# Re: Automatic reply: Automatic reply: Query publication

From: helmy.m@protonmail.com <helmy.m@protonmail.com>

To: Translational Psychiatry <TranslationalPsychiatry@us.nature.com>
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BCC: Ivan Oransky <ivan-oransky@erols.com>
Retraction Watch <team@retractionwatch.com>
retractionwatchteam@gmail.com <retractionwatchteam@gmail.com>

Date: Monday, March 29th, 2021 at 12:51 PM

Dear Professor Licinio, James, and David,

Regarding the article published in *Translational Psychiatry* in 2016, titled 'Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation' by Koe, Ashokan, and Mitra.

I believe it is unethical to publicly comment on an ongoing-investigation. However, if the investigation is closed or there is no response by the authors during a reasonable period of time and no expression of concern was made on the article, then it is my duty to the academic community to raise the issue in the public domain. Please let me know the status of the current investigation.

I look forward to your reply.

Kind regards,
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MD, PhD

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------- Original Message -------
On Monday, February 15, 2021 4:39 AM, Translational Psychiatry <TranslationalPsychiatry@us.nature.com> wrote:

> Thank you for your email.
>
> We can confirm receipt of your email and will aim to get back to you as soon as possible. Please note that as a result of the significant disruption that is being caused by the COVID-19 pandemic we are experiencing some delays.
>
> TP editorial office

------- Original Message -------
On Thursday, December 17, 2020 3:31 PM, Helmy, M. <helmy.m@protonmail.com> wrote:

Dear Professor Licinio,

cc James and David

Regarding the article published in *Translational Psychiatry* in 2016, titled 'Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation' by Koe, Ashokan, and Mitra:[1]

1. To replicate the experiment we need to know when the open-field test was done, there is no mention of that in the article. In addition, according to the text five (5) days were required to do the behavioral testing, but in Supplementary Figure 1, behavioral testing was done in only two (2) days. **May we be informed of the time of open-field testing and may Supplementary Figure 1 be corrected and perhaps elaborated to include this information?**
2. The article states: "...Time spent in the center of the field was quantified as the reciprocal proxy of the anxiety (center defined as a concentric circle to the arena with 0.33 m radius). Total distance travelled during the trial was also quantified as a measure of locomotion...":[1]
    b. In the text it states: "...In the non-MS group of animals, EE significantly increased time spent in the centre of the arena ($P$<0.001 *after* Bonferroni correction)..." but no significance is apparent nor shown in Figure 2(d). **May the Figure be corrected?**
    c. **What software was used for these analyses?**
    d. Since time and distance were known, **why was speed not calculated as an indicator of locomotion, rather than distance**?
    e. Data for distance travelled be shown in Results. The outcome was not significant but may be of value to others, and though time alone and distance alone were not significantly different between experimental groups, perhaps speed is? **May the data for distance travelled and/or speed be shown** perhaps as a Supplementary Figure 5?
3. The article states: "...On PN84, rats were killed by decapitation. Terminal trunk blood was collected...":[1]
    a. **What is the ethical and scientific justification for using a physical method of euthanasia?**
    b. **Why was blood not collected from the reportedly unanesthetized and decapitated rat?** In other words, why guillotine its head *then* slice its abdomen? Needless to say, the former procedure produces copious amounts of blood, and of higher quality than the latter.
4. Supplementary Figure 2 showing animal weight gain looks unphysiological. Regardless of conditions, the rats are not gaining weight fast enough before 7 weeks of age, and gaining weight too quickly after 9 weeks of age. **May weight gain data be confirmed?**
5. According to Supplementary Figure 3, environmental enrichment and maternal separation have no effect on "...prelimbic medial prefrontal cortex neurons..." [1]. I am not entirely certain of the histological accuracy of an prelimbic medial prefrontal cortex, by according to another article by Mitra and others, environmental enrichment *does* have an effect on medial prefrontal cortex.[2] According to yet another article by Mitra and others, deep brain

stimulation of medial prefrontal cortex *potentiates* anti-anxiety effects of environmental enrichment.[3] **How is the lack of significant effect of environmental enrichment on medial prefrontal cortex in this article, in contradiction to other articles produced in the same location and with the same paradigm, justified?**

6. It is understandable that since neuronal morphology was traced with the aid of a drawing tube (or '*camera lucida*'), digital microscopy could not be used to produce any images. I am curious if the x100 objective lens numerical aperture 1.3 oil-immersion used is one of the precious and classic Leitz apochromatic x100? That would be exciting! Or perhaps it is an Olympus Plan Apo x100...

Kind regards,

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1 Koe, A. S., Ashokan, A. & Mitra, R. Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. *Translational psychiatry* **6** (2):e729-e729, doi:10.1038/tp.2015.217 (2016).

2 Ashokan, A., Lim, J. W. H., Hang, N. & Mitra, R. Complex housing causes a robust increase in dendritic complexity and spine density of medial prefrontal cortical neurons. *Scientific reports* **8** (1):1-9, doi:10.1038/s41598-018-25399-4 (2018).

3 Bhaskar, Y., Lim, L. W. & Mitra, R. Enriched Environment Facilitates Anxiolytic Efficacy Driven by Deep-Brain Stimulation of Medial Prefrontal Cortex. *Frontiers in Behavioral Neuroscience* **12** (204), doi:10.3389/fnbeh.2018.00204 (2018).