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Manuscript Type:	Article	# Supplementary Tables:	0
		# 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 (section, e.g. Results, & paragraph number).
- 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 paragraph 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; 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?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
example	1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend
example	results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	results, section 1	paired t-test	results, section 1	3	sections from 3 rats	results, section 1	error bars are mean +/- SEM	results, section 1	p = 0.0034	results, section 1	t(2) = 17.07	results, section 1
+ -	results, section 1	paired t-test	results, section 1	3	sections from 3 rats	results, section 1	error bars are mean +/- SEM	results, section 1	p = 0.0109	results, section 1	t(2) = 9.50	results, section 1
+ -	Fig. 2e	paired t-test	Fig. legend	4	sections from 4 rats	Fig. legend	error bars are mean +/- SEM	Fig. 2e	p = 0.0011	Fig. legend	t(3) = 12.42	Fig. legend
+ -	Fig. 3e	paired t-test	Fig. legend	3	3 times of pooled vesicles from 110 rats	Methods Section 8	error bars are mean +/- SEM	Fig. 3e	p = 0.0493	Fig. legend	t(2) = 4.333	Fig. legend
+ -	results, section 2 para 5	paired t-test	results, section 2 para 5	4	sections from 4 rats	results, section 2 para 5	error bars are mean +/- SEM	results, section 2 para 5	p = 1.56e-08	results, section 2 para 5	t(3) = 527.00	results, section 2 para 5
+ -	results, section 2 para 5	paired t-test	results, section 2 para 5	3	sections from 3 rats	results, section 2 para 5	error bars are mean +/- SEM	results, section 2 para 5	p = 0.0002	results, section 2 para 5	t(2) = 75.94	results, section 2 para 5
+ -	results, section 4 para 2	paired t-test	results, section 4 para 2	4	sections from 4 mice	results, section 4 para 2	error bars are mean +/- SEM	results, section 4 para 2	p = 4.05e-06	results, section 4 para 2	t(3) = 81.65	results, section 4 para 2
+ -	Fig. 6i	paired t-test	Fig. legend	4	4 slices from 2 mice	Fig. legend	error bars are mean +/- SEM	Fig. legend	p = 0.02	Fig. legend	t(3) = 4.198	Fig. legend
+ -	Fig. 6j	paired t-test	Fig. legend	4	4 slices from 2 mice	Fig. legend	error bars are mean +/- SEM	Fig. legend	p = 0.9093	Fig. legend	t(3) = 0.1239	Fig. legend
+ -	Suppl. Fig. 4d	paired t-test	Suppl. Fig. legend	3	sections from 3 mice	Suppl. Fig. legend	error bars are mean +/- SEM	Suppl. Fig. legend	p = 0.0034	Suppl. Fig. legend	t(2) = 17.07	Suppl. Fig. legend

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 and Supplementary Figures 1-7 display representative example images.

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

   If so, where is this reported (section, paragraph #)?

   The representative examples are from group data (e.g., those include different animals and sections). Each figure displays a representative example. There are clear statements of how many times the experiments were successfully repeated in online Methods, section 5, 6, 7 para 2, 8, and 11.

Statistics and general methods

1. Is there a justification of the sample size? If so, how was it justified? Where (section, paragraph #)? Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.	Yes. The justification of the sample size for fluorescence microscopy, 3-D analysis and electron microscopy is in Methods section 4 and 10 (ref #22, 30). The justification of the sample size for synaptic vesicles preparation and western blot is in Methods section 8 (ref #12, 27, 28). The justification of the sample size for the slide recording and voltammetry is in Methods section 14 (ref # 17, 31, 33). The justification of the sample size for the single cell qPCR is in Methods section 15 (ref #11).
2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	Yes. Statistical tests were used in Fig. 2, 3, 6 and Suppl. Fig. 4 , in Methods section 17.
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Yes. The statistical methods were described in Methods section 17. For each experiment, the statistical method was clearly indicated in figure legend (Fig. 2, 3, 6 and Suppl. Fig. 4).
b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)? Where is this described (section, paragraph #)?	Normality and other assumptions of statistical tests were met. This will be described in Methods section.
c. Is there any estimate of variance within each group of data? Is the variance similar between groups that are being statistically compared? Where is this described (section, paragraph #)?	Standard error of the mean (s.e.m.) is reported with each mean in text and figures. Figure variance (s.e.m.) is described in Figure Legends.
d. Are tests specified as one- or two-sided?	Two-sided
e. Are there adjustments for multiple comparisons?	N/A
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	Yes. The criteria were established prior to data collection. The morphological criteria used for identification and classification in ultrastructural analysis section were referred to Peters et al. in Methods section 5 (ref #25). Rats and mice in anatomical, electrophysiological, and voltammetr
4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data. If no randomization was used, state so. Where does this appear (section, paragraph #)?	Image collection and analysis were according to the method of randomization (in Methods section 17). For slice recordings and voltammetry, neurons were randomly sampled (in Methods section 13, 14).

5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included? If no blinding was done, state so. Where (section, paragraph #)?	Electron microscopy and confocal analysis quantification occurred blindly (Methods section 5).
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes, Methods section 1.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes, Methods section 1.
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Yes, Methods section 1.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Yes, Methods section 1.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes, Methods section 1 and 8.
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes. Rats and mice were housed in the animal rooms at 22°C under a 12 h light/dark cycle (light on at 7 am), with ad libitum access to food and water (in Methods section 1).
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes. Rats were double housed, and all mice were housed in groups of up to four animals per cage in the animal rooms (in Methods section 1).
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	N/A
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	N/A
a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	
15. If any animals/subjects were excluded from analysis, is this reported? Where (section, paragraph #)?	Rats and mice in anatomical, electrophysiological, and voltammetric studies were excluded from data analysis if the injection sites were incorrect (in Methods section 17).

a. How were the criteria for exclusion defined?

Where is this described (section, paragraph #)?

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

Where is this described (section, paragraph #)?

Reagents

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

Yes.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

Yes.
Methods section 2, 3, 4, 6, 7, 10, and 11.

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

Where does this appear (section, paragraph #)?

We first determined the specificity of several commercially available anti-VMAT2 antibodies by western blot analysis of total protein preparations from both nAcc and from cell lysates expressing VMAT2 with Myc tag at the amino-terminus [VMAT2(Myc), Supplementary Fig. 2a]. Out of 8 tested anti-VMAT2 antibodies, only one showed selectivity for VMAT2 detection (EB06558 antibody; Everest Biotech; Supplementary Fig. 2b).

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

Where (section, paragraph #)?

Yes. HEK293 cell line was used (in Methods section 7).

a. Were they recently authenticated?

Where is this information reported (section, paragraph #)?

No.

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.

We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

2. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability**" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

Human subjects

1. Which IRB approved the protocol?

   Where is this stated (section, paragraph #)?

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

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

   Where (section, paragraph #)?

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

   Where (section, paragraph #)?

5. How well were the groups matched?

   Where is this information described (section, paragraph #)?

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

   Where (section, paragraph #)?

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

   Where (section, paragraph #)?

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?

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

    Where (section, paragraph #)?

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

    Where (section, paragraph #)?

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

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

5. Is the task design clearly described?

    Where (section, paragraph #)?

6. How was behavioral performance measured?

7. Is an ANOVA or factorial design being used?

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

    If not, state area of acquisition.

    a. How was this region determined?

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

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

    b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?

12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?

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

15. Is the contrast construction clearly defined?

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

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

17. 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?

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

19. Are statistical inferences corrected for multiple comparisons?

    a. If not, is this labeled as uncorrected?

20. 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)?

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

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

Additional comments

Additional Comments