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Modeling of neuropathology on zebrafish: traumatic brain injury

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Motivation and Aim: Traumatic brain injury (TBI) consists of a combination of pathophysiological functional and morphological changes in the brain caused by external impacts. The pathogenesis of neurotrauma includes neuroinflammation, neurodegeneration, cerebral edema, and neuroregeneration. Research of the pathogenetic mechanisms of this neurological disorder because of the high level of hospitalization of patients with TBI on the one hand and a significant percentage of mortality due to the imperfect methods of TBI curation and treatment on the other. Modern trends in translational biomedicine prioritize the task of correct choice of a model organism. Its anatomical and physiological features, the correct translation of the results obtained from a model animal to humans, the molecular genetic features of the species as well as the bioethical aspects of *in vivo* experiments should be taking into account. In this regard, the zebrafish occurs as a promising model for studying the pathogenesis of neuropathologies including neurotrauma [1]. A large number of developed behavioral tests and techniques for working with its genome, high regenerative capacity of zebrafish, high translational potential of the results of molecular genetic and even behavioral studies along with relatively low maintenance expenses make this organism extremely relevant and promising for neuroscience research and create a competitive advantage in comparison with other model animals. In this study, we propose a novel model of non-penetrating brain injury in adult zebrafish induced by a laser irradiation.

Methods and Algorithms: An important advantage of laser radiation is the distance of exposure. Transparent tissues are not affected, while opaque, deeper ones would be sensitive to laser radiation. Zebrafish strain used in experiments had transparent skin on top of the head. Thus, visible laser radiation could penetrate it without beam distortion. It allowed to selectively heat brain surface to temperatures high enough to cause hyperthermia or protein denaturation. The experimental setup included a laser diode (power – 500 mW, wavelength – 405 nm) and aiming system consisted of video camera and 3D translation stage. The radiation of laser diode was focused into a spot of 0.1 mm on the surface of the telencephalon of anesthetized zebrafish; the duration of exposure was 2 or 4 s. Neuromorphological and molecular changes were assessed using standard histological staining and immunohistochemical analysis of the telencephalon cryosections. Behavioral phenotyping was performed in the Novel tank test to evaluate

motor disturbances and anxiety. Locomotor activity of the zebrafish was measured by the total distance traveled, freezing frequency and duration, mean velocity. Time spent in the top zone and latency to enter the top zone were regarded as indices of anxiety.
Results: Damage in the brain tissue was confirmed by neuromorphological examination of the samples using histological staining. As early as 30 min after the injury, protein expression of the transcription factor HIF-1α, which is related to TBI-induced neurogenesis [2], increased in the fish telencephalon and persisted further until the Day 7 after the lesion. The expression of neurotrophic factor BDNF decreased 30 min after the injury with a subsequent elevation (the maximal increase was observed on the 3rd day after TBI. Both parameters reached control values on the Day 7. Locomotor activity in zebrafish was substantially reduced by laser-induced TBI in 30 min and on the next day after the injury while it had recovered since the Days 3–5. Laser-induced lesion of the dorsomedial telencephalon increased the anxiety of fish according to the decreased number of visits to the upper part of a tank on the day of injury (30 min after the trauma) and in one day after the injury. Moreover, TBI group significantly differed from Sham group in the latency to the first entry into the top zone on the Day 7.
Conclusion: Thus, the method of laser-induced injury is suggested as an adequate and useful tool for TBI research including the study of pathogenetic mechanisms related of TBI related to regeneration of an injury as well as preclinical screening of treatment approaches that will open new possibilities for treating patients with TBI.
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