Author’s response to reviews

Title: Magnesium administration provokes motor unit survival after sciatic nerve injury in neonatal rats

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PDF covering letter
Covering letter

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To
Editorial Team, BioMed Central

General remarks:

- New tables and figures have been added, according to the reviewers recommendations, in order to present our data more sufficiently. Thus, the numbering of Tables and Figures in the revised manuscript has changed. All changes made are listed in detail, below. (In the text they are shown in blue letters)
- One new literature reference has been added: [10] of the new manuscript.
- [10] reference of the pre-revision manuscript is [31] of the revised manuscript.
- [31] of the pre-revision manuscript is [32] of the revised manuscript.
- The necessary changes in the main text have been done as well and spelling or other mistakes have been corrected.

Dr L Greensmith’s recommendations:

- Tables in the supplementary text have been referred to, in the main body of the text.
- Figure 1: The reviewer thinks that this should be presented as a bar chart with error bars. This has been done (Figures 1a and 1b). Another figure has been also added (Fig 2, page 11, 1st paragraph 12th line).
- To avoid any misunderstanding, concerning data presentation in Tables 2&3 of the pre-revision manuscript, the Legends in both tables, has been revised as follows: ‘Muscle Weight, Number of Motor Units, Single Twitch and Maximal Tetanic Tension, expressed as a % ratio, of the operated to the control side, in the EDL/Soleus, in the four age groups tested. Axotomy has been performed in P2, in all cases’.
- Table 5, is being revised completely, according to the reviewers recommendations (appears as Table 6, in the revised manuscript).
- The first sentence of the ‘Results’ section of the Abstract, has been revised, as follows: “Axotomy resulted in 20% MU survival in EDL and 50% in soleus.”
- The 2nd sentence of the ‘Conclusion’ section of the Abstract was revised: “Magnesium administration rescues motoneurons and increases the number of motor units surviving into adulthood”.
- Background, 2nd paragraph: L.Greensmith points that it is not shown in ref [2], that fast muscle fibers are innervated by bigger nerve cells. Actually ref [2] does

- **Background, 4th paragraph:** Revised as follows: Following neonatal sciatic nerve injury, the surviving motoneurons, take at least 8 days to grow back to the hind limb muscles, whereas most of the motoneurons that die, do so by apoptosis within the first two days [5].

- **Background, last paragraph:** L.Greensmith was right: Reference [10] (Greensmith 1st author), does not prove that Magnesium crosses the blood-brain barrier. In this paper the authors assume that Magnesium ions could be acting on the nerve cells after injury, crossing the blood-brain barrier. However, magnesium has been shown to concentrate in the cerebrospinal fluid after intraperitoneal injection and increase the electrical threshold required to control seizures, in a rat model, by other investigators (reference [10]of the revised manuscript), as L.Greensmith suggested.

- **Page 6 - line 8:** Reference [12] states that signs of reinnervation are evident in 10-12 days, after sciatic axotomy, according to the authors’ experience. In our experience, (paragraph: “Magnesium administration” in page 6), signs of reinnervation were evident in 7-9 days after axotomy in Magnesium treated rats. In non-treated rats, we state that abnormal kinetic behavior was evident for about 2 weeks (our experience), which is comparable to reference’s [12] findings. We revised line 8 in page 6: ‘The first behavioral signs of reinnervation after axotomy, should be evident at about 10-12 days after injury, according to other investigators [12]’.

- **Page 7, line 5:** …‘Krebs solution’…, added.

- **Results:** The reviewer’s recommendations, were followed, in our revised manuscript.

- **Conclusions:** We revised the 1st paragraph of the ‘Conclusions’ section: “Our findings strongly support the findings of previous work [10], that has shown magnesium in vivo administration to rescue sciatic motoneurons from cell death, after axotomy”.

- **Spelling mistakes** have been corrected; line 5: we mean the resultant, not the result.

- **‘Conclusions’, paragraph 4,** revised:“In conclusion, our results show that motoneuron death occurs mostly within two weeks of axotomy, while systemic Mg²⁺ administration rescues motoneurons and increases the number of motor units surviving into adulthood. Furthermore, fast and slow muscles respond differently to axotomy, as well as, to subsequent in vivo treatment with Mg²⁺.

**Dr U Slawinska’s recommendations:**

Major Compulsory revisions

1. The reviewer is pointing on the issue of isolating soleus muscle contraction, to avoid force summation, because of concomitant gastrocnemius muscle contraction, after stimulating the sciatic nerve. It was our omission not to clear in our initial manuscript, that we used to cut the branches innervating the gastrocnemius and plantaris muscles. This is added in our ‘Methods’ section, of the revised manuscript.
2. Data presenting muscle weight and force outcome (single twitch and titanic contraction), of EDL and soleus muscle, are shown in Tables 6 and 7 of the revised manuscript.

3. This is not because we did not all the recordings (10,20,40,80 and 100 Hz), but because the traces after 80 and 100 Hz stimulation frequency were of the same amplitude (= all MUs recruited), in these occasions.

Minor Essential Revisions

1. **Page 4:** P0 (zero) is the day of birth. We revised: “The day of birth was taken as P0 (zero). At the 2nd…”

2. **Page 5:** Six animals were included in the results. We revised: “Six successfully tested animals…”

3. **Page 5:** By manipulations: The examiner, placing his index finger on the animals foot, forces it to plantar- and dorsiflex the ankle. The animal reflects actively, doing the opposite movement if able to dorsiflex and plantarflex, respectively. No reactive movements are evident after successful denervation.

4. **Page 6:** The following appear in [11].
   - Hip and knee in extension
   - Ankle in the operated side in plantar flexion
   - Adduction of the whole limb
   - Weakness of digit extension in operated side

5. **Page 6:** We shouldn’t say ‘abnormal reflexes’. We revised: “motion deficit on the denervated limb, as described above, for about 2 weeks”, instead.

6. **Page 7:** 250 msec duration

7. **Page 8:** An image of 6 single twitch recordings, elicited by incrementally increased electrical stimuli on the left sciatic nerve, indicating an axotomized EDL muscle of an adult animal, with 6 surviving MUs, is presented (Figure 2 of the revised manuscript). We should mention however that technical problems and lack of equipment does not allow us to provide a better imaging of motor unit counting.

8. **Pages 9-12:** Necessary corrections have been made

9. **Pages 13,14:** Reviewing reference [22] according to Mrs Slawinska’s comments, we realized that in fact, this paper does not refer to nerve injury. Page 13 quotation was revised (new manuscript).

10. **Page 13:** The reviewer was right/ see reference [25]: We revised stating, that: “The process of soleus’ development into a fatigue resistance muscle is stopped, after sciatic nerve axotomy. Axotomized soleus becomes less fatigue resistant in adult rats, compared to control muscles. However the process of muscle necrosis, as proposed by other authors, could contribute to this result, as well, rather than the loss of motor units alone [25].”

11. **Page 15:** [10] is the correct paper to be referred, as the reviewer, correctly, noticed. Correction was made.

Descretionary Revisions:

We do not think that we need to discuss the blood-brain barrier penetration issue that extensively. We should just assume that the blood-brain barrier is being penetrated, by
Magnesium ions, provided that there is evidence that Mg is being used as an anticonvulsant drug.