Reviewer's report

**Title:** Psychological stress decreased iron absorption in rats

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**Reviewer:** Gaelle Boudry

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This short paper investigates the effect of different durations (1d, 3d and 7d) of psychological stress (using the communication box model) on iron apparent intestinal absorption and iron transporter expression in the duodenum of rats. They observed an almost 50% decrease in iron apparent absorption after 3 days of psychological stress as well as a down-regulation of ferroportin 1 expression, up-regulation of ferritin and presence of iron in the apical poles of enterocytes. The divalent metal transporter 1 expression was not affected by stress.

**Major Compulsory Revisions**

1- There are numerous misspellings and grammatical errors that will have to be corrected.

2- The material and methods section is not complete and some precisions should be given:
- the description of the stress protocol should be improved
- the way the hypothalamus and serum was collected and then stored should be described
- the name of the companies for the ELISA kits for corticosterone, ACTH and norepinephrine as well as the CV for each assay should be given.
- How was the duodenum fixed: formalin then embedded in paraffin or frozen?
- in the Western blot section, no details on the HEPES-EDTA buffer are given (use of protease inhibitor, sucrose?). There are also no details on the electrophoresis and transfer procedures.

3- The values for ACTH are enormous (300-400 ng/mL when the usual values range between 10 and 100 pg/mL): is it a problem with the assay or a mistake in the units?

4- In the discussion and conclusion, the authors use the results of one of their recent paper to conclude on the role of hepcidin and IL-6 in the reduced iron intestinal absorption they observed. However, they did not actually measure the iron apparent absorption in rats treated with anti-IL6 antibody to draw firm conclusion. I would therefore suggest being less affirmative in their conclusions and discuss other possible mechanisms.

**Minor Essential Revisions**
1- Figure 1 B: are the iron intake in mg as written or mg/d?, Figure 1C: what is the actual unit of fecal iron content: mg/g of faeces, mg excreted / d ? Figure 1D: is it the percentage or the ratio?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests