

Manuscript Title: [Synaptic pathology in Traumatic Brain Injury and Therapeutic Insights]

Dear Reviewers,

We sincerely thank you and the reviewers for the thoughtful and constructive feedback on our manuscript. We have carefully addressed all the comments and suggestions, we believe these have strengthened the manuscript. Below, we provide a detailed point-by-point response to each comment, with corresponding changes highlighted in the revised manuscript.

We appreciate the opportunity to revise our manuscript.

Kind regards,

(on behalf of all the authors).

Reviewers' Comments to the Authors:

Reviewer #1

Comments and Suggestions for Authors

The review examines the acute, subacute and chronic phases of synaptic structure and function disorders that occur after traumatic brain injury. The loss of dendritic spines and disruption of synaptic plasticity are undoubtedly included in the main causes of pathological changes that follow such effects - therefore, the review deserves attention.

The authors propose to consider the analysis of disorders occurring after injury in three periods - during the acute phase, subacute and chronic course of processes, which is an advantage of the proposed review. I agree with the authors that during the acute phase, the main process is excitotoxicity, but it would be good to emphasize that this does not exhaust the damage to the nervous tissue, the authors in the text indicate such processes, for example, edema and mitochondrial dysfunction, this should be indicated in the abstract.

The same – it is necessary to emphasize that in the subacute phase, in addition to ongoing glutamate excitotoxicity and GABAergic disorders, along with neuroinflammatory pathology, other processes occur, such as autophagy. In the chronic phase, long-term structural remodeling of neuronal networks is observed. These chronic changes underlie persistent cognitive impairment and memory deficit, mood disorders and the development of post-traumatic epilepsy.

Response: *We agree with the reviewer's comment. The Abstract section has been revised accordingly.*

Changes made: *Main Manuscript (Abstract) Page 2, Line 32-34:* “During the acute phase of TBI, mechanical injury triggers presynaptic glutamate secretion, Ca⁺² ion-mediated excitotoxic injury, accompanied by cerebral edema, mitochondrial dysfunction, and loss of mushroom-shaped

architecture of the dendritic spines. The subacute phase is marked by continued glutamate excitotoxicity and GABAergic disruption along with neuroinflammatory pathology and autophagy.”

GENERAL COMMENTS:

The scientific significance of the review would be significantly higher if the authors analyzed in which brain structures the changes under consideration were observed.

It is necessary to indicate which brain structures suffer first after the injury, since when citing articles, in most cases, the authors do not indicate where the disorders occur (the hippocampus is often indicated, but it should be understood that the hippocampal neurons are the most vulnerable to any damaging effects).

The chronic phase is characterized too briefly. It is necessary to supplement it with data on molecular markers of the state of synapses (synaptic vesicles, post-synaptic densities). In addition, when the authors indicate the neurological consequences of the injury, indicating the brain structures associated with individual symptoms, neurotransmitter specificity and the effectiveness of pharmacological agents will

indicate more specific reorganizations of neural networks. The same applies to cognitive functions - there are good models of neural circuits that provide such functions.

When describing the pharmacological correction of disorders, the authors do not indicate in what period drugs were used (before or after the injury).

Response: We thank the reviewer for this suggestion. As for the therapeutic implications, all treatments listed in Table 1 correspond to the post-injury phase, since they have mainly been administered to animal models following a controlled cortical injury. The data in **Table 1** addresses the reviewer's comment. We have also added some potentially new neuroanatomical and molecular correlates of TBI into the sections on acute, subacute, and chronic changes post-TBI.

Changes made: Main Manuscript; Page 6, Lines 133-135; Page 8, Lines 185-186; Page 9, Lines 200-202.

MINOR COMMENTS:

#1: In an acute trauma, the shearing impact of mechanical injury can lead to disintegration of both presynaptic and postsynaptic neuronal axons. (Synapses are not only axo-axonal).

Response: This sentence has been adjusted as per reviewer's suggestion.

Changes made: Main Manuscript; Page 4, Line 71: "In an acute trauma, the shearing impact of mechanical injury can lead to disintegration of both presynaptic and postsynaptic neuronal terminals."

#2: Understanding the neuropathogenesis of TBI-induced synaptic toxicity is essential for developing effective treatment protocols. (Can a synapse be toxic?)

Response: This sentence has been adjusted as per reviewer's suggestion.

Changes made: Main Manuscript; Page 5, Line 100: "From the practical perspective, Understanding the neuropathogenesis of TBI-induced synaptic excitotoxicity is essential for developing effective treatment protocols."

#3: This subsequently results in cleavage of cytoskeletal and synaptic proteins, mitochondrial disruption and free radical production which contribute to loss of dendritic integrity (Figure 1). This is not shown in the figure, but I would like it to be so.

Response: Figure 1 has been amended to show the suggested changes.

Changes made: Main Manuscript; Pages 24-26.

Notes on the figures.

#4: Fig. 1. Although the figure is intended to explain excitotoxicity, it does not do so. The understandable desire to point to SNAP25 as the main mediator between traumatic impact and excitotoxicity has

oversimplified the overall picture. Excitotoxicity involves many more participants and components, for

example, extrasynaptic ionotropic and metabotropic receptors. At a minimum, astrocytic processes should be shown to remove excess glutamate. In order not to overload the text, relevant references should be provided.

In addition, the figure may lead one to think that apoptosis and cell swelling occur in the postsynaptic compartment.

Erroneous labeling of glutamatergic vesicles (should be glutamate-containing).

Response: We thank the reviewer for this suggestion. Figure 1 has been modified to reflect the above changes.

Changes made: Main Manuscript; Pages 24-26

#5: Fig. 2. Proinflammatory cytokines penetrate the damaged BBB, but are subsequently synthesized by many brain cells, and in response, the synthesis of anti-inflammatory cytokines is activated to resolve neuroinflammation. This important component should be mentioned, along with mitophagy and autophagy, as they serve as targets for therapy. In addition, given that the authors' main focus in the review is on synaptic changes, it is necessary to point out the participation of microglia in the removal of damaged dendritic spines and synaptic components.

Response: We thank the reviewer for this suggestion. Figure 2 has been modified to reflect the above changes depicting the role of pro-inflammatory and anti-inflammatory cytokines as well as microglial activation and phagocytosis of neuronal synapses.

Changes made: Main Manuscript; Pages 27-28