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Hypothalamic CNTF volume transmission shapes cortical noradrenergic excitability upon acute stress

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 24th Jul 2018

Thank you for transferring your manuscript with referee reports from another journal to The EMBO Journal. A good expert in the field has now reviewed your study, the referee reports from the previous journal and the point-by-point response.

As you can see from the comments below, the referee finds the study interesting and suitable for publication in The EMBO Journal. There are just a few minor comments to resolve before acceptance here - please see specific referee comments below.

When you resubmit the revised version would also take care of the following items:

- Please take a look at our author guidelines regarding supplemental figures
http://emboj.embopress.org/authorguide#expandedview

- We now encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

REFEREE COMMENTS

Referee #1:

The study by Alpár and colleagues analyzes the link between the stress-induced hypothalamus-pituitary-adrenal axis (HPA) activity and the increased cortical alertness, providing a new and comprehensive picture about the molecular chain of events connecting the two processes. In particular, the authors found that corticotropin-releasing hormone (CRH)-containing (+) neurons of the hypothalamus can stimulate (through glutamate release) ependymal cells of the third ventricle to release ciliary neurotrophic factor (CNTF) into the brain's aqueductal system. This release leads to a long-range activation of noradrenergic neurons (NA) located in the locus coeruleus (LA) innervating the prefrontal cortex, via a sequential phosphorylation including the Ca2+-sensor secretagogin. The work is excellent, very well written and relies on a combination of cutting-edge in vivo technology based on opto-/chemogenetic, electrophysiology, biochemistry and imaging approaches. Every single statement of the work is supported by compelling evidences provided by a precise manipulation of the biological system analyzed. Moreover, the authors provide evidence that the connection between HPA activity, noradrenergic neurons of LA and behavioral moderations is a molecular mechanism conserved amongst the evolution. For all these reasons, the present work deserves publication in EMBO journals.

The authors should address the following minor points:

1) In figure 1D, the authors perform electrophysiological recordings on ependymal cells to demonstrate the presence of glutamate-dependent synaptic events. It would be interesting to report the electrophysiological parameters of these events (frequency, amplitude, decay and rise times) in the supplementary figure 1.
2) I would suggest to remove the "personal communication" stating that ependymal cells is the main source of CNTF in the brain. Indeed, the singe-cell RNA-seq analyses showed in figure 1C provides a sufficient demonstration that these cells express CNTF. Evidences reported in literature showed CNTF to be expressed in other brain cells, including white matter astrocytes of the optical tract (Dallner et al., GLIA 2002).

1st Revision - authors' response 24th Jul 18

Thank you for your positive and constructive comments on our submission. We were particularly glad to learn that you have found our manuscript “comprehensive, excellent, very well written and relies on a combination of cutting-edge in vivo technology”.

In accord with your specific (and more minor) queries, we have revised the manuscript as follows.

Q1:“In figure 1D, the authors perform electrophysiological recordings on ependymal cells to demonstrate the presence of glutamate-dependent synaptic events. It would be interesting to report the electrophysiological parameters of these events (frequency, amplitude, decay and rise times) in the supplementary figure 1.”

We appreciate your attention to detail. These data were added to “Expanded View Figure 1” as its panel “D”.

Q2: “I would suggest to remove the "personal communication" stating that ependymal cells is the Main source of CNTF in the brain. Indeed, the singe-cell RNA-seq analyses showed in figure 1C provides a sufficient demonstration that these cells express CNTF. Evidences reported in literature showed CNTF to be expressed in other brain cells, including white matter astrocytes of the optical tract (Dallner et al.,GLIA 2002).”

We perhaps were unclear in phrasing this statement since it intended to specifically refer to ependymal/stem cell and neuronal sources of CNTF. We certainly do not contest glial origins for this trophic factor. Therefore, and to satisfy your query, we have removed the erroneous statement.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Tibor HARKANY
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Manuscript Number: EMBOJ-2018-100087R1

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	This was done as per our power analyses performed during the approval process of our ethical permits. In general, animal numbers were in the range of 3-8, depending on the test undertaken, requirements of particular statistical algorithms as well as experimental conventions reported earlier in the literature.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We state: "...including the size estimate of each experimental cohort." (page 19).
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	None of the animals available to us were excluded from our analysis except those rare cases whose microinjections were misplaced, as confirmed by post-hoc histochemistry.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Yes, please see below.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was ensured where possible. This is stated on page 19: "Wherever possible, animals of the same genotype were randomly assigned to experimental manipulations (control vs. various treatments) to reduce procedural bias."
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Blinding was only done for DREADD experiments (behavioral assays). We state: "In both tests, animals were handled by an experimenter blinded to the case condition to ensure objectivity." on page 28 of the manuscript.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding was only done for DREADD experiments (behavioral assays). We state: "In both tests, animals were handled by an experimenter blinded to the case condition to ensure objectivity." on page 28 of the manuscript.
5. For every figure, are statistical tests justified as appropriate?	Yes, to the best of our knowledge.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, which were tested in each case. If not, non-parametric algorhitms were used as requried.
Is there an estimate of variation within each group of data?	Yes.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
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http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

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http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	These were done in the "Reagents and Tools table".
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	This was done in "Materials and Methods" under "Cell Lines". Since INS-1E cells are proadly used, their origin and details were referenced. These cells shall be free of mycoplasma contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	These were done appropriately under "Animals" on page 19 of the manuscript (start of the "Materials and Methods" section. Husbandry conditions were explicitly stated.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This has been done in the same section, including explicit statements on regulatory approvals ("the Semmelweis University (PE/EA/1234-3/2017, Hungary) and the Medical University of Vienna (BMWFW-66.009/0277-WF/V/3b/2017, Tierversuchgesetz 2012, BGBl, Nr. 114/2012). All procedures conformed to the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (86/609/EEC)."
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Reporting of subject numbers, age, gender, phenotype/genotype relations and experimental manipulations (if any) were duly reported.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	This has been done on page 20 of the revised manuscript with the Semmelweis University (Budapest, Hungary) identified as regional authority providing approval for the study.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	This has been done in the same section (p. 20).
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	All patient material was coded to ensure anonymity throughout tissue processing.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	This has been provided since single-cell RNA-seq data were re-processed from an earlier publication (Romanov et al. Nat Neurosci) whose source data were submitted to GEO.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	This manuscript does not contain data that were to fall into this category.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A