Sciatic nerve clamp: a rat model of neuropathic pain with low inter-subject variability

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***Abstract*— Neurophysiological studies of chronic pain require animal models of pain with low inter-subject variability. Here we evaluated two experimental models of chronic pain in adult rats: (1) spinal cord contusion, and (2) sciatic nerve compression injury. The latter was found to have lower variability, so we recommend it be used in behavioral and electrophysiological experiments.**

Keywords—neuropathic pain, spinal cord injury, denervation, animal model

I. INTRODUCTION

Pain is a common complication after spinal cord injury as up to about 80% of spinal cord injury (SCI) patients experience pain. Chronic pain affects the majority of patients, and approximately one third of these patients report pain that significantly affects their quality of life [1].

Given that neuropathic pain (NP) is one of the most serious complications of SCI with the high prevalence, the selection of adequate treatment is important, but also challenging [2]. While there is a wide range of options for treatment of NP, it is difficult to achieve adequate control of NP in most patients with SCI. Pain caused by SCI, which develops in about two-thirds of patients [3], is classified as either nociceptive (pain due to nociceptors) or neuropathic (due to damage to the somatosensory nervous system) [4]. Neuropathic pain, which develops in 53% of patients with SCI, is difficult to treat, in part because the mechanisms that contribute to its development remain unknown. Symptoms typically arise from the malfunctions in the spinal cord or peripheral nerves. Because pain occurs in areas that are more likely to retain partial sensory function, this type of pain often includes allodynia (pain in response to painless stimuli such as light touch) as well as spontaneous and ongoing pain [5].

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II. METODS

A. Subjects

The study was carried out on non-linear laboratory female rats weighing 160–240 grams. In the experiments, the rats were divided into 3 groups:
1) Control group (n=20): intact rats without any intervention;
2) Spinal cord injury (contusion) group (n=10): these animals underwent laminectomy and spinal cord injury;
3) Group of animals with compression injury of the sciatic nerve (n=10): animals that underwent sciatic nerve clamping.

B. Procedure

The model of contusion spinal cord injury was standard. The injury was of moderate severity. The injury was applied at the level of T8-T9 according to the modified method of A. R. Allen (1911) [6] using a vertically falling weight of 2.5 g from a height of 5 cm. Next, the vertebral arch and/or spinous process was removed. After that, the spinal canal was exposed, became accessible for inspection and manipulation in it. After laminectomy (the dura mater remained intact), a 20 cm tube was placed on the arch of the laminectomy vertebra and a weight was lowered inside it. After injury, we observed a reflex stretching of the hind limbs. The weight was immediately removed after the impact.

Violation of nervous control was carried out according to the method of Angelis [7]. In anesthetized animals and under aseptic conditions, a mosquito-type clamp was applied to the prepared sciatic nerve for 40 seconds (as a result, some of the nerve fibers retained their properties); the length of the crushed area was 2 mm; the place of clamping was 1.5-2 cm above the knee joint. The wound was then sutured.

C. Behavior task

In the study, mechanical allodynia was determined using Von Frey test. Mechanical stimulation using calibrated von

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Frey filaments was used to determine the severity of allodynia [2].

D. Analysis

Statistical data processing was carried out using the Original Lab program. All data are presented as mean ±SD. Statistically significant differences were determined using ANOVA. The level of the test of statistical significance was set at $p<0.05$.

III. RESULTS

Animals that underwent contusion spinal cord injury (SCI) had an average hind paw reflex threshold before surgery of 88.89±59.24g, which differed from the threshold of fictitious (control) animals (65.87±45.11g), but this difference was not significant. Two weeks after the SCI, in 9 rats the thresholds were higher than in the control group. Furthermore, in 7 animals, paw sensitivity was absent and was conditionally accepted as 350g. After 6 weeks the sensitivity was restored. In 8 rats the threshold was higher than before the operation, and in 2 rats the threshold was decreased and they were considered as animals with developing tactile allodynia (neuropathic pain).

As can be seen from Fig. 1, compared with the control group, the threshold for mechanical irritation after 2 weeks was significantly higher and amounted to 400±105% ($p<0.05$) in rats without pain. Six weeks after SCI, the threshold for mechanical irritation decreased (287±116%), but was higher than before the surgery. In animals with developing tactile allodynia, the threshold significantly decreased by week 6 from 47±10%.

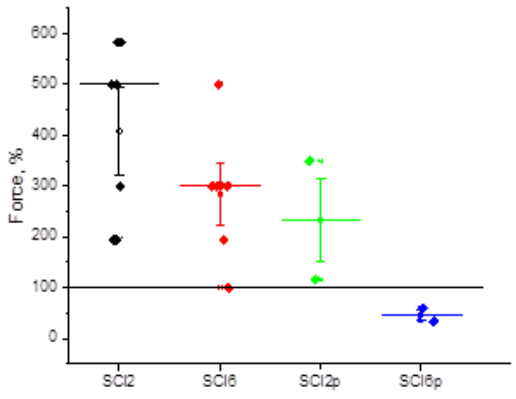

Fig. 1. Changes in mechanical irritation threshold measured with von Frey hairs after spinal cord injury (SCI). (Changes in reflex threshold in each rat are shown as a percentage of preoperative values taken as 100% at 2 (SCI2) and 6 (SCI6) weeks after spinal cord injury; p - group with pain, * - p≤0.05 significance relative to control, #- p≤0.05 significance relative to the group without neuropathic pain.

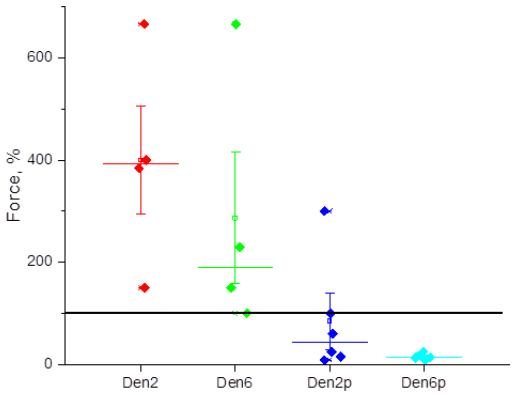

Fig. 2. Changes in mechanical irritation threshold measured with von Frey hairs after compression injury of the sciatic nerve (Den). (Changes in the reflex threshold in each rat are shown as a percentage of the values before surgery, taken as 100% at 2 (Den2) and 6 weeks (Den6) after spinal cord injury; p - group with pain,* - p≤0.05 significance relative to control, #- p≤0.05 significance relative to the group without neuropathic pain).

Animals that underwent compression injury of the sciatic nerve had an average threshold of the hind paw reflex of 62.89±41.24g before surgery, which did not differ from the threshold of the control animals (65.87±45.11g). Two weeks after injury to the sciatic nerve in 4 rats, the thresholds were higher than in the control. In 6 rats they were lower, and we considered them as animals with developing tactile allodynia (neuropathic pain) (Fig. 2). After 6 weeks, sensitivity was restored in 4 rats without pain, but the threshold was higher than before the surgery, and in 6 rats with neuropathic pain, the threshold was reduced.

As can be seen from Fig. 2, in rats without pain, the threshold for mechanical irritation after 2 weeks was significantly higher than in the control group and amounted to 395±98% ($p<0.05$). Six weeks after the operation, the threshold for mechanical irritation decreased (286±108%), but was higher than before the operation. In animals with developing tactile allodynia, the threshold significantly decreased by 6 weeks and amounted to 15±5%.

IV. DISCUSSION

There is no consensus on the best model to use in neuropathic pain research although the most commonly used model is a small contusion of the thoracic segments of the spinal cord. Chronic neuropathic pain resulting from compression injury to peripheral nerves is also a well-established model in small animals. Measuring pain in animals, even without SCI, is non-trivial simply because animals cannot report pain in the same way humans do. Researchers rely mainly on changes in evoked responses, such as lowering thresholds, increasing the amplitude and/or duration of the response. These changes in reflex behavior display prominent signs of induced pain that are often observed in humans (allodynia and hyperalgesia) [8].

Our results suggest that a much larger number of animals is needed to obtain reliable data from the SCI model. The results of the von Frey test revealed a large variability in sensitivity in rats with SCI.

V. CONCLUSIONS

Several models of neuropathic pain in sciatic nerve injury are known, with several modifications [9; 10; 11]. Our results indicate that the sciatic nerve clamp model can be used to generate hyperalgesic responses to mechanical stimulation in rats. This model provides a low inter-subject variability, so it is suitable for experimentation.

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