DTI-MRI at 2 days after spinal cord injury accurately predicts long-term locomotor function recovery in rats

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Abstract. – OBJECTIVE: Spinal cord injury (SCI) damages an individual's sensory, motor, and autonomic functions and represents a social emergency, mostly in developed countries. Accurate and timely diagnosis of the severity of SCI must be carried out as quickly as possible to allow time for drug and therapy testing in the early stages after injury.

MATERIALS AND METHODS: Male Dark Agouti (DA) rats underwent spinal cord cryo-injury at the T13 level of the spine. Under typical conditions, *in vivo* magnetic resonance imaging (MRI) T2 and echo-planar imaging - diffusion tensor imaging (EPI-DTI) examinations were conducted. This involved the reconstruction of nerve tracts and the measurement of the fractional anisotropy (FA) index, as well as measurements of the ratio of Hyper/Hypo intensive areas and spinal cord injury severity scores.

RESULTS: Our study shows that, after cryo-injury, the FA significantly decreased in all animals. An increase in FA level, derived from EPI-DTI within 2 days after SCI, accurately predicts long-term locomotor function recovery. In rats with higher FA, recorded on day 2 after injury, complete restoration of locomotor function was observed, while at low FA values, the animals maintained stable monoplegia.

CONCLUSIONS: Our results, though validating the T2 10-grade MRI scale for SCI, indicate that FA would represent the MRI technical instrument, which would better monitor the evolution of SCI and, accordingly, better objectively evaluate the impact of potentially therapeutic protocols for spinal cord traumatic injury. Despite the results achieved, significant difficulties must be overcome on the way to successful clinical implementation of the findings in humans.

Introduction

The need for a specialized technical instrument to monitor secondary injury following SCI has been acknowledged for at least 20 years[1]. The complex biological cascade of events leading to secondary pathological reactions following a spinal traumatic injury is still to be elucidated, although significant progress has been made in understanding the biomolecular mechanisms of this process[2-4]. Still, a convenient, practical way to monitor the evolution of SCI and also to validate the efficacy of possible therapeutic protocols has not been found yet. Magnetic resonance imaging (MRI) is universally recognized as having this potential capacity, and many papers, following the pioneering work of Falconer et al[1], have described MRI protocols to be used for these purposes[5-9].

In a previous paper[2], we proposed a 10-grade scale for objectively evaluating the evolution of spinal cord damage following experimental cryo-injury based on the calculation of the ratio between hypo and hyper-intense areas of the lesioned spinal cord using conventional T2 sequences. The scale has been demonstrated to correlate well with the clinical and histological results sequentially observed in traumatized animals.

Diffusion tensor imaging (DTI) is a relatively new technique that has been introduced recently

that measures the diffusiveness of water molecules along the axons. In this manner, it can reliably show the actual influx of physiological bioagents along the axons and can, in this way, prove their anatomical-physiological integrity[10,11]. It has been recently recognized as a very reliable instrument for evaluating and monitoring the consequences of SCI, as well as a prognostic indicator for neuronal functional recovery[7].

In the present study, we compared the results of these two MRI imaging techniques by evaluating the experimental animals at sequential intervals. After spinal cryoinjury, we measured the severity of spinal cord injury by T2-weighted sequences on day 2, 7, and 30, using FA and score.

Materials and Methods

Laboratory Animals

Dark Agouti (DA) male rats, weighing approximately 263±16 g, were used for the present study. All animals were housed under standard conditions in the Animal Breeding Facility of BIBCh, RAS (the Unique Research Unit Bio-Model of the IBCh, RAS; the Bioresource Collection - Collection of SPF-Laboratory Rodents for Fundamental, Biomedical and Pharmacological Studies, N° 075-15-2021-1067). The study was approved by the IACUC of the BIBCh RAS (protocol No. 913/22 of 22.12.22).

Surgical Approach to Spinal Trauma

Details of the experimental technique have been fully provided in previous publications[3,12] and will be repeated here shortly. Briefly, animals were anesthetized with 3% isoflurane to keep cardio-respiratory function and body temperature constant. Then, when an incision was performed in the lumbar midline, the T13 spinous process was carefully removed, and the underlying spinal dura was exposed under a table operating microscope (Optica, Ponteranica, Italy). Unilateral hemilaminectomy was performed using a 1 mm diamond burr. Then, with a copper cryo-conductor at -20°C, controlled spinal cord cryoinjury was performed in all groups of animals by local application for one minute on the right half of the spinal cord. A correctly performed procedure led to the development of monoplegia in the right hind paw in rats. All surgical manipulations were completed under aseptic conditions. Following careful wound closure, the animals were allowed to recover.

Magnetic Resonance Imaging

MRI studies were performed at the following intervals: 2, 7, and 30 days following the experimental procedure. Examinations were performed using a 7 Tesla MRS*DRYMAG 7017 PW machine (MR solutions, Guildford, UK) equipped with the radio frequency surface coil ASSY-RSA-2CH 7T (MR solutions, Guildford, UK). Animals were anesthetized with 3% isoflurane and fully monitored. They were placed in a ventral position in the thermos-controlled pad in order to maintain the body temperature at +37°C.

The following parameters were utilized for the study: EPI-DTI protocol; 67 DWI vectors; flip angle 90 degrees; b-values 0 and 700; TE: 27 msec. 35 axial slices 0.5 mm wide were obtained centered at the T13 level, with a 60 mm isotropic FOV, fat saturation, and read interleave programs. Nyquist ghost correction, frequency, drift, correction, and vertical motion filter programs were used for basic artifacts.

We also obtained a standard T2-weighted image with 0.5 mm slices without gaps, which were oriented in the axial plane. The FOV used was 60 mm (rectangular).

The volume of hyper/hypo intensive areas ratio measurements and spinal cord injury severity scores were performed as previously described[2]. After acquisition, the data were extracted and converted in a neuroimaging informatics technology initiative (NIFTI) format and analyzed by using a DSI Studio 0.59 for Windows software (Pittsburgh, PA, USA). To this purpose, ROIs were obtained by manual segmentation using masks to exclude soft tissues and bone elements. FA was measured as basic data on each individual animal by calculating the mean value of several rows of data.

Preliminary image preparation was performed with manual segmentation and formation of a mask of the spinal cord substance (radicles excluded) and the exclusion of other tissues from quantitative analysis. Next, using manual segmentation, regions of interest (ROIs) were formed for the volumes of the left (green) and right (red) halves of the spinal cord. Under standard conditions, reconstruction of the nerve tracts and measurement of the FA index were performed at 72 points along the main axis of the spinal cord separately for each half of it. The obtained data was exported in the form of arrays and graphs.

Statistical Analysis

The statistical analysis of the total SCI severity score and fractional anisotropy was performed

using SPSS Statistics 28 software (IBM Corp., Armonk, NY, USA). Using the Mann-Whitney U test, *p*-values lower than 0.05 were considered significant.

Results

For each rat, we analyzed the time course of the hyper/hypo ratio, total SCI severity score, and fractional anisotropy based on MRI results. Our data suggest that mean values of the hyper/hypo ratio in groups have varied greatly in different animals after injury (Table I), despite the fact that all rats had monoplegia.

The varying degrees of development of post-traumatic edema explain the differences ob-

served in the animals. Some animals (No. 1 and No. 4) had edema values exceeding 2,000, while others (No. 3 and No. 6) had much lower values. See Table I for more details. The hyper/hypo ratio did not correlate with the clinical picture.

The T2-weighted MRI images acquired 2 days after the injury demonstrated distinct hyperintense foci of craniocaudally elongated shape, indicating post-traumatic edema (Figure 1). The hyperintense area was linked latero-dorsally with the dura mater. Hypointense areas were observed only in rats with stable monoplegia throughout the experiment (Figure 1). Subsequently, by day 7, the hyperintense area gradually decreased in volume in all animals.

As we have established a normal healthy rat DA, the average FA value is 0.369 (Table I). Fractional anisotropy data obtained from DTI

Table I. Individual values of the functional state, volumes hyper/hypo intensive areas ratio, total damage degree score and fractional anisotropy in DA rats after cryoinjury.

0 days (before cryoinjury)							
N° of animal	1	2	3	4	5	6	**Mean**
Functional state	Normal	Normal	Normal	Normal	Normal	Normal	-
Hyper/Hypo ratio	0	0	0	0	0	0	**0.00**
SCI severity scores	0	0	0	0	0	0	**0.00**
FA	0.362	0.365	0.379	0.369	0.376	0.372	**0.37**
2 days							
N° of animal	1	2	3	4	5	6	**Mean**
Functional state	Mono	Mono	Mono	Mono	Mono	Mono	-
Hyper/Hypo ratio	3616	1890	17.59	2380	839	483	**1,537.60**
SCI severity scores	6	6	6	6	6	4	**5.67[#]**
FA	0.161	0.171	0.249	0.247	0.229	0.192	**0.21***
7 days							
N° of animal	1	2	3	4	5	6	**Mean**
Functional state	Mono	Mono	Recover	Recover	Recover	Mono	-
Hyper/Hypo ratio	23.91	9.22	23.3	2203	179.23	35.91	**412.43**
SCI severity scores	7	7	7	6	6	7	**6.67[#]**
FA	0.215	0.205	0.267	0.299	0.283	0.215	**0.25***
30 days							
N° of animal	1	2	3	4	5	6	**Mean**
Functional state	Mono	Mono	Recover	Recover	Recover	Mono	-
Hyper/Hypo ratio	14.51	5.15	25.66	487	75.45	41.09	**126.87**
SCI severity scores	7	3	4	2	2	4	**3.00[#]**
FA	0.259	0.240	0.302	0.321	0.303	0.241	**0.28***

[#]*p*-value <0.01; *p-value <0.05. FA, fractional anisotropy; SCI, spinal cord injury.
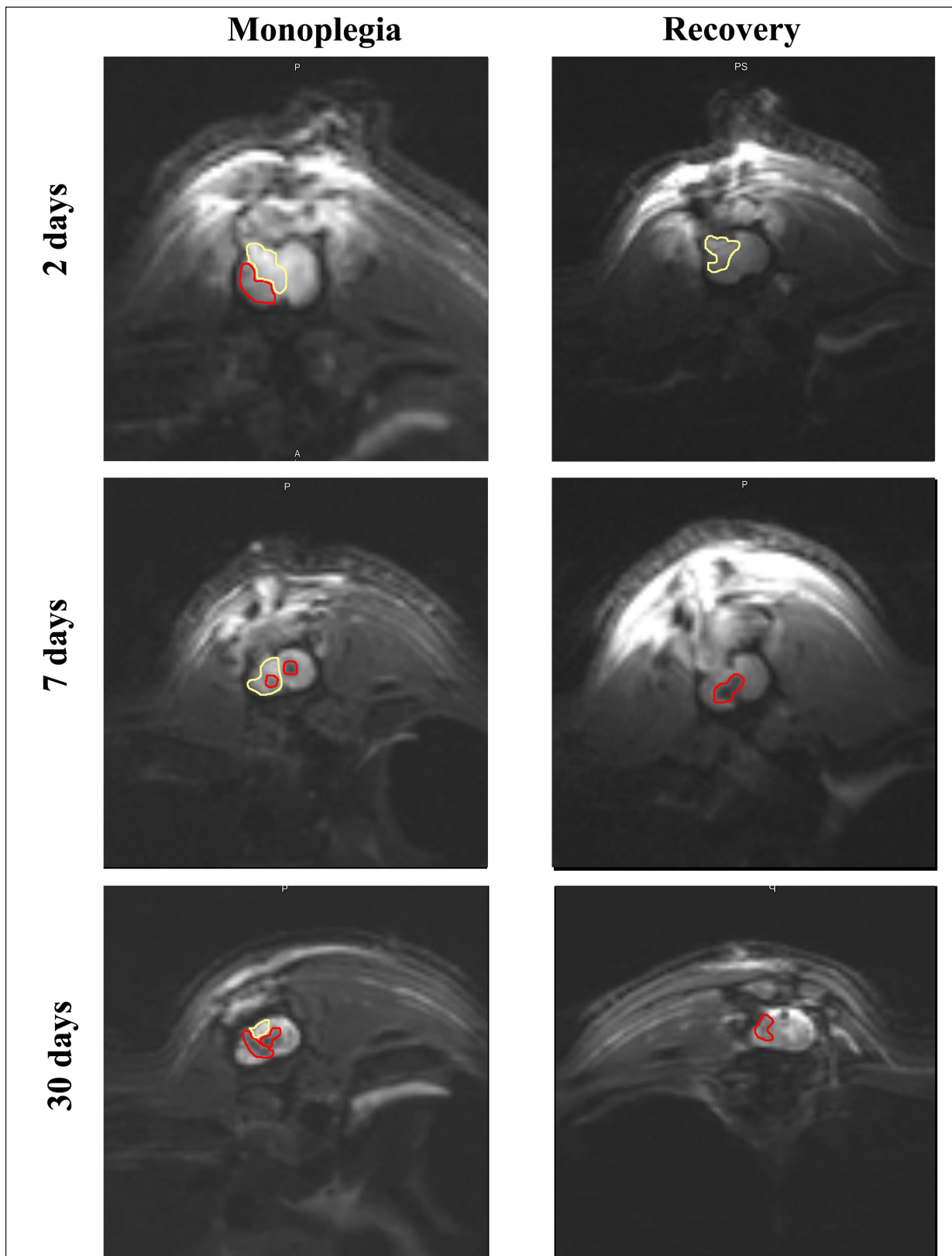
Figure 1. Comparison of T2-weighted sequences MRI results of the spinal cord of DA rats after cryoinjury with preservation of locomotor deficit and with recovery of motor function at 2, 7 and 30 days after cryoinjury. Hyperintense areas are shown as a yellow line. Hypointense areas are shown as a red line.

MRI correlated well with the observed functional recovery (Table I).In rats with fractional anisotropy (FA) of the entire spinal cord greater than 0.2, complete restoration of hind paw locomotor function was observed on the second day after cryoinjury. Conversely, at low FA values, the animals maintained stable monoplegia.

After analyzing the tractograms, it was found that by the 30th day of the study, there was minimal difference between the FA values of the right and left halves of the spinal cords (Figure 2).

This is confirmed by 3D reconstruction of tractograms: in rats with monoplegia, the area of cryoinjury in the form of missing fibers is clearly visible (Figure 2). T2-weighted sequences of the rats' spinal cord at the 30th day showed that the persistence of locomotor deficit was clearly related to the formation of cystic cavities (Figure 1). No cysts were found in recovering animals.

Discussion

This study confirms the relevant role of fractional anisotropy in the management of SCI. In particular, it suggests that FA can predict potential recovery following SCI as early as two days after trauma.
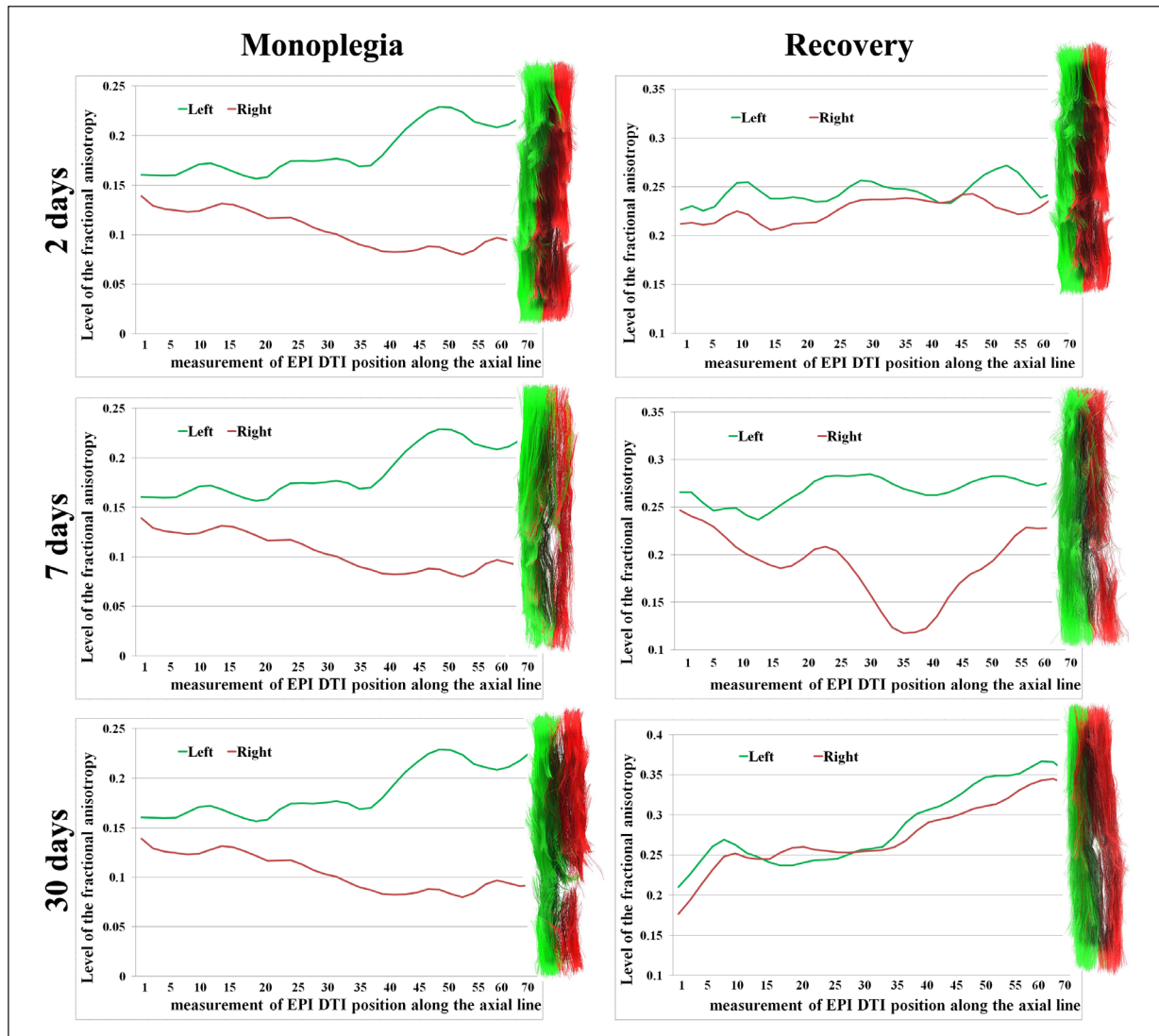

Figure 2. Comparison of EPI-DTI reconstruction MRI results of the spinal cord of DA rats after cryoinjury with preservation of locomotor deficit and with recovery of motor function by 2, 7 and 30 days after cryoinjury. On the 3D-reconstruction images: green - left (healthy) half of the spinal cord, red - right (injured) half of the spinal cord. On the graphs: green line – level of the FA of the left (healthy) half of the spinal cord, red line - level of the FA of the right (injured) half of the spinal cord.

Spinal cord injury is a devastating condition that often results in life-long, tragic consequences for the affected individual. It is a biphasic process, where the secondary phase typically worsens the actual damage caused by the traumatic event.

This fact has stimulated researchers to try to better clarify the extremely complex mechanisms of secondary damage following SCI in order to properly investigate therapeutic protocols that could potentially interfere with the cascade of events following SCI.

Almost 20 years ago, Falconer et al[1] showed the potential relevance of MRI for identifying prognostic factors early after SCI, as well as monitoring the cascade of events following the trauma. They used a non-conventional data acquisition technique in an experimental rat model of SCI using a 2-Tesla machine. In spite of the technical limitations, due to the now obsolete technology and low-field MRI machine, they were able to demonstrate convincingly that early MRI detection of the onset of the secondary phase of SCI, as well as detection of visible pathological changes with possible correlation to final outcome following trauma, was, in fact, possible using this technology.

In the last years, other non-conventional sequences have shown to be capable of providing more relevant information in SCI, such as fractional anisotropy (FA) in particular, but also apparent coefficient diffusivity (ADC)[10], water myelin diffusivity (WMD)[9], myelin water Imaging (MWI)[13]. All these sequences, which utilize specifically designed imaging acquisition protocols, are based on the principle that water molecules' motion is related to axonal integrity and follows a longitudinal course along the expected long pathways of axonal orientation[5,7,14].

The usefulness of FA has been recognized for at least one decade[5,6,8,14]. Loss of FA is considered to indicate neuronal damage[5]; however, an increase in its value is an indicator of anatomo-physiological neuronal interruption at some level. Laitinen et al[15] found an increase in FA values in the thalamus of animals with white matter lesions. We also noted in this experiment that FA could increase rostrally to the lesion area, where its value decreases to almost zero.

FA predicted functional recovery in various experimental studies. However, it can give rise to misinterpretation since myelin sheath damage and post-traumatic edema may decrease its value also in the presence of spared, thus potentially recovering, axons following trauma[11,14]. More-

over, an increase in FA score can also indicate the development of post-traumatic gliosis, which is obviously a negative prognostic factor to function recovery[6,7,10]. However, the spread of the latter is typically perpendicular to the damaged tracks. This distinguishes it from the potential longitudinal spread of physiologically neural recovery along the direction of the damaged pathways[5,7].

We conducted a study using a specialized 7-Tesla MRI machine to examine the usefulness of conventional T2-weight MRI imaging in an experimental model of SCI. We also suggested a grading scale to assess the extent of damage based on the ratio of hyperintense to hypointense lesion areas observed at different time intervals after the trauma. In the present study, we used this technique to evaluate the severity of spinal cord damage and calculated FA at the same time. Therefore, we could compare the two methods at sequential intervals following SCI.

The present result confirmed the reliability of the previously proposed T2-based evaluation grading scale and also showed the expected validity of FA as an instrument for monitoring the evolution of spinal cord injury. However, the evaluation data of anatomo-physiological damage following SCI are provided in a definitely shorter time span following trauma by using FA. This implies that this latter MRI-sequencing technique can represent the most convenient method for monitoring SCI in patients and maybe also predicting clinical outcomes, as the most recent literature seems to suggest[16-24].

Although significant obstacles must still be overcome to achieve similar data in human patients, the path to clinical translation is foreseeable and achievable. Accurate diagnosis of spinal cord injury (SCI) severity must be achieved before management in the early phases after trauma. Our data demonstrate that FA data, derived from diffusion tensor imaging (DTI) within 2 days after SCI, accurately predicts long-term locomotor behavioral recovery in rats.

It should not be overlooked that small animal models, despite their sophistication, are phylogenetically distant from actual clinical scenarios, presenting significant obstacles in their utilization.

Conclusions

The current findings, while validating the T2 10-grade MRI scale for SCI, suggest that FA may

serve as a more effective MRI tool for monitoring the progression of SCI. Consequently, it may provide a more objective assessment of the impact of potential therapeutic protocols for traumatic spinal cord injuries.

Ethics Approval

All experiments and procedures with animals were approved by the Institutional Animal Care and Use Committee IACUC No. 913/22 of 22.12.22.

Informed Consent

Not applicable due to the design of the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization, Georgii Telegin, Aldo Spallone; methodology, Aleksandr Chernov, Alexey Minakov, Elena Malyavina, Vitaly Kazakov, Maksim Rodionov; formal analysis, Georgii Telegin, Aldo Spallone; investigation, Aleksandr Chernov, Alexey Minakov, Elena Malyavina; data curation, Aleksandr Chernov, Vitaly Kazakov, Maksim Rodionov; writing-original draft preparation, Aldo Spallone, Aleksandr Chernov; writing-review and editing, Georgii Telegin; supervision, Georgii Telegin. All authors have read and agreed to the published version of the manuscript.

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