



Mohamed Helmy &lt;helmy.m@gmail.com&gt;

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## Query published articles

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**Mohamed Helmy** <helmy.m@gmail.com>

6 June 2021 at 18:41

To: Rafal Marszalek &lt;rafal.marszalek@nature.com&gt;

Cc: Rafal Marszalek &lt;scientificreports@nature.com&gt;

Dear Dr. Marszalek,

Thank you for your message, and thank you for your recommendation to raise matters concerning the academic community through the commenting facility on the article - though I am uncertain which of the two articles you are referring to, or if that was an typographical error.

Regarding Hegde *et al.* (2020; reference [1] in my original message below): I had a look through corrigenda published by *Scientific Reports* in recent years. It would appear that concern with the accuracy and specificity of the scientific record is quite tight. Small errors, which may be quickly and otherwise harmlessly elucidated by a researcher, are nevertheless corrected, for example see Bottermann *et al.* (2017). Therefore, it is not clear why it is, in this case, satisfactory for *Scientific Reports* to have in an article published an antibody that does not exist and never did exist. Importantly, findings in the article *depend* on use of this antibody, so 'just find another antibody' is not a rebuttal, it does not work towards reproducibility. Importantly, as reported in Hegde *et al.* (2020), immunofluorescent quantification of BDNF is highly experimental, and image processing is ambiguous.

I will review guidelines to see if COPE is in a position to recommend a corrigendum in *Scientific Reports* on a published antibody that never existed, and I am not certain it is worth taxing COPE's resources with an investigation of Hegde *et al.* (2020). I will also confirm that multiple use of datasets from animal experiments without reporting of the same in Ashokan *et al.* (2018; reference [3] in my original message below) is an ethical violation stated in guidelines subscribed to by *Scientific Reports*, and so in COPE's purview.

I will write frankly. Since investigating misconduct in the Singapore Dementia Consortium, and publishing [www.nanyangscandal.com](http://www.nanyangscandal.com), I have been investigating misconduct by others in related context. For example, I recently published a report on misconduct by Nitish Thakor. I will soon be publishing a report on misconduct by a notable figure, strongly and historically connected to the individuals in previously published reports, as well as others not reported. If one were to quantify the percentage of articles published in *Scientific Reports* by this group of individuals - it is very high indeed. Many multiples over significance and incomparable to, for example, the number of articles published in *any one single journal*, by the same number of individuals, working in an institute in Boston, in the same field, over the same period of time. Or by a group of individuals in an institute in the UK.

In other words, that such a high concentration of articles from a group of individuals in a field of research in an institute should be published in *only one journal* is very unusual. I wonder *why*?

My experience with Nanyang Technological University, including this unexplained uncorrecting of the scientific record by *Scientific Reports* for two articles, suggests that the reason I am looking for is, obviously, not scientific in nature. Perhaps it is political. I believe the report I will be publishing soon will show the political influence in much of what is widely believed to be the production of knowledge.

In any case, I do not agree that *Scientific Reports* transfers its responsibility to correct the scientific record for Hegde *et al.* (2020) and Ashokan *et al.* (2018) onto a member of the academic community. This is because the responsibility owed to the academic community belongs to *Scientific Reports*, and not, paradoxically, the academic community itself, in ones or *in toto*.

Kind regards,  
Mohamed Helmy  
MD, PhD

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Bottermann, Maria; Lode, Heidrun Elisabeth; Watkinson, Ruth E.; Foss, Stian; Sandlie, Inger; Andersen, Jan Terje; and James, Leo C. (2017). Corrigendum: Antibody-antigen kinetics constrain intracellular humoral immunity. *Scientific reports* 7, 45418-45418. 10.1038/srep45418

On Thu, 20 May 2021 at 17:03, Rafal Marszalek <rafal.marszalek@nature.com> wrote:

Dear Dr Helmy,

We now received a reply from the authors and found is satisfactory. We are not convinced that any further editorial action is required for these papers. Of course you are still free to share any of your concerns with our readership through the commenting facility on the article. Should you opt to do this, we ask that you please follow the community guidelines on commenting:

<https://www.nature.com/info/community-guidelines>

Sincerely,

Rafal

Dr Rafal Marszalek

Deputy Editor

*Scientific Reports*

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**From:** Rafal Marszalek <scientific.reports@springernature.com>  
**Sent:** 31 March 2021 14:41  
**To:** helmy.m@gmail.com  
**Cc:** Rafal Marszalek <rafal.marszalek@nature.com>  
**Subject:** Re: Query published articles

[External - Use Caution]

Dear Dr Helmy,

We are still looking into the further points you raised. I will let you know once this has concluded.

Please note that if you have any specific scientific concerns, you are always free to also bring them to the attention of our readers through the commenting facility on the article's page. We merely ask that community guidelines are followed when commenting:

<https://www.nature.com/info/community-guidelines>

Posting of the comments does not affect how we approach these concerns (that is to say, we will follow our editorial process and I will let you know once it's reached a resolution).

Sincerely,

Rafal

Dr Rafal Marszalek

Deputy Editor

*Scientific Reports*

On Mon, 29 Mar at 6:59 AM , Mohamed Helmy <[helmy.m@gmail.com](mailto:helmy.m@gmail.com)> wrote:

[External - Use Caution]

Dear Dr Marszalek,

Regarding articles published in *Scientific Reports* by Mitra and others, please see correspondence below.

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.

As related to the articles in question, the questionable validity of the data is fairly straight-forward to any physiologist with some background in research.

I look forward to your reply.

Kind regards,

Mohamed Helmy

MD, PhD

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On Mon, 8 Feb 2021 at 23:24, Mohamed Helmy <[helmy.m@gmail.com](mailto:helmy.m@gmail.com)> wrote:

Dear Dr Marszalek,

Thank you for your prompt reply.

The specific concerns may require correction of the scientific record. If I may trouble you to please follow up on the specific concerns with the authors and perhaps an image expert at *Scientific Reports* for the point mentioned below.

Kind regards,

Mohamed Helmy

MD, PhD

[helmy.m@gmail.com](mailto:helmy.m@gmail.com)

[helmy.m@protonmail.com](mailto:helmy.m@protonmail.com)

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On Mon, 8 Feb 2021 at 22:40, Rafal Marszalek <[rafal.marszalek@nature.com](mailto:rafal.marszalek@nature.com)> wrote:

Dear Dr Helmy,

I would ask that you please contact the authors directly with your specific concerns: this would be faster and more efficient way of reaching an understanding here, as the authors are best placed to comment on minutiae of experimental conditions etc. If you are unable to get that information from the authors, please let me know then.

Sincerely,  
Rafal

Dr Rafal Marszalek

Deputy Editor

*Scientific Reports*

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**From:** Mohamed Helmy <[helmy.m@gmail.com](mailto:helmy.m@gmail.com)>  
**Sent:** 08 February 2021 13:53  
**To:** Rafal Marszalek <[rafal.marszalek@nature.com](mailto:rafal.marszalek@nature.com)>; Scientific reports <[scientificreports@nature.com](mailto:scientificreports@nature.com)>  
**Subject:** Ticket number 1289068

[External - Use Caution]

Ticket number 1289068

Dear Dr Marszalek,

Thank you for your message and my apologies for the delay in reply.

Regarding points on Hegde et al. (2020):

1. The antibody the authors of the article in question claim was discontinued by Abcam was never produced by Abcam. Please see the email from Dr. Hung at Abcam below. The last listed article using the antibodies used by the authors and discontinued by Santa Cruz could have been published 2015 or 2017, depending on one of at least three potential antibodies I listed earlier. I do not understand what is so novel about clearly and accurately specifying the antibodies used in a study, nor why it should be impossible to publish a corrigendum for the sake of objectivity, reproducibility, and out of respect to the scientific community.
2. If what the authors write in response to point 2. is correct, then there was no control data for image analysis. May I suggest we consult with the image expert at *Scientific Reports*? The methods are on page 4 of the article under *Quantification of brain-derived neurotrophic factor (BDNF)*. If the image expert thinks it is A-OK, I will not bring it up again.
3. Since the authors agree that intranuclear GR did not co-label with DAPI in the MSEE panel, then they also agree that the image is not representative. Why was this image chosen and not a more representative one so as not to confuse the reader?

Regarding points on Ashokan et al. (2018):

1. The reference and software cited for Sholl analysis (Ferreira et al. 2014) does not produce the parameters reported in Ashokan et al. (2018). May the data processing procedure for Sholl analysis be clarified please? What is so novel about reporting how a method was carried out?
2. I assume the lens with a numerical aperture of 1.3 is the x100 one? Thank you for this information. I imagine it was an Olympus UPLFLN100XO2 or an Olympus Plan Apo? Since a drawing tube was used to trace neurons by hand, perhaps the authors may possess a Leitz apochromatic x100? I agree with the authors, sharpness with a drawing tube makes numerical aperture a secondary consideration.

3. By practical work I mean experimental work, actual experiments. Alternatively, was the dataset in the study taken from another? From the acknowledgments it appears that no experiments were done. The authors do not mention methods in their reply but there are methods in the article. If no practical work was done for this article this needs to be stated to fulfill *Scientific Reports* ethics guidelines on reporting.

I look forward to your reply.

Kind regards,

Mohamed Helmy

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----- Original Message -----

On Tuesday, January 19, 2021 10:30 AM, Abcam Scientific Support <[sg.technical@abcam.com](mailto:sg.technical@abcam.com)> wrote:

**Inquiry reference # 210118-000087**

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- Date created: Monday, Jan 18, 2021 - 09:04
- Date last updated: Tuesday, Jan 19, 2021 - 10:30

**Subject** Discontinued antibody?

**Our reply By Email Tuesday, Jan 19, 2021 - 10:30**

Dear Mohamed,

Thank you for getting in touch.

I have searched our catalog and archive, unfortunately, I could not find any record of a DyLight-488 horse anti-mouse IgG antibody. I believe the authors might have misattributed the antibody that they used to us. I would advise you to contact the authors for more information.

I am sorry for the limited help. I'll be happy to answer any further questions you have.

Best regards,

Harry

Harry Hung, PhD

Scientific Support Specialist

[www.abcam.com](http://www.abcam.com)

(852) 2603-6823 +65.6734.9252(landline)/800.  
188.5244(toll free) 1800 960 553 0800 758 929

*We are committed to continually improving our service and value your input. You may receive a 'HaveYourSay' survey email. Please share your thoughts.*

On Fri, 15 Jan 2021 at 22:09, Rafal Marszalek <[rafal.marszalek@nature.com](mailto:rafal.marszalek@nature.com)> wrote:

Dear Dr Helmy,

We have reached out to the authors with the queries you send to the journal (they indicated that if you tried to contact them with those, they did not receive your correspondence). Their replies are appended below for your information. We are not convinced that further editorial action is required at this time.

Sincerely,

Rafal

Dr Rafal Marszalek

Deputy Editor

*Scientific Reports*

### Authors replies:

Re: <https://www.nature.com/articles/s41598-020-70875-5>

1. To the best of my knowledge, Abcam does and did not produce horse anti-mouse IgG secondary antibodies used in this study for brain-derived neurotrophic factor (BDNF) quantification. Santa Cruz discontinued production of primary antibodies against glucocorticoid receptor (GR) used in this study, including rabbit anti-GR (P- 20): sc-1002, rabbit anti-GR (H-300): sc-8992, and rabbit anti-GR (M- 20): sc-1004. May the antibodies used be clarified please, for example with a product number?

- Anti-mouse IgG (“DyLight-488 horse anti-mouse IgG”) used in the paper can now be obtained from Thermo Fisher or Vector Labs. We have looked at abcam catalogue and they no longer carry this antibody. If reader insists on buying from Abcam, other DyLight-488 versions are still available and will work just fine. Otherwise please buy from Thermo Fisher or Vector Labs.
- We do not have much control over specific antibodies offered or discontinued by commercial companies. Several anti-GR antibodies and corresponding secondaries are still available from a variety of sources (including from Santa Cruz).
- In general, any antibody sequence against BDNF and GR should work after being characterised and standardised in competent hand, using methods routine in an immunohistology lab.

2. Immunofluorescent quantification of BDNF is interesting and powerful. However, I am unable to replicate the method; may we have more information on the procedure for extracting the data from confocal images?

- We did not use any custom-built tools for the quantification. A publicly available software was used and can be found at: <https://imagej.net/Welcome>.
- The website contains detailed training material for confocal image analysis. Other workflows routine in imaging labs should work equally well too.

3. On Figure 4(B) MSEE shows significantly less GR than Control (or EE), whereas Figure 4(A) shows greater number of green pixels on MSEE than EE (or Control) using several quantification methods including eyeballing. Perhaps intranuclear GR did not co-label with DAPI in the MSEE panel - I am unable to confirm given the quality available? In which case, is the image representative?

- Please note that y-axis of panel 4B depicts “% of DAPI”, in other words co-labeled particles. Green pixels are fluorescence emanating from GR staining and does not constitute what is being shown on y-axis of panel B.
- Please see the following sentence in method when reading panel 4B, “GR nuclear localization was expressed as number of nuclei showing colocalization relative to the sum of nuclei with the presence or absence of co-localization.” Please also see following on the first line of the relevant result section, “The extent of intra-nuclear localization of GR was quantified as percentage of DAPI-positive total nuclei.”



- Reader writes that perhaps, “intranuclear GR did not co-label with DAPI in the MSEE panel”. Yes, that is correct and that is what being shown in panel 4B, compared to EE. So, there is no confusion.
- We do not comment here in difference between MSEE and Control, because our experimental design precluded this comparison through use of orthogonal planned comparisons. Please see second paragraph in “statistics and analysis” section.
- We have not used ‘eyeballing’ as a quantitative method, and hence cannot comment about its use.

Re: <https://www.nature.com/articles/s41598-018-25399-4>

1. The reference and software cited for Sholl analysis [4] does not produce the parameters reported in the article [3]. May the data processing procedure for Sholl analysis be clarified?
  - We assume reader refers to two references cited for Sholl analysis, listed as #11 and #12 in the report.
  - The method cited here indeed results in segmental Sholl analysis. Please see Figure 1 of #12. And a simpler method based on #11 will work just as well.
  - Reader can use any of the routine and published methods to conduct Sholl analysis, including using pencils and transparencies.
2. What was the numerical aperture of the lenses used please?
  - NA were 1.30 and 0.75.
  - Any lens that is used for brightfield histology of glass-mounted brain slices will work when used in conjunction with camera lucida. The NA of lens is not critical for the work as far as Golgi-stained neurons can be visualized through eyepiece and a camera lucida.
3. I do not understand from the Author Information, Contributions, if any practical work was done. May we please be informed of the Animal Use Protocol number(s)?
  - Reader asks if “any practical work was done.” We find this comment too general and vague. The work is described, the introduction provides a context, and discussion lays out interpretation. It is up to the readers to decide whether they find the work practical or impractical.

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**From:** Mohamed Helmy <[helmy.m@gmail.com](mailto:helmy.m@gmail.com)>  
**Sent:** 19 December 2020 04:20  
**To:** Scientific reports <[scientificreports@nature.com](mailto:scientificreports@nature.com)>  
**Cc:** Rafal Marszalek <[rafal.marszalek@nature.com](mailto:rafal.marszalek@nature.com)>  
**Subject:** Re: Query published articles

[External - Use Caution]

Dear Dr. Marszalek,

Thank you for your message.

I look forward to clarification regarding the articles in question.

Kind regards,

Mohamed Helmy

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On Sat, 19 Dec 2020 at 01:20, Rafal Marszalek  
<[scientificreports@nature.com](mailto:scientificreports@nature.com)> wrote:

Dear Dr Helmy,

Thank you for bringing these issues to our attention. We are going to look into those and will be in touch in due course.

Sincerely,

Rafal

Dr Rafal Marszalek

Deputy Editor

*Scientific Reports*

On Mon, 14 Dec at 5:28 PM , Mohamed Helmy  
<[helmy.m@gmail.com](mailto:helmy.m@gmail.com)> wrote:

[External - Use Caution]

Dear Dr. White,

Regarding the article [1] and Author  
Correction [2] published in *Scientific Reports*, 'Early-life  
short-term environmental enrichment counteracts the

effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala' (2020):

1. To the best of my knowledge, Abcam does and did not produce horse anti-mouse IgG secondary antibodies used in this study for brain-derived neurotrophic factor (BDNF) quantification. Santa Cruz discontinued production of primary antibodies against glucocorticoid receptor (GR) used in this study, including rabbit anti-GR (P- 20): sc-1002, rabbit anti-GR (H-300): sc-8992, and rabbit anti-GR (M- 20): sc-1004. May the antibodies used be clarified please, for example with a product number?
2. Immunofluorescent quantification of BDNF is interesting and powerful. However, I am unable to replicate the method; may we have more information on the procedure for extracting the data from confocal images?
3. On Figure 4(B) MSEE shows significantly less GR than Control (or EE), whereas Figure 4(A) shows greater number of green pixels on MSEE than EE (or Control) using several quantification methods including eyeballing. Perhaps intranuclear GR did not co-label with DAPI in the MSEE panel - I am unable to confirm given the quality available? In which case, is the image representative?

Regarding the article [3] published in *Scientific Reports*, 'Complex housing causes a robust increase in dendritic complexity and spine density of medial prefrontal cortical neurons' (2018):

1. The reference and software cited for Sholl analysis [4] does not produce the parameters reported in the article [3]. May the data processing procedure for Sholl analysis be clarified?
2. What was the numerical aperture of the lenses used please?
3. I do not understand from the Author Information, Contributions, if any practical work was done. May we please be informed of the Animal Use Protocol number(s)?

Unfortunately the corresponding author did not respond to my queries. I may submit a Matter Arising if necessary.

Kind regards,

Mohamed Helmy

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[1] Hegde, A., Suresh, S., & Mitra, R. Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala. *Scientific reports*. **10**, 14053; 10.1038/s41598-020-70875-5 (2020).

[2] Hegde, A., Suresh, S., & Mitra, R. Author Correction: Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala. *Scientific Reports*. **10**, 16929; 10.1038/s41598-020-74322-3 (2020).

[3] 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**, 7308; 10.1038/s41598-018-25399-4 (2018).

[4] Ferreira, T.A., Blackman, A.V., Oyrer, J., Jayabal, S., Chung, A.J., Watt, A.J., Sjöström, P.J., & van Meyel, D.J. Neuronal morphometry directly from bitmap images. *Nature Methods*. **11**, 982-984 (2014).

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