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Plenary article

Nerve injury causes long-term attentional deficits in rats

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HIGHLIGHTS

- Rats remain hypersensitive to touch and cold over 6 months after nerve injury.
- At this late time point, rats display deficits in attentional ability.
- These deficits are not due to locomotor impairments.
- Rats also display anxiety-like behaviors 6 months after nerve injury.
- This is the first study to look at attentional ability 6 months after injury.

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ABSTRACT

Human chronic pain sufferers frequently report problems with attention and concentration that affect daily functioning and quality of life. Chronic pain is also commonly associated with anxiety and depression. It is currently not known if the pain causes these co-morbidities, or if they are pre-disposing risk factors for the development of chronic pain. Animal studies suggest a possible causative effect of pain on cognition, but usually tests are conducted during acute ongoing pain when the pain may act as a distracter to normal cognitive and emotional processing. Here we examine long-term effects of nerve injury on cognitive functioning in a rat model, which contributes to better understanding of the relationship between cognitive impairment and chronic pain experience in human populations. This study investigated attentional capability, anxiety-like behavior and sensory functioning 6 months after spared nerve injury (SNI) surgery—a time-point well beyond the acute pain phase and akin to decades of pain experience in humans. Male Long Evans rats subjected to nerve injury remained hypersensitive to sensory stimuli from the time of injury to the 6-month post-injury assessment. At 6 months they were impaired on a visual non-selective, non-sustained attention task and displayed anxiety-like behaviors in the elevated plus maze. These findings show that cognitive disturbances observed during acute pain persist for months in a rodent chronic pain model and suggest that cognitive alterations in chronic pain patients are at least partially caused by the chronic pain state.

1. Introduction

Chronic pain patients often complain of cognitive changes, particularly in the ability to concentrate and remember. Experimental evidence in a variety of chronic pain disorders substantiates these reports [see 18]. Nevertheless, it is not clear whether such co-morbidities are actually caused by the pain or are related to factors that may predispose someone to develop chronic pain. There is substantial evidence that ongoing pain disrupts the ability to focus attention in healthy individuals as well as in chronic pain patients, particularly when ongoing pain levels are high [see 18]. Studies in animals have reported that, similar to humans, ongoing pain disrupts attentional performance [4,16,20]. However, these studies tested attentional performance within 2 weeks of inflammatory injury, a time point at which there is evidence of active peripheral nociceptive input and pain chronicity may not yet be established

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[28]. Therefore it is likely that, similar to human healthy volunteer studies, pain is acting as an active distracter at this point, leading to poor performance on the attention tasks [13].

Nevertheless, in human chronic pain patients, there is evidence that attentional ability is impaired many months or years after onset of pain symptoms, even when the patient is not actively experiencing pain [8]. Thus, some of the cognitive dysfunction cannot be explained by simple distraction, but rather may be related to underlying neurobiological factors. Evidence supporting this comes from neuroimaging studies in pain patients showing less gray matter, impaired function and abnormal connectivity in brain regions involved in attentional processes, such as the anterior cingulate and prefrontal cortices [see 18]. Similar anatomical results were obtained in a rat model of neuropathic pain; 6 months after nerve injury, rats had reduced gray matter in frontal cortical regions compared to sham controls [25]. Since the rat model was a longitudinal study, in which genetics and environment were controlled, these data suggest that the anatomical differences between human chronic pain patients and controls are likely to be at least partially caused by the pain. The same injury model could be used to evaluate whether the cognitive differences observed in chronic pain patients are also at least partially caused by the chronic pain state.

Thus, the current study used a longitudinal design in a rat model to determine if deficits in attentional capability persist well beyond the acute pain phase, at a time point equivalent to many years of pain experience in a human (6 months in a rat is approx. 20% of a 2- to 3-year lifespan; 20% of a human lifespan is approx. 15–20 years). Sensory hypersensitivity was monitored for 6 months after nerve injury, and then cognitive performance was tested using the visual non-selective, non-sustained attention task described by Millecamps et al. [16]. This task is similar to other novel object recognition tasks that utilize the innate preference of rodents to explore new objects over familiar ones [11]. We also investigated anxiety-like behaviors and locomotion as indices of pain-related impairment.

2. Methods

Male Long–Evans rats (150–180 g, Charles River, QC) were housed in pairs (one experimental/one control animal per cage) in standard ventilated cages, with a 14:10 h light/dark cycle. To avoid excess weight gain, animals were fed 5 g of food (Rodent Chow 5075, Charles River) per 100 g body weight per day. All animals had ad libitum access to water. McGill University's Animal Care Committee approved all procedures.

Animals were randomly assigned to either receive spared nerve injury (SNI) surgery ($n = 13$) or sham surgery ($n = 13$) at 10–12 weeks of age. SNI surgery consists of ligation and transection of the tibial and common peroneal branches of the sciatic nerve, while sparing the sural nerve [10]. Sham animals received the same anesthesia and surgical incision but nerves were left intact.

Animals were weighed and tested for sensory sensitivity at 2, 8, 16 and 24 weeks post-surgery, and locomotion and anxiety-like behaviors tested at 24 weeks post-surgery. Testing was performed by an operator who was not told of the surgical status of the animal. The attention task was performed only once, between 25 and 28 weeks post-surgery, since performance on this task can be altered by learning and habituation if administered repeatedly [11].

2.1. Tactile and cold hypersensitivity

Animals were initially habituated to the testing apparatus for 30 min a day on 3 days prior to the initial testing session. On test days, animals were placed into the boxes and re-habituated for 10 min before testing began. Tactile hypersensitivity was measured using von Frey hairs (Stoelting, IL) using the up-down method [5]. Cold hypersensitivity was measured by applying 50 μl acetone to the plantar surface of the hindpaw, and length of response (in seconds) was timed with a stopwatch [6]. Both left (ipsilateral) and right (contralateral) paws were tested, with a minimum of 2 min between each test.

2.2. Visual non-selective, non-sustained attention task

Cognitive function was assessed using a visual non-selective, non-sustained attention task [16]. The task controls for differences in object characteristics by using objects that are similar in size, color and texture but differ in shape alone. Animals were exposed to an open field arena (90 cm × 90 cm) for 5 min on 3 consecutive days. This arena contained 4 small objects of the same size, texture and color, but different shapes, attached to the middle of the walls of the arena, 5 cm above the floor (i.e. nose height). On the test (4th) day, one object was replaced with a novel one (same color and texture but new shape). The replaced object was randomized, so it was not the same object being replaced for all animals. Behavior was recorded over 5 min by webcam hung above the arena, and the arena cleaned with diluted Windex® (1:10) between each testing session. A blinded observer scored the videos. Time spent exploring each object (defined as sniffing after interruption of ambulation) was measured, and percentage time spent exploring the novel object was calculated using the following equation:

$$\text{Attentional level}\,(\%) = \frac{\text{duration of exploration of new object}}{\text{total duration of exploration of all objects}} \times 100$$

2.3. Open field (OF) test

Locomotion was measured using an open field arena (90 cm × 90 cm) with walls 40 cm high. Animals were placed into the arena and behavior recorded over 5 min by webcam. The arena was cleaned as above between each testing session. Videos were scored by a blinded observer. Percentage time spent in either the center or perimeter of the arena was calculated, as were total number of squares (15 cm × 15 cm) traversed, and number of rears performed.

2.4. Elevated plus maze (EPM)

Anxiety-like behaviors were investigated using the elevated plus maze (EPM), consisting of a plus-shaped arena raised 50 cm from the ground, with 2 open arms and 2 enclosed arms (walls 30 cm high) opposite each other. Arms were 110 cm end-to-end. Animals were placed onto the center of the maze and recorded over 5 min by webcam, after which the maze was cleaned as above between each testing session. A blinded observer scored the videos. Percentage time spent in the closed and open arms was calculated, and entries into the center or open arms counted.

2.5. Statistical analysis

All analyses were done using GraphPad Prism 5 (GraphPad Software Inc.). Two-way repeated measures ANOVAs were performed for sensory testing, with surgical treatment and time as variables. Bonferroni's post hoc tests were performed to find the directions of any significant differences. Attention task data and behavior in the OF and EPM were analyzed at the 24-week time point with group comparisons performed by unpaired 2-tailed *t*-test (if data normally distributed, according to the D'Agostino and Pearson omnibus normality test) and Mann–Whitney test (if non-normally distributed).

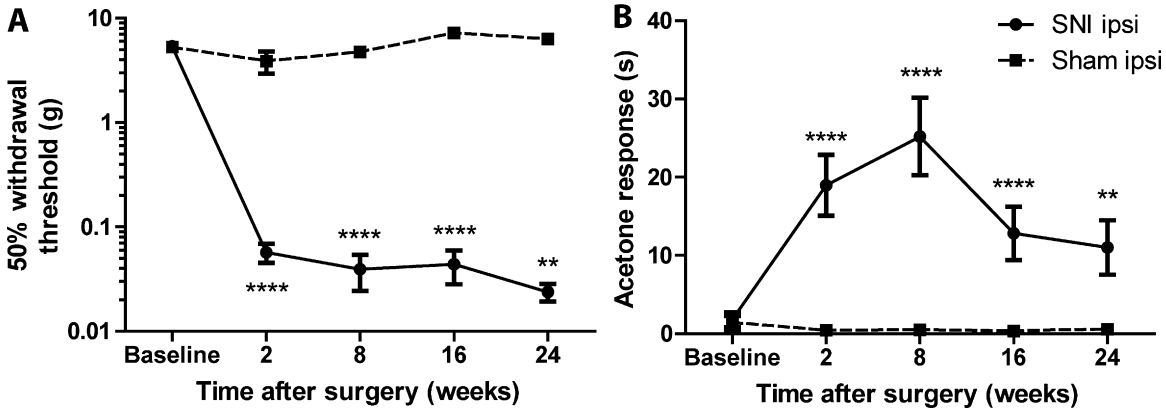

Fig. 1. Nerve injured animals remain significantly hypersensitive to mechanical and cold stimuli over the course of 6 months after surgery. The ipsilateral paw was hypersensitive to (A) mechanical stimuli at all time points after surgery (Time $F_{(4,48)} = 6.01$, $p < 0.001$) compared to sham controls (Treatment $F_{(3,48)} = 32.4$, $p < 0.0001$) and (B) acetone application compared to all other groups at all time points after surgery (Time $F_{(4,48)} = 6.88$, $p < 0.0001$; Treatment $F_{(3,48)} = 37.02$, $p < 0.0001$). There were no differences over time or between paws in sham controls ($p > 0.05$). $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ vs. controls; 2-way RM ANOVA with Bonferroni's post hoc tests. Graphs show mean ± SEM of the ipsilateral paw only.

3. Results

3.1. Nerve injury causes hypersensitivity to mechanical and cold stimuli that persists over 6 months

At baseline there were no significant differences in withdrawal thresholds between groups or paws ($p > 0.05$). At all time points post-surgery however, nerve injured animals showed ipsilateral (left) paw hypersensitivity to both mechanical (Fig. 1A) and cold stimuli (Fig. 1B). Von Frey hair stimulation elicited robust reflex withdrawals at forces of less than 1 g ($F_{(3,48)} = 32.4$, $p < 0.0001$), and acetone application produced response times of 11 ± 3.4 s, up from a control response time of 0–2 s ($F_{(3,48)} = 37.02$, $p < 0.0001$). There were no significant differences between paws or over time in the sham control group for either mechanical or cold hypersensitivity testing ($p > 0.05$). These results show that nerve-injured animals remain hypersensitive to at least 6 months after SNI surgery.

3.2. Ability to attend to a novel object is impaired 6 months after nerve injury

There was no significant difference between nerve-injured and sham operated rats in the total amount of time spent exploring objects attached to the walls of the open field arena over 5 min on the test day. Sham-operated animals ($n = 10$) spent in total 10.34 ± 1.92 s and SNI animals ($n = 13$) spent 8.65 ± 1.22 s exploring the objects ($p = 0.69$, Mann–Whitney test; data not shown).

In contrast, control animals explored the novel object for a significantly greater percentage of time (46.12 ± 5.36%) than SNI animals (30.32 ± 5.14%; $p < 0.05$, unpaired t-test; Fig. 2A). SNI animals did not explore the new object significantly above chance (25%) levels ($p > 0.05$), suggesting that the injured animals may not distinguish the new object from the familiar objects.

3.3. Cognitive impairment is not due to impaired locomotion in SNI animals

Comparing locomotion (squares traversed in the OF) between nerve-injured and control animals 6 months after injury revealed no significant differences between control and injured animals (SNI: 77.8 ± 7.31; sham: 80.4 ± 5.61; $p = 0.79$, two-tailed unpaired t-test; Fig. 2B). In addition, the percentage of time spent in the center of the OF and number of rears performed in the OF were not significantly different between treatment groups at 24 weeks post-surgery (% time—SNI: 6.46 ± 1.7%, sham: 5.39 ± 1.6%, $p = 0.65$); rears—SNI: 32.1 ± 3.9, sham: 33.5 ± 2.9; $p = 0.78$; both 2-tailed unpaired t-tests), suggesting no significant differences in exploratory behavior between groups.

3.4. Nerve injury increases anxiety-like behaviors

Consistent with other studies [19,21,22,26], we saw increased anxiety-like behavior in nerve-injured animals. Similar to a previous study [25], we saw these behaviors at 6 months after injury. SNI animals spent significantly less time in the open

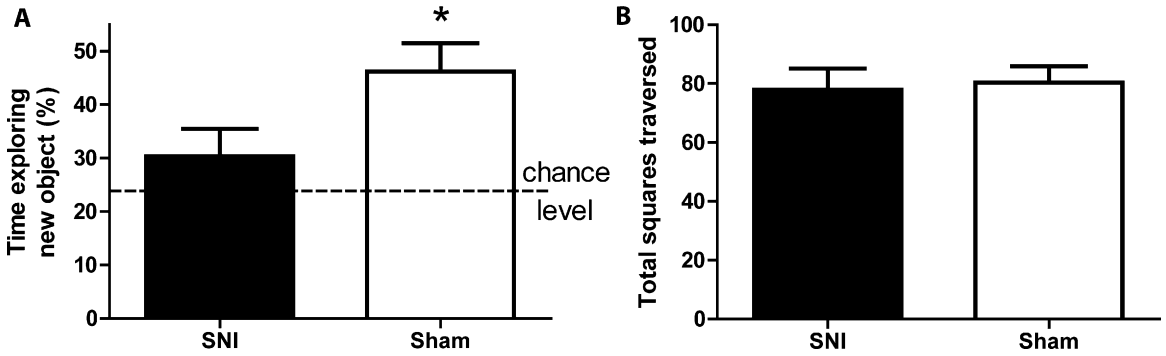

Fig. 2. Visual non-selective, non-sustained attention is impaired 6 months after nerve injury. (A) SNI animals spent significantly less time exploring the novel object on the test day than sham animals, attending to the new object no more than at chance (25%) levels. $^{*}p = 0.048$, two-tailed unpaired t-test. (B) Differences in attentional level were not due to locomotor impairments, as both groups moved around the open field equal amounts ($p = 0.79$, two-tailed unpaired t-test). Graphs show mean ± SEM.

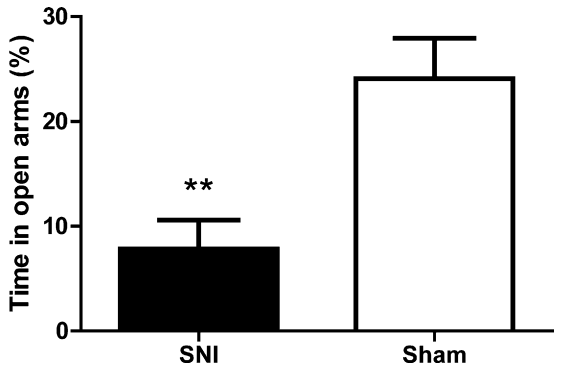

Fig. 3. Nerve injured animals show anxiety-like behaviors 6 months after nerve injury. SNI animals spent significantly less time in the open arms of the elevated plus maze compared to sham animals at 24 weeks (**$p = 0.002$; Mann–Whitney test). Graph shows mean ± SEM.

arms of the EPM at 24 weeks compared to control animals (SNI: 7.85 ± 2.7%, sham: 24.1 ± 3.9%; $p < 0.01$; Mann–Whitney test; Fig. 3A), and entered the open arm fewer times (SNI: 0.34 ± 0.3, sham: 2.77 ± 0.6; $p < 0.01$; Mann–Whitney test; data not shown), suggesting increased anxiety-like behavior.

4. Discussion

In this study, rats showed continued hypersensitivity to touch and cold, expressed anxiety-like behavior and showed reduced attention to novel objects 6 months after a nerve injury. These results demonstrate for the first time cognitive alterations in a rodent model of chronic pain long after the initial injury. Findings of cognitive differences at this late time point are similar to observations in human chronic pain patients, who show deficits in tasks involving attention and memory after having had pain for years [see 18]. However, unlike in previous human studies with cross-sectional designs, with our current longitudinal design we can deduce that the initial injury led (directly or indirectly) to the cognitive changes, since genetic, environmental, and social factors did not differ between the nerve-injured and sham control animals.

Several aspects of our findings suggest that the SNI model provides a relevant surrogate for chronic pain in humans. Like neuropathic pain conditions in humans, including diabetic neuropathy, post-herpetic neuralgia, chemotherapy-induced neuralgia, and other human conditions leading to partial nerve injury, these animals remain hypersensitive to tactile and thermal stimuli months after the initial injury [7,25]. This is in contrast to other nerve injury models in rodents that produce more transient hypersensitivity [see 17].

Nevertheless, demonstrating cutaneous hypersensitivity is not sufficient to model the human experience, since chronic pain patients frequently have co-morbid anxiety and depression [15] and altered cognitive ability [see 18]. We find in our study that rats subjected to SNI manifest similar long-term co-morbidities as human pain patients, including anxiety-like behavior and cognitive deficits, further suggesting that this is a particularly useful model for the study of chronic pain.

What is the cause of the cognitive dysfunction 6 months after a nerve injury? It is possible that, as during acute pain, animals are simply distracted and do not attend to the objects, so novel ones are not recognized. While there is currently no direct evidence of spontaneous pain experience in rats 6 months after nerve injury, it is unlikely that substantial levels of ongoing spontaneous pain are present to distract the animals from the attention task. Firstly, in our study, animals engaged in normal locomotion and continued to gain weight throughout the study. Secondly, Urban et al. [29] evaluated a number of rodent models of chronic pain, including neuropathic models, and found that for all of them, the animals resumed normal eating, grooming, social and sleep behaviors within days of the injury. Finally, chronic pain patients frequently report that their ongoing pain can wax and wane, and is seldom constant across time [2,3], suggesting that in the rat model, pain is unlikely to be constant at this late time point.

An alternative explanation for the cognitive changes described here is a disruption of cognitive circuitry in the brains of the animals subjected to nerve injury. Evidence to support this comes from human and animal studies showing neuroanatomical alterations in brain gray matter and connectivity in regions association with executive functioning in chronic pain conditions [9,10,12,14,24]. Apkarian et al. [1] first showed that gray matter density of the dorsolateral prefrontal cortex is reduced in chronic back pain patients, and these findings have been replicated and extended to a number of other brain regions and chronic pain modalities such as fibromyalgia, arthritis and complex regional pain syndrome (CRPS), and neuropathic pain [see 23]. Furthermore, brain alterations in humans have been linked to deficits in cognitive ability such as response inhibition and working memory [see 18]. Neuroanatomical changes in gray matter volume [25] and neuronal structure and function [14] are also shown in the rat neuropathy models, including in frontal cortical regions important for cognitive function. Thus, we argue that the attentional changes observed in the present study could be at least partially related to chronic pain-induced alterations in cortical structure and function.

In addition to deficits in attention, animals showed increased anxiety-like behavior 6 months after nerve injury. A number of studies have shown increases in anxiety-like behavior in rodent chronic pain models [19,22,26,30], but only one other has examined such behavior at a late time-point and shown increased anxiety 6 months after injury [25]. In that study, the onset of anxiety-like behavior began approximately 5 months after SNI injury, at the same time as cortical thinning became apparent, suggesting that the neuroanatomical changes could be an underlying cause of the late-onset anxiety increase. In the current study, we also observed increased anxiety-like behavior in SNI rats 6 months after injury, along with decreased attentional performance. Thus, it is possible that both anxiety and cognitive dysfunction are related to the neurobiological changes that are concomitant with chronic pain. Future studies are needed to examine this possibility.

Chronic pain is now beginning to be considered as a disease in itself [27], with repercussions that go far beyond the pain. The current findings show altered attentional functioning and increased anxiety-like behavior in rats 6 months after nerve injury, a delay equivalent to decades in the human lifespan. This adds evidence from an animal model to the observations in human pain patients that chronic pain can lead to emotional and cognitive effects that may reflect altered neurobiological functioning. The genetic and environmental complexity of pain patients has made it difficult to determine if the emotional and cognitive differences between pain patients and controls is the cause or effect of the pain. The current study suggests that the chronic pain condition itself is likely a causative factor in these differences. Future longitudinal studies in rodents will be critical in assessing the multitude of pain co-morbidities and their relationship to brain alterations, and may open avenues for future treatments that prevent, or reverse, these pain-related changes.

Conflict of interest

The authors declare no conflict of interest.

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