Transcranial Direct Current Stimulation for Improvement of Neurotransplantation Outcomes in Rats with 6-Hydroxydopamine-Induced Parkinsonism

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Abstract

Introduction. *With the number of patients with Parkinson's disease steadily growing, the need for novel treatment approaches is increasing. Combining transplantation of neuronal progenitors derived from induced pluripotent stem cells and transcranial direct current stimulation (tDCS) is among the promising methods.*

Aim*: to examine the effect of tDCS on the cell graft condition and motor symptoms of Parkinson's syndrome in rats.*

Materials and methods. *Parkinson's syndrome was modeled in Wistar rats by the unilateral intranigral injection of 6-hydroxydopamine (6-OHDA; 12 μg in 3 μL) The model rats underwent neurotransplantation (3×10^5 cells in 10 μL) into the caudate nuclei on the affected side. The animals underwent tDCS for 14 days. Behavioral changes were analyzed by open field and beam-walking tests. Development and morphological characteristics of the graft were assessed by the morphochemical study.*

Results. *Neurotransplantation had no significant effect on the behavior of rats with parkinsonism; however, combined with tDCS, it increased motor activity during the open field tests compared with the group of model rats ($p=0.0014$) and mitigated their anxiety-related behaviors ($p=0.048$) in tests at 3 weeks after the transplantation. These effects were not observed in tests at 3 months. The morphochemical study revealed larger graft sizes in the animals that underwent tDCS compared with the controls and cell shift to the marginal zone of the graft. Stimulation was also shown to induce division of a part of cells at early stages of differentiation and promote active synaptogenesis.*

Conclusion. *Combining neurotransplantation and tDCS in the 6-OHDA-induced model of parkinsonism demonstrated its potential to manage both motor and non-motor symptoms. Optimizing protocols of transplantation and tDCS and evaluating their long-term efficacy and safety are required to successfully implement this method into clinical practice.*

Keywords: *Parkinson's disease; animal models; neurotransplantation; transcranial direct current stimulation*

Ethics approval. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 10-7/20, 27 November, 2020).

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Conflict of interest. The authors declare that they have no apparent or potential conflicts of interest related to the publication of this article.

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Опыт применения транскраниальной электростимуляции постоянным током с целью улучшения исходов нейротрансплантации у крыс с паркинсонизмом, индуцированным 6-гидроксидофамином

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Аннотация

Введение. *Неуклонно растущее число пациентов с болезнью Паркинсона диктует необходимость поиска новых терапевтических подходов к её лечению. Одним из перспективных методов представляется сочетание трансплантации нейрональных предшественников, полученных из индуцированных плюрипотентных стволовых клеток, и транскраниальной электростимуляции (ТЭС).*

Цель *исследования: изучить влияние ТЭС постоянным током на состояние клеточного трансплантата и моторные симптомы паркинсонического синдрома у крыс.*

Материалы и методы. *Паркинсонический синдром у крыс Вистар моделировали односторонним интранигральным введением 6-гидроксидофамина (6-ГДА; 12 мкг на 3 мкл). Нейротрансплантацию (3×10^5 клеток в 10 мкл) осуществляли в хвостатые ядра мозга животных-моделей на стороне повреждения. ТЭС постоянным током проводили в течение 14 дней. Изменения поведения животных анализировали в тестах «открытое поле» и «сужающаяся дорожка». В морфохимическом исследовании оценивали развитие и морфологические характеристики трансплантата.*

Результаты. *Нейротрансплантация не оказала значимого влияния на поведение крыс с паркинсонизмом, однако в сочетании с ТЭС привела к увеличению двигательной активности крыс в тесте «открытое поле», по сравнению с группой крыс-моделей ($p = 0,0014$), и ослаблению у них неврозоподобного состояния ($p = 0,048$) в тестах через 3 нед после введения трансплантата. В тестах, проведённых через 3 мес, эти эффекты не наблюдались. Морфохимическое исследование выявило бóльшие размеры трансплантата у животных, подвергнутых ТЭС, по сравнению с контролем, и смещение клеток в краевую зону трансплантата. Показано также, что стимуляция провоцирует деление части клеток, находящихся на ранних стадиях дифференцировки, и способствует активному формированию синаптических контактов.*

Заключение. *Сочетание нейротрансплантации и ТЭС на 6-ГДА-индуцированной модели паркинсонизма демонстрирует потенциал данной технологии для коррекции как двигательных, так и недвигательных проявлений заболевания. Для успешной трансляции метода в клинику необходимы дальнейшая оптимизация протоколов трансплантации и ТЭС, оценка долгосрочной эффективности и безопасности.*

Ключевые слова: *болезнь Паркинсона; модели на животных; нейротрансплантация; транскраниальная электростимуляция*

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Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders that leads to severe disability [1]. PD pathogenesis is still poorly understood. Main motor symptoms of PD are known to be caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the subsequent nigrostriatal pathway degeneration and striatal dopamine deficiency. Nigral neurodegeneration is often linked to the accumulation of aggregated forms of the phosphorylated α-synuclein protein, which form Lewy bodies and neurites. Apart from α-synuclein accumulation, affected dopaminergic neurons are observed to have signs of mitochondrial dysfunction [2]. Numerous current studies on animal models and in patients indicate that neuroinflammation has a key role in the initiation and progression of neurodegeneration in the SNpc [3], as well as in oxidative stress development in the affected brain tissue [4, 5].

To date, there is no effective treatment that halts PD progression. Current treatment options can only alleviate numerous PD symptoms, which are classified into motor and non-motor. Non-motor manifestations tend to occur long before motor impairments, and their diagnosis can facilitate timely treatment [6, 7].

A wide range of animal models is used to elucidate causes of PD development and search for new treatment options. The most common PD model is the stereotaxic injection of neurotoxins into certain brain structures, thus avoiding their systemic effects [8].

Unilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the SNpc, which selectively affects dopaminergic neurons, is an optimal model to test neurotransplantation (NT) methods in PD [9]. Transplantation of dopaminergic neuronal progenitor cells into the caudate nuclei allows to replenish the dopamine deficiency in this structure, which may affect the neurodegenerative process to some extent. Transplantation of induced pluripotent stem cells (iPSC) and their derivatives, including autologous ones, reduces the recipient's immune response, eliminates ethical concerns, and has no limitation on the number of transplanted cells [10]. It should be noted that iPSC transplantation increases the percentage of progenitor cells that adapted and differentiated into healthy dopaminergic neurons. However, the issue of transplanted cell survival and function has not been fully addressed [10–13].

Transcranial direct current stimulation (tDCS) is a non-invasive and safe neuromodulation technique, which is successfully used in neurology to manage some pathologies [14]. The literature data confirm that various types of electrical stimulation can alleviate motor and non-motor symptoms of PD and also demonstrate that tDCS has a beneficial effect on differentiation and survival of transplanted cells [15, 16]. Thus, combining tDCS and NT may be a promising approach for PD therapy.

To expand the range of experiments with tDCS, we had to develop and build a multichannel electrical stimulator for small laboratory animals. The staff of the Laboratory of Experimental Nervous System Pathology and Neuropharmacology (Brain Science Institute, Research Center of Neurology) and engineers from the Bauman Moscow State Technical University jointly designed and engineered a multichannel prototype for tDCS, which operates in different modes.

The study **aims** to examine the effect of tDCS on the cell graft condition and motor symptoms of 6-OHDA-induced Parkinson's syndrome in rats that underwent NT using human iPSC derivatives.

Materials and Methods

Animals

All experiments were conducted in line with bioethical standards for proper handling of laboratory animals, including minimizing the number of animals used. The study was approved by the ethics committee of the Research Center of Neurology (Protocol No. 10-7/20 dated November 27, 2020).

Male Wistar rats ($n = 40$) 3.5 months old and weighing 300–350 g at the beginning of the experiment were taken from the Stolbovaya Branch of the Scientific Center for Biomedical Technologies of the Federal Medical-Biological Agency.

Animal procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS No. 170), Order of the Ministry of Health of the Russian Federation No. 119H dated April 1, 2016 "On Approval of the Rules of Laboratory Practice", and the national standard "Species-Specific Provisions for Laboratory Rodents and Rabbits" (GOST 33216-2014). The animals were kept under standard vivarium conditions, with a 12-hour light/dark cycle and ad libitum access to food and water. The rats were quarantined for 14 days before the beginning of the experiment.

Surgical Procedures

For stereotactic surgery, the animals were secured in a stereotaxic frame (Stoelting Co., RWD Life Science Co. Ltd.); the scalp was incised, and burr holes were drilled in the skull using a portable drill to access specific brain structures. A cotton gauze pad was placed between the work surface and the animal to prevent hypothermia during and after surgery.

Zoletil 100 (Valdepharm; solvent, Delpharm Tours) at 3 mg/100 g and xyla (Interchemie werken 'De Adelaar' B.V.) at

3 mg/kg were administered intramuscularly to maintain anesthesia. Atropine (Dalkhimpharm) at 0.04 mg/kg was given subcutaneously 10–15 minutes before xyla administration.

For a model of Parkinson's syndrome, the animals ($n = 32$) were injected with 6-OHDA (Sigma), a selective toxin for dopaminergic neurons, at a dose of 12 μg in 3 μL of 0.05% ascorbic acid solution in the right SNpc (Paxinos Atlas coordinates [17]: AP = –4.8; L = 1.9; V = 8.0) (Fig. 1). The same volume of the solvent was administered in the left substantia nigra. Sham-operated (control) animals ($n = 8$) were injected with the same volume of the solvent bilaterally.

On day 25 after the 6-OHDA injection, the animals ($n = 24$) underwent transplantation of neural progenitor cells into the caudate nuclei (Paxinos Atlas coordinates: AP = 1.5; L = 2.2; V = 4.5). The anesthesia technique was described above. The control animals that did not receive the neurotoxin (group C1; $n = 8$) and a part of the 6-OHDA-injected animals did not undergo transplantation; group C2 ($n = 8$) was bilaterally injected with the same volume of the normal saline into the caudate nuclei.

Cell transplantation was performed unilaterally, on the affected side. A suspension (3×10^5 cells in 10 μL of the normal saline) was injected at a constant rate for 5 minutes via the Hamilton microliter syringe into the caudate nuclei. After the injection, the syringe was left in place for 2 minutes and then slowly withdrawn. The same volume of the normal saline was injected in the left caudate nuclei. The animals received 12 mg/kg of cyclosporine one day before cell transplantation and then daily during the entire experiment.

Cell cultures were obtained in the cell biology laboratory of the Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine. Neurons were differentiated from iPSCs, which were derived from skin fibroblasts and obtained from a healthy donor (a 60-year-old man without any neurological pathology) after the informed consent. The iPSC line IPSRG4S was characterized according to generally accepted standards [18]. The cell line has a normal karyotype. IPSRG4S pluripotency was confirmed at molecular and functional levels. The iPSCs were directed to differentiate into early neuronal progenitors that were later differentiated into ventral mesencephalic neuronal progenitors, which were used for transplantation on day 24 of differentiation. The method of IPSC differentiation and media composition are available upon request.

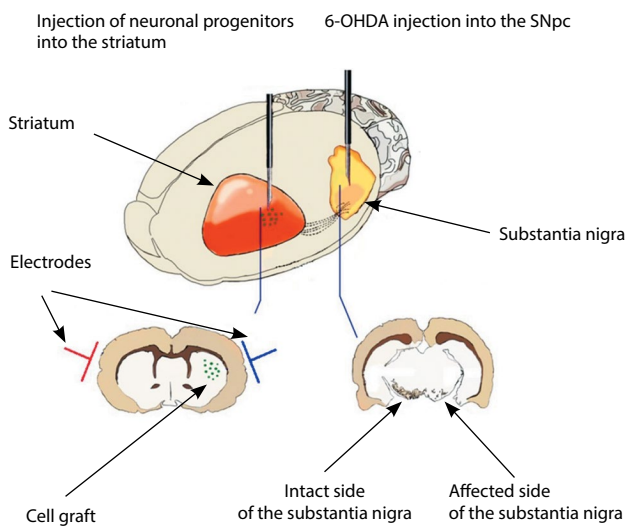

Fig. 1. Schematic representation of modeling Parkinson's syndrome and subsequent NT.

The 6-OHDA-injected rats that underwent NT were divided into 3 groups (8 animals each). The rats from group T + tDCS underwent tDCS with the new stimulator; group T + S underwent sham stimulation and sedation, and group T had neither stimulation nor sedation.

Bilateral tDCS

tDCS began on day 5 after the transplantation of ventral mesencephalic neuronal progenitors into the dorsolateral caudate nucleus.

The designed autonomous electrical stimulator is a microprocessor-based programmable device, which can be considered a generator of various stable current types used for tDCS in laboratory animals. The device consists of a programmable master oscillator, a multichannel voltage-to-current converter, a power supply, and control hardware. The master oscillator, based on a microprocessor of the selected series, uses software to generate a pulse-code modulation data stream, describing the current's waveform, amplitude, and time characteristics (frequency and duration). All stimulation parameters are set via the control panel and displayed on the screen.

Data are transferred between the device blocks and circuit elements via a common I2C interface, an industry-standard solution with low cost but sufficient speed and reliability. Then the data stream through the galvanic isolation based on ADuM microcircuits goes to the MCP4725 digital-to-analog converter. Galvanic isolation is needed to ensure the electrical safety of the device and improve noise immunity.

The digital-to-analog converter converts the data stream into an analog signal, a voltage that varies with the data stream and is used as the control signal for the stable current generator. Then the signal is fed to the input of the stable current generator, designed to form the actuating signal, a time-varying current of the parameters set by an experimenter.

The use of a microprocessor enabled to flexibly change the stimulation current parameters according to the experiment aims.

Device specifications:
- up to 16 channels;
- frequency range of 0 (DC) to 80 Hz;
- current range of 0 to 1 mA;
- various pulse waveforms, including rectangular, triangular, sinusoidal, and noise-like signals.

Prior to tDCS the rat was immobilized by the intramuscular injection of 0.5 mL/kg of 0.5% dexmedetomidine solution (Dexdomitor, Orion Pharma) and placed on a pad with thermal insulation properties to prevent hypothermia. We used a 0.5% solution of hypromellose (Iskusstvennaya sleza, Firn M) to prevent damage to the cornea. The fur from the temporal regions was carefully removed to improve adhesion and reduce electrical resistance; the skin of the temples was degreased, and a part of MedTab electrodes (23×34 mm, Ceracarta) was symmetrically placed on the temporal regions so that an imaginary line through their centers intersected the geometric center of the cell graft (Fig. 2). The anode was placed on the contralateral side of the graft and the cathode on the ipsilateral side. Using a stimulating device, a direct current of 0.5 mA was applied to the electrodes for 20 minutes; then the electrodes were disconnected, and the residual adhesive layer of the electrodes was removed from the temporal regions with water.

The rat was returned to its home cage and 30 minutes later injected with 0.2 mL/kg of a 0.5% solution of atipamezole (Antisedan, Orion Corporation) intramuscularly to accelerate recovery from sedation. The time between the end of the stimulation session and the atipamezole injection is needed to prevent rats from scratching the skin under the electrodes, which may be caused by paresthesia at the electrode sites and is a very common adverse effect [19].

The stimulation sessions were performed once a day at the same time for 14 consecutive days. During the first stimulation procedures, the temperature of the electrodes and surrounding skin was monitored by an infrared pyrometer (Raytek).

Fig. 2. Simultaneous tDCS in 4 rats.

In sham tDCS, all procedures were performed similarly to those described above, but no electric current was applied to the electrodes.

Behavioral effects

Behavioral effects of exposure to a toxicant followed by NT and tDCS were assessed by changes in the rats' motor activity during open field (OF) and beam-walking (BW) tests. The OF test duration was 3 minutes, and the test was performed three times: before cell injection, at 3 weeks, and at 3 months. Rat behavior was recorded using the ANY-maze video tracking system (Stoelting Inc.).

In the BW test, the animal had to walk across an elevated beam from one end to the home cage. We recorded the walking time and the percentage of slips in relation to the total number of steps to cross the beam. In this experiment, we also assessed the psychoemotional state of the rats with an anxiety scale [20, 21]. We recorded non-standard behavioral activities that could be attributed to external signs of anxiety-related behaviors: compulsive head turns, chewing movements, active sniffing and licking of the beam, circling, backward gait, grooming, diaphragm contractions, ptosis, etc. The rats were trained to perform the BW test for 3 days, 2 sessions per day, 1 hour apart, before stereotactic brain surgery. The maximum test time was 100 seconds.

Factorial analysis of variance (ANOVA) was used to determine the statistical significance of differences, and Fisher's post hoc test was employed to compare the groups. The differences were considered significant at $p<0.05$. The data are presented as the mean ± standard error of the mean.

At 3 months after NT and at the end of physiologic examination, half of the rats from each group were decapitated, and the brains were extracted for immunohistochemistry.

Immunohistochemistry and Morphometry

The brain specimens of 4 rats from each group were used in the immunomorphologic study. For morphologic evaluation of the graft 3 months after the cell injection, the animals were decapitated. The brains were fixed for 24 hours in 10% formalin. Frozen frontal sections (10 μm thick) were used for the study. Antigen retrieval was performed by heating in citrate buffer (0.01 M, pH 6.0). The sections were incubated with primary antibodies for 18 hours at room temperature, and corresponding secondary antibodies labeled with Atto 488 or Atto 555 fluorochromes (Invitrogen) were used to detect binding. The sections were further stained with DAPI. Antibodies against human nuclear antigen (HNA) and species-specific antibodies against human neuron-specific enolase (NSE) were used to de-

tect graft cells. Furthermore, antibodies against synaptophysin (SYP) were used to assess graft integration. Transplantation outcomes were previously characterized using an expanded panel of neuronal and glial marker proteins [13].

On the frontal sections using a ×4 objective, we estimated the cross-sectional area of the graft in the striatum by NSE detection. We selected at least 3 sections that showed the needle track at the full depth of insertion. The NIS-Elements software was used to calculate the area in the images.

The data are presented as median and interquartile ranges. The Mann–Whitney test was used to compare the groups.

Results

All the animals tolerated the surgical procedures and tDCS well, and their condition was satisfactory throughout the study. Regular daily examinations by a veterinarian did not reveal any changes in bowel and bladder functions, porphyrin discharge around the eyes and nose, or alopecia. No neoplasms were found during autopsy after the decapitation.

Behavioral tests were performed before the administration of ventral mesencephalic neuronal progenitors (test 1: 25 days after the 6-OHDA administration in the SNpc), at the end of the tDCS course (test 2: 3 weeks after NT), and at 3 months after NT of ventral mesencephalic neuronal progenitors (test 3).

Fig. 3 shows the distance traveled in the OF test by the control animals from groups C1 and C2 that did not receive a cell graft. 6-OHDA administration resulted in a statistically significant decrease in motor activity, which was observed in all the tests: 13.990 ± 0.881 and 6.387 ± 1.112 (ANOVA, p (pA) = 0.0005) in test 1, 13.469 ± 1.572 and 6.439 ± 1.406 (pA = 0.0007) in test 2, and 13.076 ± 1.406 and 6.404 ± 1.575 (pA = 0.0013) in test 3 in groups C1 and C2, respectively.

Fig. 4 shows changes in motor activity of the model rats following neuronal progenitor transplantation into the dorsolateral caudate nucleus. It should be noted that by the time of test 2, a part of the rats (group T+tDCS) had undergone a tDCS course. Fig. 4 shows that locomotor activity remained at the level recorded before cell administration in group T+S receiving dexdomitor for sham tDCS (5.946 ± 1.011 and 5.233 ± 1.229; pA = 0.9436), in contrast to the significantly decreased in the rats without sedation (3.006 ± 0.601 and 6.996 ± 1.178; pA = 0.0227). In the rats after tDCS, the distance traveled in the OF test more than doubled: 14.069 ± 1.094 and 5.635 ± 1.511 (pA = 0.0014). The motor activity at 3 months after NT remained unchanged in all the groups with the graft compared with the test 1 results.

The BM test at 3 weeks after NT also revealed significant differences between groups C1 and C2, most of which were the refusal of the 6-OHDA-injected rats to walk along the beam and

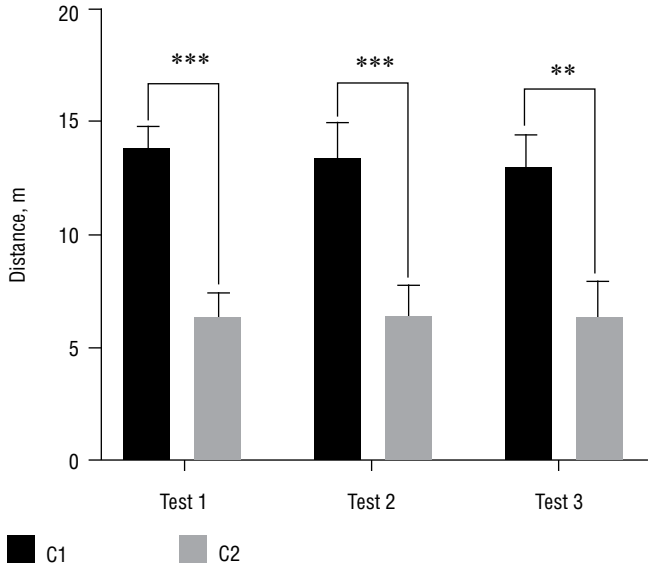

Fig. 3. Motor activity assessment by the OF test in the rats. *p_A < 0.05 compared with group C2.

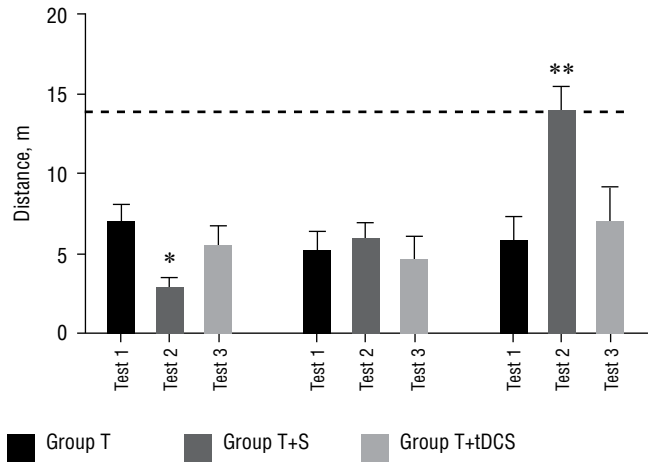

Fig. 4. Distance traveled in the OF test by the model animals after NT. *p_A < 0.05 compared with test 1.

distinct (pA = 0.01) signs of anxiety-related behaviors (Fig. 5, *A*). Therefore, it was not possible to process the numerical values of the number of stumbles using statistical methods. The ventral mesencephalic neuronal progenitor administration and tDCS course had no effect on the rats' movement along the beam. However, while groups T and T+S exhibited anxiety-related behaviors, which scores were significantly different compared with group C1 (8.00 and 7.83 vs 2.67 scores; pA = 0.0005 and pA = 0.001, respectively), group T+tDCS had significantly lower scores and no statistically significant differences with group C1 (4.71 and 2.67; pA = 0.139). This parameter was also significantly different compared with groups T and T+S (pA = 0.017 and pA = 0.029, respectively). In test at 3 months after NT, the difference in these parameters between the groups leveled off, which is consistent with the data of the OF test (Fig. 5, *B*).

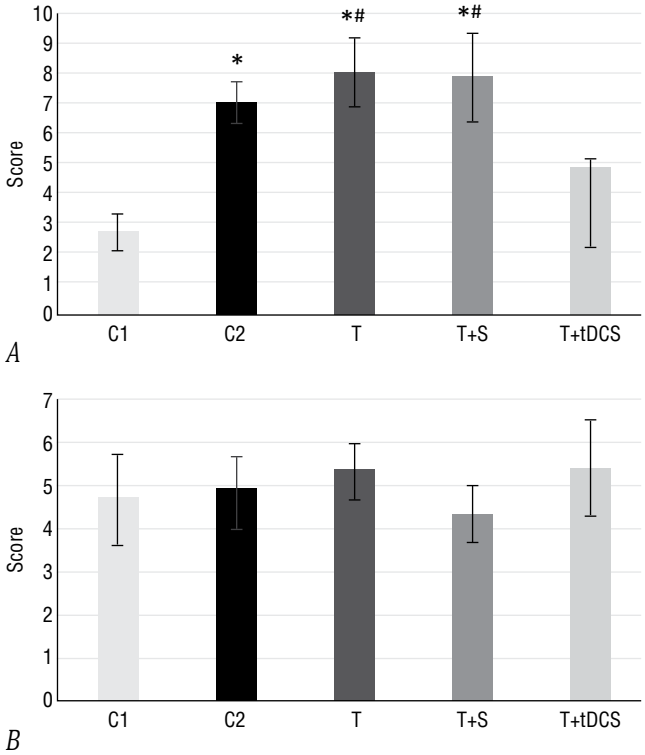

Fig. 5. Anxiety-related behavior score in the BW tests at 3 weeks (*A*) and 3 months (*B*) after NT.
***$p_A < 0.05$ compared with group C1; #$p_A < 0.05$ compared with group T+tDCS.**

Previous morphologic studies of grafts have shown decreased staining for tyrosine hydroxylase on the side of 6-OHDA injection [12, 13], indicating damage to SNpc neurons. Also, by month 3, 3% to 5% of dopaminergic neurons were detectable in the graft, and we did not observe migration of cells expressing mature neuronal markers outside the graft area.

The animals subjected to tDCS had larger graft sizes compared with the controls (Fig. 6). Previously we showed the zonal structure of grafts in animals without tDCS exposure [13] with predominant localization of NSE+-cells (mature neurons) in the central zone and formation of glial sheath around the graft. Due to tDCS the graft morphology changed: after the stimulation there was a shift of NSE staining to the marginal zone of the graft, which was located outward. The graft size in the striatum was significantly greater ($p = 0.002$, Mann-Whitney test) after tDCS. In the control group, the median area of NSE^+ staining was 1.695 [1.45; 1.89] mm^2, and it was 4.04 [3.08; 6.03] mm^2 as a result of tDCS. The central regions in the group after tDCS comprised HNA^+ cells with low NSE expression. The stimulation was likely to provoke division of some cells in the early stages of differentiation, which should be further investigated. The detection of SYP may indicate the synaptogenesis in the graft by month 3. We have previously shown an increase in SYP expression as neurons mature [13]. The more pronounced staining for SYP

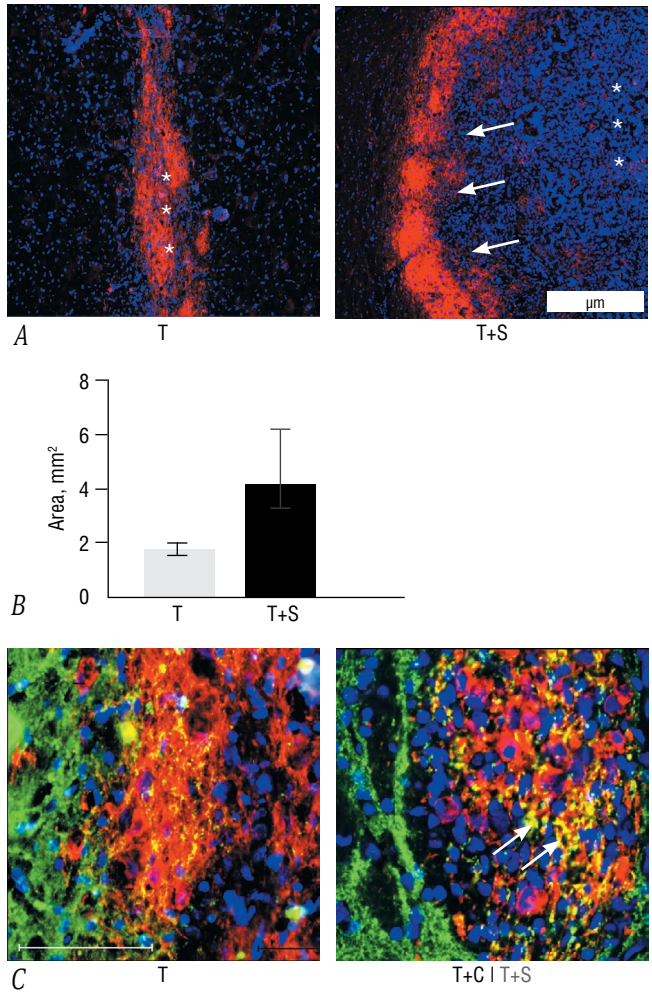

Fig. 6. Localization of transplanted neurons in the control group (day 24 of differentiation) and after tDCS at 3 months following the transplantation.
***A* – shift of NSE^+ cells (shown in red) to the marginal zone of the graft (arrows), the central zone is indicated by asterisks; *B* – graft size; *C* – increase in SYP (shown in green) and NSE (shown in red) colocalization areas caused by tDCS (arrows). Cell nuclei were counterstained with DAPI (shown in blue).**

and overlap with NSE+ structures may reflect the effect of tDCS on the formation of synaptic contacts with transplanted neurons.

Thus, the morphologic study showed the effect of tDCS on development and morphologic characteristics of the graft and cell migration within the graft area. No pathologic changes in the structures surrounding the graft were detected. tDCS appears to have an effect on both differentiation and migration as well as integration of graft neurons, which should be further studied.

Discussion

NT is one of the promising therapies for PD. Alleviation of motor symptoms in PD is its main expected behavioral ef-

fect. This effect has been mostly shown in studies on NT of embryonic ventral mesencephalic dopaminergic neurons [22, 23]; however, the injection of such cells raised ethical concerns and caused severe graft-induced dyskinesias. Another source of cell grafts with autologous dopaminergic neurons is iPSCs obtained via reprogramming fibroblasts using expression of peptide pluripotency factors in them [24], followed by in vitro differentiation of iPSCs into neurons according to different protocols [11]. The following parameters serve as criteria of morphofunctional correspondence of dopaminergic neurons differentiated from iPSCs to native dopaminergic neurons: survival of transplanted neurons, intensity of neurite growth from the graft, formation of a diffuse network of dopaminergic terminals in the striatum, dopamine release, their bioelectrical activity, as well as recovery of lost motor functions in animals with a PD model [25]. Our studies using a similar differentiation protocol have previously shown the development of dopaminergic neurons and the formation of their outgrowths in the graft by month 3-6 [12, 13]. Transplantation of neuronal progenitors in animals with PD models has shown certain advantages over fetal cell transplantation, but the positive results achieved are still not well reproducible [25, 26] due to a number of factors: the type and quality of transplanted cells, the PD model used, and individual characteristics of recipient animals. Optimizing these factors will improve treatment efficacy and stability of behavioral effects.

An independent promising therapeutic approach in neurodegenerative diseases is the use of non-invasive neuromodulation methods [14, 27]. They include various forms of low-intensity transcranial electrical stimulation; direct current stimulation is the most studied, and its effects on neuroplasticity in the motor cortex are polarity-dependent. In this study, we focused on cathodal polarity, in which the resting membrane potential is hyperpolarized (in contrast to anodal polarity, in which the resting membrane potential is depolarized) [28]. Cathodal tDCS using standard protocols reduces cortical excitability and can induce homosynaptic long-term depression in case of sufficiently long stimulation duration. Apart from duration and intensity, the stimulation repetition is a crucial factor in cathodal tDCS efficacy, affecting the duration of the neuroplastic effect. The mechanisms underlying the beneficial effects of tDCS are not yet fully understood; animal models, especially those involving rodents, facilitate their studying, testing the method safety, and optimizing stimulation parameters [29–31]. When selecting stimulation parameters, we were guided by the literature data because we have not previously conducted such study [15, 16, 32].

tDCS was shown to have a beneficial effect on differentiation and survival of transplanted cells [15, 16]. In our previous studies [12, 13] we found that functional maturation of transplanted neurons occurred within 3 months after transplantation, and the greatest changes in the expression of cell differentiation proteins were observed within 1 month and continued up to 3 months after transplantation, which determines the possible time frame of the tDCS effect on the graft in terms of neuronal maturation improvement. It should be noted that in some experiments [12], the graft contained a mixed glioneuronal culture, and part of the cells yielded an astrocyte population. Researchers discuss possible mechanisms for the tDCS effect on astrocytes [33], which may have a significant impact on both the host astroglia response in transplantation and donor astrocytes when mixed cultures are used. Thus, combining tDCS and NT may be a promising approach for PD therapy.

The observed increase in graft size and changes in graft morphology may indicate the direct effect of tDCS on the transplanted cells, their maturation and integration into the recipient's striatum. That being said, the tDCS effects on the graft behavior and development may be caused by a number of factors: the effect of the striatum, neocortex, and other brain structures, involved in the motor activity regulation in animals, on neurons via changing the balance between excitatory and inhibitory inputs [34], the effect on glial cells, including anti-inflammatory effects [35], increased expression of BDNF [36] involved in plastic changes in the nervous system, etc.

Although animal models are a powerful tool in identifying neurobiological mechanisms of tDCS action, finding a current generator, which is easy to use and allows for a wide range of stimulation parameters, can be challenging and/or expensive [37]. In most cases, Russian researchers use foreign devices, for example, Alpha-Stim (Electromedical Products International, Inc.), when studying the effects of tDCS. Such devices are designed for tDCS procedures to treat anxiety, insomnia, depression, and pain. They are effective, safe, easy to use, and received necessary regulatory approvals. However, some design features limit their use in laboratory setting: the waveform of the generated pulses, frequency range, current range, and pulse duration.

Experimental conditions for studying transcranial electrical stimulation require a much wider range of stimulating current parameters: eg, a current in the form of sinusoidal pulses with a constant component or a noise-like signal. The technical limitations of transcranial electrical stimulation devices dictated the need to develop an original device designed primarily for laboratory use and free from the disadvantages of existing and commercially available devices. Specialists from the Bauman Moscow State Technical University designed and engineered a prototype of such stimulator. The Beta-Stim device is a programmable stable current generator with the frequency range from DC to 80 Hz, an arbitrary (set by the experimenter) form of the signal, and a current range from 1 μA to 1 mA. It was designed for experiments on small rodents. The device is easy to operate. It is built with many freely available Russia-produced components, free from license and patent restrictions.

In this study, 6-OHDA-induced Parkinson's syndrome was modeled in Wistar rats. This model is most convenient for studying the potential of NT of dopaminergic neuronal progenitors into the caudate nuclei of the rat brain. NT is known to temporarily worsen symptoms in the early postoperative period [38], which seems to explain the decreased motor activity in group T detected 3 weeks after reoperation. The motor activity later returned to the preoperative levels. The maintained level of motor activity in group T + S might be linked to the anti-inflammatory effect of dexdomitor used for sedation. NT was combined with a tDCS course. We observed positive effects of tDCS on motor activity and emotional state in group T + tDCS. The rats from groups T + S, C2, and T showed signs of anxiety-related behavior, which allows to rule out the possibility of a dexdomitor effect on this parameter. Behavioral testing 3 months after NT did not reveal any differences between the groups, which may indicate that our chosen mode for tDCS has short-term effects.

Conclusion

The model rats with PD tolerated well tDCS and transplantation of dopaminergic neuronal progenitors using the developed specialized laboratory stimulator.

The findings of physiologic and morphochemical studies indicate the tDCS effects on graft development and structure, as well as on changes in motor and non-motor symptoms in rats after NT.

Thus, the combination of NT and tDCS in PD models, particularly those induced by the 6-OHDA injection into the SNpc, demonstrates the potential to manage both motor and non-motor symptoms. However, further optimizing protocols of transplantation and tDCS and evaluating their long-term efficacy and safety are required to successfully implement this method into clinical practice.

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