

Spontaneous Behavior in the Home Cage: a New Test Environment for Measuring Neuropathic Pain

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INTRODUCTION

A sensitive animal model requires: 1) the distinction between novelty-induced and base line behavior, 2) the exclusion of confounding factors such as human handling, the continuous presence or preceding stressors; 3) the disentanglement of distinct motivational systems such as exploration, cognition involved in complex behavior. Thus, the activation of various motivational systems must be possible in a test situation allowing assessment of a distinct contribution of each of them to certain parameters.

A very good example of allowing the animal to display emotional behavioral expressions and to detect interactions between emotional states, for instance pain and anxiety, is the monitoring of the development of neuropathic pain measured in the home cage.

Neuropathic pain is usually indirectly measured by applying temperature (hot, cold stimuli > allodynia) or mechanical stimuli and assessing the animal's hypersensitivity to those stimuli.

In those tests involving handling, novelty and exposure to a challenging stimulus, stress as a result of the mechanical or temperature stimulus may inhibit sensory input and, thus, the animal's perception of and/or expression of neuropathic pain.

We now monitor the spontaneous behaviors of animals developing neuropathic pain induced using a standard surgical procedure (constriction of the *N. Ischiadicus*).

The rationale is that animals suffering from pain will adapt their voluntarily movements to avoid pain and that pain influences behavioral patterns over time and develop behavioral changes as a result on the interaction of pain perception and other emotional expressions, i.e., anxiety. We expect that animals meet their primary demands for food, water and exploration, but that they will modify their spontaneous behaviors to avoid the use of the painful body

part and due to the pain seek more shelter (safety, sleep, rest).

Thus, the home cage may allow the detection of a slowly developing chronic pain based on spontaneous reactions of the animal, most probably a direct consequence of the neuropathy and not an indirect reaction via hypersensitivity.

We aim to:

- Measure (the development of) symptoms of (chronic) pain by continuous automated monitoring of detailed potential changes in spontaneous behavior in the home cage after surgery that inflicts chronic neuropathic pain.
- Assess whether chronic pain affects other emotional states such as anxiety by measuring a possibly sensitized behavioral responses of animals having a constricted nerve in the hind paw to an aversive (light) stimulus in the home cage.
- Controlling the efficacy of the protocol used by assessing pain in a well known standard test to measure mechanical hypersensitivity, using the von Frey method.

We expect:

- to find changes in the distribution of time spent on various behavioral activities, such as time spent sleeping/sheltering, eating, exploring;
- that pain affects other emotional states such as anxiety, and thus, that animals subjected to the constriction injury will show altered behavioral responses to an anxiogenic stimulus.

METHODS

Subjects were twenty-four rats (Wistar, CharlesRiver Germany), that were housed socially in pairs in a standard macrolon cage containing bedding, nesting material and tubes. Water and food were available ad libitum. The animals were housed under a regular 12/12 day/night rhythm (light period: 6:00-18:00; dark period: 18:00-6:00) and were allowed to adjust to housing and management procedures for 2.5 weeks before the start of the experiment. The animals were randomly assigned to the CCI-group (n=12), or Control-group (n=12).

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Day	Measurements
-4	START PhenoTyper [24hr behavioral monitoring]
-4 0	continuous Behavioral monitoring
0	SURGERY: Chronic constriction injury (CCI) at the sciatic nerve
0 7	continuous Behavioral monitoring
7	weighing, cage cleaning
7 14	continuous Behavioral monitoring
14	weighing, cage cleaning
14 21	continuous Behavioral monitoring
22	ANXIETY-test: Light spot on Food hopper during first 3hrs of dark period
24 25	PAIN-test, mechanical hyper-sensitivity: Von Frey Pre-test
26	PAIN-test, mechanical hyper-sensitivity: Von Frey, 1 PAIN-test, mechanical hyper-sensitivity: Von Frey, 2

Table 1. Time schedule of the experimental protocol.

Phenotyper Anxiety Test: Light Spot At Day 22 on Feeding Area

After the start of the dark (i.e. active) period, during which rats normally spend a lot of time on feeding, a bright light spot (500 lux) was focused on the food trough for 3 hours. Parameters such as latency to enter the illuminated food zone, frequency of visits and time spent in this zone can be indicative of an aversive response and related to the level of anxiety. After the light spot has been switched off the latency to return and number of frequencies to this previously illuminated zone can be indicative of a long-term effect or sensitization.

Measurement of Mechanical Hypersensitivity

Mechanical hypersensitivity was measured using a set of graded von Frey Filaments (Somedic, Sweden). The animals are placed in a Plexiglas chamber (16x24x14 cm) with a grate bottom and left to adapt for at least 15 min; the animals have been habituated to the transport procedure and boxes (app. 20-30 min) the day before. The test is conducted during the light (inactive) phase between 9:30 and 13:00 hr. Mechanical stimuli are generated by touching the plantar region of the animals hind paw with von Frey filaments (glass fiber) of differential thickness, causing different levels of pressure.

The filament is pressed against the hind paw until it bends and is held steady for 5-6 seconds, scoring if the animal responds or not. The animals were measured randomly three times on the operated (left) paw. The measurements on the left paw of the non-operated control group serves as a control.

RESULTS

The results show acute consequences of the constriction injury of the sciatic nerve (CCI) on various parameters during the first week after surgery: e.g. Time spent on eating, distance moved, velocity and frequency of moving.

The following weeks more long-term effects of the constriction, and presumably indicating chronic pain, were noticed on how the animals spent their time and explored their home cage. In this abstract we limit ourselves to the effects of CCI on the time spent near the feeder and anxiety.

Time Spent Near Feeder: Increase

During the active period of their day CCI animals spent more time in the vicinity of the feeder (Figure 1). Body weight did not change over the weeks between the groups.

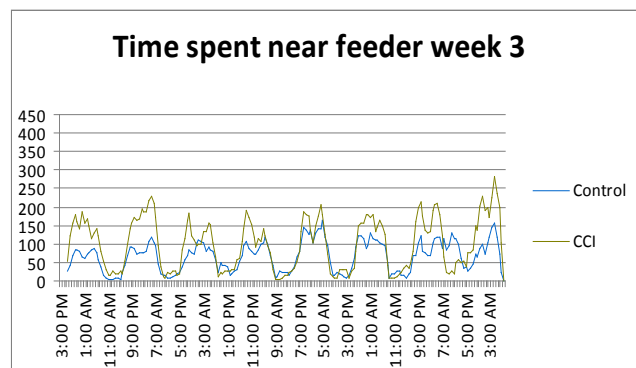


Figure 1. On the y-axis time spent in the feeding area is displayed per hour. The X-axis represents time over 7 days.

CCI animals showed a longer latency (Figure 2) before entering the food-area during the test and spent less time in that area after the test indicating a prolonged aversive effect or even sensitization to an aversive stimulus in CCI-animals.

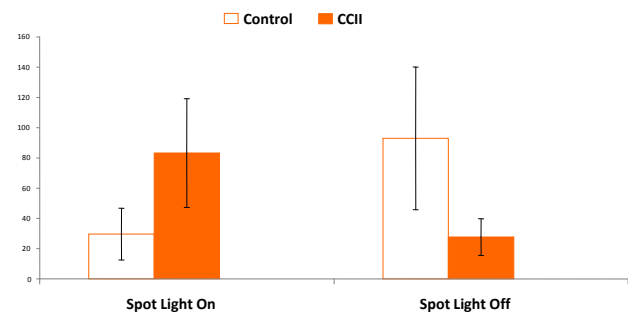


Figure 2. Latency to enter the illuminated zone.

In addition, if the time spent near the feeder during the LightSpot is compared per animal to the time spent in that zone 24 hrs before (when light was off), a decrease in time spent near feeder is seen (data not shown).

At the end of the home cage-monitoring period, the von Frey test clearly verified the presence of neuropathic pain as assessed by this standard method.

DISCUSSION

It seems that the animals were capable of displaying all kinds of movements, and behaved differently in response to the feeder and an aversive light beam. Other parameters not shown here such as distance moved, velocity and time spent on shelter, immediately after surgery and after 3 weeks changed as well and are now statistically being analyzed and will be presented. Effects of pain on anxiety measured in the home cage have been demonstrated, which seems

indicative of a change at the emotional level in the animals with neuropathic pain. The effects of the aversive stimulus on anxiety seems to be long lasting as these animals still showed a change in behavior (time spent in the previously lighted area) the next day. The effects of pain on a sensitized anxiety response underline the notion that the behavioral changes are due to the experience of pain and not a mere nociceptive response.

Notwithstanding the fact that the study was limited to 4 weeks, whereas it might be that a longer time-window and a more in-depth analysis is necessary to detect symptoms of (developing) chronic pain, the results are promising and opens avenues for assessing symptoms of neuropathic pain in the home cage.