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Main Figures: 8

Supplementary Figures: 11

Supplementary Tables: N/A

Supplementary Videos: N/A

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
+ -	1b	Two-way ANOVA	Fig leg 1	5, 6; 6, 7; 6, 8; 24, 20; 7, 8; 6, 7	rats recorded per age/group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.0038	Fig leg 1	F(5, 36) = 4.255	Fig leg 1
+ -	1c	Two-way ANOVA	Fig leg 1	2, 3; 3, 3	P17 rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.4453	Fig leg 1	F(1, 7) = 0.6538	Fig leg 1
+ -	1d	Two-way ANOVA	Fig leg 1	3, 3; 3, 4	P20 aged rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.208	Fig leg 1	F(1, 9) = 1.702	Fig leg 1

		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	
+ -	1e	Two-way ANOVA	Fig leg 1	3, 3; 4, 4	P26 aged rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.7148	Fig leg 1	F(1, 9) = 0.1421	Fig leg 1
+ -	1f	Two-way ANOVA	Fig leg 1	12, 12; 8, 12	P35 aged rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.003	Fig leg 1	F(1, 40) = 10.204	Fig leg 1
+ -	1g	Two-way ANOVA	Fig leg 1	3, 4; 3, 5	P45 aged rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.4960	Fig leg 1	F(1, 11) = 0.4955	Fig leg 1
+ -	1h	Two-way ANOVA	Fig leg 1	2, 4; 2, 5	P75 aged rats recorded per group	Fig leg 1	Error bars are mean +/- SEM	Fig leg 1	0.7308	Fig leg 1	F(1, 9) = 0.1259	Fig leg 1
+ -	2a	Two-way ANOVA on ranks	Fig leg 2	6, 6; 6, 10; 8, 6; 32, 14; 6, 6; 4, 9	slices recorded for age /group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.00203	Fig leg 2	F(5, 101) = 2.2508	Fig leg 2
+ -	2b	Student's t-test	Fig leg 2	6, 6	slices recorded from 3, 3 P17 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.92143	Fig leg 2	t(10) = -0.10115	Fig leg 2
+ -	2c	Student's t-test	Fig leg 2	6, 10	slices recorded from 4, 4 P20 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.91100	Fig leg 2	t(14) = -0.11382	Fig leg 2
+ -	2d	Student's t-test	Fig leg 2	8, 6	slices recorded from 4,3 P26 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.52825	Fig leg 2	t(12) = 0.64950	Fig leg 2
+ -	2e	Mann-Whitney Rank Sum Test	Fig leg 2	32, 14	slices recorded from 18, 6 P35 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	<0.00001	Fig leg 2	T(14, 32) = 473.00000	Fig leg 2
+ -	2f	Student's t-test	Fig leg 2	6, 6	slices recorded from 4, 4 P45 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.99092	Fig leg 2	t(10) = -0.011665	Fig leg 2
+ -	2g	Student's t-test	Fig leg 2	4, 9	slices recorded from 1, 2 P75 aged rats per group	Fig leg 2	Error bars are mean +/- SEM	Fig leg 2	0.87431	Fig leg 2	t(11) = 0.16191	Fig leg 2
+ -	3b total dLGN area	Student's t-test	Fig leg 3	3, 5	rats processed per group	Fig leg 3	Error bars are mean +/- SEM	Fig leg 3	0.831	Fig leg 3	t(6) = 0.223	Fig leg 3
+ -	3b contra area	Student's t-test	Fig leg 3	3, 5	rats processed per group	Fig leg 3	Error bars are mean +/- SEM	Fig leg 3	0.16996	Fig leg 3	t(6) = -1.559	Fig leg 3
+ -	3b ipsi area	Student's t-test	Fig leg 3	3, 5	rats processed per group	Fig leg 3	Error bars are mean +/- SEM	Fig leg 3	0.616	Fig leg 3	t(6) = 0.529	Fig leg 3
+ -	3b overlap	Student's t-test	Fig leg 3	3, 5	rats processed per group	Fig leg 3	Error bars are mean +/- SEM	Fig leg 3	0.666	Fig leg 3	t(6) = -0.454	Fig leg 3
+ -	3f	Two-way ANOVA on ranks	Fig leg 3	19, 11	slices processed from 6, 3 rats per group	Fig leg 3	Error bars are mean +/- SEM	Fig leg 3	0.42479	Fig leg 3	F(2, 84) = 1.33106	Fig leg 3
+ -	4a	Chi-square	Fig leg 4	14, 11	rats processed per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.199	Fig leg 4	chi (4) = 6.00	Fig leg 4
+ -	4b	Two-way RM ANOVA	Fig leg 4	7, 3	rats processed per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.9683	Fig leg 4	F(5, 40) = 0.1805	Fig leg 4
+ -	4c	Two-way RM ANOVA	Fig leg 4	4, 4	rats processed per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.1509	Fig leg 4	F(4, 20) = 1.8935	Fig leg 4

+ -	4d	Student's t-test	Fig leg 4	5, 8	rats recorded per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.4660	Fig leg 4	t(11) = 0.7551	Fig leg 4
+ -	4e	Student's t-test	Fig leg 4	5, 7	rats recorded per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.3557	Fig leg 4	t(10) = 0.96824	Fig leg 4
+ -	4f	Student's t-test	Fig leg 4	7, 7	rats recorded per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	Bin: 0.3850 Contra: 0.5447 Ipsi: 0.7574	Fig leg 4	t(12) = -0.901457 t(12) = -0.623307 t(12) = -0.315991	Fig leg 4
+ -	4g	Student's t-test	Fig leg 4	7, 7	rats recorded per group	Fig leg 4	Error bars are mean +/- SEM	Fig leg 4	0.9928	Fig leg 4	t(12) = 0.00920693	Fig leg 4
+ -	5a	One-way ANOVA	Fig leg 5	24, 20, 7, 8	rats recorded per group	Fig leg 5	Error bars are mean +/- SEM	Fig leg 5	0.000022	Fig leg 5	F(3, 55) = 10.0427	Fig leg 5
+ -	5b	One-way ANOVA	Fig leg 5	28, 14, 14, 9	slices recorded per group	Fig leg 5	Error bars are mean +/- SEM	Fig leg 5	0.00057	Fig leg 5	F(3,61) = 6.67813	Fig leg 5
+ -	5c	Student's t-test	Fig leg 5	7, 7	serum samples per group	Fig leg 5	Error bars are mean +/- SEM	Fig leg 5	0.02423	Fig leg 5	t(12) = -2.57709	Fig leg 5
+ -	6a	Student's t-test	Fig leg 6	9, 9	cells recorded per group	Fig leg 6	Error bars are mean +/- SEM	Fig leg 6	0.04512	Fig leg 6	t(16) = 2.17333	Fig leg 6
+ -	6b	Student's t-test	Fig leg 6	9, 9	cells recorded per group	Fig leg 6	Error bars are mean +/- SEM	Fig leg 6	0.42577	Fig leg 6	t(16) = 0.81728	Fig leg 6
+ -	6c	Student's t-test	Fig leg 6	6, 5	rats processed per group	Fig leg 6	Error bars are mean +/- SEM	Fig leg 6	0.03938	Fig leg 6	t(9) = 2.40794	Fig leg 6
+ -	6e WFA	Mann-Whitney rank sum test	Fig leg 6	42, 34	slices from 6, 5 rats per group	Fig leg 6	box chart	Fig leg 6	0.019	Fig leg 6	T(34, 42) = 1083.500	Fig leg 6
+ -	6e PV	Student's t-test	Fig leg 6	48, 40	slices from 6, 5 rats per group	Fig leg 6	Error bars are mean +/- SEM	Fig leg 6	0.511	Fig leg 6	t(86) = 0.660	Fig leg 6
+ -	6e WFA-PV	Mann-Whitney rank sum test	Fig leg 6	46, 35	slices from 6, 5 rats per group	Fig leg 6	box chart	Fig leg 6	0.000000000000000001	Fig leg 6	T(35, 46) = 630.000	Fig leg 6
+ -	7b	Two-way ANOVA	Fig leg 7	12, 15, 9, 15	triplicates from 4,5,3,5 rats per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.00317	Fig leg 7	F(1,47) = 9.67641	Fig leg 7
+ -	7c	Two-way ANOVA	Fig leg 7	4, 5, 3, 3	rats processed per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.013	Fig leg 7	F(1, 11) = 1.713	Fig leg 7
+ -	7e	Two-way ANOVA	Fig leg 7	12, 12; 8, 12; 4, 6	rats recorded per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.0020	Fig leg 7	F(2, 48) = 7.0822	Fig leg 7
+ -	7f	Student's t-test	Fig leg 7	8, 4	rats recorded per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.0304	Fig leg 7	t(10) = 2.519	Fig leg 7
+ -	7g	One-way ANOVA	Fig leg 7	32, 14, 9	slices recorded per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.00007	Fig leg 7	F(2, 52) = 11.50863	Fig leg 7
+ -	7h	Student's t-test	Fig leg 7	14, 9	slices recorded per group	Fig leg 7	Error bars are mean +/- SEM	Fig leg 7	0.00542	Fig leg 7	t(21) = 3.09991	Fig leg 7
+ -	8a	Student's t-test	Fig leg 8	9, 14	cells recorded per group	Fig leg 8	Error bars are mean +/- SEM	Fig leg 8	0.01232	Fig leg 8	t(21) = -2.73804	Fig leg 8
+ -	8b	Mann-Whitney rank sum test	Fig leg 8	5, 6	rats processed per group	Fig leg 8	Error bars are mean +/- SEM	Fig leg 8	0.004	Fig leg 8	T(5, 6) = 15.000	Fig leg 8

+ -	8c	Student's t-test	Fig leg 8	34, 32	slices from 5, 5 rats per group	Fig leg 8	box chart	Fig leg 8	0.019	Fig leg 8	t(64) = -2.409	Fig leg 8
+ -	suppl.fi g. 2	Mann-Whitney rank sum test	supplementary, 2	8, 12	rats recorded per group	supplementary, 2	box chart	supplementary, 2	0.002	supplementary, 2	T(37, 274) = 7312.50	supplementary, 2
+ -	suppl.fi g. 3c	Student's t-test	suppl.fi g. 3	22, 21	GFP positive cells from 3,4 rats per group	suppl.fi g. 3	Error bars are mean +/- SEM	suppl.fi g. 3	0.50160	suppl.fi g. 3	t(41) = 0.67796	suppl.fi g. 3
+ -	suppl.fi g. 3d	Student's t-test	suppl.fi g. 3	22, 21	GFP positive cells from 3,4 rats per group	suppl.fi g. 3	Error bars are mean +/- SEM	suppl.fi g. 3	0.64579	suppl.fi g. 3	t(41) = 0.46303	suppl.fi g. 3
+ -	suppl.fi g. 4	Two-way RM ANOVA	suppl.fi g. 4	6, 6	rats per group	suppl.fi g. 4	Error bars are mean +/- SEM	suppl.fi g. 4	0.65971	suppl.fi g. 4	F(5, 50) = 0.02086	suppl.fi g. 4
+ -	suppl.fi g. 5a	Student's t-test	suppl.fi g. 5	7, 8	cells per group	suppl.fi g. 5	Error bars are mean +/- SEM	suppl.fi g. 5	0.42634	suppl.fi g. 5	t(13) = -0.82120	suppl.fi g. 5
+ -	suppl.fi g. 5b	Student's t-test	suppl.fi g. 5	7, 8	cells per group	suppl.fi g. 5	Error bars are mean +/- SEM	suppl.fi g. 5	0.06780	suppl.fi g. 5	t(13) = 1.99202	suppl.fi g. 5
+ -	suppl.fi g. 5c	Mann-Whitney rank sum test	suppl.fi g. 5	7, 8	cells per group	suppl.fi g. 5	Error bars are mean +/- SEM	suppl.fi g. 5	0.77887	suppl.fi g. 5	T(7, 8) = 53.50000	suppl.fi g. 5
+ -	suppl.fi g. 5d	Mann-Whitney rank sum test	suppl.fi g. 5	9,7	cells per group	suppl.fi g. 5	Error bars are mean +/- SEM	suppl.fi g. 5	1.00000	suppl.fi g. 5	T(7, 9) = 60.00000	suppl.fi g. 5
+ -	suppl.fi g. 5e	Mann-Whitney rank sum test	suppl.fi g. 5	9, 7	cells per group	suppl.fi g. 5	Error bars are mean +/- SEM	suppl.fi g. 5	0.09034	suppl.fi g. 5	T(7, 9) = 76.00000	suppl.fi g. 5
+ -	suppl.fi g. 6b	Student's t-test	suppl.fi g. 6b	4, 5	rats per group	suppl.fi g. 6b	Error bars are mean +/- SEM	suppl.fi g. 6b	0.025	suppl.fi g. 6b	t(7) = -2.832	suppl.fi g. 6b
+ -	suppl.fi g. 7b	Mann-Whitney rank sum test	suppl.fi g. 7	6, 5	rats per group	suppl.fi g. 7	Error bars are mean +/- SEM	suppl.fi g. 7	0.93074	suppl.fi g. 7	T(5, 6) = 31.00000	suppl.fi g. 7
+ -	suppl.fi g. 8a	Student's t-test	suppl.fi g. 8	8, 6	cells per group	suppl.fi g. 8	Error bars are mean +/- SEM	suppl.fi g. 8	0.36698	suppl.fi g. 8	t(12) = -0.93752	suppl.fi g. 8
+ -	suppl.fi g. 8b	Student's t-test	suppl.fi g. 8	8, 6	cells per group	suppl.fi g. 8	Error bars are mean +/- SEM	suppl.fi g. 8	0.56289	suppl.fi g. 8	t(12) = -0.59501	suppl.fi g. 8
+ -	suppl.fi g. 8c	Student's t-test	suppl.fi g. 8	3, 3	rats per group	suppl.fi g. 8	Error bars are mean +/- SEM	suppl.fi g. 8	0.7563	suppl.fi g. 8	t(4) = -0.33237	suppl.fi g. 8
+ -	suppl.fi g. 9a	Student's t-test	suppl.fi g. 9	9, 7	cells per group	suppl.fi g. 9	Error bars are mean +/- SEM	suppl.fi g. 9	0.17965	suppl.fi g. 9	t(14) = -1.41247	suppl.fi g. 9

+ -	suppl.fi g. 9b	Student's t-test	sup pl.fi g. 9	9, 7	cells per group	sup pl.fi g. 9	Error bars are mean +/- SEM	sup pl.fi g. 9	0.71180	sup pl.fi g. 9	t(14) = -0.37703	sup pl.fi g. 9
+ -	suppl.fi g. 9c	Student's t-test	sup pl.fi g. 9	4, 5	rats per group	sup pl.fi g. 9	Error bars are mean +/- SEM	sup pl.fi g. 9	0.5330	sup pl.fi g. 9	t(7) = -0.65565	sup pl.fi g. 9
+ -	suppl.fi g. 9d	Student's t-test	sup pl.fi g. 9	4, 4	rats per group	sup pl.fi g. 9	box chart	sup pl.fi g. 9	0.76653	sup pl.fi g. 9	t(35) = 0.29924	sup pl.fi g. 9
+ -	suppl.fi g. 10b	Student's t-test	sup pl.fi g. 10	8, 6	slices from 4, 3 rats per group	sup pl.fi g. 10	Error bars are mean +/- SEM	sup pl.fi g. 10	0.43334	sup pl.fi g. 10	t(12) = 0.81068	sup pl.fi g. 10
+ -	results, pg 7, P35 AMPA	Student's t-test	resu lts, pg 7	9, 10	cells per group	resu lts, pg 7	mean +/- SEM	resu lts, pg 7	0.50074	resu lts, pg 7	t(17) = 0.68798	resu lts, pg 7
+ -	results, pg 9, P26	Student's t-test	resu lts, pg 9	8, 6	cells per group	resu lts, pg 9	mean +/- SEM	resu lts, pg 9	0.67098	resu lts, pg 9	t(12) = 0.43543	resu lts, pg 9
+ -	results, pg 9, P75	Student's t-test	resu lts, pg 9	9, 7	cells per group	resu lts, pg 9	mean +/- SEM	resu lts, pg 9	0.64366	resu lts, pg 9	t(14) = 0.47277	resu lts, pg 9

► Representative figures

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

If so, what figure(s)?

Representative images are shown in Fig. 2b, 2c, 2d, 2e, 2f, 2g, 3b, 3d, 3e, 6a, 6b, 6c, 6d, 7g, 8a, 8b, 8c, Suppl. Fig.1, Suppl. Fig.3b, Suppl. Fig.7a, Suppl. Fig.8a-c, Suppl. Fig.9a-d, Suppl. Fig. 10a.

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, on what page(s) is this reported?

With exception of Fig 3d, each of the images is followed by a summary graph with all experimental points, the average, and standard error of the mean.
For figure 3d, the data sample is the same of Suppl. Figure 3 (4 rats for bumetanide, 3 for controls)

► Statistics and general methods

1. Is there a justification of the sample size?

If so, how was it justified?

On what page(s)?

Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

Our laboratories have a long-standing experience in monocular deprivation, visual cortical LTP, intraocular injection, and in utero electroporation. Thus, we have a clear idea of the variability of data samples in these kinds of experiments. Sample size was calculated by the sample-size calculator on Sigma-plot 11.0, which takes into account the expected difference of the means, the expected standard deviation, and the desired power (we set the power to be higher than 0.05), and the alpha value (set for our experiments at 0.05).

2. Are statistical tests justified as appropriate for every figure?

On what page(s)?

The criteria for choosing a specific statistical test has been described on page 32 (Statistical analysis).

- a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?
- Yes, as described on Page 32.
- 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?
- Yes, as described on page 32.
- 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?
- Variance of all averages for each group is estimated by SEM. Yes. We performed all statistical tests with Sigma-plot 11.0. Before performing any comparison test, the program performs an estimate of variance for each group of data, to ensure that the variance is similar between groups. This has been reported on page 32.
- d. Are tests specified as one- or two-sided?
- The tests are two sided (reported on page 32).
- e. Are there adjustments for multiple comparisons?
- Yes
3. Are criteria for excluding data points reported?
- Was this criterion established prior to data collection?
- On what page(s) is this described?
- N/A.
No data point were excluded from the analysis.
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.
- On what page(s) does this appear?
- Method for sample randomization:
- For image acquisition at the confocal microscope, all slides were acquired in a random order and in a single session for each litter of animals to minimize errors caused by fluctuation in laser output and degradation of fluorescence.
- For electrophysiological recordings, care was used to record from one bumetanide- and one vehicle-injected animal on alternating days.
- For LTP, MD and behavioral experiments, animals treated with bumetanide or vehicle were litter mates.
- This info was reported in the manuscript on page 32.
- Where not otherwise specified, data collection and analysis were not performed blind to the conditions of the experiments.
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, is a statement to this effect included?
- On what page(s)?
- Data analysis of VEP, and image acquisition and data analysis of confocal images, and behavioral experiments were performed by an experimenter blind to the phenotype, as described on page 23, 28, 29.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
- On what page(s)?
- Yes, on page 23.

7. Is the species of the animals used reported?
On what page(s)?
- Yes, the species is reported in the title, throughout the manuscript, and in details in the Method section (page 23).
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
On what page(s)?
- Yes, the strain is reported in the Method section on page 23.
9. Is the sex of the animals/subjects used reported?
On what page(s)?
- Yes, both males and females were used for the study. The info is reported on page 23.
10. Is the age of the animals/subjects reported?
On what page(s)?
- Yes, throughout the manuscript and in the Figures.
11. For animals housed in a vivarium, is the light/dark cycle reported?
On what page(s)?
- Yes, on page 23.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
On what page(s)?
- Our cages hosted only one dam/cage. For weaned (P21-75) animals/cage, 3-4 animals were usually hosted.
- This information is not reported in the manuscript.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
On what page(s)?
- Behavioral experiments were performed in the morning. This information is not reported in the manuscript.
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
On what page(s)?
- Yes, this is reported for each figure in a schematic cartoon.
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
On what page(s)?
- N/A
15. If any animals/subjects were excluded from analysis, is this reported?
On what page(s)?
- N/A. No animals were excluded from the analysis
- a. How were the criteria for exclusion defined?
Where is this described?
- 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.

(1) mouse anti-NKCC1 was previously described (Ge et al., 2006 Nature), and it was validated by experiments on NKCC1 KO, and by biological validation (Supplementary. Fig.1).
 (2) rabbit anti-KCC2 was previously described (Ge et al., 2006 Nature) and by biological validation (Supplementary. Fig.1).
 (3) rabbit anti-BDNF antibody was validated loading 0.05 ng of pure Human recombinant BDNF
 (4) guinea pig anti-PV and WFA was previously described (Antonucci, F., et al. J Neurosci, 2012; Pizzorusso, T., et al., Science, 2002)
 (5) mouse anti-trkB (Jovanovic JN, et al., Nature neurosci, 2000)
 (6) mouse anti-GAD67 (Sgado', P., et al. Exp Neurol, 2013)

- a. Is antibody catalog number given?

On what page(s) does this appear?

Mouse anti-NKCC1 #T4, rabbit anti-KCC2 #07-432, rabbit anti-BDNF # sc-546, guinea pig anti-PV #195004, WFA #L1516-2mg, mouse anti-trkB #610101. mouse anti-GAD67 #MAB5406 . Catalog numbers, host species, dilution and company are reported in the manuscript.

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

On what page(s) does this appear?

(1) mouse anti-NKCC1, Acknowledgments on page 16, supplementary Fig.1; Ge et al., 2006, Nature
 (2) rabbit anti-KCC2, Supplementary Fig.1; Ge et al., 2006, Nature
 (3) guinea pig anti-PV, Antonucci, F., et al. J Neurosci, 2012
 (4) WFA, Pizzorusso, T., et al., Science, 2002
 (5) mouse anti-trkB (Jovanovic JN, et al., Nature neurosci, 2000)
 (6) mouse anti-GAD67 (Sgado', P., et al. Exp Neurol, 2013)

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:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- 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)?

Primers for RT-PCR are reported on page 31.

▶ 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.

Yes. For analysis of in vivo recordings we used a custom-made software written in LabView. The software is not available online because it is copyrighted.

▶ 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