

# Rat Sciatic Nerve Crush Injury and Recovery Tracked by Plantar Test and Immunohistochemistry Analysis

---

Pavić, Roman; Pavić, Michele L.; Tvrdeić, Ante; Tot, Ozana Katarina; Heffer, Marija

Source / Izvornik: **Collegium antropologicum, 2011, 35, 93 - 100**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:239:481797>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-11-22**



Repository / Repozitorij:

[Repository UHC Osijek - Repository University Hospital Centre Osijek](#)

# Rat Sciatic Nerve Crush Injury and Recovery Tracked by Plantar Test and Immunohistochemistry Analysis

Roman Pavić<sup>1</sup>, Michele L. Pavić<sup>2</sup>, Ante Tvrdeić<sup>3</sup>, Ozana K. Tot<sup>4</sup> and Marija Heffer<sup>2</sup>

<sup>1</sup> »J. J. Strossmayer« University, Osijek University Hospital Center, Department of Surgery, Osijek, Croatia

<sup>2</sup> »J. J. Strossmayer« University, School of Medicine, Department of Medical Biology, Osijek, Croatia

<sup>3</sup> University of Zagreb, School of Medicine, Department of Pharmacology, Zagreb, Croatia

<sup>4</sup> »J. J. Strossmayer« University, Osijek University Hospital Center, Department of Anesthesia, Osijek, Croatia

## ABSTRACT

An experimental crush injury to the sciatic nerve, with a crush force of 49.2 N (pressure  $p=1.98 \times 10^8$  Pa), was inflicted in 30 male rats (Wistar). A control group (sham), with the same number of rats, was also operated upon exactly as the experimental group but without the crush injury. We tested the sensory and motor recovery of the sciatic nerve with Hargreaves method, using an apparatus from Ugo Basile, Italy. Testing was continued for both legs of each rat, injured and uninjured, starting preoperatively (0 day), and then 1, 7, 14, 21, and 28 days postoperatively. The same experiment was run simultaneously with the sham group. The Plantar test showed recovery of the sensory and motor function of the sciatic nerve, though not complete recovery, by 28 days. An immunohistochemical experiment was run in parallel with the plantar test on L3-L6 segments of the spinal cord from where the sciatic nerve extends. We used antibodies for Myelin-associated glycoprotein (MAG), and gangliosides GD1a and GT1b on the aforesaid part of the spinal cord. The immunohistochemical methods showed changes in sensory and motor axons in the spinal cord segment L3-L6 which suggest correspondence with the results of the Plantar test, in terms of recovery of the sensory and motor function after injury of the sciatic nerve. The immunohistochemical results also show ipsilateral and contralateral changes following injury. Results of the plantar test are suggestive that the rat shows compensation for an injury in its contralateral leg.

**Key words:** sciatic nerve injury, regeneration, Plantar test, Myelin-associated glycoprotein, MAG, ganglioside GD1a, ganglioside GT1b

## Introduction

Peripheral nerve injury, specifically sciatic nerve injury, is a widely used approach to study nerve regeneration ability. When a standardized crush injury is surgically inflicted upon the sciatic nerve in experimental rats<sup>1,2</sup> the crush lesion causes loss of axonal continuity which results in Wallerian degeneration distal to the lesion. But most importantly the myelin sheath integrity is saved. Thereafter motor and sensory regeneration spontaneously occurs and may be monitored.

Withdrawal is a basic motor response. One of the more selective ways to illicit this response is to stimulate cutaneous receptors with heat. The Plantar test (Hargreaves' method) was introduced as a scientific experi-

ment in 1988<sup>3,4</sup>. Using Hargreaves' method, the animal can move freely within the closed space. This freedom allows for a more accurate picture of withdrawal latency. This method allowed us to follow the functional regeneration of the crushed sciatic nerve in the rat.

Modern immunohistochemical (IHC) techniques allow the tracking of various putative regeneration markers in the spinal cord<sup>5,6</sup>. Grey matter in the rat is divided into 10 cytoarchitecture regions, from these studies we know where motor and sensory nerves are located in various levels of the spinal column<sup>7</sup>. In rats the sciatic nerve is formed from segments L4 and L5. It has been found however L6 in 54% of cases is also connected to the sci-

atic nerve by a thin thread, likewise, in 25% of cases, there is a narrow connection between the spinal nerves of L4 and L3<sup>8</sup>.

Researchers give much attention to the differences in the central nervous system and the peripheral nervous system because the latter has the ability to regenerate damage to its axons while the former can not. It has been established that lack of regeneration in the central nervous system is explained by an unfavorable growth environment and by the presence of growth inhibitory molecules<sup>9</sup>. Myelin-associated glycoprotein (MAG) is one of the inhibitors of nerve regeneration or axon growth. MAG is a member of the Siglec family of sialic acid (NeuAc) binding lectins, which binds preferentially to a specific glycan structure which is prominently expressed on certain gangliosides. Additionally gangliosides GD1a and GT1b have been associated with MAG as specific functional ligands on nerve cells responsible for MAG-mediated inhibition of nerve regeneration<sup>10</sup>.

To see if there were any changes in these putative regeneration markers of the spinal cord as functional recovery proceeded, the experiment tracked regeneration of the damaged sciatic nerve via the paw withdrawal plantar test comparing it with the not operated leg. In performing IHC analysis for MAG, GD1a and GT1b on vertebral segments L3-L6, where the sciatic nerve forms, we watched for changes on motor and sensory axons in these vertebral segments following the sciatic nerve damage.

## Materials and Methods

### Animals

The animals used in this experiment consisted of 57 (30 crush group and 27 sham group) male Wistar rats, 3 months old and weighing between 250–350 g. They were kept in a temperature controlled room (24°C) with 12 hour light/dark cycle (lights on at 8:00am) with free access to water and food. Experiments were performed between 4–7 pm. The experiments were carried out according to the Ethical Committee guidelines, Medical School at the »J.J. Strossmayer« University in Osijek, Croatia and in accordance with Croatian law regarding the handling and treatment laboratory animals.

### Plantar test (functional test)

The plantar test was performed before trauma infliction (day 0) on the left sciatic nerve, then 1, 7, 14, 21, and 28 days after this procedure. Testing was consistently made in the same room at approximately the same time of day. The time (in seconds) taken by the rat to withdraw its hind paw in response to a radiant heat source was measured using commercially available Plantar test device (Ugo Basile, Italy). The number of rats used for this section of the experiment was 15 crush operated and 15 sham operated.

To measure latency a cylindrical aluminum pipe with an infrared (IR) emitter is situated under the glass floor. Also located in the cylinder is an IR sensor, which stops

IR emission when animal moves its foot therefore recording latency and stopping the noxious heat. Maximum time to which a rat was exposed was set to 30 seconds. Temperature on the glass bottom was not higher than 53°C in 30 seconds. IR stimulation which we used in this experiment, 80 on control machine, corresponds to an emission of  $282.5 \pm 0.71$  mW/cm<sup>2</sup>. The animal was placed 15 minutes before evaluation in the plastic box with glass bottom for acclimation. Evaluation of both hind legs was repeated three times with a pause for the glass bottom to cool down between tests and to allow the animal to desensitize from the heat.

### Surgery

Using anesthetic Isofluran (Foran, Abbott, Queens borough, Great Britain) with concentration from 0.75 to 1.5 vol % and with intraperitoneal injected solution of Ketanest (Pfizer, Vienna, Austria) diluted 10x with 0.9% NaCl (25 mg/mL Ketanest with 9 mL 0.9% NaCl) 27 rats were induced and kept in general anesthesia. After the animal was anesthetized, shaving and washing the field for operation with factory made solution Plivasept (5g chlorhexidine gluconate in 70% propylalcohol, Pliva, Zagreb, Croatia). An incision was made in the medial left thigh. Then, carefully, with a blunt instrument, the muscles were moved without lesion to reveal the sciatic nerve<sup>11</sup>. A lesion was made 1 cm above the bifurcation the left sciatic nerve into the tibial and peroneal nerve (Figure 1) delivered with Martin's microsurgical forceps creating pressure force of 49.2 N. The pressure this force created upon the sciatic nerve was calculated using the area of the nerve measured before and after the crush was delivered and the measurement of the forceps, so that the pressure (p) was calculated to be  $1.98 \times 10^8$  Pa. This pressure was held for 60 seconds. A loose marking suture was placed around the damage sciatic nerve (non-absorbable suture 4-0, Sof silk, United States Surgical Corp, Norwalk, CT, USA). This was done so that the injured nerve could be easily identified in IHC dissection.

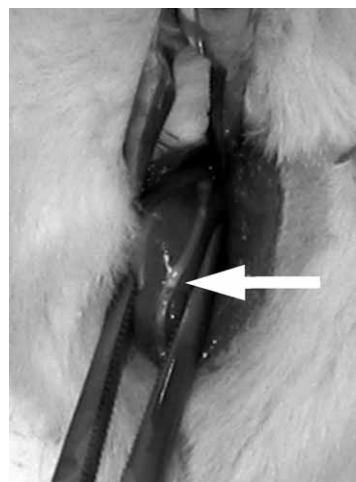


Fig. 1. Damage to the left sciatic nerve created by the 60 second crush with microsurgical forceps.

The operative wound was then closed with an absorbable suture 4–0 (VicrylPlus, Johnson&Johnson Int'l, St Stevens-Woluwe, Belgium). The opposite leg's sciatic nerve was not operated upon and served as the control.

A sham study with 27 rats was also conducted, identical to the crush inflicted group except in the surgical part of the experiment which consisted of sciatic nerve exposure, placement of the non-absorbable suture and wound closure. There are 27 rats in this group, because the original preoperative sacrificed animals for IHC are the same regardless of group, operated or sham.

### *Immunohistochemical (IHC) analysis*

Animals (3 rats) were sacrificed in deep anesthesia for IHC analysis prior to operation 0 day (for control), and then on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day after the operation (6 animals each day: 3 injured and 3 sham). After the animal was anaesthetized and intracardially perfused with PBS (phosphate buffer saline) followed by perfusion of 4% paraformaldehyde in PBS buffer (Figure 2). Following this preparation the animal is dissected and the spinal cord placed in the fixative for 24 hours. The samples were cryo-protected in PBS containing 10% (w/v) sucrose for 24 hours at room temperature and snap frozen with isopentane at  $-80^{\circ}\text{C}$ . The frozen isolated segment of spinal cord (L3-L6) was taken and sliced to a thickness of  $35\mu\text{m}$ . The IHC analysis was performed on free floating tissue slices<sup>5</sup>. The entire IHC method was completed at  $4^{\circ}\text{C}$ , and all incubations steps were on a shake table. First the specimens were blocked with a nonspecific blocker with 1% BSA (bovine serum albumin) and 5% goat anti-serum in 50 mM Tris-HCl, 1.5% (w/v) NaCl, pH 7.4 (TBS) buffer for 2 hours. Treatment with 1% Triton in blocking step is necessary for trans-membrane protein MAG because the detergent allows penetration of the antibody to the isotope exposed on interface between axon and oligodendrocyte and covered with compact myelin layers of internode. On the other hand the same treatment during blocking in case of ganglioside IHC causes rearrangement of the lipid domains in the plasma membrane and creates an artifact. Triton in 1% concentration

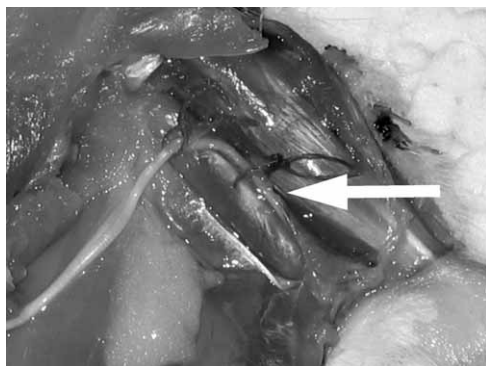


Fig. 2. Damage seen in the sciatic nerve 1 week postoperatively. Lower suture is the marking suture the upper one is marking of the body side used later for immunohistochemistry placed during dissection.

was included just in the blocking step in the case of MAG IHC and was completely excluded in the case of ganglioside IHC. The next step is incubation in primary antibody for 16 hours. Primary antibodies were diluted in blocking solution: IgG-class anti-ganglioside mouse monoclonal antibodies were diluted 0.25 mg per mL; anti-MAG was diluted 1:500. Sections were then washed three times with TBS, 10 min each. Incubation with secondary antibody lasted for 4 hours and was performed with 2 mg/mL biotin-conjugated goat anti-mouse. Non-specific bound secondary antibodies were rinsed three times, 10 min each, with TBS. Tertiary complex was comprised of avidin (component A) and biotinylated enzyme alkaline phosphatase (Vector Lab., Burlingame CA, USA). The incubation process lasted 2 hours. Rinsing was identical to the proceeding two rinsing steps. Finally, sections were developed with Vector BCIP/NBT substrate (Vector Laboratories, Burlingame, CA, USA) according to manufacturer's instructions, rinsed, mounted on silanized glass slides, dried and covered with Vecta Mount™ permanent medium.

All reagents used for IHC method buffers were analytically clean (Sigma-Aldrich, Steinheim, Germany). Primary antibody for ganglioside GD1a and GT1b were gifts from Dr. Ronald Schnaar (John Hopkins School of Medicine, Baltimore, MD, USA). Anti-MAG antibodies were from Calbiochem, San Diego, CA, USA. Biotinylated secondary antibody (anti-mouse IgG) purchased from Jackson Immuno Research Laboratories (West Grove, PA, USA). Vectastain ABC kit and substrate Vector BCIP/NBT were produced by Vector Laboratories (Burlingame, CA, USA).

### *Statistical analysis*

Statistical analysis consisted of the descriptive statistics: Mean, median and standard deviation, as well as ANOVA (one-way). In the statistical analysis of the hind leg latency each group (crush operated, crush not operated, sham operated and sham not operated) for each day was compared to the respective preoperative (0) day value. For every day latency was measured, the 1-way ANOVA confirmed statistical significance between all groups (crush operated, crush not operated, sham operated and sham not operated). Post Hoc (LSD and Scheffe) tests were then calculated to identify if any individual group was significantly different from any of the other groups. All statistics were completed on the results of the plantar test using Statistica (StatSoft). For all statistical tests a p value of less than 0.05 was considered significant ( $p < 0.05$ ). IHC results are qualitative, seen or not seen changes.

## **Results**

The operations were well tolerated and all wounds healed. There were no signs of un-stimulated pain or discomfort observed over the evaluation period. However there were 3 rats (of 30 operated) eliminated from the crush group. Two were eliminated before the 1 day test-

ing – they died unexpectedly without any signs of trauma, the third was eliminated prior to 14 day testing with an apparent lesion to its back.

The average latency of foot movement in seconds, for both legs (with lesion and without lesion on the sciatic nerve) is shown in Figure 3. The standard deviations of the mean for each of the followed groups are shown in separate graphs in Figure 4. The first day after operation latency increased from about 5 sec, preoperatively, to 12.86 sec. Each day latency was measured overall significance was found (1, 7, 14, 21 and 28 days) between the four groups measured: crush operated, crush not operated, sham operated and sham not operated ( $p < 0.05$ ). On day 1, 7 and 21 the crush operated group was found to be significantly different from all the other groups using the Post Hoc (LSD and Scheffe) tests ( $p < 0.05$ ). On day 14 all groups were found to be significantly different to one another except for sham operated compared to sham not operated ( $p = 0.978$  and  $p = 0.999$ ). On day 28, crush operated and crush not operated were no longer significant compared to each other ( $p = 0.123$  and  $p = 0.487$ ), however the crush operated and crush not operated groups were significantly different from the sham operated and sham not operated groups ( $p < 0.05$ ). Even though many of these differences are numerically small, the statistical analysis showed significance. (Table 1)

The surgical technique used allowed for quick wound healing (no crosscut muscle, etc) so that the sham operated animals had, as expected, no statistically significant differences to the preoperative latency values for sham animals. (Figure 3)

MAG: Preoperatively anti-MAG staining is accentuated more than in any of the slices after lesion to the sciatic nerve visible in white and grey matter (Figure 5). Postoperatively, anti-MAG staining is seen in white matter. However, above the central canal, in the dorsal funiculi, there is an increase 14 days following lesion of the sciatic nerve. This change can still be observed in day 21 but it is definitely fading.

GD1a: Anti-GD1a stains specifically Lamina I and II (Figure 5). Additionally there is staining present of the grey matter in the central canal and bordering the dorsal

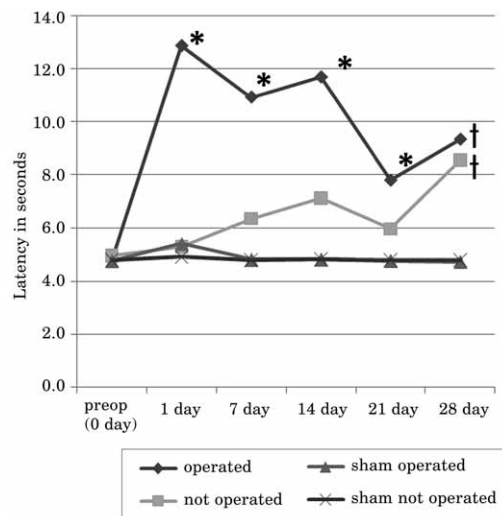


Fig. 3. Showing the average latency in seconds: Crush operated (left) leg; crush not operated (right) leg; sham operated (left) leg; and sham not operated (right) leg. Preop represents preoperative measurement (0 days). Statistically significant measured using ANOVA with LSD and Scheffe post hoc tests; \* statistically significant ( $p < 0.05$ ) compared to crush not operated, sham operated and sham not operated groups at the particular time point, † statistically significant ( $p < 0.05$ ) compared to sham operated and sham not operated groups at 28 days.

funiculi. However, in 7 days after lesion to the sciatic nerve there is a visible weakening in the staining.

GT1b: Anti-GT1b staining is found dispersed in the grey matter, little stronger in Lamina I and II compared to the preoperative 0 day (Figure 5). However, in 7 days following lesion to the sciatic nerve there appears a band in the ventral horn. There is a slight appearance of this staining 1 day following lesion though not as pronounced as at 7 days. Anti-GT1b also stain a portion of the axons which crossover at the central canal. The staining on the sides of the white matter are artifacts of the staining process.

The left side of the spinal cord is ipsilateral to the injured sciatic nerve in all images. While crush operated animals showed a dramatic change in the expression in

TABLE 1  
THE X±SD LATENCY IN SECONDS OF THE PLANTAR TEST EVALUATION

Latency in seconds	Preop (0 day)	1 day	7 days	14 days	21 days	28 days
Crush operated (left leg)	4.84±0.90	12.86±6.47*	10.92±3.42*	11.68±2.81*	7.79±3.28*	9.33±1.95†
Crush not operated (right leg)	4.95±1.19	5.29±1.18	6.34±1.79	7.12±1.94	5.96±1.51	8.55±2.73†
Sham operated (left leg)	4.76±0.82	5.40±1.00	4.81±0.83	4.82±0.80	4.77±0.85	4.73±0.81
Sham not operated (right leg)	4.79±0.95	4.91±0.94	4.80±0.95	4.83±0.94	4.79±0.95	4.80±0.97
Number in crush group (N)	15	13	13	12	12	12
Number in sham group (N)	15	15	15	15	15	15

Using ANOVA with LSD and Scheffe post hoc tests, \* statistically significant ( $p < 0.05$ ) compared to crush not operated, sham operated and sham not operated groups at the particular time point, † statistically significant ( $p < 0.05$ ) compared to sham operated and sham not operated groups at 28 days.

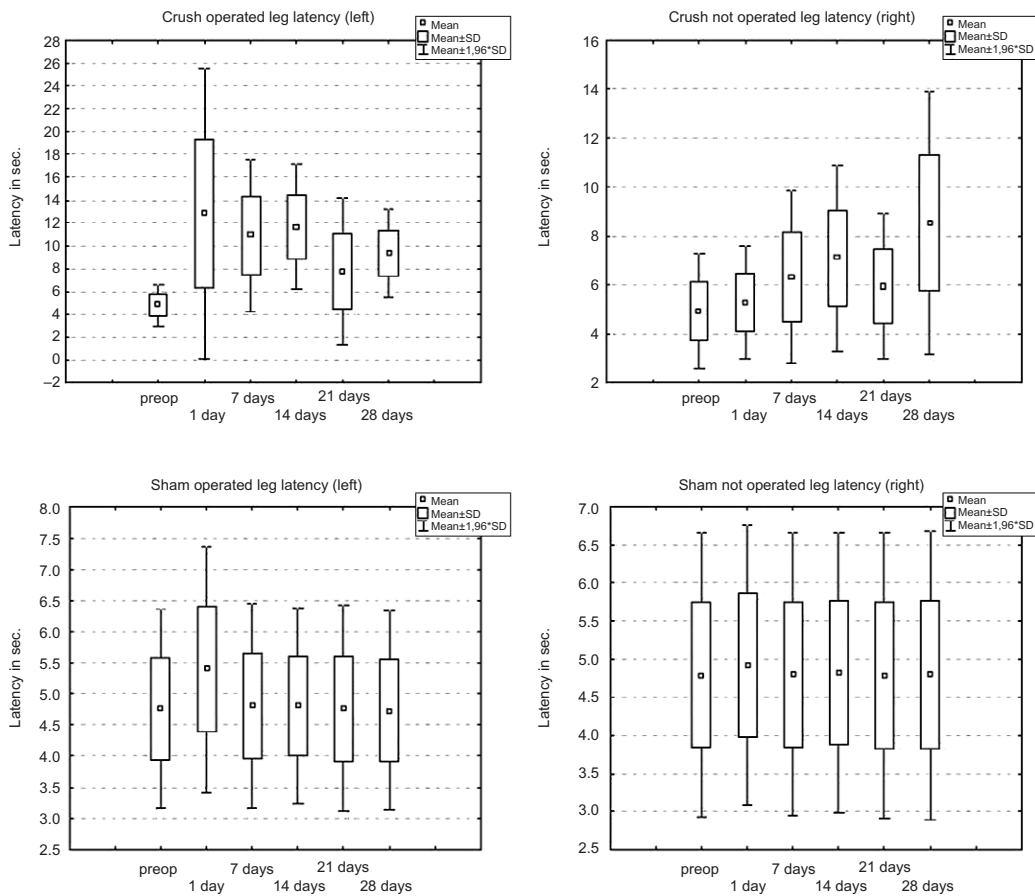


Fig. 4. Standard deviation box and whisker plot graphs for all groups followed (crush operated (left) and not operated (right) legs; sham operated (left) and not operated (right) legs). Latency measured in seconds.

markers GD1a, GT1b and MAG, sham animals for the same markers were continuously equal to preoperative (day 0) results without any dynamics throughout the study period. The images labeled control slices show background staining for the secondary antibody, the different primary antibodies are omitted. A single rat specimen is shown in Figure 5 for each day to allow for comparison between staining, however all three specimens exhibited similar changes.

## Discussion

Standardization of the pressure applied in the crush injury is one of the major issues which have not been overcome in sciatic crush injury research. The crush must be standardized between animals and even though each laboratory uses a similar apparatus to produce the crush the pressure exerted upon the nerve must be measured so that comparison between papers can be made<sup>2,12</sup>. We evaluated our microsurgical forceps pressure to be  $p = 1.98 \times 10^8$  Pa on the measured area of the sciatic nerve.

Heat withdrawal testing is not foreign to peripheral nerve regeneration experiments however usually in the hotplate test the animal is covered and held in place. The apparatus produced by Basel allows the animal freedom

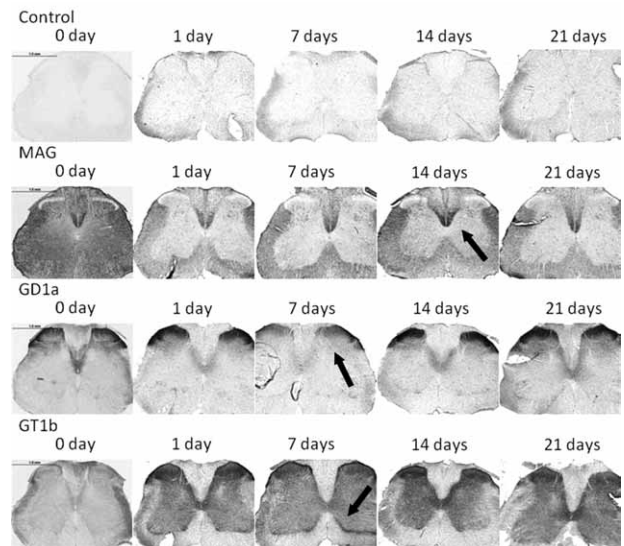


Fig. 5. Immunohistochemistry results showing sciatic nerve slices preoperative (0 day), 1, 7, 14, and 21 days after lesion of control, anti-MAG, anti-GD1a, and anti-GT1b stained sections, magnified 5x, bar is equal to 1.0 mm. Control slices show background staining for the secondary antibody, the primary antibody is omitted.

to move about and allows investigating behavioral responses to hyperalgesia. We used it as a tool to monitor the sensory and motor recovery of the sciatic nerve instead of the traditional withdrawal reflex latency (WRL) testing which involves holding the animal. It is assumed that when the peripheral nerve spontaneously recovers both motor function and sensory function recover. This appears to be the case in our study because the reflex behavior to stop the noxious heat could have manifested itself in other ways than a mere lifting of the paw. The animal for example, if it felt pain could have moved in a horizontal direction. We did not see this type of escape. This type of escape is not feasible to observe in the traditional WRL testing. The sham results also suggest this as there is hardly any change in values, except for 1 day postoperatively, when it can be suggested that the change in latency was due to the freshly sutured wound.

In our experiment we recognize the decrease in latency the third week after injury as recovery of sciatic nerve function. Functional equalization of operated and non operated hind legs was shown 4 weeks after injury. It has been shown that there is a contralateral compensation in rats in nerve crush experiments<sup>13</sup>. Motor recovery is frequently monitored as sciatic functional index (SFI) calculations in crush nerve models. Using a similar method of nerve crush and evaluation timetable in testing, de Souza et al. (2004) evaluated three calculations for gait studies and open arena after sciatic nerve crush injury, with male Wistar rats<sup>1</sup>. Nerve regeneration was denoted in 21 days. We have also seen similar results with sciatic functional indexing<sup>14</sup>. From the sciatic functional index we find functional (motor) recovery at 21 days, however sensory recovery as shown by Volgelhaar (2004) takes much longer<sup>15</sup>. The Plantar test shows the motor recovery of the damaged sciatic nerve 21 days after injury and at 28 days equalization between injured and not injured sides. Although using standard statistical methods this shows as significant as compared to preoperative latency levels, this equalization of latency times suggests that there is another phase of regeneration. In a novel re-examination of data Pavić et al.<sup>14</sup> found that the rat compensates with the contralateral paw for deficits in the injured leg in walking track analysis. The expected findings are that the contralateral leg remains as it was preoperatively. In rats, the contralateral toe spread increases after injury and the ipsilateral leg toe spread diminishes – neither leg remains similar to preoperative measurements. This, therefore, should be taken into account when performing any functional tests which assume the contralateral leg is »normal«, meaning unaffected by the ipsilateral crush injury<sup>14</sup>. It can be seen in the plantar test latency measurements a similar phenomenon – the latency (in seconds) increases in the crush operated (left) leg, however the contralateral leg latency also increases, though more slowly (in days), while sham operated rats latency results remained fairly constant (Figure 3). Luís et al. in 2009 state that full recovery is not achieved by 4 weeks (the time frame for this study) but closer to 12 weeks or more<sup>12</sup>. This can be seen in sciatic functional in-

dexing; 28 days following injury there is again a slight fall in function<sup>1,14</sup>. It has also been shown in long term studies that after functional nerve recovery, there exists pain as a residue causing the animal not to put full pressure on the recovering limb<sup>15</sup>.

The Plantar test has been used in human experiments<sup>16</sup> using the middle finger pad for the thermal stimulation; it was shown to be a very sensitive method. The results of that experiment reinforce the results which are done on laboratory animals as an accurate reflection of pain. In our experiments with the plantar test, the noxious heat used caused repeatable results for each evaluation session. The rats never tried to escape noxious heat with any movement other than lifting the isolated paw, regardless that this was an option. There are various studies distracting from Hargreaves' method as only a reflex<sup>17</sup>, however as our animals did not attempt any other escape from the IR beam we feel that Hargreaves' method is a very specific way of delivering pain to an unbound animal.

Behnam-Rasouli et al. (2000) investigated the post-operative time effects following sciatic crush injury on the numerical density of alpha motoneurons<sup>18</sup>. They noted the decrease in spinal ventral horn motoneurons. The reduction of cell numbers appeared complete at three weeks, with no additional loss noted at 8 weeks<sup>18</sup>. They postulate that since motoneurons of the unoperated side also receive inputs, at least partially, via crossed interneurons, the transneuronal degeneration might affect cell bodies of the unoperated side. They also put forth that although degeneration processes are completed as early as week 3 after compression, a partial recovery in numerical density of motoneurons in the unoperated side may be possible<sup>18</sup>. This observation may also explain why we see an equalizing of plantar latency, operated to unoperated, but do not see a full recovery to preoperational latency, within 28 days.

With the IHC results we show a correlation between plantar test results and IHC staining on the slices of the spinal cord level L3-L6 where the sciatic nerve formation begins. In each of the control slices a small amount of unspecific staining can be seen. This type of staining is caused by nonspecific binding of goat anti-mouse antibody or avidin complex and biotinylated alkaline phosphate. MAG is present on the interface between axon and oligodendrocyte and covered with internodal compact myelin layers in a virtually identical manner on all myelinated fibers of telencephalon. MAG is also present on oligodendrocytes of the white column in rats. It can be seen that MAG is present in the entire white column (Figure 5), as well as its absence in the unmyelinated fibers of the substantiae gelatinosae (lamina I and II). GD1a and GT1b expression is widespread in the nervous system<sup>9</sup>. In rodents anti-GD1a stained only lamina I and II in the dorsal horn<sup>5</sup>. In our results, in addition to the staining found by Gong et al (2002)<sup>5</sup> we also observed staining around the central canal suggesting that this staining is particular to recovery after nerve damage. On the seventh postoperative day there appears a lessening

in the staining of anti-GD1a especially in Lamina I and II. Ganglioside GT1b is a marker of motor neurons in the ventral horn of the spinal cord of the rat and we see a band which appears there 7 days after injury. This appearance and the slight appearance of staining 1 day after injury occurs in a similar location suggesting it correlates with sciatic nerve recovery, as plasticity induce by injury. It is important to emphasize that GD1a and GT1b staining is reduced in the substantiae gelatinose around 7 days when it is thought that the continuing process of damage distal axon removal and the neurons either survive or die. Lessening of these two regeneration inhibitor molecules occurs prior to the regeneration process. Both then return to their pre-damage states after axonal growth, this may be seen clearly in pictures for 14 and 21 days. Fourteen days following sciatic nerve lesion there is an increase of staining in the crossover region of the dorsal funiculi that appears to be caused by the recovery of the peripheral nerve. Around day 14, MAG is most distinctive in the crossover fibers, however afterwards in the same place appears the amplification of GD1a and GT1b, which probably is related to the limiting process plasticity in the course of regeneration and directing axons towards a lesser number of target neurons. MAG binds to GD1a and GT1b present in the growth cone<sup>9</sup> this may be why we see a change in the IHC slices later in MAG than in GD1a or GT1b. MAG is also reported to bind to axonal gangliosides GD1a and GT1b to inhibit neurite outgrowth<sup>19</sup>. This too seems to correspond with our results. The peripheral nerve heals and then MAG inhibits further growth, of activated growth simulation, in the white matter of the spinal cord. If Wallerian degen-

eration did not extend into the spinal cord proper then the nerve recovery appears clinically complete.

It is known that the various markers of regeneration appear in different concentrations in the central nervous system versus the peripheral nervous system. Therefore, it would be beneficial to repeat this experiment with IHC on the upper sciatic nerve itself to see if there is a difference in markers in the central nervous system and the peripheral nervous system.

The plantar test results show that the rat recovers from the functional deficiency suffered following sciatic nerve crush; though by 28 days the withdrawal latency has not returned to 0 day measurements. The sham operated animals latency measurements returned to 0 day values around the 7<sup>th</sup> day. The operated rats' contralateral leg at 7 days showed an increase in latency, which continued through to the end of the study (28 days), suggesting a compensational change in the contralateral leg. The IHC results show that after an ipsilateral injury, bilateral and contralateral changes may be seen in the spinal cord segments with the punitive regeneration markers MAG, GD1a and GT1b.

## Acknowledgements

This work was done within projects financed by Croatian Ministry of Science, Education and Sport (219-0061194-2158).

## REFERENCES

1. DE SOUZA A, DA SILVA CA, DEL BEL EA, J Neurotrauma, 21 (2004) 627. — 2. VAREJÃO ASP, MELO-PINTO P, MEEK MF, FILIPE VM, BULAS-CRUZ J, Neurological Research, 26 (2004) 186. — 3. HARGREAVES KM, DUBNER R, BROWN F, FLORES C, JORIS J, Pain, 32 (1988) 77. — 4. LEBARS D, GOZARIU M, CADDEN SW, Pharmacol Rev, 53 (2001) 597. — 5. GONG Y, TAGAWA Y, LUNN MP, LAROY W, HEFFER-LAUC M, LI CY, GRIFFIN JW, SCHNAAR RL, SHEIKH KA, Brain, 125 (2002) 2491. — 6. HEFFER-LAUC M, LAUC G, NIMRICHTER L, FROMHOLT SE, SCHNAAR RL, Biochimica et Biophysica Acta, 1686 (2005) 200. — 7. MOLANDER C, GRANT G, Spinal Cord Cytoarchitecture. In: Paxinos G (Ed) The Rat Nervous System. 2nd ed. (Academic Press, 1994). — 8. ASATO F, BUTLER M, BLOMBERG H, GORDH T, J Peri Nerv Sys, 5 (2000) 19. — 9. MCKERRACHER L, PNAS, 99 (2002) 7811. — 10. VYAS AA, PATEL HV, FROMHOLT SE, HEFFER-LAUC M, VYAS KA, DANG J, SCHACHNER M, SCHNAAR RL, PNAS, 99 (2002) 8412. — 11. PAVIĆ R, TVRDEIĆ A, TOT OK, HEFFER-LAUC M, Somatosens Mot Res, 24 (2007) 213. — 12. LUÍS AL, AMADO S, GEUNA S, RODRIGUES JM, SIMÕES MJ, SANTOS JD, FREGNAN F, RAIMONDO S, VELOSO AP, FERREIRA AJ, ARMADA-DA-SILVA PA, VAREJÃO AS, MAURÍCIO AC, J Neurosci Methods, 163 (2007) 92. — 13. DELLON ES, DELLON AL, Plast Reconstr Surg, 88 (1991) 686. — 14. PAVIĆ R, PAVIĆ ML, TOT OK, BENŠIĆ M, HEFFER-LAUC M, Somatosens Mot Res, 25 (2008) 163. — 15. VOGELAAR CF, VRITEN DH, HOEKMAN MF, BRAKKEE JH, BURBACH JP, HAMERS FP, Brain Res, 1027 (2004) 67. — 16. MONTAGNE-CLAVEL J, OLIVERAS JL, Somatosens Mot Res, 13 (1996) 215. — 17. BALIKI M, CALVO O, CHIALVO DR, APKARIAN AV, Molecular Pain, 1 (2005) 18. — 18. BEHNAM-RASOULI M, NIKRAVESH MR, MAHDAVI-SHAHRI N, TEHRANIPOUR M, Iran Biomed J, 4 (2000) 45. — 19. LEHMANN HC, LOPEZ PH, ZHANG G, NGYUEN T, ZHANG J, KIESEIER BC, MORI S, SHEIKH KA, J Neurosci, 27 (2007) 27.

R. Pavić

»J. J. Strossmayer« University, Osijek University Hospital Center, Department of Traumatology, Surgery Clinic, J. Huttlera 4, 31000 Osijek, Croatia  
e-mail: roman.pavic@os.t-com.hr



## **OZLJEDA NAGNJEČENJEM ISHIJADIČNOG ŽIVCA KOD ŠTAKORA I PRAĆENJE OPORAVKA ISTOG PUTEV PLANTARNOG TESTA I IMUNOHISTOKEMIJSKIM PUTEV**

### **S A Ž E T A K**

Na 30 muških štakora (Wistar) učinjeno je eksperimentalno ozljeđivanje ishijadičnog živca nagnječenjem, upotrebom sile od 49,2 N (tlak  $p=1.98 \times 10^8$  Pa). Također je na jednakom broju štakora učinjen kontrolni dio pokusa (sham) gdje je prikazan neozljeđeni ishijadični živac uz prethodno razmicanje muskulature. Iza toga smo ispitivali osjetilni i motorički oporavak ozljeđenog živca upotrebom Hargreavesove metode plantarnog testa, uz upotrebu aparata Ugo Basile, Italija. Testiranje je vršeno kontinuirano za obje noge štakora, ozljeđenu i neozljeđenu, prijeoperacijski (0 dan), prvi dan nakon ozljeđe, 7, 14, 21 i 28 dan poslije ozljeđe. Istovjetno je učinjeno i za kontrolnu skupinu životinja (sham). Plantarni test je pokazao oporavak motoričke i osjetilne funkcije ozljeđenog ishijadičnog živca 28 dan ali nije u potpunosti dosegao prijeoperacijske vrijednosti. Paralelno sa plantarnim testom radili smo imunohistokemijski pokus na dijelu kralježnične moždine L3-L6, gdje je polazište ishijadičnog živca. Koristili smo protutijela na mijelinom udruženi glikoprotein (MAG), i gangliozide GD1a i GT1b u navedenom dijelu kralježnične moždine. Imunohistokemijskom metodom pokazali smo promjene na motoričkim i osjetilnim aksonima u kralježničnom dijelu L3-L6 i usporedili sa rezultatima plantarnog testa, u smislu oporavaka motorike i osjeta nakon ozljeđe ishijadičnog živca. Imunohistokemijskom analizom dobiveni rezultati također pokazuju ipsilateralne i kontralateralne promjene nakon ozljeđe. Rezultati plantarnog testa pokazuju motoričku kompenzaciju ozljeđene noge štakora neozljeđenom.