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RETRACTED: Spermatogonial stem-cell-derived neural-like cell transplantation enhances the functional recovery of a rat spinal cord injury model: characterization of evoked potentials

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Severe spinal cord injuries (SCIs) usually result in the temporary or permanent impairment of strength, sensation or autonomic functions below the sites of injuries. To date, a large number of therapeutic approaches have been used to ameliorate SCIs, and subsequent stem cell transplantation appears to be a promising strategy. The aim of this study was to evaluate the therapeutic effect of stem cells by changes in the evoked potentials at different time points after a transplantation of spermatogonial stem cells (SSCs) to differentiate the source neurons in a rat model with SCIs, as well as through histopathology. A modified Plemel spinal cord lateral compression model was used. The experiment was divided into a blank, a control and a SSC transplantation group. Motor activity scores, sensory evoked potentials (SEPs) and motor evoked potentials (MEPs) were assessed through motor resuscitation as well as histologic evaluation on each experimental group to determine the improvement. Consistent with our results, motor scores and evoked potentials were significantly improved in the SSC transplantation group. In addition, a histologic assessment showed that the transplanted stem cells had a significant restorative effect on the reconstruction of tissue cells. 1 week after the stem cell transplantation, the SSC transplantation group showed improvement in spinal cord functions and spinal cord pathologic injuries. After 2 weeks and beyond, the SSC transplantation group showed significant improvement in spinal cord functions and spinal cord pathology compared to the control group, meanwhile the evoked potentials and motor function of the hind limbs of rats in the SSC transplantation group were significantly improved. Therefore, the therapeutic strategies for spermatogonial stem cells will be an effective program in the study on SCIs, and we suggest the somatosensory evoked potentials as a tool to assess the degree of recovery from SCIs after the transplantation of stem cells.

KEYWORDS

spermatogonial stem cells, transdifferentiation, neuronal cells, cell transplantation, spinal cord injury, evoked potentials

Introduction

Spinal cord injuries (SCIs) are highly-disabling, which lead to not only the impairment or loss of the sensory and motor function, but also multi-organ dysfunctions. The high treatment cost, long recovery treatment cycle and the serious consequences of the complete loss of labor always bring great impact to individuals and families, which also bring heavy burden to the society (Inman, 1999; Kang et al., 2018). SCIs can be classified into traumatic and nontraumatic SCIs according to the etiology (Fehlings, 2013). Traumatic SCIs are acute injuries to the spinal cord caused by an external physical shock, such as car accidents, falls, extreme sports or violence; whereas nontraumatic SCIs are injuries resulting from a disease process, usually a chronic one, and they can be caused by both extramedullary and intramedullary lesions, such as tumors, infections or degenerative disc diseases (Ganau et al., 2012; Ahuja et al., 2017; Ganau et al., 2018). The incidence of SCIs varies around the world: traumatic SCIs have an incidence of 39 cases/ million in North America, 16 cases/ million in Australia and 15 cases/ million in Western Europe (Brodke et al., 2003); in contrast, non-traumatic SCIs have an incidence of 1,227 cases/ million in Canada and 364 cases/ million in Australia (Daniels et al., 2017; Kashkoush et al., 2019; Reddy et al., 2021). Restoring the limb functions of patients is the primary concern and the main goal of medical treatments. With the development of medicine, exogenous stem cell transplantation has become a new strategy to repair the structure and function of SCIs.

Since most of the stem cells have various problems such as cell sources, ethical issues, alloimmune rejection, cell survival and differentiation after the transplantation and integration with host neurons, the clinical application still faces many insurmountable difficulties and great challenges. Spermatogonial stem cells (SSCs) are a class of adult stem cells located in the basement membrane of the inner lumen of the seminiferous tubules capable of maintaining self-renewal, which is also capable of directional differentiation into spermatogonia and spermatogenesis through meiosis (Ohta et al., 2000). These specialized stem cells are a type of adult stem cells in the body, which can accompany the organism throughout life and pass on genetic materials to offspring. With the extensive development of regenerative medicine research, the cytological properties of SSCs have become a hotspot of research and attention in many fields, based on the unique cellular characteristics of SSCs – stem cell properties and the unparalleled genetic amplification effect. The function of SSCs is no longer limited to the traditional role of assisting in reproduction for the production of spermatozoa, but rather, the biological function has been greatly expanded. To date, a large number of studies have shown that SSCs are pluripotent, and that mammalian SSCs can be induced to produce hepatocytes, cardiomyocytes, neuronal cells, osteocytes, pancreatic islet cells as well as prostate epithelial cells under special conditions (Guan et al., 2007; Seandel et al., 2007; Conrad et al., 2008; Payne and Braun, 2008; Simon et al., 2009; Streckfuss-Bömeke et al., 2009; Qasemi-Panahi et al., 2011; Zhang et al., 2013; Yang et al., 2015). These pioneering studies have provided new ideas for the application of SSCs as a new source of therapeutic cells in clinical medicine, as well as a new means of the treatment and functional reconstruction of human neurological injuries as well as neurodegenerative lineage diseases. Currently, it has been found that SSCs, compared with embryonic stem (ES) cells, induced multipotent stem (iPS) cells and mesenchymal stem cells (MSCs), have advantages such as being easy to come from (SSCs are generated throughout the life span of human beings), being free of ethical problems, non-tumorigenicity and being free of immune rejection, of which the most critical is that the differentiation of SSCs into neurons does not require the complex induction procedure of genetic modification. Since spermatogonial stem cells have an easy source and are continuously produced throughout life, spermatogonial stem cells are more cost-effective in treating spinal cord injuries than various stem cells such as embryonic stem cells (ES), induced pluripotent stem cells (iPS), and mesenchymal stem cells (MSCs). In our previous project, a simple procedure was used to induce SSCs into neurons, and the induction efficiency was also relatively high. Therefore, the use of SSC transplantation is expected to provide a new idea for the treatment of SCIs.

In addition, clinical data on limb recovery after SCIs will be critical to the success of stem cell therapies. Electrophysiology is a functional means of assessing the integrity of all aspects of the nervous system, including the spinal cord. Clinical applications of electrophysiology include the diagnosis of peripheral or central nervous system injuries and the monitoring of the integrity of the central nervous system during surgeries (Cruccu et al., 2008; Wang et al., 2008; Malhotra and Shaffrey, 2010). Somatosensory-evoked potential (SEP) monitoring has been applied in various rat models with peripheral nerve injuries and SCIs to detect minor injuries and assess ultrastructural damage as well as functional recovery. In contrast, motor-evoked potentials (MEPs) provide a reproducible, noninvasive and objective assessment of axonal conduction in the downstream motor pathways of normal and injured spinal cords. The MEP response is able to show the degree of the functional integrity of specific muscle bundles and their effect on reflexes as well as voluntary motor function. Therefore, neurophysiology has been used as a subclinical tool to determine the degree of preservation of spinal cord functional pathways in the injured regions as well as predict functional outcomes after human and experimental SCIs. In the present study, neurophysiological data was collected during the treatment of a SCI model through a stem cell treatment of rats with SCIs in the hope of providing an effective support for clinical work.

Materials and methods

All experimental protocols and animal handling procedures were approved by the Animal Care and Use Committee of Hong Hui Hospital affiliated Xi'an Jiaotong University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Adult female SD rats (220-250 g, provided by the Experimental Animal Center of Xi'an Jiaotong University) were used for the experiments. A SCI model was modified from the lateral spinal cord extrusion model of Plemel et al. (2008). Animals were anesthetized with 2% sodium pentobarbital (40 mg/kg) intraperitoneally to reduce pains. After T9 laminectomy, an aneurysm clip was applied and the spinal cord was pinched at the same level, resulting in an aneurysm

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clip contusion-compression model. The injured model was cultured for 7 days and then transplanted with differentiated SSCs to fill the SCI areas. At the end of the surgery, the overlying muscles and skins were sutured sequentially. Rats received extensive postoperative care, including intramuscular penicillin (50,000 U/kg/d) for 3d, with manual urination 3 times per day until their automatic urinary function was restored. Cyclosporin A was administered once daily for 2 months.

Culture and differentiation of spermatogonial stem cells

Primary SSCs were isolated from the testes of male SD rats on Postnatal Day 6 using a two-step enzymatic digestion and purification method according to our previously-described procedure (He et al., 2015). These purified and concentrated SSCs were maintained at 37°C with 5% CO2. When the SSCs reached a fusion of 85%, they were passaged to cover slips or 24-well plates at a density of 1 × 104cell/cm2 for further experiments. In the induction experiments, primary SSCs were inoculated on 24-well plates or coverslips at a density of 1 × 104 cells/cm2, and the growth medium was replaced with a conditioned induction medium of DMEM/F12 + 5 μ-MA + 10-μM SB431542 + 0.5 mM IBMX +250 ng/mL SHH + 20 ng/mL FGF2 containing 1% fetal bovine serum medium. The culture medium was changed every 3 days, and the cells were used for further transplantation after 21 days of differentiation.

Transplantation

Phosphate buffer solution (PBS) or SSC-differentiated neuronal cells were then transplanted into the injured areas after SCIs. In this study, we represented 3 experimental groups. Group I contained normal SD rats, Group II contained untreated SD rats with SCIs, and Group III contained SD rats with a cell therapy after injuries. The rats were placed in a stereotactic frame under the effect of isoflurane and anesthetized with 2% sodium pentobarbital (40 mg/kg) intraperitoneally. Saline and wounds were carefully re-exposed. A suspension of neural stem cells suspended at 5,000 cells/ml was injected into 4 sites of 0.5–1.0 mm on either side of the midline of the spinal cord. Injections were made at a rate of 0.5 mL/min and left for 3 min.

Immunocytofluorescence staining

Specific proteins were detected using immunofluorescence staining (IFS). Briefly, cultured cells were fixed with 4% paraformaldehyde for 15-20 min, rinsed 3 times with 0.01 M phosphate buffer solution (PBS), permeabilized with 0.5% Triton X-100 in phosphate-buffered saline (PBS) for 30 min, blocked with 1% BSA for 30 min, and incubated with the primary antibody overnight at 4°C. After rinsing with PBS, the cells were incubated with the secondary antibody and visualized through fluorescence microscopy observation using a fluorescence microscope. The antibodies used are summarized as follows: TUJ1 (CST#4466, mouse, 1:300) and MAP2 (CST#8707, rabbit, 1:300).

Tissue

A number of animals (4 per group) were randomly selected to be executed at the end of the treatment, which were perfused with 4% paraformaldehyde. Each segment of their central spinal cord was excised at the sites of lesions with a length of 1 cm. Tissues were preserved in 4% paraformaldehyde and 30% sucrose PBS solution to treat the tissue blocks. Paraffin-embedded sections were made. Tissue sections were hematoxylin–eosin (H&E) stained to observe the morphology of the injured sites.

Electrophysiology

MEPs of the animals were recorded 1, 2, 3 and 4 weeks postoperatively, and motor nerve conduction was detected with a Nicolet EDX EMG evoked potential system from the United States. After general anesthesia (2% sodium pentobarbital 30 mg/kg), the sensorimotor cortex (SMC) and sciatic nerve were exposed, to which stimulating and recording electrodes were connected, respectively. MEPs were induced *via* the electrical stimulation of the SMC located 2 mm lateral to the midline and 2 mm posterior to the bridge. A single-pulse stimulation with a duration of 50 ms was used. The voltage was adjusted to produce the maximum amplitude of MEPs. Under normal conditions, it was appropriate to adjust the voltage to 6-10 V. The amplitude and latency of the MEPs were then obtained.

All rats were subjected to the SEP of the left upper limb (i.e., the injured side) as a baseline before operation, and the SEP was monitored 1, 2, 3 and 4 weeks after modeling. The specific operation steps were as follows: the anesthetized animals were placed prone on an experimental stage, whose head was fixed and prepared, and recording electrodes were inserted into the periosteum of their skull along the interparietal bone and the intersection of the parietal bone on both sides (note that the recording electrodes of the left SEP were located on the right side of the brain), the reference electrodes were inserted into the nose tip of the rats, the ground electrodes were inserted from the back of their ears, and the stimulating electrodes were inserted into the left upper extremity of their toes, with an interval of about 1 cm among electrodes. The electrodes were connected to the amplifier interface and the stimulation interface sequentially, as is shown in Figures 1, 2. After connecting the stimulation device with the computer, the impedance was normal after debugging, after which a stimulation was given and the SEP signal was recorded sequentially. The parameters of electrical stimulation were set to stimulate the square wave frequency of 4.1 Hz, the width of 0.1 ms and the stimulation intensity of 8-15ma. An appropriate slight jitter appeared at the upper limb, with a magnification of 2000 times, a filtering of 20-2000 Hz and a sampling rate of 50 kHz, meanwhile waveforms were superimposed for 500 times to record the waveforms of evoked potentials, together with the latency and amplitude of those waveforms sequentially.

Behavioral measurements

Behavioral tests were performed using a BBB motor rating scale to assess the motor function once preoperatively, as well as 1 week, 2 weeks, 3 weeks and 4 weeks postoperatively. The scale was sensitive

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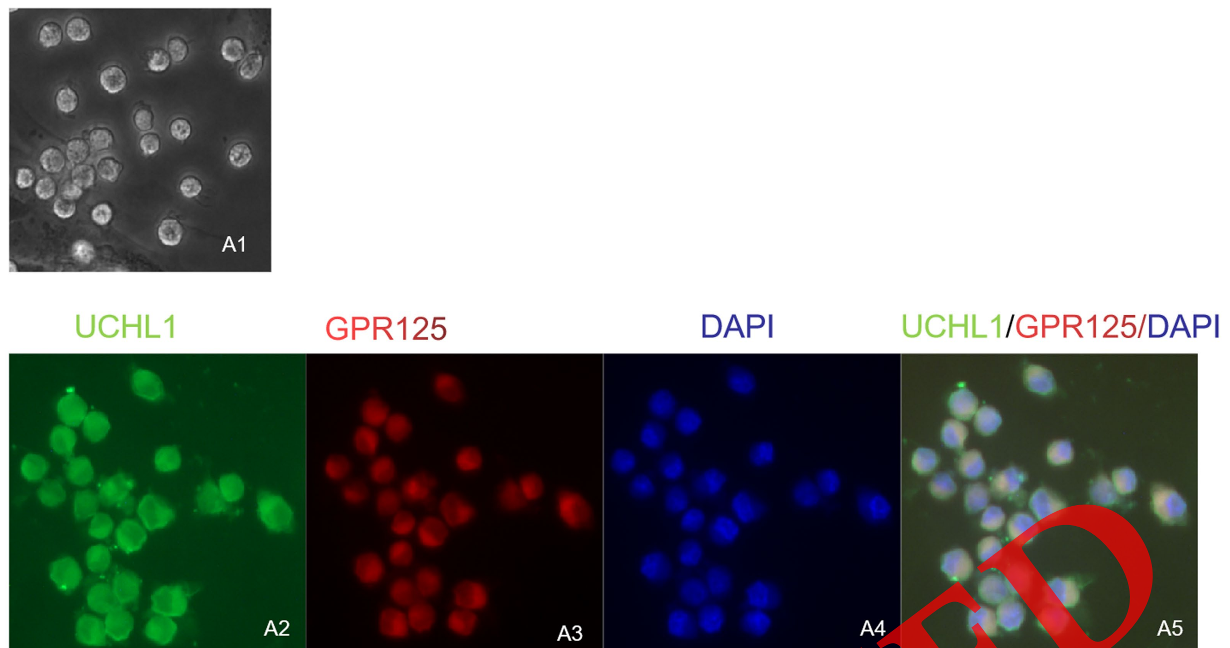

FIGURE 1
Phase contrast images and immunostaining micrographs of primary SSCs. **(A1)** Primary SSCs. **(A2–A5)** Immunofluorescence micrographs representing these corresponding cells identifying specific markers. The antibodies shown are depicted.

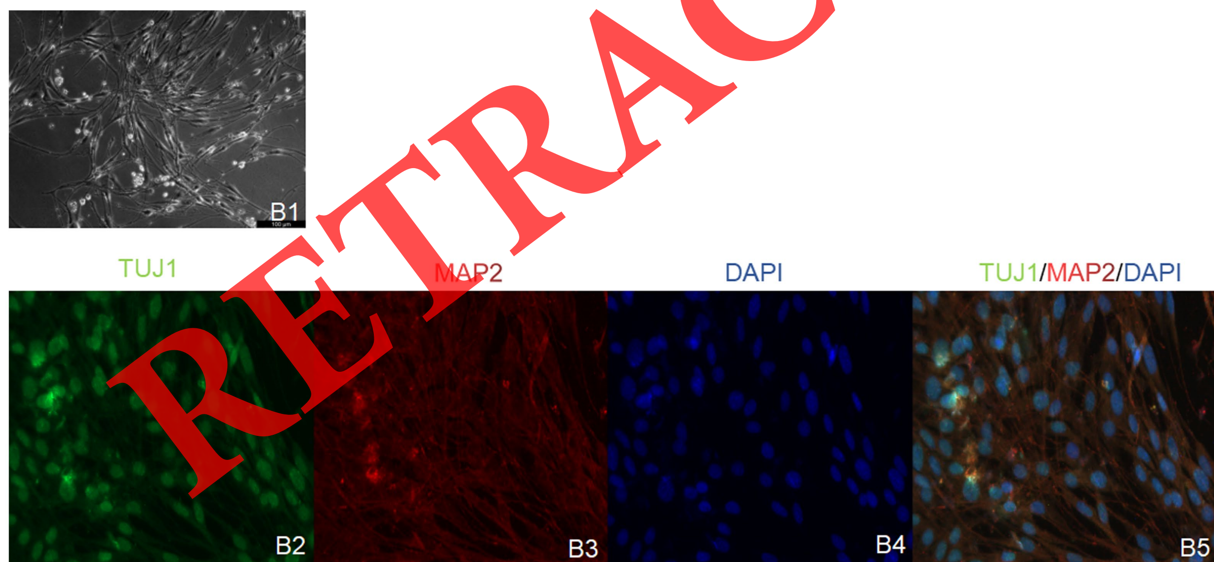

FIGURE 2
Morphological and biochemical characteristics of primary SSC when cultured in conditioned medium. **(B1)** Phase contrast photographs of neural cells differentiated from primary SSC after 3 weeks of induction. **(B2–B5)** Immunofluorescence assay showed upregulation of neuronal and glial markers Tuj-1, Map2 expression in neuronal cells differentiated from primary SSC.

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to the rats' lower limb joint movements, hindlimb movements, trunk position and stability, coordination, gait, paw placement and tail position; details of the scoring scheme can be found in Muheremu et al. (2016), Mohammadshirazi et al. (2019), and Mcdonald et al. (1999). Increases in BBB scores corresponded to those in the degree of locomotor recovery, which could be categorized into 3 phases: early, intermediate and late. Rats were individually placed on a 90 cm plastic clearing. 2 examiners observed and scored each rat for exactly 4 min, scoring each hindlimb individually. The final score of each limb was the lowest score obtained by the 2 observers. Rats were scored on a scale ranging from 0 to 21, with 0 indicating no hindlimb movements and 21 indicating complete hindlimb movements with a wide range of joint movements, including the extension of hindlimbs, knees and hips, consistent plantar pedaling, consistent toe clearance, no rotation

during pedaling, and sustained upward movements of the tail (Mcdonald et al., 1999; Muheremu et al., 2016; Mohammadshirazi et al., 2019). All behavioral assessments were performed by 2 researchers who were blind to the severity of the injuries. Mean scores were calculated based on injury groups, with BBB scores as a function of the time since injuries.

Statistical methods

All data of the statistical analysis was reported and expressed as mean ± standard deviation structure. A statistical analysis was performed using commercial software (Stata, StataCord LP, College Station, TX). A t-test was used to compare the BBB score, mean MEP amplitude, mean SSEP amplitude and latency at each time point between the SCI group and the control group. Differences were considered statistically significant at $p < 0.05$.

Results

In order to design an ideal protocol to induce SSCs to functional spinal cord neurons, we first cultured and characterized primary rat SSCs, whose morphology was observed using phase contrast microscopy (Figure 1A1). Immunostaining showed that these purified cells were positive for GFR125/UCHL1 (Figures 1A2–A5). In order to choose an effective method to induce the transformation of stem cells into spinal cord neurons, we cultured the stem cells in the following conditions: a DF12 medium plus chemicals IBMX and SB431542, with various defining factors such as RA, SHH and FGF2, which is an effective pathway for inducing the transformation of rat SSCs into neurons. This was demonstrated through phase contrast microscopy observation of the morphology of SSCs differentiated to neuronal cells and the expression of neuronal markers Tuj-1 and Map2 (Figures 2B1–B5).

A histological examination was performed using histological staining with H & E to verify the presence and extent of SCIs. The result of cross-sectional HE staining microscopy of the SSC group showed that the morphology of the spinal cord was restored to a normal form compared to the control group, with structural integrity, and that the boundary of the gray matter, white matter as well as central canal was clear. In the anterior horn of the spinal cord, motor neurons and Nystrom's bodies could be seen, and through high-magnification microscopy, it could be seen that there were more motor neuron cells on the injured side and less glial cell infiltration compared with that in the control group (Figures 3C1,C2). In the control group, the cross section of spinal cord specimen could be seen as a non-complete oval shape, the spinal cord tissues were deformed due to compression, the central canal was deformed or even disappeared, the structure of gray matter and white matter was changed, no hemorrhage was seen; in the gray matter, Nieri's vesicles could be observed to be dissolved, ruptured and disappeared, and neuron cell apoptosis was observed. A decrease in the number of motor neuron cells in the anterior horn of the injured side was seen under a high magnification (Figures 3C3,C4).

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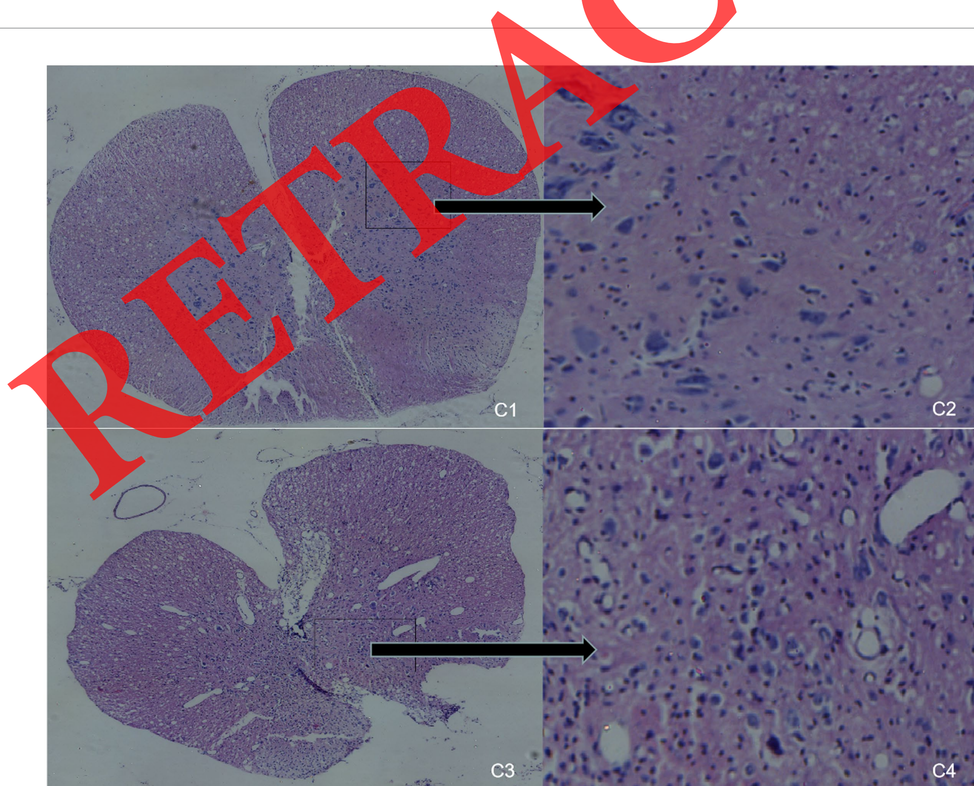

FIGURE 3
Spinal cord cross-sectional HE staining microscopic view of the control group and SSC group, **(C1)** is the SSC group spinal cord compression center cross-sectional section microscopic, **(C2)** is the SSC spinal cord anterior horn HE staining high magnification microscopy, can see the motor neurons, Nyctal microsomes, **(C3)** is the control group spinal cord compression center cross-section microscopic, **(C4)** is the control group spinal cord anterior horn HE staining high magnification microscopy, can see neuronal cell apoptosis, few glial cells. Neuronal cell apoptosis and more glial cell infiltration can be seen.

TABLE 1 BBB scores between the three groups.

Group	Pre-operation	Postoperation 1 week	Postoperation 2 weeks	Postoperation 3 weeks	Postoperation 4 weeks
normal	21.00 ± 0.00	21.00 ± 0.00	21.00 ± 0.00	21.00 ± 0.00	21.00 ± 0.00
control	21.00 ± 0.00	13.40 ± 1.84[a]	15.30 ± 1.67[a]	16.85 ± 1.78[a]	17.91 ± 1.78[a]
SSC	21.00 ± 0.00	15.70 ± 2.79[ab]	17.57 ± 1.42[a]	18.33 ± 1.67[ab]	19.26 ± 1.93[ab]
P		<0.05	<0.05	<0.05	<0.05

a means $p < 0.05$ for comparison of other group with the normal group at the same time point, b means $p < 0.05$ for comparison of the experimental group with the control group at the same time point.

For a behavioral assessment to check the functional recovery after SCIs, a blood–brain barrier test was performed weekly (5 animals in the sham-operated group, 10 animals in the control group, and 10 animals in the SSC group). A blood–brain barrier score of 21 was determined for all animals before the SCI surgery, which shifted to 0 the day after surgery and remained constant for at least 3 days. The BBB score of the SSC group was elevated 1, 2, 3 and 4 weeks postoperatively compared to the control group, with statistically-significant differences ($p < 0.05$), and there was a difference in the BBB score of the SSC group 1 week postoperatively compared to that of the control group ($p < 0.05$); the BBB score of the groups was statistically different from those of the control group 1, 2, 3 and 4 weeks postoperatively. Comparing the BBB scores 4 weeks postoperatively with those 1 week postoperatively, the difference was statistically significant ($p < 0.05$); BBB scores of the SSC group and the control group were the lowest1 week after modeling, and then gradually increased (Table 1; Figure 4).

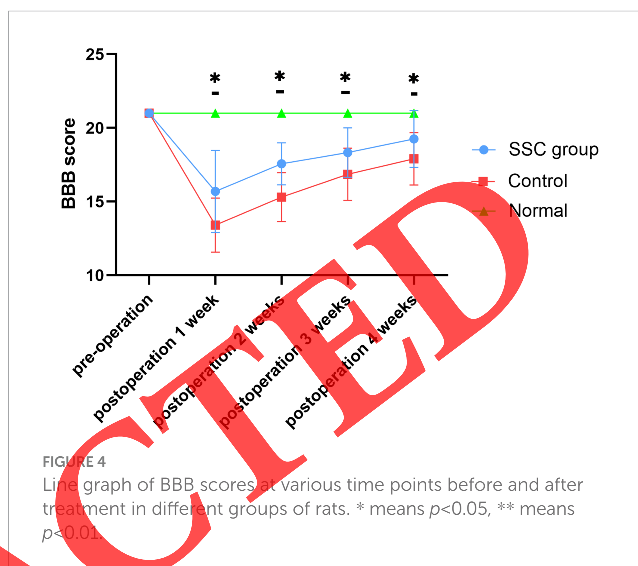

FIGURE 4
Line graph of BBB scores at various time points before and after treatment in different groups of rats. * means $p<0.05$, ** means $p<0.01$

To confirm whether the remyelination and axonal regeneration at the sites of SCIs/grafting are associated with functional recovery, we measured MEPs and SEPs of model rats. In the normal group, the stimulus elicited a larger response amplitude and a shorter latency for MEPs. Notably, in the SCI group, the response amplitude and latency of MEPs were significantly improved compared with those of the control group ($p < 0.05$). The results suggest that the SSC group showed an increased remyelination at the injured/grafting sites, which might be associated with the improved nerve conduction (Tables 2, 3; Figures 5E1–10).

When measuring SEPs, we found that 500 iterations of a 4.1 Hz square wave stimulation for 0.1 ms were performed, and the latency as well as amplitude recovery measurements were compared with the normal standardized values during this period, which are shown in a bar graph. Comparisons were then made in the control group.

SEP signal: The latency period of SEPs was prolonged to varying degrees in all experimental groups, and the amplitude of the signal was generally reduced, so this experiment was chosen for observation after the cell therapy. With a prolongation of the treatment time, the amplitude of the SEP signal gradually increased, and the latency period was gradually shortened, which approached to the normal shape of the SEP signal curve but failed to return to normal.

The recovery of P-wave peaks was significantly higher in the SSC group than in the control group ($p < 0.05$). In addition, the recovery of P-wave peaks in the SSC group was statistically significant compared to that of the control group ($p < 0.05$), which indicated that the differentiated SSCs had a repairing effect on SCIs, restoring the P peak to a larger wave amplitude with its waveform close to that of the normal group.

In terms of the P-wave latency time of the 2 groups, the amplitude of latency recovery in the SSC group was significantly higher than that in the control group, with a statistically--significant difference ($p < 0.05$). It indicated that after the treatment for the experimental group, a shorter latency time and a larger wave amplitude were recovered with the P peak, whose waveform was close to that of the normal group (Tables 4, 5; Figures 6F1–F10).

Discussion

Regarding recent studies, neural stem cell transplantation in SCIs is established to encompass a wide range of goals. Examples include replacing lost cells such as neurons and oligodendrocytes, maintaining remaining neurons, as well as preventing apoptosis. In addition, transplanted neural stem cells are thought to provide a right number of neurotrophic factors to regenerate axons, promote synapse formation, and encourage remyelination (Muheremu et al., 2016). In a study conducted by Mohammadshirazi et al. (2019), the efficacy of lithium transplanted with human neural stem cells in rat spinal cord injury was found to be favorable. Although neural stem cells are directly differentiated into neural cells without going through transcellular lineage, there are still many insurmountable difficulties and great challenges facing the cell source of NSCs, ethical issues, alloimmune rejection, cell survival and differentiation after transplantation, as well as the integration with host neurons make the clinical application of NSCs. In contrast, the source of spermatogonial

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TABLE 2 MEP latency in experimental and control groups (ms).

Group	Pre-operation	Postoperation 1 week	Postoperation 2 weeks	Postoperation 3 weeks	Postoperation 4 weeks
SSC	11.90 ± 0.45	19.22 ± 1.03[a]	18.21 ± 1.57[a]	15.59 ± 2.31[a]	14.79 ± 1.33[a]
control	12.07 ± 0.50	20.88 ± 2.26	20.00 ± 2.12	19.18 ± 1.85	17.36 ± 2.04

a means $p < 0.05$ for comparison of the experimental group with the control group at the same time point.

TABLE 3 MEP amplitude in experimental and control groups (μv).

Group	Pre-operation	Postoperation 1 week	Postoperation 2 weeks	Postoperation 3 weeks	Postoperation 4 weeks
SSC	5.39 ± 2.26	0.51 ± 0.06[a]	1.16 ± 0.35[a]	2.58 ± 0.50[a]	3.22 ± 1.26[a]
control	5.51 ± 2.07	0.28 ± 0.09	0.59 ± 0.18	1.79 ± 0.62	2.73 ± 0.46

a means $p < 0.05$ for comparison of the experimental group with the control group at the same time point.

stem cells in this study is significantly better than that of human neural stem cells, and they are produced continuously throughout life, showing simplicity and efficiency in differentiation.

In this experiment, neuronal cells induced and differentiated from rat primary SSCs were transplanted into a SCI contusion model. Our data showed a significant improvement in the recovery of the limbs of the rat model after SCIs compared to that of the control group. The improvement in electromyography of the transplanted group was also consistent with our expected research hypothesis.

From our findings, SSCs appeared to be able to differentiate into cells expressing prostate, uterine and skin epitheliums, suggesting their ability to differentiate into different lineages. In the present study, the expression level of other neurotransmitters is not examined, which is focused only on the ability of neuronal cells to differentiate and their effect on the functional recovery of animals. In 1999, McDonald and his colleagues claimed that BBB scores increased after the transplantation of ESC-derived neural stem cells (Mcdonald et al., 1999). Other studies have also shown that transplanted neural stem cells can migrate and differentiate into the astrocytes, oligodendrocytes as well as neurons of the damaged spinal cord of mice, rats and primates (Mcdonald et al., 1999; Iwanami et al., 2005; Muheremu et al., 2016). In a study conducted by Qiu et al. (2015), it was found out that the neural-like cells derived from msc and transplanted in GS scaffolds could trans-differentiate into myelin-forming cells in the fully transected rat spinal cord and improve function. However, it is widely known that the differentiation of stem cells into neurons is accompanied by the defects such as the risk of viral infection, the cellular mutation after gene transfection, and the instability of gene integration into the cells. Also, the susceptibility of host cells to viral infection can lead to the potential pitfalls of tumor generation, the instability of gene integration, the uncertain efficiency of cell transdifferentiation, and the return of cells to their initial phenotype or intermediate state when they leave their induced environment. In contrast, the SSCs used in this study are advantageous as they cause no ethical problems, tumorigenicity or immune rejection. Most importantly, the neurons obtained by SSCs differentiation do not require complex induction such as gene modification.

The present tissue results indicate that the damaged spinal cord can be significantly repaired through the transplantation of SSC-derived neuronal cells into the damaged spinal cord. Thus, the transplantation of SSC-derived neuronal cells may favor axonal regeneration, the survival of host damaged neurons and the improvement of locomotion. On the other hand, given that functional improvement seems to be obvious, therefore, further studies on the potential mechanisms guiding the trans-differentiation of SSCs *in vivo* are desirable.

Electrophysiological studies on SCIs with different animal models have become increasingly popular. Multiple hypotheses about the process of spinal cord dysfunctions following injuries or diseases involving human spinal cord dysfunctions can be functionally tested using this relatively simple model. Measuring the physiologic integrity of the spinal cord in a rapid, objective and quantitative manner is highly desirable. Although further research is needed, the results of this study and earlier reports suggest that a reproducible, noninvasive and fairly readily-available signal can be induced in peripheral muscles through cortical stimulation. This signal is an electrical measure of functions that is dependent on an intact motor system. Neuromyoelectricity has many possible applications, such as the monitoring during tissue manipulation or the assessment of progressive neurological disorders. With further studies on neuromuscular electricity, especially with regard to SCIs and diseases, it should greatly add to the conclusions drawn from many of the earlier studies.

As revealed by All et al. (2020), their use of SEP in conjunction with BBB scores in their study of SCI allowed for a more effective assessment of injury progression, which is consistent with the conclusion of the present study and is reaffirmed by the post-transplantation injury modeling in the present study. In the present study, we observed that 2 weeks after SCIs, the SSC group presented improved locomotor performance of injured rats compared to the control group. A significant improvement in wave amplitude as well as latency was shown in the neurophysiological results, which implied that the downstream spinal tracts, especially the subcortical tracts, had been repaired after cell transplantation. Moreover, in the SCI model of the stem cell transplantation group, N1 wave amplitude significantly increased, indicating improved motor units. We also observed a significant reduction in N1 latency compared with the control group, which confirmed the reconnection of the downstream spinal cord bundles.

In the present study, all rats that received cell transplants showed motor improvements. Although our results suggest that the "placing reflex" of the hindlimbs as well as the coordinated movement of the anterior and posterior limbs is initiated by

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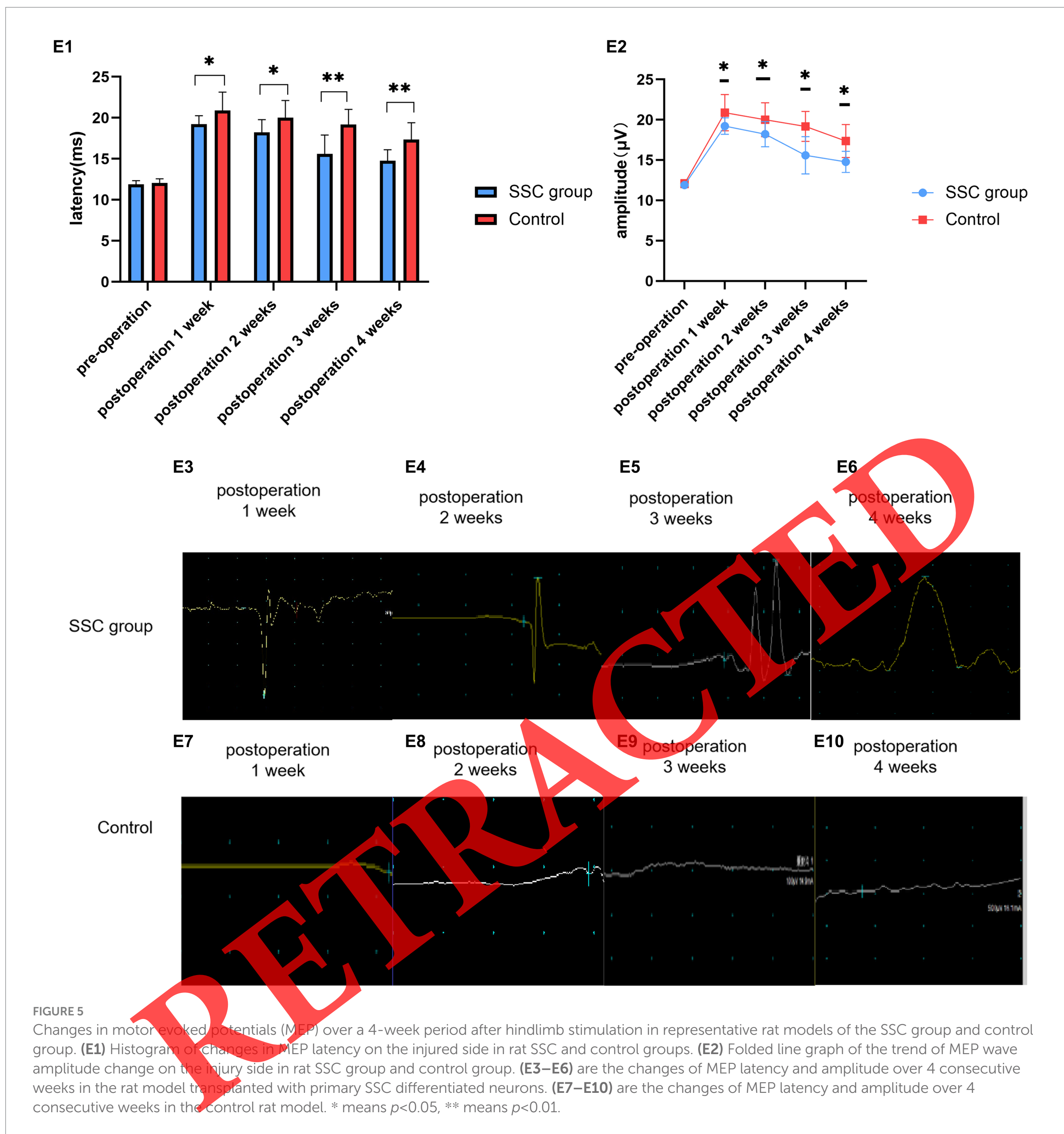

FIGURE 5
Changes in motor evoked potentials (MEP) over a 4-week period after hindlimb stimulation in representative rat models of the SSC group and control group. **(E1)** Histogram of changes in MEP latency on the injured side in rat SSC and control groups. **(E2)** Folded line graph of the trend of MEP wave amplitude change on the injury side in rat SSC group and control group. **(E3–E6)** are the changes of MEP latency and amplitude over 4 consecutive weeks in the rat model transplanted with primary SSC differentiated neurons. **(E7–E10)** are the changes of MEP latency and amplitude over 4 consecutive weeks in the control rat model. * means $p<0.05$, ** means $p<0.01$.

TABLE 4 SEP latency in experimental and control groups (ms).

Group	Pre-operation	Postoperation 1 week	Postoperation 2 weeks	Postoperation 3 weeks	Postoperation 4 weeks
SSC	13.17 ± 0.90	18.51 ± 0.67[a]	17.91 ± 0.83[a]	16.87 ± 0.48[a]	15.62 ± 0.69[a]
control	13.21 ± 0.75	20.19 ± 0.33	18.25 ± 0.47	17.47 ± 0.52	17.05 ± 0.19

a means $p < 0.05$ for comparison of the experimental group with the control group at the same time point.

locomotion after transplantation, it does not necessarily mean that there is sufficient "information transfer" between the cerebral cortex and the caudal side of the injured/ transplanted parts of the spinal cord. Therefore, the improvement in electrophysiological characteristics, evidenced by a higher MEP and SEP amplitude as well as a shorter latency, may be beneficial

TABLE 5 SEP amplitude in experimental and control groups (μv).

Group	Pre-operation	Postoperation 1 week	Postoperation 2 weeks	Postoperation 3 weeks	Postoperation 4 weeks
SSC	6.51 ± 1.98	1.55 ± 0.83[a]	2.18 ± 0.96[a]	3.09 ± 0.89[a]	4.66 ± 0.12[a]
control	6.69 ± 2.03	0.78 ± 0.17	1.28 ± 0.42	2.42 ± 0.97	3.15 ± 0.44

a means $p < 0.05$ for comparison of the experimental group with the control group at the same time point.

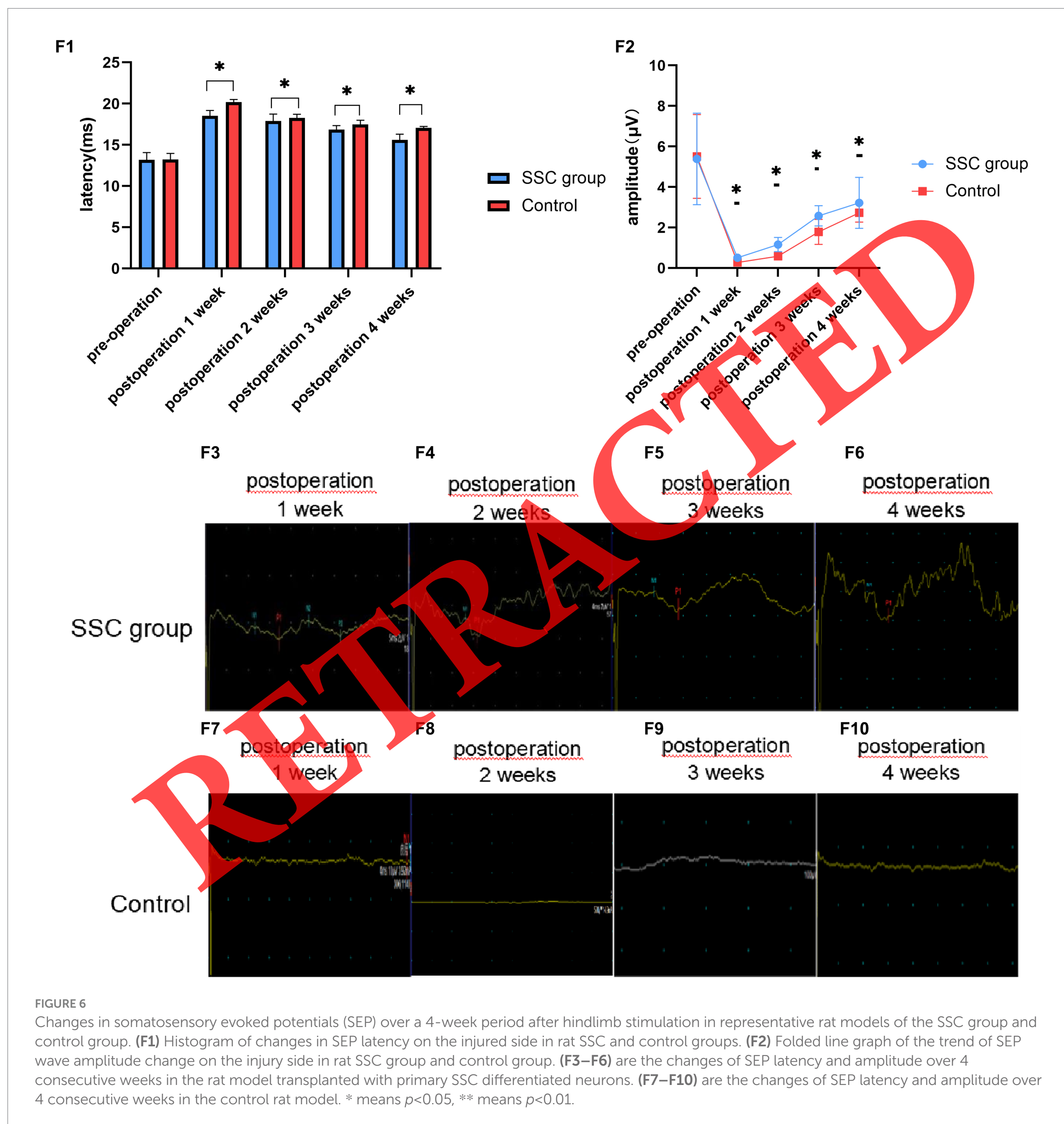

FIGURE 6
Changes in somatosensory evoked potentials (SEP) over a 4-week period after hindlimb stimulation in representative rat models of the SSC group and control group. **(F1)** Histogram of changes in SEP latency on the injured side in rat SSC and control groups. **(F2)** Folded line graph of the trend of SEP wave amplitude change on the injury side in rat SSC group and control group. **(F3–F6)** are the changes of SEP latency and amplitude over 4 consecutive weeks in the rat model transplanted with primary SSC differentiated neurons. **(F7–F10)** are the changes of SEP latency and amplitude over 4 consecutive weeks in the control rat model. * means p<0.05, ** means p<0.01.

to demonstrating the reorganization of nerve fibers and myelin sheath formation after axotomy.

The main concern in this study is the therapeutic effect of transplantation of neuronal cells derived from spermatogonial stem cells through diffrentiation in a rat model of spinal cord injury, but there are traumatic and nontraumatic spinal cord injuries. Although the results of the study are considered applicable to nontraumatic spinal cord injuries, nontraumatic

spinal cord injuries are more specific and heterogeneous. Also, it is difficult to replicate such a model in a laboratory. Therefore, the use of spermatogonial stem cells to treat non-traumatic spinal cord injuries is worthwhile in future research and is a promising solution to clinical treatment.

In conclusion, neurophysiology is a promising tool for assessing the performance of the spinal cord after injuries and the extent of damage. Moreover, through the observation of the effect of neurophysiology on the intervention of stem cell therapy after SCIs, this method was proved able to be used for evaluating the spinal cord functions and the relative degree of recovery, which was a reliable quantitative analysis strategy. Therefore, we suggest neurophysiologic monitoring as a valuable method for monitoring stem cell therapy after SCIs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal studies were approved by the Animal Care and Use Committee of Xi'an Jiaotong University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

XG: Writing – original draft, Writing – review & editing, Conceptualization, Investigation. CJ: Writing – review & editing, Formal analysis, Software. YZ: Writing – review & editing, Visualization. ZC: Writing – review & editing, Data curation, Methodology. DH: Writing – original draft, Formal analysis, Project administration. HZ: Writing – original draft, Resources, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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