

Morphine and gabapentin decrease mechanical hyperalgesia and escape/avoidance behavior in a rat model of neuropathic pain

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Abstract

A behavioral test paradigm that measures the aversive quality of stimulus-evoked pain in an animal model of neuropathic pain (L5 ligation) was tested for sensitivity to (1) different forces (476 and 202 mN) and frequencies (once every 15 or 30 s) of mechanical stimulation to the hyperalgesic paw and (2) different doses of the common antinociceptive compounds morphine (1 and 10 mg/kg) and gabapentin (30 and 90 mg/kg). Compared to non-ligated controls, the greater force (476 mN) and frequency (every 15 s) of mechanical stimulation of the hyperalgesic paw was associated with the greatest degree of escape/avoidance behavior. There was not a significant degree of escape/avoidance behavior at the lowest force (202 mN) and frequency (every 30 s) of mechanical stimulation. Compared to ligated vehicle treated controls, morphine (1 mg/kg) and gabapentin (90 mg/kg) decreased mechanical hyperalgesia and also attenuated the escape/avoidance behavior. The antinociceptive and antiaversive effects were found at doses that did not produce evidence of decreased motor activity. It is concluded that the behavioral test paradigm used to measure the aversiveness of stimulus-evoked nociceptive behavior is sensitive to different degrees of evoked pain and traditional analgesic compounds. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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Traditional nociceptive tests typically measure the response or a change in threshold to a noxious or non-noxious mechanical or thermal stimulus, such as a change in mechanical threshold following nerve damage or a response to radiant heat (tail-flick, paw withdrawal). These tests have been useful to elucidate spinal and supraspinal mechanisms of nociception and screen compounds for analgesic efficacy. For instance, both morphine and gabapentin have been extensively studied and demonstrated to possess analgesic properties in many clinical pain states as well as animal models of inflammatory and neuropathic pain [1,3,4,8,10,13,15,17,18].

We have recently developed a behavioral test paradigm that measures the aversiveness of nociceptive stimuli as an attempt to model the affective/motivational aspect of clinical pain states [12]. The behavioral paradigm allows animals to 'choose' an environment associated with the application of a mechanical stimulus to the hyperalgesic paw or to the non-operated contralateral paw. Our previous findings indicate that the test paradigm is sensitive to measure the aversive

nature of evoked pain in animal models of neuropathic and inflammatory conditions. However, it remains to be determined if the behavioral paradigm is sensitive to differing forces and frequencies of mechanical stimulation and traditional analgesic compounds. It is expected that if mechanical stimulation of the hyperalgesic paw is aversive, then as the force and frequency of the stimulation decreases, there should be a decrease in the aversive nature of evoked pain caused by the stimulus that should be reflected as a decrease of escape/avoidance behavior. It is also expected that compounds that decrease mechanical hyperalgesia should be associated with an attenuation of the aversive nature of the noxious stimulus. Therefore, the purpose of the present experiment was to (1) examine the effect of different forces and frequencies of mechanical stimulation on the place avoidance behavior; and (2) to determine, in a dose dependent manner, if the behavioral test paradigm is sensitive to morphine and gabapentin.

One hundred and forty one male Sprague–Dawley rats (UTA vivarium) were housed in pairs and allowed free access to food and water throughout the study. Room temperature and humidity were maintained at 21°C and

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70%, respectively. All procedures were approved by the UTA Institutional Animal Use and Care Committee.

Nerve injury was produced by tightly ligating the L5 spinal nerve ($n = 94$) [11,12]. Forty-seven additional animals served as sham surgery control without ligation of the L5 spinal nerve. Behavioral testing was performed on the second day following the surgical procedure which involved measures of mechanical paw withdrawal thresholds (MPWT) using the up/down technique [6,12] immediately followed by further behavioral testing described for experiment 1 (force/frequency) or experiment 2 (morphine/gabapentin).

Experiment 1 examined different forces and frequencies of mechanical stimulation on escape/avoidance behavior. Sham surgery and L5 ligated animals were randomized to one of four groups (476 mN/15 s, 476 mN/30 s, 202 mN/15 s, 202 mN/30 s) and tested for escape/avoidance behavior using methods previously reported [12]. Immediately following MPWT testing, animals were placed within a 30 × 30 × 30-cm Plexiglas chamber (half painted black and the other half painted white) and allowed unrestricted movement for the duration of a 20-min test period. The mechanical stimulus (either 202 or 476 mN) was applied to the plantar surface of the hindpaws at a constant interval of time (either 15 or 30 s). When the animal was within the dark side of the chamber, the mechanical stimulus was applied to the hyperalgesic paw; when the animal was within the light side of the chamber, the mechanical stimulus was applied to the non-ligated control paw. The amount of time that each animal stayed within the light side of the chamber was recorded.

The second experiment examined the effect of morphine and gabapentin on MPWT and escape/avoidance behavior. Immediately following pre-drug administration baseline MPWT measurement, L5 ligated animals were randomized to receive one of five (two doses of morphine and gabapentin and a vehicle control) coded drug solutions. Morphine and gabapentin (Sigma Chemical Co., St. Louis, Mo) were prepared on the day prior to the injection. Morphine was dissolved in a 0.9% saline solution to form either a 1 or 10 mg/ml solution and was delivered s.c. (10 ml/kg). Gabapentin was dissolved in a 0.9% saline solution to form either a 30 or 90 mg/ml solution and was delivered s.c. (10 ml/kg). The vehicle solution consisted of the 0.9% saline solution delivered in the same manner. A sham surgery group that did not receive injection served as an additional control group. At 20-min post-injection, animals were tested in the escape/avoidance test using the same methods as described for experiment 1. Based on the results from experiment 1, testing was performed for 20 min using the 476 mN force applied at 15 s intervals. Quantification of motor behavior consisted of counting the number of center-line crossings, as defined as all four paws crossing the line, during the 20-min test period. Following the escape/avoidance test, animals were tested for MPWT. The experimenter was blind to the content of each solution.

For experiment 1, the analysis of time spent within the light side of the chamber for the different force/frequency combinations revealed a significant group × time interaction for the 476 mN force applied at 15 s intervals ($P < 0.01$), with the L5 ligated group spending significantly more time within the light side of the chamber at 20 min compared to the sham surgery group (Fig. 1). Analysis of the other force/frequency combinations revealed no significant group differences or group × time interactions. However, visual inspection of Fig. 1 indicates a strong trend towards a group × time interaction for the 202 mN force applied at 15 s intervals ($P < 0.15$), with the L5 ligated group spending significantly more time in the light side of the chamber at 20 min compared to the sham surgery group. All L5 ligated groups demonstrated a significant decrease of MPWT that did not differ among the groups (data not shown).

For experiment 2, the analysis of MPWT for animals that received L5 ligation or sham surgery revealed a significant main effect for group at the pre-injection time point ($P < 0.001$), with all five L5 ligated groups demonstrating a significant decrease of left hindpaw MPWT compared to sham surgery controls (Fig. 2A) with no significant pre-injection MPWT differences among the five L5 spinal nerve ligated groups. The analysis of MPWT following the escape/avoidance test revealed a significant main effect for group ($P < 0.001$), with the sham surgery group, both groups that received morphine (1 and 10 mg/kg) and both groups that received gabapentin (30 and 90 mg/kg) demonstrating significantly less mechanical hyperalgesia than the

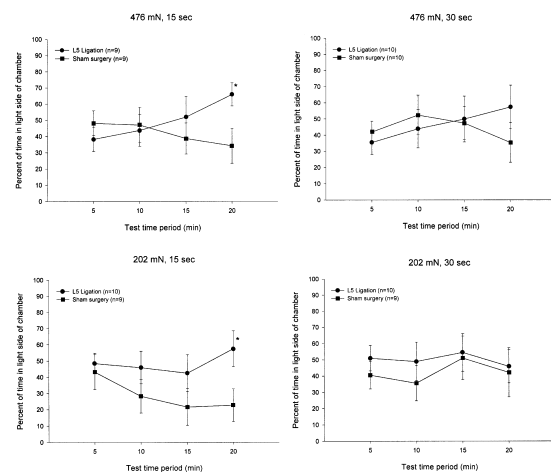


Fig. 1. Mean (\pm SEM) percent of time spent within the light side of the chamber for animals that had L5 spinal nerve ligation or received a sham surgery without ligation of the L5 spinal nerve. Groups of animals were tested for 20 min in the escape/avoidance test while being administered a von Frey force of either 476 or 202 mN at a frequency of once every 15 or every 30 s. The duration of time spent within the light side of the chamber for each force/frequency combination was analyzed separately for group differences using repeated measures ANOVA followed by post-hoc comparison (LSD) of group differences at each test time period. * $P < 0.05$ compared to vehicle control.

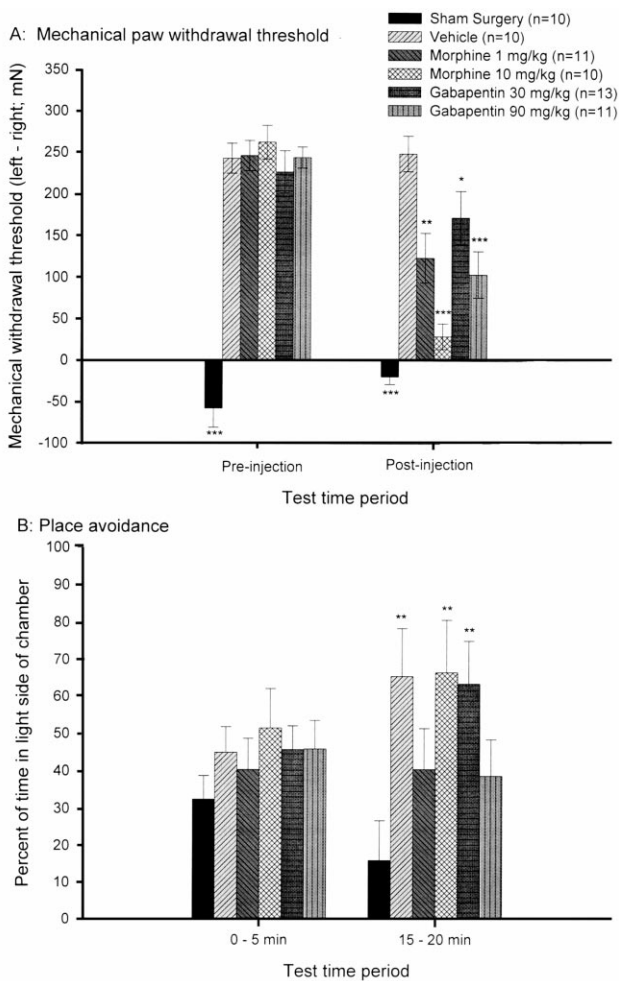


Fig. 2. (A) Mean (\pm SEM) mechanical paw withdrawal threshold (left paw–right paw) for animals that did not receive L5 ligation (Sham Surgery) or received different doses of different compounds following L5 ligation. Mechanical paw withdrawal thresholds for the pre-injection and post-injection time periods were analyzed using one way ANOVA on the right–left paw difference score for each animal at each time point followed by post-hoc comparison (LSD) for group differences. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle control. (B) Mean (\pm SEM) percent of time spent within the light side of the chamber from 0–5 and 15–20 min for animals that did not receive L5 ligation (Sham Surgery) or received different doses of different compounds following L5 ligation. The duration of time spent within the light side of the chamber was analyzed for group difference using one-way ANOVA for the 0–5 min and the 15–20 min time periods followed by post-hoc comparison (LSD) for group differences. ** $P < 0.01$ compared to sham surgery control.

L5 ligated vehicle treated group. The overall analysis of percent time spent within the light area of the test chamber during the first 5 min of the test period revealed no significant difference among the groups ($P > 0.05$). However, at 15–20 min, there was a significant main effect for group ($P < 0.05$), with no significant difference in the amount of time spent within the light side of the chamber for animals treated with 1 mg/kg morphine and 90 mg/kg gabapentin compared to sham surgery treated animals (Fig. 2B).

The overall analysis (one-way ANOVA followed by post-hoc comparison (LSD) of total crosses from the light to dark side of the chamber revealed a significant main effect for group ($P < 0.01$). Animals treated with 10 mg/kg morphine made significantly fewer line crosses than all other ligated groups (data not shown). In addition, there was no significant difference in line crosses between the 10 mg/kg morphine and sham surgery groups ($P > 0.05$).

The rationale for developing the present behavioral escape/avoidance test paradigm is based on the need for a method to quantify the negative affective dimension of pain in various animal models. The present results confirm our previous report indicating that animals quickly begin to spend less time in the naturally preferred environmental area (i.e. dark) that is associated with mechanical stimulation of the hyperalgesic paw [12]. In the present experiment, both the L5 ligated vehicle treated and sham surgery groups started out with an equal preference for the light side of the chamber (Fig. 2B, 0–5 min). However, by 15–20 min the amount of time spent within the light side of the chamber was approximately 15% for the sham surgery group versus 70% for the L5 ligated vehicle treated group. This finding is a clear indication that animals find mechanical stimulation of the L5 spinal nerve ligated paw aversive, and when given a choice, will perform purposeful behavior to minimize stimulation of the hyperalgesic part. In addition, our prediction that as the force and frequency of mechanical stimulation decreased, there would be an associated decrease in the shift from the dark area of the chamber to the light side of the chamber was confirmed (Fig. 1).

A second purpose of the present experiment was to examine if the behavioral test paradigm to measure the aversive nature of mechanical stimulation following nerve ligation was sensitive to known analgesic compounds. First, we ensured that morphine and gabapentin reversed mechanical hyperalgesia following L5 nerve ligation. Our results confirm that mechanical hyperalgesia produced by L5 spinal nerve ligation can be attenuated with gabapentin at doses previously found to be effective in neuropathic conditions [1,17]. It should be noted that the dose of gabapentin that was observed to decrease mechanical hyperalgesia did not produce any obvious effect on motor activity. In addition, both doses of morphine (1 and 10 mg/kg) were found to possess anti-allodynic properties. Although there is controversy as to the clinical utility of morphine for the treatment of neuropathic pain [5], our finding was not entirely surprising considering reports that morphine can be an effective treatment for neuropathic pain [4,7,18]. The attenuation of hyperalgesia at a dose of 1 mg/kg morphine occurred in the absence of significant sedative effects as revealed by normal motor behavior reflected by total number of line crossings, while the dose of 10 mg/kg morphine significantly decreased mechanical hyperalgesia and motor activity.

It was hypothesized that if animals were less hyperalgesic, then mechanical stimulation during the escape/avoidance test should be less aversive which should be reflected as an

attenuation in the amount of time that animals spent in the light side of the chamber. Indeed, morphine and gabapentin, two drugs that are able to attenuate mechanical hyperalgesia (Fig. 2A) also attenuate the aversive nature of the mechanical stimulus (Fig. 2B). The lack of a significant effect of the 1 mg/kg morphine and 90 mg/kg gabapentin on total number of line crossings rules out the possibility that sedative properties account for the lack of shift from the dark side to the light side of the chamber. Rather, it seems more likely that mechanical stimulation of the L5 ligated paw is less aversive in animals treated with morphine and gabapentin. The failure of the 10 mg/kg morphine group to show attenuation in the shift from the light side to the dark side of the chamber most likely reflects impaired motor activity.

In the present paradigm, animals must acquire an associate between the applications of the mechanical stimulus to the hyperalgesic paw with some external cue (i.e. dark vs. light area of the test chamber). It is possible that the attenuation of time spent within the light side of the chamber seen with morphine and gabapentin is caused by an interference with acquisition and retention of this relationship rather than a change in the negative hedonic value of the mechanical stimulus. Indeed, morphine has been found to impair performance on the Morris water maze [14] and the radial arm maze [19], which is a test of spatial memory [16]. However, it should be noted that the effect of morphine on the radial arm maze requires chronic high dose administration (up to 40 mg/kg) and most likely is related to impaired acquisition of procedures necessary to perform the task rather than with interference of working memory [19]. Other investigators report biphasic results in rats such that lower doses of morphine enhance while higher doses impair memory [2,9]. Avoidance responding has been reported to be unaltered following morphine administration at doses that inhibit reflexive withdrawal responding in non-human primates [20]. The effect of gabapentin on the acquisition and retention of spatial memory tasks in animals remains unknown. Taken together, the most parsimonious interpretation of the present results is that a decrease of mechanical hyperalgesia is associated with a decrease in the aversiveness of a mechanical stimulus applied to the hyperalgesic body region.

In conclusion, the present experiment provides additional support that a behavioral paradigm based on a shift in the amount of time that animals spend in an environmental location associated with mechanical stimulation of the hyperalgesic paw can be used to measure the affective dimension of pain in rats. In addition, it is concluded that two commonly prescribed analgesic compounds are directly affective against stimulus-evoked nociceptive responses (mechanical paw withdrawal threshold) as well as the aversive nature of neuropathic pain. Future studies will examine additional compounds and also explore supraspinal structures related to the limbic system to dissociate sensory from affective nociceptive processing.

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