically, rats receive a reinforcer for each response that is emitted at least 72 seconds after the previous response (IRT). Responses with IRT's less than 72 seconds do not receive a reinforcer, and the IRT requirement is reset to 72 seconds. Efficiency is recorded as number of reinforced responses ÷ total number of responses. After stable baseline responding is achieved, defined as responding for 4 consecutive sessions with no more than 10% variability, animals begin drug testing. Animals receive drug no more than 1x per week.

Differential reinforcement at low rates-72s– Validation data

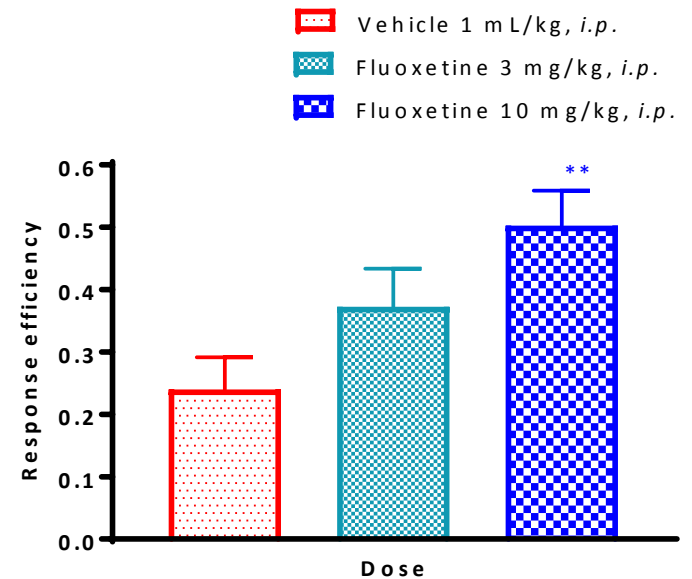
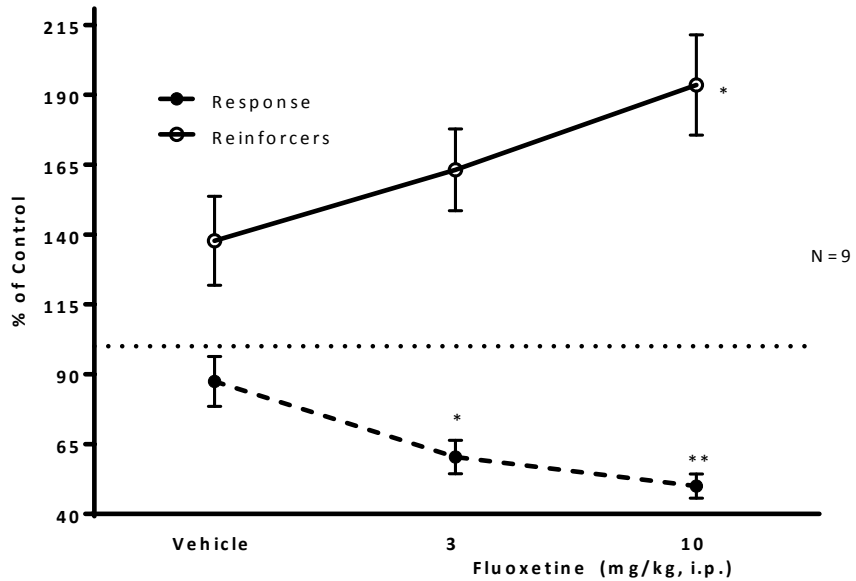


Data represent Mean \pm SEM. **P<0.01, *P<0.05 Vs Vehicle

Differential reinforcement at low rates-72s– Validation data

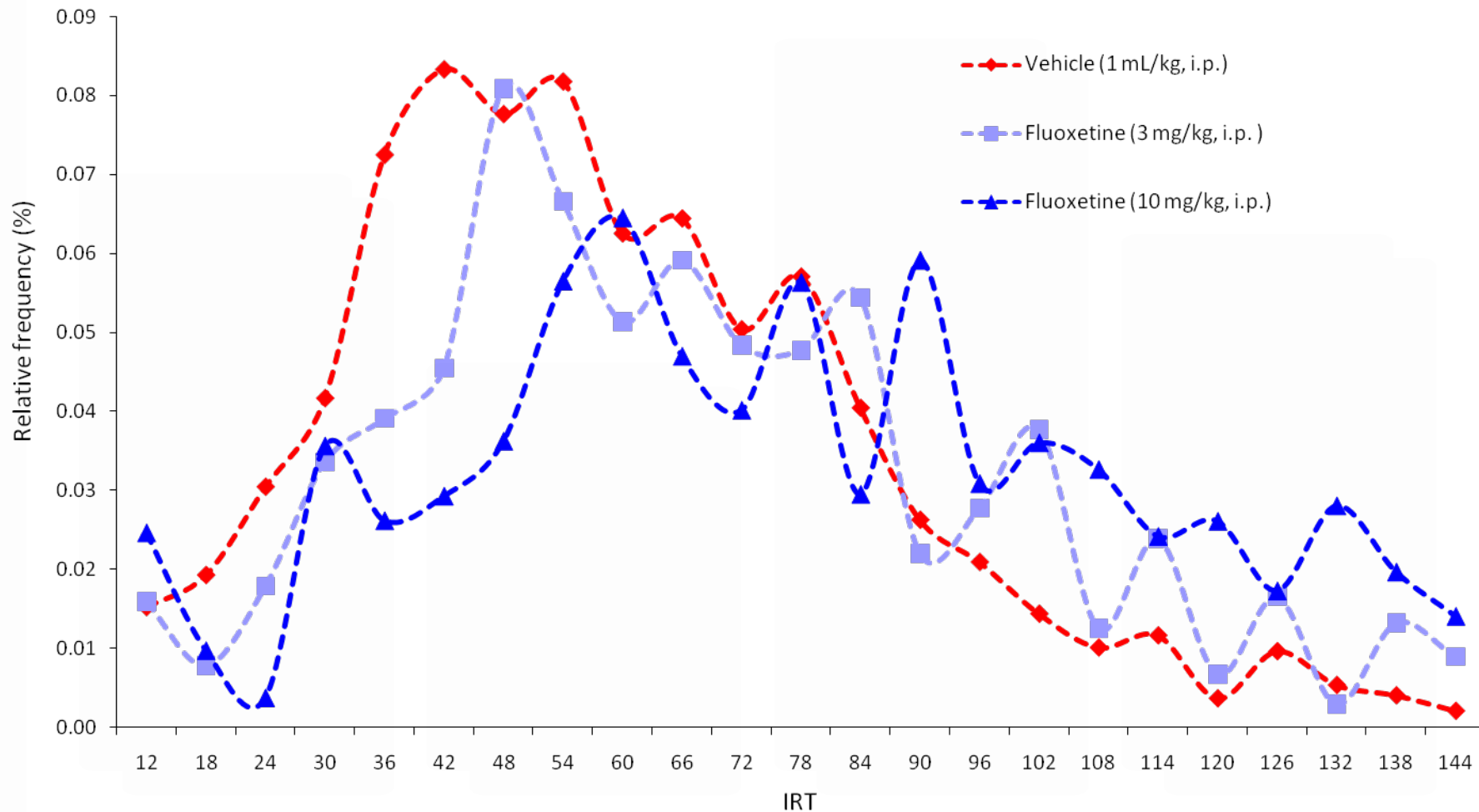


Differential reinforcement at low rates-72s– Validation data



Data represent Mean \pm SEM. **P<0.01, *P<0.05 Vs Vehicle

Differential reinforcement at low rates-72s– Validation data





Summary:

- **Imipramine** decreased the immobility time in the mouse as well as the rat forced swim assay (Characteristic property of an antidepressant).
- **Imipramine and Fluoxetine** dose dependently increased the reinforcement and reward efficiency. Similarly a dose dependent decrease in the responses was observed. A coherent shift in the IRT interval was also observed (Characteristic property of an antidepressant).
- **Imipramine and Fluoxetine** significantly reduced the submissive behavior in the dominant submissive assay.
- **Fluoxetine** decreased the immobility time in the tail suspension test (Characteristic property of an antidepressant).

Rodent models for pain disorders



Acetic acid induced writhing, Formalin Induced Nociception, & Hot plate

Complete Freund's adjuvant - induced mechanical hyperalgesia

Chemotherapy - induced neuropathic pain

Streptozotocin - induced diabetic neuropathic pain

Spinal nerve (L5) ligation - induced neuropathic pain

Chronic constricted injury (CCI) - induced neuropathic pain

Partial sciatic nerve ligation (PSNL) - induced neuropathic pain

Capsaicin induced secondary mechanical allodynia in rats

Mono iodoacetate induced osteoarthritis

Complete Freund's adjuvant induced rheumatoid arthritis

Medial meniscal tear model of osteoarthritis

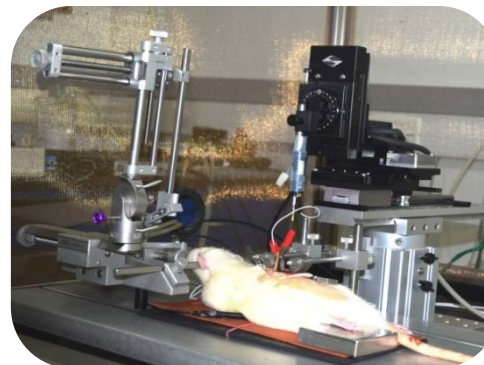
Reserpine induced myalgia in rats

Chronic post-ischemia pain

Arachidonic acid induced ear edema

Spontaneous burrowing behavior in pain models

In vivo electrophysiology in rats



Acetic acid induced writhing – Study Outline



Species: Mice

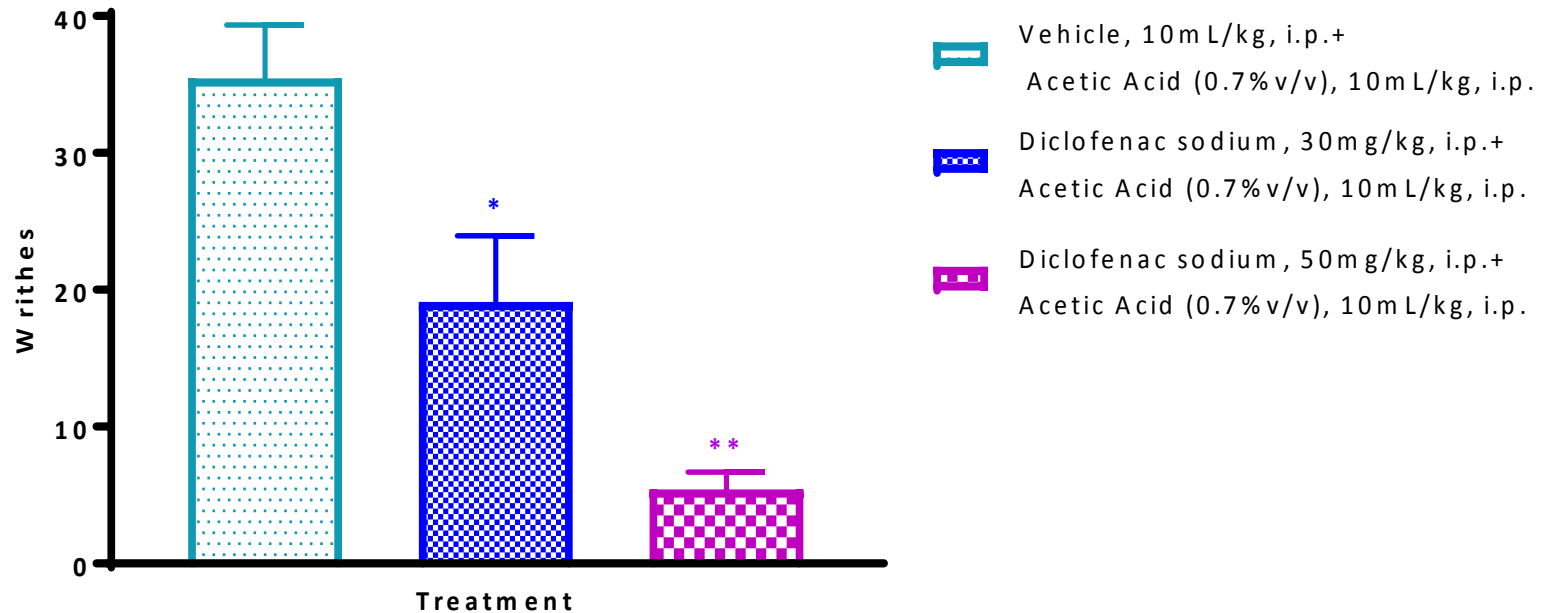
Strain: NMRI

Experimental Procedure:

0.7% v/v acetic acid was administered intraperitoneally in a dose volume of 10 mL/kg to all the groups of animals. Vehicle or Diclofenac sodium was administered 20 minutes prior to acetic acid administration to their respective group. Number of writhes were recorded after discarding the first 5 minutes for the period of 20 minutes.



Acetic acid induced writhing – Validation data



Data represent Mean ± SEM. *P<0.05,**P<0.01 Vs Vehicle



Formalin induced nociception – Study Outline

Species: Rat

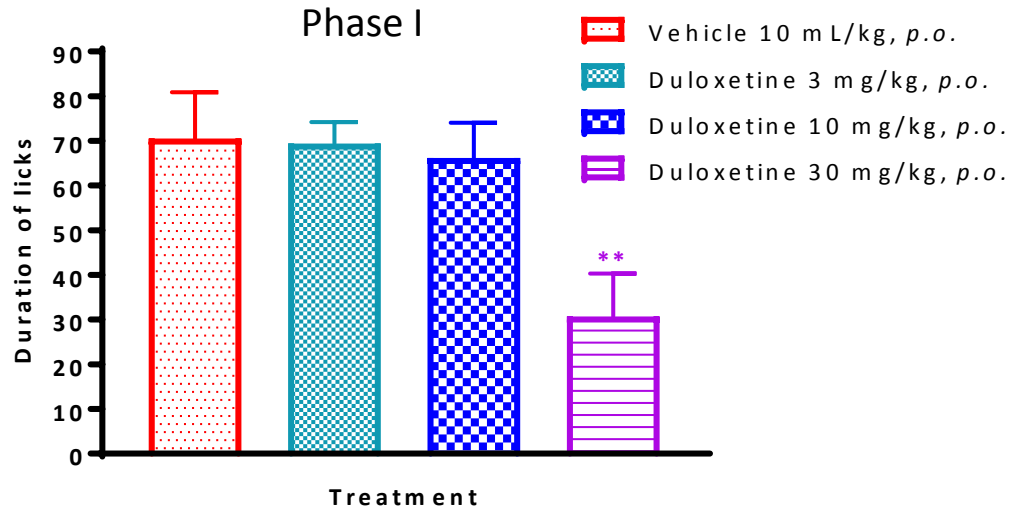
Strain: Sprague Dawley

Experimental Procedure:

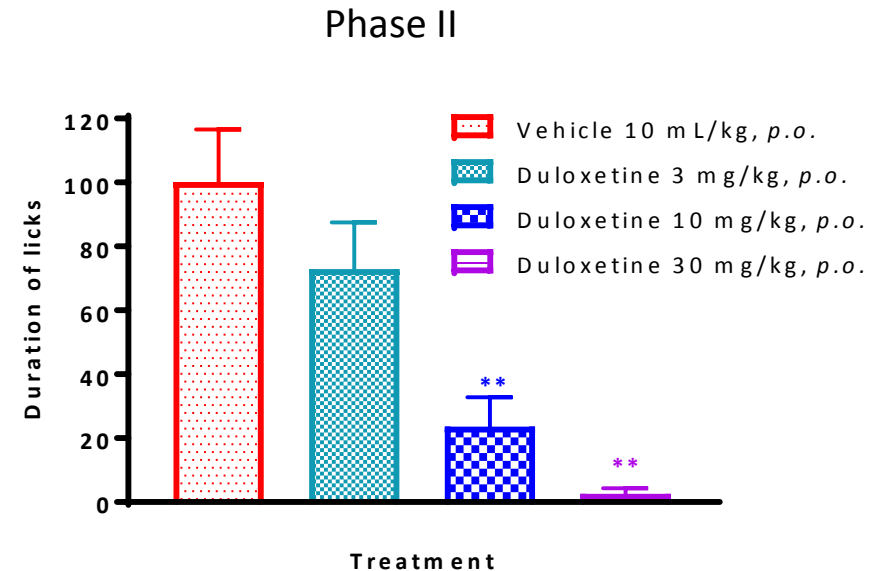
One hour (h) before the trial, the animals were administered vehicle or test drug. Then rats were habituated to the arena before the trial. Just prior to the commencement of the trial, 50 μ L of formalin was injected into the right hind paw. Duration of licks were noted using stop watch for 35 min.



Formalin Induced Nociception – Validation data

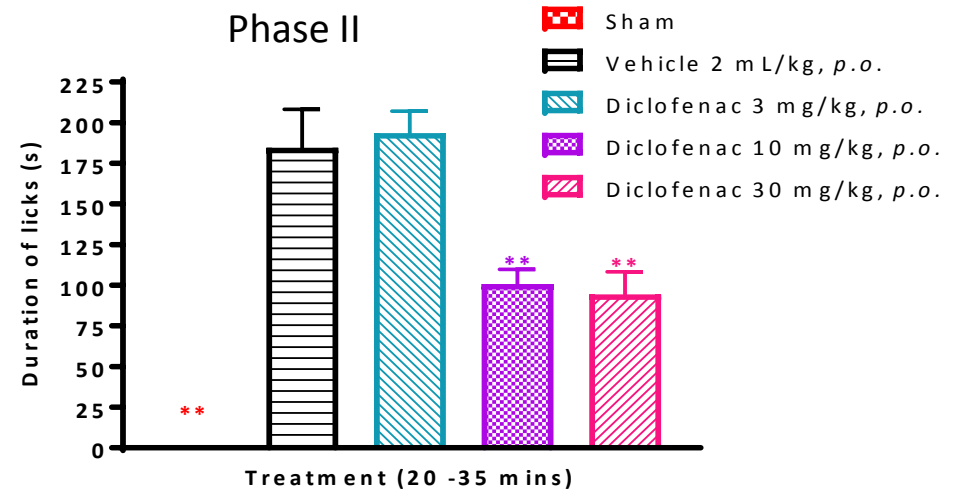
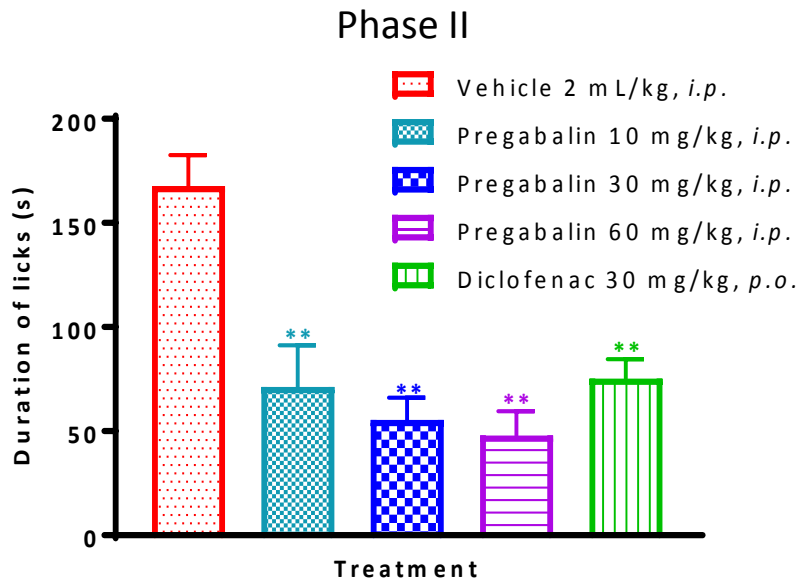


Data represent Mean ± SEM. **p<0.01 Vs Vehicle





Formalin induced nociception – Validation data



Data represent Mean \pm SEM. ** $p < 0.01$ Vs Vehicle

Hot plate – Study Outline



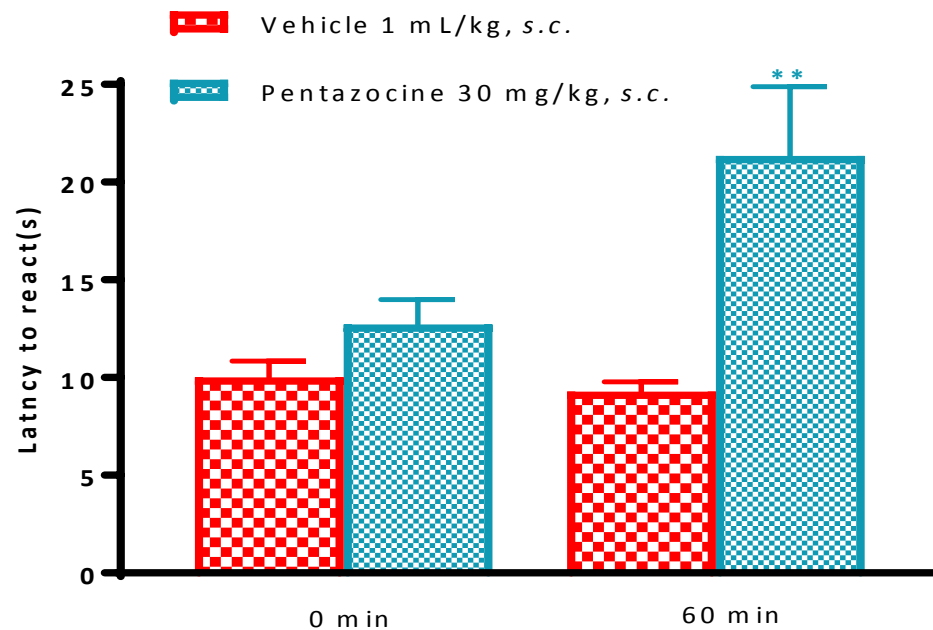
Species: Rat

Strain: Sprague Dawley

Experimental Procedure:

Rats of 240-300g were randomized according to body weight. The basal latency of the rats to react were measured in the hot plate at 52 ± 0.5 °C. Pentazocine was administered 30mg/kg, *s.c.* and latency to react was measured at 0 and 60 minutes after treatment. Experimenter was blinded to treatment groups.

Hot plate – Validation data



Data represent Mean \pm SEM. **p<0.01 Vs Vehicle

Complete Freund's adjuvant-induced mechanical hyperalgesia –



Study Outline

Species: Rat

Strain: Sprague Dawley

Experimental Procedure:

Baseline Paw withdrawal Threshold (PWT) was measured on left paw using Randall-selitto. The cut off was set at 250g. The rats were anesthetized using isoflurane and received an intraplantar injection of, Complete Freund's adjuvant (CFA, 150µl) to the left hind paw. Twenty four hours after CFA, pre-dose PWT was recorded. PWT was again determined 1, 2, 3, 4, 5 and 24hr after the drug administration. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams and percentage reversal of hyperalgesia.

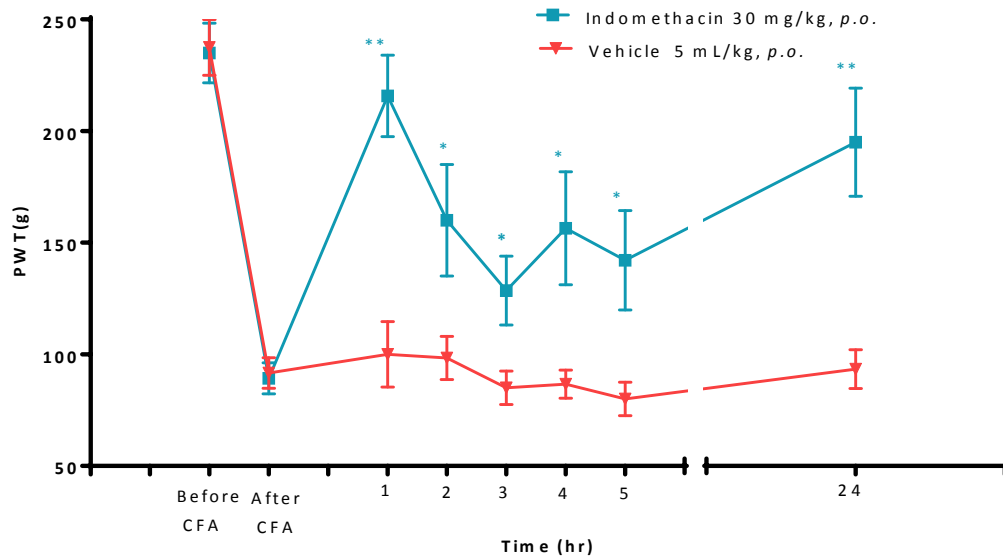
$$\frac{(\text{post dose PWT} - \text{pre dose PWT})}{(\text{Basal} - \text{pre dose PWT})} \times 100$$

Complete Freund's adjuvant-induced mechanical hyperalgesia –

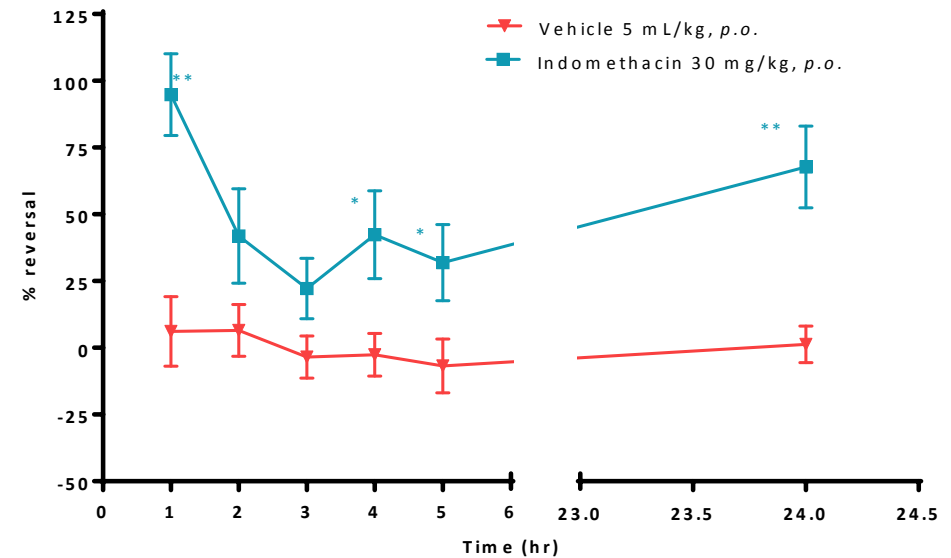


Validation data

Analgesymeter



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle



Complete Freund's adjuvant-induced mechanical allodynia –



Study Outline

Species: Rat

Strain: Wistar

Experimental Procedure:

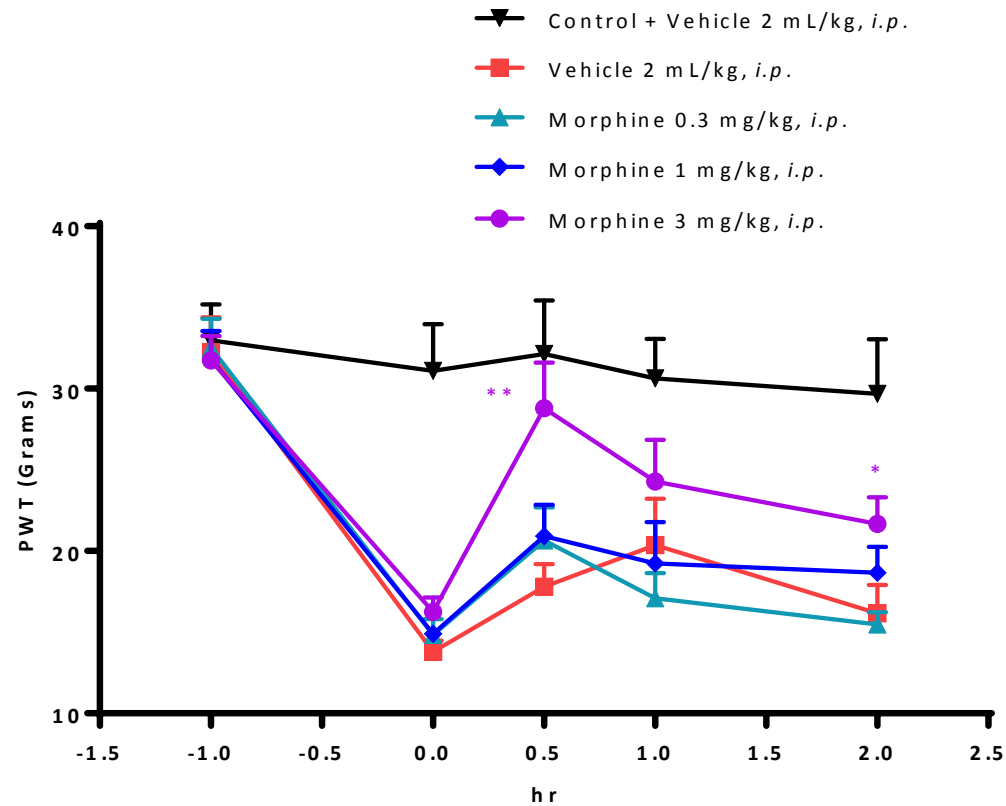
Baseline Paw withdrawal Threshold (PWT) was measured on left paw using Dynamic plantar Aesthesiometer. Cut off was set at 50 gram/ 20sec. The rats were anesthetized using isoflurane and received an intraplantar injection of Complete Freund's adjuvant (CFA, 50 μ l) to the left hind paw. Twenty four hours after CFA, pre-dose PWT was recorded. PWT was again determined at 0 (basal), 1 and 2 hr after the drug administration. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams.

Complete Freund's adjuvant-induced mechanical allodynia –



Validation data

Dynamic Plantar Aesthesiometer



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Chemotherapy induced neuropathic pain – Study Outline



Species: Rat

Strain: Sprague Dawley

Experimental Procedure:

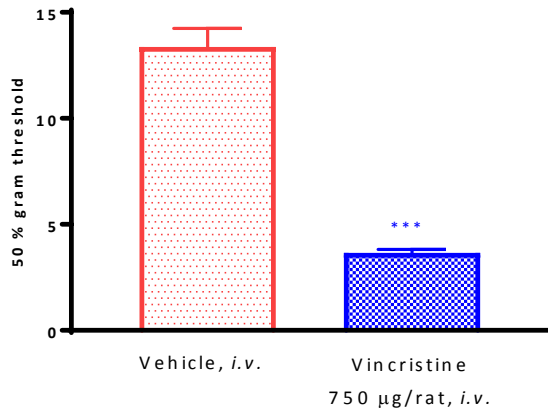
Animals received Vincristine 50 µg/kg from day 1 to 5, 100 µg/kg from day 8 to 12 injected through tail vein (cumulative dose 750 µg/kg, *i.v*) or vehicle 1 mL/kg, *i.v*. Paw withdrawal responses were observed by using Von Frey mono filaments (0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, 15 grams) and Dixon up and down method. Animals were randomized and efficacy was determined pre-dose, 30, 60 and 120 min after the drug administration. Experimenter was blinded to treatment groups. Each response was calculated by using formulae $50\% \text{ g threshold} = (10^{(xf+kd)})/10,000$. Data was expressed as 50% g threshold.

Chemotherapy induced neuropathic pain – Validation data

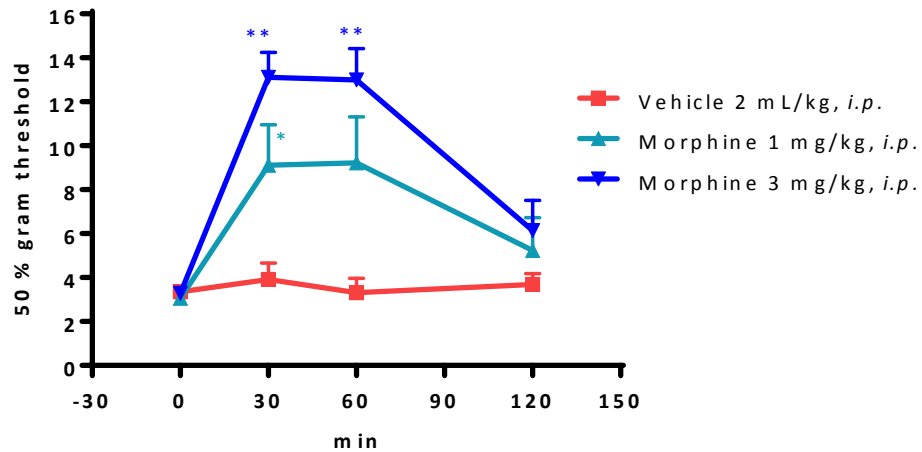
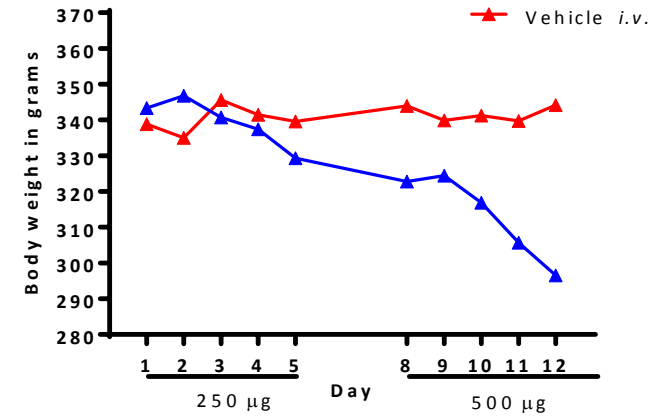


Von Frey filaments

Effects of Vincristine on PWT (Day-13)



Effect of Vincristine on Body weights



Data represent Mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 Vs Vehicle

Streptozotocin induced diabetic neuropathic pain – Study Outline



Species: Rat

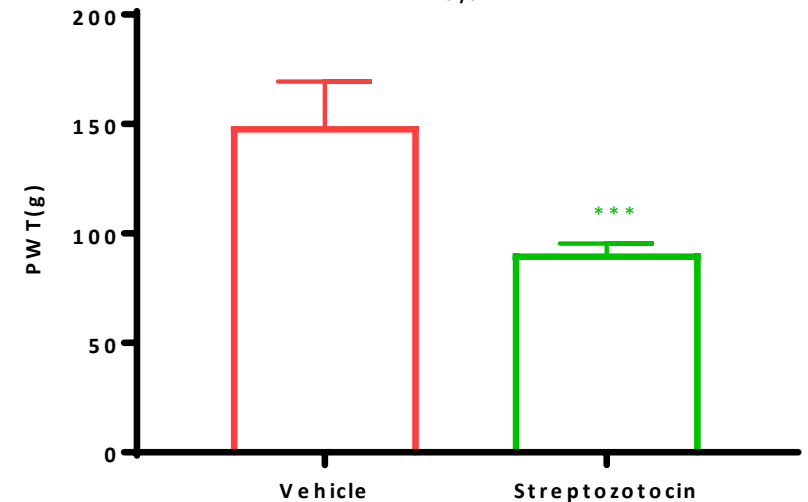
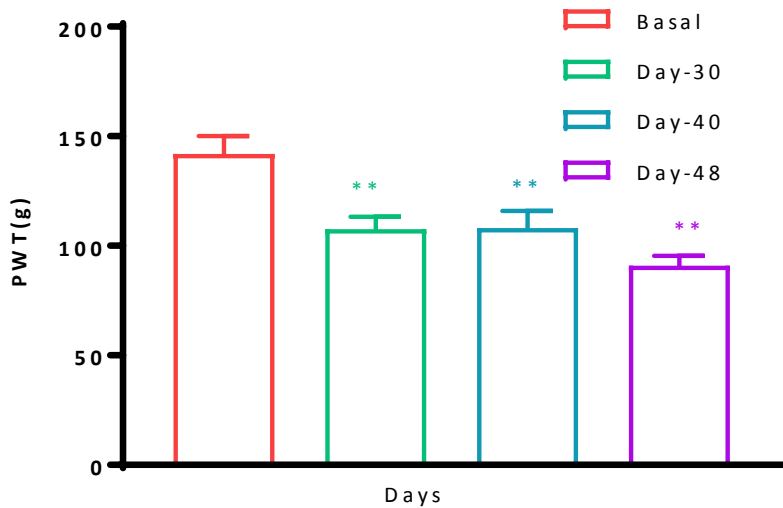
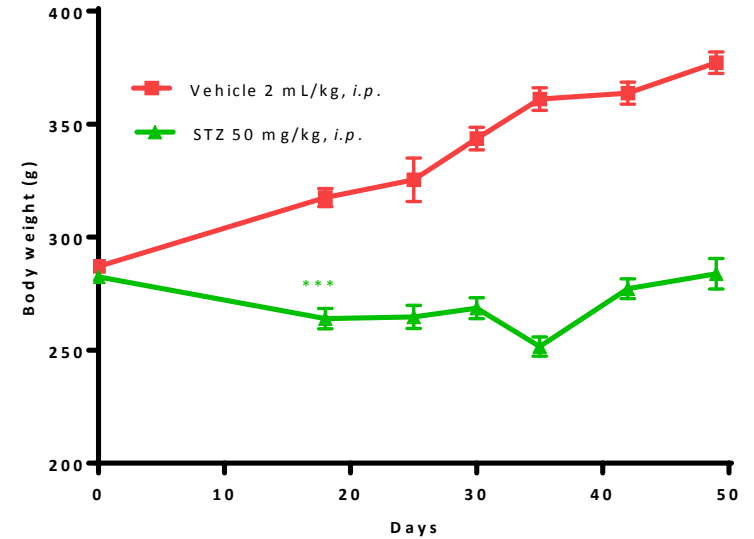
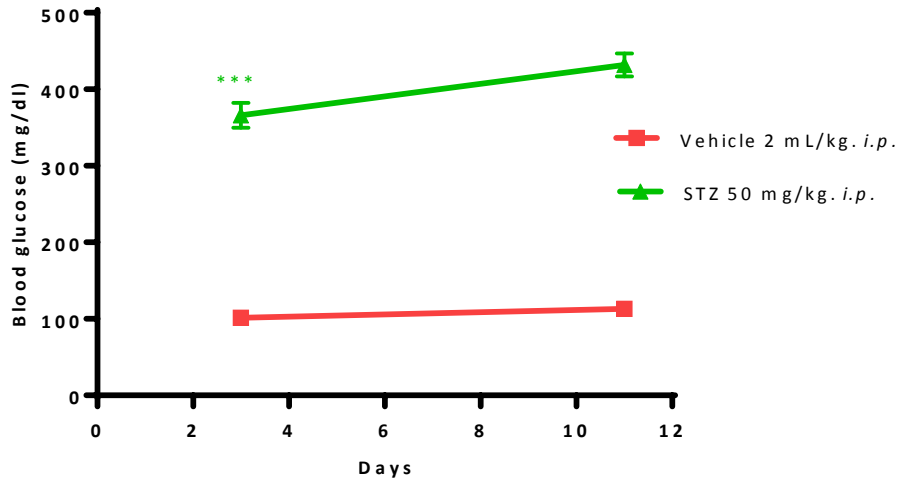
Strain: Wistar

Experimental Procedure:

Basal Paw Withdrawal Thresholds (PWTs) were determined on both paws using Randall-selitto. Animals were randomized based on PWT on day-01. The cut off was set at 250g. Animals were received streptozotocin (STZ) 50 mg/kg, *i.p* or vehicle 2 mL/kg.*i.p*. Diabetes were confirmed on Day-03. Blood glucose >260 mg/dl were considered as hyperglycemic. PWTs were determined on Day-30, 40 and 48 after the STZ administration. Animals were randomized and efficacy was determined predose, 30, 60, 120, and 180 min after the drug administration. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams and Maximum Possible Effect (% M.P.E)

$$\frac{(\text{Post drug thresholds} - \text{Pre-drug thresholds})}{(\text{Cut off- Pre-drug thresholds})} \times 100$$

Streptozotocin induced diabetic neuropathic pain – Validation data

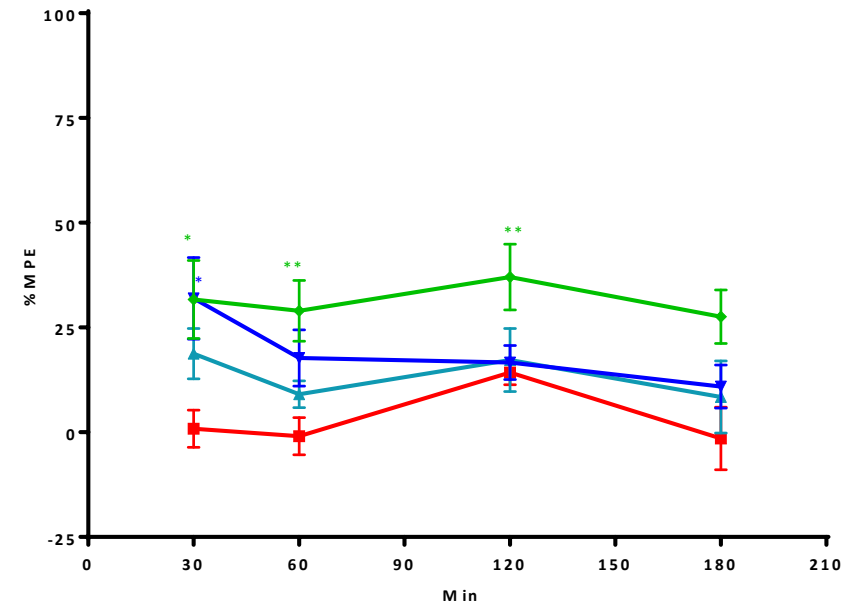
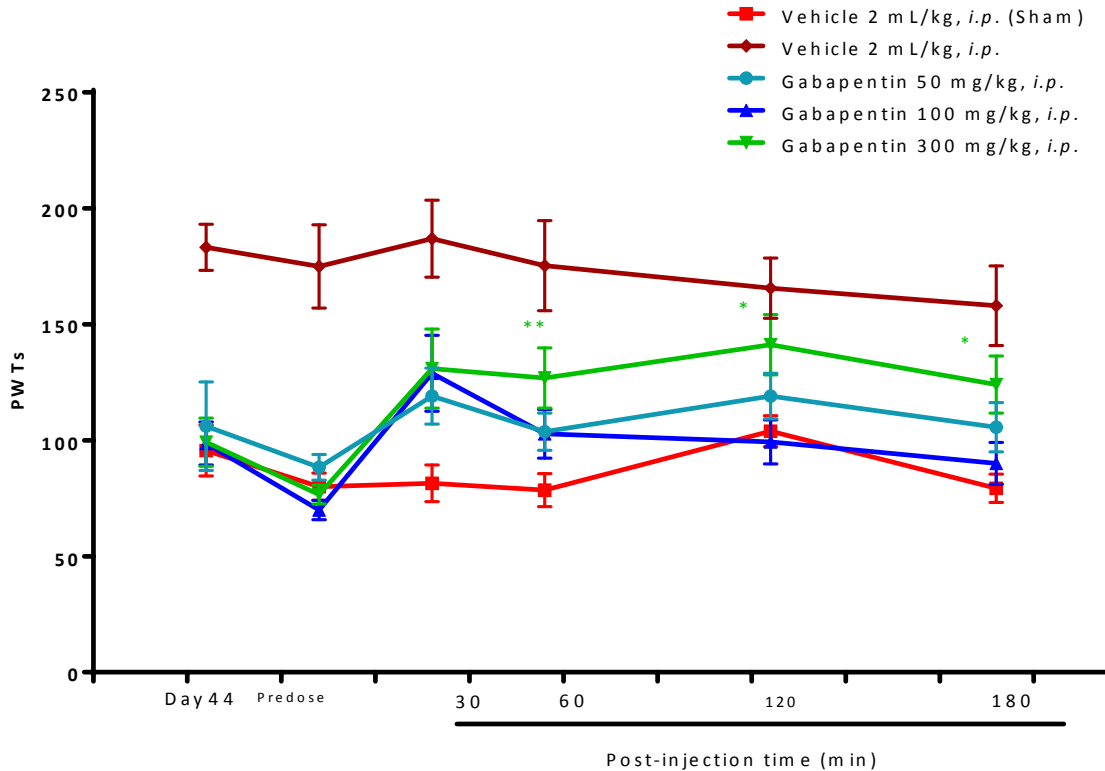


Data represent Mean ± SEM. **p<0.01, *p<0.05 Vs Vehicle

Streptozotocin induced diabetic neuropathic pain – Validation data



Analgesymeter

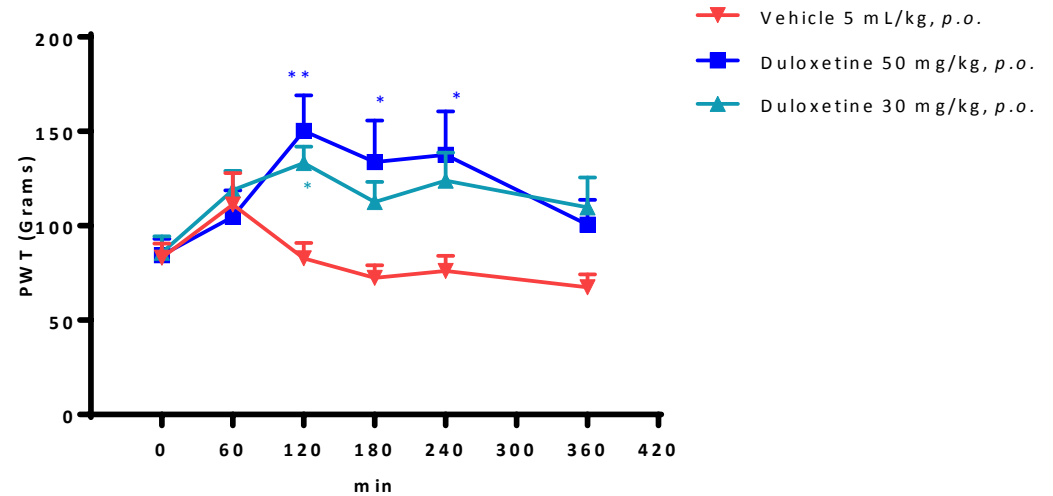
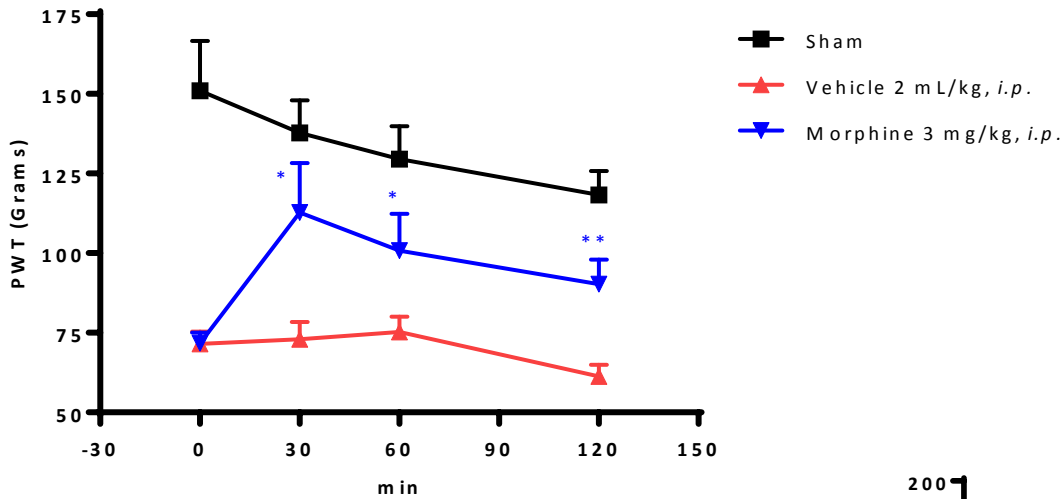


Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Streptozotocin induced diabetic neuropathic pain – Validation data



Analgesymeter



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Spinal nerve (L5) ligation induced neuropathic pain – Study outline



Species: Rat

Strain: Wistar

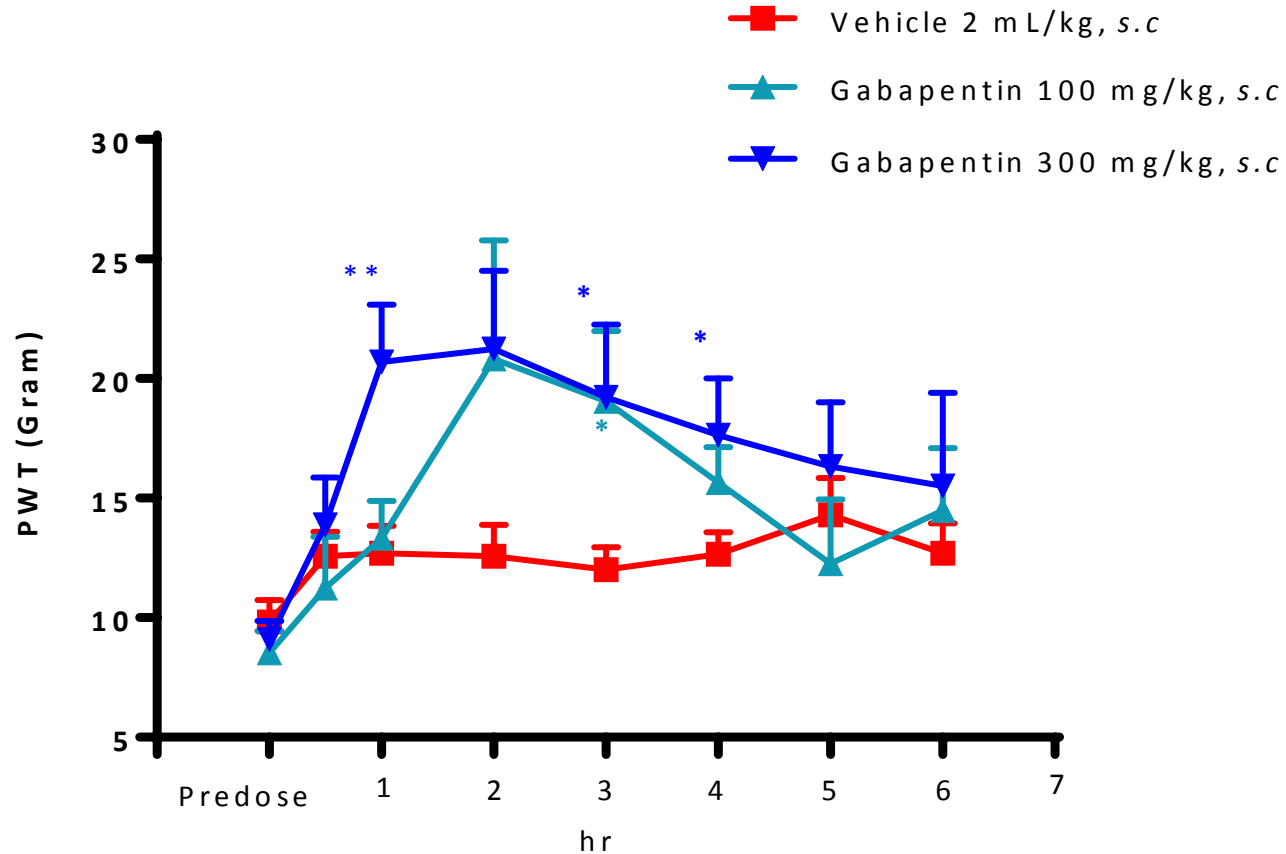
Experimental Procedure:

Rats were anaesthetized and placed in a prone position and the left paraspinal muscles were separated from the spinous processes at the L6-S2 levels. The L6 transverse process carefully removed to identify visually the L4-L6 spinal nerves. The left L5 spinal nerves were isolated and tightly ligated with 6-0 silk thread. Skin was sutured back to close the opened tissue and animals were allowed to recover for at least 1 to 2 weeks before testing. Paw withdrawal thresholds (PWTs) were determined on ipsilateral paw using Dynamic Plantar Aesthesiometer. The cut off was set at 50 g/20 sec. Only rats with threshold scores \leq 12 g were considered allodynia and utilized for testing. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as 50% g threshold.

Spinal nerve (L5) ligation induced neuropathic pain – Validation data



Dynamic Plantar Aesthesiometer

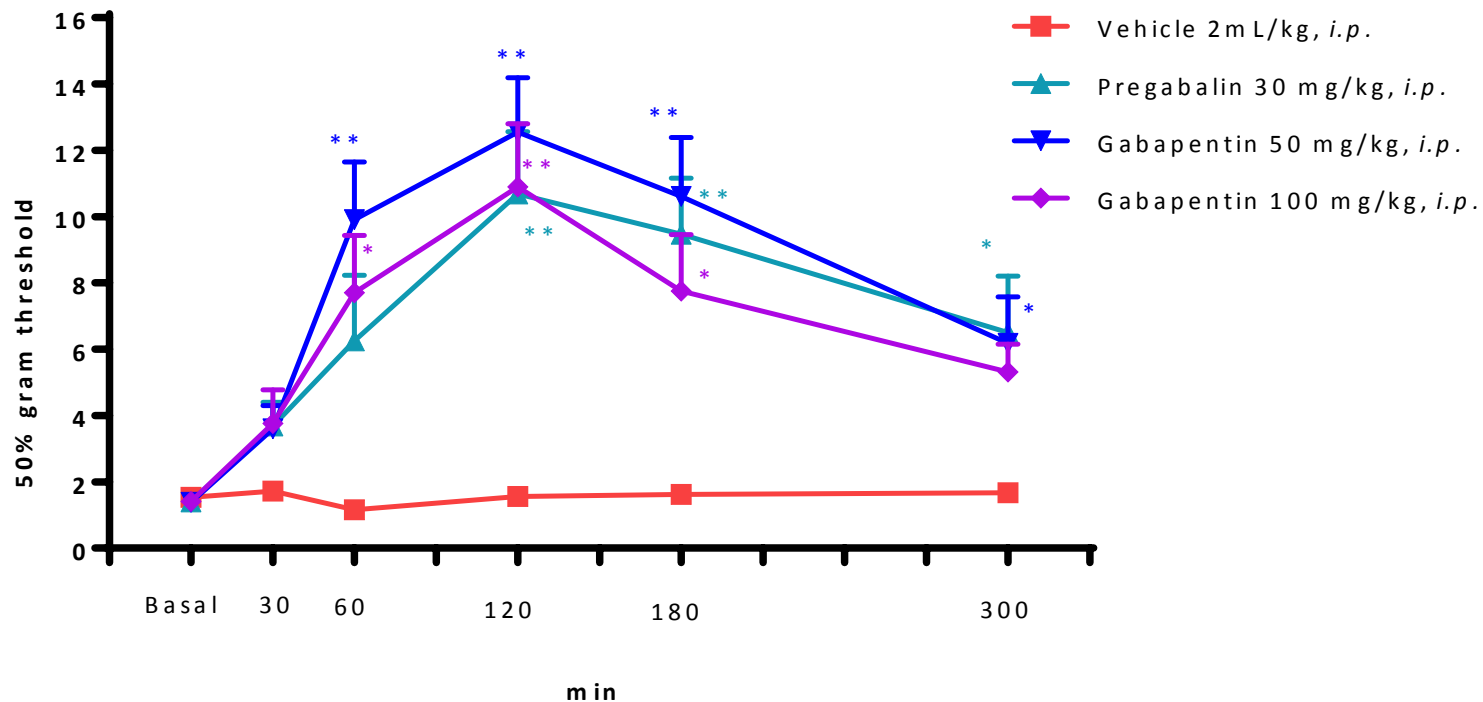


Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Spinal nerve (L5) ligation induced neuropathic pain – Validation data



Von Frey filaments



Data represent Mean \pm SEM. **p<0.01, *p<0.05 Vs Vehicle



Outline

Species: Rat or Mice

Strain: Wistar or Swiss

Experimental Procedure:

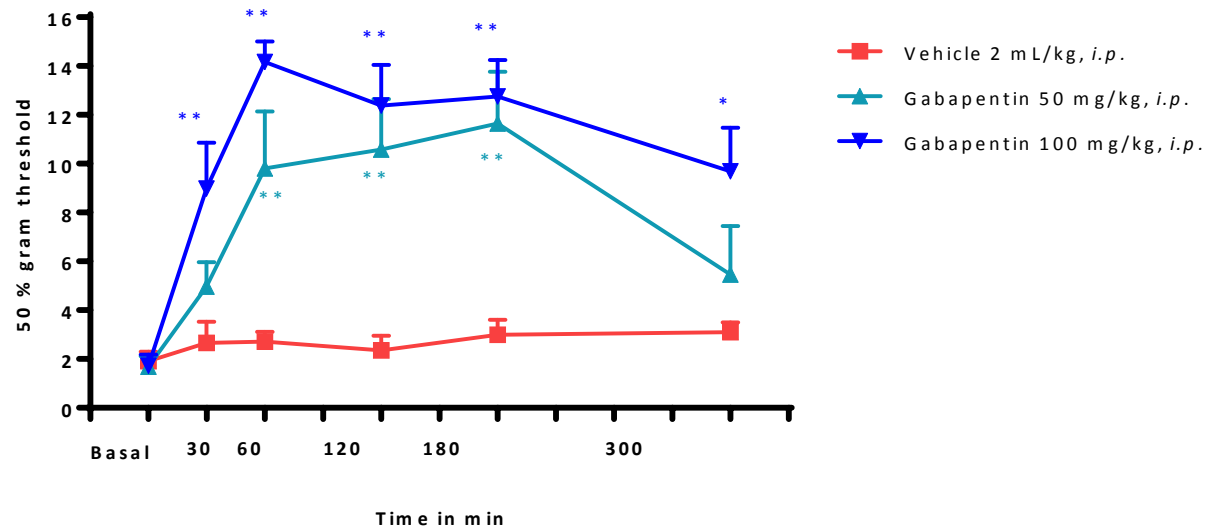
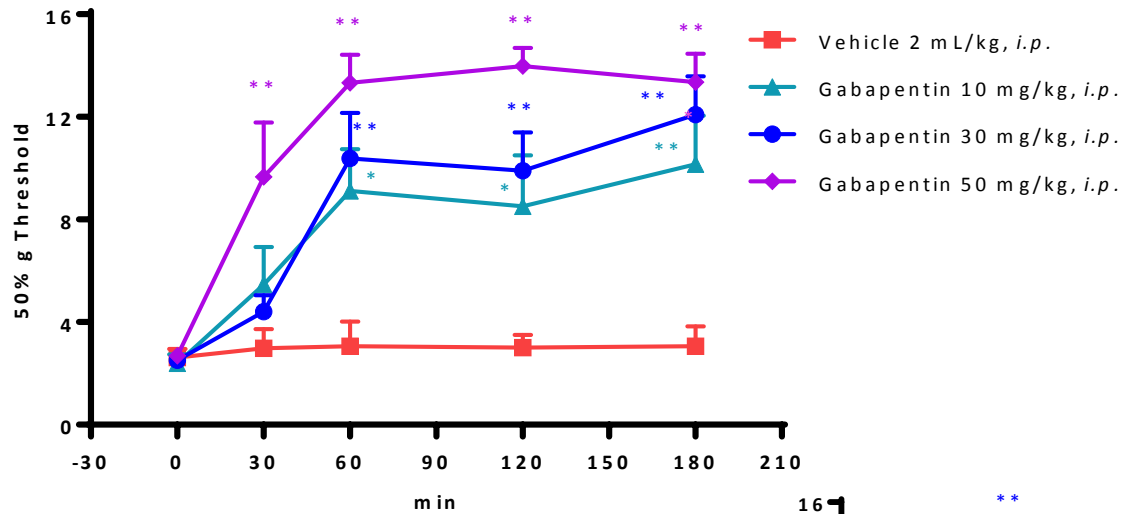
Rats or mice were anaesthetized. Sciatic nerve was exposed by a blunt cut at the mid thigh region of the left hind limb and then four loose ligatures (chromic cat gut) were tied around the sciatic nerve. Skin was sutured back to close the opened tissue and rats were allowed to recover from surgery for 1-2 weeks. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as 50% g threshold.

Chronic constricted injury (CCI) induced neuropathic pain (rats)



– Validation data

Von Frey filaments



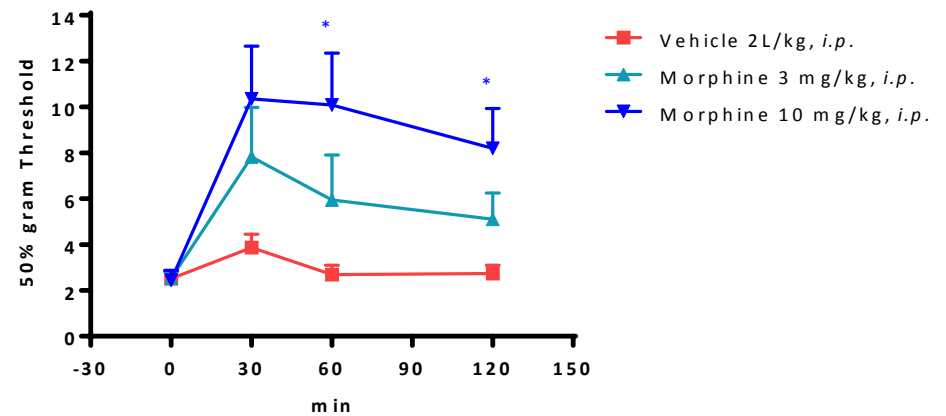
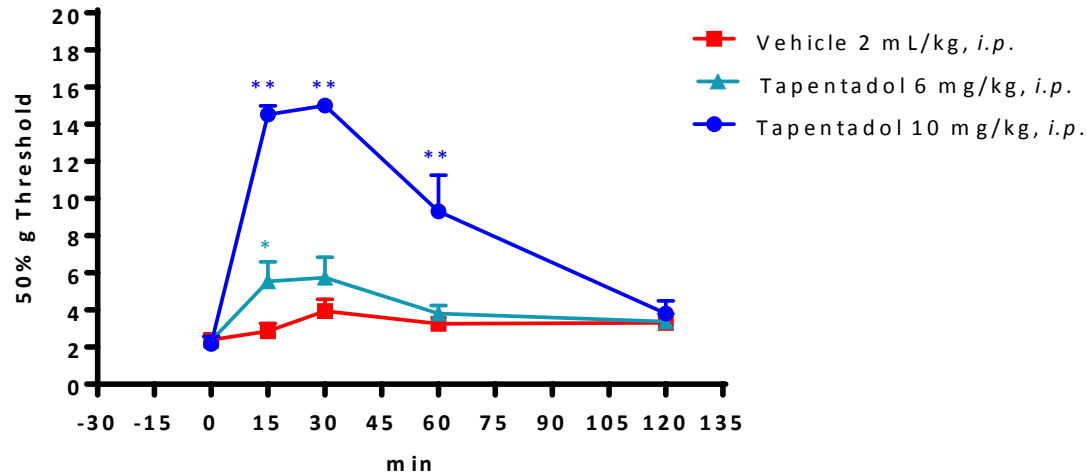
Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Chronic constricted injury (CCI) induced neuropathic pain (rats)



– Validation data

Von Frey filaments



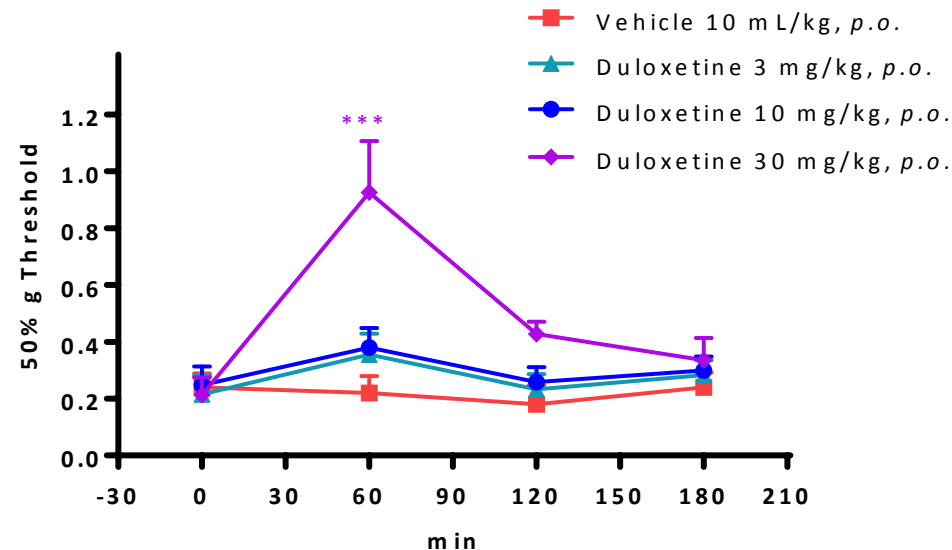
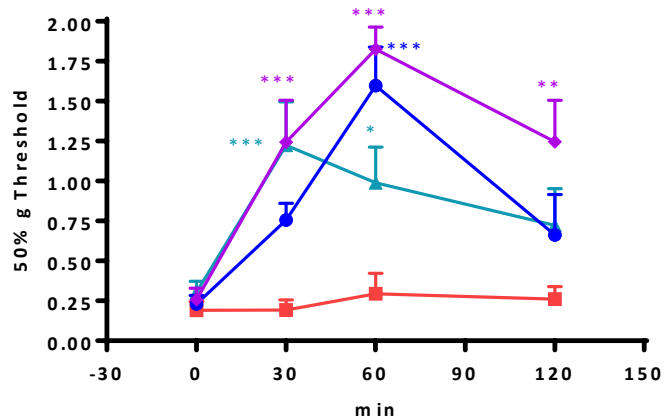
Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Chronic constricted injury (CCI) induced neuropathic pain (mice)



– Validation data

Von Frey filaments



Data represent Mean \pm SEM. *** p <0.001 ** p <0.01, * p <0.05 Vs Vehicle

Partial sciatic nerve ligation (PSNL) induced neuropathic pain



– Study Outline

Species: Rat or Mice

Strain: Wistar or Swiss

Experimental Procedure:

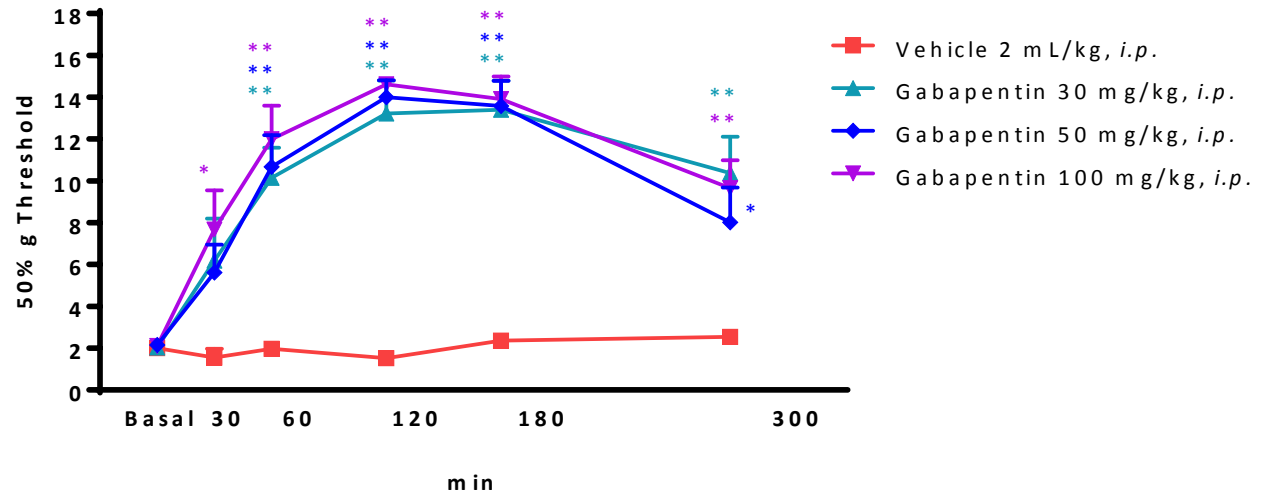
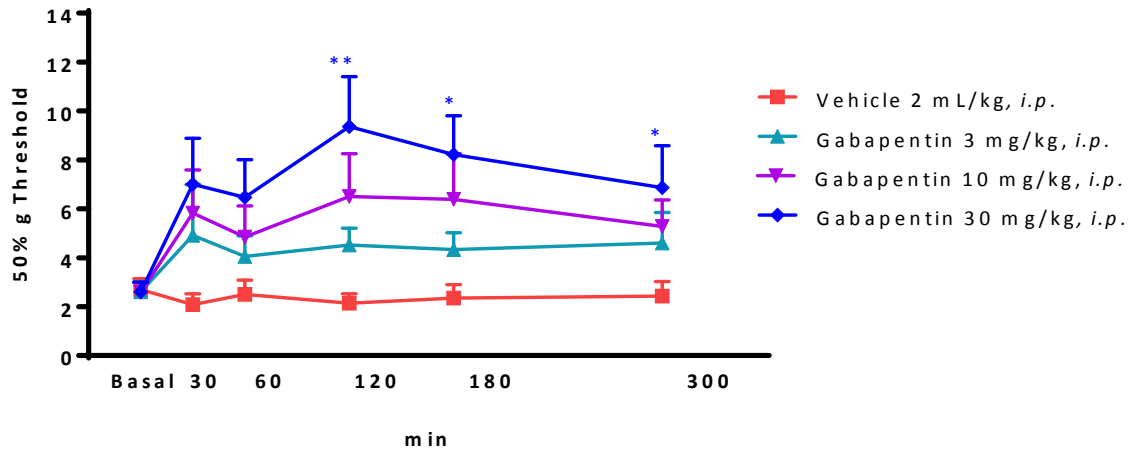
Rats or mice were anaesthetized using pentobarbitone. Sciatic nerve was exposed by a blunt cut at the upper thigh region of the left hind limb and then 1/3 to 3/4 of sciatic nerve was ligated with silk thread 6-0. Skin was sutured back to close the opened tissue and rats were allowed to recover from surgery for 1-2 weeks. Mechanical hyperalgesia was assessed using Analgesymeter. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as 50% g threshold.

Partial sciatic nerve ligation (PSNL) induced neuropathic pain (rats)



– Validation data

Von Frey filaments



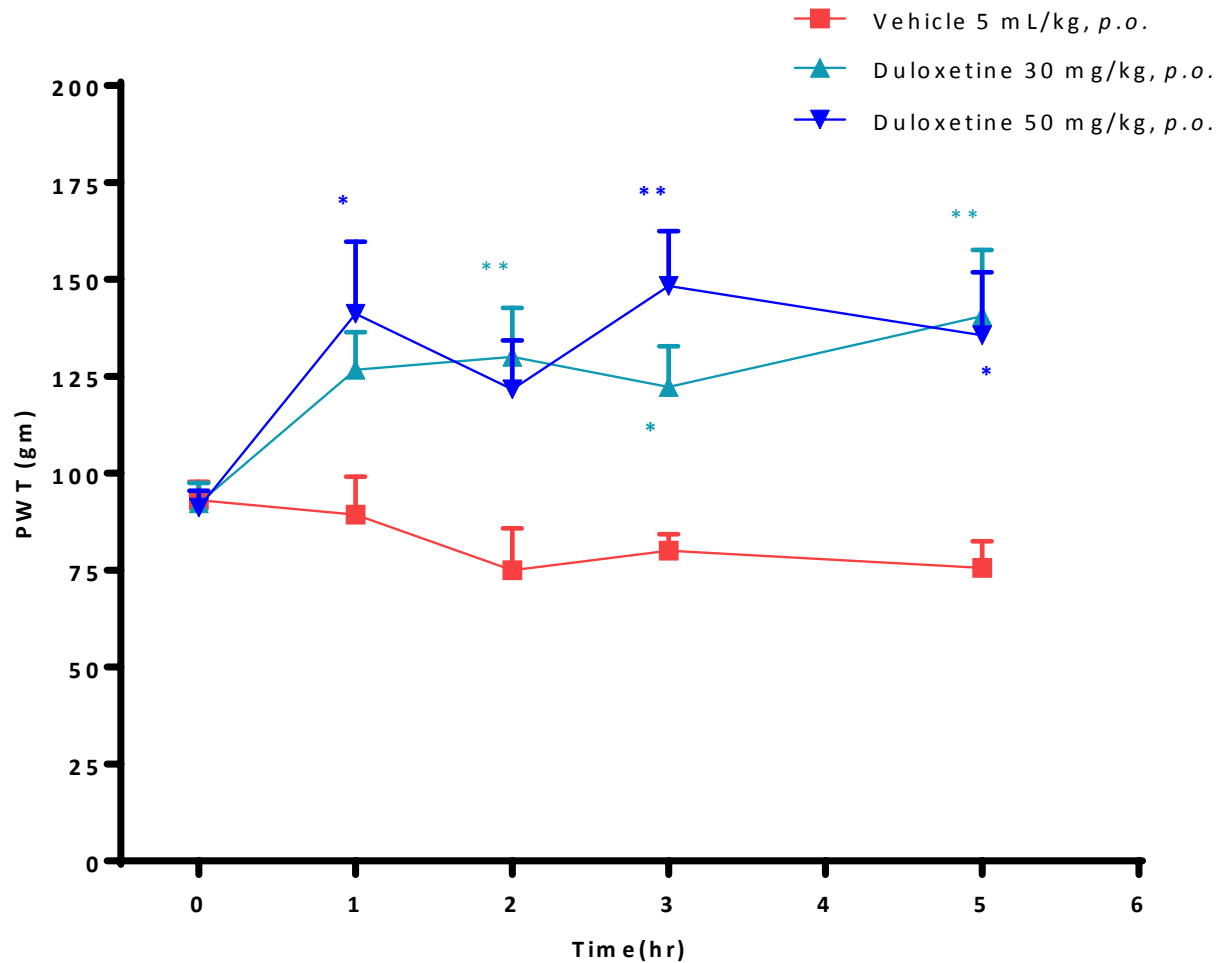
Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Partial sciatic nerve ligation (PSNL) induced neuropathic pain (rats)



– Validation data

Analgesymeter



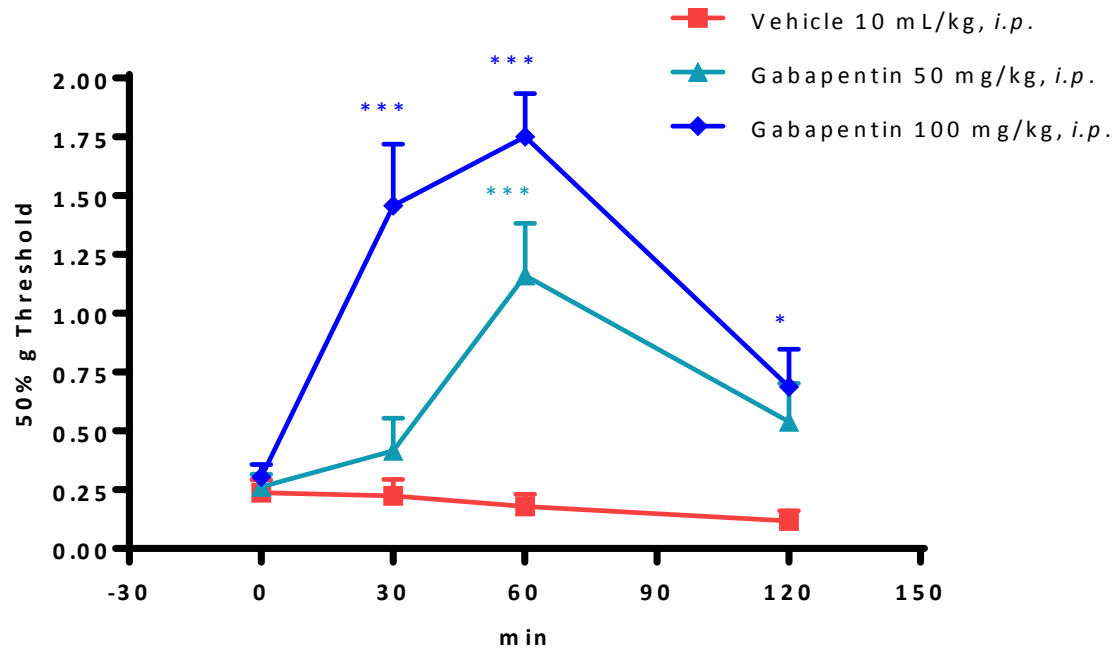
Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Partial sciatic nerve ligation (PSNL) induced neuropathic pain (mice)



Validation data

Von Frey filaments



Data represent Mean \pm SEM. *** $p < 0.001$, * $p < 0.05$ Vs Vehicle



Outline

Species: Rat

Strain: Wistar

Experimental Procedure:

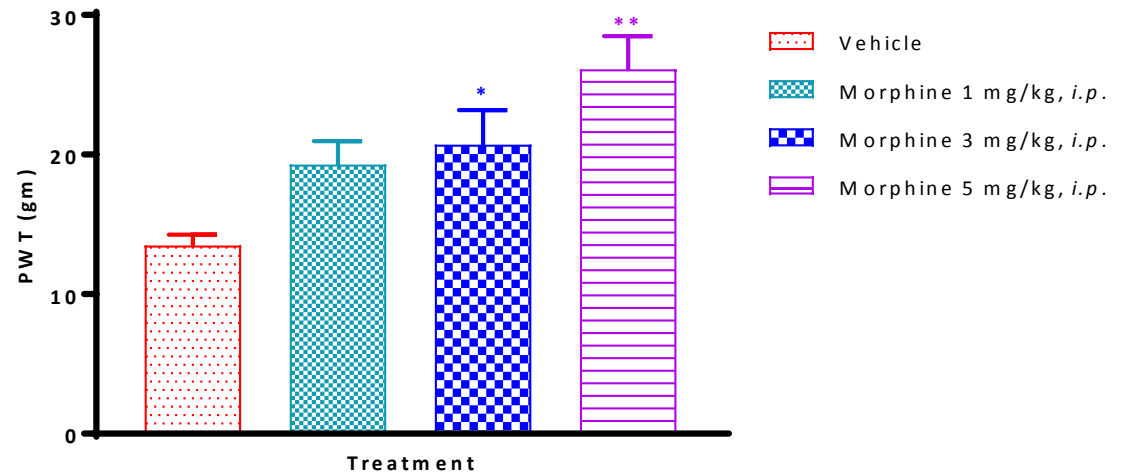
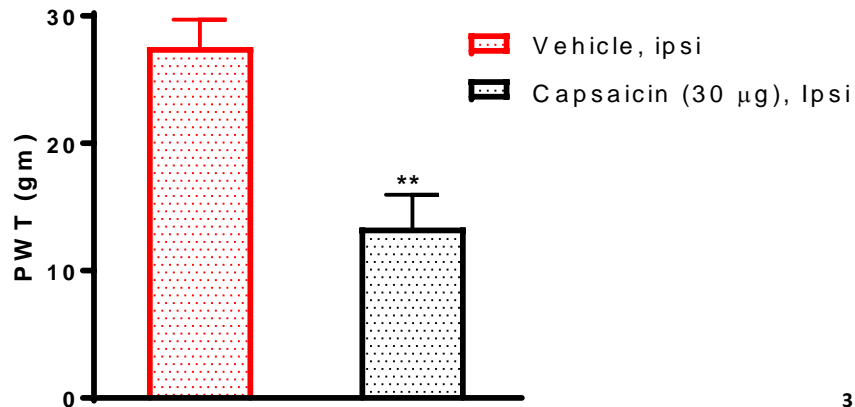
Rats injected with Capsaicin 30 μ g/ μ L intra-dermally in the hind paw and after 15 min interval paw withdrawal threshold was recorded. Rats were placed on an elevated screen in a clean testing chamber and allowed to acclimate to the testing environment before any measurement were taken. To assess mechanical allodynia paw withdrawal threshold (PWT) to a non-noxious tactile stimuli were determined using an automated Von-Frey device. The electronic von-Frey device employs a single non flexible filament which applies an increasing force (0 to 50 gm) in the plantar surface over a period of 20 sec. Vehicle or compound was administered intraperitoneally 15 min before capsaicin injection. The endpoint was taken as a nocifensive paw withdrawal. Data were expressed as withdrawal threshold in grams.

Capsaicin induced secondary mechanical allodynia in rats—



Validation data

Dynamic Plantar Aesthesiometer



Data represent Mean ± SEM. **p<0.01, *p<0.05 Vs Vehicle

Mono iodoacetate (MIA) induced osteoarthritis – Study Outline



Species: Rat

Strain: Wistar

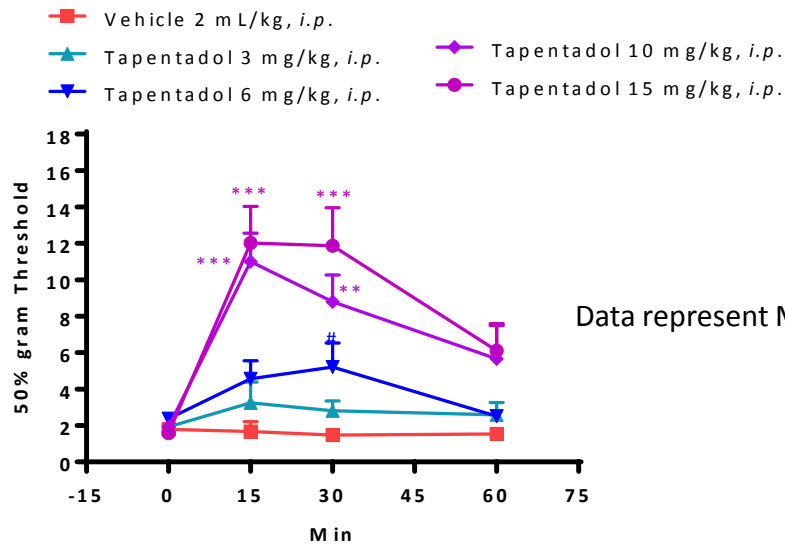
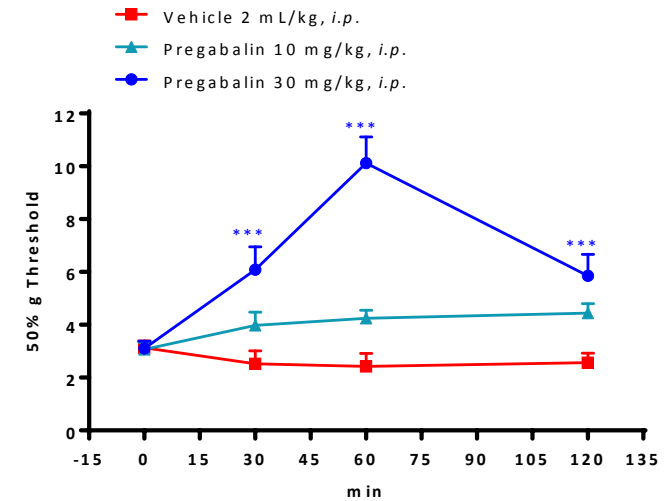
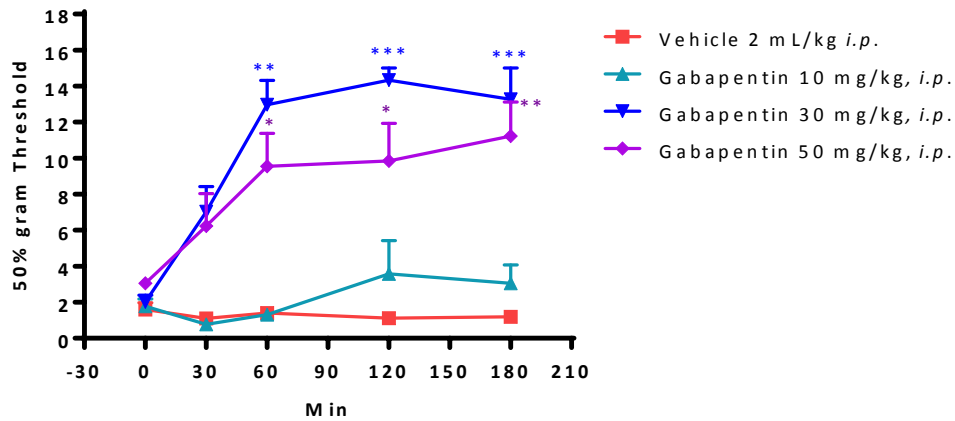
Experimental Procedure:

Rats were anesthetized using isoflurane and mono iodoacetate (MIA) was injected into left hind knee at a dose of 2mg/rat *i.a.* Two weeks after administration of MIA, paw withdrawal thresholds (PWTs) were measured on left hind paw (ipsilateral paw) using Von Frey Monofilaments. Animals which showed PWTs $\leq 4g$ were selected. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Paw withdrawal thresholds (PWTs) were determined on ipsilateral paw using Dynamic Plantar Aesthesiometer. The cut off was set at 50 g/20 sec. Only rats with threshold scores ≤ 12 g were considered allodynia and utilized for testing. Paw withdrawal thresholds (PWTs) were determined on ipsilateral paw using Analgesy meter. Animals which showed PWTs $\leq 140g$ were selected.

Mono iodoacetate (MIA) induced osteoarthritis (mechanical allodynia) – Validation data



Von Frey filaments

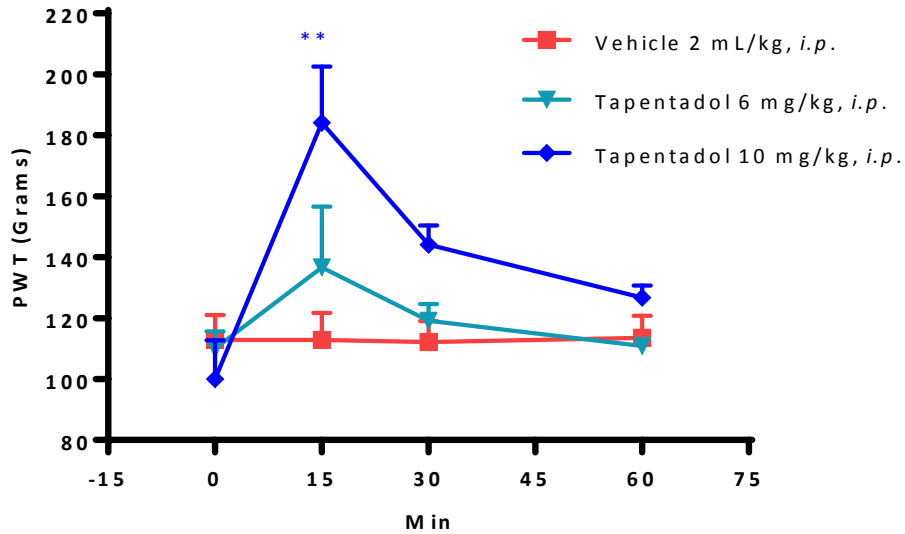


Data represent Mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 Vs vehicle

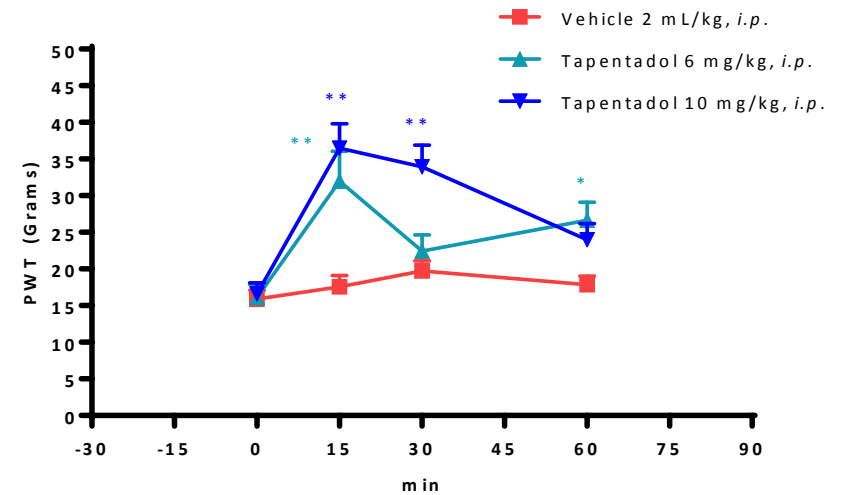
Mono iodoacetate (MIA) induced osteoarthritis (mechanical allodynia) – Validation data



Analgesymeter



Dynamic Plantar Aesthesiometer



Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Complete Freund's adjuvant (CFA) induced rheumatoid arthritis –



Study Outline

Species: Rat

Strain: Wistar

Experimental Procedure:

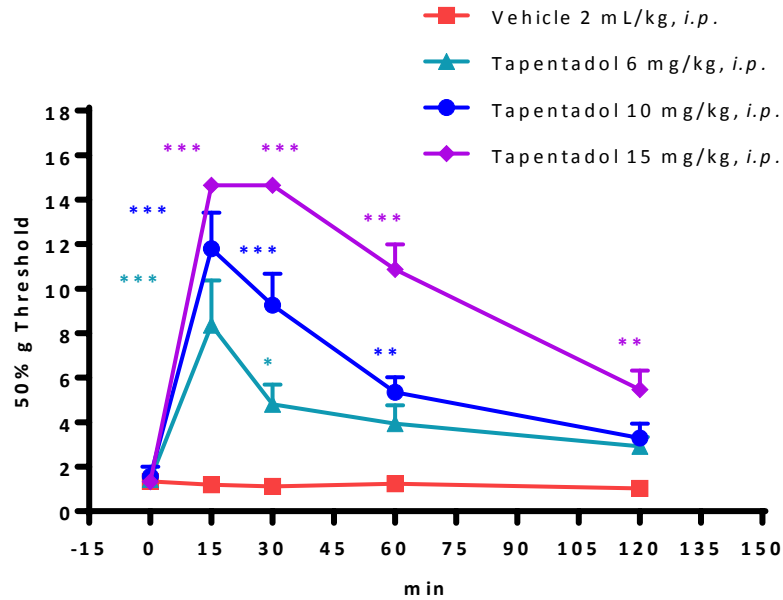
Rats were anesthetized using isoflurane and received Complete Freund's adjuvant into left hind knee at a dose of 100 μ L/rat *i.a.* After two weeks of CFA administration paw withdrawal thresholds (PWTs) were measured on left hind paw (ipsilateral paw) using Von Frey Monofilaments. Animals which showed PWTs \leq 4g were selected. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as 50% withdrawal threshold in grams

Complete Freund's adjuvant (CFA) induced rheumatoid arthritis –

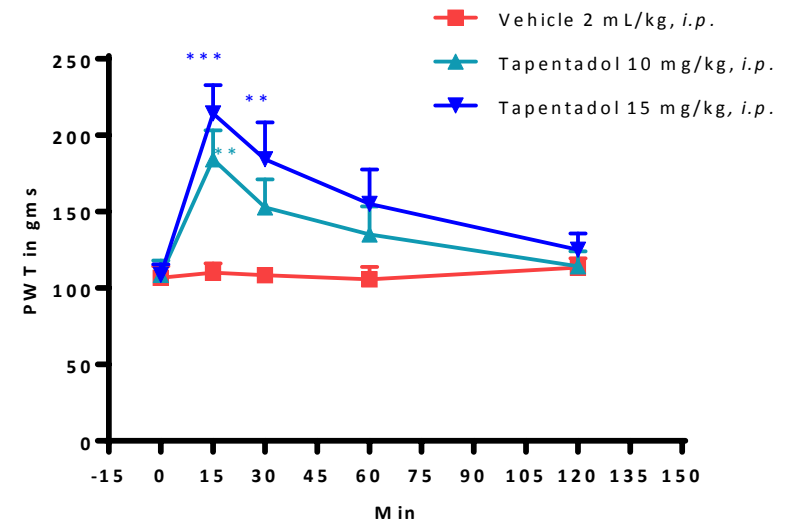


Validation data

Von Frey Monofilaments



Analgesymeter



Data represent Mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ Vs Vehicle

Medial meniscal tear (MMT) induced osteoarthritis – Study Outline



Species: Rat

Strain: Wistar

Experimental Procedure:

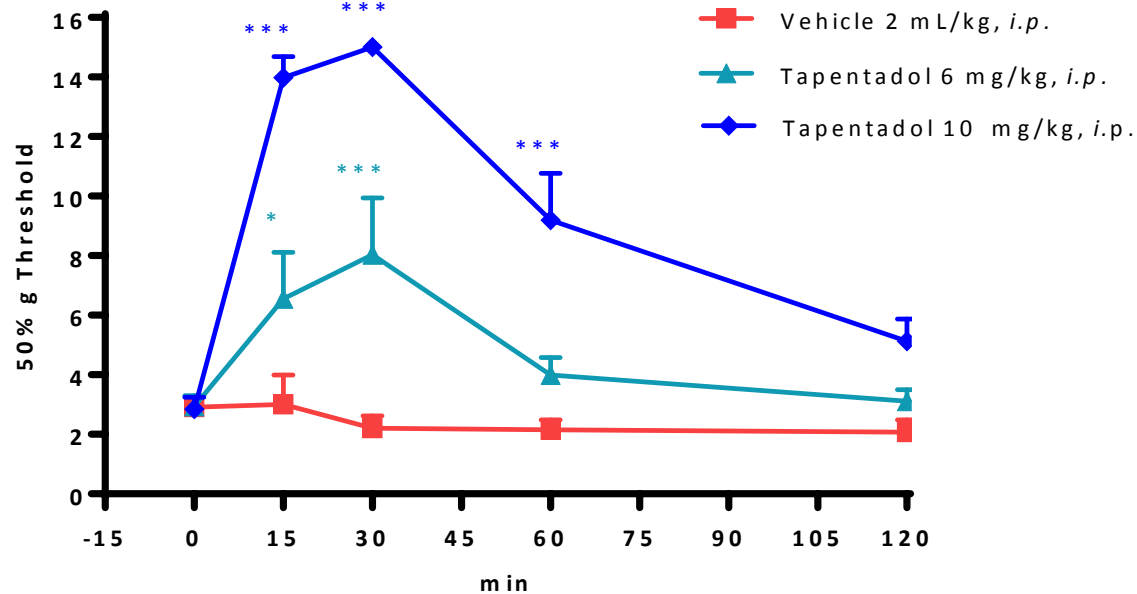
Rats were anaesthetized with pentobarbitone 35mg/kg i.p. The knee joint region of left leg a small cut was given and medial collateral ligament was transected, then medial meniscal tear (MMT) was performed .The opened tissue and skin was sutured back to recovery . After four weeks of MMT surgery paw withdrawal thresholds (PWTs) were measured on left hind paw (ipsilateral paw) using Von Frey Monofilaments. Animals which showed PWTs $\leq 4g$ were selected. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams.

Medial meniscal tear (MMT) induced osteoarthritis – Validation



data

Von Frey Monofilaments



Data represent Mean \pm SEM. *** p <0.001, ** p <0.01, * p <0.05 Vs Vehicle

Reserpine induced myalgia in rats – Study Outline



Species: Rat

Strain: Wistar

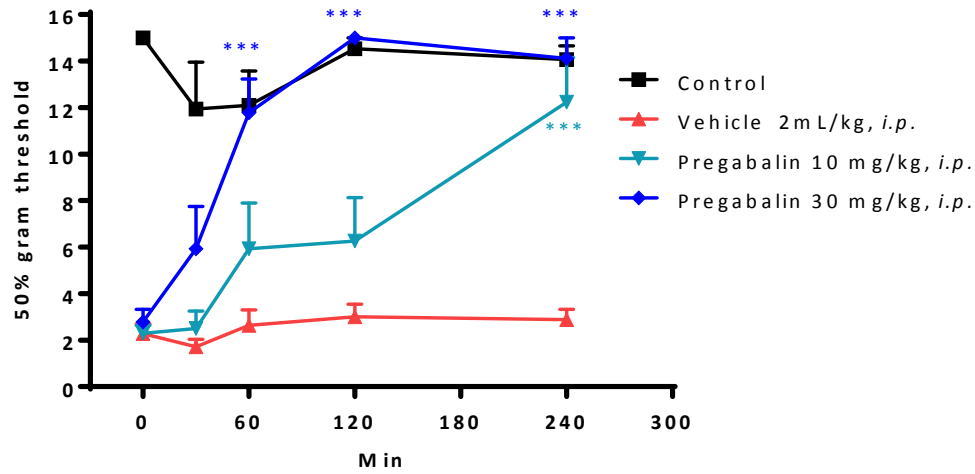
Experimental Procedure:

Rats were anaesthetized with pentobarbitone 35mg/kg i.p. The knee joint region of left leg a small cut was given and medial collateral ligament was transected, then medial meniscal tear (MMT) was performed .The opened tissue and skin was sutured back to recovery . After four weeks of MMT surgery paw withdrawal thresholds (PWTs) were measured on left hind paw (ipsilateral paw) using Von Frey Monofilaments. Animals which showed PWTs ≤ 4 g were selected. Mechanical allodynia was assessed using Von Frey filaments according to the Dixon up and down method. Experimenter was blinded to treatment groups. Data were expressed as withdrawal threshold in grams.

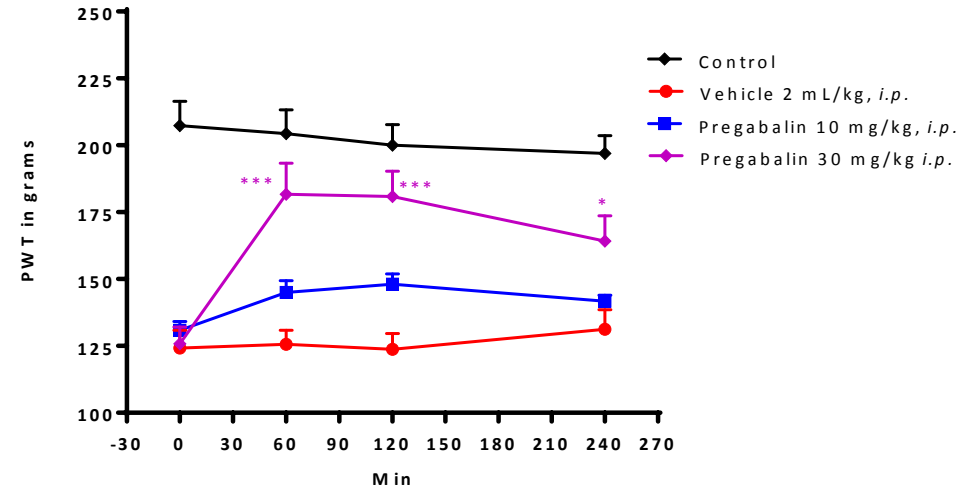


Reserpine induced myalgia in rats – Validation data

Von Frey Monofilaments



Analgesymeter



Data represent Mean \pm SEM. *** $p < 0.001$, * $p < 0.05$ Vs Vehicle

Chronic post-ischemia pain (CPIP) – Study Outline



Species: Rat

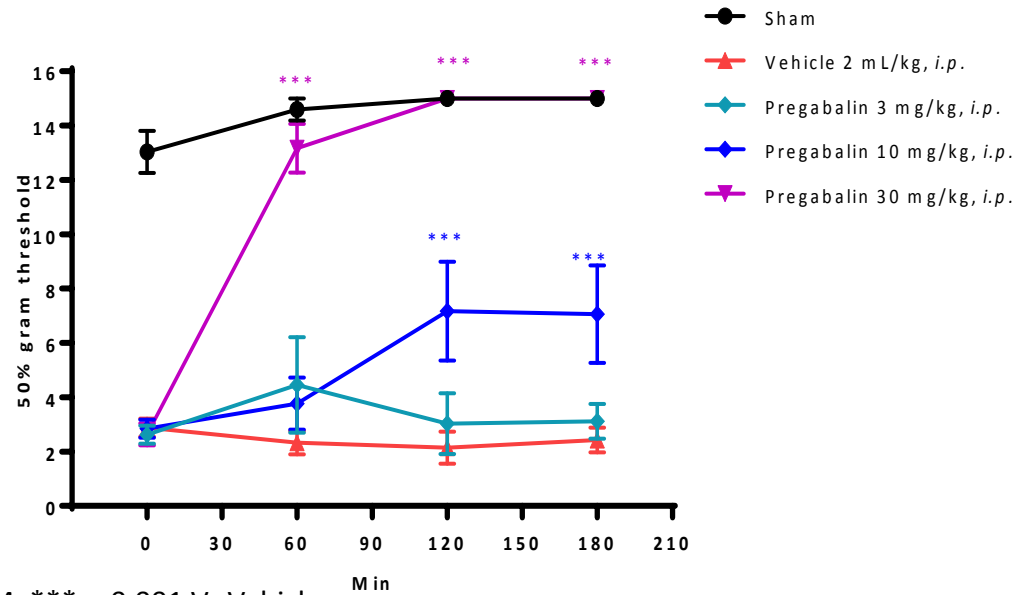
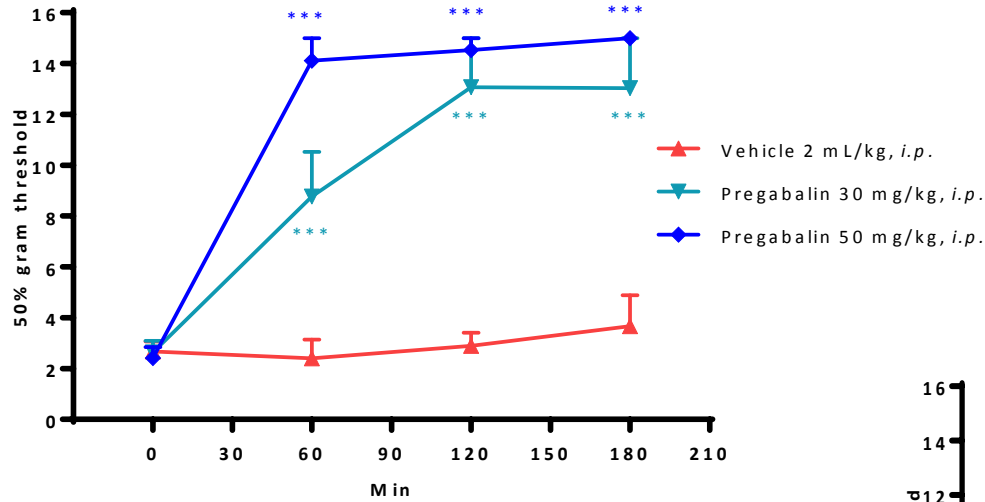
Strain: Wistar

Experimental Procedure:

Rats (300-350g) were anesthetized over a 3 h period with a bolus (250mg/kg, i.p.) followed by substitute dose of 35mg/kg, i.p. after 90min of bolus dose of Avertin. After induction of anesthesia, a O-ring with 5 mm internal diameter was placed around the rat's left hindlimb proximal to the ankle joint. The tight-fitting of O-ring produces a complete blockade of arterial blood flow, then ring was left in place for 3 h, and rats were allowed to recover from anesthesia after reperfusion. Sham rats were anesthetized for the same period and had a cut (loose) O-ring placed around their ankles (Each rat received 40% w/v dextrose of 1ml intraperitoneal injections at basal, 60 and 120 min after the induction of ischemia). Rats were tested for allodynia condition on day 2, 4 and 6 using Von Frey monofilaments. Rats which showed paw withdrawal threshold of ≤ 4 grams were selected for testing. Animals selected for allodynia condition were divided in to three groups (n=6), received vehicle, pregabalin 30 or 50mg/kg. Rats tested for analgesic activity of pregabalin at 60, 120 and 180 Min post treatment.



Chronic post-ischemia pain (CPIP) – Study Outline



Data represent Mean \pm SEM. ***p<0.001 Vs Vehicle



Outline

Species: Rat

Strain: Wistar

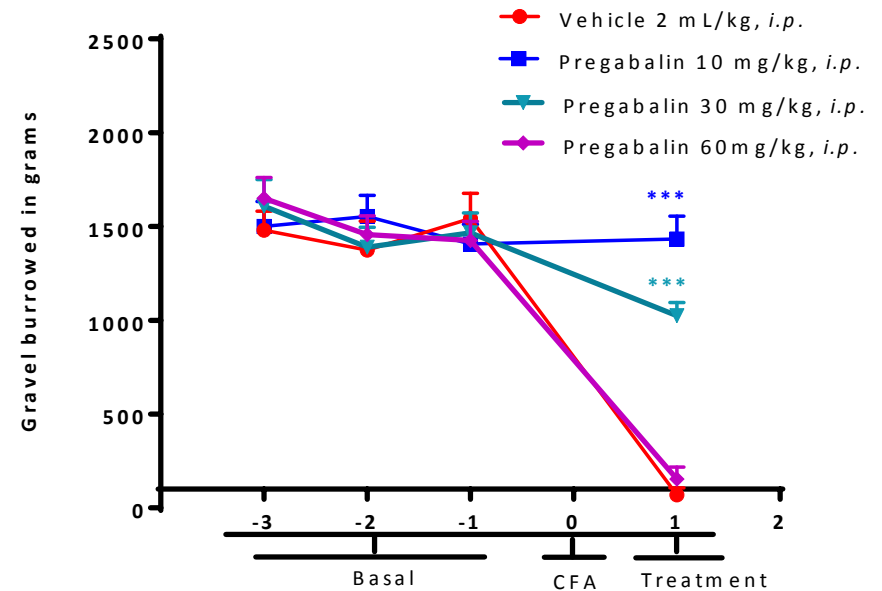
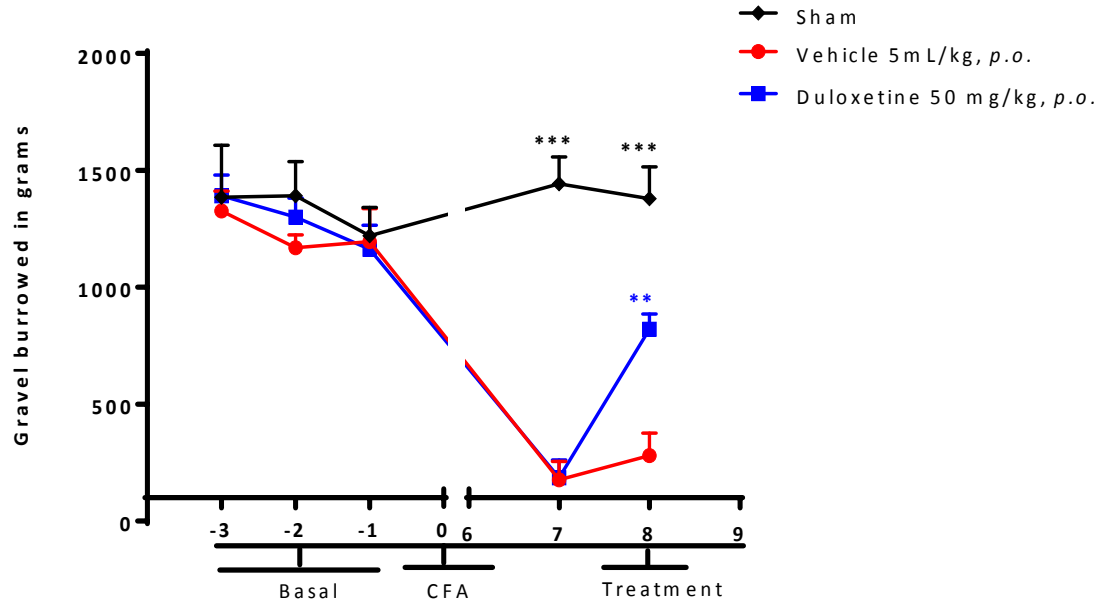
Experimental Procedure:

On day-1 pairs of rats were habituated to empty cage for 1 hr. A burrowing tube which was filled with 2.5 kg gravel was placed in the cage, and the rats were allowed to burrow for 2 hrs. The same procedure was repeated on second day of training. In case if rats burrowed less than 90% of burrowing substrate, technique of social facilitation was used i.e. if any rat pair did not burrow at all or burrow <90%, then one rat from this pair was swapped with a rat from a pair that burrowed >90%. Pairs of rats which show around 90% of burrowing were selected. Rats were allowed to burrow for 2 hrs after 1 hr habituation. 2 hrs later, amount of burrowed gravel was calculated. Similar procedure was done for three days. Rats that burrowed on average <1000 g or had a standard deviation (SD) >450 g after the three individual training days were excluded from the experiment. On the day of testing, rats were individually placed in the empty cages (same as used for training) for habituation. Test compounds or vehicle were administered prior to habituation. After 1 hr habituation, the rats were allowed to burrow for 2 hrs. At the end of the test period, the leftover gravel in the burrow was weighed and the amount of the displaced gravel calculated. At the end of the experimentation all rats were returned to their home cages.

Spontaneous burrowing behavior in CFA induced arthritis



(rats) – Validation data

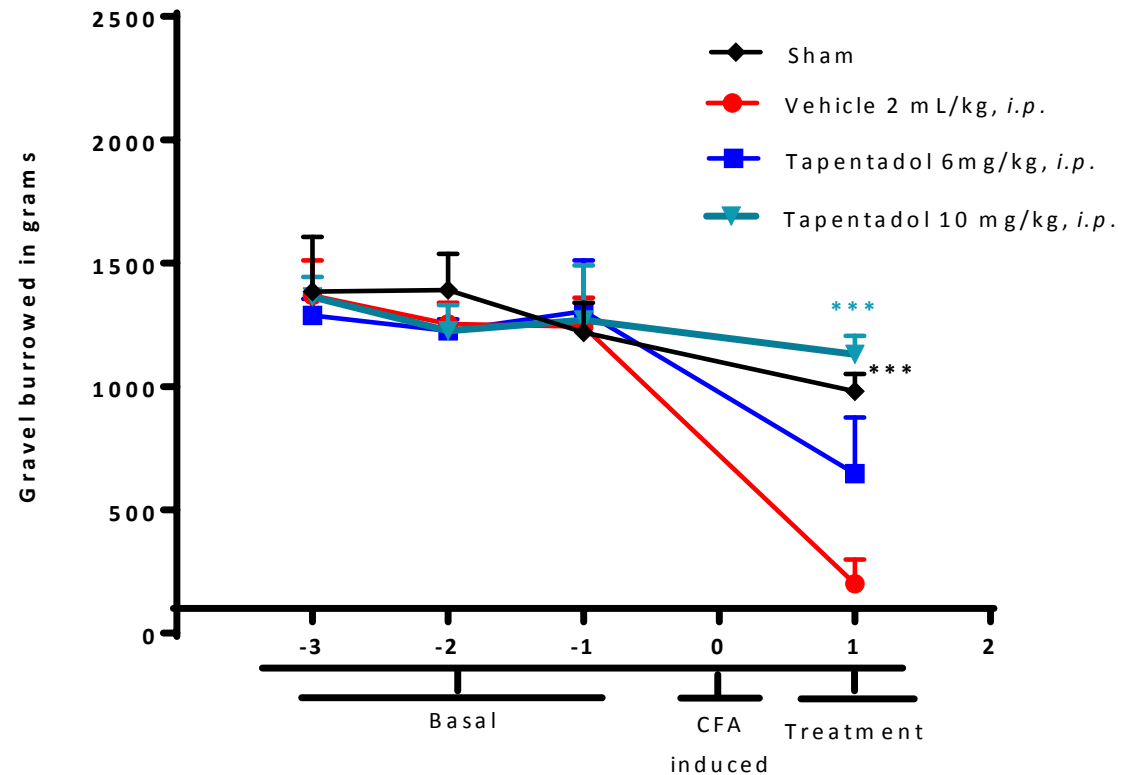


Data represent Mean ± SEM. ****p*<0.001, ***p*<0.01 Vs Vehicle

Spontaneous burrowing behavior in CFA induced arthritis



(rats) – Validation data

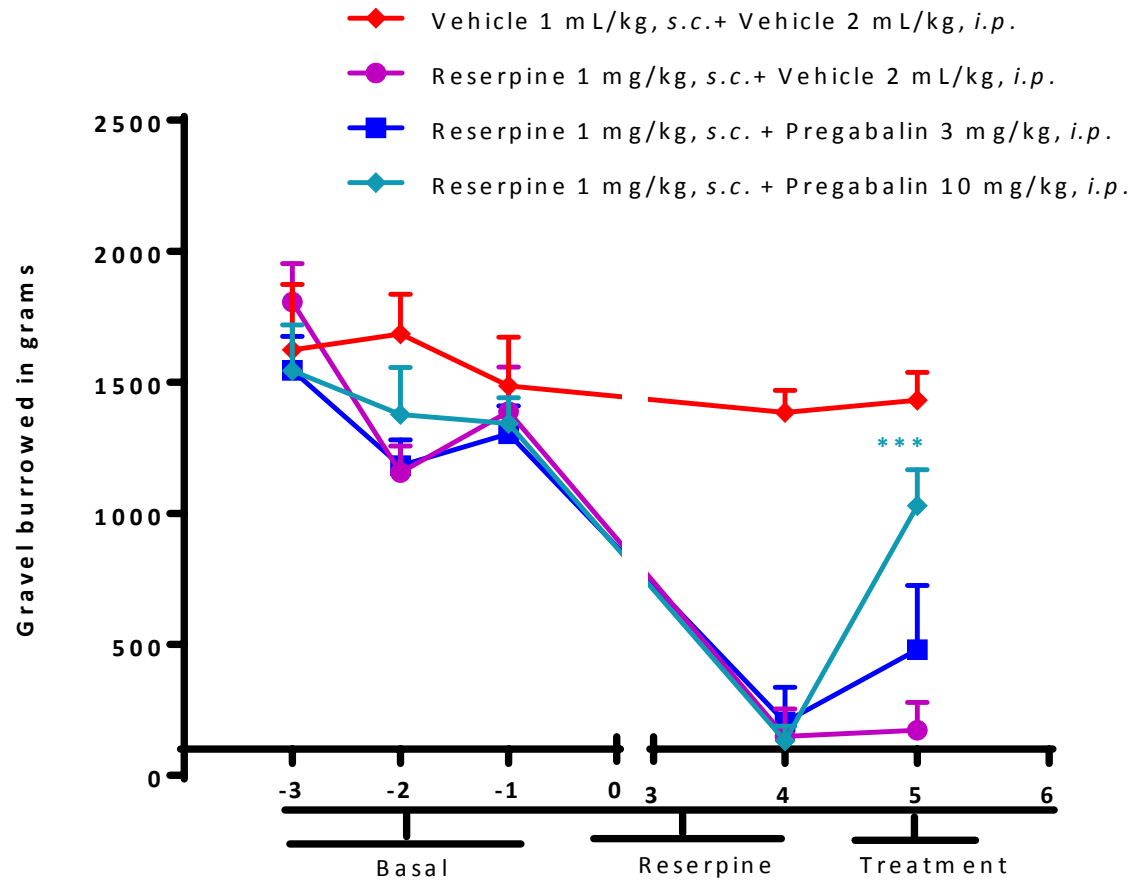


Data represent Mean \pm SEM. *** p <0.001, ** p <0.01 Vs Vehicle

Spontaneous burrowing behavior in reserpine induced myalgia



(rats)– Validation data



Data represent Mean \pm SEM. *** $p < 0.001$ Vs Vehicle

Arachidonic acid induced ear odema in mice – Study Outline



Species: Mice

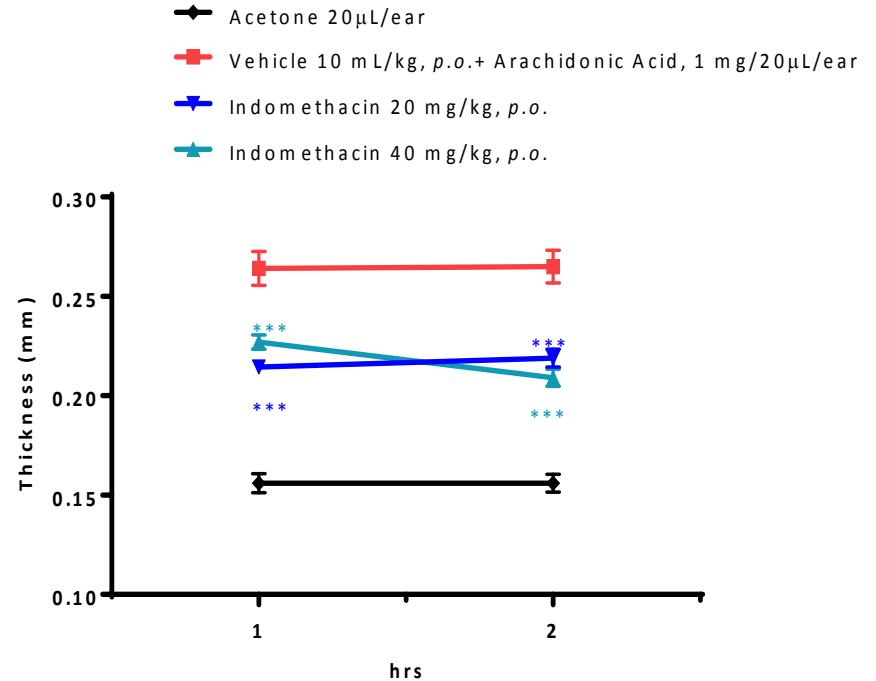
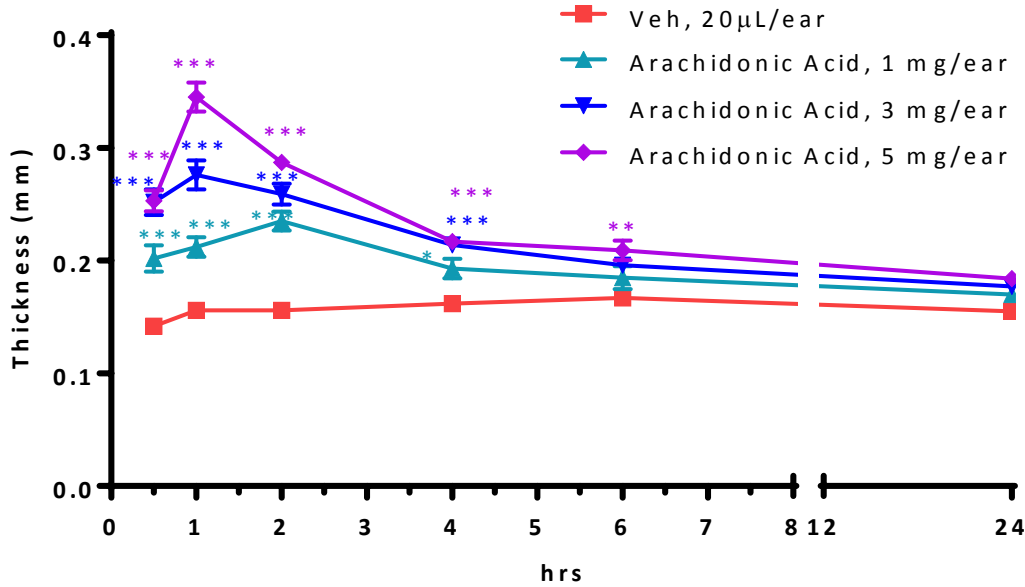
Strain: Swiss

Experimental Procedure:

Arachidonic acid (1 mg/ 20 μ L/ear) was applied topically inside and out side of the right ear of mice. Ear thickness was measured using automated vernier caliper. Ear thickness was recorded. Indomethacin was administered 60 min prior to the arachidonic acid.



Arachidonic acid induced ear odema in mice – Validation data



Data represent Mean ± SEM. ***p<0.001, **p<0.01 Vs Vehicle

In-vivo electrophysiology in rats – Study Outline



Species: Rat

Strain: Wistar

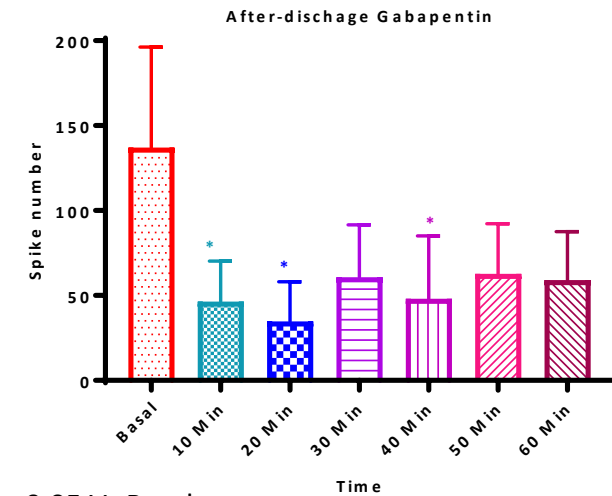
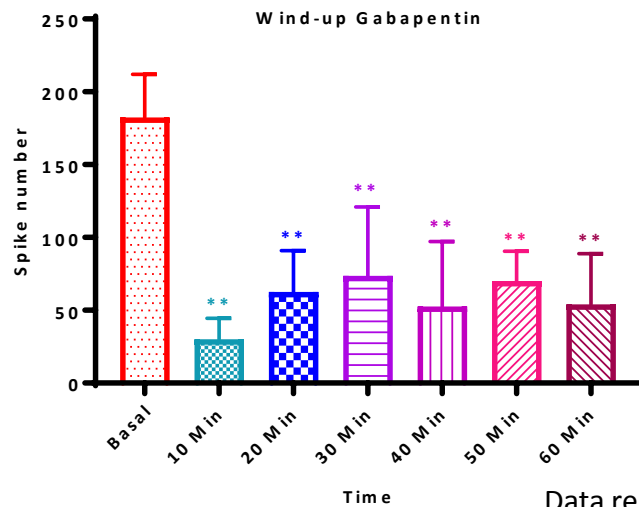
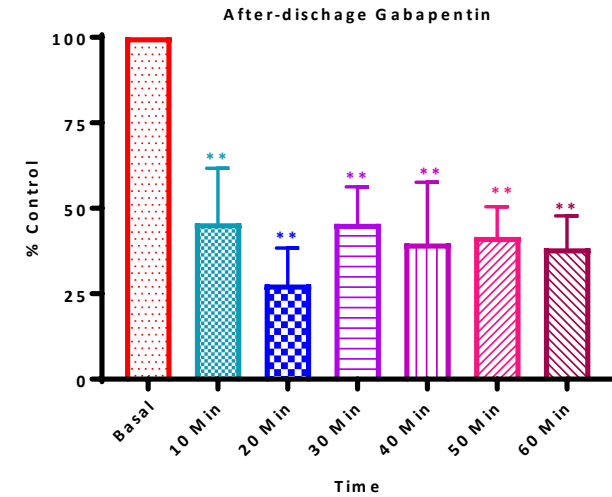
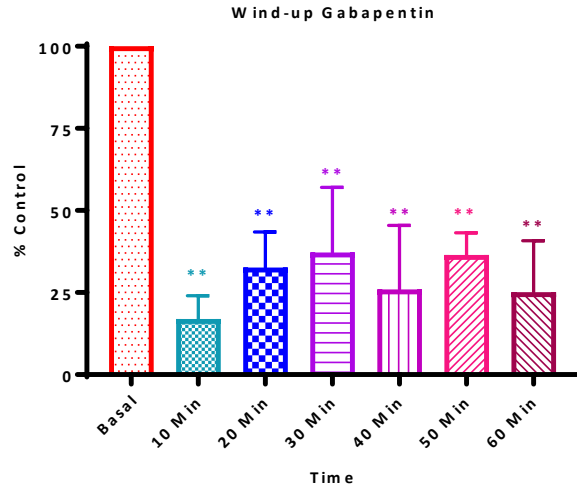
Experimental Procedure:

Anaesthesia was induced with urethane. Segments L4-L5 of the spinal cord were exposed. Extracellular recordings of dorsal horn neurons were recorded with parylene coated tungsten electrode which were descended through the micromanipulator, the depth of the neuron from the surface of the dorsal horn of the spinal cord was recorded.. Responses of dorsal horn neurons to transcutaneous electrical stimulation (16 stimuli, 0.5 Hz, 2 ms pulse-width at two times the threshold for C-fiber evoked responses) of the center of the receptive field were recorded at 10 min intervals. Effect of compound was studied on wind-up and after-discharge of the spikes in wide-dynamic range neurons up to 60 min. Trials were repeated every 10 min with electrical stimulation of 10 sec. Response of dorsal horn neurons to mechanical stimulation was obtained from single fiber isolated by tapping or Von Frey filaments in CCI rats. Isolated neuronal firing was observed for stability. After treatment response was recorded for 1hr by giving mechanical stimulation for every 20 min. Responses of dorsal horn neurons to formalin induced neuronal hyperexcitability was obtained after isolation of wide dynamic range neurons. Isolated neuronal firing was observed for stability. Formalin was administered in the receptive area. The effect of treatment was recorded for 90 mins.

In-vivo electrophysiology in rats – Validation data



Electrical stimulation

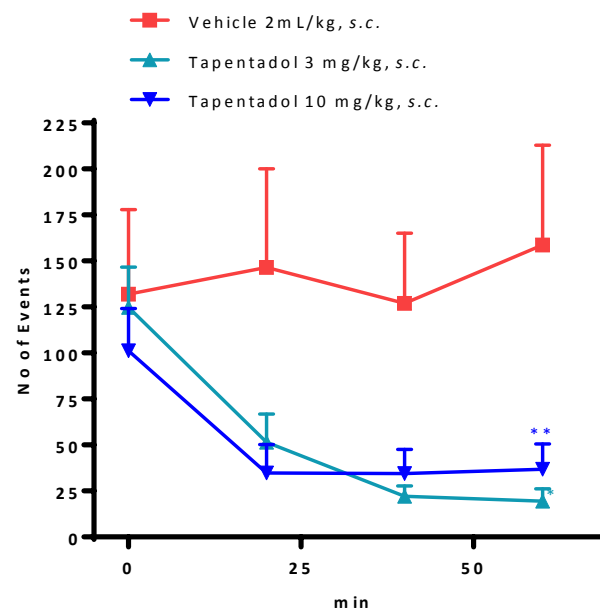
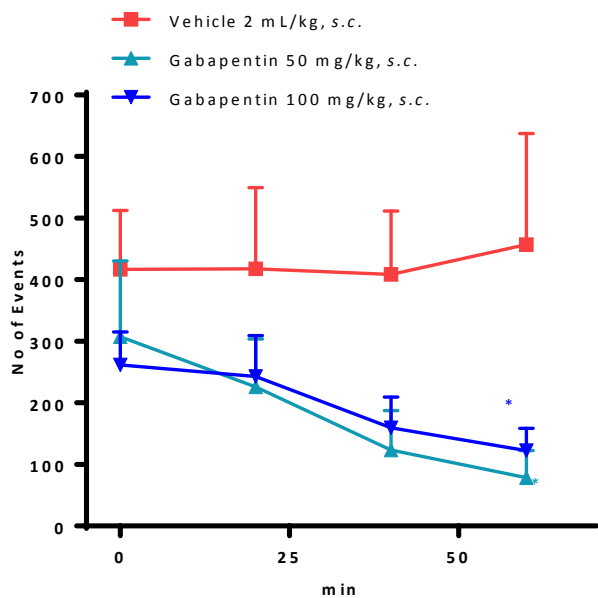


Data represent Mean \pm SEM. ** $p < 0.01$, * $p < 0.05$ Vs Basal

In-vivo electrophysiology in rats – Validation data



Mechanical stimulation

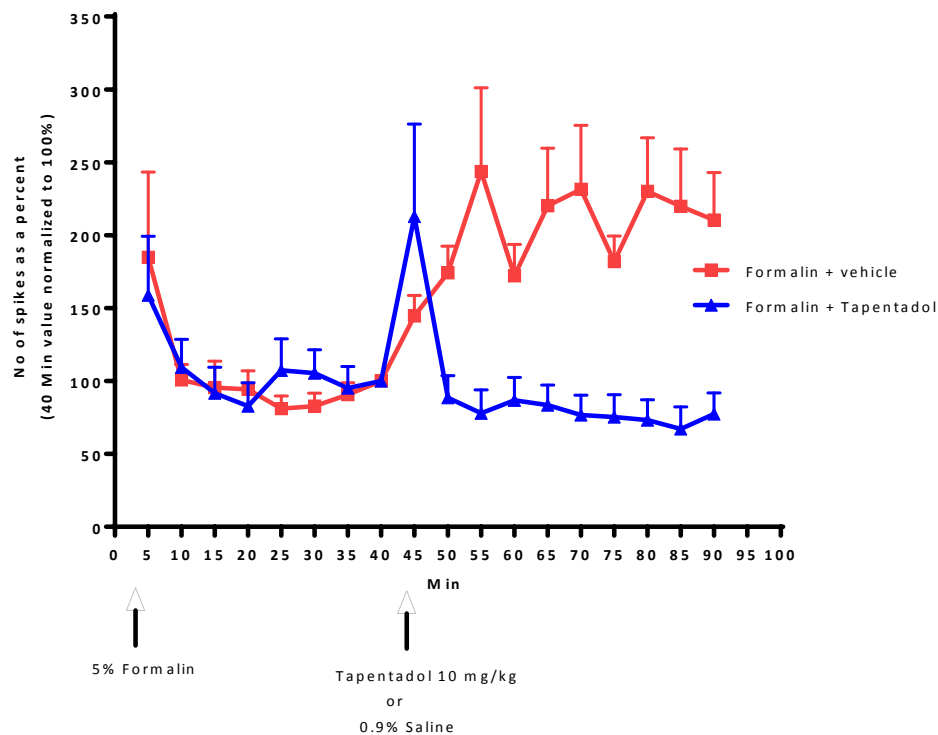
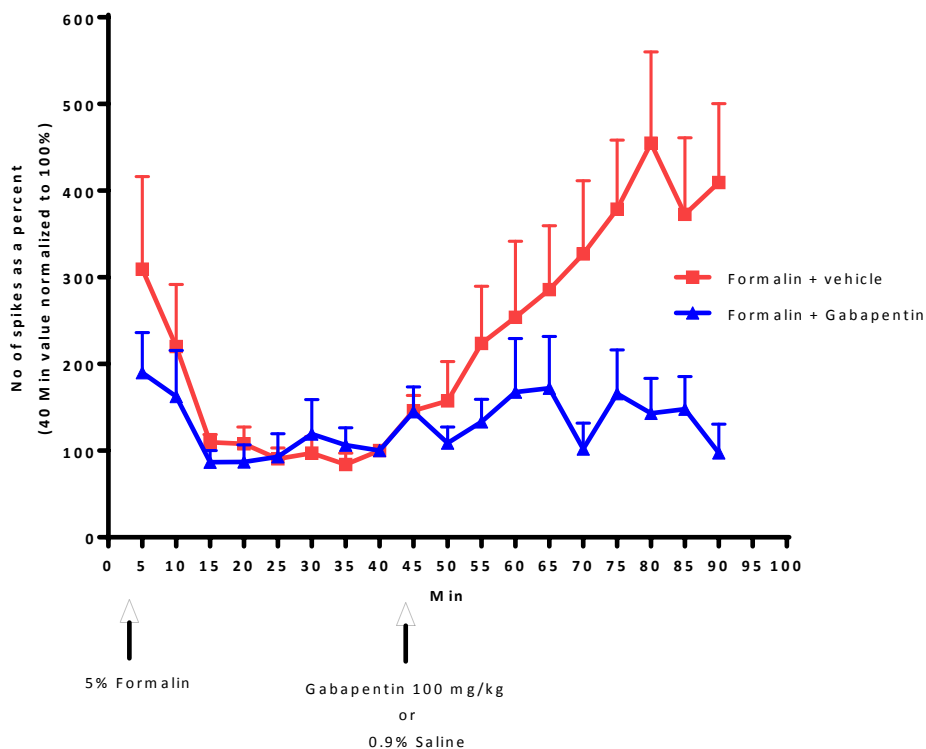


Data represent Mean \pm SEM. ** $p < 0.001$, * $p < 0.05$ Vs Vehicle

In-vivo electrophysiology in rats – Validation data



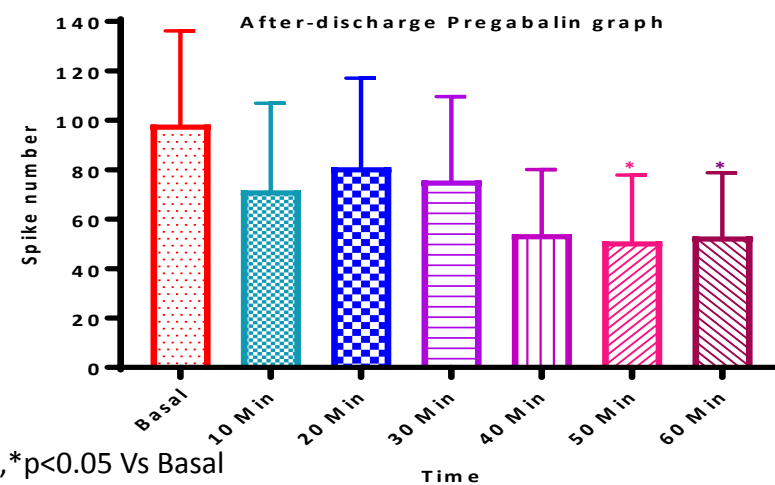
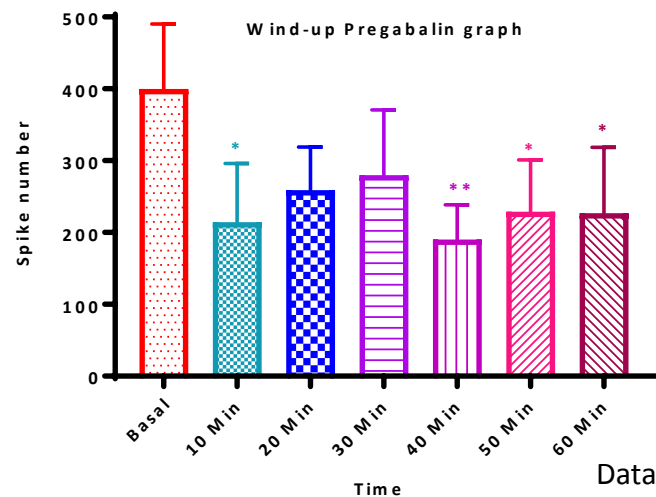
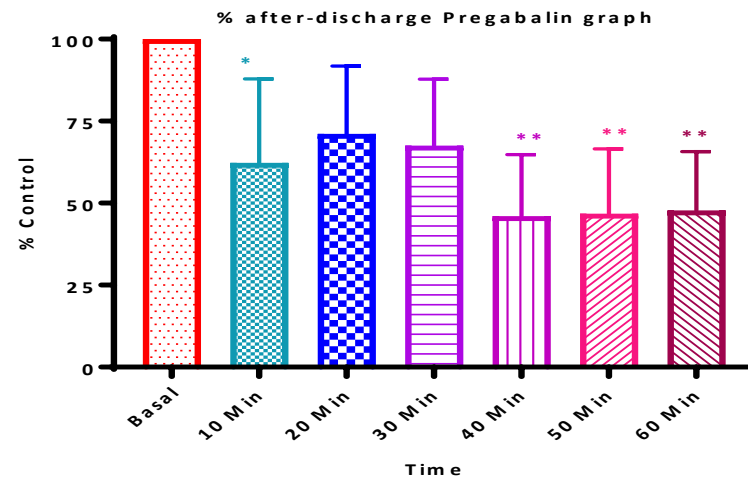
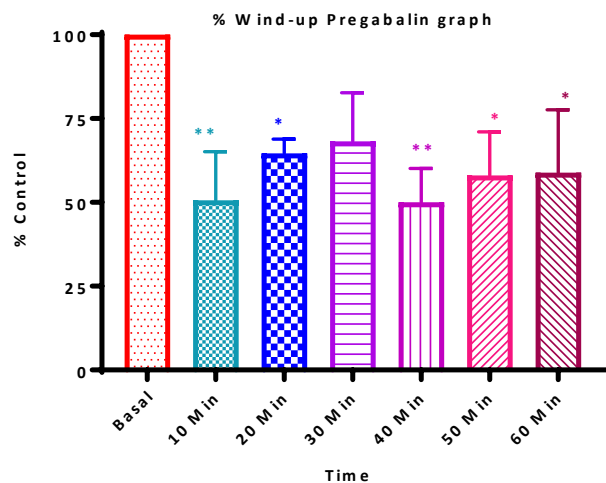
Formalin induced neuronal hyperexcitability





data

Electrical stimulation



Data represent Mean ± SEM. **p<0.01, *p<0.05 Vs Basal

Animal models of pain: Summary



Summary:

Diclofenac evoked dose-dependent reductions in the writhing responses after acetic acid injection.

Diclofenac and **Pregabalin** evoked dose-dependent reductions in the nocifensive responses after formalin injection to the hind paw.

Penatzocine significantly increased the pain threshold in the hot plate model .

Indomethacin significantly reduced the mechanical hyperalgesia produced by CFA.

Morphine significantly reduced the pain produced by CFA .

Morphine significantly reversed the allodynia in animal model of vincristine induced Neuropathic Pain.

Gabapentin, Morphine and Duloxetine significantly reversed the hyperalgesia in animal model of diabetes induced Neuropathic Pain.

Gabapentin dose dependently reversed tactile allodynia in rats with spinal nerve ligation (L5) induced neuropathic pain.

Pregabalin and Gabapentin significantly reversed mechanical allodynia in rats with spinal nerve ligation (L5) induced neuropathic pain.

Gabapentin dose dependently reversed mechanical allodynia in rats and mice with CCI induced neuropathic pain.

Animal models of pain: Summary



Summary:

Morphine and Tapentadol dose dependently reversed mechanical allodynia in rats with CCI induced neuropathic pain.

Duloxetine dose dependently reversed mechanical hyperalgesia in rats with PSNL induced neuropathic pain.

Gabapentin dose dependently reversed mechanical allodynia in rats and mice with PSNL induced neuropathic pain.

Morphine dose dependently reversed secondary mechanical allodynia in rats with Capsaicin induced pain.

Duloxetine dose dependently reversed mechanical allodynia in mice with CCI induced neuropathic pain.

Pregabalin, Gabapentin and Tapentadol significantly reversed mechanical allodynia in rats with Mono iodoacetate induced osteoarthritis pain in rats.

Tapentadol significantly reversed mechanical allodynia in rats with CFA induced Rheumatoid arthritis pain in rats.

Tapentadol significantly reversed mechanical allodynia in rats with MMT induced osteoarthritis pain in rats.

Pregabalin significantly reversed mechanical allodynia and hyperalgesia in reserpine induced myalgic pain in rats.

Pregabalin significantly reversed mechanical allodynia associated with chronic post-ischemia in rats.

Pregabalin, Tapentadol, Duloxetine significantly reversed burrowing deficits induced by CCI and Reserpine induced myalgic pain models.

Gabapentin, Tapentadol, pregabalin significantly reversed dorsal horn neuron firing in pain models.

Reversal of MPTP induced locomotor deficit by carbidopa and L-Dopa – Study Outline



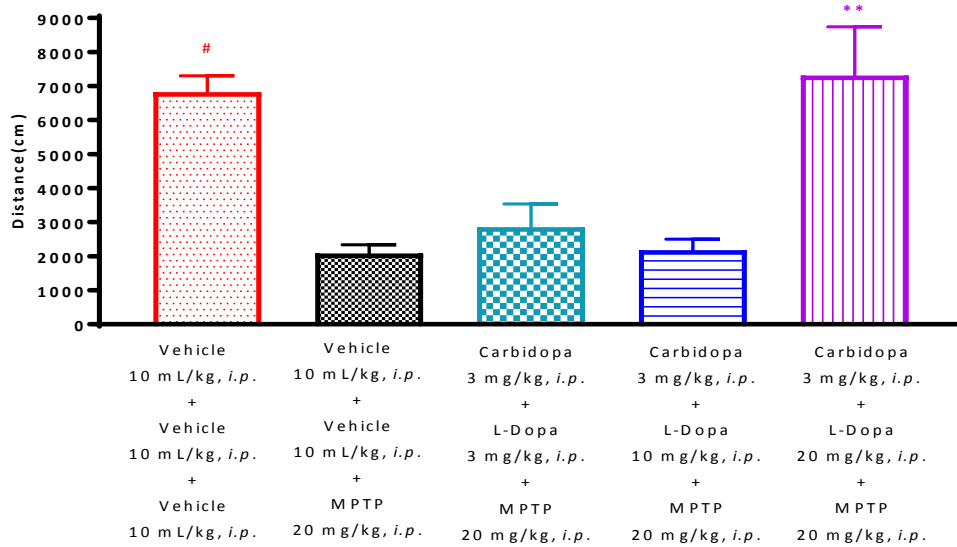
Species: Mice

Strain: C57BL6J

Experimental Procedure:

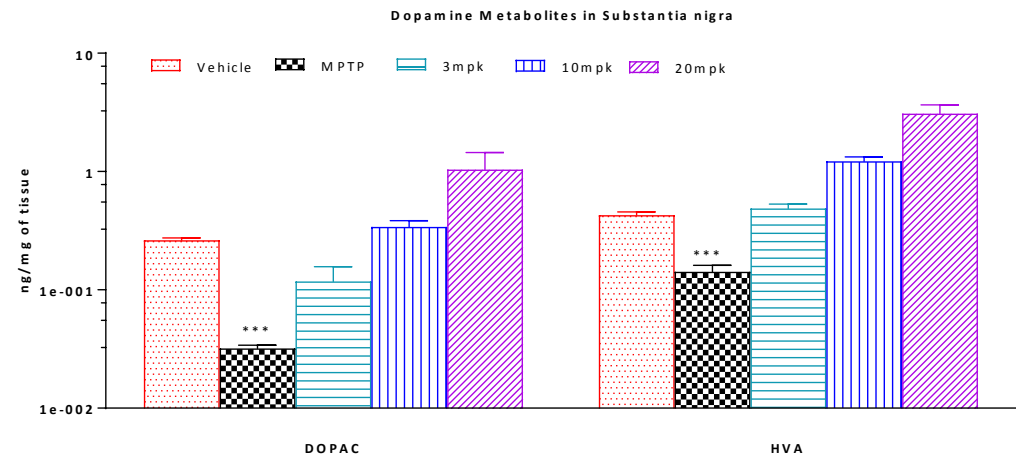
Mice were grouped according to body weight. The mice were dosed with Carbidopa followed by L-Dopa which was then followed by MPTP as base for a period of 5 days . Once the mice were dosed , they were kept under table lamp on all the days. On day 5, the mice were dosed with MPTP or vehicle and one hour later placed in the open field. The distance travelled for a period of 30 minutes were recorded. Immediately after the trial the animals were sacrificed and the brain samples were collected for the estimation of dopamine turnover.

Reversal of MPTP induced locomotor deficit by carbidopa and L-Dopa – Validation data



Data represents Mean \pm SEM. ** $p < 0.01$ Vs Vehicle

Levodopa and carbidopa reversed the deficits induced by MPTP



Erectile dysfunction – Study Outline



Species: Rat

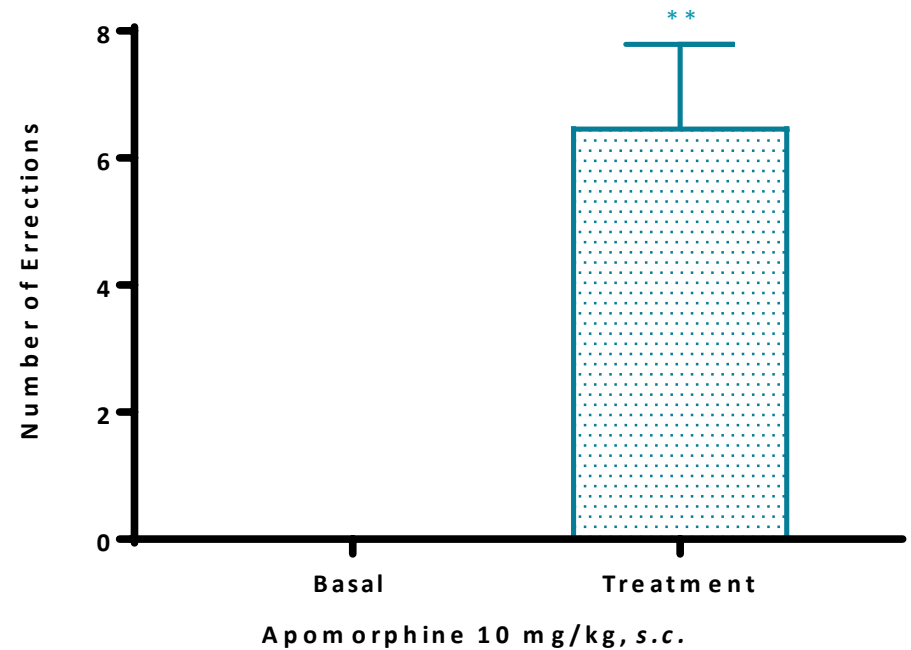
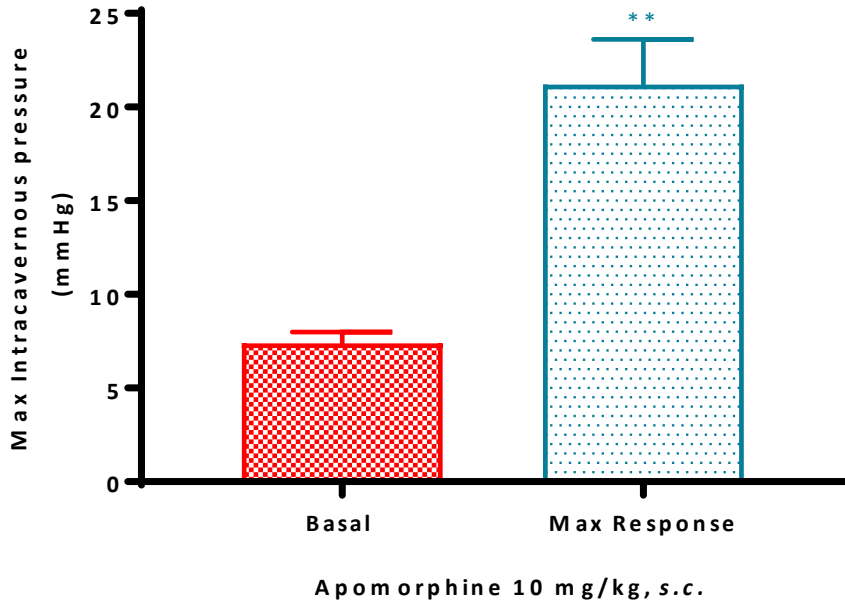
Strain: Wistar

Experimental Procedure:

Rats were anesthetized with 12% of urethane, i.p. Anesthetized rats were placed in supine position on the thermoregulatory pad (37 °C). A midline incision was made in the perineum. The base of the penis, enclosed by striated muscles, was exposed. Ischiocavernosus muscle covering the crus corpus cavernosum was divided on right side of the rat and entrance was given to the underlying tunica albuginea. A 25-gauge needle attached to a heparinized polyethylene catheter (PE-50) was inserted into the crus corpus cavernosum. Free end of catheter was connected to the pressure transducer to record pressure from crus corpus cavernosum. After a stabilization of 30 min, test item was administered and the intra-cavernous pressure, number of erections was recorded.



Erectile dysfunction – Validation data



Data represents Mean \pm SEM. **p<0.01 Vs basal



Safety Pharmacology

CNS safety pharmacology

(as per ICH S7 safety guidelines)



Test	Species	Compound Used For Standardization
IRWIN test / Functional Observation Battery (FOB)	Rat or Mouse	MK-801 and Diazepam
Rota Rod	Rat or Mouse	Chlordiazepoxide
Open Field (Locomotor Activity)	Rat or Mouse	MK-801 and Haloperidol
Proconvulsant effect	Rat or Mouse	Pentylentetrazole
Antagonism of Seizures induced by pentetrazole (PTZ)	Rat or Mouse	Diazepam
Catalepsy	Rat or Mouse	Haloperidol

Models under validation



- Mouse Model of Fracture Pain
- Slice electrophysiology
- In-vivo electrophysiology – LTP

Quality Assurance



- Independent Quality Assurance team
- Quality System Procedures (QSP's) for Quality System Management and Standard Operating Procedures (SOP's) for Operation, Calibration, Maintenance of Equipment's
- Accredited by ISO/IEC 17025:2005 Quality System since 2005
- Document and Data Control, Conducting Internal Audits, Study Specific Audits
- Dedicated Archive facility for the retention of the records
- Facility audited and approved by many global pharmaceutical companies and majority of Indian Pharma Companies
- Integrity of studies is maintained by following critical components of GLP compliance like SOP, test and control article handling, data handling, training documents, archives etc.



Venkat Jasti

Chairman & CEO

E-mail: info@suven.com

Ramakrishna Nirogi

Vice President, Discovery Research

E-mail: nvsrk@suven.com