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# Main Figures: 5

# Supplementary Figures: 8

# Supplementary Tables: 2

# Supplementary Videos: 0

# Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

**Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.**

## Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported.
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the page number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process, and it is misleading not to state this clearly.

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FIGURE NUMBER | WHICH TEST? | PAGE | EXACT VALUE | DEFINED? | PAGE | REPORTED? | PAGE | EXACT VALUE | PAGE | VALUE | PAGE |
| example | 1a | one-way ANOVA | 4 | 9, 9, 10, 15 | mice from at least 3 litters/group | 4 | error bars are mean +/- SEM | 4 | p = 0.044 | 4 | F(3, 36) = 2.97 | 4 |
| example | results, pg 6 | unpaired t-test | 6 | 15 | slices from 10 mice | 6 | error bars are mean +/- SEM | 6 | p = 0.0006 | 6 | t(28) = 2.808 | 6 |
| + - | Figure 1 | representative data | 13 | | | | | | | | | |
| + - | Figure 2 | representative data, same mouse received vehicle and CNO injections | 14 | | | | | | | | | |

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FIGURE NUMBER | WHICH TEST? | PAGE | EXACT VALUE | DEFINED? | PAGE | REPORTED? | PAGE | EXACT VALUE | PAGE | VALUE | PAGE |
| + - | Figure 3 | two-way ANOVA Paired T test | Suppl Methods p3 | 13 hM3Dq+ used for sleep-wake quantity, 8 hM3Dq + used for spectral analysis | same mouse received vehicle and CNO injection, one time point | 14-15 | error bars are mean +/- SEM | 14-15 | * p<0.05 | 14-14 | see excel file "Figure3" | |
| + - | Figure 4 | two-way ANOVA Paired T test | Suppl Methods p3 | 13 hM3Dq+ used for sleep-wake quantity, 7 hM3Dq + used for spectral analysis | same mouse received vehicle and CNO injection, one time point | 15 | erros bars are mean +/- SEM | 15 | * p<0.05 | 15 | see excel file "Figure4" | |
| + - | Figure S2 | two-way ANOVA | Suppl Methods p3 | 13 hM3Dq+ and 13 control were used for hourly sleep-wake amounts. 8 hM3Dq+ and 9 control were used for spectral analysis | hM3Dq+ and control littermates were recorded in baseline condition | Suppl Methods p7 | erros bars are mean +/- SEM | Suppl Methods p7 | No significance | | see excel file "FigS2 & TableS1" | |
| + - | Figure S3 | two-way ANOVA | Suppl Methods p3 | 12 control used for sleep-wake quantity, 7 control used for spectral analysis | same mouse received vehicle and CNO injection, one time point | Suppl Methods p7 | erros bars are mean +/- SEM | Suppl Methods p7 | No significance | | see excel file "FigureS3" | |
| + - | Figure S4 | two-way ANOVA | Suppl Methods p3 | 8 control mice | same mouse received vehicle and CNO injection, one time point | Suppl Methods p7 | erros bars are mean +/- SEM | Suppl Methods p7 | No significance | | see excel file "FigureS4" | |
| + - | | | | | | | | | | | | |
| + - | Table S1 | two-way ANOVA Paired T test | Suppl Methods p3 | 13 hM3Dq+ and 12 control mice | same mouse received vehicle and CNO injection, one time point | Suppl Methods p10 | +/- SEM | Suppl Methods p10 | No significance | | see excel file "FigS2 & TableS1" | |
| + - | Table S2 | two-way ANOVA Paired T test | Suppl Methods p3 | 13 hM3Dq+ and 12 control mice | same mouse received vehicle and CNO injection, one time point | Suppl Methods p11 | +/- SEM | Suppl Methods p11 | a: p<0.05<br>b: p<0.01<br>c: p<0.001 | Suppl Methods p11 | see excel files "TableS2" | |

## Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

   Yes. Figures 1, 5, S5 and S7

2. For each representative image, is there a clear statement of how many time s this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, on what page(s) is this reported?

   Yes. the extent of the transfected hM3Dq somas (DsRED Ab) is shown for all animals in S1. The Abs used in the study have been in routine use in our laboratory for many years. These validated Abs can be obtained from commercial sources and the sequence information is available. Repeatability has not been an issue and we can make a statement to this effect, if necessary.
   Page 3, Suppl Methods page 7

## Statistics and general methods

| | |
|---|---|
| 1. Is there a justification of the sample size?<br>If so, how was it justified?<br>On what page(s)?<br>Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size. | Sample size and power calculations were performed post-hoc at http://www.biomath.info, using means and standard deviations derived from our analysis. The present study was sufficiently powered to detect effect sizes.<br><br>Please see Suppl Methods page 3 |
| 2. Are statistical tests justified as appropriate for every figure?<br>On what page(s)? | Yes<br>We provide the n for all tests and an alpha of <0.05 was considered significant for all test.<br>Please see Suppl Methods page 3 |
| a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined? | Statistical analysis was performed using Prism v6 (GraphPad Software, San Diego, CA, USA). Following confirmation that the data met the assumptions of the ANOVA model, a two-way ANOVA followed by a post hoc Bonferroni test were used to compare the effects of the genotype or the drug injection on sleep-wake parameters.<br>Please see Suppl Methods page 3.<br><br>The for each figure and table, an excel file containing the average and SEM as well as the ANOVA table has been submitted. |
| b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?<br>Where is this described? | Statistical analysis was performed using Prism v6 (GraphPad Software, San Diego, CA, USA). Following confirmation that the data met the assumptions of the ANOVA model, a two-way ANOVA followed by a post hoc Bonferroni test were used to compare the effects of the genotype or the drug injection on sleep-wake parameters.<br>Please see Suppl Methods page 3. |
| c. Is there any estimate of variance within each group of data?<br>Is the variance similar between groups that are being statistically compared?<br>Where is this described? | Statistical analysis was performed using Prism v6 (GraphPad Software, San Diego, CA, USA). Following confirmation that the data met the assumptions of the ANOVA model, a two-way ANOVA followed by a post hoc Bonferroni test were used to compare the effects of the genotype or the drug injection on sleep-wake parameters.<br>Please see Suppl Methods page 3. |
| d. Are tests specified as one- or two-sided? | We did not perform one-sided test. |
| e. Are there adjustments for multiple comparisons? | Yes,<br>Statistical analysis was performed using Prism v6 (GraphPad Software, San Diego, CA, USA). Following confirmation that the data met the assumptions of the ANOVA model, a two-way ANOVA followed by a post hoc Bonferroni test were used to compare the effects of the genotype or the drug injection on sleep-wake parameters.<br>Please see Suppl Methods page 3. |

| | |
|---|---|
| 3. Are criteria for excluding data points reported?<br>Was this criterion established prior to data collection?<br>On what page(s) is this described? | We did not exclude data point in our analysis. |
| 4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.<br>If no randomization was used, state so.<br>On what page(s) does this appear? | The injections were performed using a cross-over design.<br>Please see Suppl Methods page 2. |
| 5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?<br>If no blinding was done, is a statement to this effect included?<br>On what page(s)? | The individuals performing the saline/CNO injections and sleep-wake analysis did not perform the genotyping or initial immunohistochemical assessment of the injection sites.<br>Please see Suppl Methods page 3. |
| 6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?<br>On what page(s)? | Mice were bred at our animal facility and underwent genotyping both before and after experiments and all procedures were approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center.<br>Please see Suppl Methods page 1. |
| 7. Is the species of the animals used reported?<br>On what page(s)? | Adult male Vgat-IRES-cre and Vglut2-IRES-cre (129/C57/FVB) mice37 and non-cre-expressing littermate mice [8-12 weeks, 20-25g; n = 29 in vivo and n = 17 in vitro] were used in this study.<br>Please see Suppl Methods page 1. |
| 8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?<br>On what page(s)? | Adult male Vgat-IRES-cre and Vglut2-IRES-cre (129/C57/FVB) mice37 and non-cre-expressing littermate mice [8-12 weeks, 20-25g; n = 29 in vivo and n = 17 in vitro] were used in this study.<br>Please see Suppl Methods page 1. |
| 9. Is the sex of the animals/subjects used reported?<br>On what page(s)? | Adult male Vgat-IRES-cre and Vglut2-IRES-cre (129/C57/FVB) mice37 and non-cre-expressing littermate mice [8-12 weeks, 20-25g; n = 29 in vivo and n = 17 in vitro] were used in this study.<br>Please see Suppl Methods page 1. |
| 10. Is the age of the animals/subjects reported?<br>On what page(s)? | Adult male Vgat-IRES-cre and Vglut2-IRES-cre (129/C57/FVB) mice37 and non-cre-expressing littermate mice [8-12 weeks, 20-25g; n = 29 in vivo and n = 17 in vitro] were used in this study.<br>Please see Suppl Methods page 1. |
| 11. For animals housed in a vivarium, is the light/dark cycle reported?<br>On what page(s)? | the mice were housed individually in transparent barrels in an insulated sound-proofed recording chamber maintained at an ambient temperature of 22 ± 1°C and on a 12 hrs light/dark cycle (lights-on at 7 A.M., Zeitgeber time: ZT0) with food and water available ad libitum.<br>Please see Suppl Methods page 1. |
| 12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?<br>On what page(s)? | the mice were housed individually in transparent barrels in an insulated sound-proofed recording chamber maintained at an ambient temperature of 22 ± 1°C and on a 12 hrs light/dark cycle (lights-on at 7 A.M., Zeitgeber time: ZT0) with food and water available ad libitum.<br>Please see Suppl Methods page 1. |

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

    On what page(s)?

Mice were recorded for 24h baseline period followed by injections of Clozapine-N-oxide (CNO, Sigma-Aldrich; 0.3 mg/kg in saline, IP) injections at 7 P.M. (ZT12, lights-off, time of high waking drive) and at 10 A.M. (ZT3, light period, time of high sleeping drive).
Please see Suppl Methods page 1.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

    On what page(s)?

The animals were all naive prior to the experimental surgeries (AAV injections and EEG implants).
Please see Suppl Methods page 1.

    a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

       On what page(s)?

Mice were recorded for 24h baseline period followed by injections of Clozapine-N-oxide (CNO, Sigma-Aldrich; 0.3 mg/kg in saline, IP) injections at 7 P.M. (ZT12, lights-off, time of high waking drive) and at 10 A.M. (ZT3, light period, time of high sleeping drive). As an injection control mice were injected with saline at 10 A.M. and 7 P.M.. CNO and saline injections were performed in a random sequence and separated by 3-5 days washout period. Mice recordings (baseline, saline and CNO injections) were performed on four at a time and each time including two PZ hM3Dq-expressing mice and two non-hM3Dq-expressing littermate mice. The injections were performed using a cross-over design.
Please see Suppl Methods page 1-2.

15. If any animals/subjects were excluded from analysis, is this reported?

    On what page(s)?

No animal was excluded.

    a. How were the criteria for exclusion defined?

       Where is this described?

N/A

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

       Where is this described?

N/A

## Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes. Pre-absorption controls, tested in uninjected mice, previous publications by our lab.

a. Is antibody catalog number given?

On what page(s) does this appear?

Yes,
Antibody characterization.
The rabbit polyclonal Fos antibody (Oncogene Sciences; catalog number 4188) was raised against a synthetic peptide including residues 4-17 from human c-Fos. This antibody stained a single band of 55 kD m.w. on Western blots from rat brain (manufacturer's technical information). c-Fos staining with the Ab5 antiserum is found in many CNS structures45,46 only when neurons within these structures have recently been physiologically stimulated.
The goat polyclonal antibody against mCherry was raised against DsRED (Clontech; catalog number 632496) and the specificity of immunostaining for DsRED was indicated by the lack of detectable immunostaining in uninjected mice.
For all secondary antibody immunohistochemical controls, the primary antibodies were omitted and the tissue showed no immunoreactivity above background.

Suppl Methods page 3

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

On what page(s) does this appear?

Yes,
Antibody characterization.
The rabbit polyclonal Fos antibody (Oncogene Sciences; catalog number 4188) was raised against a synthetic peptide including residues 4-17 from human c-Fos. This antibody stained a single band of 55 kD m.w. on Western blots from rat brain (manufacturer's technical information). c-Fos staining with the Ab5 antiserum is found in many CNS structures45,46 only when neurons within these structures have recently been physiologically stimulated.
The goat polyclonal antibody against mCherry was raised against DsRED (Clontech; catalog number 632496) and the specificity of immunostaining for DsRED was indicated by the lack of detectable immunostaining in uninjected mice.
For all secondary antibody immunohistochemical controls, the primary antibodies were omitted and the tissue showed no immunoreactivity above background.

Suppl Methods page 3

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

On what page(s)?

N/A

a. Were they recently authenticated?

On what page(s) is this information reported?

## 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. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?

   On what page(s)?

N/A

## Computer code/software

1. Is there any custom algorithm/software that is integral to the study that has not been previously reported?

   If so, is this algorithm/software provided in a usable and readable form for the referees?

   Indicate in what form this is provided.

No

## Human subjects

1. Which IRB approved the protocol?

   Where is this stated?

N/A

2. Is demographic information on all subjects provided?

   On what page(s)?

3. Is the number of human subjects, their age and sex clearly defined?

   On what page(s)?

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   On what page(s)?

5. How well were the groups matched?

   Where is this information described?

6. Is a statement confirming that informed consent was obtained from all subjects included?

   On what page(s)?

7. For publication of patient photos, is a statement confirming that consent to publish was obtained included?

   On what page(s)?

## fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected? N/A

   a. If yes, is the number rejected and reasons for rejection described?

      On what page(s)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   On what page(s)?

3. Is the length of each trial and interval between trials specified?

4. Is a blocked design used?

   If so, is length of blocks specified?

5. Is an event-related design being used?

   If so, how was the design optimized?

6. Is the task design clearly described?

   Where?

7. How was behavioral performance measured?

8. Are any planned comparisons being used?

   a. Are they clearly described?

   b. Is an ANOVA used?

9. For data acquisition, is a whole brain scan used?

   If not, state area of acquisition.

   a. How was this region determined?

10. Is the field strength (in Tesla) of the MRI system stated?

a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?

11. Is the software used for data processing and pre-processing clearly stated?

12. For any anatomical imaging, is the coordinate space defined?

13. How was the brain image template space, name, modality and resolution determined?

14. How were anatomical locations determined?

15. Is the statistical model and estimation method clearly described?

16. Were any additional regressors (behavioral covariates, motion etc) used?

17. Is the contrast construction clearly defined?

18. Is a mixed/random effects or fixed inference used?

a. If fixed effects inference used, is this justified?

19. Were repeated measures used (multiple measurements per subject)?

a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?

20. If the threshold used for inference and visualization in figures varies, is this clearly stated?

21. Are statistical inferences corrected for multiple comparisons?

a. If not, is this labeled as uncorrected?

22. Are the results based on an ROI (region of interest) analysis?

a. If so, is the rationale clearly described?

b. How were the ROI's defined (functional vs anatomical localization)?

23. Is there correction for multiple comparisons within each voxel?

24. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

## Additional comments

Additional Comments