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Assessment of the functional return of an injured limb in a mouse model with Bcl-2 gene absent, following the application of locomotor training

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Abstract

Introduction and Objective. The impact of physical activity on human health is invaluable. Studies confirm an increase in neurotrophins in response to locomotor training. The Bcl-2 family of proteins, due to their diversity and pleiotropic actions, places damaged nerve cells on the path to apoptosis, making them a promising prospect in the treatment of neurodegenerative diseases. The aim of the experiment was to demonstrate the effect of the absence of the bcl-2 gene on the process of peripheral nerve regeneration in animals subjected to locomotor training.

Materials and Method. The first group consisted of animals with an absent bcl-2 gene, 129S1/SvImJBcl2tm1Mpin/J (N=40). Group E consisted of animals subjected to sciatic nerve damage with absent bcl-2 gene (N=20), which were subjected to locomotor training, while mice in which the sciatic nerve was cut (N=20) but no training programme was applied represent group K. The evaluation used an automatic gait analysis system for mice and rats from Nodus system Cat&Walk. The study analyzed footprints on a treadmill controlling the mechanism of locomotor movements for analysis before sciatic nerve damage and at 7, 14 and 28 days after treatment.

Results. Both strains showed statistically significant differences in the experimental group compared to the untrained group, as well as a statistically significant difference in trained mice between the Bcl-2 and bl6 strains at day 14 after sciatic nerve injury, and at day 28 after sciatic nerve injury in the Bcl-2 group compared to the bl6 group, both subjected to training.

Conclusions. Regeneration of the sciatic nerve in the locomotor-trained animals studied on the basis of the Cat&Walk study showed that significant improvement occurred in the group of Bcl-2 mice subjected to locomotor training on day 14 after nerve injury.

Key words

sciatic nerve, peripheral nervous system, Bcl-2 proteins, locomotor training

INTRODUCTION

The currently accepted relationship between exercise and its beneficial effects on the human body is increasingly becoming the mainstream in scientific research. Many studies have suggested changes in sympathetic nervous system activity in response to lack of physical activity-induced stimulation in both health and disease [1, 2]. Increasing attention is being paid to the role of physical activity in improving memory and cognitive abilities due to changes in the number, structure and function of neurons [3–5]. This may be related to neuroplasticity, where depending on the intensity of exercise, neurotrophic factors are produced within the central nervous system, including Brain-Derived Neurotrophic Factor (BDNF) and Insulinlike Growth Factor (IGF) [6–8]. One possibility is to look for proteins that are a response to inflammatory processes in both the central and peripheral nervous systems. Their absence or presence would be an important molecular marker confirming this relationship. Exercise and activity-dependent interventions have real effects on the neurobiological mechanisms of peripheral nerve regeneration. Most research is based on an animal model, with rodents in particular being used for this purpose.

Over the past 20 years, transgenic animals have become an important research tool in the biological and medical sciences. Mice, compared to other model organisms, have a number of characteristics that make them most commonly used in molecular research. A key feature is their genome in which 99% of genes contain their counterparts in the human genome. Therefore, transgenic mouse models make good models for preclinical studies after appropriate validation, with the goal of reflecting the results as reliably as possible. The small size and ease of cultivation make it possible to conduct research on a large scale, which makes it a highly economical model [9]. Transgenic models are now an important topic in biology and medicine because, thanks to them, it is possible to understand the molecular pathways underlying the development of human diseases and to develop effective and targeted therapies [10, 11].

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A mouse model lacking the Bcl-2 gene, commonly used in studies within the central and peripheral nervous systems, was chosen for the study. Mice lacking the bcl-2 gene develop normally during foetal life, without disturbances in embryogenesis. Significant differences in the appearance of mutants compared to wild-type mice only become apparent after birth. Mutants are characterised by growth retardation and early mortality – they significantly shorter lives (up to 19 weeks), due to impaired renal cell differentiation and increased apoptosis, leading to polycystic kidneys. The animals are finer, have smaller ears and whitish fur due to a defect in melanin synthesis.

The bcl-2 family of proteins is involved in regulating the process of apoptosis. Moreover, a correlation is found between the absence of BDNF factor and increased expression of Bax proteins intensifying the process of programmed cell death. Regeneration within the damaged nerve promotes the expression of a number of genes whose protein products may contribute to its neuroprotection, and thus its restoration. The 4 expression levels of Bcl-2 and Bax genes are in close relationship with the regulation of neurotrophic factors leading to the prevention of Wallerian degradation [12]. Research on the process of apoptosis within the central and peripheral nervous systems often uses transgenic animals with the absence of the Bcl-2 family protein gene, indicating the relationship between them and other nerve growth factors. Changes in the expression of Bcl-2 family genes in neurons suggest or demonstrate the role of these proteins after their damage. Over-expression of Bcl 2 or Bcl XL in neurons after axotomy (axon cutting) has a neuroprotective effect in many experimental models [13]. However, the question remains: do peripheral nerves with Bcl-2 knockout retain the ability to spontaneously regenerate? The development of genetic engineering methods has contributed to the study of molecular mechanisms occurring within a single neuron cell. Currently, new solutions and therapies are being sought that could significantly improve the results of treatment of peripheral nerves after their damage. The process of surgical treatment of patients after neurological injuries is complemented by rehabilitation. One of the ways to improve the regeneration of peripheral nerves, and thus restore the function of the target organ, is locomotor training [14, 15]. Its impact on the process of rebuilding the damaged neurite is being intensively studied at the cellular level, and the main goal of the research is to explain the beneficial effects of physical exercises on the nervous system.

The aim of the experiment was to evaluate the effect of locomotor training in the different groups of mice studied, with a special focus on bcl-2 mice, and assessment of gait patterns of study mice 7, 14, and 28 days after sciatic nerve injury.

MATERIALS AND METHOD

The study material consisted of 80, 2-month-old male mice, with an average body weight of 22.3 +/- 1.85 g (20.01 – 26.20 g). The first group consisted of animals with an absent bcl-2 gene, 129S1/SvImJ-Bcl2tm1Mpin/J (N=40), which included subgroup E – sciatic nerve-injured animals with an absent bcl-2 gene (N=20) that underwent locomotor training, and group K – mice in which the sciatic nerve was cut (n=20), but no training programme was applied to them. Another group consisted of Bl6: C57BL/6N mice (N=40). These mice were divided into 2 groups: group E (N=20) – nerve-injured animals subjected to locomotor training. Group K (N=20) – mice with damage but not subjected to training. The mice were raised in the breeding facilities of the SUM Centre for Experimental Medicine under standard conditions, with a 12-hour light cycle, and received standard feed and water *ad libitum*. All experiments were carried out in accordance with the current Law on Animal Experiments of 21 January 2005, with prior approval of the Local Ethical Committee for Animal Experiments of the Silesian Medical University in Katowice, Poland (Resolution No. 88/2015, dated 1.07.2015).

The article covers the research and part of the results, which are the subject of a PhD thesis, 'The effect of locomotor training on the process of sciatic nerve regeneration in mice with Bcl-2 gene absent.' The animals were housed individually in cages. They were provided with constant access to food and water. Access to light depended on the diurnal cycle. The experiments were conducted between 09:00 – 14:00, with the surgical procedures performed under sterile conditions. Damage was inflicted on the right sciatic nerve of the animals, specifically, under general anesthesia, a crush injury of the neuropraxia type was performed using a vascular clip. After surgery, paracetamol (120mg/5ml suspension) – a drug with generally known analgesic effect (5mg/kg c. c.) – was administered to animals in drinking water in all groups for 3 days. All experiments were performed to minimize the number of animals used, in a way that reduced their suffering.

Model of sciatic nerve injury. The sciatic nerve injury procedure was carried out under the conditions of the operating room of the SUM Center for Experimental Medicine. Damage to the sciatic nerve was performed using an Aesculap YASARGIL vascular clip with a specific strength and duration of the clip. Under general anesthesia Thiopental, administered intraperitoneally at a dose of 420 mg/kg c.c., (under aseptic conditions after shaving and disinfecting the skin with Skinsept antiseptic fluid) on the outer surface of the mouse thigh, the skin was incised (incision length – 1 cm). The sciatic nerve was exposed using surgical instruments and prepared 'bluntly', approximately 5 mm in front of the trifurcation – the point where the nerve divides into the fibular, tibial and calf nerves. The dissected nerve was crushed using a Yasargil Aneurysm Clip mini temporary with a force of 70.0 gms/0.69 N and a duration of 60 seconds. The wound was then sutured with 4/0 sutures. Neomycin ointment (5mg/g) was applied topically. The photo below shows the dissected sciatic nerve from a mouse animal model and the moment of compression of the nerve with a vascular clip.

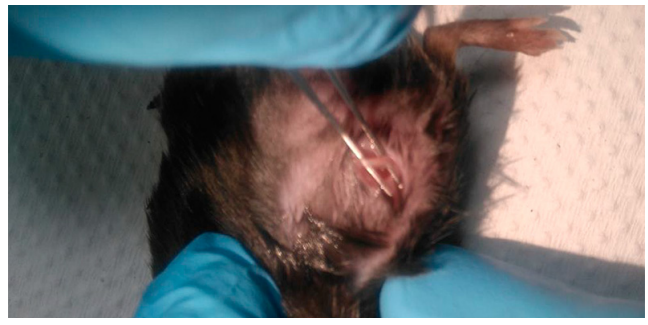

Figure 1. AESCULAP vascular clip, photograph showing the moment of compression of the sciatic nerve in bcl-2 mice.
Source: own materials

Genotyping. Genotyping was performed to confirm the existing mutation. Animal genotypes were confirmed by PCR (Polymerase Chain Reaction). DNA for PCR was isolated from blood taken from the mouse tail. Based on the genotyping results, the animals were classified into appropriate study groups.

Locomotor training. All animals were subjected to locomotor training on a treadmill in the same order after adequately long acclimatization on the device. Training began on the second day after nerve injury. The animals ran on a treadmill with a running speed of 15 m/min continuously for 20 minutes a day, five days a week.

Functional tests – 'Cat&Walk' gait analysis. The CatWalk™ system (Noldus Information Technology, Wageningen, The Netherlands) is designed for rodent gait analysis. This device consists of a catwalk with a 1,300 mm long green backlit glass floor, above which there is a red-lit ceiling that serves as a contrasting background. On the underside, a high-resolution high-speed camera is positioned that records and transmits images to a computer with dedicated software (CatWalk XT 9.1). The operation of the device is based on the effect of light dispersion at the point of contact between the animal's paw and the surface of the glass, resulting in a reflection that reproduces the shape of the paw. The test involves recording the marks left by the mouse under examination. Functional tests were conducted before the sciatic nerve injury and on days 7, 14 and 28 after surgery.

Prior to the evaluation, several test cycles were carried out to accustom the animals to the test conditions. Gait analysis with the CatWalk using a computerized CatwalkXT device evaluates and records the steps taken by a test animal as it moves across the glass floor. Each mouse made at least 2 uninterrupted passes over the area observed by the camera, which were then subjected to semi-automatic analysis using dedicated software. The experiment consisted of walking the animal twice through a tunnel with a transparent, illuminated floor. The emitted light is completely reflected, except in those areas where the animal's are in contact with the glass floor plate, where the light is refracted. A camera located under the glass plate records the illuminated areas. The image is automatically sent to a computer and analyzed. If the animal exerts more pressure in a given area, the resulting image is proportionally brighter, thus allowing the detection of even subtle parameter differences between individual paws. The computer programme automatically recognizes individual mouse paws (dividing them into front and back and right and left), memorizes and analyzes the individual toes of each paw during gait. Gait analysis was performed with the following detection parameters: Camera gain=28.51.01, Intensity threshold=0.32. During functional gait testing with the Cat&Walk system, the following parameters were obtained and then selected for assessment.

The average values of the parameters were calculated for the damaged paw. Selected parameters are explained and defined below.

1) **Max Contact Mean Intensity (s)** – the mean intensity during maximum paw contact, calculated as the average of all recorded impressions for a given paw. The 'Max Contact Mean Intensity' parameter refers to the average intensity of paw contact with the ground at the moment of peak pressure during a rodent's step. This intensity is measured by the brightness of the fluorescence emitted by the paw when in contact with the illuminated surface of the treadmill. A higher value of this parameter indicates stronger paw pressure on the ground, which may reflect normal motor function or compensatory mechanisms in response to pain or injury.
   Changes in 'Max Contact Mean Intensity' can be useful for assessing the extent of nervous system damage, and evaluating the effectiveness of therapeutic interventions in preclinical studies.
2) **Swing (s)** – a parameter in the **CatWalk XT** gait analysis system that defines the time (in seconds) during which the paw remains in the air between consecutive recorded footprints while the animal is walking. In other words, it represents the period when the limb is not in contact with the ground during the gait cycle. This is crucial in studies on neurological disorders, post-injury regeneration, and the evaluation of therapeutic interventions. Its analysis helps to better understand movement mechanics and adaptive strategies used by animals in response to injury or neuromuscular dysfunction.
3) **Stride Length (cm)** – is a parameter in the CatWalk XT gait analysis system that measures the distance between consecutive imprints of a given paw. This distance is calculated from the centre of one imprint to the centre of the next imprint of the same paw during the gait cycle. It is an essential metric for evaluating locomotion and coordination in animal models.
4) **Min Intensity [0–255]** – minimum recorded trace intensity during paw contact – the average of all traces.
5) **Max Intensity [0–255]** – maximum recorded latent intensity during pawprint contact – the average of all latents.
6) **CatWalk XT system** – records the animal's movement on a transparent, illuminated treadmill, analyzing the order and timing of paw prints. Figure 2 shows a mouse walking on the CatWalk. treadmill.

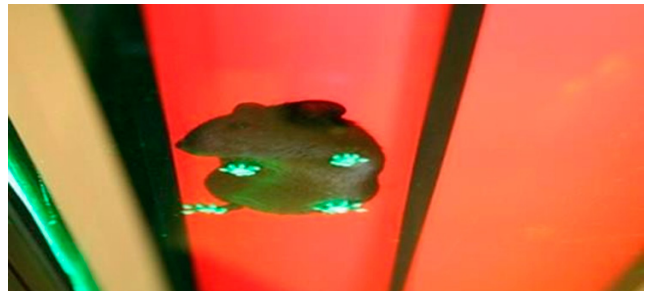

Figure 2. A mouse while walking on a Catwalk treadmill. The device during the test analyzes footprints on the treadmill, controlling the mechanism of locomotion. The green colors of the limbs are the result of diffraction of light emitted by diodes placed parallel to the glass surface of the treadmill, occurring at the point of contact between the limb and the glass surface.
Source: own materials

Statistical analysis. The collected data was subjected to the verification process in the Statistica program by Statsoft. Descriptive statistics were performed. The examined variables were described numerically by means of mean value, maximum, minimum, standard deviation, standard error, median and modal, and presented in tabular and/or graphical form. The Shapiro-Wilk W test and the Lilliefors test were used to test the normality of distribution of quantitative variables. Two-way tables and observed frequencies were

used to characterize ordinal qualitative variables. Hypothesis testing using statistical significance tests was performed against both groups in the experimental model, and in the order performed during the study. The Kruskall-Wallis test (in the case of non-compliance with the normal distribution) or the ANOVA analysis of variance (in the case of the normal distribution) was used to test the statistical significance of differences in quantitative variables between the groups.

To verify the hypotheses and determine the level of statistical significance of differences in paired data, quantitative variables from dependent measurements (subsequent studies), Tukey's RIR test (in the case of non-compliance with the normal distribution) or the Student's t-test for pairs (in the case of non-compliance with the normal distribution) was used.

RESULTS

Average values of all studied parameters in each group and observation periods of the parameters.

In order to present the obtained research results, the research material was divided into 4 groups:

- *bcl-2 (n=40)* – the first group consisted of animals with an absent bcl-2 gene, divided into groups E and K;
- *bcl – 2 E (n=20)* – sciatic nerve-injured animals with an absent bcl-2 gene that underwent locomotor training;
- *bcl – 2 K (n=20)* – mice in which the sciatic nerve was cut (n=20) but no training programme applied;
- *bl6* – C57BL/6N mice (n=40), divided into 2 groups:
- *bl6 E (n=20)* – nerve-injured animals subjected to locomotor training;
- *bl6 K (n=20)* – mice with nerve damage but not subjected to training.

Table 1. Comparative analysis of mean intensity during maximum contact of the paw with the ground – mean of all recorded impressions for a given paw – right hind paw (Max Contact Mean Intensity[s])

Animal groups	Before injury	7 days after injury	14 days after injury	28 days after injury
		[s]		
bcl-2 E	213.608	214.067	161.272	216.723
bcl-2 K	221.338	221.592	219.453	213.842
bl6 E	225.998	222.048	220.262	235
bl6 K	224.293	226.881	226.258	226

A statistical correlation was obtained for the parameter Max Contact Mean Intensity._Mean on days 7, 14 and 28 after sciatic nerve injury in the experimental group in Bcl-2 mice, relative to trained Bl6 mice (day 7 – p=0.042426; day 14 – p= 0.0276; day 28 – p= 0.03213). In addition, it was shown that the improvement in both trained versus untrained groups was statistically significant (bcl-2 p=0.033150; bl6 p= 0.032172).

A statistical correlation was also obtained for the parameter Swing_(s)_Mean on day 7 after sciatic nerve injury – right hind foot in the group of bcl-2 mice subjected to training to the group of bl6 mice also subjected to training (p=0.026090).

Another parameter is StrideLength_(cm)_Mean at day 7 after sciatic nerve injury. A statistically significant difference was observed between trained Bcl-2 strain mice, compared to trained Bl6 mice (p=0.034769). It is worth noting that in trained versus untrained Bl6 mice, the improvement was also statistically significant (p= 0.03276).

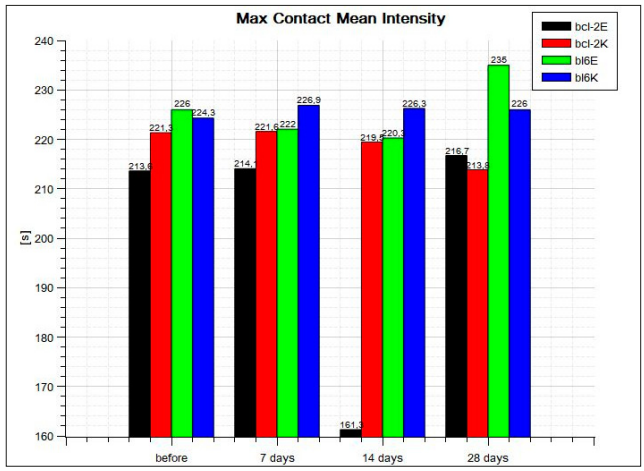

Figure 3. Mean intensity during maximum contact of the paw with the ground – average of all recorded impressions for a given paw – right hind paw (Max Contact Mean Intensity [s])

Table 2. Comparative analysis of the time of keeping the paw in the air between recorded traces. (Swing [s])

Animal groups	Before injury	7 days after injury	14 days after injury	28 days after injury
		[s]		
bcl2 E	0.111	0.085	0.106	0.125
bcl2 K	0.103	0.106	0.114	0.128
bl6 E	0.116	0.117	0.137	0.054
bl6 K	0.117	0.131	0.105	0.114

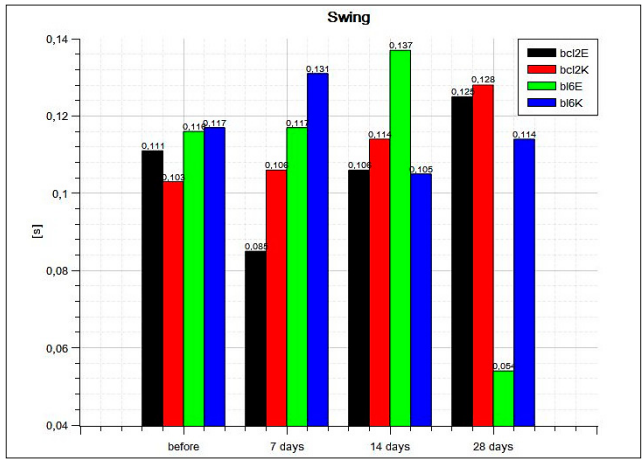

Figure 4. Time to keep the paw in the air between recorded traces (Swing [s])

A statistical correlation was obtained for the Mean Intensity parameter at day 14 after sciatic nerve injury (right hind paw) in the group of trained Bcl-2 mice compared to trained Bl6 mice (p=0.02134).

Another parameter tested was Max Intensity_Mean – for both strains showed statistically significant differences in the experimental group compared to the untrained group (bcl-2 p=0.028921. bl6 p= 0.025341), as well as a statistically significant difference in trained mice between the Bcl-2 and Bl6 strains (p= 0.01423) at day 14 after sciatic nerve injury, and at day 28 after sciatic nerve injury in the Bcl-2 group, compared to the Bl6 group. where both groups were trained (p=0.034561). Figure 8 below graphically shows trends in gait improvement over time.

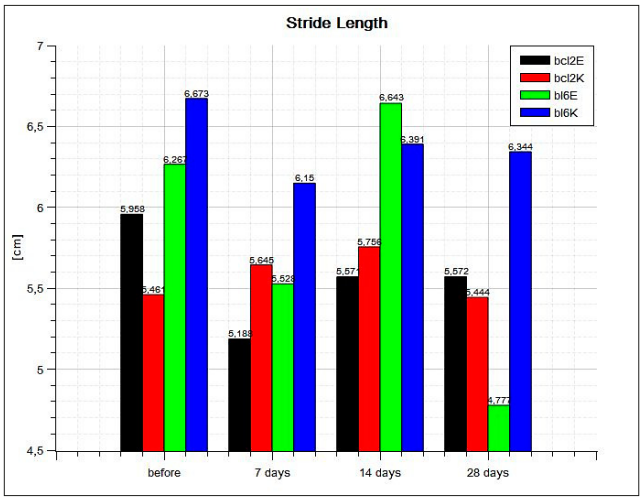

Figure 5. Stride length: distance between successive prints of a given paw, counted from the centre of the print (Stride Length [cm])

Table 3. Comparative analysis of step length. Distance between consecutive imprints of a given paw – calculated from the center of the imprint (Stride Lenght [cm])

Animal groups	Before injury	7 days after injury	14 days after injury	28 days after injury
		[cm]		
bcl2 E	5.958	5.188	5.571	5.572
bcl2 K	5.461	5.645	5.756	5.444
bl6 E	6.267	5.528	6.643	4.777
bl6 K	6.673	6.15	6.391	6.344

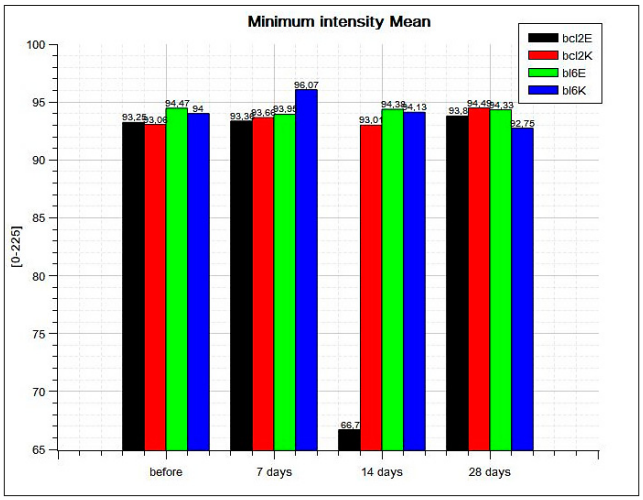

Figure 6. Minimum recorded trace intensity during paw contact with the ground – average of all qualified traces. (Min Intensity Mean [0–255])

Table 4. Comparative analysis of minimum recorded trace intensity during paw contact with the ground – the average of all qualified traces (Min Intensity Mean [0–255])

Animal groups	Before injury	7 days after injury	14 days after injury	28 days after injury
bcl2 E	93.248	93.361	66.697	93.801
bcl2 K	93.059	93.66	93.008	94.487
bl6 E	94.466	93.946	94.375	94.333
bl6 K	94.003	96.071	94.126	92.75

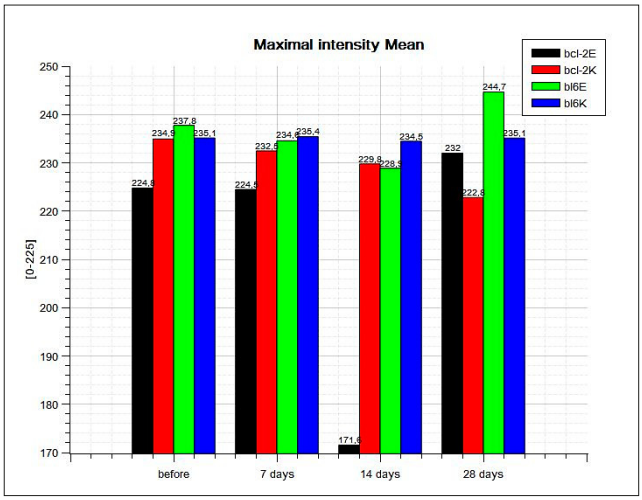

Figure 7. Maximum recorded intensity of the trace during contact of the paw with the ground – average of all qualified traces (Max Intensity Mean [0–255])

Table 5. Comparative analysis of maximum recorded trace intensity during paw contact with the ground – average of all qualified traces (Max Intensity Mean [0–255])

Animal groups	Before injury	7 days after injury	14 days after injury	28 days after injury
bcl-2 E	224.793	224.486	171.568	232.011
bcl-2 K	234.933	232.457	229.76	222.817
bl6 E	237.758	234.562	228.887	244.66
bl6 K	235.139	235.426	234.462	235.125

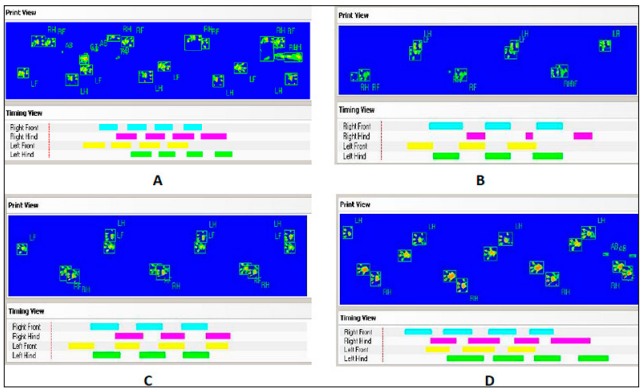

Figure 8. Trends in gait improvement over time (A – before sciatic nerve damage. B – after 7 days. C – 14 days and D – 28 days after the training) for Bl-6. Mouse limb markings: RF – Right Front. RH – Right Hind. LF – Left Front. LH – Left Hind

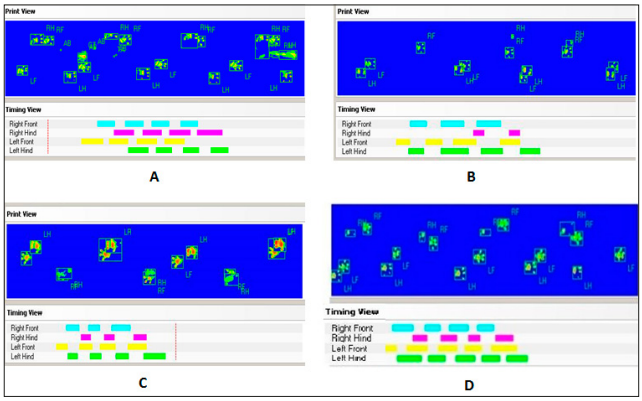

Figure 9. Trends in gait improvement over time. A – before sciatic nerve damage; B – after 7 days; C – 14 days; D – 28 days after training for Bcl-2 mice. Mouse limb markings: RF – Right Front. RH – Right Hind. LF – Left Front. LH – Left Hind

DISCUSSION

There can be many causes leading to damage to the peripheral nervous system. Currently, peripheral nerve injuries are one of the most common injuries in the population and the largest group of injuries involve nerves in the limbs. Unfortunately, as a result of the slow or ineffective process of axonal regeneration, full functional return is a rare phenomenon. Improving and accelerating the process of axonal regeneration is therefore a key goal of many scientific studies [16]. The results of scientific experiments confirm that regular training on the tested animal model leads to over-expression of neurotrophins and adhesion proteins, which may contribute to the strengthening of natural repair mechanisms. For example, in a rat animal model with a damaged spinal cord, the applied locomotor training significantly contributed to functional improvement. The mechanism underlying these effects remains unknown and is under constant research; however, the benefits of exercise may be related to an increase in brain-derived neurotrophic factor [17]. According to a review of the literature, moderate daily walking on a horizontal treadmill improves axonal regeneration and leads to a gradual improvement in locomotor function [18].

The results of the above study, were confirmed in the present experiment, as an improvement in gait function was observed in various parameters of the CatWalk test in the experimental group subjected to daily training, compared to the control group (without training) [19]. It is known that the intensity and duration of stimulation can affect neuroprotection in different ways by inducing an increase or decrease in the secretion of neurotropic factors. Expression or inhibition of neurotropic factors, such as BDNF, can promote or inhibit pain and recovery. depending on whether physical activity activates neurotrophin signalling and stimulates neurons at the peripheral or central level [17]. According to a review of the literature, moderate daily walking on a horizontal treadmill improves axonal regeneration and leads to a gradual improvement in locomotor function [18]. The results of the above study were confirmed in the current experiment, as an improvement in gait function was observed in various parameters CatWalk study in the experimental group subjected to daily training, compared to the control group without training) [19].

It is known that the intensity and duration of stimulation can affect neuroprotection in different ways by inducing an increase or decrease in the secretion of neurotrophic factors. Expression or inhibition of neurotrophic factors, such as BDNF, can promote or inhibit pain and recovery depending on whether physical activity activates neurotrophin signalling, and excites peripheral or central neurons [17].

One of the main advantages of locomotor training after peripheral nerve injury is its beneficial effect on the transport of neurotrophins, which contribute to the reconstruction of the damaged neurite. Their presence in the extracellular space is essential for the initiation of neuronal growth. Neurotrophins are recognized by specific receptor proteins located on the membranes of the neuron, which leads to the growth of neurites towards their release sites; therefore, these proteins are also called growth factors. One of the best known neurotrophins is NGF (Nerve Growth Factor). Its role is to direct the neurite growth cone to the right destinations, which increases the probability of survival of these neurons [20]. It has been proven that the level of concentration of brain-derived neurotrophic factor in the nervous system depends on the activity of neurons. BDNF increases the activity of the neurotransmitter secreted from the excited neuron, and thus improves its effectiveness on the target cell. This is possible because the binding of BDNF to the TrkB receptor triggers appropriate intracellular processes that may ultimately bring about significant neuroplastic changes, such as increasing the area of the synapse and improving the efficiency of signal transmission through these synapses. It can also increase the number of axonal branches while increasing the surface of the neuron, creating conditions for increasing the number of synapses, which improves the efficiency of neural networks [21].

Different types of neurons have different sensitivity to BDNF and neutrophin-3. For example, dorsal root ganglion sensory neurons belonging to group Ia, which innervate muscle spindles, are particularly sensitive to NT-3, while nociceptor neurons innervating the skin are sensitive to NGF. Certain classes of neurons are also influenced by BDNF. Sometimes growth factors that specifically activate the growth of non-neural cells can manifest neurotrophic features [22].

While analyzing scientific studies on animal models, it was noted that spontaneous exercise on a spinning wheel for a period of only 3 and 7 days, led to a marked increase in the rate of neurite growth as well as an increase in the number of sensory neurons regenerating after sciatic nerve injury. In the current study, the mice which received locomotor training had higher levels of brain-derived neurotrophic factor compared to untrained animals [23–25]. Tthe improvement of the gait function was not linear, but showed an abrupt character. The greatest improvement in relation to the group without training was observed on day 7 after experimental damage to the sciatic nerve. In the following days, the functional advantage of the trained mice in relation to the control group was still visible. However. over time, it became less and less visible. In mice of the Bl6 strain subjected to training. a greater and faster improvement in gait function was shown, but without a clear time interval (linear character). The abrupt improvement can only be explained by the altered expression of neurotrophic factors in mice lacking the bcl2 gene. It is worthwhile to further demonstrate the differences between C57/bl6 and Bcl-2 mouse strains. The C57BL/6 strain is a standard mouse strain often used as a control in biomedical research. In contrast. Bcl-2 deletion mice (mutants) are genetically modified, leading to specific differences due to the lack of expression of the Bcl-2 gene which is crucial for cell survival and regulation of apoptosis, therefore its deletion can lead to increased apoptosis in peripheral and central neurons. They may be characterized by delayed or impaired sciatic nerve regeneration as a result of increased death of Schwann cells and motor neurons.

The differences observed in the presented experiment related to the recovery time of individual parameters, reflect the dynamic changes in the balance of the regenerating nerve (e.g. neurotrophic factors, the survival capacity of individual neurons, and rate of re-growth of individual axons or subsequent myelination). The determination that manipulation of apoptosis pathway genes by increasing or inhibiting neurotrophins can prevent post-nerve injury disorders, is a promising prospect, and further research will contribute to the neuromotor improvement of patients with neurological injuries.

CONCLUSIONS AND CLINICAL IMPLICATIONS

1) Locomotor training significantly affects the functional recovery of the damaged limb in a group of mice subjected to training.
2) Functional recovery of the sciatic nerve in animals subjected to locomotor training based on the Cat&Walk study, showed that a significant improvement occurred in the group of Bcl-2 mice subjected to locomotor training on the day 14 after nerve damage.

The experiment demonstrated that locomotor training has a significant impact on the regeneration process of the injured sciatic nerve. Additionally, analysis of the results obtained from the CatWalk system showed a noticeable improvement in motor function in the Bcl-2 mouse group, particularly on day 14 after sciatic nerve injury, which could suggest potential benefits of early rehabilitative intervention.

The obtained results contribute significantly to the understanding of peripheral nerve regeneration mechanisms and the impact of physical activity on the restoration of motor functions. From a clinical perspective, the findings of this experiment may have important implications for the development of new rehabilitative strategies for patients with peripheral nerve injuries. Locomotor training as a form of therapeutic intervention could be a promising method to support the regeneration process in cases of peripheral nerve damage, such as sciatic nerve injuries. which often occur as a result of mechanical trauma or chronic neurological conditions.

Further studies should focus on identifying the optimal training parameters (frequency. intensity. or duration) that could maximize rehabilitation effectiveness in the context of various types of nerve damage. Moreover, considering the specific Bcl-2 mouse model, in the future, it would be worthwhile assessing the influence of other genes and molecular mechanisms that may support the regeneration process. In particular, research on the interactions between training and biological mechanisms responsible for nerve damage repair could provide crucial information that might be applied in human therapies. The next steps in this field could also include testing the combination of this type of training with other therapies, such as pharmacological treatments or the use of assistive technologies, to develop comprehensive rehabilitation approaches for patients with peripheral nerve injuries.

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