



## Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 1

A traumatic brain injury (TBI) is common among professional athletes engaged in contact and collision sports. Although mild TBI is characterized by a transient disturbance in brain function manifested with neurological symptoms, such as headache, dizziness, and confusion, 10–25% of patients develop persistent post-concussion symptoms and long-term cognitive deficits. In this study, the authors from the United States examined changes in the expression of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 1 (NHE1), an important pH-regulating plasma membrane protein, and the markers of axonal damage, neuroinflammation, and oxidative stress in a mouse model of repetitive mild TBI (r-mildTBI). They also investigated whether selective inhibition of NHE1 reduces brain injury and sensorimotor and cognitive deficits in a mouse model of repetitive mild TBI.

A key pathology after mild TBI is a diffuse axonal injury (DAI), characterized by axonal stretching, mitochondrial swelling, cytoskeletal disorganization, and axonal bulb formation. This leads to disruptions in neuronal transport and protein accumulation, as evidenced by the accumulation of amyloid precursor protein (APP), which occurs rapidly after the inciting event. Early following TBI, astrocyte and microglial activation results in heterogeneous responses, such as altered gene expression, cytokine secretion, and reactive oxygen species formation.

The microglial NHE1 protein is a vital pH-regulatory plasma membrane protein that facilitates the efflux of H<sup>+</sup> in exchange for the influx of Na<sup>+</sup> and maintains optimal intracellular pH homeostasis. The authors emphasize that post-traumatic cerebral acidosis corresponds to slower recovery and elevated risk of poorer outcomes and mortality. Moreover, it is believed that extracellular acidic conditions following a TBI contribute to the accumulation of tau and amyloid- $\beta$  peptide aggregates observed in chronic traumatic encephalopathy (CTE). Long-term exposure to repeated head impacts can lead to persistent cognitive and neuropsychiatric symptoms and progressive, neurodegenerative disease known as CTE, which is a histopathological diagnosis that can only be diagnosed *postmortem*. It is based on neuronal perivascular accumulation of hyperphosphorylated tau aggregates in the depths of the cortical sulci, comprising pathognomonic lesions of p-tau immunoreactive neurofibrillary tangles and dotlike neurites, oriented around small vessels. A recent study has shown that CTE has been found in 41% of deceased young athletes who played contact sports and died before 30. <https://discovermednews.com/chronic-traumatic-encephalopathy-deceased-young-athletes/>

The same research team reported before that selective inhibition of NHE1 reduced neuroinflammation, enhanced remyelination, and improved neurological functional outcomes in a mouse model of TBI with open-skull injury. Accordingly, correction of the



## Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 2

pathologic H<sup>+</sup> homeostasis may be a promising strategy to modulate pathology after mild TBI.

### **About the study**

Adult male C57BL/6J wild-type mice, aged 2-3 months, were randomly assigned to either the repetitive-mild TBI group (r-mildTBI) or the sham control group. The mice got five closed-skull concussions, mimicking a mild TBI, on 0, 2, 4, 6, and 8 days, with an inter-concussion interval of 48 hours.

Sensorimotor and cognitive impairments were evaluated blindly with the rotarod accelerating test, open field test, y-maze spontaneous alternation test, and y-maze novel spatial recognition test. The rotarod accelerating test was performed a day before and 10-15 days post-injury (dpi). The sham control mice underwent the same procedures.

To investigate whether the NHE1 inhibitor HOE642 could attenuate the neurological deficits after mild TBI, mice with r-mildTBI were assigned to two groups: the NHE1 inhibitor HOE642 or vehicle control (DMSO) at a dose of 0.3 mg/kg body weight/day, twice daily for 7 days starting 24 hours after the last impact.

At 60 dpi, the cohort of mice that underwent behavioral assessments was humanely euthanized and decapitated, keeping the brains intact within the skull. The *corpus callosum*, hippocampal CA1, internal capsule, and external capsule in both hemispheres were identified as regions of interest (ROI). The levels of astrogliosis and microgliosis were evaluated by immunostaining for the expression of the reactive astrocyte marker protein GFAP (glial fibrillary acidic protein) and the microglial marker protein IBA1 (ionized calcium-binding adaptor molecule 1). Immunofluorescence staining was used to examine the alterations in the NHE1 protein.

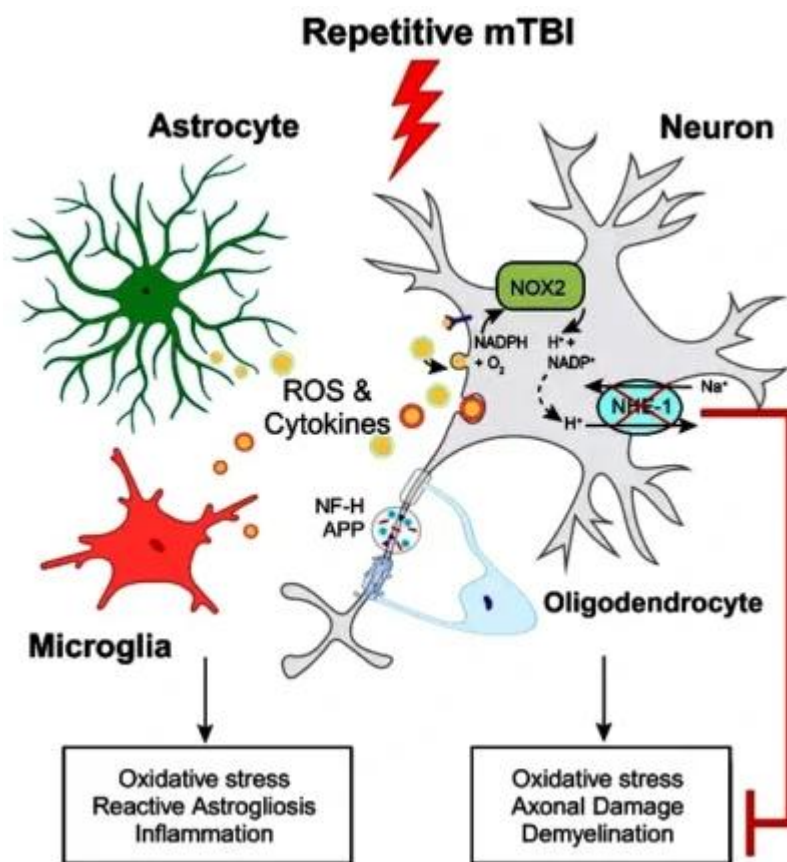
The white matter damage in *ex vivo* brains of the sham control, vehicle control (DMSO-treated), and HOE642-treated mice with r-mildTBI was evaluated using diffusion magnetic resonance imaging (MRI) and MRI diffusion tensor imaging (DTI). The authors stressed that diffusion MRI estimates brain fiber structures using water diffusion properties, whereas MRI DTI evaluates diffusivity across multiple directions and provides precise details about structural characteristics and directional alignment of white matter pathways. The DTI metrics of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were calculated for each region of interest. Lower FA values indicate a loss of integrity in conditions that cause axonal damage or demyelination, while RD values

# D

## Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 3

increase in conditions such as demyelination or changes in axonal density.

To evaluate changes induced by oxidative stress in brains with r-mild TBI, the researchers performed immunostaining for phosphor-p47-phox (p-p47), a crucial active subunit in the NOX2 complex activation, and of 4-hydroxy-2-nonenal (HNE), a well-characterized marker of oxidative stress.



Original illustration from the study of Bielamin JP, et al.

### Results

All animals exposed to repetitive mild TBI temporarily stopped breathing after each impact, followed by an extended period of immobility. In contrast to the sham mice, the mice with r-mildTBI exhibited a significantly higher righting time, the time necessary to return to the upright position after the anesthesia. These results show that injured animals needed a longer time to restore neurological functions. At 10 and 13 dpi, motor function in the rotarod accelerating test was significantly poorer in the r-mildTBI mice than in the sham group. At 40 dpi, mice with r-mildTBI demonstrated poor spatial recognition memory



## Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 4

function, as evidenced by a worsened performance in the y-maze novel spatial recognition test. These findings indicate motor and cognitive deficits in both acute and chronic stages after mild TBI.

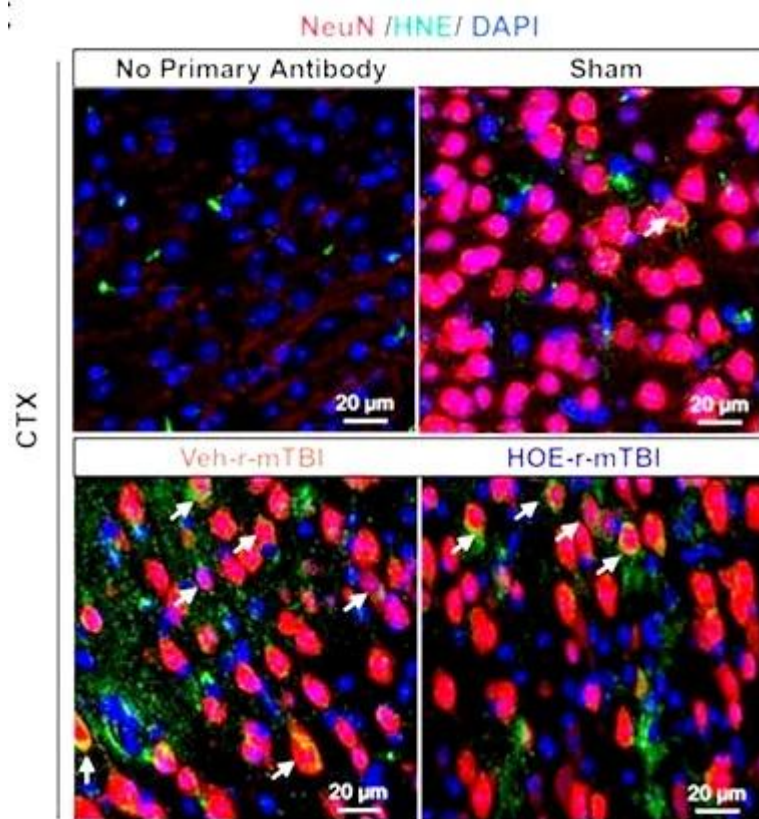
At 60 dpi, in contrast to the “resting” cellular morphology of astrocytes and microglia observed in the brains of sham mice, the brains of r-mildTBI mice displayed activation of GFAP+ astrocytes and IBA1+ microglial cells, especially in the peri-lesion areas throughout the cortex, *corpus callosum*, and hippocampal CA1 regions. The microglia morphology was amoeboid, whereas reactive astrocytes exhibited hypertrophy and elongated processes. An increase in the deposition of APP in the cortex and hippocampal CA1 neurons of r-mildTBI brains, compared to the sham brains, suggested a dysregulation of axonal transport. According to the authors, astrogliosis, microgliosis, and axonal damage occurred early in the pathogenesis of repetitive mild TBI.

NHE1 protein expression was significantly increased in the GFAP+ astrocytes, OLIG2+ oligodendrocytes, and IBA1+ microglia across the cerebral cortex and *corpus callosum* of r-mild TBI brains. In contrast, NHE1 protein expression was low in the same cells throughout the same regions of interest in the sham control brains.

These findings prompted researchers to investigate whether blocking NHE1 protein would impact brain damage induced by repetitive mild TBI. The results showed that locomotor and cognitive neurological functions improved after the administration of HOE642, an inhibitor of the NHE1 protein. Furthermore, immunostaining for GFAP+ and IBA1+ expression in the cortex, *corpus callosum*, and hippocampal CA1 neurons of mice with r-mild TBI treated with HOE642 was similar after 60 dpi as in sham brains after 15 dpi. This decrease in the number of GFAP+ and IBA1+ cells in all three brain regions indicates reduced activation of reactive astrocytes and microglia. In cortical neurons from mice exposed to repetitive mild TBI and treated with HOE642, immunostaining showed a decrease in the expression of p-p47, an active subunit of NOX2 complex activation, and HNE, a marker of oxidative stress. According to these findings, astrogliosis, microgliosis, and neurons’ oxidative stress response, were reduced by delayed pharmacological blockade of NHE1 protein after repetitive mild TBI.

# D

Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 5



Original illustration from the study of Bielani JP, et al.

At 60 dpi, MRI DTI, which was used to evaluate the white matter damage in *ex vivo* brains, revealed reduced DTI FA values in the *corpus callosum* of the vehicle control mice subjected to r-mildTBI, indicating a loss of integrity caused by axonal damage or demyelination. In contrast, the FA values were preserved in mice with r-mildTBI treated with HOE642. Of note, the DTI metric RD increased significantly in both cohorts exposed to r-mildTBI, in the vehicle control and the HOE642-treated mice, compared to sham animals, suggesting demyelination or changes in axonal density. There were no differences in FA, AD, MD, or RD values between the three groups in the hippocampal CA1 regions and the internal and external capsules. These results demonstrated reduced axonal damage and preserved white matter integrity in the *corpus callosum* following pharmacological inhibition of the NHE1 protein.

## Conclusion

This study found that repetitive mild TBI in C57BL/6J mice stimulated neuroinflammation, caused axonal damage, upregulated the expression of the NHE1 protein, and induced



## Selective inhibition of NHE1, a pH-regulatory protein, improved locomotor and cognitive recovery in a mouse model of repetitive mild TBI | 6

neurological functional deficits. The administration of a potent NHE1 inhibitor, HOE642, following repetitive mild TBI, resulted in a reduction of pathological signatures, such as gliosis, oxidative stress, axonal damage, and white matter damage (as detected by MRI DTI) and improved locomotor and cognitive functional recovery. The authors concluded that targeting the NHE1 protein could be a potential therapeutic strategy for reducing mild TBI pathology and improving neurological functions.

This article was published in Antioxidants.

### ***Journal Reference***

Bielanin JP, et al. NHE1 Protein in Repetitive Mild TBI-Mediated Neuroinflammation and Neurological Function Impairment. *Antioxidants* 2024, 13, 836. (Open Access)

<https://www.mdpi.com/2076-3921/13/7/836>

