THE ROLE OF MCT1 TRANSPORTERS IN THE IMPLEMENTATION OF THE NEUROPROTECTIVE EFFECT OF GLIAL CELL-DERIVED NEUROTROPHIC FACTOR

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Abstract. The search for new molecular targets to protect brain cells from ischemic damage has remained one of the most urgent tasks of neurobiology and medicine over the past decades. The modern concept of the glucose-lactate shuttle, the main mechanism for providing energy support to neurons under conditions of increased physiological activity, implies a functional fusion of neuron and astrocyte metabolism, which becomes particularly important under energy or oxygen starvation conditions. The transfer of energy substrates in response to increased glutamate release by the presynaptic terminal occurs through monocarboxylate transporters (MCTs) and depends on a large number of endogenous mechanisms of homeostasis. Our work examined the role of MCTs in the implementation of the neuroprotective effect of glial cell-derived neurotrophic factor (GDNF), one of the key participants in the regulatory system capable of maintaining the viability and functional activity of neurons under conditions of energy starvation. It has been shown that the application of the MCT1 transporter inhibitor, even under normal conditions, significantly affects the parameters of spontaneous bioelectrical activity of neural networks and cell viability of primary dissociated hippocampal cultures. However, the authors' data on the role of this type of transporters in glucose deprivation and the effect of MCT blockade on the neuroprotective effects of GDNF are of the greatest interest.

Keywords: ischemia, MCT, GDNF, astrocytes, primary hippocampal cultures, functional neural network activity, neuroprotection, astrocyte-neuron lactate shuttle.

List of Abbreviations

ANLS – astrocyte-neuron lactate shuttle
DIV – day in vitro
GD – glucose deprivation
GDNF – glial cell-derived neurotrophic factor
GLUTs ¬glucose transport proteins
MCT – monocarboxylate transporter
SLC – solute carrier

Introduction

Cerebral infarction is a common pathology that remains one of the leading causes of death and disability in developed countries. The study of the pathogenetic mechanisms of ischemia attracts the attention of researchers around the world. The research of the last decades aimed to find new effective ways to preserve the viability of brain cells during the development of ischemic damage. One of such promising molecules of endogenous origin is the glial cell-derived neurotrophic factor (GDNF). To date, the main molecular cascades of the neuroprotective action of this neurotrophic factor have been disclosed; however, the role of GDNF in the regulation of energy metabolism under stress is a complex, currently unsolved problem (Li *et al.*, 2019).

It is known that one of the most important functions of astrocytes is to provide neurons with energy substrates, the supply of which is impaired under ischemic damage. Astrocytes are able to store glycogen, which can be rapidly converted into lactate or pyruvate, metabolized in the tricarboxylic acid cycle, or used for glutamate biosynthesis and glucose synthesis. There is a molecular mechanism in which astrocytes and neurons are metabolically linked. This mechanism is called the astrocyte-neuron lactate shuttle (ANLS), the concept of which was formulated in 1994 (Pellerin & Magistretti, 1994). According to the lactate shuttle model, lactate released from astrocytes enters nerve cells, is converted to pyruvate, and then directly used in the tricarboxylic acid cycle for energy production (Belanger *et al.*, 2011). Glucose and lactate transport between brain cells is per-

formed by glucose transport proteins (GLUTs) and monocarboxylate transporters (MCTs), respectively; these are transmembrane proteins belonging to the solute carrier (SLC) transporter superfamily. Lactic acid produced by astrocytes has been shown to be important for maintaining neuronal activity during glucose deficiency (Wyss *et al.*, 2011). The most energy-consuming process is synaptic transmission; therefore, energy-intensive astrocytic support is a key condition for maintaining the functioning of neural networks. Thus, astrocytes are the key compensatory mechanism for neurons under conditions of reduced energy exchange during the development of ischemic injury.

On the other hand, activation of glycolysis during ischemic injury and, as a consequence, increased production of lactate by glial cells leads to an increase in its concentration from 1-3 mmol/L in the norm to 10–50 mmol/L in the area of infarction (Banerjee *et al.*, 2016). Lactate from this area diffuses into the penumbra region, where it acts as a signaling molecule by binding to orphan lactate receptors. Increased lactate concentration leads to tissue acidosis in the area of infarction; pH decreases from 7.0 to 6.6 (Regli et al., 1995), which results in apoptosis of neurons and astrocytes (Shen *et al.*, 2015). In addition, lactate can induce the production of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin (IL)-6, and IL-8 by glial cells. Thus, the role of lactate and lactic acid in ischemic injury is quite multifaceted.

Disruption of the neuron-glial interaction, in particular, due to disruption of the MCTs, can lead to the development of a number of damaging reactions and, ultimately, disruption of the neurons' functioning and death. To date, the question of the ability of astrocytes through these transporters to have a protective effect on neurons in conditions of acute glucose deficiency, as well as the search for substances that can support this process, remains open (Curcio *et al.*, 2015).

In this regard, this work aims to study the effect of the MCT1 transporter blockade on the functional activity of neural networks in the model of glucose deprivation in vitro and to evaluate the potential role of MCT1 in the implementation of the neuroprotective effect of GDNF.

Materials and Methods

Research object

Primary hippocampal cultures obtained from 18-day-old embryos of C57BL/6 mice were used in the study. The basic rules for keeping and caring for animals corresponded to the bioethical standards specified in the Guide for Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press), National state standard of Russian Federation (GOST) No. 33044-2014 «Principles of good laboratory practice», Order of the Ministry of Health and Social Development of the Russian Federation No. 708n «On Approval of the Rules of Laboratory Practice» dated August 23, 2010, and approved by the Ethics Committee of Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation. The biological material was disposed of in accordance with the Sanitary Rules and Norms N2.1.7.2790-10 dated December 12, 2010, «Sanitary and Epidemiological Requirements for the Management of Medical Waste».

Cultivation of primary hippocampal cultures

Hippocampal cells were obtained from 18-day-old embryos of C57BL/6 mice. The cells were dissociated by treating hippocampal tissue with 0.25% trypsin (25200-056, Invitrogen, USA) with subsequent resuspension in Neurobasal medium (21103-049, Invitrogen, USA) supplemented with bioactive supplement B27 (17504-044, Invitrogen, USA), glutamine (17504-044, Invitrogen, USA), fetal calf serum (K055, PanEco, Russia), and cultured on coverslips and multielectrode arrays pre-treated with polyethyleneimine (P 3143, Sigma-Aldrich, Germany). The viability of cells was maintained in a CO2 incubator at a temperature of 35.5 °C and a gas mixture containing 5% CO2. The culture medium was changed day after culture's preparation and then every two days (Vedunova *et al.*, 2013).

Modeling of glucose deprivation

Glucose deprivation was modeled at 14 DIV by replacing the culture medium with a medium containing no glucose, pyruvate, and lactate for 90 minutes. A specially designed medium matching the composition of Neurobasal™ but not containing the listed energy substrates was used (Vedunova *et al.*, 2013). GDNF at concentration of 1 ng/ml (GF030, Merk KGaA, Germany) and/or MCT1 transporter inhibitor SR13800 (5.09663, Merk KGaA, Germany) were added to the culture medium twenty minutes before the modeled stress.

Cell viability assessment

Cell culture viability was evaluated by staining with bisbenzimide (Hoechst33342, Sigma) and propidium iodide (P4170, Sigma-Aldrich, Germany). Visualization was performed using a Zeiss Axio Observer A1 fluorescent microscope (Zeiss, Germany). The number of dead cells was defined as the ratio of bisbenzimide- to propidium iodide-positive cells. Cell counts were performed in at least ten fields of view for each culture. The number of biological repeats in each group of cultures was at least three.

Analysis of spontaneous bioelectrical activity

Multielectrode arrays MEA60 (Multichannel system, Germany) were used to assess the spontaneous bioelectrical activity of primary hippocampal cultures. Multielectrode arrays are based on planar microelectrode technology and allow long-term recording of extracellular field potentials of neuronal cultures. Registration of neural network bioelectrical activity was carried out before glucose deprivation modeling, and on days 1, 3, 5, and 7 after the modeled stress.

The MC Rack™ software (Multichannel Systems, Germany) was used to obtain data. The obtained data were analyzed in the MATLAB software environment using the original MEAMAN algorithm set (state registration certificate for computer program No. 2012611190, Vedunova *et al.*, 2013) with subsequent statistical assessment in Sigma Plot 11.0 software (Systat Software, Inc.).

Statistical analysis

Data are presented as the Mean ± standard error of the mean (SEM). Statistical analyses were performed using two-way ANOVA implemented in Sigma Plot 11.0 software (Systat Software, Inc.). The Tukey post hoc test was used as a post hoc test following ANOVA. Differences between groups were considered significant if the corresponding p-value was less than 0.05.

Results

First, we analyzed the effect of different concentrations of the MCT1 transporter inhibitor on the cell viability of primary hippocampal cultures in normal conditions. The viability assessment was performed on day 3 after the application of the inhibitor (Table 1).

The MCT1 inhibitor SR13800 concentration of 15 nM was shown to have a pronounced toxic effect (15.1 ± 4.75% of viable cells). When the inhibitor is added at a concentration of 7.5 nM, a significant ($p < 0.05$, ANOVA) decrease in the number of viable cells to 63.8 ± 5.91* was observed. When exposed to the MCT1 inhibitor at a concentration of 5.5 nM, the number of dead cells also significantly ($p < 0.05$, ANOVA) increased relative to the «Intact» group; when using a concentration of 2.5 nM, there were no significant differences in viability from intact cultures. Since the glucose-lactate shuttle is necessary for the normal functioning of neural networks, concentrations of 5.5 and 7.5 nM were chosen for further studies as they have a physiological effect and minimal toxicity under normal conditions.

Next, we studied the effect of the MCT1 inhibitor on spontaneous bioelectrical activity of primary hippocampal cultures. Registration of the main parameters of neural network activity was carried out before a single addition of the MCT1 inhibitor, for 1.5 hours during its exposure and on days 1, 3, 5, 7 after the experiment. Three main parameters were evaluated: the number of small network bursts, the number of spikes in a burst, and the duration of small network bursts. Each the studied time point was compared to the initial level of bioelectrical activity.

Table 1

Cell viability of primary hippocampal cultures three days after application of MCT1 transporter inhibitor (17 DIV)

Group of cultures	Share of viable cells, %
Intact	95.5 ± 1.24
MCT1 inhibitor 2.5 nM	88.4 ± 6.31
MCT1 inhibitor 5.5 nM	75.5 ± 3.01*
MCT1 inhibitor 6.5 nM	63.8 ± 5.91*
MCT1 inhibitor 15 nM	15.1 ± 4.75*

* – the differences are significant compared to the "Intact" group, $p \leq 0.05$, ANOVA

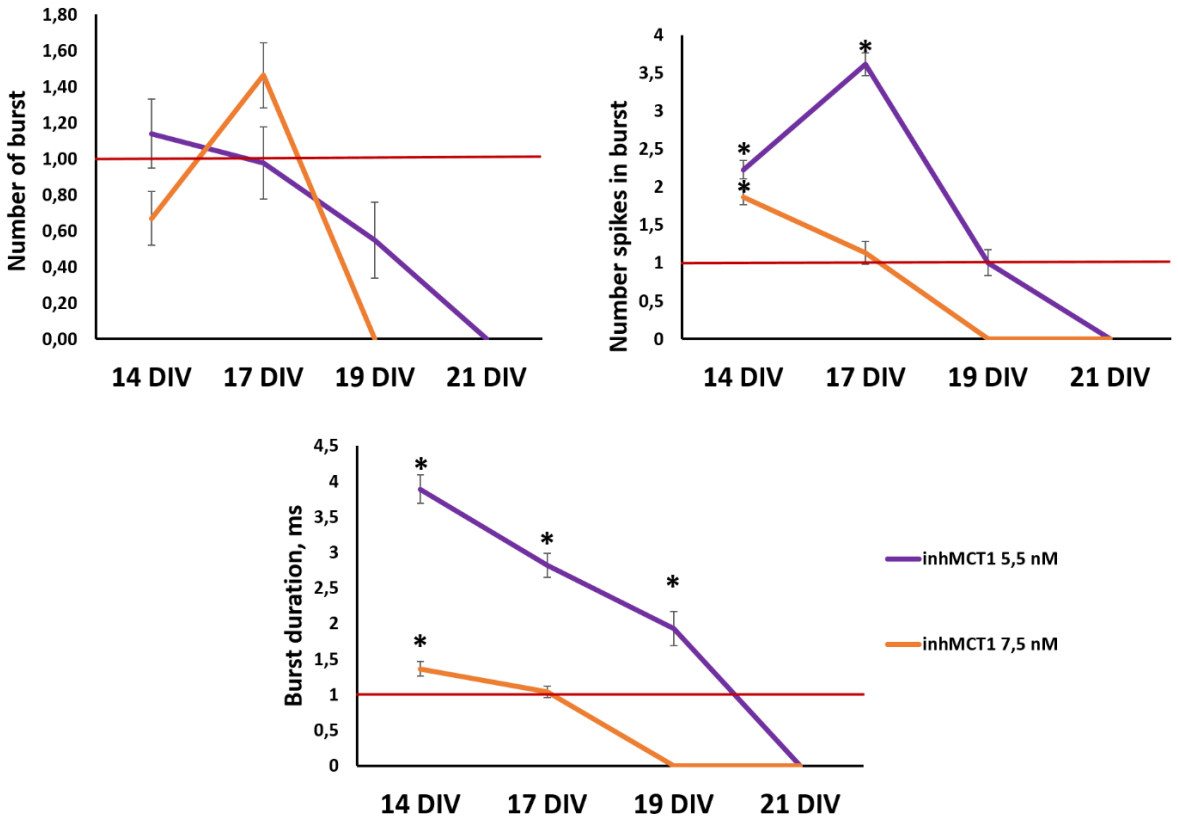

Fig 1. Changes in the main parameters of spontaneous bioelectrical activity of primary hippocampal cultures within 7 days after the application of the MCT1 inhibitor; the data are normalized to the activity of «Intact» group. * – statistically significant difference from the initial level, $p \leq 0{,}05$, ANOVA

It was shown that MST1 blockade with the SR13800 inhibitor at a concentration of 5.5 nM increased the number of small network bursts by 40% on the next day after treatment. Further, there was a gradual decrease in bursting activity, which ceased completely 7 days after the application of the inhibitor (Fig. 1A). When a high concentration of the MCT1 transporter inhibitor (7.5 nM) was added to the culture medium, the activity of neural networks ceased already after 3 days. At the same time, the activity of the «Intact» group of cultures remained at a constant level.

It was shown that the blockade of MCT1 transporters with an inhibitor at a concentration of 7.5 nM on the first day after exposure leads to a significant ($p < 0.05$, ANOVA) increase in the average number of spikes in a burst – by 1.8

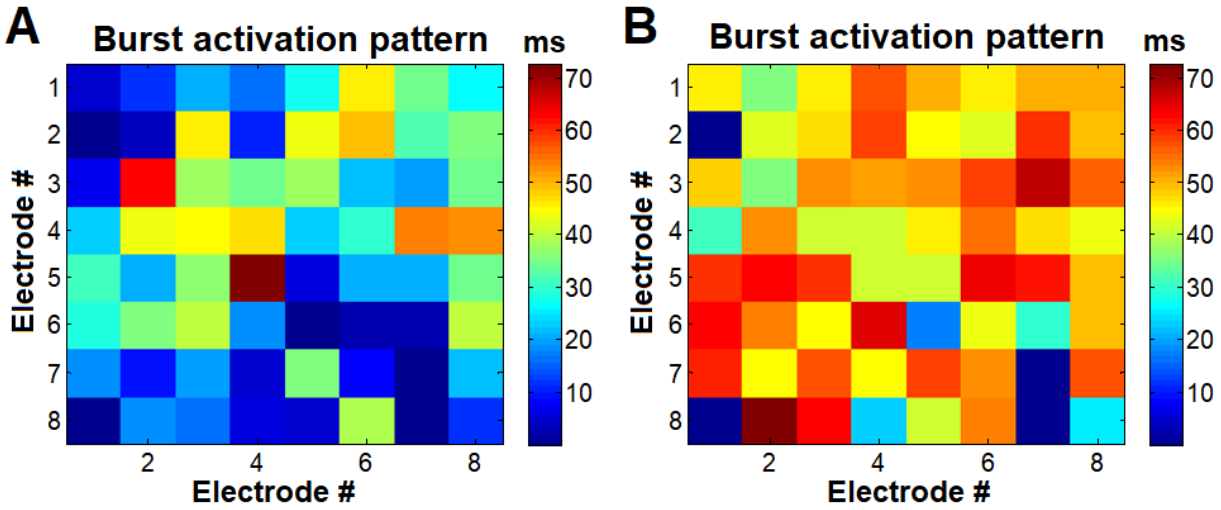

Fig. 2. Typical examples of changes in burst activation pattern in primary hippocampal neurons under the inhibition of MCT1 transporters. The color scale corresponds to the time of occurrence of the first spike in the network burst and is presented in squares according to the electrodes of the multielectrode array. A – before application of MCT1 inhibitor (5,5 nM); B – day 3 after application of MCT1 inhibitor (5,5 nM)

Table 2

Cell viability of primary hippocampal cultures 3 days after the modeling of glucose deprivation against the background of MCT1 transporter inhibition (17 DIV)

Group of cultures	Share of viable cells, %
Intact	94.1 ± 0.98
GD	82.8 ± 2.12*
GD + MCT1 inhibitor 5.5 nM	85.2 ± 2.97*
GD + GDNF	92.3 ± 1.91#
GD + GDNF + MCT1 inhibitor 5.5 nM	87.1 ± 2.75*

* – statistically significant difference compared to the «Intact» group, $p \leq 0,05$ (ANOVA)
# - statistically significant difference compared to the «GD» group, $p \leq 0,05$ (ANOVA)

times; the neural network activity returns to its initial level on day 3 and completely negates by day 5 after exposure (Fig. 1B). Application of the inhibitor at a concentration of 5.5 nM causes a more pronounced increase in the number of spikes in a burst (by 2.18 times on the first day and 4.1 times on the 3rd day) and a significant increase in their duration (Fig. 1C), which persists for 5 days, after which the neural network activity also completely ceases.

The analysis of the activation pattern of the network bursts revealed that on day 3 after the application of the inhibitor (5.5 nM), there was a restructuring of the network activity, which manifested in a delay in signal propagation (Fig. 2).

Thus, blockade of MCT1 transporters leads to a sharp increase in the activity of neural networks in dissociated hippocampal cultures and then to its complete suppression. This indicates that the neuron-glial interaction, which ensures the maintenance of the energy status of neurons, is necessary to maintain the functional activity of neural networks.

Next, we studied the role of MCT1 transporters in maintaining the viability and functional activity of primary hippocampal cultures in glucose deprivation modeling. Cell cultures

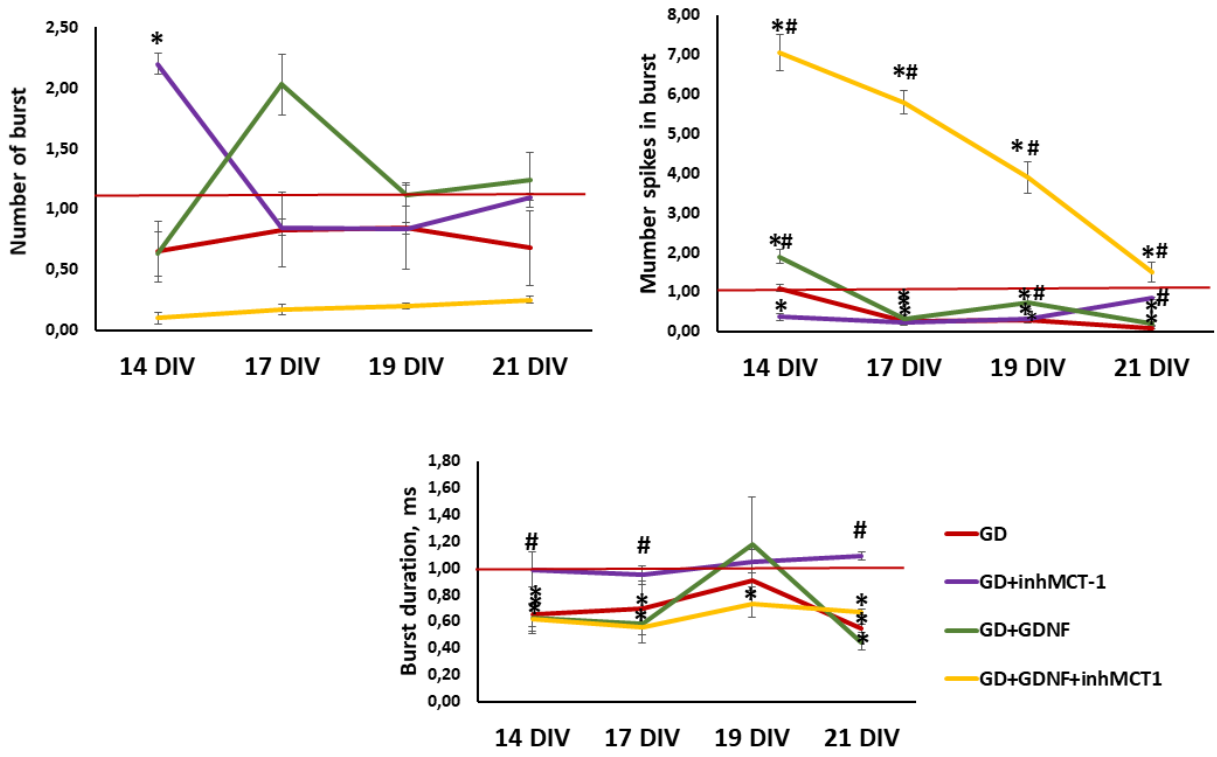

Fig. 3. Changes in the main parameters of spontaneous bioelectrical activity of primary hippocampal cultures within 7 days after the modeling of glucose deprivation (GD). * – statistically significant difference from the initial level, $p < 0.05$ (ANOVA); # – statistically significant difference compared to «GD» group, $p < 0.05$ (ANOVA)

viability was assessed on the third day after exposure.

The studies have shown that glucose deprivation leads to a significant decrease in cell viability in culture (85.8 ± 2.12%, Table 2). In the group with MCT1 transporter inhibition, the viability of cells was amounted to 89.2 ± 2.97. Preventive application of GDNF at a concentration of 1 ng/mL has a pronounced neuroprotective effect, maintaining the viability of primary hippocampal cultures at the level of intact values. Simultaneous application of the MCT1 inhibitor and GDNF completely eliminates the neuroprotective effect of GDNF.

An analysis of the bioelectrical activity of primary hippocampal cultures showed that glucose deprivation has a moderate inhibitory effect on neural network activity manifested in a decrease in the number of spikes in a burst and a decrease in the burst duration (Fig. 3). There is a splitting of normal bursts into smaller ones. Blockade of MCT1 transporters during glucose deprivation caused a short-term increase in the number of bursts on day 1 after GD. Further, the number of small network bursts normalized and remained at the baseline activity level throughout the entire observation period. The number of spikes in a burst in the «GD+MCT1 inhibitor» group decreased significantly relative to the initial level; however, at 21 DIV, there was a trend towards normalization of this indicator. There were no significant changes in the network burst duration in this group.

Preventive administration of GDNF makes allows to partially preserve the neural network activity. On day 1 after GD modeling, there was a 1.9-fold increase in the number of spikes in a burst compared to the initial level. Further, the number of spikes and the duration of bursts significantly decrease compared to the initial level but remain higher than in the «GD» group. It should be noted that the duration of network

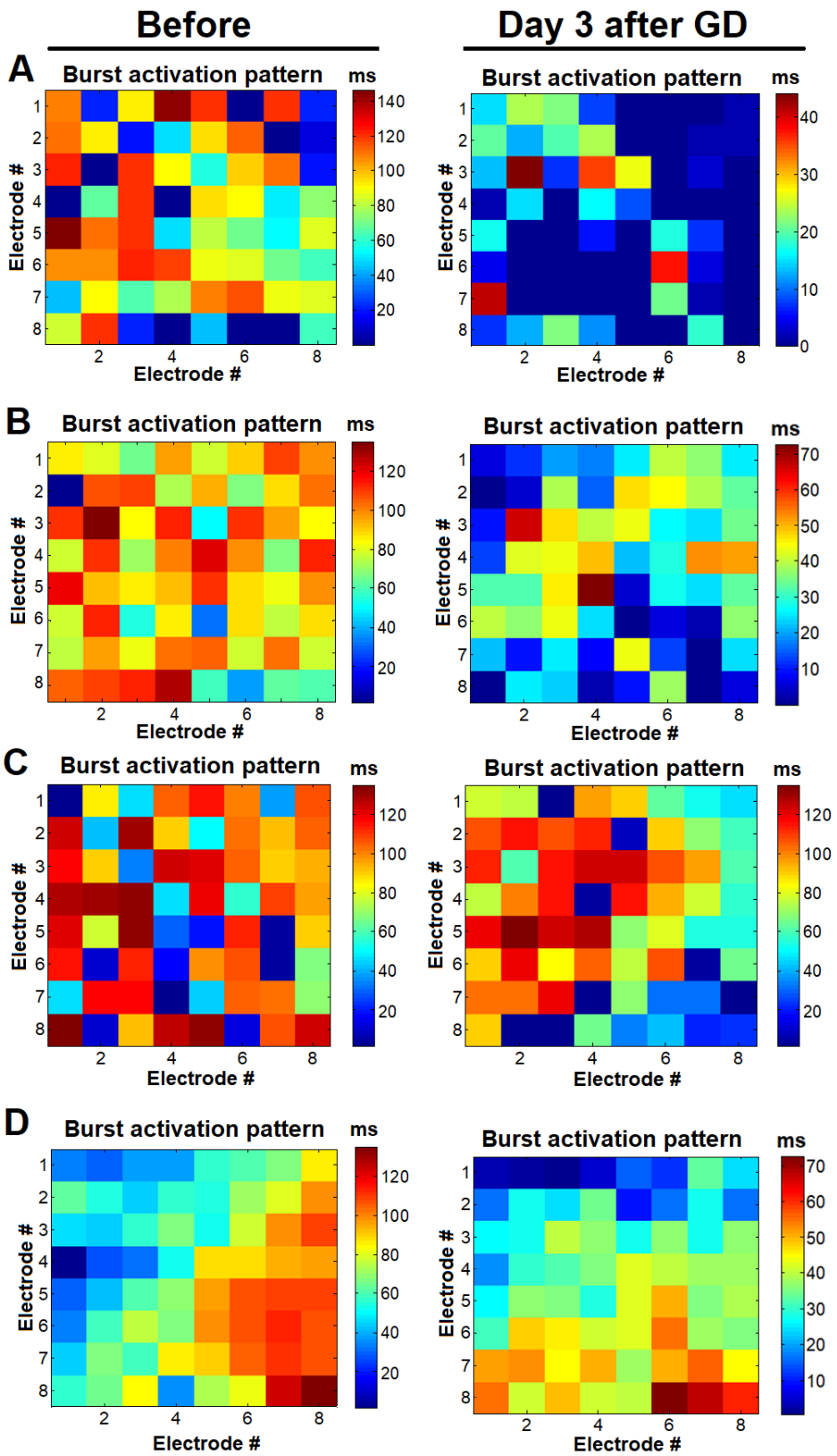

Fig. 4. Typical examples of changes in burst activation pattern in primary hippocampal neurons after glucose deprivation modeling. The color scale corresponds to the time of occurrence of the first spike in the network burst and is presented in squares according to the electrodes of the multielectrode array. A – GD; B – GD+inhMCT (5.5 nM); C – GD+GDFN (1 ng/mL); D – GD+GDFN (1 ng/mL) + inhMCT (5.5 nM)

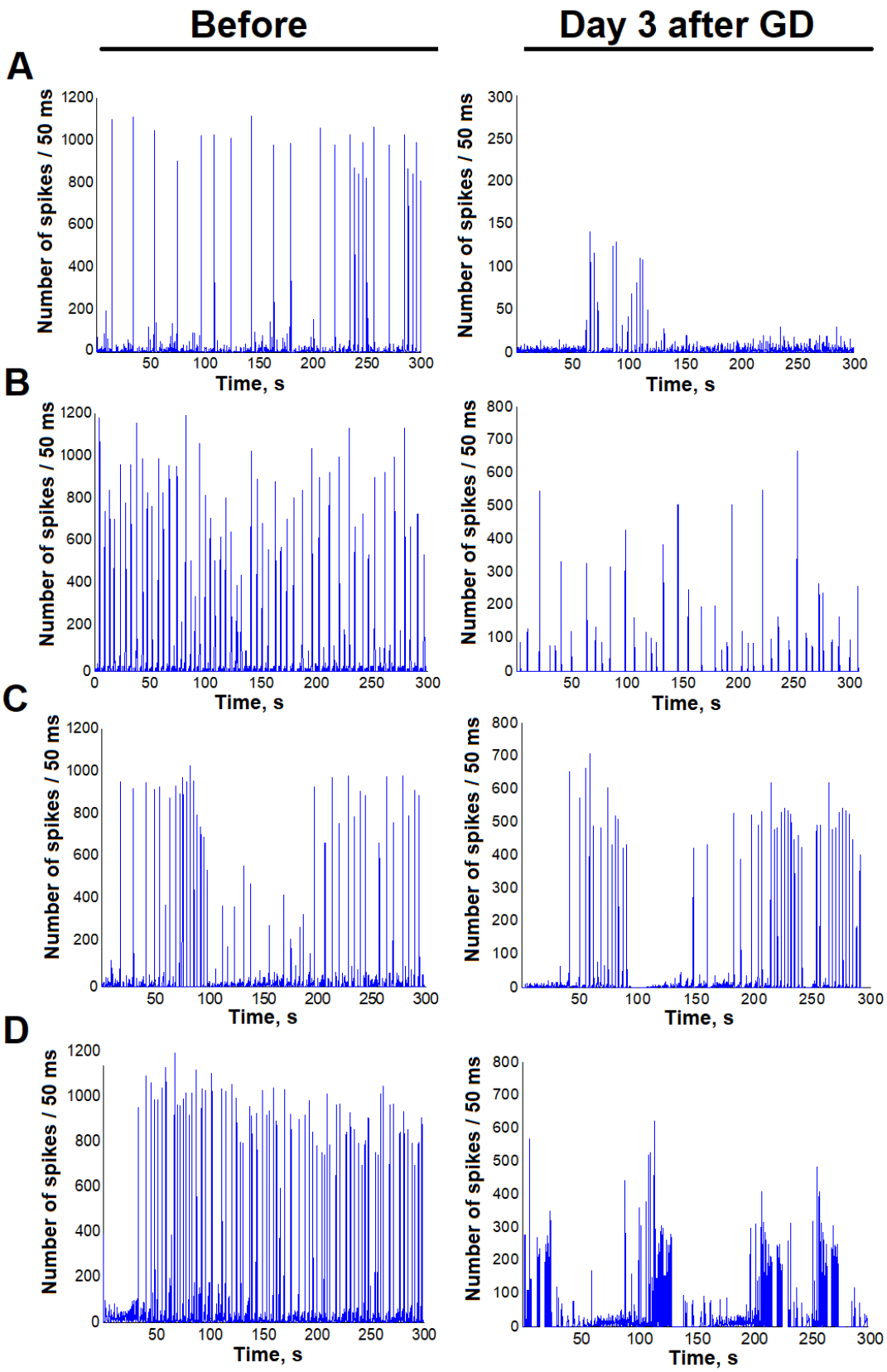

Fig. 5. Typical examples of changes in the spontaneous bioelectrical activity profile in primary hippocampal cultures after glucose deprivation modeling. A – GD; B – GD+inhMCT (5.5 nM); C – GD+GDFN (1 ng/mL); D – GD+GDFN (1 ng/mL) + inhMCT (5.5 nM)

bursts in the «GDNF» group on the first day after GD is significantly lower compared to the «Intact» group and does not differ from the parameters of the «GD» group; on day 7 it significantly decreases relative to both the "Intact" and «GD» group.

Simultaneous addition of GDNF and inhibition of MCT1 under glucose deprivation leads to a sharp increase in the number of spikes in a burst compared to all experimental groups. In the late observation period, the number of spikes in a burst gradually decreases and on day 7 is comparable with the initial values. However, the number of small network bursts in the «GD+GDNF+inhMCT1» group was significantly lower than in the «GD+GDNF» group throughout the entire observation period. The duration of network bursts was also significantly reduced relative to the initial values and did not differ from the parameters of the group subjected to glucose deprivation.

An analysis of raster diagrams of spontaneous bioelectrical activity and burst activation patterns showed that glucose deprivation leads to a significant simplification of the network burst structure, a reduction in the number of spikes in a burst, and a 2.3-fold decrease in the signal propagation speed (Fig. 4, 5).

It was shown that a single injection of the MCT1-transporter blocker under conditions of glucose deprivation, on the third day, a change and simplification of the network burst structure is observed, but not as pronounced as under GD. It is interesting to note that GDNF application against the background of GD makes it possible to preserve the speed of signal propagation, although the activity of some of the electrodes stops (Fig. 4, 5).

Thus, the modeling of glucose deprivation and inhibition of MCT1 leads to inhibition of spontaneous bioelectrical activity of primary hippocampal cultures. Preventive application of GDNF (1 ng/mL) partially reduces the induced damaging effect, increasing the viability of cultured cells and maintaining neural network activity. Inhibition of MCT1 transporters simultaneously with GDNF application leads to hyperactivation of neural networks within 3 days, followed by an irreversible decrease.

Discussion

According to the astrocyte-neuron lactate shuttle hypothesis, astrocytes play an important role in providing neurons with energy in the form of lactate during intense neuronal activity, when the energy demand of neurons exceeds the supply of blood glucose supply. Thus, lactate can be considered a potential neuroprotector and a key signal in the blood flow regulation (Pellerin & Magistretti, 2012).

Glutamate produced during the development of excitotoxicity in ischemic and hypoxic brain injury causes the activation of Na+/K (+)-ATPase in astrocytes and promotes their uptake of extracellular glucose (Kitano et al., 2002). At the same time, there is an activation of lactate dehydrogenase, which converts pyruvate into lactate. The transfer of lactate from astrocytes to neurons is provided by monocarboxylate transporters present in every cell. The main types of these transporters in astrocytes are MCT1 and MCT4. Once transported by these transporters, lactate is transferred by neuronal transporters MCT2 and used as an energy substrate. It was shown that after transient ischemia, both lactate production and expression of MCT1, MCT2, and MCT4 increased in astrocytes (Rosafio *et al.*, 2016).

Lactate shuttle dysfunction has a negative effect on the central nervous system; for example, it can negatively affect neurite growth (Chen *et al.*, 2018). The metabolic connection between neurons and astrocytes influences learning and memory. The astrocyte-neuron lactate shuttle is thought to play an important role in the development of nerve cells and the formation of neural networks (Chen *et al.*, 2018). It has been shown that lactate secreted by astrocytes is not only used as an energy substrate for nerve cells but is also a signaling molecule that regulates such functions as nerve cell excitability and synaptic transmission plasticity. We identified hydroxycarboxylic acid receptor 1 (HCAR1), an orphan G protein-coupled receptor that is a sensor for extracellular lactate and modulates cell functions under oxygen-deficient conditions (Cai *et al.*, 2008; Hoque). In addition, it has been shown that lactate can affect some homeostatic functions of

neurons (Magistretti & Allaman, 2018). Thus, the regulation of lactate supply from astrocytes to neurons through MCTs may be an important factor in the adaptation of nerve cells to oxygen and glucose deprivation (Gao et al., 2015).

Our studies have shown that the MCT1 inhibition stimulates spontaneous bioelectrical neural network activity in the short term and leads to its irreversible inhibition in the long-term period. The effect of a short-term increase in the neural network activity is associated with the mechanisms of glucose-lactate shuttle activation. Astrocytes are known to intensify glycolysis when the concentration of glutamate in the synaptic cleft grows, which in turn stimulates the work of MCT transporters (Rosafio *et al.*, 2016). When the concentration of monocarboxylates in the area of the synaptic terminal decreases, there may be a short-term increase in the release of glutamate in the presynaptic area to compensate for the decrease in energy substrates. However, this process can only exacerbate the effects of excitotoxicity if the supply of energy substrates is impaired.

The data on the role of MCT transporters in the implementation of the neuroprotective action of GDNF are of particular interest. Numerous studies show the pronounced protective effects of this neurotrophic factor in the development of neurodegenerative processes of various origins (Shishkina et al., 2018; Conway et al., 2020; Zhang et al., 2021). Some of the molecular mechanisms of GDNF action are widely known. Our study shows that possible mechanisms of protection of neurons from the action of glucose deprivation can be associated with the modulation of the work of MCT transporters. Since their blockade not only completely eliminates the positive effect of GDNF but also leads to an increase in the processes of disassociation of functional network activity.

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