Response to Comments by Reviewer 2

Comment #1: The phraseology occasionally seems awkward, reflecting the fact that English is likely not the authors’ primary language. For example, on p. 1, I recommend changing “constant form of HO isoforms” to “constitutive isoform of heme oxygenase”; on p.6, “the mouse blinded model” should be re-phrased.

Response: We have changed those sentences per your suggestion.

(Lines 34–35) [HO-2, a constitutive isoform of HO, is present in high levels in the liver, brain, and testes.]

(Lines 254–255) [Müller cells can be reprogrammed to generate rod photoreceptors, leading to restored visual responses in a mouse model of congenital blindness]

Comment #2: On p. 1, the authors state that “HO-3, another form of HO, is found in the rat brain”. More accurately, HO-3 is a pseudogene (retrotransposition of Hmox2) specific to rats (Scapagnini et al., 2002).

Response: We have changed that sentence per your suggestion.

(Lines 36–37) [HO-3 is a pseudogene found in the rat brain and does not have enzymatic activity [1, 2].]

Comment #3: This reviewer’s most pressing concern is that the authors entirely neglected to present the other ‘Janus’ face of HO-1—viz., that sustained up-regulation of HO-1 in brain may exacerbate, rather than mitigate, neural injury in a host of human neurological conditions. A balanced literature should acknowledge that, in a host of chronic human CNS afflictions, the glial HO-1 response may serve as a transducer of noxious stimuli and an important driver of relevant neuropathology. Specifically, sustained over-expression of HO-1 in astroglia, with attendant liberation of intracellular free iron and CO, may contribute to the pathological iron deposition, oxidative damage and mitochondrial insufficiency documented in multiple sclerosis – an important neuroimmunological disorder - as well as several neurodegenerative conditions such as Alzheimer disease and Parkinson disease (recently reviewed in Prog Neurobiol, in press.
On p. 8 the authors state that “HO-1 overexpression increased the survival rate of dopaminergic neurons” in an MPP+ rat model of Parkinson’s disease”. Yet, the authors fail to mention that overexpression of astroglial HO-1 (to levels seen in human PD brain) in middle-aged mice results in a robust phenotype highly reminiscent of idiopathic PD (Neurobiol Aging 58: 163-179, 2017). The disparate behaviour of HO-1 under various neuropathological conditions may be reconciled by the fact that intracellular heme degradation may exert net antioxidant or pro-oxidant effects contingent upon the intensity and temporal profile of HO-1 induction and its interplay with the prevailing redox microenvironment. The authors should seriously consider the ongoing controversy regarding the potential benefits and liabilities of chronic HO-1 expression in brain before advocating induction of the enzyme as a therapeutic modality in neuroinflammatory and other CNS conditions.

Response: We changed these sentences per your suggestion.

(Lines 55–61) [However, HO and its metabolites are Janus-faced. In this regard, HO-1 in astrocytes of the aging and diseased central nervous system (CNS) can be an effector of deleterious stimuli, leading to neuronal injury [3]. Administration of the HO inducer hemin can increase pro-inflammatory prostaglandin E2 levels in rat hypothalamic explants and in primary cultures of rat hypothalamic astrocytes [4]. CO and BR may also have dual roles (i.e., pro-inflammatory and anti-inflammatory) in several different organs and tissues [5, 6], depending on the concentration and the signaling pathway involved.]

(Lines 182–189) [In reactive glia, astrocyte-specific overexpression of HO-1 in conditions of oxidative stress leads to neuronal injury by releasing neurotoxic molecules such as IL-1β and TNF-α [3, 7]. In transgenic mice overexpressing human HO-1 in their astrocytes, HO metabolites promote mitochondrial sequestration of non-transferrin iron, oxidative stress-mediated substrate modification within the mitochondria, and subsequent mitophagy [7]. Co-culture of PC12 cells with HO-1 overexpressing astrocytes induced PC12 cell death, which was reduced by treatment with deferoxamine, an iron chelating agent [8]. Therefore, the neurotoxic role of HO-1 in oxidative stress-conditioned astrocytes may stem from excessive iron deposition.]

(Lines 427–430) [However, sustained upregulation of HO and its metabolites in the brain may exacerbate neural injury. Therefore, the disparate properties of HO and its
metabolites under various neuropathological conditions must be carefully considered for clinical approaches.

References