NeuroDetective©
INTERNATIONAL
Expert Neuroscience Research that Advances Drug Development

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 MODELS:	• tail-flick, • hot-plate
ACUTE INFLAMMATORY MODELS:	• formalin, • carrageenan (subcutaneous, intra-articular)
CHRONIC INFLAMMATORY MODELS:	• Freund's adjuvant (subcutaneous, intra-articular)
NEUROPATHIC PAIN:	• Chung model
POST-SURGICAL PAIN:	• Brennan model
DIABETIC NEUROPATHY:	• STZ-induced
BACK PAIN:	• Muscle relaxant model (Straub tail), with control tests for muscle weakness and overall sedation
VASCULAR PAIN:	• EMG Recording of flexor reflexes
IN VIVO ELECTROPHYSIOLOGICAL RECORDINGS	• Dorsal horn recording • Single teased fiber recording • Single fiber recording from the dorsal root

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…see [5] below).

Electrophysiological measures are also possible following carrageenan injection of the hindpaw. Recordings are made from individual C-fibers in the saphenous nerve, following sub-threshold, threshold, and supra-threshold mechanical stimulation.

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 intro 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 publications: 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. Wilson, H.D., Toepfer, V.E., Senapati, A.K., Wilson, J.R., and Fuchs, P.N. Hyperbaric oxygen treatment is comparable toacetylsalicylic acid treatment in an animal model of arthritis. The Journal of Pain, 2007, 8, 924-930.

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 isoflourane 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 (UgoBasile, 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. CHUNG MODEL OF NEUROPATHIC PAIN

Method: Tight ligation of the L5 spinal nerve (Chung model) is performed following induction of anesthesia with isoflurane 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: [Xth]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 |he 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.

5. 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.

6. BACK PAIN

Back pain is most often treated with muscle relaxants, and their efficacy as relaxants needs to be distinguished from a muscle weakening effect and possible sedative effects. A set of three behavioral assays is used for this purpose: Straub tail (muscle relaxant assay), grip strength (muscle weakening assay), locomotor activity (general sedation assay). The so-called Straub tail is a stiffening of the normal flaccid tail of either a rat or a mouse in response to morphine. Grip strength assessment and general activity evaluation use standard methodologies.

Representative publication: Fuchs, P.N. (2010) Muscle relaxant property of Company X Compound X (Study No. YYY). (Confidential report, NeuroDetective International; redacted version available on request.)

7. DIABETIC NEUROPATHY

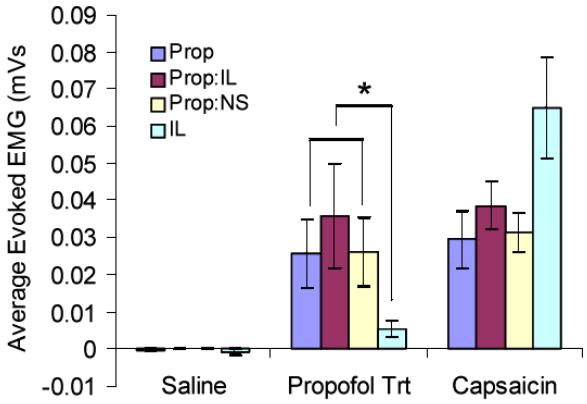
Method: Adult male Sprague-Dawley rats are first assayed for their paw-withdrawal thresholds following standard mechanical stimulation (von Frey hairs). The animals then receive injections of streptozotocin to induce insulin-dependent diabetes mellitus. This condition is confirmed by assay of blood glucose 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 prediabetes 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)

8. VASCULAR PAIN MODEL

Vascular pain can be inferred from electromyographic (EMG) activity, following injection of test substances (Ando and Wattanabe, 1995). The magnitudes of the flexor reflexes in response to arterial administration of different substances is measured using an EMG of the left posterior biceps femoris/semitendinosus muscle. For quantitative analysis, the area of the waveform within the EMG is calculated (mVs) and used as the EMG response. In addition, the latency and duration of the EMG responses are measured after drug injection. Mechanical stimuli (brush, pressure, and pinch) are also applied at the left hindpaw as controls. (See example graph, below)



Summary of evoked responses by intra-arterial injection of saline, mixtures of propofol, and capsaicin. *: $p<0.05$.

9. IN VIVO ELECTROPHYSIOLOGICAL RECORDINGS

Direct recordings of neuronal activity in response to peripheral electrical, mechanical, thermal, and chemical stimuli can be achieved by recording singleteased fiber in the primary afferent fibers (sural nerve, saphenous nerve, and dorsal root), as well as in the spinal cord dorsal horn neurons. The efficacy of potential drugs can be evaluated by using these methods after local or systemic administration in normal animals and in acute, chronic, and neuropathic pain models mentioned above.

Examples of the use of this technique for evaluating potential pain therapeutics can be found in the following references.

A. **Dorsal horn recording** (Senapati AK, Huntington PJ, Peng YB. Spinal dorsal horn neuron response to mechanical stimuli is decreased by electrical stimulation of the primary motor cortex. Brain Res.,2005, Mar 2;1036(1-2): 173-9)

B. **Single teased fiber recording** (Peng YB, Ringkamp M, Meyer RA, Campbell JN. Fatigue and paradoxical enhancement of heat response in C-fiber nociceptors from cross-modal excitation. J Neurosci., 2003, Jun 1;23(11):4766-74)

C. **Single fiber recording from the dorsal root** (Peng YB, Kenshalo DR, Gracely RH. Periaqueductal gray-evoked dorsal root reflex is frequency dependent. Brain Res., 2003, Jun 27;976(2):217-26)

An example of how this technique can be applied towards efficacy testing, click **here**.

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