Author's response to reviews

Title: Role of astrocytes in manganese mediated neurotoxicity

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Author's response to reviews: see over
Dear Dr. Morrey,

We are pleased to resubmit our manuscript entitled “Role of astrocytes in manganese mediated neurotoxicity” for consideration as a review article in *BMC Pharmacology and Toxicology*. Below are the Reviewer’s comments and our replies with the changes we amended in the article. We appreciate the Reviewer’s comments and hope that we have addressed their concerns and suggestions.

Thank you for considering our manuscript.

Reviewer 1.

1). There is, however, one point where the discussion is one-sided and therefore needs additional information to provide the reader with a more balanced view of the status of the hypothesis. This concerns the discussion of the astrocyte-neuron lactate shuttle (end of section 2.1, p. 6). From the presentation provided the reader will get the impression that this hypothesis is already fully proven and accepted, a notion which is far from being correct. There are several opposing views that based on experimental evidence would point to the possibility of lactate flux in the opposite direction, i.e. from neurons to astrocytes depending on the actual lactate concentrations.

Response: We agree with the reviewer that ALSH hypothesis was described in somewhat of a biased way. We added information showing alternative hypothesis for the ALSH. In the current version, we also discuss a role for lactate as neuronal energy fuel (Section 2.1, p.6, l.9-20).

2). Section 1, p. 3, l. 18: have instead of ‘has’
   Section 2.1, p. 5, l. 12: to instead of ‘for’
   Section 2.1, p. 5, l. 21: than instead of ‘then’
   Section 6, p. 10, l. 9: all of instead ‘of all’
   Section 7, p. 11, l. 14, ‘taken up by’
**Response:** We corrected all suggested changes (Section 1, p. 3, l. 19; Section 2.1, p. 5, l. 15; Section 2.1, p. 5, l. 24; Section 6, p. 11, l. 22; Section 7, p. 12, l. 28).

**Reviewer 2:**

1) **Among different effects of Mn in the CNS, it should be discussed better why it can cause an oxidative stress.**

**Response:** We added the missing information about mechanistic aspect of Mn-mediated oxidative stress, including glutathione and taurine depletion (Section 3, p.8, l.24-30).

2) **It is not true that neurons lack the capacity to synthesize glutamate from glucose. There is a huge controversy as to the relative contribution of glial and neuronal oxidative metabolism, but it is clear now that neurons can oxidize glucose in the Krebs cycle.**

**Response:** We corrected these sentences and added data showing the role of the glial-specific pyruvate carboxylase for de novo synthesis of glutamate and neuronal energy support by astrocytes. In the current version we also describe dependency of Glu neurotransmission from astrocytic metabolism (Section 2.1, p.4, l.29-30; p.5, l.1-5).

3) **Because of the involvement of Mn to the activity of PC and its important role for anaplerosis and glutamate replenishment, a paragraph discussing PC should be essential in Chapter 2.1, e.g. when discussing the synthesis of glutamine. In general, the role of Mn for PC and glutamine synthetase, and the consequences for neurotransmission, should be discussed in much more detail.**

**Response:** We included the missing information about Mn-mediated disruption of activity of PC and glutamine synthetase and its role for glucose metabolism and neurotransmission (Section 3, p.8, l.31-32; p.9, l.1-6).

4) **The effect of Mn on astrocytic pathology should also be discusses as this is an important point. This includes the relative effect on astrocyte swelling and the appearance of Alzheimer type II astrocytosis, which are observed in patients, animal models and cell culture. Both pathological changes in astrocytes by Mn may represent an important aspect of manganese neurotoxicity. In this regard it encephalopathy (HE), in addition to ammonia. Astrocyte swelling and Alzheimer**
type II astrocytosis are also observed in HE. It will be important to include the role of Mn in HE, also considering the effects of ammonia on neuron-glia metabolic shuttles and neurotransmission.

Response: In the revised version, we describe the ability of Mn to induce pathological changes in astrocytes, including Alzheimer type II astrocytosis, astrocyte swelling and gliosis in relation to the Alzheimer diseases. In regards to similar possible contribution of Mn –induced astrocyte pathology, we discuss the role for Mn in hepatic encephalopathy (HE). Additionally, we provide evidence for the synergistic effects of manganese and ammonia in the etiology of HE, including effects on astrocyte morphology and neurotransmission (Section 2.2, p.8, l.1-19, we changed the title of Section 2.2: Astrocytes and manganese in some neuropathological conditions: focus on Alzheimer’s disease (AD) and chronic hepatic encephalopathy (HE)).