Original article

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Model of spinal cord lateral hemi-excision at the lower thoracic level for the tasks of reconstructive and experimental neurosurgery

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Purpose. To test the model of spinal cord lateral hemiexcision in young rats.

Materials and methods. Animals – male rats (age about 1 month, body weight about 50 g, inbred derivatives of the Wistar line); the number of experimental groups is: 1) lateral spinal cord hemisection at the level of segments about T12–T13 (**Sect**; n=11); 2) lateral spinal cord hemiexcision about 1 mm long at the similar level (**Exc**; n=8). Assessment of motor Function Index (FI) and the Spasticity Index (SI) of the paretic hindlimb was carried out using the Basso–Beattie–Bresnahan (BBB) scale and Ashworth scale, respectively, in our technical modifications. The non-inclusion criteria: the BBB score above 9 points of FI for the ipsilateral hindlimb in a week after injury –and / or BBB score less than or equal to 14 points of FI of the contralateral hindlimb during a long follow-up period (in general, 2 animals in the **Sect** group, 3 animals – in the **Exc** group). Asymptotic differences in the timing of testing between subgroups and groups were revealed during the first three weeks of follow-up. Interpolation reproduction of individual values of FI and SI was used in exceptional cases. The total follow-up period was 5 months. Statistical analysis was performed using the Mann-Whitney U Test, Wilcoxon Matched Pairs Test, Spearman*'s* Rank Order Correlation. For pathomorphological study, the method of silver impregnation of the spinal cord longitudinal sections of the **Exc** group animals obtained in 5 months after the simulation of injury was used.

Results. One week after injury, the FI in the **Sect** group was 5.9±1.1 according to BBB points, a statistically significant increase in the FI lasted for the first 3 weeks ($p<0.05$; Wilcoxon Matched Pairs Test), the FI maximum in the group was 10.1±1.1 BBB points, and the FI value at the end of the study was 9.5±1.0 BBB points. In the **Exc** group, 1 week after injury, the FI was 0.9±0.5 BBB points, during the next week it reached the actual maximum (1.9±0.7 BBB points), by the end of the 5th month it significantly decreased to 0.8±0.3 BBB points ($p<0.05$; Wilcoxon Matched Pairs Test). One week after injury, the SI value in the **Sect** group was 0.3±0.1 points according to Ashworth scale, in the **Exc** group – 0.7±0.1 Ashworth points, a significant increase ($p<0.05$; Wilcoxon Matched Pairs Test) in SI in the **Sect** group was noted during the 2nd week and the 2nd month, in the **Exc** group – during the 2nd and 6th week, as well as the 3rd and 5th month after injury. The SI final and maximal score for the **Sect** group was 0.8±0.2 Ashworth points, and for the **Exc** group – 3.6±0.3 Ashworth points. For both groups, there was no correlation between the mean FI value and a significant positive correlation of the mean SI value with the value of the follow-up period ($p<0.05$; Spearman*'s* Rank Order Correlation), as well as the absence of correlation between the mean FI and SI values during the total follow-up period. A significant negative correlation ($p<0.05$; Spearman*'s* Rank Order Correlation) between individual FI and SI values was found after 1 and 4 weeks, 3 and 5 months after the injury for the **Sect** group, as well as after 5, 7, 8 weeks and after 3 and 4 months for the **Exc** group. At all periods of follow-up, the difference in both FI and SI mean values of both groups was significant ($p<0.05$; the Mann-Whitney U Test).

Conclusions. The studied model of spinal cord injury in young rats is the means of choice for testing solid neural transplantation means for the spinal cord injury restorative treatment. The interpretation of data obtained using the BBB scale on models of lateral half spinal cord injury should be carried out with caution, and the methodology for verifying spasticity requires significant improvement. We recommend that the optimal timing for the FI and SI monitoring after lateral half spinal cord injury is 7 days, 14 days and in 1, 2, 3, 4, 5, 6, and 7 months.

Key words*: lateral spinal cord hemisection; lateral spinal cord hemiexcision; autogenous restoration of motor function; post-traumatic spasticity*

Introduction

Spinal cord injury (SCI) in many respects is a significant type of damage to the nervous system with high mortality, frequent disability, reduced life expectancy and deterioration of its quality [1-5]. The estimated global incidence for SCI, according to 2016 epidemiological data, was about 1 million cases with an age-adjusted relative index in 13 cases per 100 thousand population, and the estimated global prevalence for this type of injury in the same period was about 27 million people [6]. SCI occurs more often in men, the oldest group is people under 30 years [7], the main causes are traffic road accidents and falls [7,8]. Complications of SCI significantly increase the annual costs associated with the treatment and care of spinal patients [9,10]. The most famous of them are spasticity [11–14], chronic pain [15,16], autonomic imbalance [17,18] and related disorders of cardiovascular [19], digestive [20], urinary [21,22] and reproductive systems [23, 24], as well as, probably, affective [25–28] and cognitive disorders [29–33].

The cause of clinical symptoms in SCI should be considered the death of motoneurons at the level of injury and the exclusion of descending supraspinal innervation of motor and autonomic efferent neurons located below the level of injury. Consequently, in case of localization of the focus of trauma outside the cervical or lumbosacral enlargement of the spinal cord, the primary task of rehabilitation treatment is the restoration of long supraspinal projections on neurons devoid of descending synaptic effects. The solution of this problem is associated with the development of neuroengineering technology [34-41], in particular with the development of tissue scaffolding, or matrices [36,37,39,40,42], which could be implanted in the area of traumatic or special surgical defect, for example, after the spinal cord scar removal. It is assumed that the implantation of matrices associated with stem or progenitor cells of a certain phenotype will be accompanied by better clinical results [35,37]. In addition, the following facts motivate the development of neuroengineering means of restoring supraspinal projections onto the neural networks of the injured spinal cord: the existence of voluntary stepping locomotion in a cat correlates with the preservation of only 10% of the transection of white matter, in a monkey satisfactory restoration of the function of hindlimbs is observed while preserving 25 % of the transection of the spinal cord, in a human - <50% [43].

Limited spontaneous functional regeneration of the spinal cord is a well-known clinical fact [44,45]. Thus, about 20% of spinal patients of the AIS-A category (American Spinal Injury Association Impairment Scale grade A) during the first year after injury move to another clinical category: 10–17% - in AIS – B, 4–7% - in AIS– C, 2–4% - in AIS – D [44]. According to other data [46], 4–10% of AIS-A category patients are transfered to AIS – B category within the period > 1 month after injury. Such autogenous restoration for obvious reasons cannot be fully explained by regression of spinal shock. Most likely, it is provided by rearranging the topology of neural networks of the motor system of the brain [47–54] and spinal [47,48,52,54–56] cord, in particular with the participation of propriospinal neurons [54,57]. This process depends on many factors, for example, on the mechanism of spinal cord injury, the degree of primary preservation of its substance, the level of damage and the patient's age.

Paradoxically, the phenomenon of spontaneous regeneration of the spinal cord, in addition to the obvious positive, has a negative meaning. It is precisely because of its presence in lower mammals, the development of a satisfactory model of SCI, which when reproduced by any research group would give a similar level of neurological deficit in low mortality and the ability to assess the effectiveness of restorative treatment, is an urgent problem of experimental neurosurgery [58]. For example, the models of spinal cord injury or compression closest to clinical conditions [58] can be used only for testing liquid agents, such as cell suspensions. In addition, due to significant autogenous restoration of the rat spinal cord [59], the uniformity of neurological deficit in experimental groups with such trauma can be achieved by increasing its severity [60], which will inevitably affect the mortality and suffering of experimental animals and require high quality veterinary care. The complete transection model is quite simple to perform [58], is characterized by high reproducibility, the same type of neurological deficit and poor autogenous restoration of motor function (see, for example, [61]). Thus, it is the most complementary study of the effectiveness of solid neural transplantation means of restorative treatment of SCI [58]. However, its use critically depends on the availability of complex veterinary care for animals. In this sense, the lateral hemisection model seems to be a compromise variant of the experimental SCI. However, its disadvantage is a significant autogenous restoration of the rat spinal cord [62–66], which, along with other difficulties (spatial precision, delicacy and fundamental impossibility of intraoperative assessment of hemisection accuracy) turns the model from acceptable to problematic.

The expediency of searching for a model of SCI that is optimal for testing of restorative neural transplantation interventions is evidenced by unsatisfactory clinical translation of encouraging results obtained experimentally [48,58,67]. In this regard, some authors point to the need to standardize the protocols of such experimental studies [67-70].

Given the widespread belief about the dependence of the regenerative potential of the nervous system on the age of the organism [71–76], we investigated the features of autogenous restoration of motor function in a young rat after lateral hemisection and lateral hemiexcision of the spinal cord at the lower thoracic level. The data obtained give grounds to propose a SCI model, optimal for testing solid neural transplantation means of restoring of injured spinal cord.

Purpose: To test the model of spinal cord lateral hemiexcision in young rats.

This article contains some figures that are displayed in color online but in black and white in the print edition

Materials and methods

Experimental animals. The study was carried out on white outbred rats (inbred derivatives of the Wistar line) of the vivarium of Institute of Physiology named after O.O. Bogomolets aged about 1 month with a body weight of about 50 g. The animals were kept at a temperature of 18-22 ° C with a natural circadian light cycle, with a balanced diet with a combined feed ad libitum in a room with the permanent ventilation.

Ethical regulations. The study was carried out in accordance with the principles of bioethics and humane treatment of animals regulated by the EU Council Directive 86/609/ EEC «On the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes» (1986)., The European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (1986), and the Law of Ukraine № 3447-IV "On the Protection of Animals from Cruelty" (2006). The study design was considered for compliance with current requirements of bioethics and approved by the Commission on Bioethics and Research Ethics of the National Medical University named after O.O. Bogomolets of the Ministry of Health of Ukraine (Minutes № 114 dated October 10, 2018).

Experimental groups and general study design. During the study, two experimental groups were formed: the Sect group (sectio) - lateral hemisection of the spinal cord in the lower thoracic region (n = 11), the Exc group (excisio) - lateral hemiexcision of the spinal cord in the lower thoracic region about 1 mm long (n = 8).

Simulation of trauma. In animals of the Sect group, a model of the left sided hemisection of the spinal cord in the lower thoracic region was reproduced ***(Fig. 1)***. Surgical interventions were performed under general anesthesia, which was achieved by intraperitoneal administration of a mixture of solutions of xylazine ("Biowet", Poland; about 15 mg/kg body weight) and ketamine ("Farmak", Ukraine; about 70 mg/kg body weight), with mild aseptic regulations and in most cases

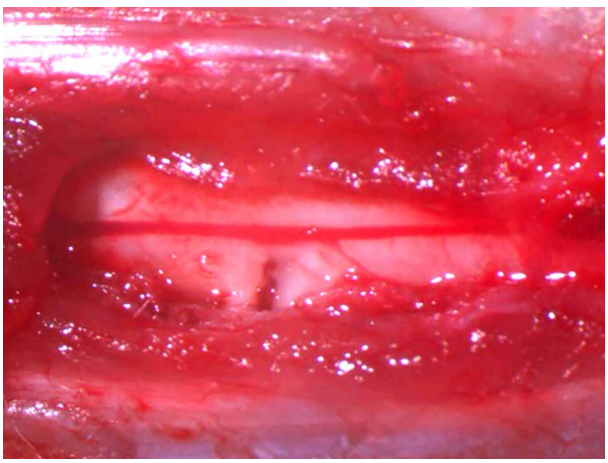

Fig. 1. View of the area of the left sided transection of the half of the spinal cord in the lower thoracic region in an animal with biometric indicators characteristic of animals of both experimental groups, immediately after drug overdose

- provided that the operating table is heated. In this and previous studies ([77–85], etc.), palpation of the caudal edge of the chest of a deeply anesthetized animal at the site of its fixation to the spine was used to select the injury simulation area. Given the theoretically less ossification, greater flexibility and mobility of the last pairs of ribs (costae fluctuantes) in rodents [86,87], especially in young individuals, the place of stopping the surgeon's fingers when detecting a rigid apex of the angle between the chest and spine may be above the middle vertebra T13. This circumstance, along with the lack of special visualization of the cervical parts of the ribs and places of their fixation to the spine and radiological control allows localizing the laminectomy site approximately, most likely - at the level of T11-T12 vertebrae, ie taking into account approximate skeletal data [88,89], age-adjusted of the animal [90,91], - at the level of spinal cord segments T12 – T13. After a linear incision of roughly shaved and antiseptic-treated skin at the level of T9 – L2 vertebrae and skeletization of the posterior surface of the spine, the interarcual space was perforated with a blunt spear-shaped steel rod. Limited from the right laminectomy was performed with a mosquito-shaped clamp curved along the rib. Determination of the condition of the dura mater within the bone window and the release of the spinal cord from adjacent nerve trunks before modeling SCI was not performed. The needle of the insulin syringe in the ventral direction was pierced the spinal cord as close as possible to the left edge of the posterior median artery [92], trying to maintain a perpendicular location of the needle to the dorsal surface of the spinal cord. One of the branches of ophthalmic scissors was inserted into the spinal cord wound, the second branch covered the left half of the diameter of the spinal cord together with the trunks of the nerve roots transecting it in several steps. The jointed branches of ophthalmic tweezers curved along the rib were immersed in the intersection and, resting the working end on the anterior surface of the spinal canal, passed along its inner surface in the direction from the midline to the fracture edge of the root arch, thereby the likelihood of incomplete transection of lateral parts of white matter of the spinal cord was reduced and the trunks of the anterior roots, which was often accompanied by synchronous motor responses were injured. The use of a special surgical means of controlling of the completeness of the transection is also described by other authors [61].

In animals of the Exc group, a model of lateral spinal cord hemiexcision on the left in the lower thoracic region was reproduced ***(Fig. 2)***. As in the previous model, after performing a limited to the right laminectomy in deeply anesthetized animals without special determination of the condition of the dura mater and the release of the spinal cord from adjacent nerve trunks using an insulin syringe needle through the ventral direction the spinal cord was pierced as close as possible to the left edge of the posterior median artery [92]. Similarly, through punctures were performed at 0.5 mm more rostral and caudal. One branch of open ophthalmic scissors was inserted into each two adjacent punctures and a longitudinal paramedian opening of the spinal cord was formed in several steps on a segment about 1 mm long. One of the branches of ophthalmic scissors was inserted

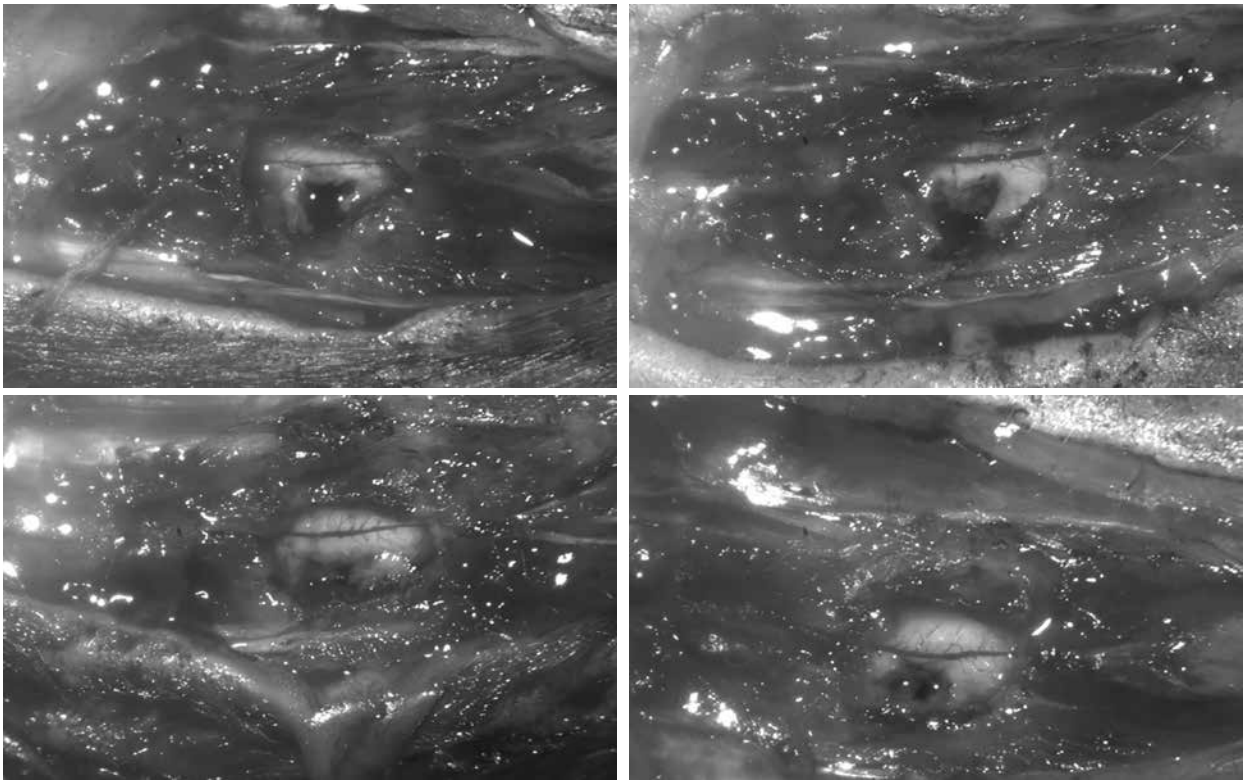

Fig. 2. The most typical intraoperative observations after lateral hemiexcision of the spinal cord in the lower thoracic region (group Exc)

into the rostral and caudal end (the sequence was not specially controlled) of the spinal cord wound thus formed, the second branch covered the left half of the diameter of the spinal cord together with the nerve root trunks and transected it in several steps. The spinal cord substance within the formed fragment was removed with curved and straight ophthalmic tweezers under variable (if necessary) magnification of the operating microscope for 20-30 minutes. In some cases, visually non-viable fragments of the ends of the spinal cord were removed. Quite often the rostro-caudal size of the wound slightly exceeded the transverse one.

In animals of both groups, after spontaneous arrest of bleeding and wound toilet of the spinal cord, the window of access to the spinal canal was covered with a fragment of subcutaneous connective tissue specially removed in the access area, soft tissues and skin were sutured with two rows of interrupted sutures. The wound site was treated with a povidone-iodine solution (EGIS, Hungary). Considering the organization of only mild aseptic regulation during surgery for the prevention of infectious complications a solution of bicillin-5 ("Kyivmedpreparat", Ukraine) was injected in the posterior cervical region subcutaneously at a dose of about 0.5 million IU / kg of live weight. Intraperitoneal administration of a solution of dexamethasone ("KRKA", Slovenia) at a dose of about 5 mg / kg of live weight was used for anti-inflammatory and antiedematous therapy. After surgery, until complete awakening, the animals were kept at increased air temperature. In order to prevent ophthalmic complications in most cases, the outer part of the eyeball after surgery was moistened with saline. Subsequently, the animals were kept in special plastic cages with an external bottom size of 40 × 30 cm and an external height of about 16 cm (the size of the upper plane of the cage is about 50 × 36 cm) with a horizontal covering lattice 3 individuals each (subgroup of the Sect group, n = 6, 2 cages) or individually (other animals of both groups) in cages of similar design with an external bottom size of about 38 × 22 cm and an external height of about 16 cm (the size of the upper plane of the cage is about 43 × 28 cm; in the article [93] the wrong size of these cages is given).

Evaluation of motor function of the paretic limb. The function index (FI) of the posterior ipsilateral relative to the area of limb injury was determined according to the scale proposed by D.M. Basso, M.S. Beattie and J.C. Bresnahan (BBB) [59] ***(Table 1)***, in its own technical modification, while observing the motor activity of an animal on a horizontal open solid surface of an unregulated shape, area, size (sufficient for the study of the long-term unidirectional step locomotion), smoothness, without video recording , recording the daily time and duration of study of each animal.

In order to detail the dynamics of the recovery process the absolute weekly increment or rate of change of FI (V_{FI}) was determined by:

$$\{\mathbf{V_{FI}}\}_n \cdot = \cdot \frac{\mathbf{FI_n} - \mathbf{FI_{n-1}}}{k},$$

where **n** is the ordinal number of the observation within the standardized timescale for displaying the result (see below); **k** is the number of weeks in the estimated time interval (it was conditionally assumed that 3 months correspond to 12 weeks, 4 months - 16

Table 1. Locomotor rating scale of hindlimbs of a rat after spinal cord injury, proposed by D.M. Basso, M.S. Beattie and J.C. Bresnahan [59] with our comments

Score	Leading signs of motor activity of the hindlimbs (hindlimb) during unidirectional step locomotion
0	No observable hindlimb (HL) movement
1	Slight movement of one or two joints, usually the hip and/or knee
2	Extensive movement of one joint or extensive movement of one joint and slight movement of one other joint
3	Extensive movement of two joints
4	Slight movement of all three joints of the HL
5	Slight movement of two joints and extensive movement of the third
6	Extensive movement of two joints and slight movement of the third
7	Extensive movement of all three joints of the HL *Note*: the next level of the indicator (8 points) also states common movements in three joints, but organized in quasi-step synergies. Consequently, at values <8 points, we found mobility in the joints in any spatial-positional conditions, for example, in the ankle joint, the movement amplitude was often assessed when the animal was lifted by the tail.
8	Sweeping with no weight support or plantar placement of the paw with no weight support *Note*: We usually used visual identification of the abdominal separation at the pelvic level (ie corresponding posterior quadrant of the body) from the surface of locomotion in the penetrating light
9	Plantar placement of the paw with weight support in stance only (i.e., when stationary) or occasional, frequent, or consistent weight supported dorsal stepping and no plantar stepping
10	Occasional weight supported plantar steps, no forelimb (FL)-HL coordination *Note*: assessment of the coordination of step movements of the limbs is probably one of the most difficult criteria to determine the scale (see [94]). Usually (except of cases of obvious discoordination) we took into account the frequency of «prolapses» during unidirectional locomotion of the animal - visible «failures» in the synchronization of locomotor cycles, of the estimated limb and three other limbs, reminiscent of moments of «light jumping», when paired, a person changes the type of step collaboration from mirror (the left leg of one partner is concurrent to the right leg of the other) to syntopic, «synlateral» (both partners «walk in step» - step movements of both right and both left limbs are synchronous). During such a «prolapse», a jump-like synchronization of non-harmonized cycle of the paretic limb of the animal probably occurs
11	Frequent to consistent weight supported plantar steps and no FL-HL coordination
12	Frequent to consistent weight supported plantar steps and occasional FL-HL coordination
13	Frequent to consistent weight supported plantar steps and frequent FL-HL coordination
14	Consistent weight supported plantar steps, consistent FL-HL coordination; and predominant paw position during locomotion is rotated (internally or externally) when it makes initial contact with the surface as well as just before it is lifted off at the end of stance or frequent plantar stepping, consistent FL-HL coordination, and occasional dorsal stepping
15	Consistent plantar stepping and consistent FL-HL coordination; and no toe clearance or occasional toe clearance during forward limb advancement; predominant paw position is parallel to the body at initial contact *Note*: the absence of toe clearance during the swing phase is easy to determine by auscultation when testing the animal on a cardboard surface in the presence of a distinct sound of scraping - shuffling fingers over the surface. According to our observations, as the frequency of shuffling steps decreases, the duration and volume of the characteristic «rustling sound» diminish, which significantly complicates the verification of the frequency of symptom manifestation. Only acoustic analysis can help determine the statistical significance of such a statement

Continuation of ***Table 1***

16	Consistent plantar stepping and consistent FL-HL coordination during gait; and toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off
17	Consistent plantar stepping and consistent FL-HL coordination during gait; and toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and lift off
18	Consistent plantar stepping and consistent FL-HL coordination during gait; and toe clearance occurs consistently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off
19	Consistent plantar stepping and consistent FL-HL coordination during gait; and toe clearance occurs consistently during forward limb advancement; predominant paw position is parallel at initial contact and lift off; and tail is down part or all of the time
20	Consistent plantar stepping and consistent coordinated gait; consistent toe clearance; predominant paw position is parallel at initial contact and lift off; tail consistently up; and trunk instability
21	Consistent plantar stepping and coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, consistent trunk stability, tail consistently up
Slight: partial joint movement through less than half the range of joint motion Extensive: movement through more than half of the range of joint motion Sweeping: rhythmic movement of HL in which all three joints are extended, then fully flex and extend again; animal is usually sidelying, the plantar surface of paw may or may not contact the ground; no weight support across the HL is evident No Weight Support: no contraction of the extensor muscles of the HL during plantar placement of the paw; or no elevation of the hindquarter Weight Support: contraction of the extensor muscles of the HL during plantar placement of the paw, or elevation of the hindquarter Plantar Stepping: The paw is in plantar contact with weight support then the HL is advanced forward and plantar contact with weight support is reestablished Dorsal Stepping: weight is supported through the dorsal surface of the paw at some point in the step cycle FL-HL Coordination: for every FL step an HL step is taken and the HLs alternate Occasional: less than or equal to half; <50% Frequent: more than half but not always; 51-94% Consistent: nearly always or always; 95-100% Trunk Instability: lateral weight shifts that cause waddling from side to side or a partial collapse of the trunk Note: according to our experience, the figures given are quite conditional in the sense of the authors' stated accuracy of their clarification, which is also evident from the explanations presented, for example, to determine the frequency of steps with shuffling the toes: "Examiners had difficulty distinguishing between frequent and consistent toe clearance; therefore, consistent toe clearance was defined as four or less toe-drags during the 4-minute period rats with five or more toe-drags per session were rated as having frequent toe clearance" [59]	

weeks, 5 months - 20 weeks). Considering the close to zero values of V_{FI} during these follow-up periods, such a simplification most likely did not affect the probability of the results obtained.

Evaluation of spasticity in the paretic limb. the Spasticity Index (SI) of the posterior ipsilateral relative to the area of injury of the limb was evaluated according to the Ashworth scale adapted to the experimental conditions [95] in our technical modification ***(Table 2)*** without acclimatization training and relaxation blinding of animals, holding the animal by the tail end in a close to horizontal position during different types of locomotor behavior (the urge to escape, the state of locomotor rest or the desire for adduction-flexion release of the paretic limb from the hand of the experimentor). Given the difficulty of differentiating between «voluntary» and «involunyary» resistance to manual muscle stretching against the background of mild spastic paresis, the original criteria for verification of this condition have been used (***Table 2***, line «0.5 points»). Usually all values of SI 0 and ≥1 points were detected at the level of the ankle joint (examining the force of resistance to manual extension, ie dorsiflexion of the foot at different rate), SI value of 0.5 points was found mainly at the level of the knee and hip (for example, when pulling the limb for the foot to the side). Zero points were recorded in the absence of resistance, 1 point - with slight resistance, 2 points - with greater resistance and preservation of mobility with a sufficiently light effort, 3 points - with more or less complete passive mobility with a sufficiently strong but not algogenic effort, 4 points - when limiting passive mobility with such an effort. The absolute weekly increment in SI (V_{SI}) was determined according to the method described for V_{FI}.

Determination of FI and SI was carried out by the same experimentor, conditionally blinded both in relation to the individual characteristics of all animals operated by him, and in relation to the previous values of FI and SI. FI and SI values were measured not earlier than in a conditional week after injury (see below) in view of the ethical regulations for working with experimental animals. The values of FI and SI immediately after

Table 2. Modified Ashworth scale for assessing the degree of spasticity of the paretic limb at the level of the studied joint of a rat

Score	Clinical equivalent
0	There is no increase in muscle resistance to stretching in the passive movement in the joint. *Note*: in the work of H.W. Dong et al. (2005) [95] "resistance against the passive movement" is briefly denoted by the term "tone"
0,5	More noticeable in the paretic limb than in the conditionally intact (in this study - contralateral), trembling when pulling it for the foot to the side and / or hypermetric extension of the paretic limb during postural resistance to sharp traction of the animal by the tail on a horizontal surface
1	Slight increase in muscle resistance during passive movement in the joint, the presence of the «catch» phenomenon - a dramatic increase in muscle resistance to rapid passive movement in the joint; in slow movement – resistance is normal [96]
2	Significant increase in muscle resistance throughout the entire volume of passive movement; passive movements in the joint are possible in full volume with a fairly light effort
3	Significant increase in muscle resistance during passive movement in the joint; passive movements in the joint are significantly hampered and, in some cases, limited to a certain extent, however, without verification by additional effort ***NB!*** In the original scale [95], limitation of joint mobility at this level of the SI is not mentioned
4	Severe contracture - a significant limitation of passive mobility in the joint (within the limits that are determined, in particular, by the painful response of the animal), in some cases – ankylosis

simulation of the injury were conventionally taken equal to zero, given the state of spinal shock. Subsequently, in case of doubt about to the exact integer value of the FI and SI, the half- value was recorded.

Non-inclusion criteria. The declared experimental groups did not involve animals that during the first follow-up period (see below) showed FI of the paretic limb > 9 points on the BBB scale (in the Sect group - 2 animals, in the Exc group - 1), as well as animals with a persistent motor deficits functions of the posterior contralateral limb (≤14 points, in the Exc group - 2 animals) and animals with obvious signs of persistent peripheral paresis, ie with damage to motoneurons of the lower extremities, localized in the L3 – L6 segments [97]. Cases of obvious ipsilateral paresis of the abdominal wall muscles, ie excessively rostralized spinal cord injury (see below), were not specifically recorded.

Hourly regulations of monitoring and presentation of FI and SI values. The condition of the motor system was reflected according to a standardized time scale within the experiment: during the first 2 months - weekly (1–8 weeks), as well as in 3, 4 and 5 months after injury. When displaying the duration of follow-up in weeks, 7 days were considered the minimum unit of time, when displaying the duration in months - the interval between the same numbers (dates) of two adjacent months (ignoring the options for the length of the month during the year). Among all observations with recorded calendar data, asymptotic differences in period of testing between subgroups and groups were detected during the first 3 weeks of follow-up period (≤29% of the value of the corresponding term of the scale of presenting the results); at other times they did not exceed 11%. In the Exc group, the individual values of FI and SI 6 weeks after injury were reproduced by interpolation as the arithmetic mean of the values of two adjacent time points of follow-up. Similarly, the values of FI and SI in one of the subgroups of the group Sect (n = 5) were reproduced 7 weeks after injury. In the same subgroup, the individual values of FI and SI after 3 months were reproduced in the same way from the test results after 2 months and 12 days and 3 months and 12 days after the injury, and the value after 4 months was reproduced from the values 3 and 5 months after injury . A similar algorithm was previously used by default in the context of bringing temporarily dissimilar results to a standardized time scale [78–85,93,98–112]. Assessing the values of V_{FI} and V_{SI} from the standpoint of the above adjustments, as well as the results of comparative statistical analysis during the first month of follow-up and in 6 and 7 weeks, 3 and 4 months after the injury, it should also be taken into account that starting from the 5th week of the post-traumatic period long-term unidirectional dynamics in the models of SCI that have been studied are usually absent [62,64,94,113–115].

Statistical analysis. Comparative statistical analysis of FI, SI, V_{FI} and V_{SI} values in both groups was carried out up to and including the 5th month, by means of the Statistica 10.0 software package using the Mann-Whitney U-Test, Wilcoxon Matched Pairs Test and Spearman's Rank Order Correlations (correlation coefficient - r_s). The mean values are given as M ± SE, where M is the mean value, SE is the standard error of the mean value. In all cases, the assumption about the statistical significance of the obtained result was considered correct if the probability of realization of the alternative statement was less than 0.05 ($p < 0.05$).

Pathomorphological study. Material for pathomorphological examination in animals of the Exc group (n = 4) was obtained immediately after the follow-up completion (see above). The method of impregnation with silver nitrate was used to visualize nerve fibers in the spinal cord substance [116]. After euthanasia the animal by the overdose of the above drugs, the area of the spine that contained the site of injury was removed and placed in a 4% paraformaldehyde solution. A few weeks later, the spinal cord was isolated by micropreparation, the excess part of the caudal and rostral areas was dissected away with the blade, narrowing the area around the injury. During

histological processing, the material was placed on the surface of the metallic operative unit of the microtome cryostat (MK-25, USSR), frozen to -20 ° C and longitudinal sections of 15 μm thick were made, which were placed in a vessel with tap water at room temperature, visually the highest quality samples were selected and with the help of a glass hook were transferred for storage in a 1% of acidic formalin solution. After 1 day to increase the permeability of myelin shells for silver nitrate, the sections were transferred to an organic solvent pyridine, after a day - into a vessel with tap water (three times for 10 minutes), then - into a vessel with distilled water (three times for 5 minutes), then - into 30% silver nitrate solution. After 24 h, the sections were transferred into distilled water for a period of less than 1 min, then into 1% acid formalin solution (three times for 4 min), then into ammonia silver solution (for 2 min), which was prepared ex tempore from 30% silver nitrate solution titration with 25% ammonia solution until complete dissolution of the precipitate with filtration. The sections were placed in 1% solution of acidic formalin until they turned brown, then in 0.5% solution of acidic formalin with a glucose content of 1-2% (15-20 s), a weak ammonia solution (for 30s) and distilled water (10-15 minutes). At the end, the sections were transferred to a 99.8% solution of isopropyl alcohol for a few minutes, after which a slide was immersed in the solution, which was used to pick up and take out the required section. Excess alcohol was removed from the glass with filter paper, without touching the section, until it was completely dry. One or two drops of Canadian balsam were applied to the section and covered with a thin glass. After drying for 1 day, the sections were considered ready for microscopy. Viewing was carried out on an Axiophot light optical microscope (Opton, Germany) with an objective magnification of 2.5, 10, 20 and 40 times, an optical adapter – of 2 times, an eyepiece – of 10 times. Images were photographed with a Canon 600 digital camera (Canon Inc., Taiwan). Images were calibrated on a computer image analyzer CAI-01ABH ("Selmi", Ukraine) using "Kappa opto-electronics GmbH" software (Germany) and Carl Zeiss micrometer object (0.01–1.0). mm, the value of δ was ± 15%). Prepressing of images was carried out in the software package Adobe Photoshop.

Results and discussion

Technical features of models of unilateral lacerated injury of the rat spinal cord

In mature rats, the diameter area of the spinal canal in the lower thoracic region significantly exceeds the transection area of the spinal cord. Symmetrical spread of the spinal canal cavity is usually found on the transverse sections of the spine in the lateroventral directions from the central axis [117–120]. Vertebral arches at this and other levels have a peculiar anatomy: only a small part of their dorsal surface can be easily released from adjacent muscles and removed without damaging the intervertebral joints, ie complete destruction of the arch usually requires resection of articular surfaces and for obvious reasons can lead to a decrease in spine stability, which will complicate the course of the injury. Thus, the lateral location of the fibers of the descending pathways important for locomotion in rats (primarily the reticulospinal tract (tr. Reticulospinalis) [121]), given the described anatomical feature of the spinal canal, may be the reason of their incomplete transection. That is why blind control of the completeness of the transection is an important point in the reproduction of this SCI model.

Another condition for the correct performing of a unilateral hemisection of the spinal cord is to preserve the integrity of the posterior middle artery of the spinal cord. In case of its damage a rapid increase of edema - swelling of a spinal cord at the level of intervention with the formation of a deep permanent lower paraplegia is observed. Therefore, the area of myelotomy should be limited parasagittally.

The question of the exact determination of the site of injury along the rostro-caudal axis, despite the apparent obviousness of the answer, is difficult primarily due to the age and probably intraspecific (linear) features of the skeletotopy of the rat spinal cord. Palpation of the caudal edge of the chest of a deeply anesthetized animal at the site of its fixation to the spine is usually used to select the area of injury, due to the greater mobility of the last pairs of ribs (costae fluctuantes; in rodents [86,87]), as well as without visualization of the cervical parts of ribs and places of their fixation to the spine (possibly by micropreparation with a high probability of pneumothorax) or without X-ray control allows localizing the laminectomy site approximately: in adult animals, most likely - at the level of T11-T12 vertebrae, in young animals - more often at the T11 level. Given the somewhat ambiguous literature information [88,89], it can be assumed that in our previous studies laminectomy in adult animals was performed at the level of T10 – T13 vertebrae, most often at the level of T11 – T12 vertebrae, and the injury was inflicted at the segments level of the spinal cord T12 – L2, most often at the level of T13 – L1 segments. At the same time, the nerve roots of T12, T13 segments, possibly T11, which were not specifically separated from the surface of the spinal cord, were also transected. In young animals, the body weight of which is at least 4-5 times less than that of mature animals, the skeletotopy of the spinal cord is different. It is known that in an adult human the caudal end of the spinal cord is localized at the level of the lower third of the L1 vertebra - the upper third of the L2 vertebra [122], in newborn infants - approximately one vertebra below [123,124]. In an adult rat, the caudal end of the spinal cord is found at the level between the L3 and L4 vertebrae or between the L4 and L5 vertebrae [90], in a 20-day-old rat (Sprague-Dawley, n = 1) - at the level of the L6 vertebra [91], therefore, according to the authors [91], the skeletotopic difference in the location of the planned injury should be 15–20 mm more caudal compared to adult specimens. One should also take into account the significantly lower ossification, and therefore the greater mobility and flexibility of costae fluctuantes in one month old rats, which may mean a higher location of the place of stopping the surgeon's fingers when a rigid apex of the angle between the thorax and spine is detected (level of probable T13 vertebral location). Thus, the area of spinal cord injury in a similar procedure for determining the location of the T13 vertebra in young animals will be located higher, probably by 1 segment. In our case, the probable location of the injury site in young animals was the area of T12 – T13 segments (most likely T12) and the associated nerve roots of T12 segments and possibly T11 and T13 segments.

The innervation of the rat abdominal wall muscles is generally provided by the spinal nerves T6 – L1, and its medial part, the paresis of which should be best visible, is provided by the T7 – T10 nerves [125]. Damage to the spinal nerves during the control of completeness of the transection of the half of the spinal cord while performing laminectomy in the area of vertebrae T11-T12 can not result in visible paresis of the abdominal wall muscles, since spinal nerve trunks T11-L2 are located at this level in the spinal canal [89]. Thus, paresis of the abdominal wall (under such conditions, most likely, peripheral, flaccid paresis) indicates primarily to the infliction of the injury at a level above T11, or to a significant rostral spread of secondary alteration reactions in the substance of the injured spinal cord. According to our preliminary observations, the neurological deficit in the paretic limb under such conditions, most likely, does not differ significantly from the cases of the usual localization of the injury area.

In our experience, paresis of the abdominal wall muscles significantly affects the symmetry of the body and the quality of the step locomotion of the rat. Perhaps this is also due to the expected in such conditions violation of the innervation of the colon in SCI, a decrease in the propulsion of its content, ie a tendency to overflow of it and an increase in the volume of intra-abdominal contents. In our opinion, this makes it difficult to determine the level of motor function of the paretic hindlimb.

It is known that motoneurons of the main muscles of the hindlimb of a rat are localized at the level of spinal cord segments L3 – L6 [97], therefore the presence of persistent peripheral paresis in the animal probably indicates an excessively caudalized injury or the spread of secondary alteration reactions to the main motoneuron groups of the hindlimb.

Therefore, both symptoms (expressive ipsilateral flaccid paresis of the abdominal wall or hindlimb) should be used as additional non-inclusion criteria, ie as tools for increasing the homogeneity of the experimental groups in terms of injury accuracy infliction at the optimal level for clinical verification - within segments T12-L1.

Restoration of motor function and time course of spasticity within the tested models

One week after the injury simulation according to the standardized time scale, the mean value of FI in the Sect group was (5.9 ± 1.1) points on the BBB scale ***(Fig. 3)***, the period of statistically significant increase in FI lasted first 3 weeks: when comparing the FI values after 1 week with indicators at other follow-up periods, the probability of a statistically significant difference varied from p = 0.003 to p = 0.005, when comparing the value of FI after 2 weeks with indicators at other follow-up periods - from p = 0.005 to p = 0.029 (Wilcoxon test). The output of FI values on the conditional "plateau" was observed during the first month. The actual maximum (10.1 ± 1.1) points on the BBB scale was recorded 6 weeks after injury (see ***Fig. 3***). The average value of FI at the end of the experiment was (9.5 ± 1.0) points on the BBB scale. There was no significant correlation between the FI value and the duration of follow-up during the experiment (rs = 0.45, p> 0.05).

The level of V_{FI} in the Sect group during the first week of the standardized time scale was (5.9 ± 1.1) points / week, during the second and third weeks - (1.9 ± 0.4) and (1.2 ± 0.4) points / week, respectively ***(Fig. 4)***. The value of V_{FI} in the group after 1 week differed significantly from the indicators in other follow-up periods (the range of statistical significance of the difference was from p = 0.003 to p = 0.033, here and until the end of the sentence Wilcoxon test was used), the value after 2 weeks differed from the indicators after 4 (p = 0.033), 5 (p = 0.008), 7 (p = 0.005) and 8 weeks (p = 0.005), 3 (p = 0.004), 4 (p = 0.004) and 5 months (p = 0.003), and the values after 3 weeks differed from the values after 7 (p = 0.037) and 8 weeks (p = 0.009), 3 (p = 0.037), 4 (p = 0.013) and 5 months (p = 0.022) of the experiment. Further changes in V_{FI} were insignificant (p> 0.05, Wilcoxon test): starting from the 4th week, the value of this indicator was close to zero. The average value of V_{FI} during the first 8 weeks was (1.2 ± 0.1) points / week, throughout the entire experiment - (0.9 ± 0.1) points / week.

In the Exc group, 1 week after the injury simulation according to the standardized time scale, the mean value

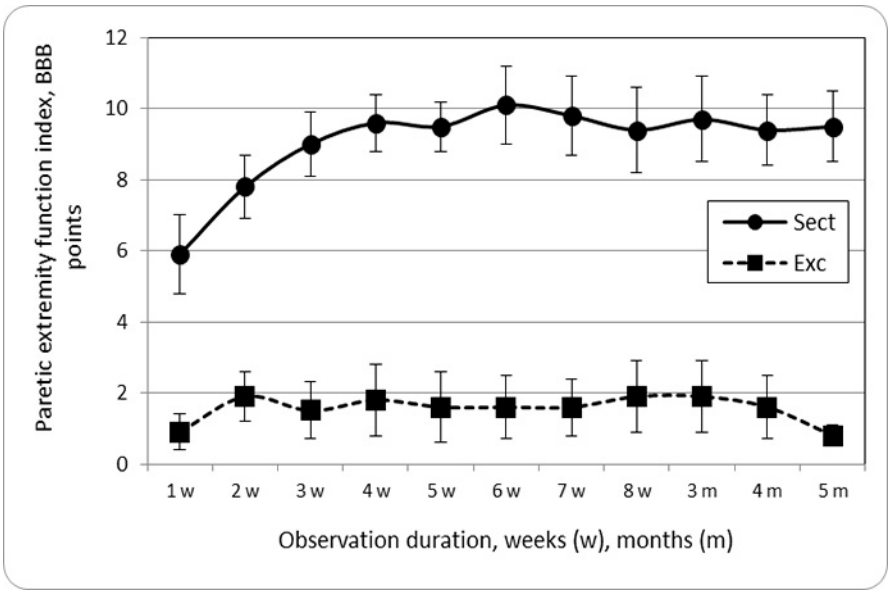

Fig. 3. The time course of motor function index in the Sect and Exc groups during the experiment, is given according to a standardized time scale. A statistically significant difference between the values in the groups was revealed in all follow-up periods (p <0.05, Mann – Whitney U-test)

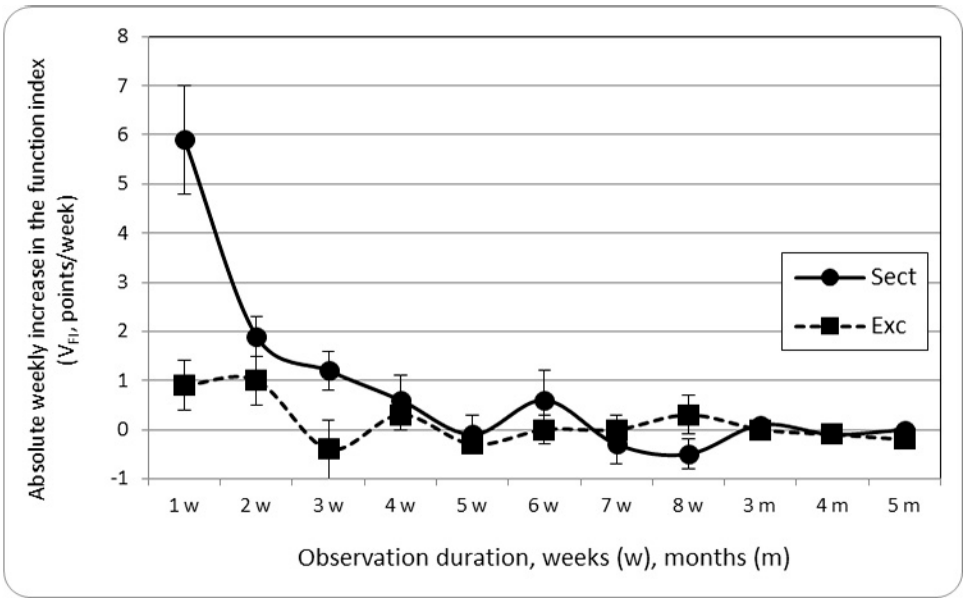

Fig. 4. The time course of absolute weekly increment in the function index (VFI) in the Sect and Exc groups. A statistically significant difference between the values in the groups was revealed only after 1 and 3 weeks of follow-up (p <0.03, Mann – Whitney U-test)

of FI was (0.9 ± 0.5) points on the BBB scale (see ***Fig. 3***), during the next week it reached the actual but statistically insignificant maximum (1.9 ± 0.7) points on the BBB scale; p = 0.105 when compared with values after 1 week and p = 0.500 when compared with values after 3 weeks, Wilcoxon test). Subsequently, the values of FI were about 2 points on the BBB scale, and during the 4-5th month it decreased to (0.8 ± 0.3) points (see ***Fig. 3***), significantly yielding to the level of the indicator at the end of the 2nd week of follow-up (p = 0.035, Wilcoxon test). As in the Sect group, during the follow-up, the mean FI value in the Exc group did not show a significant correlation with the duration of follow-up (rs = 0.02, p> 0.05).

The maximum values of V_{FI} in the Exc group were detected during the first 2 weeks of follow-up - (0.9 ± 0.5) and (1.0 ± 0.5) points / week respectively. After 3 weeks, this indicator was (0.4 ± 0.6) points / week, later it was close to zero (see **Fig. 4**). The decrease in V_{FI} value during the experiment became statistically significant only when comparing the values at 5 weeks or for the last 2 months with the indicator 1 week after injury (respectively p = 0.043, p = 0.043, p = 0.046, Wilcoxon test,). The mean V_{FI} value during the first 8 weeks of follow-up in the group was (0.2 ± 0.1) points / week, and throughout the entire experiment it was close to zero (0.1 ± 0.1) points / week).

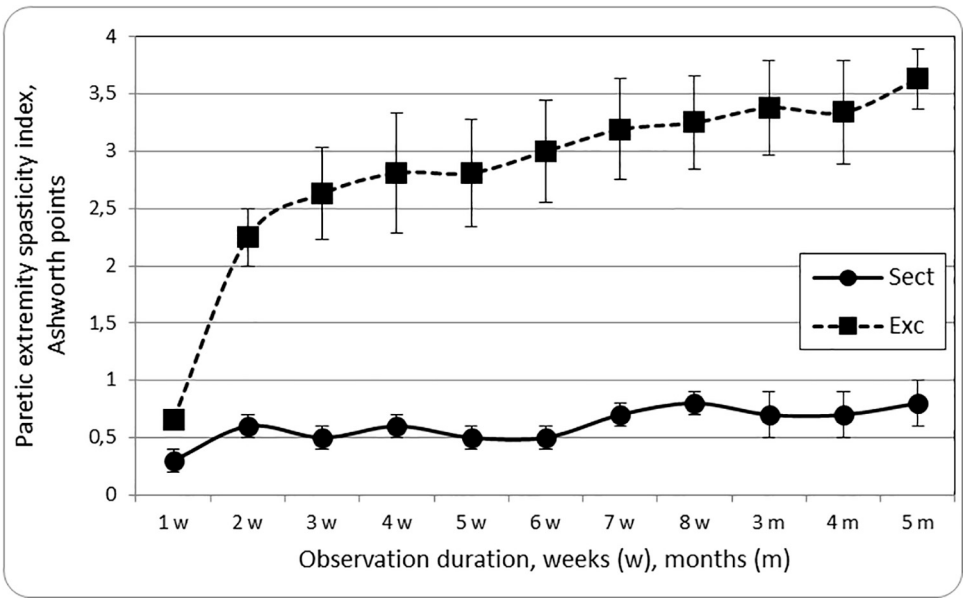

Fig. 5. Spacticity index dynamics in the Sect and Exc groups. A statistically significant difference between the values in the groups was revealed in all follow-up periods (p <0,05, Mann – Whitney U-test)

The time course of SI in the Sect group was characterized by two statistically significant extrema ***(Fig. 5)***: on 2–4 weeks (0.6 ± 0.1) points on the Ashworth scale, p = 0.017, p = 0.043, p = 0.028 at comparison the values after 2, 3 and 4 weeks, respectively , with the indicator 1 week after injury, Wilcoxon test) and after 8 weeks of the standardized time scale (0.8 ± 0.1) points on the Ashworth scale, p = 0.011, p = 0.046 , p = 0.028 when compared with the indicator, respectively, 1, 4 and 5 weeks after injury simulation, Wilcoxon test). The final value of SI was (0.8 ± 0.2) points on the Ashworth scale and was the actual maximum for the group (see ***Fig. 5***). During the follow-up, a significant positive correlation was revealed between the mean value of SI and the duration of follow-up (rs = 0.83, p <0.05).

1 week after injury simulation according to the standardized time scale, the V_{SI} value in the Sect group was (0.3 ± 0.1) points / week ***(Fig. 6)***, a statistically significant decrease in V_{SI} to zero was recorded after 3 weeks (p = 0.018 when compared with the value after 1 week). Significantly lower values of V_{SI} in the group relative to the indicators of the first 2 weeks were found after 5 weeks (relative to the value after 2 weeks, p = 0.018, here and until the end of the sentence - Wilcoxon test) and after 3-5 months (p = 0.008, p = 0.037, p = 0.017 when compared with the value after 1 week, p = 0.019, p = 0.009, p = 0.033 when compared with the value after 2 weeks). In general, the average value of V_{SI} during the first 8 weeks and throughout the entire experiment in the group was (0.1 ± 0.0) points / week.

One week after injury simulation according to the standardized time scale, the value of SI in the Exc group was (0.7 ± 0.1) points on the Ashworth scale (see ***Fig. 5***), during the next week there was a significant dramatic increase of the indicator to (2, 3 ± 0.3) points (p = 0.01 when compared with the indicator after 1 week, Wilcoxon test). Subsequently, a steady increase in SI was observed, significant - after 7 weeks (p = 0.050 when compared with the value after 2 weeks, here and until the end of the sentence - Wilcoxon test), after 3 (p = 0.043, compared with the value after 3 weeks), 4 (p = 0.043 when compared with the value after 5 weeks) and 5 months (p = 0.043 when compared with the value after 6 weeks). The final value of SI in the group was (3.6 ± 0.3) points on the Ashworth scale (see ***Fig. 5***): 6 out of 8 animals showed the maximum value of SI (4 points). Progressive increase in SI of the paretic limb in animals of this group during the experiment was also verified by the value of the correlation coefficient between the mean value of SI and the duration of follow-up (rs = 0.99, p <0.05).

The highest values of V_{SI} in the Exc group according to the standardized time scale were observed during the first 2 weeks of the experiment (see ***Fig. 6***), and a statistically significant maximum occurred in the 2nd week (1.6 ± 0.2) points / week), significantly different from the indicator after 1 week (p = 0.017, Wilcoxon test). Starting from the 3rd week, in all subsequent periods of follow-up, the values of V_{SI} in the group were significantly lower than after 2 weeks (range of probability of statistical significance of the difference – was from p = 0.012 to p = 0.025, Wilcoxon test). During the 3rd week, the value of V_{SI} was (0.4 ± 0.3) points / week, then it was close to zero (see ***Fig. 6***). Statistically significantly less than in the first week, the values of V_{SI} were recorded from the 5th week until the end of the experiment (range of probability of statistical significance of the difference was from p = 0.012 to p = 0.036, Wilcoxon test). The average value of V_{SI} during the first 8 weeks was (0.4 ± 0.1) points / week, throughout the entire experiment - (0.3 ± 0.0) points / week.

For the Sect group, no correlation was found between the mean FI and SI values throughout the entire experiment (rs = 0.19; p> 0.05). Statistically significant negative correlation of individual values of FI and SI was registered after 1 (rs = –0.64), 4 weeks (rs = –0.69),

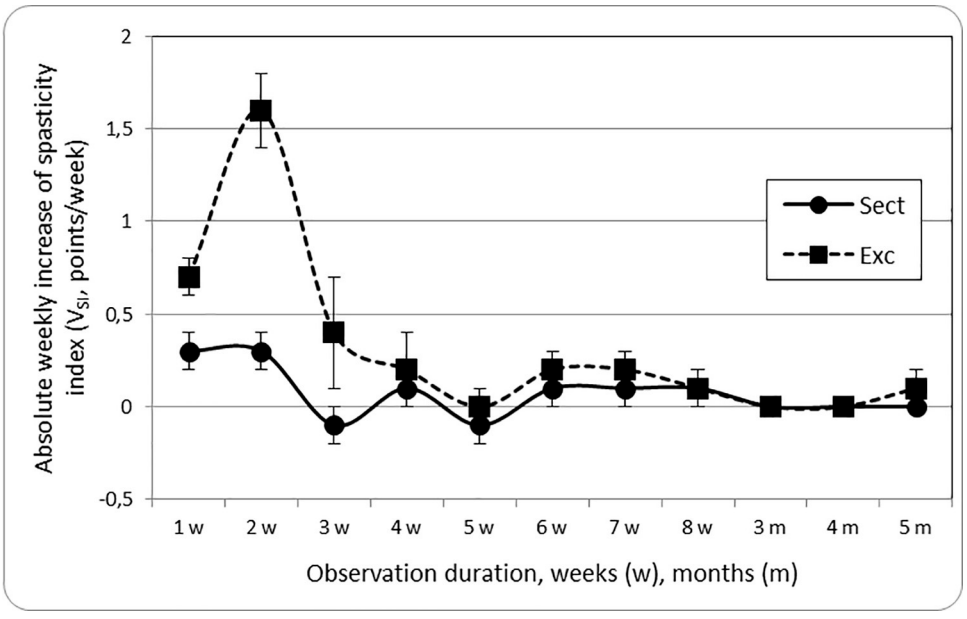

Fig. 6. Dynamics of absolute weekly increment of spasticity index (VSI) in the Sect and Exc groups. A statistically significant difference between the values in the groups was found during the first 3 weeks of the experiment (p <0.03, Mann – Whitney U-test)

3 (rs = –0.78) and 5 months (rs = –0.86) after injury (all p <0.05). A similar picture was observed in the Exc group: in the absence of correlation between the mean values of FI and SI throughout the entire experiment (rs = 0.07; p> 0.05), between individual values of FI and SI statistically significant negative correlation was found after 5 (rs) = –0.78), 7 (rs = –0.81), 8 weeks (rs = –0.81), 3 (rs = –0.92) and 4 months (rs = –0.88) after injury (all p <0.05).

When comparing the values of both FI and SI, in the Sect and Exc groups, a statistically significant difference was found in all follow-up periods (range of probability of statistical significance of the difference - from p = 0.0002 to p = 0.021, Mann – Whitney U-test). The value of V_{FI} in both groups differed significantly 1 and 3 weeks after injury (respectively p = 0,007 and p = 0,021, here and until the end of the sentence U-test Mann – Whitney), the mean value of V_{FI} in both groups - during the first 2 months and during the entire experiment (respectively p = 0.0009 and p = 0.0008), the value of V_{SI} in both groups - during the first 3 weeks of the experiment (respectively, p = 0.021, p = 0.002, p = 0.022) against the background of significant difference averaged during the first 2 months and throughout the experiment values (respectively p = 0.0005 and p = 0.0004).

Pathomorphological picture in the area of lateral spinal cord hemiexcision

For animals of the **Exc** group at least three patterns of organization of injury site are characteristic: 1) without filling the defect with scar tissue or without its strong association with the spinal cord substance ***(Fig. 7A)***, 2) with moderate filling of the defect with scar tissue and its rather stable association with the spinal cord substance ***(Fig. 7B)***, 3) with volumetric filling of the defect area with cicatricial conglomerate, but with its weak association with the substance of the spinal cord and slight segregation during micropreparation ***(Fig. 7C)***.

In the first two cases (especially see ***Fig. 7A***) symmetrical, rostral and caudal post-traumatic atrophy of the left half of the spinal cord was observed: from the site of the injury epicenter in both directions the thickness

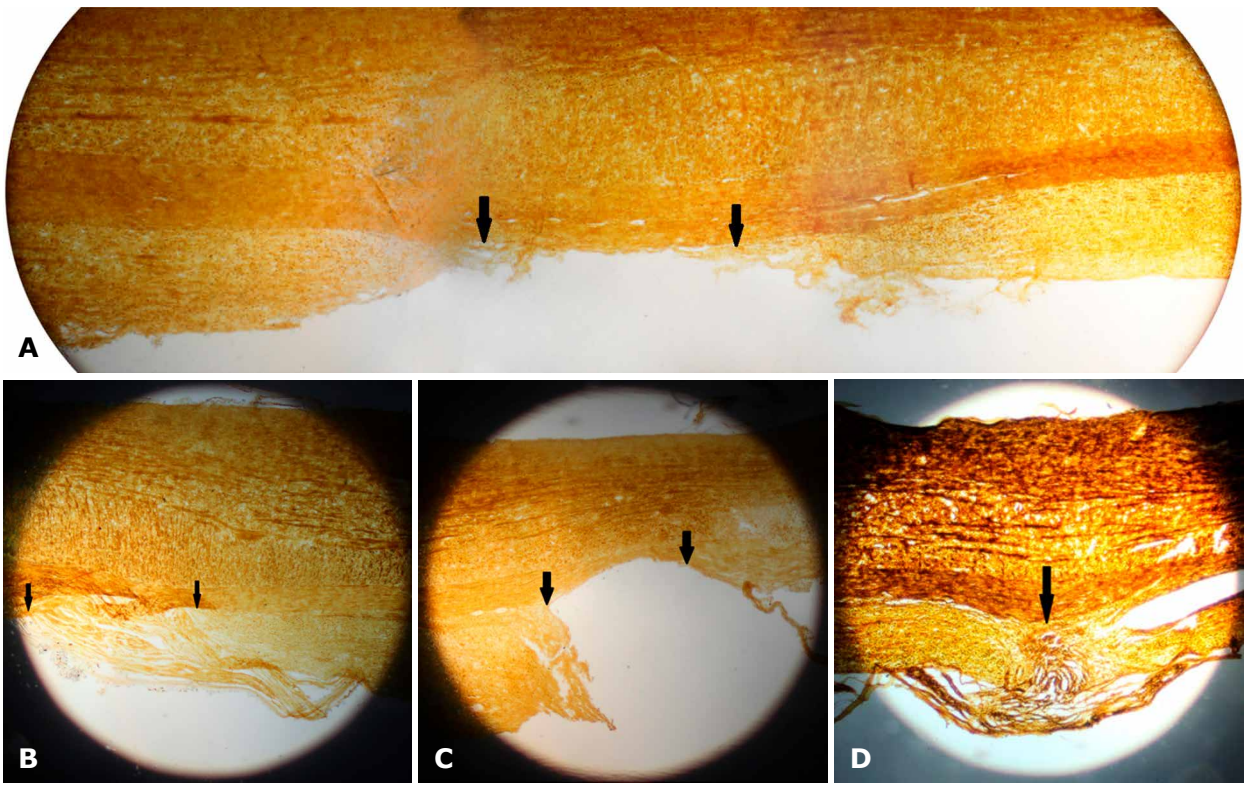

Fig. 7. Pathomorphological picture of the epicenter of spinal cord injury and organization in the long-term follow-up period: A - view of the area of excision of the fragment of the spinal cord substance (Exc group) 5 months after the simulation of the injury, panoramic reconstruction of the fragment, made from three consecutive images; B - a variant of the organization of the area of lateral spinal cord hemiexcision (Exc group) 5 months after the simulation of injury with the filling of the defect area with connective tissue associated with the spinal cord membrane; C – an example of the organization of the site of excision of a fragment of spinal cord substance (Exc group) 5 months after the simulation of injury, probably after complete segregation of a dense conglomerate of scar tissue within the defect area during micropreparation. The caudal part of the ipsilateral half of the spinal cord section (on the right) is less thick than the rostral and contralateral part, dissociated during the section and did not get into the field of view; D - view of the lateral hemisection of the spinal cord, similar to that performed in the Sect group, but performed in an adult animal, about 7 months after injury: FI at the time of euthanasia was 2 points on the BBB scale, SI - 2 points on the Ashworth scale. In all cases, impregnation with silver. Microphotograms are placed taking into account the most probable location of the rostral (left) and caudal (right) part of the spinal cord. Magnification within each circular field of view, in particular within the panoramic reconstruction - 50 times. A, B, C - arrows cover the approximate rostro-caudal location of the excision zone; D - arrow indicates the area of hemisection

of the substance of the injured half of the spinal cord slowly increased. In our opinion, the epicenter of the injury should be considered a flat area, the bottom of which on longitudinal sections rests on an expressive wide strand of central, parallel to the main axis of the spinal cord thick nerve fibers (see ***Fig. 7A***). This strand, most likely should be correlated with the corticospinal tract, the main part of which in a rat, as is known [121, 126], lies paramedian and in the dorso-ventral dimension - near the central canal. If this assumption is correct, then we can state the absence of visual signs of transection of the part of this path, at least in the case shown in ***Fig. 7A***. Despite this, in the studied animal, the values of FI and SI at the time of injury were 0 points on the BBB scale and 4 points on the Ashworth scale respectively. The interpretation of this discrepancy may be a much more modest role of the rat corticospinal tract in locomotion [121]. It is most likely that in this case the connective tissue membrane, which should have covered the area of the spinal cord wound, was separated at the stage of micropreparation.

In the second case (see ***Fig. 7B***; the FI and SI values of the animal at the time of injury were 0.5 points on the BBB scale and 4 points on the Ashworth scale, respectively) changes in the thickness of the transected part of the spinal cord along the rostro-caudal axis are similar, but the epicenter of injury filled with a node of dense multilayered connective tissue conglomerate, which rostrally and caudally extends into the thickened membrane and does not compensate for the deficit of spinal cord volume in the focus of injury.

In the third case (see Fig. ***7C***; the FI and SI values of the animal at the time of injury were 2 points on the BBB scale and 3 points on the Ashworth scale, respectively), the cicatricial conglomerate was accidentally and easily detached from the spinal cord defect bed during micropreparation. The presented histological section clearly shows that only a quarter of the middle band of the longitudinal fibers of the wound bottom remained conditionally intact. In another longitudinal section (not shown) obtained from the same animal, the central strand of longitudinal fibers in the area of injury is absent, but immediately rostral (clearly) and caudally (moderately) is present. Despite this, during the experiment, severe disorders of motor function of the posterior contralateral limb were not detected in the animal.

When comparing the described options for organizing the area of hemiexcision of lateral fragment of the spinal cord with the area of organization after lateral hemisection of the spinal cord of an adult rat ***(Fig. 7D)***, the differences are obvious: in case of hemiexcision the probability of autogenous sprouting of fibers through the area of posttraumatic organization is significantly lower (if at all possible) than in case of a hemisection.

Discussion

The search for effective means of restorative treatment of SCI revealed a typical biomedical problem - frequent unsatisfactory clinical transmission of observation data [48,58,67]. The reason for this situation is seen, among other things, in the irrelevance of the used models of the studied pathological processes [58,67–70]. For example, the most common pathomorphological form of SCI in clinical settings is contusion [58,69,70,127], in which the use of solid macroscopic grafts will be possible only when their restorative effect is guaranteed to exceed the most successful autogenous spinal cord regeneration in the presence of post-traumatic scar which will allow bringing up the question of the validity of its removal and the formation of a bed for a bioengineered graft. That is why laceration models of SCI remain key for studying the effectiveness of neural transplantal interventions [58]. In this case, the modeling of the complete transection of the spinal cord, despite the technical simplicity [58], is associated with the obvious difficulties of postoperative care of experimental animals. Thus, the model of unilateral hemisection (UHS), or lateral hemisection of the spinal cord, is optimal in terms of domestic financial restrictions and taking into account the ethical requirements. Moreover, it is widespread and well studied in experimental neurosurgery.

However, the most paradoxical given the well-known clinical concepts and experimentally annoying feature of the UHS of the spinal cord of the rat is the rapid and significant autogenous restoration of the motor function of the paretic limb [58]. C.D. Mills et al. (2001) [62] note that when performing UHS at the T13 level (young male rats, body weight - 100–125 g), the function of the ipsilateral hindlimb on the 35th day was (15.0 ± 0.4) points on the BBB scale (Long – Evans line), (15.6 ± 1.6) points (Wistar line) and (17.0 ± 1.2) points (Sprague – Dawley line). The contralateral hindlimb in animals involved in the calculation group was considered intact from the point of view of the locomotion. The data obtained by other authors agree with these results [63,64,66]. Similar time course of autogenous restoration of motor function is found for dorsal hemisection of the spinal cord of a rat [121]. It is also known that in case of damage to the corticospinal tract, recovery up to 13 points occurs within a week, and the rubrospinal tract - within 7 weeks after injury [121]. Damage to other descending tracts is accompanied by neurological deficits, which significantly regress within 1–4 weeks [121], with the exception of destruction of the reticulospinal tract [121]. This pathway in rats is located in the ventrolateral areas of the white matter of the spinal cord and plays a leading role in the implementation of step locomotion [121]. In particular, the preservation of even <5% of the white matter area of the ventrolateral bundle at the T9 – T11 level is a prerequisite for a significant restoration of locomotor function of the hindlimb ([121], with reference to [128]). By the way, in case of modeling UHS in an adult rat at the C4 – C5 level, the ipsilateral forelimb experiences an irreversible deep motor deficit, which is probably associated with the destruction of motoneurons m. spinodeltoideus, m. biceps brachii, m. extensor pollicis longus and m. extensor carpi radialis longus, located at a similar level [65]. FI deficiency of the ipsilateral hindlimb in the same model is moderate and significantly regresses during the first week of the follow-up.

It is doubtful that these examples of rapid recovery of the function of the hindlimb of a rat after UHS are the result of compensatory rearrangement of intraspinal connections or growth of descending axonal endings *de novo*, since, in our opinion, both components of the regeneration process are characterized by a significantly longer duration. For example, sprouting of corticospinal tract fibers in adult animals after its transection is registered not earlier than in 2 weeks [101, p. 428–429]. Given the absence of neurological deficits

in the contralateral limbs in UHS, it can theoretically be assumed that a number of these studies reproduce a variant of injury in which a temporary reverse dysfunction of some main descending pathways, and the field of distribution of secondary alteration reactions does not exceed the limit of the damaged half of the spinal cord diameter. Probably, when performing UHS, the most laterally and ventrally located fibers (primarily of the reticulospinal tract) experienced only a secondary, demyelinating lesion.

An alternative position involves the absence of a doubt about the completeness of transection of ipsilateral part of the spinal cord whereas the main reason for the decrease in the success of the process of autoregeneration in UHS sees damage to the contralateral part of the spinal cord, due to the interneuronal and fibrous apparatus of which, most likely, is the establishment of supraspinal effects on denervated ipsilateral motoneurons [101, p. 427–428]. For example, if we ignore the extremely low quantitative characteristics of the experimental groups and the difference in the localization of the lesion, according to A.A. Webb and G.D. Muir (2002) [63], on the 40th day after performing UHS (n = 5) at the level of the C3 vertebra without concomitant morphological signs of damage to the contralateral part of the spinal cord in adult female rats the FI of ipsilateral hindlimb was 21 points on the BBB scale, and after performing UHS at the level of T9 – T10 vertebrae (n = 4) with morphological signs of paramedian damage to the opposite part of the spinal cord was only 15 points. V.L. Arvanian et al. (2009) [64] as of the 42nd day after UHS at the level of the spinal cord segment T10 recorded the value of FI of the ipsilateral hindlimb 13–14 points on the BBB scale, and the contralateral part of the spinal cord was most likely also damaged since FI of the contralateral hindlimb was only 15-16 points, increasing to this level during the first week from a value of 5 points. Undoubtedly, in this interpretive paradigm, the lesion of the contralateral part of the spinal cord at the level of UHS is secondary or as a result of vascular damage with contralateral spread of vascularization fields, therefore the features of immune and local tissue reactivity of the spinal cord of animals, which probably depends on a wide range of factors may play a major role in the recovery process. Note also that in our opinion, low values of FI of the contralateral hindlimb 40 days after injury can be attributed to the irrelevance of the BBB scale against the background of the lateral hemisection (see below).

Analysis of the primary material from our previous studies [78–85] found (unpublished data) that when simulating an isolated trauma in adult animals similar to that reproduced in the Sect group of animals, in almost every third case distinct signs of damage to the contralateral part of the spinal cord were observed and in almost every fifth - signs of peripheral paresis, which indicates either too caudal level of performance of UHS (see above) or a significant caudal spread of the damaging inflammatory process. The FI values of the ipsilateral hindlimb of a rat in these observations were close to the FI values of the hindlimbs of classically close experimental animals after modeling the complete transection of the spinal cord [61].

It is known that lateral hemiexcision of the spinal cord substance of the rat is generally accompanied by a generally limited autogenous restoration of motor function of the paretic limb. For example, R. Jian et al. (2015) [129] after modeling the lateral hemiexcision of the spinal cord in adult female rats at the T8 level of 3 mm in length after 8 weeks, the FI value of the ipsilateral hindlimb was about 6 points on the BBB scale (the presence of bilateral deficiency in animals from the publication could not be clarified). In the study by Q. Zhang et al. (2016) [115], performed on mature male rats (Sprague-Dawley line, body weight - 180–200 g), after hemiexcision of the fragment of the spinal cord 2 mm long at the level of T9 – T10 vertebrae, the results are close to our previous data obtained using the UHS model (see, for example, [78–85]): the FI of the ipsilateral hindlimb on the 70th day after injury simulation averaged 4.8 points on the BBB scale. Note that from the photos given by the authors in ***Fig. 4A – D***, it can be concluded that there is a deficit of motor function of the posterior contralateral limb of the studied animals on the 30th day of follow-up. V. Pertici et al. (2013) [114] in adult animals (Sprague-Dawley line, 8 months, body weight - 300 g, n = 15) for unilateral 1-millimeter excision of the spinal cord at the T10 level after 14 weeks the value of FI of ipsilateral hindlimb was recorded (8.66 ± 1.25) points on the BBB scale, without reporting the state of motor function of the contralateral hindlimb. Instead, T.H. Hsieh et al. (2010) [113] observed autogenous restoration of motor function of the ipsilateral hindlimb up to 11 (!) points on the BBB scale 55 days after lateral hemiexcision of the spinal cord (1-2 mm long) in adult male rats at the level of T7 – T9 vertebrae (at the level of the T8 segment of the spinal cord) against the background of a pronounced contralateral motor deficit: the next day after injury, the function of the contralateral hindlimb was assessed by 3 points on the BBB scale, on the 56th day - 15 points.

In the context of the data presented, it can be assumed that in previous studies [78–85] we performed a more severe version of UHS. Perhaps this is due to an increase in the incidence of damage to the contralateral half of the spinal cord or expansion of the trauma zone along the rostro-caudal axis during control of the completeness of the transection performed with ophthalmic tweezers inserted into the wound of the spinal cord curved along the rib with closed branches, which is often wrapped in medical gauze. It should be noted that we are not original in terms of involving an additional surgical means of monitoring the completeness of transection of the white matter pathways when modeling laceration SCI, [61].

From a surgical point of view, more limited autogenous restoration of motor function in lateral hemiexcision of the fragment of the spinal cord can be interpreted in the context of completeness of UHS performance: during excision in contrast to the usual hemisection, a wider field of view of epicenter of trauma is formed, that facilitates the detection of the remnants of the non-transected spinal cord substance. If we discard the suspicion about incomplete transection of the ipsilateral pathways of the spinal cord, then what can be the mechanisms described by most authors of the dramatic autogenous restoration of motor function of the paretic limb after UHS?

In our opinion, the most likely explanation is the establishment of polysynaptic pathways of descending transmission of nerve impulses «bypassing» the area of transection due to contralateral propriospinal

interneurons [57]. Since such a mechanism completely depends on the length of the damage zone (in the lateral excision of the fragment in contrast to the UHS the autogenous restoration is negligible) it can be assumed that short-axon interneurons play a leading role in its implementation [57], the length of the processes of which is sufficient to provide a «bypass». It is not difficult to guess what this length should be, estimating the rostro-caudal size of the scar after the UHS (see ***Fig. 7D***). We assume that short-axon interneurons with a small rostro-caudal proliferation of proceses in the spinal cord are much greater than long-axon interneurons, which could reproduce the «bypass» for excitation transmission after excision of a large rostro-caudal fragment of the lateral half of the spinal cord. In addition, within the framework of this model, the interpretation of the dependence of the success of autogenous restoration of motor function of the ipsilateral hindlimb on the state of the contralateral part of the spinal cord is obvious.

Despite the proportionally larger size of neuronal processes in humans compared to rats, we assume that in this case the area of human SCI of the same relative size as the UHS in a rat is too large to form an alternative pathway by population of short-axon interneurons of the spinal cord. Although the time course of the recovery process after UHS in humans is hardly known. Probably, such cases of knife injury or fragmentary half-damage are worth demonstrating as an interesting incident, but not involving in the cohort of a statistical analysis. Taking into account the equally significant autogenous restoration of the motor function of the hindlimbs in a rat against the background of contusion SCI characteristic of humans, it is likely the above assumption regarding the cause of the interspecies difference in spontaneous recovery after this type of injury is correct.

Whether the population of short-axon interneurons is really much greater than the population of long-axon spinal cord interneurons, and whether the described mechanism of establishing an alternative pathway of transmission of descending influences «bypassing» the area of injury is key in autogenous restoration of motor function after UHS, remains to be clarified in the future.

Technical limitations of the study

The BBB scale used by us to account for the motor function of the paretic limb was developed to study the consequences of bilateral, for example, spinal cord contusion injury [59]. Despite the widespread use of the scale for monitoring the motor function of the hindlimb in unilateral lesions [62–64,66,94,113–115,130], its verification potential in such conditions, in our opinion, is limited. Firstly, the accurate determination of FI within 0–7 and 10–14 points is impossible without kinematic, and in case of 15–18 points - also audiological analysis of video-recorded material. Secondly, according to our observations, animals with unilateral spinal cord injury due to satisfactory function of the contralateral limb, can «put» the paretic limb with a function on the level of 8 points foot downward, «squatting» on it, which significantly complicates the differentiation with the clinical picture which is characteristic for 9 points on the BBB scale. Thirdly, in some cases, with sufficient spastic fixation of the ankle and / or knee joint against the background of satisfactory motor activity of the contralateral limb, the animal can use the paretic limb in the «crutch» mode, resting on it, although when assessing locomotor mobility in the joints the level of FI of such a limb is likely to be lower to 10 points on the BBB scale (perhaps, partly, this type of locomotion is covered by one of the signs of 9 points). Fourthly, there is no doubt that the way the animal moves against the background of deep paresis of one of the hindlimbs is compensatory transformed, for example, into «tripedal» locomotion, which is characterized by the transfer of the posterior part of the body by a «jump», with a support on conditionally intact limb. At the same time, the deficit of supporting the mass of the posterior part of the body will in fact remain undetectable: the partial failure of this function on the part of the paretic limb will be compensated by the contralateral limb with a corresponding gait deformity. In this case, anteroposterior desynchronization of motor patterns will express the compensatory mechanism of supporting the posterior half of the body above the surface of locomotion, rather than the discoordination of the rostral and caudal part of the locomotor pattern generator of the spinal cord associated with post-traumatic deficiency of connections between these parts. Despite this, in order to accurately determine the FI of the paretic limb at the level of 10–14 points on the BBB scale, it is proposed to differentially assess the ability to support the corresponding posterior quadrant of the body above the movement surface and the consistency of its locomotor movements with the movements of the forelimbs. Compensatory convergence of the vertical of the center of mass of the posterior part of the animal's body and the vertical, performed through the conditional center of support of the «intact» hindlimb can theoretically change the position of the feet of both hindlimbs on the locomotion surface and the completeness of toe clearance of the "intact" limb from the surface of movement during stepping phase of transfer, making it difficult to determine the level of function within 14-18 points. Under such conditions, the correct accounting of values of motor function of the conditionally intact hindlimb at the level of 19-21 points (holding the tail above the surface of movement and ensuring locomotor translateral stability of the torso) is generally impossible, since the implementation of these signs completely depends on satisfactory function of both limbs.

In general, it is obvious that the determining capacity of the BBB scale at the level of> 8 points against the background of unilateral spinal cord injury is limited. The negative consequences of this shortcoming at the present stage of research can be partially compensated for only by following the same methodological and technical conditions for assessing the FI in all compared experimental groups. Perhaps, in this regard, the motor function of the posterior contralateral limb injury site is neglected in most studies on the UHS model.

To differentiate the level of FI 16 and 17 points, during the entire time of using the UHS model ([77-85,93,98-112,141-144], etc.) we accidentally used our own criterion - positioning the foot relative to the rostro-caudal axis when raising sharply the posterior part of the animal's body by the tail and its immediately returning to its previous position without raising of the forelimbs from the horizontal surface. Parallel positioning of the feet relative to the axis when raising and in contact with the surface was regarded as a sign of 17 points,

the rotation of the foot at raising from the surface - as a sign of 16 points. Differentiation of these two values within the original protocol, according to its authors, is problematic [59]. The uncertainty of the situation with the use of a differential sign is evidenced by the fact that in the original work [59] and in other works where this scale is used, for example, J. Šedý et al. (2008) [131], at the level of 15 points the positioning of the foot is described only for the beginning of the static phase of the stride cycle, and in one of the important methodological works on this topic [132] the manifestation of the sign at the level of 15 to 19 points differs significantly from that given in the original scale [59]. G.A.S. Metz et al. (2000) [94] in general propose to evaluate the positioning of the foot and toe clearance from the surface of the step movement separately.

It was also found that the estimated ability of the BBB scale depends on the severity of the spinal cord injury in rats (Wistar line, male rats, biometric indicators are not given): T.E.P. de Barros Filho and A.E.I.S. Molina (2008) [60] note that "The Basso, Beattie, Bresnahan scale showed high reproducibility and satisfactory sensitivity for identifying mild injuries; satisfactory reproducibility and non-satisfactory sensitivity for moderate injuries; and reduced reproducibility and non-satisfactory sensitivity for severe injuries ".

The Ashworth scale is also not free from methodological shortcomings. First of all, there is the difficulty of verifying mild spastic paresis, ie the differentiation of «voluntary» and «involuntary» components of muscle tone. The «relaxation» ability of such techniques as «blinding» the animal by the experimentor's hand and previous acclimatization [95, 133], the possibility of a significant reduction with their help of the «involuntary» muscle tone of the joint under study, in our opinion, is doubtful. Therefore, we used the original clinical symptoms of verification of mild paresis (see above). Another difficulty of the scale is the subjective differentiation of SI levels from 1 to 3 points. The differential criterion of spasticity at the level of 1 point - 'catch'-phenomenon, we observed extremely rarely. With significant difficulty of passive movement in the joint (3 points), we often noted a certain limitation of the range of motion in it in non-algogenic effort. Finally, 4 points were recorded in case of significant contracture, which as is known to be not identical to the complete exclusion of passive movements in the joint: if passive movement in full or close to full volume is impossible for any reasonable effort (within the limits which are determined in particular by the animal's pain response), then the SI was recorded at the level of 4 points, if more or less complete passive mobility was realized with a significant but non-algogenic effort of the experimentor, then the SI was recorded at the level of 3 points. In addition, when working with experimental animals, the main sign of spasticity is the dependence of muscle resistance force on the rate of its stretching [11] due to the size of the animal and permanent motor activity is almost impossible to identify, especially at the level of the knee or hip joint. Therefore, only the force of muscle resistance to stretching remains the verifier at a relatively low and more or less the same rate throughout the experiment. Despite this, the choice of verifying the spasticity of the Ashworth scale is due to the limited information content of a technically much more complex alternative instrument -electroneuromyography (ENMG). It is known that there is a weak positive correlation between the level of spasticity assessed by the Ashworth scale and the ratio of the H-wave and M-wave amplitudes (N / M) measured using ENMG (r=0.51, p=0.03) [95], and the mean index of interindividual and temporal (in each individual animal) variability (mean coefficients of variability) of the N/M ratio under experimental conditions is about 21% (p <0,05) and about 13%, respectively, moreover the the correlation coefficient between measurements is 0.59 (p <0.1) [134]. The limited accuracy of ENMG is due to several technical aspects, for example, the impossibility of detecting in small experimental animals the so-called motor point of the muscle - the area of entry of motor nerve trunks into the muscle [135] or the place where the threshold of muscle excitability during point electrical stimulation is smallest [136] or muscle contraction is greatest at the minimum intensity of electrical stimulation [135], in particular due to direct electrophysiological excitation in such conditions of motor nerve branching [137]. In our opinion, this is especially important given the decomposition and change in the fiber composition of the paretic muscle against the background of SCI [138] and the small size of the studied rat muscles.

To a certain extent, the statistical significance of the data presented in this article decreases the variability of the actual observation period in groups and subgroups (see above). In our opinion, this factor is of greatest importance during the first month of the recovery process, in particular for the interpretation of the values of V_{FI} and V_{SI}. Despite this, the dramatic difference between FI and SI in the study groups indicates a sufficient reliability of the overall conclusions of the study.

Another methodological drawback of our work is the keeping of animals of some subgroups in cages of different sizes (see above). However, it should be noted that there is no statistically significant difference between the values of both FI and SI, when comparing them in animals that were kept in cages of various sizes aginst the background of neuroengineering interventions after modeling UHS [93]. On the other hand, differences in the course of the recovery process under such conditions could be detected only by means of complex analysis, which is reflected in a rather careful formulation «limiting the spontaneous locomotor activity of the animal under conditions of tissue-engineering restorative intervention after a spinal injury complicates the course of the regeneration process, accelerates the formation of a stable syndrome of spasticity» (highlighted by us – V.M.) [93].

Another factor worth paying attention to when organizing an experimental study using models of partial laceration damage to the spinal cord (as well as contusion [139, 140]) is a certain dependence of the result of autogenous restoration of motor function on the line of animals involved [62]. In particular, this may explain the data obtained by us on the efficiency of implantation in the UHS area of neurogenic or mesenchymal stem cells associated with a hydrogel based on N-(2-hydroxypropyl) methacrylamide [93,104,106,108-111,141-144], or, for example, were primarily interpreted by us as a manifestation gender mismatch between donor and recipient; differences in the effect of some types of neural transplantation of this type [109,111].

Numerous data indicate that the maximum activity of the autogenous neuroplastic process occurs in the first weeks after SCI [48]. However, when choosing the time period for restorative intervention, one should also take into account the time course of local inflammatory reactions [58,145–147], which are inevitably accompanied by an increase in the concentration of cytokines and other chemical factors affecting the state of transplanted cells. Infiltration of the epicenter of the injury by neutrophils reaches a maximum on the 3rd day after SCI, and by vascular macrophages, whose role in the development of further inflammatory process is undoubted [58] - in the second half of the first week [145]. According to other data [58], the first phase of inflammation in SCI (up to two days after injury) is the activation of resident microglia cells, astrocytes and the involvement of circulating neutrophils, the next phase is in the attraction of macrophages, B- and T lymphocytes from the bloodstream to the focus. In our opinion, taking into account these data, the choice of the the scheme of their immediate use for verifying the effectiveness of restorative interventions after modeling the laceration spinal cord injury is fully justified.

Conclusions

The model we have tested for the the spinal cord lateral hemiexcision in the lower thoracic region in young animals allows reproducing a deep unilateral motor deficit, which at least within the above data is not accompanied by high mortality or adverse neurological disorders, and therefore the model is promising for approbation solid neural transplantation means of restorative treatment of SCI.

Interpretation of the data on the restoration of motor function of the paretic hindlimb, obtained using the BBB scale, in models of lateral half spinal cord injury should be carried out with caution, especially at values of FI> 8 points.

The methodology and means of verifying the spasticity syndrome on SCI models require significant improvement, possibly involving chronic *in vivo* ENMG monitoring [148,149] or recording electrical and dynamic parameters of muscle function [113] in sedated animals without deep muscle relaxation and anesthesia.

Taking into account our own experience and results obtained by other authors [62,64,66,113-115], the points of the optimal time scale for detailed monitoring of the motor function of the paretic limb against the background of lateral half spinal cord injury, we suggest considering 7, 14 days, 1, 2, 3, 4, 5, 6 and 7 months after injury simulation.

Disclosure

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving experimental animal are in accordance with ethical standards and approved by the ethics committee of the scientific institution where the study was conducted.

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