Author's response to reviews

Title: Structural and functional consequences of buserelin-induced enteric neuropathy in rat

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Version: 2
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Author's response to reviews: see over
Answer to BMC Gastroenterol October 2014

Dear Editor,

Thank you for considering our manuscript: MS: 1075674467133928
Structural and functional consequences of buserelin-induced enteric neuropathy in rat by Elin Sand, Bodil Roth, Björn Weström, Peter Bonn, Eva Ekblad, and Bodil Ohlsson for reviewing.

We have now answered all the questions and comments raised by the reviewer, and we think that the manuscript has been further improved after revision. All changes are marked in yellow, and we submit one marked and one unmarked version of the manuscript. Please consider this revision for publication in BMC Gastroenterology.

Reviewer's report
Title: Structural and functional consequences of buserelin-induced enteric neuropathy in rat
Version: 1 Date: 10 October 2014
Reviewer: Bridget Southwell

Reviewer's report:
Busrelin and enteric neuropathy
MS: 1552626293142224
Structural and functional consequences of buserelin-induced enteric neuropathy in rat Elin Sand, Bodil Roth, Björn Weström, Peter Bonn, Eva Ekblad and Bodil Ohlsson BMC Gastroenterology
Review of revision 1.
The authors have greatly improved the manuscript and answered the questions. There are some small editing changes. I would like to see the manuscript again after these changes
Page 2- abstract. The results for VAChT are not in the results now. So this should be removed from the abstract line 42.

Reply: We have not removed VAchT from the results, VAcht is shown in Table 2. All subpopulations are referred to together in the main text, page 15, line 323-324. Thus, we want to keep it in the abstract.

Page 7 line 129- what does ‘x R x’ mean?

Reply: \((1.12 \times R \times (\text{RPM}/1000)^2)\) is the formula for calculating rcf, where R = radius.

Page 12 line 250. Add ‘Analysis of’ before ‘antibodies’

Reply: This is now added, page 12, line 249.

Page 14 line 308-. Figure 1 shows Submucosal ganglia and then myenteric ganglia. Change the order in the text or figure so that they are the same.

Reply: The order is now changed in the text, page 14, line 307-310.

Page 14 line 311. Says the loss of neurons in the myenteric ganglia was greater
than in the submucosal ganglia. But there was a reduction of 61% in colon submosal neurons. As this is larger, change text to say ’loss of neurons in the colonic submucosal ganglia was greater than in the myenteric ganglia’.

Reply: Although the difference in median values is greater in submucous ganglia, the difference is statistically more significant in mucous ganglia, as the values in each group are more scattered in results from the submucous ganglia. We have reformulated the sentence, page 14, line 310.

Page 15- there are no results for VAChT now. If they are not included remove the antibody information from the abstract and methods. Or add results.

Reply: The results are shown in Table 2. All subpopulations are referred to together in the main text, page 15, line 323-324. Thus, we want to keep it in the text and table.

Page 17 lines 359 to 362-’However in a complementary study....(unpublished data)’. Add this data to results and methods. You can’t add data into the discussion.

Reply: The data from the complementary/other study is not added since it was quite another study with different design and methodology. In that separate study, feces were collected from 8 controls and 12 buserelin-treated rats during 12 h of fasting in metabolism cages and analyzed for weight in both wet and dry condition. Wet feces were weighed before and after being dried in a 40 °C heating box during 48h. The rats were very stressed by the treatment in the metabolism cages, which may affect feces weight and content. In the present study, the rats were not as stressed as they were not put in metabolism cages, but were in their home cages. The feces was dried in a room temperature during 3 months. As the 2 experiments are not comparable in design and handling of the animals, we do not want to include it in the present study. That study will be published in another publication. Unfortunately, the dried weight was not weighted in the present study, why we just can speculate on the reasons to increased wet weight, page 17, line 349-358. The result of wet weight is not highlighted in the conclusions because of uncertainty whether depending on food intake or water content or something else. Furthermore, test of feces weight is never used in the daily clinical practice, whereas fat in feces can be used in patients undergoing examination of GI dysfunction, page 20, line 422-425.

Page 18 line 380- VACht is listed here but not in results- add to results or delete Here

Reply: The results are shown in Table 2. All subpopulations are referred to together in the main text, page 15, line 323-324. Thus, we want to keep it in the text and table.

Page 25 line 532- delete ‘s’ from ‘indicates’

Reply: The “s” is deleted.

Page 25 line 536- change ‘magnification bar’ to ‘scale bar’, delete ‘in micrograph (j)’
Reply: Performed.

Page 33- table 2: you have combined saline and buserelin treated rat data in this table. This is awkward as the aim is to show they are the same. Make this clear in the heading: change to ‘Nerve fiber density and distribution in colon was identical in saline and buserelin-treated rats’.

Reply: The heading has been changed.

Page 33- table 2 line 778 and 780. Move last sentence and add to end of sentence in line 778 to read ‘...in colon of saline- and buserelin-treated rats are shown combined as no differences in nerve fibre.....’.

Reply: The sentences have been changed.

Figure 2: shows high numbers of NOS neurons in the submucosal ganglia and very few SP neurons. There are no NOS nerve fibers in the mucosa but many SP nerve fibers in the mucosa and many SP neurons in the SG. Is this data correct or miss labelled?

Reply: The results are as written on page 15: In colon, a small number (fewer than 10%) of submucous nerve cell bodies were immunoreactive to CGRP, CART, galanin, NPY or SP, while GRP-, NOS-, and VIP immunoreactivity were found in 10%–43% of submucous neurons. The relative number of somatostatin-containing submucous nerve cell bodies was 11% in colon from controls, but showed a tendency to increase to 14% after buserelin treatment (p = 0.062; Figure 2). In both controls and buserelin-treated rats, few colonic myenteric nerve cell bodies were found to contain CGRP-, galanin-, NPY-, SP-, or VIP immunoreactivity, and moderate numbers of neurons were found to contain CART, GRP, and somatostatin, whereas myenteric NOS immunoreactivity was abundant. All data from immunocytochemistry are shown in Table 2 and Figure 2.

The conclusion in the question above is not correct.

Figure 3. the text in the scale bar is not readable. Make it larger or leave it out. The text can go above the scale bar in white.

Reply: This is now corrected.

Figure 5- galactose is misspelt

Reply: This is now corrected.