



Pain and Analgesia Research

NDI scientists are among the world's most active researchers in the field of pain and analgesia. We offer expertise in the following models:

Acute Inflammatory Models:

formalin,
carrageenan (subcutaneous, intra-articular)

Chronic Inflammatory Models:

Freund's adjuvant (subcutaneous, intra-articular)

Acute Nociceptive Models:

tail-flick, hot-plate

Chronic Neuropathic or Post-surgical Pain:

Chung, Bennett and Brennan models,
using separate measures of pain threshold vs. affective (perceived) component of pain.

Diabetic Neuropathy

1. Carrageenan-induced acute or chronic inflammation in rats

There are two ways in which carrageenan can be used as a model of inflammatory pain.

a). Subcutaneous injection into the hindpaw: An acute inflammatory condition is produced by a subcutaneous injection of 3% lambda CARR (0.12 ml) into the plantar surface of one hindpaw under light isoflurane anesthesia. Usually, there is an additional control group that receives an equal volume of saline. In most studies, animals then receive a drug compound 3 ½ hours after the CARR injection, but the timing depends on the particular design of the experiment and the properties of the compound being tested. Quantification of pain behavior is performed using the same procedures as outlined for quantification of pain behavior in the Chung and CCI models of neuropathic pain (mechanical paw withdrawal threshold and, if needed, PEAP testing...see [6] below).

b) Intra-articular injection: A longer lasting state of inflammation is produced by performing intra-articular injection of CARR (0.1 ml, 3%) into the tibial joint under isoflurane anesthesia. This route of administration induces an inflammatory condition that can last for up to 7 days following injection and is an established model of arthritic inflammatory pain. Quantification of pain behavior is performed using the same procedures as outlined under (6), below. Open Field activity is an additional measurement that can be performed. The open field consists of a circular base (100-cm diameter) with aluminum sheet metal wall (height of 45 cm). The surface is divided by 0.5 cm black lines into 24 partitions (12 outer, 12 inner) and illuminated by a central light. Animals are individually placed in the center of the open field and the following indices are recorded: total number of partitions entered, latency to enter the outer circle, revolutions around the open field, grooming, rearings, and total defecation.



Representative publication: LaBuda, C.J., and Fuchs, P.N. Low dose aspirin attenuates escape/avoidance behavior, but does not reduce mechanical hyperalgesia in a rodent model of inflammatory pain. *Neuroscience Letters*, 2001, 304, 137-140.

2. CFA-induced acute inflammation and paw edema in rats

Method: The inflammatory condition is produced by a subcutaneous injection of CFA into the plantar surface of one hindpaw under light isoflurane anesthesia. Behavioral testing of mechanical paw withdrawal threshold takes place within a 24 – 48 hour period following the carrageenan injection. This method is described under (6) below. In addition hindpaw thermal paw withdrawal (TPW) latency is measured using an infrared heat source (Ugo Basile, Italy, Plantar Test) applied to the plantar surface of both hindpaws. During behavioral testing, animals are placed in plastic chambers (8 cm x 8 cm x 20 cm) and allowed a 20-minute habituation period to the apparatus for two consecutive days. Animals are then allowed 30 minutes to habituate to the test chamber on the day of behavioral testing. During testing, the experimenter activates a thermal stimulus aimed at the plantar surface of the hindpaw, which remains activated until the animal withdraws its paw or a cutoff of 30-seconds to avoid tissue damage. Threshold testing is performed twice per paw and the mean value of the four scores is calculated to determine the TPW threshold for each animal. Each measure is separated by 20-seconds. Measurement of paw edema is performed following behavioral testing.

Representative publication: LaBuda, C.J., and Fuchs, P.N. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. *Experimental Neurology*, 2000, 163, 490-494.

3. Formalin Model of Acute Inflammatory Pain:

Method: Animals are placed in a 30 x 30 x 30 cm Plexiglas chamber and allowed to habituate for at least fifteen minutes. To allow for easy viewing of behavioral responses, a mirror is placed below the test chamber at a 45-degree angle. Subjects are typically administered a 0.05-ml injection of 1% formalin solution into the plantar or dorsal surface of one hindpaw. Behavioral testing begins immediately after injection and lasts for 45 – 60 minutes. The amount of time the animals spend with the injected paw down, elevated, or licking the paw is recorded using customized software. The test period is divided into 5 minute time bins. A weighted pain score for each animal is calculated using the following formula: Pain Score = (time spent with inflamed paw elevated + 2 x (time spent licking inflamed paw)) / 300. A pain score of zero reflects the entire duration of the 5-minute period being spent with the paw down, while a pain score of 2 indicates that the entire duration of the 5-minute period was spent licking the injected paw. In addition to weighted pain scores, the raw scores for each of the behavioral categories (i.e. paw down, paw elevated, and paw licking) are also summed over each 5-minute time interval.

Representative publication: LaBuda, C.J., Donahue, R., and Fuchs, P.N. Enhanced formalin nociceptive responses following L5 ligation in the rat reveals neuropathy-induced inflammatory hyperalgesia. *Pain*, 2001, 94, 59-63.

4. Brennan model of post-surgical pain in rat

Method: Rats are anesthetized with isoflourane and a 1-cm longitudinal incision is made in the plantar surface of the hind paw beginning 0.5 cm from the end of the heel. The skin, planar fascia, and underlying muscle is incised. The skin is closed with 5-0 nylon suture. Behavioral testing of mechanical paw withdrawal threshold takes place within a 4-day period following the incision, using the method described under (6) below.

Representative publication: Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision. *The Journal of Pain*, 2004, 5, 157-163.

5. Chung and Bennett models of neuropathic or post-surgical pain

Method: Either tight ligation of the L5 spinal nerve (Chung model) or loose ligature of the sciatic nerve (Bennett model) is performed following induction of anesthesia with isoflourane in 100% O₂ (3% induction, 2% maintenance). For L5 ligation, under magnification approximately one-third of the transverse process is removed and the L5 spinal nerve is identified and carefully dissected free from the adjacent L4 spinal nerve. The L5 spinal nerve is either tightly ligated or loosely tied using 6-0 silk suture. In most experiments, additional animals serve as sham

surgery controls in which the spinal nerves are exposed without ligation/ligature of the L5 spinal nerve.

Mechanical Paw Withdrawal Threshold: Typically, we allow for a three-day post-surgical recovery period. Animals are then placed within a Plexiglas chamber (20 x 10.5 x 40.5 cm) and allowed to habituate for 15-min. The chamber is positioned on top of a mesh screen so that mechanical stimuli can be administered to the middle plantar surface of both hindpaws. Mechanical threshold measurements for each hindpaw are typically obtained using the up/down method with eight von Frey monofilaments (4, 6, 11, 18, 45, 74, 131, and 193 mN) (although an ascending series can also be utilized to provide the full stimulus-response



function). Each trial begins with a von Frey force of 11 mN delivered to the right hindpaw for approximately 1-sec, and then the left hindpaw. If there was no withdrawal response, the next higher force is delivered. If there is a response, the next lower force is delivered. This procedure is performed until no response is made at the highest force (193 mN) or until four stimuli are administered following the initial response. The 50% response probability for each paw is calculated using the following formula: $[X_{th}]_{\log} = [vFr]_{\log} + ky$ where $[vFr]$ is the force of the last von Frey used, $k = 0.2501$ which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal does not respond to the highest von Frey hair (193 mN), then $y = 1.00$ and the 50% response probability for that paw is calculated to be 343.29 mN. Mechanical paw withdrawal threshold testing is performed three times and the 50% response probability values were averaged over the three trials to determine the

mean mechanical paw withdrawal threshold for the right and left paw for each animal.

Place/escape Avoidance Testing: Under certain circumstances (largely depending upon the specific question and experimental design), the place escape/avoidance test is also performed. In general, PEAP testing is performed immediately following mechanical paw withdrawal threshold testing. Animals are placed within a 16 x 40.5 x 30.5 cm Plexiglas chamber positioned on top of a mesh screen. One half of the chamber is painted white (light area) and the other half of the chamber is painted black (dark area). During behavioral testing, animals are allowed unrestricted movement throughout the test chamber for the duration of a 30-min test period. Testing begins immediately with suprathreshold mechanical stimulation (476 mN von Frey monofilament) applied to the middle plantar surface of the hindpaws at 15-sec intervals throughout the test period. The mechanical stimulus is applied to the injured paw when the animal is within the preferred dark area of the test chamber and the uninjured paw when the animal was within the non-preferred light area of the test chamber. Based on the location of the animal at each 15-sec interval, the mean percentage of time spent in each side of the chamber is calculated for the entire test period.

Representative publication: LaBuda, C.J., and Fuchs, P.N. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. *Experimental Neurology*, 2000, 163, 490-494.

6. STZ-induced diabetic neuropathy

Method: Adult male Sprague-Dawley rats are first assayed for their paw-withdrawal thresholds following standard mechanical stimulation (von Frey hairs). The animals then receive injections of streptozotocin to induce insulin-dependent diabetes mellitus. This condition is confirmed by assay of blood glucose levels. Once the condition is confirmed, the animals' paw withdrawal thresholds are re-determined, and only animals with significant decreases in withdrawal threshold compared to their pre-diabetes level are included.

Induction of diabetes: Animals are injected with streptozotocin, dissolved in 0.9% sodium chloride, twice on successive days (75 mg/kg each day, i.p.). Blood glucose level is assayed at one week post-injection, from samples taken from the tail vein, using standard test strips and colorimeter. Only animals with a blood glucose level > 15 mM are considered diabetic.

Behavioral testing: Animals are first habituated to a 30 x 30 x 30-cm Plexiglas test chamber on top of a mesh screen for 15 min. The size of the chamber allows for free movement of the animal and the mesh screen allows for application of calibrated von Frey monofilaments to the plantar surface of each hindpaw. The animals are then tested to determine mechanical paw withdrawal threshold using the up/down technique, as described in (6), above.

Representative publication: Fuchs, P.N. (2003) Evaluation of three COMPANY compounds in a rat model of diabetes-induced neuropathic pain. (Confidential Report, NeuroDetective International; sanitized version available upon request)

7. Peripheral afferent activities following noxious mechanical and thermal stimuli

Physiological responses to noxious stimuli may be evaluated using the teased-fiber technique. This study is typically done using one of the above pain models, i.e. after the appropriate lesion and behavioral confirmation of the lesion's effectiveness.

Method: Rats are initially anesthetized with sodium pentobarbital sodium (50 mg/kg ip). A catheter is placed in the jugular vein for continuous administration of anesthetic; a tracheotomy allows insertion of a tracheal cannula for artificial ventilation. A 4-cm-long laminectomy is then performed over the lumbosacral enlargement to expose the spinal cord and dorsal roots. For this and subsequent procedures the rat is held in a stereotaxic frame to prevent any movement during recording. The skin over the laminectomy is elevated to form a pool that is filled with light mineral oil. Continuous anesthesia is accomplished by giving a mixture of 50 mg sodium pentobarbital in 9 ml 0.9% NaCl at a rate of 1.0 ml/h. The end-tidal

CO₂ is maintained at 30 mmHg. The animal's body temperature is maintained at 37°C by a feedback-controlled electric heating blanket.

Single teased fiber recording. Extracellular single-fiber recordings are made from the distal stump of the dorsal root. A silver wire hook electrode is used to record extracellular single-unit discharges in filaments of the L4 or L5 dorsal roots. A small strand of the dorsal root is teased distally from the main trunk and is further separated into a filament containing a single active fiber. The dorsal root filament is wrapped around the recording electrode. Electrical stimulation is applied at the receptive field for recordings of orthodromic spikes to determine the conduction velocity. Single fibers can then be classified as into A- β , A- δ , or C-fibers according to their conduction velocity; this allows drug effects to be ascertained for each class of pain fibers. Following classification of the fibers, recordings from single fibers are obtained again after application of graded intensities of mechanical stimuli delivered to a receptive field on the skin, the receptive field being determined by the initial electrical stimulation recordings. Both background activity and discrete responses are recorded for each type of stimulus; recordings are obtained for 10 sec each, with a 20-sec inter-stimulus interval. Two types of mechanical stimuli are applied to the receptive field: (1) innocuous stimulation (brush) is delivered by repeated brushing in a stereotyped manner with a camel's hair brush; and (2) noxious stimulation (pinch) is applied with an arterial clip. At the discretion of the client, responses to heat stimulation may be tested instead of mechanical stimulation, using a feedback controlled Peltier device that is lightly placed on the receptive field. The size of the thermal probe is 10 mm in diameter, and the baseline temperature is set at 30° C. Neuronal responses to increasing temperatures between 37°C and 51°C are recorded in increments of 2°C. Stimulus duration is 10 sec with an inter-stimulus interval of 30 sec.

After drug application (via local or intraperitoneal injection), the same series of mechanical or thermal tests are repeated, at post-dosing time-points determined by the client. Responses before and after drug administration are compared.

Data analysis: The SPIKE2 computer software program and CED 1401Plus, a multichannel data-acquisition system (Cambridge Electronic Design), are used to record the timing and amplitude of action potentials and to analyze data on- or off-line. The advantage of this program is that it can differentiate between action potentials of different dorsal root units by amplitude and assign a colored and numbered template to each spike. Each channel can be duplicated to demonstrate various plotting modes simultaneously. The stored digital record of unit activity is retrieved and analyzed off-line. For single-fiber recordings, responses to mechanical stimuli applied to the receptive field for 10 sec are calculated by subtracting the preceding 10 sec of background activity to yield a net increase in discharge rate. Statistical significance is tested using one-way ANOVA followed by Dunn's post-hoc test.



Representative publication: Ringkamp M, Peng YB, Wu G, Campbell JN, and Meyer RA: Capsaicin responses in heat-sensitive and heat-insensitive A-fiber nociceptors. J. Neurosci. 21(12): 4460-8, 2001.

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