Author's response to reviews

Title: Hypericum perforatum treatment: effect on behaviour and neurogenesis in a chronic stress model in mice

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Author's response to reviews: see over

Prof. Diana Marshall

Senior Scientific Editor

BMC Complementary and Alternative Medicine

RE: MS: 1458689704287049

Dear Prof. Diana Marshall,

Thank you very much for sending us the comments of the reviewers to our article "Hypericum perforatum treatment: effect on behaviour and neurogenesis in a chronic stress model in mice".
We have now revised the manuscript according to their suggestions and in particular:

Reviewer: Oliver Grundmann

Discretionary revisions:

BrdU as a key word does not seem to be very specific - the authors may consider to eliminate this keyword

In agreement to the suggestion of the reviewer we have eliminated BrdU as a key word in our paper.

The Latin name of St. John's wort should also be italicized in the abstract

The Latin name of St. John's wort has been italicized in the abstract, as suggested.

The last sentence of the background section should probably end with "correlations" instead of "implications" since behaviour is correlated to the morphological changes observed but not an implication of these changes.

Ok corrected.

Figure 4A may not be necessary - the application of BrdU 2 hours prior to sample collection can be mentioned in the methods section.

Ok eliminated.

Minor Essential Revisions:

In the Conclusions section of the Abstract, the authors state that morphological adaptations occurred in both newborn and mature hippocampal neurons. In the paper and the methods section, only adult mice are mentioned that were actually analyzed. This should therefore be clarified.

In agreement to this observation we modified the statement by deleting the term "newborn".

In the background section, the authors do not mention flavonoids as potential active components in Hypericum extracts. This has been shown by Butterweck et al. in mice and rat models and should therefore be included (specifically hyperoside and isoquercitrin).

According to the suggestion, we have included flavonoids as potential active components in *Hypericum* extracts. The full statement was modified and previous reference n. 6 (Vitiello et al.) has been substituted by Butterweck et al. (2007).

Under "Animals" the care guidelines should probably state NIH instead of NHI.

Ok corrected.

Usually, standardized Hypericum extracts contain a certain amount of flavonoids that are quantified by quercetin and its conjugates - this should be clarified in the manuscript. Kaempferol should be written correctly.

Already modified (see above when listing active components in *Hypericum* extract).

In the immunohistochemistry section, when the term DG is first mentioned, it should be written as dentate gyrus followed by the abbreviation (DG). Thereafter it can be written as DG.

Ok corrected.

On figure 4H, the single star above the cort- vehicle group should be removed.

Ok removed.

Major compulsory revisions:

Under "drugs and treatment schedule" the vehicle control should be defined - was it simply water? Did the animals who did not receive Hypericum still receive DMSO/saline injections?

The vehicle control as now been defined.

The authors state later that each group actually had n=8 animals - but in the methods section, each group had n=16 animals. The assumption that the intial two groups, cort- and cort+ each have n=16 animals, are then each split up into Hyp+ and Hyp- to give the final n=8 animals/group should be clarified here.

Ok, this point has been clarified.

The explanation of the 4-point scale for fur condition is insufficient - only 2 conditions have been mentioned (0 and 1) - what about the other 2?

Right, we did not used a 4- point scale but a 2-point scale as now indicated in the text. The specific reference was added .

How were the hippocampal samples retrieved? Which method was used and was the whole brain or only the hippocampal region fixed in formalin solution? Which magnification was used for detection?

We specified that whole brain was fixed in formalin solution and indicated the magnification used for detection.

The "Statistical analysis" section is not sufficient. There are overall four groups of animals and as such, a simple t-test is not suitable to compare between these groups. An ANOVA test needs to be included, either with a pair-wise Bonferroni adjustment for the number of pairwise comparisons, or an overall multiple comparison test such as Newman-Keuls, HSD, or Tukey's LSD

In agreement to this observation, we have now used a one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons.

Under results, the deterioration of the coat state was not shown in the figures - this should be mentioned or data included. Also, figure 1 only compares 3 groups - cort-, cort+, and cort+/Hyp+. Overall, there were 4 groups and the group cort-/Hyp+ is not included. The authors should give a justification why this group has not been included or change figure and correct the results and discussion section accordingly.

Whit regard to this point, we included the deterioration of the coat state in the figure 2 and we have modified the figure 1 as requested.

The DCX+ cells were not significantly different between cort+/vehicle and cort-/vehicle - this is not stated correctly in the paragraph "immunohistochemistry results"

Ok corrected.

Figure 2 - as mentioned before - should include the non-stressed Hyp+ group.

Ok corrected.

In general, figure legends should be descriptive, figure 3A does only offer interpretation without any description of what is displayed.

Figure 3A is now descriptive.

In figure 4I and 4L, the two photomicrographs shown should be identified as to which group they belong.

Ok corrected

With regard to the final observation, we indicated the route and the dose of administration in according to the study by Di Paola et al.

Reviewer: Nicolas Singewald

Major compulsory revisions:

The author's have used unpaired t-test to conduct statistical analysis. However, since there are 2 independent variables (group and treatment), 2 way ANOVA should be used because an unpaired t-test will not give the group X treatment interaction. In addition, the results of these tests have to be reported with degrees of freedom.

In agreement to the observation of the reviewer, we have used a one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons.

The graphs have not been adequately labeled. It is difficult to interpret which groups are being compared. The authors are advised to use different symbols for different comparisons and state these comparisons clearly in the legend.

According to the suggestion, different symbols were used in the graphs.

The authors show in Fig 1 that the behavior was assessed 3 times. Which behavior was assessed specifically when? Please add.

Ok corrected.

The images are not clear. There are no visible cells as indicated by arrows in fig 4E and 4g. In addition, the dendritic and spine processes of a DCX positive cell in fig 4g are completely missing. Therefore, it's difficult to accept these cells as DCX+ cell. A high magnification inset could be provided in the same figure for a better illustration. In short, the images have to be considerably improved in quality.

We modified the figure 4 as requested.

Some references are missing. For instance, under `background´ section: Stress causes alterations in hippocampal networks, including altered patterns of neurogenesis and remodeling of dendrites. Ref?

As suggested we added two references concerning these topics.

In the discussion section the authors mention: Our data are in accordance with those reporting an anti-anxiety effect induced by hypericum perforatum administration in models of restraint stress and sleep deprivation. The effect of hypericum perforatum on the anxiogenic and depression-like behavior induced by feeding a magnesium-deficient diet needs to be mentioned here and discussed.

According to the suggestion, we added two studies concerning the effects of *Hypericum perforatum* administration in models of restraint stress and sleep deprivation in the discussion section.

Hypericum perforatum increased the total number of BrdU positive cells in Cort- group but had no effect on DCX+ cells. The authors should comment on this.

In agreement to the suggestion of the reviewer we added in Discussion a comment regarding the effect of *Hypericum perforatum* treatment on DCX+ cells.

Minor Essential Revisions

In fig 3a, the authors report that there is decrease in vehicle treated Cort+ group in the center time (p<0.05). Furthermore, this decrease in center time is reversed in the hypericum perforatum treated Cort+ group (p<0.001). However, by looking at the graphs it appears that there is a greater magnitude of difference in the graphs with p<0.05 in comparison to p<0.001, together with lower standard error. Is the labeling correct?

Yes, the labeling is correct

Please check grammar. For instance, ´Different Hypericum perforatum components as pseudohyperforin', 'its efficacy in the treatment mild-to-moderate depression', 'which can migrate into the granular cell layer where differentiate into granular neurons and being then functionally integrated into the hippocampal circuitry.'

Right, we checked grammar and corrected.

In addition to the points raised by the reviewers, we addressed the suggested editorial points. In the revised manuscript, changes are indicated in yellow.

We wish to thank the reviewers for constructive criticism and we hope that the paper is now acceptable for publication

We look forward to hearing from you at your earliest convenience.

With best regards

Prof. Edoardo Spina