



Double Decker Enrichment cages have no effect on long term nociception in neuropathic rats but increase exploration while decreasing anxiety-like behaviors

by Pascal Vachon

*Correspondence: Pascal Vachon

University of Montreal, Faculty of Veterinary Medicine, Department of Veterinary Biomedicine, Saint-Hyacinthe, Québec, Canada J2S 2M2

Tel + 1 (450) 773-8521 ext. 8294

E-mail pascal.vachon@umontreal.ca

Summary

In this present study, we investigated the impact of environmental enrichment in Sprague Dawley rats up to three months after a chronic or sham nerve injury. Sprague Dawley rats were housed in either standard polycarbonate cages or rat enrichment cages. Following 2 weeks of training and the recording of baseline behavioral values, half of the animals underwent a right sciatic nerve chronic constriction injury (CCI) surgery under general anaesthesia to induce chronic neuropathic pain. The other animals underwent a sham surgery. Animals were then evaluated once a month for 3 consecutive months in different behavioural tests for of mechanical and heat sensitivities as well as for exploration and anxiety-like behaviors. Mechanical and heat sensitivities were also tested at 15 days following the surgery. One month following the surgery, half of the rats in each group (CCI and sham) were either left in the standard rat cages or placed in the Double Decker cages. Environmental enrichment did not affect the mechanical or heat sensitivity of neuropathic animals; however exploration increased, and anxiety-like behaviours decreased, significantly ($p < 0.01$). These results clearly show that environmental enrichment can have a significant impact on exploratory and anxiety-like behaviour in neuropathic rats without modifying pain hypersensitivity.

Introduction

Enrichment of both social and physical life is gaining interest for the treatment of chronic pain, since an enriched environment may be protective against the development of chronic pain. For example, studies in animals (Tagerian *et al.*, 2013; Vachon *et al.*, 2013; Gabriel *et al.*, 2009, 2010a, 2010b; Tall 2009) and humans (Smith *et al.*, 2003; Ulrich, 1984) looking at the effects of the environment on pain recovery following surgery showed a significant alleviating effect with environmental enrichment. Environmental enrichment may also protect against the development of depression and anxiety disorders, both of which are commonly associated with chronic pain in animals (Tagerian *et al.*, 2013; Seminowicz *et al.*, 2009; Suzuki *et al.*, 2007) and humans (Campbell *et*

al., 2003; Daniel *et al.*, 2008; Dick *et al.*, 2008; Haythornthwaite *et al.*, 2000; Kewman *et al.*, 1991; Petrak *et al.*, 2003). Social isolation on the other hand has unclear effects on pain sensitivity. Adler *et al.* (1975) and DeFeudis *et al.* (1976) reported that isolation had no effect on pain behaviors; however Panksepp (1980) suggested that it may increase pain sensitivity.

Rodents living in enriched environments show less pain behavior following a peripheral nerve or central nervous system injury than those living in standard or restricted environments (Tall, 2009; Gabriel *et al.*, 2009; 2010a; 2010b, Berrocal *et al.*, 2007; Lankhorst *et al.*, 2001). However in these studies animals were kept in the enriched environment from the time of injury onward. Most studies have been conducted with healthy non-injured animals (Chourbadji *et al.*, 2008; Rossi & Neubert, 2008; Smith *et al.*,

2003, 2004 & 2005) as well as animals with inflammatory (Gabriel *et al.*, 2009; 2010a,b; Tall, 2009) and neuropathic pain (Stagg *et al.*, 2011). It has recently been shown that pain can be alleviated by environmental enrichment well after the establishment of chronic pain in mice (Vachon *et al.*, 2013; Tagerian *et al.*, 2013). In this study, we investigated whether leaving neuropathic rats in standard polycarbonate cages or putting neuropathic rats into enrichment cages one month after surgery would alter pain sensation as well as associated anxiety-like and exploratory behaviors.

Materials and methods

Animals and husbandry

Twenty-four Sprague Dawley rats (CRL:CD[SD]; Charles River, St-Constant, Canada) weighing between 225-250 g were purchased for this study. Following their arrival, they were kept in a standard laboratory animal environment (fresh filtered air, 15 changes/ hour; temperature, $21 \pm 2^\circ\text{C}$; humidity $50 \pm 20\%$; and light-dark cycle, 12:12h). Rats were housed on hardwood bedding (Beta chip, Northeastern Products, USA) with only black PVC tubing for environmental enrichment and they were placed 2 per cage in large polycarbonate cages (48x38x21 cm; Tecniplast) up to one month following surgery. Rats received tap water and a standard laboratory rodent diet (Charles River Rodent Chow 5075, Canada) *ad libitum*. The Faculty of Veterinary Medicine Institutional Animal Care and Use Committee approved the experimental protocol prior to animal use in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Following their arrival and a one-week acclimation period, rats were trained for two weeks to the different behavioral tests and baseline values were taken. A peripheral mononeuropathy was then produced in 12 animals using the chronic sciatic nerve constriction (CCI) model (Bennett and Xie, 1988). Briefly, following anesthesia with isoflurane (AErrane; Baxter), the right common sciatic nerve was exposed via blunt dissection at the level of the thigh (*biceps femoris*). Four loose ligatures were placed around the right sciatic nerve with 4.0 Catgut suture material. The overlying muscles were then sutured with 3.0-vicryl and the skin was closed with a 2.0 silk suture. Animals were then tested in all behavioral tests once a month for 3 consecutive months following the surgery. In addition, mechanical (von Frey test) and heat sensitivity (Hargreaves test) were also evaluated 15 days following the surgery. The other 12

animals underwent a sham surgery (same procedures without the sciatic ligatures).

One month following surgery (and behavioral evaluations at one month), CCI rats were randomly assigned to either of 2 groups: 1) nerve injury and environmental enrichment (NE, n=6) or 2) nerve injury and standard housing (NS, n=6), while sham animals were assigned to either 1) sham surgery in environmental enrichment (SE, n=6) or 2) sham surgery and standard housing (SS, n=6). Environmental enrichment consisted of Double Decker enrichment cages (Tecniplast Inc.) and for the standard housing animals were left in the large polycarbonate cages.

Behavioral evaluations

For all behavioral tests, each apparatus was washed with a very diluted cleaning solution between two consecutive animals to minimize odor interference.

Mechanical sensitivity Calibrated von Frey filaments (Stoelting Co.) were applied for 4 sec or until withdrawal. The stimulus force ranged from 1 - 26 g, corresponding to filament sizes 4.08 - 5.46 g. For each animal, the actual filaments used within the aforementioned series were determined based on the lowest filament to evoke a positive response using the up-down method. Animals were acclimated to the experimental setup for 15 minutes prior to testing. The mechanical sensitivity was assessed on the plantar surface of both hind paws.

Hargreaves thermal sensitivity test: Thermal sensitivity was evaluated using a Hargreaves apparatus (IITC Life Science) as previously described (Hargreaves *et al.*, 1988). Each animal was placed in a Plexiglas chamber with the ground floor made of heated glass ($29-31^\circ\text{C}$). Animals were allowed to acclimate to the experimental set up for 15 min prior to testing. Then radiant heat generated by a high intensity light bulb (40W) was directed to the plantar surface of a hind paw. The lamp generated noxious heat stimulus. The time the animal took to lift its paw from the floor was recorded and noted as the thermal threshold. Rats were tested in groups of 4 animals. The test began alternatively with the right or the left hind paw to prevent any anticipatory behavior. A cut-off time for the radiant stimulation was set at 20 sec to minimize tissue injury.

Open field test: Spontaneous exploratory behavior was evaluated in a transparent open field apparatus (60 x 60 cm), placed in a quiet room. The floor of the apparatus was divided equally into nine squares (20 x 20 cm²). Rats were individually placed into the open field on the central square, and their spontaneous behavior was videotaped for 5 minutes

before being scored by an observer blinded to the experimental protocol. Subsequent analysis of the total number of squares visited, as well as the number of times rats stood on their hind paws, was used to assess general motor activity and exploration.

Elevated plus maze: An elevated plus maze apparatus was placed in a quiet room. The apparatus consisted of two open arms (100 cm long x 10 cm wide) across from each other and perpendicular to two closed arms (2 x 45 cm long x 10 cm wide x 30 cm high). The entire apparatus was positioned 60 cm above the floor. Rats were individually placed at the

intersection of the four arms, head towards an open arm. Their spontaneous behavior was videotaped for 5 minutes before being scored by a blinded observer. The total time where partial (at least two front paws in contact with the open arm) or total body was on an open arm was measured.

Statistical analysis

A 2-way repeated measures ANOVA was performed for all behavioral tests. *Post hoc* Tukey tests were performed to assess the difference between groups at different time points. The level of significance was set at 0.05. Data are presented as mean \pm SD.

Results

The von Frey filaments and Hargreaves results are presented in s 1 & 2. Results from non-enriched, as well as enriched, environments were pooled together since no significant differences occurred with environmental conditions. Only the ipsilateral hind paw of nerve-injured animals was hypersensitive to mechanical stimuli up to 60 days following the surgery ($p < 0.05$ at 15 days and $p < 0.01$ at 30 & 60 days). The same general findings were obtained for the Hargreaves test ($p < 0.01$ at 15, 30 & 60 days for the ipsilateral hind paw in CCI animals). At 90 days following the surgery, CCI animals were no longer neuropathic for either mechanical or heat sensitivities.

When looking at the total number of squares entered in the open field test (Figure 3) and the number of times the animals stood on their hind

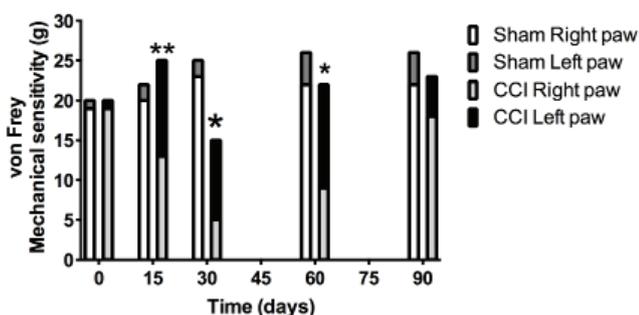


Figure 1. Mechanical sensitivity (g) evaluated with von Frey filaments for the different groups (SS sham surgery standard cage; SE sham surgery enrichment cage; NS CCI standard cage; NE CCI enrichment cage) at different time points following the surgery (surgery performed at time 0). Values shown at time 0 correspond to baseline results taken prior to the surgery. Results are pooled for enrichment conditions since this parameter had no effect on mechanical sensitivity. ** $p < 0.05$, * $p < 0.01$

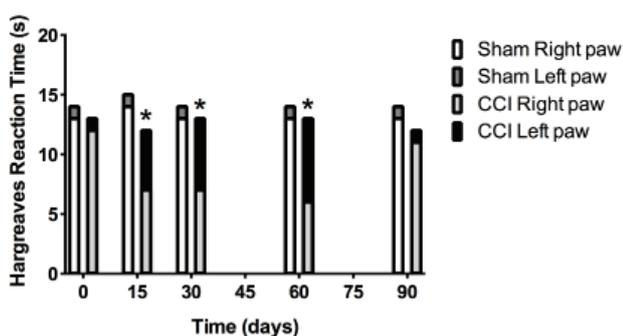


Figure 2. Heat sensitivity evaluated with Hargreaves test for the different groups (SS sham surgery standard cage; SE sham surgery enrichment cage; NS CCI standard cage; NE CCI enrichment cage) at different time points following the surgery (surgery performed at time 0). Response presented is a reaction time (s) to the heat stimulus. Values shown at time 0 correspond to baseline results taken prior to the surgery. Results are pooled for enrichment conditions since this parameter had no effect on heat sensitivity. * $p < 0.01$

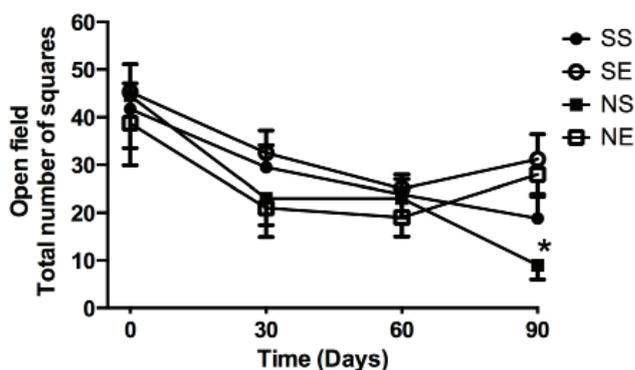


Figure 3. Exploratory behavior evaluated with the open field test (total number of squares explored over 5 minutes) for different groups (SS sham surgery standard cage; SE sham surgery enrichment cage; NS CCI standard cage; NE CCI enrichment cage) at different time points following the surgery (surgery performed at time 0). Values shown at time 0 correspond to baseline results taken prior to the surgery. * $p < 0.01$

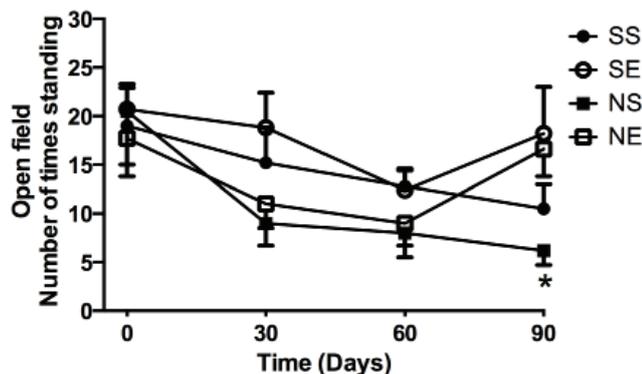


Figure 4. Standing behavior evaluated with the open field test (number of time animals are standing on their 2 hind paws during 5 minutes) for different groups (SS sham surgery standard cage; SE sham surgery enrichment cage; NS CCI standard cage; NE CCI enrichment cage) at different time points following the surgery (surgery performed at time 0). Values shown at time 0 correspond to baseline results taken prior to the surgery. * $p < 0.01$

paws (Figure 4) over a 5 minute period, there were no differences between the experimental groups up to 60 days, however a decrease in exploration occurred over time, which could be associated with learning. At 90 days a clear difference in both behaviors occurred for the neuropathic animals: enrichment cages had a clear benefit over the standard environment ($p < 0.01$). Although not significant, a trend to increased exploration and standing behavior at 90 days occurred for sham animals placed in enrichment cages.

With the elevated plus maze (Figure 5) all groups explored the open arm for the same percentage of time up the 30 days post-surgery. At 60 days, sham animals in enriched environment were clearly less anxious than the sham animals in the standard environment ($p < 0.01$), with no significant difference seen amongst other groups. At 90 days post-surgery, neuropathic animals in the enriched environment clearly showed less anxiety-like behavior (greater exploration of the open arm) than neuropathic animals in the standard environment ($p < 0.01$).

Discussion

Our results clearly show that environmental enrichment has a significant impact on exploratory and anxiety-like behaviors, but doesn't affect pain hypersensitivity in a rat model of neuropathic pain. At 60 days post-surgery, environmental enrichment did not affect pain threshold to mechanical and heat stimuli in rats, an effect that was clearly seen in mice (Vachon *et al.*, 2013). At 90 days post-surgery, the loss

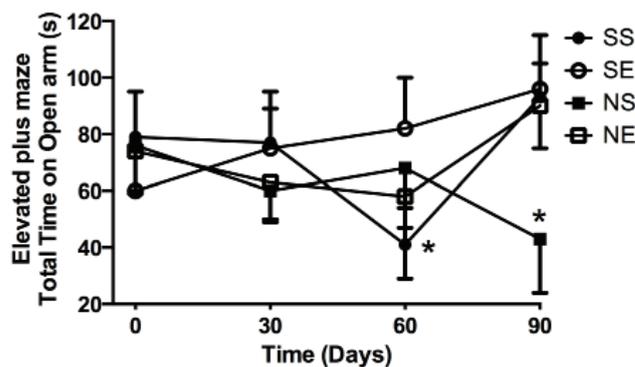


Figure 5. Anxiety-like behavior evaluated with the elevated plus maze for different groups (SS sham surgery standard cage; SE sham surgery enrichment cage; NS CCI standard cage; NE CCI enrichment cage) at different time points following the surgery (surgery performed at time 0). Figure presents the total time where half (at least 2 front paws on an open arm) or the whole body was on an open arm of the elevated plus maze over a 5 minute period. Values shown at time 0 correspond to baseline results taken prior to the surgery. * $p < 0.01$

of heat and mechanical pain sensitivities in neuropathic animals is a normal finding when using the CCI model (Coggeshal *et al.*, 1993). Interestingly these animals benefited the most from the enrichment cages (decreased anxiety and increased exploration) even though they were no longer neuropathic. Since standing and exploratory behaviors increased, and anxiety-like behaviour decreased, in neuropathic animals when housed in the Double Decker cages, this strongly suggests that their well-being increased. Importantly, cage size would appear to influence some higher brain functions without affecting chronic pain processing.

We have previously shown (Vachon *et al.*, 2013; Tajerian *et al.*, 2013) that environmental enrichment in mice had an alleviating effect on well established chronic neuropathic pain and anxiety-like behaviors. One of the main components of the enriched environment in this previous study was a running wheel, and exercise is known to decrease pain symptoms and improve motor function in chronic pain models. Stagg *et al.* (2011), demonstrated that thermal hyperalgesia in a rat model of neuropathic pain is reversed with exercise. Interestingly, reversal of sensory hypersensitivity was seen even when exercise was initiated 4 weeks after spinal nerve ligation, and returned 5 days after discontinuing exercise. In the present study, the main focus was the effect of cage size on pain-related behaviors and therefore the addition of exercise toys could very well provide an additional benefit, if the objective is to treat

pain. Exercise appears sufficient to have a significant alleviating effect on neuropathic pain symptoms, and clinical studies in humans suggest that exercise decreases pain symptoms in chronic pain patients (Malmros *et al.*, 1988; Ferrel *et al.*, 1997; Gowan *et al.*, 2004; Hayden *et al.*, 2005; Robb *et al.*, 2006; Chatzitheodorou *et al.*, 2007).

In conclusion, the present findings suggest that environmental enrichment cages appear to be a very interesting option when studying neuropathic pain in a rat model, since pain behaviors didn't appear to be affected but there was evidence for increased animal welfare.

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