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# Viewpoint

# A Promise to Keep, but Miles to Go Before We Sleep …

In this issue of the *Journal of Pharmacology and Experimental Therapeutics*, Al Yacoub et al. (2024) report new findings in their paper "Recovery from Traumatic Brain Injury (TBI) Is Nociceptin/Orphanin FQ Peptide (NOP) Receptor Genotype-, Sex-, and Injury Severity-Dependent", which suggest that nociceptin/orphanin FQ peptide (NOP) may play an adverse role in the pathogenesis of functionally significant deficits following traumatic brain injury (TBI) (Fig. 1). TBI is a major worldwide public health problem (GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019), for which many candidate interventions have been identified and tested in preclinical models (Lerouet et al., 2021). Unfortunately, no therapeutic agent has been shown to benefit humans with TBI. This disconnect between preclinical efficacy and the human condition has been attributed to a range of potential factors (Alves et al., 2019; Hoogenboom et al., 2019a,b; National Academies of Sciences, Engineering, and Medicine, 2022). Al Yacoub et al. (2024) designed their study to determine whether absence of the NOP receptor reduces pathologic, biochemical, and neurobehavioral adverse effects of mild and moderate controlled cortical impact (CCI). Their findings suggest that downregulation of NOP function could prove to be a new means to attenuate TBI-induced pathology and dysfunction. The world needs an effective intervention to improve outcomes for more than 53 million patients who sustain TBI each year. Al Yacoub et al. (2024) point to a potential treatment target, which the field must now contextualize to assess its ultimate potential.

Al Yacoub et al. (2024) stratified male and female wild-type (WT) and NOP receptor knockout (KO) rats into groups, which underwent actual or sham open skull CCI. The CCI protocol was administered to separate groups at two different severities. A wide range of outcomes was examined at acute (day 1) and subacute (day 8) time points, including neurologic function, nociception, and biochemistry (serum, cerebrospinal fluid, and homogenized brain tissue). The study confirmed that moderate CCI induced a larger focal brain lesion than mild CCI. However, the moderate CCI brain lesions were smaller in KO compared with WT animals. Moreover, at each injury severity, NOP-KO animals exhibited smaller lesion volume compared with the same injury in WT animals (sexes were pooled for the lesion volume assessments). Neurobehavioral function was altered acutely in all injury groups compared with sham. As expected, dysfunction was greater in moderate compared with mild CCI. Sex and genotype did not show a significant interaction with injury severity at the day 1 acute time point. At day 8, however, interactions of injury severity and genotype and of sex and genotype were identified. Neurobehavioral dysfunction in WT animals was greater for both male and female moderate CCI compared with mild CCI and to sham. This indicates that the injury paradigm induced persistent dysfunction at day 8. Male moderate CCI KO animals exhibited dysfunction, but less than WT at day 8. Among female moderate CCI animals, sham and KO animals were not distinguishable at day 8, consistent with full recovery. In summary, KO animals exhibited greater (male) or complete (female) recovery at day 8 compared with WT.

Rotarod performance over the 8 days of follow-up showed a decline from baseline at day 1 for both injury severities, regardless of sex or genotype. Male KO animals exhibited greater recovery compared with male WT, but both male WT and male KO exhibited persistently diminished performance at day 8 compared with sham. Female animals recovered more completely than males, with day 8 performances not significantly different from sham regardless of severity or genotype. Female KO animals, however, recovered to the sham level more rapidly than female WT animals.

On measures of tactile and thermal nociception, compared with sham, TBI animals exhibited allodynia on day 2 regardless of injury severity, sex or genotype. The only exception to this pattern was a de minimis effect of the thermal stimulus on KO animals, regardless of injury severity or sex. Group differences in recovery of allodynia, compared with sham, were incomplete in WT animals regardless of injury severity and sex, but were complete in KO animals regardless of injury severity or sex.

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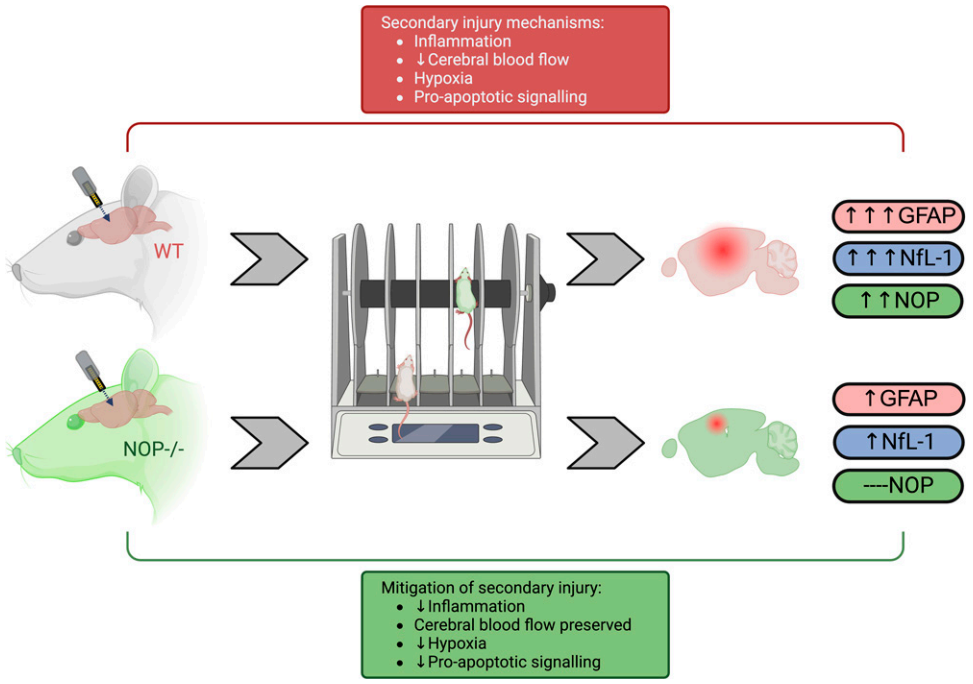


**Fig. 1.** Schematic depiction of the evolution, following CCI in WT and NOP-KO animals, of behavioral dysfunction (only rotarod shown), injury severity (brain lesion size depicted in red), and injury markers (pink and blue ovals). Putative secondary injury mechanisms (red rectangle) related to N/OFQ activity (green oval) are attenuated in the KO animals (green rectangle). Note that all groups and sex differences described in the text are not shown in this schematic. Created with BioRender.com.

Similar patterns were seen on biochemical assays related to the NOP system. NOP levels and NOP receptor expression in the cerebral hemisphere ipsilateral to the impact were higher in WT males compared with sham, but only reaching significance in moderate CCI. In female WT, however, NOP levels and NOP receptor expression in the ipsilateral hemisphere were lower than in female sham regardless of injury severity. Assays for markers of tissue injury, including axonal injury (NfL-1) and astrogliosis (glial fibrillary acidic protein) showed elevation of injury biomarkers ipsilateral to the CCI in WT animals, regardless of injury severity, and sex, whereas biomarker expression was not significantly different from sham for KO animals, regardless of injury severity or sex. Testing with the elevated plus maze, interestingly, showed no emergence of anxiety-like behavior in any group.

Al Yacoub et al. (2024) have provided a wide-ranging look into the impact of the NOP system on a range of TBI outcomes. Important strengths of their study include its use of multiple comparison groups and inclusion of male and female animals, a surgical sham condition, a longitudinal design and inclusion of multiple, and varied outcomes probing both tissue and function. The findings demonstrate a high degree of convergence across multiple outcome measures that points to a potentially adverse effect of NOP, which in turn appears to interact with sex, where female animals benefit most from absence of the NOP receptor. This pattern itself is interesting, in that women are generally found to be more vulnerable to persistent effects of TBI in human studies. The looming question, as with all preclinical studies, is how these findings might inform approaches to human TBI.

The authors employed a standardized and widely described CCI model, which facilitates comparison across groups, thereby mitigating variance due to the injury protocol itself. The CCI approach, however, also confers important limitations on interpretation and translation of the findings (Zhao et al., 2023). As the authors acknowledge, TBI entails a combination of focal and diffuse injury. Assessment of the diffuse injury component, however, was beyond the scope of this broad study. The induction of a frank focal injury also confounds assessment of the most common form of TBI. Mild TBI (mTBI), also referred to as concussion, accounts for the vast segment of TBI cases, as much as 75% or more (Centers for Disease Control and Prevention, 2017). Because mTBI is so prevalent, it plausibly accounts for more morbidity and societal cost worldwide than more severe TBI (Miller et al., 2021). Although still controversial, mounting evidence raises the concern that mTBI may confer risk for later life neurodegeneration (Barnes et al., 2018).

In mTBI, the skull remains intact and focal injury does not occur. In this regard, the authors were precise in describing their mild injury condition as “mild CCI” and not mTBI. The craniotomy and direct

impact onto the brain surface, resulting in gross focal loss of brain tissue, are not consistent with human mTBI. Additional limitations of the TBI model include the lissencephalic rat brain, use of anesthesia, and fixation of the head in a stereotaxic frame during CCI, among others (Hoogenboom et al., 2019a). These carefully standardized CCI methods have, of course, facilitated much progress in the understanding of TBI pathogenesis, and the use of this model is a strength of the carefully executed study of Al Yacoub et al. (2024). At the same time, the implications of their findings for human TBI remain limited due to the model system employed and should motivate more and different approaches to investigating the potential relevance of the NOP system to human TBI.

Many interventions identified as beneficial in preclinical TBI studies—most famously progesterone (Wright et al., 2014)—later failed when tested in clinical trials of human TBI. This disconnect could be due to fundamental differences in the pathologic condition used to derive the candidate therapy (Nichol et al., 2015). It also may be that interventions characterized in the context of focal TBI do not address the diffuse injury that likely underpins much TBI morbidity (Chen et al., 2020). Finally, significant focal injury with tissue loss may not be amenable to meaningful repair (Maas et al., 2005). Notably, the investigation by Al Yacoub et al. (2024) showed, through a loss of function variant, that recovery is better after CCI when NOP is absent from before the time of injury. Whether downregulating NOP after the time of injury (when treatment is likely to be feasible in humans) is also effective remains untested and an important target for future study. An additional open question is whether the effects demonstrated could alternatively arise from a compensatory mechanism operative in the KO animal. To assess the potential efficacy of NOP as a candidate treatment target for TBI, other experimental approaches that more closely approximate mechanisms in human TBI will be useful, such as closed skull impact models without fixation of the head, models employing head acceleration–deceleration without head impact and adoption of gyrencephalic mammals such as ferrets and piglets, which more closely reproduce the biomechanics of human TBI and could approximate mTBI (Hoogenboom et al., 2019a). The work by Al Yacoub et al. (2024) in this issue provides a broad basis to further explore a potential role for downregulation of NOP function, perhaps through NOP receptor blockade, as an intervention to improve TBI outcomes. Investigation of receptor blockade could also address the concern that compensatory mechanisms in the transgenic variant might underlie the outcome identified in the KO animal. Al Yacoub et al. (2024) point us toward a potentially effective TBI intervention. Like Robert Frost's winter traveler (Frost, 2019), however, there is a long road ahead before we define its potential. Further investigation for effects (e.g., diffuse injury to white matter) and in experimental in contexts (e.g., closed head mild impact or non-impact models) relevant to the human condition, as well as application of NOP blockade prior to and following injury, could further illuminate the path toward clinical efficacy.

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