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Research report

Enduring attentional deficits in rats treated with a peripheral nerve injury☆

Guy A. Higgins[a,b,*], Leo B. Silenieks[a], Annalise Van Niekerk[a], Jill Desnoyer[a], Amy Patrick[a], Winnie Lau[a], Sandy Thevarkunnel[a]

[a] InterVivo Solutions Inc., 120 Carlton Street, Toronto, ON Canada M5A 4K2
[b] Dept. Pharmacology & Toxicology, U. Toronto, ON, Canada M5S 1A8

HIGHLIGHTS

- Rats prepared with spared nerve injury (SNI) as model of neuropathic pain.
- Rats evaluated daily for 3 months post surgery for food motivation (PR schedule) or attention/reaction time (serial 5-choice task).
- SNI rats show similar motivation for food compared to sham operated controls.
- SNI rats show deficits in attention and response speed compared to sham operated controls from 2 weeks post surgery.
- Implications for translational research into study of attention based deficits in neuropathic pain.

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ABSTRACT

The present study investigated the impact of a spared nerve injury (SNI) on the daily performance of rats tested in two instrumental conditioning procedures: the progressive ratio (PR) schedule of food reinforcement to study motivation for an appetitive stimulus, and the 5-choice serial reaction time task (5-CSRTT), a test of attention and reaction time. Separate groups of male, Sprague-Dawley rats of age 8–10 months were trained to asymptotic performance in either task, before undergoing either SNI or sham surgery. After a recovery period of 3–4 days the animals were run 5 days/week for 3 months in either task. Tests of responsivity to evoked tactile (Von Frey) and thermal (acetone) stimuli were also conducted over this period to check integrity of the model. Post SNI surgery, rats showed equivalent responding to sham controls for food available under a PR schedule throughout the test period, implying a similar level of motivation for a food reward. In contrast, a performance deficit emerged in SNI treated rats run in the 5-CSRTT, consistent with an attentional deficit. This deficit emerged during the second month post-surgery and was characterized by slower response speed, reduced accuracy and increased trial omissions. Both SNI groups showed equivalent hypersensitivity to evoked sensory stimuli compared to controls. Since attention based deficits have been reported in individuals with clinical forms of neuropathic pain, the present studies suggest a novel approach to study this phenomena and a means to study the effect of treatments against this cognitive endpoint.

1. Introduction

Gaps in the translation between preclinical to clinical findings for new chemical entities (NCE's) is a topic of significant concern, because failure to demonstrate clinical efficacy has become the most significant reason for program termination [1,2]. This trend is apparent across all therapeutic areas including NCE's developed for pain management [2]. One counter approach is to reevaluate the animal models themselves and identify ways to improve their predictive power. Several key articles have been written about how pain models can be refined with a major theme being

Abbreviations: NCE, new chemical entity; SNI, spared nerve injury; SNL, spinal nerve ligation; PR, progressive ratio; 5-CSRTT, 5-choice serial reaction time task; SD, stimulus duration; ITI, inter trial interval; LH, limited hold; ICSS, intracranial self-stimulation; NbM, nucleus basalis of Meynert; VTA, ventral tegmental area; FCA, Freunds complete adjuvant.

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* Corresponding author at: InterVivo Solutions Inc., 120 Carlton Street, Toronto, ON, Canada M5A 4K2. Fax: +1 416 920 1876.
E-mail address: guyh@intervivo.com (G.A. Higgins).

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the type of endpoint used to measure pain [3–10]. A significant ongoing research effort is to identify endpoints beyond the traditional evoked sensory responses which remain a mainstay yet do not capture the chronicity of a pain state or reflect clinical endpoints which tend to be measures of continuous spontaneous pain.

Reflective of the fact that depression and cognitive decline are often associated with clinical forms of chronic pain [11–13], indirect measures of affect such as place conditioning and sucrose preference [14–19] and cognitive performance across multiple test designs [20–25] have been studied as endpoints in both neuropathic and inflammatory pain models. While many of these studies have identified behavioural deficits consistent with clinical experience, most of these tests are acute i.e. conducted on limited occasion, and in tests that are subject to both environmental factors, and critical details/variations in protocol. Taken together, these factors make consistent replication over time and between laboratories problematic [26]. One way to reduce these inconsistencies is to utilize instrumental conditioning procedures in which animals are trained to perform a specific task for food reinforcement. In addition to generating stable baselines within experiments enabling the detection of subtle performance changes, such tests can also be conducted daily to continuously measure performance in the same animals over days/weeks to establish reliability to any change [27,28]. Task contingencies can also be manipulated to measure specific aspects of behaviour.

Examples of instrumental conditioning tasks include the progressive ratio (PR) and the 5-choice serial reaction time (5-CSRT) tasks. The progressive ratio schedule is an approach used to measure motivation to respond for a rewarding stimulus [29]. By training rats to lever press for a reward (e.g. food pellet) and progressively increasing the number of lever presses necessary for each subsequent reward, an index of the amount of effort that an animal will commit can be determined, with the final ratio achieved (i.e. the "break point") providing an objective measure of the test subjects motivation to work for that reward. Since reduced motivation is a core symptom of depression [30,31], the PR test has been used to characterize animal models of depression, including chronic mild stress procedures [32–35]. In contrast, the 5-choice serial reaction time task measures the ability of a test subject to detect and respond to a brief visual stimulus presented in random location, and has become a task widely used to study attention and reaction time in rodents [36–38]. Primary outcome measures in this test include choice accuracy, response speed (reaction time), and premature responding, an index of response control. Both the PR and 5-CSRT tasks can be conducted across multiple species including humans raising a possibility for translational study.

The purpose of the present studies was to adopt the PR and 5-CSRT tasks as a means to measure the performance of rats following induction of a Spared Nerve Injury (SNI; [39]) model of neuropathic pain. This approach was selected due to the long term hypersensitivity to evoked sensory stimuli, consistent with the clinical pain state, and it was a specific purpose of these studies to continuously monitor performance of rats trained to either the PR or 5-CSRT for an extended post-surgical time period (3 months). It was thought that such studies could shed useful information about the long term impact of the SNI model on motivation for food reward (an index of affect), and on a core aspect of cognitive function. In recognition of the fact that neuropathic pain conditions are more prevalent in the ageing population [5,7,26], these studies were conducted in rats of mid-age, i.e. 8–12 months age. A previous study identified rats of this age group to be more susceptible to chronic pain-induced affective and cognitive deterioration compared to young (3 months) and aged (22 months) cohorts [24].

2. Methods

2.1. Animals and housing

Test subjects were male Sprague-Dawley rats (source: Charles River, St. Constant, Quebec, Canada) of approximate age 8 months at the study start. Previous studies have shown this strain to give reliable tactile and thermal allodynia following SNI surgery [40,41]. Animals were singly housed in polycarbonate cages with sawdust bedding with water freely available. Food (LabDiet, 5001) availability was restricted to approximately 18–20 g at the end of each day, plus that earned during the daily operant session. The housing room was maintained at a constant temperature of $22 \pm 2\,^{\circ}C$, under a 12 h light-dark cycle (lights on: 06:00–18:00 h). Testing was conducted under the light phase of the animals light/dark cycle. All studies were approved by an Institutional Animal Care and Use committee and conducted in accordance with guidelines established by the Canadian Council of Animal Care (CCAC).

2.2. Preparation of animals: spared nerve injury

Following anaesthesia with ketamine (75 mg/kg IP) and xylazine (10 mg/kg IP), the skin on the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle to expose the sciatic nerve and its three terminal branches: the sural, common peroneal and tibial nerves. The common peroneal and the tibial nerves were tight ligated with 5–0 or 6–0 silk sutures and sectioned distal to the ligation, removing 2–4 mm of the distal nerve stump. Care was taken to avoid any contact with, or stretching of, the intact sural nerve. Sham controls involved exposure of the sciatic nerve without any lesion or further manipulation. At the completion of surgery, the muscles were sutured and the skin closed with silk sutures. Animals were returned to their home cage lined with soft sawdust bedding for the duration of study [42].

Post surgery, the animals tended to develop a change to the posture of the hindpaw ipsilateral to the nerve injury reflecting an avoidance of weight bearing on the lateral portion of the affected paw. Autotomy was not detected throughout the 3-month post-surgery period. A preliminary test was undertaken to assess mechanical allodynia (see below). Any non-responders (typically <5%) were removed from the study and any adjustments are highlighted in the relevant experimental results sections.

2.3. Measurement of evoked responses to sensory stimuli

For the measurement of mechanical static allodynia, the animals were singly placed in clear elevated chambers on a Perspex grid floor and allowed 10–15 min to settle. The lateral plantar surface of the paw was stimulated with a series of ascending force Von Frey hair filaments (0.4, 1, 2, 4, 6, 8, 10, and 15 g). Application of filament to plantar surface was applied beginning with the lowest force filament (0.4 g). The threshold was taken as the lowest force that evoked a brisk withdrawal response. A filament with the next highest force was applied to confirm the threshold. The average score from three separate assessments was taken as the final measure for that animal [41].

The measurement of cold allodynia (acetone drop test) was conducted in the same chamber approximately 5 min following the Von Frey test. A drop of acetone solution was carefully dropped onto the lateral plantar surface of the paw, using a blunt needle connected to a syringe, without touching the skin. The magnitude of the withdrawal response was scored according to a 4 point rating scale where 0 = no visible response, 1 = response but without paw withdrawal, 2 = clear withdrawal of the paw, 3 = withdrawal combined with flinching and licking of the paw, 4 = prolonged withdrawal