
EXPERIMENTAL PAPERS

Bilateral Synchronization of Hippocampal Theta Oscillations in vitro

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Received March 30, 2023; revised April 21, 2023; accepted April 22, 2023

Abstract—Hippocampal theta oscillations are pivotal for hippocampal functions of spatial navigation, learning and memory. In freely behaving animals, hippocampal theta oscillations display bilateral synchronization. Internally generated oscillations in the theta frequency range have also been described in the intact hippocampal preparation in vitro. However, it remains elusive how theta oscillations are synchronized between the left and right hippocampi. We used a preparation of the intact hippocampi interconnected by the ventral hippocampal commissure, prepared from juvenile and adult mice and rats in vitro. Local field potentials and multiunit activity were recorded using extracellular electrodes from the pyramidal cell layer and stratum radiatum of the left and right hippocampi. Neuronal network activity in the left and right hippocampi was found to be organized in theta oscillations, which strongly modulated the firing of CA1 neurons. Both neuronal activity and field potential theta oscillations demonstrated high levels of bilateral synchronization. Theta oscillations persisted on both sides, but their bilateral synchronization was abolished after surgical transection of the ventral hippocampal commissure. Thus, theta oscillations are synchronized in the left and right hippocampi in vitro, and their bilateral synchronization is provided by the ventral commissural connections.

DOI: 10.1134/S0022093023030067

Keywords: hippocampus, theta oscillations, bilateral, synchronization, ventral hippocampal commissure

INTRODUCTION

Interhemispheric (bilateral) synchronization is an important property of neural network activity in the hippocampal system. Simultaneous recording of the activity in the right and left hippocampi revealed a high level of synchronization between different patterns of electrical activity in these structures [1–12]. Bilateral synchronization of neuronal activity is also characteristic of theta oscillations in the hippocampal system in vivo [3].

Theta oscillations are generated in the hippocampus during animal movement (as well as during fast or REM sleep) and represent a tool for linking hippocampal neuronal ensembles (so-called place cells) during space navigation [13–16].

Presumably, this process is based on comparing sensory information entering the hippocampus from the entorhinal cortex, as well as from the internal models encoded in the neuronal networks of the hippocampus itself, in each cycle of theta oscillations. Given the contralateral organization

of sensory streams, the bilateral synchronization of theta activity in the hippocampal system is a necessary prerequisite for their integration during spatial navigation. It has been suggested that this can be achieved by a synchronous input from the entorhinal cortex, as well as due to bilateral synchronization in the entorhinal cortex via intercortical connections [9, 17–19]. It has also been hypothesized that bilateral synchronization of internal models can, in turn, be implemented via CA3–CA3 and CA3–CA1 bilateral connections within the hippocampal commissure [20]. However, the mechanisms of bilateral synchronization of theta oscillations still remain only hypothetical and require direct experimental verification.

It has been previously demonstrated that the *in vitro* preparation of the isolated hippocampus of juvenile and adult mice generates autonomous spontaneous oscillations in the frequency range approximating that of theta oscillations *in vivo* [21–24]. We hypothesized that a more comprehensive *in vitro* model of the hippocampal system, which includes both isolated intact hippocampi interconnected by the ventral commissure [25–28], could be used to study interhippocampal synchronization of theta oscillations through commissural connections, which was implemented in the present study.

MATERIALS AND METHODS

Experiments were carried out on preparations of the whole hippocampi interconnected by the ventral hippocampal commissure and isolated from P13–P15 Wistar rats ($n = 4$) and P22, P57 and 1-year-old C57BL mice ($n = 3$). The procedures of making hippocampal preparations have been described in detail elsewhere [26]. After decapitation under isoflurane anesthesia, the brain was rapidly extracted and immersed in the ice-cold oxygenated (95% O₂/5% CO₂) solution of artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 126, KCl 3.5, CaCl₂ 2.0, MgCl₂ 1.3, NaHCO₃ 25, NaH₂PO₄ 1.2, glucose 11 (pH 7.4). After removal of the cerebellum and frontal lobes, the complex comprising two hippocampi interconnected by the hippocampal commissure was isolated using the following procedure: the brain stem, midbrain, and

striatum were carefully separated from the hippocampi by two spatulas. The neocortex was then cut off by sliding along the corpus callosum along the dorsal septo-hippocampal surface with a spatula inserted into the lateral ventricle. The dual hippocampal preparation was then isolated from the septo-hippocampal complex. The preparation retained small fragments of surrounding tissues allowing it to be pinned to a sylgard-coated bottom of a chamber using entomological needles. The complete procedure of making a hippocampal preparation took 8–10 min. The preparation was then carefully transferred to ACSF and incubated therein at room temperature (20–22°C) for at least 1 h before recording. For recordings, the preparation was placed into a special chamber with a nylon mesh bottom and continuously perfused with ACSF at a rate of ~15 mL/min. Extracellular recordings of local field potentials (LFPs) and multiple unit activity (MUA) were performed using electrodes made of tungsten wire (50 μm in diameter, California Fine Wire, Grover Beach, CA, USA) and placed in the pyramidal layer or stratum radiatum of the hippocampal CA1 region. The recording electrodes were placed in the middle part of the hippocampus at an equal distance from the septal pole. The recorded signals were amplified and digitized using a DAM-8A amplifier (×1000, band 0.1 Hz–3 kHz) and a Digidata 1440A ADC (Molecular Devices, CA, USA) with a frequency of 10 kHz. The raw data were pre-processed using the functions we developed on the basis of the Matlab software package. The original signal was downsampled to 1 kHz and used further for LFP analysis. To detect action potentials, the original signal was low-pass filtered at >200 Hz, and the negative events with an amplitude exceeding 3 standard deviations were considered as action potentials. The LFP and MUA were analyzed using the functions we developed based on the Matlab software package (MathWorks, USA). The following operations were applied for theta phase determination. The LFP was decomposed into real and imaginary parts through the Hilbert transform. Then, to obtain the angle in radians in the $[-\pi, \pi]$ interval, the four-quadrant arctangent operation was applied over the resulting real and imaginary parts. To analyze phasic modulation of the MUA

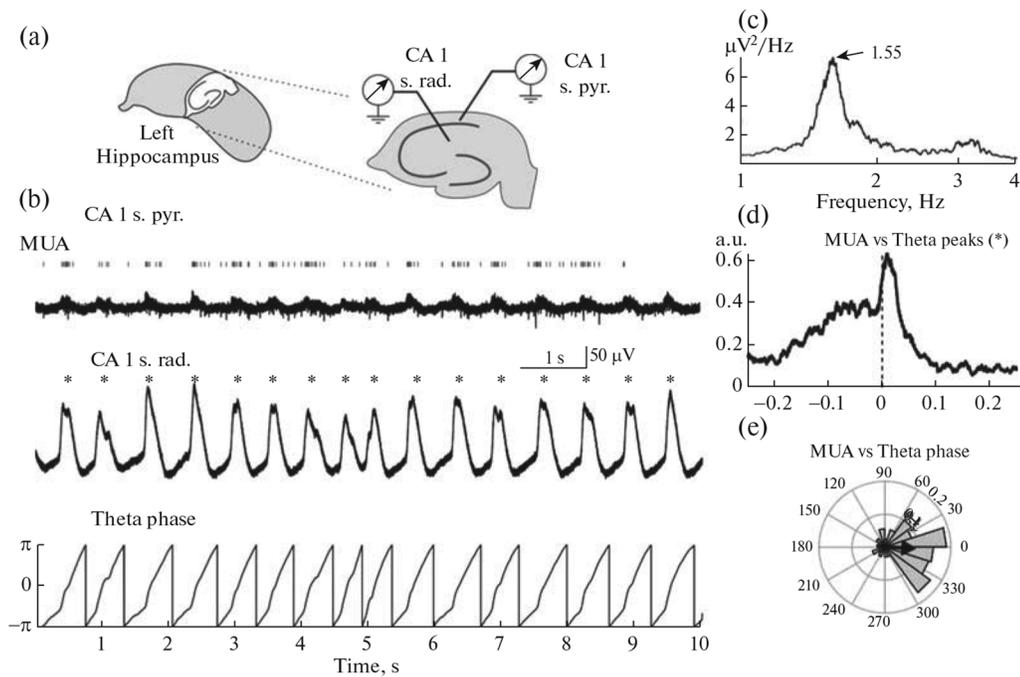


Fig. 1. Theta oscillations in the isolated hippocampus in vitro. (a) Electrode placement layout in the intact hippocampus in vitro. (b) Representative simultaneous records of LFPs and MUAs in the strata pyramidale and radiatum of the hippocampal CA1 subfield. MUA is indicated by vertical gray bars. *—Peaks of theta oscillations. Under the LFP record, in the stratum radiatum — the result of the LFP signal Hilbert transformation into theta phases. (c) Plot of the LFP power spectrum in the CA1 stratum radiatum. (d) Temporal cross-correlation of MUA in the CA1 pyramidal layer with the peak of the LFP theta oscillations recorded in the stratum radiatum. (e) Circular histogram of the MUA frequency relative to the theta phase. Rayleigh vector is marked in black.

frequency by theta oscillations, the Rayleigh test from the Matlab toolbox for circular statistics was used. The amplitude of the resulting Rayleigh vector shows the degree of MUA frequency modulation by theta oscillations, while its angle shows the theta phase of the resulting Rayleigh vector. Group data are presented as the median and (Q1–Q3) interquartile range. To compare theta frequencies before and after transection of the commissural connections, the Wilcoxon test for paired samples was used. The level of significance was taken at 5% (0.05).

RESULTS

Spontaneous activity in the isolated hippocampus of rats and mice older than 2 weeks was characterized by oscillations in the theta frequency range with cyclic changes in the LFP with a maximum amplitude in the CA1 stratum radiatum (Fig. 1). By the results of the analysis using fast Fourier transform, theta peak power values of

1.8 (0.6–8.0) $\mu\text{V}^2/\text{Hz}$ were observed at 2.0 (1.5–2.4) Hz ($n = 7$; minimum 1.5 and maximum 8.1 Hz). Spiking activity in the CA1 pyramidal layer was significantly modulated by theta oscillations, which was characterized by high values of cross-correlation between MUA and LFP of theta oscillations (Fig. 1d). Consistent with these observations, circular statistical analysis of neuronal activity revealed that the amplitude of Rayleigh vector values (0.075 (0.054–0.112)) is reached immediately before the theta peak (Rayleigh vector angle -32.8 ((-35.7)–(-5.1)) degrees) ($n = 7$) (Fig. 1e).

To study the bilateral organization of theta oscillations, we simultaneously recorded the activity in the left and right hippocampi with preserved commissural connections (Fig. 2). It was found that theta oscillations were highly synchronized between both hippocampi, as evidenced by a high level of cross-correlation between LFPs in both hippocampi, whose values reached 0.77 (0.55–0.93), with a delay of 4.4 ((-10.3)–

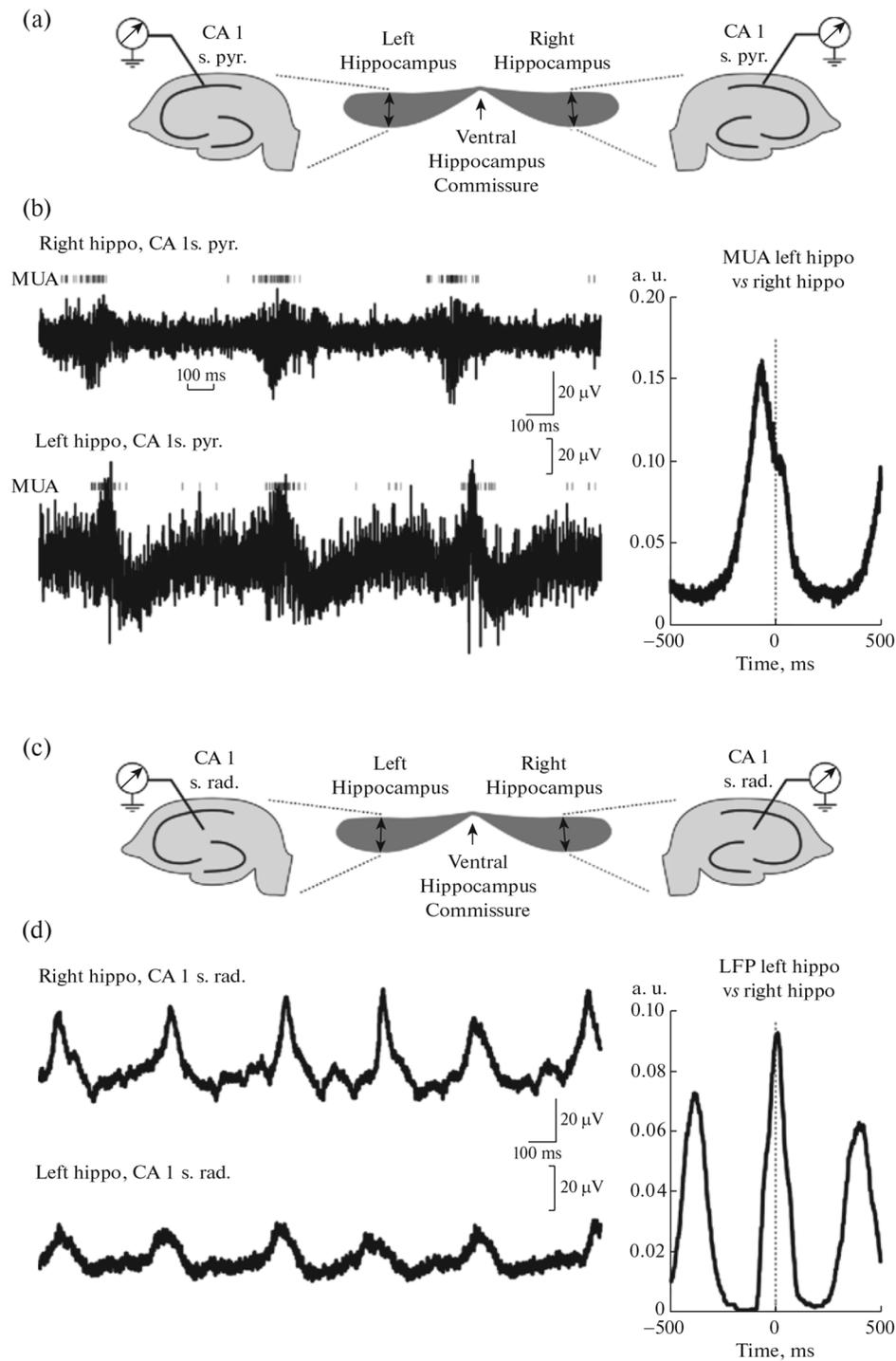


Fig. 2. Bilateral synchronization of theta oscillations in the left and right commissure-bound hippocampi in vitro. (a) Electrode placement layout in the preparation of commissure-bound left and right hippocampi in vitro. (b) Representative simultaneous records of LFPs and MUAs in the CA1 pyramidal layer of the left and right hippocampi. MUA is indicated by vertical gray bars. Right, plot of MUA cross-correlation in the CA1 pyramidal layer of the left and right hippocampi. (c) Electrode placement layout in the preparation of commissure-bound left and right hippocampi in vitro to record LFPs. (d) Representative simultaneous records of LFPs in the CA1 stratum radiatum of the left and right hippocampi. Right, plot of LFP cross-correlation in the left and right hippocampi.

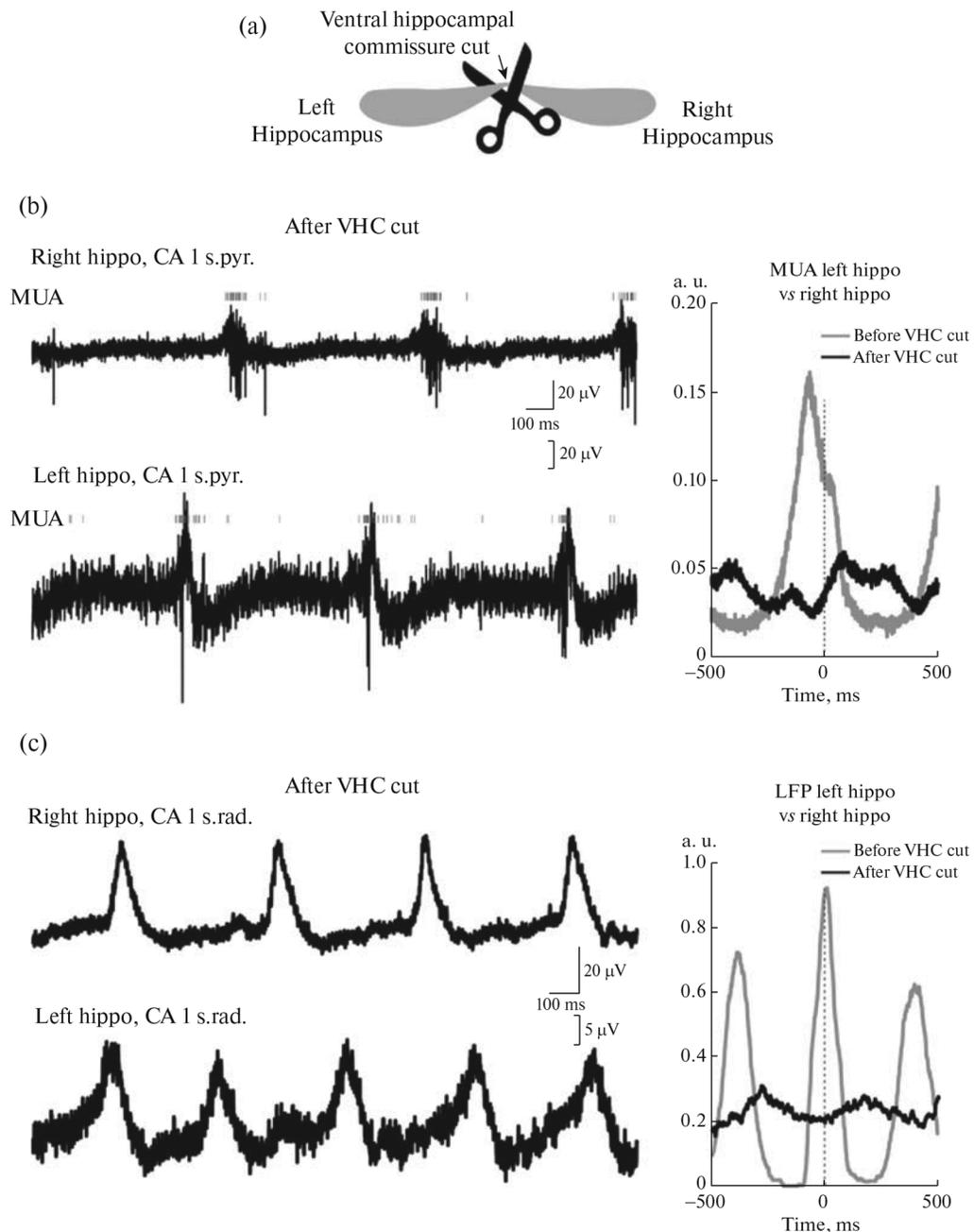


Fig. 3. Transection of the hippocampal commissure abolishes bilateral synchronization of theta oscillations. (a) Electrode placement layout in the dual hippocampal preparation after complete transection of the ventral and dorsal hippocampal commissures. (b) Representative simultaneous records of LFPs and MUA in the CA1 pyramidal layer of the left and right hippocampi. Right, plot of MUA cross-correlation in the CA1 pyramidal layer of the left and right hippocampi before and after ventral commissure transection. (c) Representative simultaneous records of LFPs in the CA1 stratum radiatum of the left and right hippocampi. Right, plot of LFP cross-correlation in the left and right hippocampi before and after ventral commissure transection.

6.6) ms of peak cross-correlation values between the right and left hippocampi ($n = 7$). A high level of bilateral theta synchronization was also evidenced by a high correlation between MUA in the

right and left hippocampi, reaching the values of 0.15 (0.12–0.18) with a delay of 2.9 ((–43.7)–19.8) ms of the peak MUA cross-correlation values between the right and left hippocampi ($n = 5$).

In different experiments, there was observed a predominant leadership of either the left or the right hippocampi, however, no significant leadership of either side in the generation of theta oscillations was detected at the group level.

Transection of the commissural connections led to a complete desynchronization of theta oscillations in the left and right hippocampi, as evidenced by the disappearance of the significant bilateral correlation between LFP and MUA oscillations ($n = 4$; Fig. 3). The frequency of theta oscillations after transection of the commissural connections did not change significantly in either the right or the left hippocampi ($p = 0.93$; $n = 4$).

DISCUSSION

The major findings of the present study are the demonstration of a high level of theta synchronization in the preparation of the left and right hippocampi derived from juvenile and adult rats and mice *in vitro*, as well as the proof that bilateral synchronization of these theta oscillations is provided by inter-hippocampal commissural connections.

An *in vitro* model of the hippocampal system, which comprises both isolated intact hippocampi interconnected by the ventral commissure [25–28], was originally developed for ontogenetic studies. This model allowed to characterize bilateral synchronization via commissural connections during giant depolarizing potentials [28] and the propagation of paroxysmal discharges [25, 27, 29–31] in the developing hippocampal system of rats and mice. It has initially been assumed that this model has age limits and can only be used in the postnatal period (before postnatal day P10) because of hippocampal thickening and extracellular space narrowing, which worsens the penetration of metabolites from the bath solution deep into the preparation as animals mature, and due to the age-related increase in energy consumption [26]. However, optimization of the experimental conditions, primarily a significant increase in the flow rate of the bath solution, made it possible to achieve the conditions, which preserved the neuronal membrane potential, functional synaptic connections, and network interactions in the intact hippocampi of even adult animals [21]. It was found that under these conditions, auto-

nous spontaneous oscillations in the isolated hippocampus of juvenile and adult mice arise in the frequency range close to that of theta oscillations *in vivo*. This model of theta oscillations has provided important insights into the cellular, synaptic, and network mechanisms of theta oscillations [21–24]. Although this model seems to have limits due to the absence of some pivotal cortical and subcortical structures involved in theta genesis *in vivo*, it is quite adequate for studying the intrahippocampal component of theta oscillations.

Theta oscillations in the isolated hippocampi *in vitro*, which are described in the present study, are self-generated within the hippocampal neuronal networks, since the entorhinal cortex is absent in this preparation. Thus, theta oscillations in the isolated hippocampi can be relevant to the component of “internal models” of theta oscillations *in vivo*, and also correspond, for example, to theta oscillations generated in the CA3 networks in urethane-anesthetized animals or after bilateral resection of the entorhinal cortex [32–34]. Accordingly, bilateral theta synchronization in the *in vitro* preparation of the two hippocampi, which was found in the present study, may indicate the presence of the mechanism for internal model integration, based on bilateral synchronization of the hippocampal neuronal networks in theta oscillations through direct connections organized in the ventral hippocampal commissure. In order to find out the significance of the commissural connections in the bilateral synchronization of physiological theta oscillations *in vivo*, it would be interesting to test the effect of severing the commissural connections on bilateral theta synchronization in the intact animal in further studies.

AUTHORS' CONTRIBUTION

Conceptualization and experimental design (R.Kh.), data collection (I.Kh.), data processing (A.G.), writing and editing the manuscript (R.Kh., A.G., I.Kh.).

FUNDING

This work was supported by the National Research Agency (Project No. ANR-21-CE16-

0005-01 “DevHippo”), and as part of the Priority 2030 research program at Kazan Federal University.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed with the involvement of animals complied with ethical standards approved by legal acts of the Russian Federation, the principles of the Basel Declaration, and recommendations of the Directive 2010/63/EU for animal experiments. All animal use protocols were approved by the French National Institute of Health and Medical Research (APAFIS #16992-2020070612319346 v2) and Local Ethics Committee of Kazan Federal University (#24/ 22.09.2020).

CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest related to the publication of this article.

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Translated by A. Polyansky