DOI 10.1007/s11055-019-00840-x
*Neuroscience and Behavioral Physiology, Vol. 49, No. 8, October, 2019*

# Early Electrophysiological Sequelae of Dosed Craniocerebral Trauma in Rats

**I. G. Komoltsev,[1,2] S. O. Frankevich,[1] N. I. Shirobokova,[1] A. A. Volkova,[1] I. P. Levshina,[1] M. R. Novikova,[1] A. O. Manolova,[1] and N. V. Gulyaeva[1,2]**

*Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 118, No. 10, Iss. 2, Epilepsy, pp. 21–26, October, 2018.*

**Objectives.** To identify and analyze pathological activity in the acute period of craniocerebral trauma (CCT) and to seek possible morphological correlates of this activity in the cortex and hippocampus. **Materials and methods.** Studies were performed using Sprague–Dawley rats. CCT was modeled using lateral hydrodynamic blows to the sensorimotor cortex. Electrocorticograms were recorded one week before application of CCT and one week after CCT. Histological analysis was run one week after CCT. Sections were stained by the Nissl method and immunohistochemically for an astrocyte marker (GFAP) and microglia (isolectin B4). The extents of damage in the cortex and hippocampal were evaluated. **Results and conclusions.** Slowing of baseline activity was seen 1 and 6 h after CCT, and epileptiform activity appeared in 50% of the animals one week after CCT. The number of discharges correlated with the area of astrocyte gliosis in the cortex and the number of dark "ischemic" neurons in the hippocampus. Microglial activity in the hippocampus did not correlate with epileptiform activity. these data are important for understanding the early mechanisms of posttraumatic epileptogenesis.

**Keywords:** craniocerebral trauma, EEG, epileptiform activity, epileptogenesis, hippocampus.

Craniocerebral trauma (CCT) in the acute period is accompanied by symptomatic convulsive seizures induced by primary and secondary damage to brain tissue – cell death, impairments to the blood–brain barrier, neuroinflammation, and changes to neurotransmitter and ion release. Acute convulsive seizures in patients with CCT are known to be a significant risk factor for the occurrence of posttraumatic epilepsy (PTE) [1]. In the late period after onset of the first unprovoked convulsive seizure, the probability of subsequent seizures is extremely high [2], which suggests, according to the ILAE criteria, that the patient can be diagnosed with epilepsy [3]. In most cases, clinical and EEG data and MRI scan results show that these patients develop medial temporal epilepsy [4]. The mechanisms of PTE include delayed cell death, astrocyte dysfunction, and reorganization of neural networks as a result of cellular and synaptic plasticity. The key anatomical structure for these changes is the hippocampus. Hippocampal sclerosis accompanying neuron death in hippocampal field CA1 and CA3 and the dentate gyrus, along with astrocyte gliosis, is the most frequent pathomorphological substrate of this disease [5]. Pathological networks with spontaneous synchronous activation of large pools of neurons form in the altered hippocampus; this hypersynchronization is apparent as late unprovoked convulsive seizures [6]. In the long-term period of CCT, spontaneous convulsive seizures develop in 10–20% of patients [1], though clinical practice does not yet include any reliable markers for the development of PTE or any means of preventing it [7]. This is mainly because the primary mechanisms triggering these changes have received insufficient study.

Studies of the mechanisms of origination of PTE and the search for a treatment strategy for patients are conducted using a variety of animal models. The most valid (close to

[1] Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia; e-mail: komoltsev.ilia@gmail.com.
[2] Solov'ev Scientific and Applied Psychoneurology Center, Moscow Health Department, Moscow, Russia.

the features of CCT in humans) is the lateral hydrodynamic blow model (liquid percussive brain trauma) in rats [8, 9]. PTE has been shown to develop in the late period in this model, with occurrence of unprovoked convulsive seizures and interictal epileptiform activity. The late period is also characterized by marked changes in the hippocampus – sprouting of mossy fibers, gliosis, and neuron death [10]. The acute period of CCT, which is of key importance in triggering the mechanisms of posttraumatic epileptogenesis, has received insufficient study. In particular, little is yet known about the origination and nature of early epileptiform activity and its potential relationship with early changes in the hippocampus.

The aim of the present work was to identify and analyze pathological electrophysiological and behavioral activity in the acute period of CCT and to seek possible morphological correlates of this activity in the cortex and hippocampus.

**Materials and Methods.** Studies were performed on 18 male Sprague–Dawley rats aged about six months (weight 450–550 g) from the Pushchino supplier (Moscow District). The CCT group included 11 rats and the sham operation (SO) group included seven.

*Experimental protocol and surgery*. All surgical procedures were carried out under inhalational anesthesia with 2% isoflurane. After scalping and removal of the temporal muscles, stainless steel epidural electrodes were implanted at symmetrical points over the frontal (AP = 1, L = 3) and occipital (AP= –7, L = 3) cortex with preservation of the area for subsequent craniotomy. The reference electrode was implanted in the caudal part of the occipital bone in the midline. After surgery, rats were placed in individual chambers with free access to water and food in conditions of natural illumination and electrocorticograms (ECoG) were recorded for seven days simultaneously with continuous video recording.

CCT was modeled by trepanning a 3-mm aperture in the right parietal bone (AP = 3 mm, L = 3 mm) seven days after electrode implantation. The head of a Luer-type injection needle was fixed at the margin of the trepanned aperture with cyanoacrylate glue. One hour after craniotomy and recovery from anesthesia, lateral hydrodynamic blows (3.4 ± 0.1 atm) were applied to rats in conditions of free behavior. The only difference between rats of the SO and CCT groups was that the former did not receive hydrodynamic blows. Animals were returned to their home cages after CCT. Video and ECoG recordings were made for seven days after trauma. Animals were then anesthetized with chloral hydrate (450 mg/kg i.p.) and sacrificed by intracardiac perfusion with 4% paraformaldehyde solution in 0.1 M phosphate buffer pH 7.4.

*Analysis of slow-wave activity*. Trace segments at 1, 6, 12, 18, and 24 h after CCT were selected in EDF browser 1.57. A Butterworth filter with a lower limit of 1 Hz and an upper limit of 30 Hz was used. The spectral power density of the ECoG signal at the lower frequencies had two peaks, at 1.3–1.6 and 2.2–3.0 Hz. Spectral power density was measured in the cortex of the ipsilateral and contralateral hemispheres with the rats in the state of passive waking, separately for each peak. Three different fragments of duration 3–5 sec were analyzed for each time point and data for each were averaged.

*Analysis of epileptiform activity*. 24-Hour trace fragments were analyzed at baseline and on posttrauma day 6. Traces were divided into 20-sec epochs; epochs lacking epileptiform activity were selected for further analysis (1100 epochs were selected out of 78000). The assignment of each epoch to phases in the sleep–waking cycle was noted, along with the rats' behavior in the home chamber.

*Histological methods*. Vibratome sections of thickness 50 μm were prepared from rat brains. Sections located 600 μm apart with coordinates between 2.1 and 5.8 mm from the bregma were selected for further analysis. Sections were stained with cresyl violet by the Nissl method. Immunohistochemical staining for glial fibrillary acid protein (GFAP), an astroglial marker, used polyclonal rabbit-anti-GFAP (Dako, Denmark) diluted 1:500 with secondary antibodies (goat anti-rabbit IgG, Alexa Fluor, USA) diluted 1:500. Sections were also stained for isolectin B4, a marker for microglia (biotinylated *Griffonia simplicifolia* lectin I isolectin B4, Vector Laboratories, USA) diluted 1:100, detected with streptavidin 488 (Alexa Fluor, USA) diluted 1:500. Microphotographs of sections stained by the Nissl method were made using a Keyence BZ-X700 microscope by immersion microscopy (magnification ×60). Four sections were selected for counting of the total number of dark neurons with ischemic morphology (typical signs: dark cytoplasm, concave outlines, perifocal edema zones, tortuous processes) in the polymorphic layer of the dentate gyrus of the hippocampus on both sides; data were averaged for each hemisphere. Microphotographs (magnification ×20) of immunohistochemically stained sections were made for further calculations in ImageJ. Sections were used to determine the areas of cortical damage and the numbers of all microglial and astroglial cells in the polymorphic layer of the dentate gyrus of the hippocampus, as well as the area of the dentate gyrus of the hippocampus; cell densities were then calculated as the ratio of these two parameters.

Animal experiments were performed in compliance with the requirements of Directives 2010/63/EU of the European Parliament and Council, September 22, 2010, and decree No. 267 of June 19, 2003 for the protection and use of animals in experimental research. The experimental protocol was approved by the Ethics Committee of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences. All measures were taken to reduce the number of animals used and to minimize their suffering.

*Statistical methods*. All calculations were run in Statistica 10 (StatSoft, Inc., USA). The CCT and SO groups were compared in the electrophysiological and morphological parts of the study using the Mann–Whitney test; the

hemispheres in the morphological part of the study were compared using the Wilcoxon test. Data are presented as mean and standard error of the mean ($M \pm SEM$).

**Results and Discussion.** Seven out of 10 animals in the CCT group showed rapid tonic-clonic convulsions lasting 30 sec (three rats were excluded from the experiment), followed by loss of posture and loss of responses to external stimuli for 3–5 min. The remaining rats showed a hypermotor state, also with loss of posture for 3–5 min.

*Slowing of baseline electrophysiological activity.* Focal slowing of baseline activity was not seen in baseline traces or in rats of the SO group,. All rats after CCT showed slowing of baseline activity in the hemisphere ipsilateral to the trauma 1 h after trauma, 70% of rats showing this at 6 h after trauma. At 12, 18, and 24 h after trauma, slowing of baseline activity was seen in only 20% of rats.

Comparison of the frequencies at which peaks were seen showed that there were no significant differences between groups and time points.

The spectral power density in the selected ECoG signal fragments was significantly greater for the ipsilateral hemisphere 1 and 6 h after trauma for both peaks: in the frequency range 1.3–1.6 Hz 1 h after CCT the level in the contralateral hemisphere was $730 \pm 180$ $\mu V^2$/Hz vs. $2840 \pm$ $\pm 590$ $\mu V^2$/Hz in the ipsilateral ($p < 0.005$), compared with values at 6 h of $1050 \pm 260$ $\mu V^2$/Hz vs. $3230 \pm 750$ $\mu V^2$/Hz ($p < 0.05$); values in the frequency range 2.2–3.0 Hz were $450 \pm 100$ $\mu V^2$/Hz vs. $2640 \pm 1070$ $\mu V^2$/Hz ($p < 0.005$) and $910 \pm 230$ $\mu V^2$/Hz vs. $3760 \pm 1210$ $\mu V^2$/Hz ($p < 0.005$) at 1 and 6 h, respectively.

*Epileptiform activity.* Epileptiform activity consisted of high-amplitude rhythmic (about 7 Hz) bursts consisting of multiple spikes in the frontal areas on both sides. The structure of discharges periodically showed low-amplitude slow waves before individual spikes. Epileptiform activity was encountered in all rats, both in baseline and after CCT or SO. There were no differences in the structure of epileptiform activity between groups or time points.

Of the 18 rats taking part in the experiment, three (17%) showed a mean number of epochs with epileptiform activity in baseline conditions which was 20 times greater than that seen in the other animals, such that they were excluded from study.

The number of epochs with epileptiform activity in rats of the SO group remained at the same level before and after craniotomy. In 50% of rats, CCT increased the mean number of epochs with epileptiform activity by a factor of 20 from the baseline level. Rats were divided into two groups on the basis of the increase in the number of epochs with epileptiform activity after CCT: animals with increased epileptiform activity and those with no increase; this division was used for further analysis.

Overall, 650 epochs with epileptiform activity were analyzed. Mean discharge duration was $2.2 \pm 0.2$ sec and was no different in rats of the different subgroups. The mean number of discharges per day in rats with increased epileptiform activity was $86.4 \pm 22.2$, which was greater than baseline ($9.0 \pm 2.5$, $p < 0.01$) and also higher than that in rats in the subgroup without an increase in epileptiform activity after CCT ($4.6 \pm 1.2$, $p < 0.01$) and animals of the SO group ($14.4 \pm 6.4$, $p < 0.01$).

A total of 70% of all discharges were grouped (mean $2.8 \pm 0.1$ discharges); the numbers of discharges in groups did not differ between the SO and CCT groups. Grouped and single discharges appeared predominantly during the transition from waking to NREM sleep, though a significant increase in the number of discharges was seen separately in each phase of the cycle: during waking ($p < 0.05$ compared with the subgroup without an increase in epileptiform activity, with rats of the SO group, and with baseline) and during the transition from waking to NREM sleep ($p < 0.05$) and during NREM sleep ($p < 0.05$). No discharges were seen during REM sleep.

The animals did not move during any of the epochs analyzed. Episodes were selected during waking which were accompanied by cessation of movement activity immediately before the occurrence of discharges and renewal of motor activity immediately after the end of the discharge (or group of discharges). The mean number of episodes of this freezing was significantly greater in rats with an increase in epileptiform activity after CCT ($10.4 \pm 3.0$) than in baseline ($0.2 \pm 0.2$, $p < 0.01$) and rats without an increase in epileptiform activity after CCT ($1.2 \pm 0.6$, $p < 0.05$).

Detection of epileptiform activity in the early post-CCT period and its comparison with baseline measures is new, as this is the first use of this experimental approach. Previous studies have demonstrated the presence of spike-wave discharges in adult Sprague–Dawley rats [11], the discharge frequency depending on age [12]. Some authors [13] link epileptiform activity with whisker movement, though this question has not been completely resolved as activity may also occur without being accompanied by movement of the perioral muscles in rats [14].

In the present work, discharges were accompanied by freezing of the animals, which can be regarded as an analog of focal seizures in humans. Most authors exclude this type of epileptiform activity from analysis of posttraumatic discharges in rats on the basis that is it not trauma-associated [6, 15]. Recording of the ECoG before CCT in these experiments provides for precise determination of the dynamics of epileptiform activity as a result of trauma. In addition, the experimental protocol allowed animals with initially high numbers of discharges (17%) to be excluded, which cannot be done in most studies of PTE.

Thus, bifrontal discharges at 7 Hz in rats are controversial, though there are no studies providing detailed descriptions of increases in the numbers of discharges in the early post-CCT period. Synchronization of the operation of neural networks responsible for forming this type of epileptiform activity may be important, though this element of PTE has yet to be studied in detail in rats.

*Histological results. Damage in the neocortex*. The focus in the cortex of the ipsilateral hemisphere was defined in terms of intense staining for GFAP and isolectin B4. Microglial cells were round in shape and marked increases in their numbers could be identified visually at the center of the lesion. Astrocytes had thickened processes and intense staining and were located at the center and periphery of the focus of microcyte gliosis, exceeding it in terms of area: the area of astrocyte gliosis in the cortex of the ipsilateral hemisphere in rats of the CCT group was $2.00 \pm 0.32$ mm$^2$, while the area of microcyte gliosis was $0.24 \pm 0.13$ mm$^2$. Rats of the SO group showed only superficial astrocyte gliosis in the cortex in the craniotomy area.

*Remote damage in the hippocampus*. In rats of the CCT group, as compared with those of the SO group, the dentate gyrus of the hippocampus of both hemispheres showed increased numbers of dark cells with ischemic morphology. The increase in the number of dark cells in the ipsilateral hemisphere was at the level of a statistical trend ($23 \pm 10$ cells vs. $41 \pm 7$ cells, $p = 0.08$). The difference in the contralateral hemisphere was significant ($22 \pm 7$ cells vs. $48 \pm 9$ cells, $p < 0.05$).

The mean area of the dentate gyrus showed no difference between rats of the different groups or between hemispheres. The density of microglial cells in the dentate gyrus of the hippocampus was significantly greater in the ipsilateral hippocampus of rats of the CCT group ($1364 \pm 118$ cells/mm) as compared with the SO group ($919 \pm 73$ cells/mm, $p < 0.05$). Significant differences were also seen on comparison of the ipsi- and contralateral hemispheres in the CCT group ($p < 0.05$). There were no between-group differences in astroglial density in the dentate fascia of the hippocampus.

*Comparison of electrophysiological and histological results*. No correlations were found between the number of epochs with epileptiform activity six days after trauma with the duration, frequency, or spectral power density of slowing of baseline activity on posttrauma day 1. Comparison of data obtained in the electrophysiological and morphological studies revealed a positive correlation between the area of astrocyte gliosis and the number of epochs containing epileptiform activity in rats of the CCT ($r = 0.70$, $p = 0.04$). In addition, a positive correlation was found between the number of dark neurons in the contralateral dentate gyrus of the hippocampus with the number of epochs containing epileptiform activity ($r = 0.77$, $p = 0.001$). Correlation of cell density in the dentate fascia of the ipsilateral and contralateral hippocampus and the number of epochs with epileptiform activity was not significant.

These experiments provided the first analysis of the morphological correlates of the activity identified. A direct relationship was found between the area of astrocyte gliosis in the cortex and the number of discharges. Formation of a focus of damage in the cortex is known to be accompanied by activation of the glia, cytokine expression, astrocyte dysfunction, and the accumulation of extracellular $K^+$ – all of which lead to hypersynchronization of neurons in the cortex [16] and the occurrence of epileptiform activity. The neural mechanisms of this epileptiform activity require further study.

Hippocampal sclerosis is the pathology typical of medial temporal epilepsy [5]. Unfortunately, studies of humans cannot follow pathomorphological changes over time – in particular, nothing is known of the early changes in the hippocampus after CCT. In the present study, the hippocampus showed increases in the number of dark neurons with ischemic morphology. In the ipsilateral hemisphere, these differences were seen at the level of statistical trends and can be explained in terms of cell death in the dentate gyrus in the ipsilateral hippocampus [17, 18], as demonstrated in previous studies [19].

The fact that rats with high levels of epileptiform activity also had a greater number of ischemic neurons in the contralateral hemisphere is interesting. Epileptiform activity in the cortex may be one of the factors triggering the mechanisms of cell death in the hippocampus and may also take part in triggering the mechanisms of epileptogenesis.

Activation of the microglia in the dentate fascia of the ipsilateral hippocampus is important in the development of posttraumatic pathology, including PTE [10]. In these experiments, activation of the microglia was seen in all rats of the CCT group and did not correlate with the number of discharges. In other words, all rats, regardless of the level of epileptiform activity in the cortex, developed neuroinflammation process in the hippocampus. These results may be evidence for different mechanisms underlying the occurrence of epileptiform activity and remote damage to the hippocampus in the acute phase of CCT and require further experimental confirmation.

Thus, the results of this experiment lead to the following conclusions:

1) Sprague–Dawley rats showed regional slowing of the baseline activity 1 h after CCT, this persisting to 6 h in 70% and to 18–24 h in only 20%. The spectral power density of the ECoG signal at the lower frequencies at 1 and 6 h was greater in rats after CCT in the ipsilateral hemisphere as compared with the contralateral;

2) epileptiform activity consisting of rhythmic high-amplitude spikes at 7 Hz was recorded in the rostral parts of the cerebral hemispheres on both sides. The number of discharges in 17% of the animals was many times greater than the mean level in the other rats. These animals with large amounts of epileptiform activity on baseline conditions were excluded from further analysis;

3) one week after CCT, half of the rats showed an increase in the number of discharges which was many times greater as compared with baseline values and the other post-CCT rats and SO rats;

4) in the ipsilateral hemisphere, the cerebral cortex of rats of the CCT group showed a focus of damage one week after trauma, with astrocyte activation and microglial activation; the polymorphic layer of the dentate gyrus of the

ipsilateral hippocampus showed an increase in the density of microglial cells. An increase in the number of dark neurons occurred in the dentate gyrus of the hippocampus of both hemispheres;

5) a positive correlation was found between the area of astrocyte gliosis in the cortex of the ipsilateral hemisphere and the number of discharges in the group of rats with CCT. In addition, a positive correlation was found between the number of dark neurons in the dentate gyrus of the contralateral hemisphere and the number of discharges one week after CCT. Activation of the microglia in the dentate fascia of the ipsilateral hippocampus occurred in all rats after CCT and did not correlate with the appearance of epileptiform activity.

This study was supported by the Russian Foundation for Basic Research (Grant Nos. 16-04-00855 (EEG analysis) and 18-315-00146 (histological analysis)).

The authors have no conflicts of interests.

## REFERENCES

1. J. Christensen, "The epidemiology of posttraumatic epilepsy," *Semin. Neurol.*, **35**, 218–222 (2015), https://doi.org/10.1055/s-0035-1552923.
2. A. M. Haltiner, N. R. Temkin, and S. S. Dikmen, "Risk of seizure recurrence after the first late posttraumatic seizure," *Arch. Phys. Med. Rehabil.*, **78**, 835–840 (1997), https://doi.org/10.1016/S0003-9993(97)90196-9.
3. R. S. Fisher, C. Acevedo, A. Arzimanoglou, et al., "ILAE Official Report: A practical clinical definition of epilepsy," *Epilepsia*, **55**, 475–482 (2014), https://doi.org/10.1111/epi.12550.
4. P. Gupta, K. Ding, N. Sayed, et al., "Subtypes of post-traumatic epilepsy: clinical, electrophysiological, and imaging features," *J. Neurotrauma*, **31**, 1439–1443 (2014), https://doi.org/10.1089/neu.2013.3221.
5. I. Blümcke, R. Coras, H. Miyata, and C. Ozkara, "Defining clinico-neuropathological subtypes of mesial temporal lobe epilepsy with hippocampal sclerosis," *Brain Pathol.*, **22**, 402–411 (2012), https://doi.org/10.1111/j.1750-3639.2012.00583.x.
6. A. Pitkänen, J. Kyyriainen, P. Andrade, et al., *Epilepsy after Traumatic Brain Injury. Model Seizures Epilepsy*, Elsevier, Academic Press, New York (2017), https://doi.org/10.1016/B978-0-12-804066-9.00046-8.
7. A. Pitkänen and J. Engel, "Past and present definitions of epileptogenesis and its biomarkers," *Neurotherapeutics*, **11**, 231–241 (2014), https://doi.org/10.1007/s13311-014-0257-2.
8. S. C. Cortez, T. K. McIntosh, and L. J. Noble, "Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations," *Brain Res.*, **482**, 271–282 (1989), https://doi.org/10.1016/0006-8993(89)91190-6.
9. H. J. Thompson, J. Lifshitz, N. Marklund, et al., "Lateral fluid percussion brain injury: a 15-year review and evaluation," *J. Neurotrauma*, **22**, 42–75 (2005), https://doi.org/10.1089/neu.2005.22.42.
10. E. Aronica, A. Mühlebner, E. A. van Vliet, and J. A. Gorter, *Characterization of Pathology. Model. Seizures Epilepsy*, Academic Press, New York (2017), 2nd ed., https://doi.org/10.1016/B978-0-12-804066-9.00011-0.
11. P. S. Pearce, D. Friedman, J. J. Lafrancois, et al., "Spike-wave discharges in adult Sprague–Dawley rats and their implications for animal models of temporal lobe epilepsy," *Epilepsy Behav.*, **32**, 121–231 (2014), https://doi.org/10.1016/j.yebeh.2014.01.004.
12. K. M. Rodgers, F. E. Dudek, and D. S. Barth, "Progressive, seizure-Like, spike-wave discharges are common in both injured and uninjured Sprague–Dawley rats: implications for the fluid percussion injury model of post-traumatic epilepsy," *J. Neurosci.*, **35**, No. 24, 9194–204 (2015), https://doi.org/10.1523/JNEUROSCI.0919-15.2015.
13. K. Semba and B. R. Komisaruk, "Neural substrates of two different rhythmical vibrissal movements in the rat," *Neuroscience*, **12**, 761–774 (1984), https://doi.org/10.1016/0306-4522(84)90168-4.
14. F.-Z. Shaw and Y.-F. Liao, "Relation between activities of the cortex and vibrissae muscles during high-voltage rhythmic spike discharges in rats," *J. Neurophysiol.*, **93**, 2435–2448 (2005), https://doi.org/10.1152/jn.00999.2004.
15. R. D'Ambrosio, J. S. Fender, J. P. Fairbanks, et al., "Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat," *Brain*, **128**, 174–188 (2005), https://doi.org/10.1093/brain/awh337.
16. A. Vezzani, S. Auvin, T. Ravizza, and E. Aronica, "Glia-neuronal interactions in ictogenesis and epileptogenesis: role of inflammatory mediators," in: *Jasper's Basic Mechanisms of the Epilepsies* (2012), 4th ed.
17. M. S. Grady, J. S. Charleston, D. Maris, et al., "Neuronal and glial cell number in the hippocampus after experimental traumatic brain injury: Analysis by stereological estimation," *J. Neurotrauma*, **20**, 929–941 (2003), https://doi.org/10.1089/089771503770195786.
18. L. D. Tran, J. Lifshitz, B. M. Witgen, et al., "Response of the contralateral hippocampus to lateral fluid percussion brain injury," *J. Neurotrauma*, **23**, 1330–1342 (2006), https://doi.org/10.1089/neu.2006.23.1330.
19. I. G. Komoltsev, A. A. Volkova, I. P. Levshina, et al., "The number of IgG-positive neurons in the rat hippocampus increases after dosed craniocerebral trauma," *Neirokhimiya*, **35**, No. 3, 250–255 (2018), https://doi.org/10.1134/S1027813318030056.