nature research

Corresponding author(s): Amelia J. Eisch

☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Samples sizes were determined by calculations performed on empirical data and verified via power analysis. The number of animals used enabled detection of differences with 80% power and alpha set (Type I error rate) at 0.05. We consult routinely with our biostatistician on site at each step of the project. Supplementary Table 1 provides the exact subject number for each group in each experiment.

2. Data exclusions

Describe any data exclusions.

For experiments where virus is infused intracranially, accurate virus targeting (EGFP+ soma in Ent II/III and GFP+ terminals in perforant path/middle and outer DG molecular layer, Mol) was verified after brain collection, and mistargeted mice were not analyzed for cellular or behavior data. However, the mistargeted mice are presented in the supplementary data (Supplementary Fig. 5) as this allows a complementary approach to testing our hypothesis that stimulation of the Ent is antidepressive.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Behavioral and cellular results were replicated via at least one independent experiment, and results were combined and are presented in these figures: Fig. 1, 2h, j, n-w, 3i-j, 4b-g, 5g-t; Supp Figs. 1b-m, 3a-l, 4a-e, 5i-k, 7a-b, 8a-b, 9a-f, and 11e-g. No experimental replications failed to reproduce original results. Supplementary Table 1 provides detail on which experiments were replicated, and how many times.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For all molecular, cellular, and behavioral studies, mice were randomly assigned to groups. EEG data for power analyses were sampled in the form of 10 pseudo-randomly selected, noise- and artifact-free, 2-min epochs for each designated time period and for each day of recording.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were blinded to the treatment group until all data had been collected.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

For western blot analysis, Scion image software (Scion Corporation) or LI-COR Image studio 5.x. were used. For quantification of total DCX+ cell number or dendritic arborization of DCX+ immature cells, Stereo Investigator software (ver. 11.03) and NeuroLucida Explorer (ver. 10, MBF Bioscience, Williston, VT, USA) were used. For assessment and quantification of immunoreactive soma and terminals, Cellsens standard software (ver. 1.16, Olympus America, Center Valley, PA, USA) was used. For SI test, open field, and EPM test, Ethovision software (Noldus Information Technology) was used. For LM test, a computer-controlled photobeam activity system (San Diego Instruments) recorded total movement of mice. Scoring of freezing behavior was automatically performed by the Med Associates software for CFC test. Statistics were performed using Prism software (ver. 6.0, ver. 7.0). For EEG power spectra analyses, NeuroExplorer (ver. 5, Nex Technologies, Madison, AL) in conjunction with a custom-made Matlab code (developed by RC Ahrens-Nicklas) were used. MatLab code for spike and frequency analysis of in vivo electroelectroencephalogram experiments can be accessed via contacting Rebecca Ahrens-Nicklas at AhrensNicklasR@email.chop.edu.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials used in this work are readily available from the authors or standard commercial sources (which are provided in the text).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Primary antibodies purchased from vendors were validated by vendor, and manufacturer's product sheet provides western blot or immunohistochemistry data along with references supporting specificity of the antibody. In addition, TRIP8b and TRIP8b isoform (1a5, exon4, and 1b) antibodies were validated in Lewis et al. J. Neurosci. 31, 7424–7440 (2011).

For IHC, the following primary antibodies were used: rabbit anti-Ki67 (1:500; Thermo Scientific, Cat. #RM-9106), goat anti-DCX (1:500; Santa Cruz, Cat. #SC-8066), rat anti-BrdU (1:800; Accurate Chemicals Cat. #Obt0030), chicken anti-GFP (1:3000; Aves Cat. #GFP-1020), guinea pig anti -TRIP8, mouse anti-TRIP8b1a5 (both from D. M. Chetkovich), mouse anti-NeuN (1:500; Millipore, Cat. #MAB377), and rabbit anti-mCherry (1:1000; Clontech, Cat. #632496). Secondary antibodies used for IHC include: biotinylated-donkey anti-rabbit IgG (Cat. #711-065-162), biotinylated-donkey-anti-goat IgG antibody (Cat. #705-065-003), biotinylated-donkey-anti-rat-IgG (Cat. #712-065-153), biotinylated-donkey-anti-chicken-IgY (Cat. #703-065-155), biotinylated-donkey-anti-guinea pig IgG (Cat. #706-065-148) , biotinylated-donkey-anti-mouse-IgG (Cat. #715-065-150) and Cyanine 5-donkey-anti-mouse secondary antibody (Cat. #715-175-150), all 1:200 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). These secondary antibodies have been tested by ELISA and/or solid-phase adsorbed method to ensure minimal cross-reaction with bovine, chicken, goat, guinea pig, syrian hamster, horse, human, rabbit and sheep serum proteins, but per manufacturer's instructions they may cross-react with immunoglobulins from other species.

For western blot, the following primary antibodies were used: rabbit anti-TRIP8b (total, from D. M. Chetkovich), Guinea pig anti-TRIP8b (1a5, from D. M. Chetkovich), mouse anti-TRIP8b (exon 4, Neuromab, Cat. #73-208). mouse anti-TRIP8b (1b, Neuromab, Cat. #73-245) or mouse anti-GAPDH (Millipore, Cat.# MAB374). Secondary antibodies used for western blotting include: for TRIP8b isoforms, donkey anti-rabbit IgG-HRP (Calbiochem, Cat. #401393), donkey anti-guinea pig IgG-HRP (Jackson ImmunoResearch Laboratories, Cat. #106-035-003), and donkey anti-mouse IgG-HRP (Calbiochem, Cat. #401253). The specificity of these secondary antibodies is listed in the manufacturers' instructions, and is noted as follows. Donkey anti-rabbit IgG-HRP (Calbiochem, Cat. #401393) is monospecific for rabbit IgG, heavy and light chains, as immunoelectrophoresis against normal rabbit serum. Cross-reactivity with normal bovine, horse, human, and mouse serum: <2% by direct solid phase immunoassay. Based on immunoelectrophoresis and/or ELISA, donkey anti-guinea pig IgG-HRP (Jackson ImmunoResearch Laboratories, Cat. #106-035-003) antibody reacts with whole molecule guinea pig IgG. It also reacts with the light chains of other guinea pig immunoglobulins. No antibody was detected against non-immunoglobulin serum proteins. The antibody may cross-react with immunoglobulins from other species. Donkey anti-mouse IgG-HRP secondary antibodies (Calbiochem, Cat. #401253) is monospecific for mouse IgG, heavy and light chains, as determined by immunoelectrophoresis against normal mouse serum. Cross-reactivity to normal bovine, horse and human sera: <1% by direct solid phase immunoassay.
For assessment of in vitro knockdown efficiency of pAAV-TRIP8b shRNA, IRDye® 680LT Goat anti-Mouse IgG (H + L, LI-COR Bioscience, Cat# P/N 925-68020) and IRDye® 800CW Donkey anti-Rabbit IgG (H + L, LI-COR Bioscience, Cat# P/N 926-32213) secondary antibodies were used. Per manufacturer's information, based on immunoeletrophoresis these antibodies react with heavy chains and light chains on mouse IgG and rabbit IgG, respectively. No antibody was detected against non-immunogliobulin serum proteins. These antibodies were tested in ELISA and/or solid-phase adsorbed method to ensure minimal cross reaction with bovine, chicken, goat, quinea pig, Syrian hamster, horse, human, rat and sheep serum protein. These antibodies may cross-react with immunoglobulin from other species.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293T cells were purchased from American Type Culture Collection (ATCC).

b. Describe the method of cell line authentication used.

ATCC uses morphology, karyotyping, and PCR based approaches to confirm the identity of human cell lines and to rule out both intra- and interspecies contamination. These include an assay to detect species specific variants of the cytochrome C oxidase I gene (COI analysis) to rule out inter-species contamination and short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination.

c. Report whether the cell lines were tested for mycoplasma contamination.

At the ATCC, both the Hoechst and direct culture method techniques are used several times while a cell line is accessioned and again when the culture is expanded for distribution.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used in this work.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

7 or 8 weeks old C57BL/6J male or female mice were purchased from Jackson Laboratory (stock number: 000664). Nestin-GFP mice were bred at UTSW, and CamKIIα-icre mice were bred at UTSW and PennMed/CHOP, resulting in hemizygous transgenic mice. CamKIIa-icre and nestin-GFP mice were originally from Schutz's lab and Mori's lab, respectively, and original references are provided in the text. Trip8b germline knock-out mice were generated by the Chetkovich Laboratory by breeding heterozygous Trip8b+/- mice.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.