nature neuroscience

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| Corresponding Author: | Jason Aoto | # Main Figures: | 8 |
| Manuscript Number: | NN-A48879 | # Supplementary Figures: | 8 |
| Manuscript Type: | Article | # Supplementary Tables: | 1 |
| | | # 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 | |
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| | 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 | |
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| | FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # |
| + - | 1c | chi-squiared | fig. legend and methods | for males: 42 wt, 105 het, 20 KO For Females: 57 wt, 116 het, 17 KO | # of animals | fig. legend | error bars are mean +/- SEM | legend | p<0.0001 calculated by hand using chi-squiared table | legend | df: 2 | |
| + - | 1d | single factor ANOVA | fig. legend and methods | WT: 38; Het: 78 KO: 12 | # of animals | fig. legend | error bars are mean +/- SEM | legend | P5: p = 0.144; P7: p = 0.00375; P9: = 0.003; P11: p = 0.00134; P13: p < 0.0001; P15: p < 0.0001; P17: p = 0.0001; P19: p = 0.000178; P21: p = 0.0151; P23: p = 0.305; P25: p = 0.0807; P27: p = 0.0115; P29: p < 0.0001; P31: p = 0.00999 | legend | df: 11 | |
| + - | 1e | single factor anova | fig. legend | 3 male littermate WT and KO | # of animals | fig legend | error bars are mean +/- SEM | fig legend | p < 0.0001 | legend | dF: 2 F = 54 F-crit = 6.6 | |
| + - | 2b, d | For bar graphs: single factor ANOVA For cumulative distributions : K-S test | fig. legend and methods | b; control: 40 cells from 3 expts, KO 39 cells/3 expts d; control: 44/3 cre: 42/3 | # of expts | fig. legend | error bars are mean +/- SEM | fig. legend | b: mEPSC frequency cumulative probability: p = 0.972, frequency: p = 0.128; amplitude cumulative probability: p < 0.0001, mEPSC amplitude bar graph: p = 0.0216 c: mIPSC frequency cumulative probability: p = 0.481, frequency: p = 0.879; amplitude cumulative probability: p = 0.392, amplitude: p = 0.467 | legend | dF: 2 F=13.359 F-crit = 7.7 | |

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| + - | 2f | single-factor ANOVA | fig. legend and methods | control: 36cells from 3 experiments cre: 37 cells from 3 expts | # of experiments | fig. legend | error bars are mean +/- SEM | fig. legend | p = 0.0036 | legend | df: 2 F=37.41 F-crit = 7.7 | |
| + - | 2e | single factor ANOVA | legend / methods | control: 36 cells form 3 expts cre: 37 cells from 3 expts | # of experiments | fig. legend | error bars are mean +/- SEM | legend | p = 0.752 | legend | df: 2 F=0.121; F-crit = 7.7 | |
| + - | 2g | single factor anova | legend / methods | control: 40 cells, 3expts cre: 35 cells from 3 expts | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.822 | legend | df: 2 F=0.057; F-crit = 7.7 | |
| + - | 2h | single factor ANOVA | legend / methods | control: 40 cells, 3expts cre: 35 cells from 3 expts | # of experiments | legend | error bars are mean +/- SEM | legend | 80 ms ISI: p = 0.969 | legend | df: 2 F-crit = 7.7 | |
| + - | 3b-d | single factor ANOVA | legend / methods | control and cre: 5 independent expts with at least 15 cells per experiment for GluA1, PSD95 and vGluT1. control and cre: 3 independent experiments for GluA2 | # of experiments | legend | error bars ar emean +/- SEM | legend | Density: GluA1, p = 0.698; GluA2, p = 0.631; PSD95, p = 0.818; vGluT1, p = 0.758. Size: GluA1, p = 0.018; GluA2, p = 0.0387, PSD95, p = 0.186; vGluT1, p = 0.421. Intensity: GluA1, p = 0.579; GluA2, p = 0.267; PSD95, p = 0.821; vGluT1, p = 0.796 | legend | dF: 4 F = 10.31 F-crit = 7.7 | |
| + - | 3f | single factor ANOVA | legend / methods | control and KO: 5 independent experiments with at least 15 cells per experiment | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.0034 | legend | dF: 4 F=6.45 F-crit = 5.98 | |

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| + - | 4a | single factor ANOVA | legend / methods | Nrxn3aSS4- control: 45/3, cre: 47/3, control +rescue: 46/3, cre +rescue: 46/3 Nrxn3SS4+: control: 46/3, cre: 45/3, control +rescue: 41/3, cre +rescue: 48/3 | # of experiments | legend | error bars are mean +/- SEM | legend | Nrxn3αSS4– (left: cre: p = 0.0014; + Nrxn3αSS4–: p = 0.962). Nrxn3αSS4+ (right: cre: p = 0.00836; + Nrxn3αSS4+: p = 0.00450 | legend | dF=2 F=12.76 F-crit = 7.7 | |
| + - | 4b | single factor ANOVA | legend / methods | Nrxn3bSS4-: contro: 41/3, cre: 42/3, control +rescue: 43/3, cre +rescue: 39/3 Nrxn3bSS4+: control: 43/3, cre: 42/3, control +rese: 40/3, cre +rescue: 41/3 | # of experiments | legend | error bars are mean +/-SEM | legend | Nrxn3β SS4– (left: cre: p = 0.00785; + Nrxn3βSS4–: p = 0.647). Nrxn3βSS4– (right: cre: p = 0.0138; + Nrxn3βSS4+: p = 0.0118) | legend | dF=2 F = 18.511 F-crit = 7.7 | |
| + - | 4c | single factor ANOVA | legend / methods | control: 32/3, cre: 34/3, control +GPI: 32/3, cre +GPI: 34/3 | # of experiments | legend | error bars are mean +/- SEM | legend | cre: p = 0.0009; cre + GPI- Nrxn3αSS4–: p = 0.742 | legend | dF: 2 f = 77.3 F-crit = 7.7 F = 0.125 F = 7.7 | |
| + - | 4d | single factor ANVOA | legend / methods | control: 39/3, cre: 39/3, control+ sNrxn3: 41/3, cre +solNrxn 3: 43/3 | # of experiments | legend | error bars are mean +/- SEM | legend | cre: p = 0.003; cre + sNrxn3βSS4–: p = 0.007 | legend | dF=2 F=45.241 F-crit = 7.7 | |
| + - | 4e | single factor ANOVA | legend / methods | control: 43/3, cre: 42/3, cre +1b: 39/3, cre +2b: 38/3 | # of experiments | legend | error bars are mean +/- SEM | legend | cre: p = 0.0025; cre + Nrxn1βSS4–: p = 0.717; cre + Nrxn2βSS4–: p = 0.697 | legend | dF=2 F=45.241 F-crit = 7.7 | |
| + - | 4f | single factor ANOVA | legend / methods | control: 38/3, cre: 37/3, control +pdis: 43/3, cre +pdis: 38/8 | # of experiments | legend | error bars are mean +/- SEM | legend | cre: p = 0.0073; cre + Nrxn1βSS4–- PDGFR: p = 0.535 | legend | dF=2 F=45.241 F-crit = 7.7 | |

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| + - | 4g | single factor ANOVA | legend / methods | control: 40/3, cre: 40/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.100 | legend | dF=2 F = 4.5 F-crit = 7.7 | |
| + - | 4h | single factor ANOVA | legend / methods | control: 43/3, cre: 47/3, control +transf: 40/3, cre +transf: 38/3 | # of experiments | legend | error bars are mean +/- SEM | legend | cre: p = 0.00112; cre +transfection p = 0.0186 | legend | dF=2 F = 16.21 F-crit = 7.7 | |
| + - | 5c | single factor ANOVA | legend / methods | control: 22/3, cre: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | 10µA: p = 0.0728; 25µA: p = 0.177; 50µA: p = 0.122; 75µA: p = 0.0198; 100µA: p = 0.00748 and slope: p = 0.0362 | legend | df: 22/F = 7.88 | |
| + - | 5d | single-factor ANOVA | legend / methods | control: 22/3, cre: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | 60ms ISI: p = 0.422 | legend | df: 22 /F = 7.88 | |
| + - | 5e | single factor ANOVA | legend / methods | control: 10/3, cre: 6/3 | # of cells | legend | error bars are mean +/- SEM | legend | 10µA: p = 0.132; 25µA: p = 0.188; 50µA: p = 0.0248, 75µA: 0.0349, 100µA: 0.035 and slope: p = 0.0172 | legend | dF: 5 F=6.31/5.53 | |
| + - | 5f | single factor ANOVA | legend / methods | control: 10/3, cre: 6/3 | # of cells | legend | error bars are mean +/- SEM | legend | 60ms ISI: p = 0.954 | legend | dF: 5 F = 5.5 | |
| + - | 6c | single factor ANOVA | legend / methods | control: 31/3, cre: 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | frequency: p = 0.603; amplitude: p = 0.276 | legend | dF: 2 F = 134.1 F-crit = 7.7 | |
| + - | 6d | single factor ANOVA | legend / methods | control: 34/3, cre; 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.469 | legend | dF: 2 F = 0.637 F-crit = 7.71 | |
| + - | 6e | single factor ANOVA | legend / methods | control: 32/3, cre: 26/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 537 | legend | dF: 2 F = 0.455 F-crit = 7.7 | |
| + - | 6f | single factor ANOVA | legend / methods | control: 32/3, cre:26/3 | # of cells | legend | error bars are mean +/- SEM | legend | 100 ms ISI: p = 0.300 | legend | dF = 25 F = 1.1 F-crit = 4.01 | |
| + - | 6g | single factor ANOVA | legend / methods | Nrxn3SS4 + 38/4, Nrxn3SS4 -: 39/4 | # of experiments | legend | error bars are mean +/- SEM | legend | frequency: p = 0.883; amplitude: p = 0.181 | legend | dF = 3 Freq F = 0.231 F-crit = 5.98 Amp: F = 2.28 F-crit = 5.98 | |
| + - | 6h | single factor ANOVA | legend / methods | Nrxn3SS4 +: 33/3, Nrxn3SS4 - 32/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.881 | legend | dF: 2 F = 0.0255 F-crit = 7.7 | |

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| + - | 6i | single factor ANOVA | legend / methods | Nrxn3SS4+: 24/3, Nrxn3SS4- 22/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.721 | legend | dF: 2<br>F = 0.146<br>F-crit = 7.7 | |
| + - | 6j | single factor ANOVA | legend / methods | Nrxn3SS4+: 24/3, Nrxn3SS4- 22/3 | # of cells | legend | error bars are mean +/- SEM | legend | 100ms ISI: 0.807 | legend | dF: 21<br>F = 0.06<br>F-crit = 4.06 | |
| + - | 7a | single factor ANOVA | legend / methods | control: 26/3, cre: 27/3 | # of experiments | legend | error bars are mean +/- SEM | legend | frequency: p = 0.039; amplitude: 0.419 | legend | dF: 2<br>F=9.15 | |
| + - | 7b | single factor ANOVA | legend / methods | control: 19/3, cre: 20/3 | # of cells | legend | error bars are mean +/- SEM | legend | p = 0.024 | legend | df: 2<br>F=15.869 | |
| + - | 7c | single factor ANOVA | legend / methods | control: 19/3, cre: 20/3 | # of cells | legend | error bars are mean +/- SEM | legend | 20ms ISI: p = 0.00264; 40 ms ISI: p = 0.0365; 60ms ISI: p = 0.450 | legend | df: 18<br>F= 10.62 (20ms ISI), 4.75 (40 ms ISI), 4.15 (60ms ISI) | |
| + - | 7d | single factor ANOVA | legend / methods | WT: 38/3, KO, 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | frequency: p = 0.0435; amplitude: p = 0.367 | legend | dF: 2<br>F=8.48 | |
| + - | 7e | single factor ANOVA | legend / methods | WT: 25/3, KO: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | p = 0.0005 | legend | dF:2<br>F=13.759 | |
| + - | 7f | single factor ANOVA | legend / methods | WT: 25/3, KO: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | 20ms ISI: p = 0.00805, 40ms ISI: 0.00744, 60ms ISI: p = 0.0327 | legend | df: 23<br>F=7.69 (20ms ISI), 7.857 (40ms ISI), 4.85 (60ms ISI) | |
| + - | 7g | single factor ANOVA | legend / methods | Nrxn3SS4+: 49/4 Nrxn3SS4-: 45/4 | # of experiments | legend | error bars are mean +/- SEM | legend | frequency: p = 0.536, amplitude: p = 0.614 | legend | dF: 3<br>Freq<br>F =0.455<br>F-crit = 7.7<br>Amp<br>F = 0.297<br>F-crit = 7.7 | |
| + - | 7h | single factor ANOVA | legend / methods | Nrxn3SS4+: 24/3 Nrxn3SS4-: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | p = 0.983 | legend | dF: 23<br>F = 0.00465<br>F-crit = 7.7 | |
| + - | 7i | single factor ANOVA | legend / methos | Nrxn3SS4+: 24/3 Nrxn3SS4-: 24/3 | # of cells | legend | error bars are mean +/- SEM | legend | 60 ms ISI: p = 0.130 | legend | dF: 23<br>F = 2.37<br>F-crit = 4.05 | |
| + - | 7j | single factor ANOVA | legend / methods | control: 17/3, cre: 15/3, control +rescue: 23/3, cre +rescue: 22/3 | # of cells | legend | error bars are mean +/- SEM | legend | cre: p =0.0034; cre + Nrxn3αSS4+: p = 0.0217 | legend | dF: 14<br>F = 10.1<br>F-crit = 4.17<br>dF: 21<br>F = 5.66<br>F-crit = 4.07 | |

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| + - | 7k | single factor ANOVA | legend / methods | control: 17/3, cre: 15/3, control +rescue: 23/3, cre +rescue: 22/3 | # of cells | legend | error bars are mean +/- SEM | legend | ; cre: 20ms ISI: p = 0.000515, 40ms ISI: p = 0.0363, 60 ms ISI: p = 0.02891, + Nrxn3αSS4+: 20ms ISI: p = 0.0334, 40ms ISI: p = 0.128, 60ms ISI: p = 0.284 | legend | 40 ms ISI dF: 14 F =4.79 F-crit =4.17 F = 2.4 F-crit = 4.06 | |
| + - | 7l | single factor ANOVA | legend / methods | control: 22/3, cre: 20/3, control +GPI: 20/3, cre +GPI: 22/3 | # of cells | legend | error bars are mean +/- SEM | legend | cre: p < 0.001; + GPI-Nrxn3αSS4+: p = 0.00165 | legend | dF: 19 F = 14.9 F-crit = 4.08 F = 11.5 F-crit = 4.11 | |
| + - | 7m | single factor ANOVA | legend / methods | control: 22/3, cre: 20/3, control +GPI: 20/3, cre +GPI: 22/3 | # of cells | legend | error bars are mean +/- SEM | legend | cre: 20 ms ISI: p = 0.0006, 40 ms ISI: p = 0.0107, 60 ms ISI: p = 0.0055; + GPI-Nrxn3αSS4+: 20 ms ISI: p = 0.0231, 40 ms ISI: 0.0213, 60 ms ISI: 0.219 | legend | 40 ms ISI dF: 19 F = 7.16 F-crit = 4.08 dF: 18 F = 5.77 F-crit = 4.1 | |
| + - | 8d | single factor ANOVA | legend / methods | control: 23/3, cre: 20/3 | # of cells | legend | error bars are mean +/- SEM | legend | 10 µA: p = 0.0749; 25µA: p = 0.111; 50 µA: p = 0.00578; 75 µA: p = 0.00052; 100 µA: p = 0.0026; slope: p = 0.00370 | legend | dF: 19 F = 9.4788 | |
| + - | 8e | single factor ANOVA | legend / methods | control: 8 animals, cre: 7 animals | # of animals | legend | error bars are mean +/- SEM | legend | p = 0.0162 | legend | dF: 5 F= 8.04 | |
| + - | Supplementary 1b | Student's t-test | legend | WT: 3, KO: 3 | # of animals | legend | error bars are mean +/- SEM | legend | p<0.05 | legend | | |
| + - | Supplementary 1c | single factor ANOVA | legend | WT: 3, KO: 3 | # of animals | legend | error bars are mean +/- SEM | legend | Nrxn3a: p < 0.0001 Nrxn3b: p < 0.0001 | legend | dF: 2 F = 793 F-crit = 7.7 F = 5.51 F-crit = 7.7 | |
| + - | supplementary 2a | single factor ANOVA | legend | control: 50/4, cre: 47/4 | # of experiments | legend | error bars are mean +/- SEM | legend | Rm: p = 0.612; Cm: p = 0.646 | legend | dF: 3 Rm: F = 0.286 F-crit = 5.98 Cm F = 0.232 F-crit = 5.99 | |
| + - | Supplementary 2b | single factor ANOVA | legend | control: 40/3, cre: 38/3 | # of experiments | legend | error bars are mean +/- SEM | legend | rise: p = 0.995; decay: p = 0.861 | legend | dF: 2 Rise F < 0.0001 F-crit = 7.7 Decay F = 0.0348 F-crit = 7.7 | |

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| + - | Supplementary 2c | single factor ANOVA | legend | control 44/3, cre: 42/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mIPSCs: rise: p = 0.876; decay: p = 0.800 | legend | dF: 2<br>Rise:<br>F = 0.0274<br>F-crit = 7.7<br>Decay:<br>F = 0.0735<br>F-crit = 7.7 | |
| + - | Supplementary 2d | single factor ANOVA | legend | control: 3, cre: 3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.710 | legend | dF: 3<br>F = 2.59<br>F-crit = 4.96 | |
| + - | Supplementary 2e | single factor ANOVA | legend | WT: 33/3, KO 31/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.569; Cm: p = 0.670 | legend | dF: 2<br>Rm<br>F = 0.383<br>F-crit = 7.7<br>Cm<br>F = 0.209<br>F-crit = 7.7 | |
| + - | Supplementary 2f | single factor ANOVA | legend | WT: 35/3, KO: 36/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mEPSC frequency (p = 0.0676) and amplitude (p = 0.613). | legend | dF: 2<br>freq<br>F = 0.201<br>F-crit = 7.7<br>amp<br>F = 0.300<br>F-crit = 7.7 | |
| + - | supplementar 2g | single factor ANOVA | legend | WT: 27/3, KO: 27/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p - 0.842 | legend | dF: 2<br>F = 0.044<br>F-crit = 7.70 | |
| + - | Supplementary 2h | single factor ANOVA | legend | WT: 21/3, KO: 20/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.767 | legend | dF: 2<br>F = 0.100<br>F-crit = 7.7 | |
| + - | Supplementary 2i | single factor ANOVA | legend | WT: 9/3, KO: 9/3 | # of cells | legend | error bars are mean +/- SEM | legend | 100 ms ISI: 0.553 | legend | dF: 8<br>F = 0.367<br>F-crit = 4.49 | |
| + - | Supplementary 3a-c | single factor ANOVA | legend | control: 5, cre: 5<br>GluA2: control: 3, cre: 3 | # of experiments | legend | error bars are mean +/- SEM | legend | density (a; GluA1: p = 0.694; GluA2: p = 0.714; PSD95: p = 0.819; vGluT1: p = 0.758), size (b; GluA1: p = 0.0213; GluA2: p = 0.0424; PSD95: p = 0.188; vGluT1: p = 0.421) and intensity (c; GluA1: p = 0.579; GluA2: p = 0.267; vGluT1: p = 0.796 | legend | dF: 4<br>GluA1/GluA2/<br>PSD95/vGluT1<br>Density:<br>F = 0.163<br>F-crit = 4.84<br>F = 0.153<br>F-crit = 7.7<br>F =0.00552<br>F-crit = 4.84<br>F = 0.105<br>F-crit = 4.96<br>Size:<br>F = 8.98<br>F-crit = 4.84<br>F = 17.3<br>F-crit = 7.7<br>F = 1.98<br>F-crit = 4.84<br>F = 0.703<br>F-crit = 4.96<br>Intensity:<br>F = 0.326<br>F-crit = 4,84<br>F = 1.65<br>F-crit = 7.7<br>F = 0.0072<br>F-crit =4.84<br>F = 0.0072<br>F-crit = 4.96 | |

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| + - | Supplementary 3d | Single factor ANOVA | legend | control : 5, cre: 5 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.0110 | legend | dF: 4<br>F = 10.7<br>F-crit = 5.31 | |
| + - | supplementary 5c | single factor ANOVA | legend | Control: 19/3, cre: 23/3 | # of cells | legend | error bars are mean +/-SEM | legend | Rm: p = 0.719<br>Cm: p = 0.796 | legend | dF: 5<br>Rm:<br>F = 0.131<br>F-crit = 4.072<br>Cm<br>F = 3.22<br>F-crit = 4.072 | |
| + - | supplementary 5d | single factor ANOVA | legend | control: 10/3, cre: 6/3 | # of cells | legend | error bars are mean +/- SEM | legend | Rm: 0696<br>Cm: 0.929 | legend | dF: 5<br>Rm:<br>F = 0.161<br>F-crit = 4.96<br>Cm<br>F = 0.819<br>F-crit = 4.96 | |
| + - | supplementary 6a-c | single factor ANOVA | legend | control: 36/3, cre: 35/3 | # of experiments | legend | error bars are mean +/- SEM | legend | Puncta density (b; gephyrin: p = 0.884; vGAT: p = 0.176; vGluT1: p = 0.465), size (c; gephyrin: p = 0.262; vGAT: p = 0.170; vGluT1: p = 0.645) and intensity (d; gephyrin: p = 0.737; vGAT: p = 0.851; vGluT1: p = 0.693) | legend | dF: 2<br>Gephyrin/VGAT/VGluT1 density<br>F = 0.024<br>F-crit = 7.7<br>F = 2.96<br>F-crit = 7.7<br>F = 0.496<br>F-crit = 7.7<br>size<br>F = 1.70<br>F-crit = 7.7<br>F = 2.79<br>F-crit = 7.7<br>F = 0.0153<br>F-crit = 7.7<br>intensity<br>F = 0.129<br>F-crit = 7.7<br>F = 0.0402<br>F-crit = 7.7<br>F =0.180<br>F-crit = 7.7 | |
| + - | supplementary 6d | single factor ANOVA | legend | control: 32/3, cre: 36/3 | # of experiments | legend | error bars are mean +/- SEM | legend | input resistance: p = 0.728; capacitance: p = 0.583 | legend | dF: 2<br>Rm<br>F = 0.139<br>F-crit = 7.7<br>Cm<br>F = 0.355<br>F-crit = 7.7 | |
| + - | supplementary 6e | single factor ANOVA | legend | control: 31/3, cre: 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mEPSC rise: p = 0.816; decay: p = 0.286 | legend | dF: 2<br>Rise:<br>F = 0.614<br>F-crit = 7.7<br>decay<br>F = 1.51<br>F-crit = 7.7 | |
| + - | supplementary 6f | single factor ANOVA | legend | Nrxn3SS4+: 42/4 Nrxn3SS4-: 45/4 | # of experiments | legend | error bars are mean +/- SEM | legend | input resistance: p = 0.921; capacitance: 0.894 | legend | dF: 3<br>Rm<br>F = 0.0106<br>F-crit = 5.98<br>Cm<br>F = 0.192<br>F-crit = 5.98 | |
| + - | supplementary 6g | single factor ANOVA | legend | Nrxn3SS4+: 38/3 Nrxn3SS4-: 39/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mEPSC rise: p = 0.626; decay: p = 0.693 | legend | dF: 2<br>rise<br>F = 0.277<br>F-crit = 7.7<br>decay<br>F = 0.179<br>F-crit = 7.7 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + - | supplementary 7a | K-S test | legend | control: 26/3, cre: 27/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p < 0.0001 | legend | | |
| + - | supplementary 7b | single-factor ANOVA | legend | control: 26/3, cre: 27/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mIPSC rise: p = 0.462; decay: p = 0.991 | legend | dF: 2<br>rise<br>F = 0.660<br>F-crit = 7.7<br>decay<br>F = 0.00017<br>F-crit = 7.7 | |
| + - | supplementary 7c | single factor ANOVA | legend | control: 29/3 cre: 42/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.303 | legend | dF: 2<br>F = 1.39<br>F-crit = 7.7 | |
| + - | supplemntary 7d | K-S test | legend | WT: 38/3, KO: 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p < 0.0001 | legend | | |
| + - | supplementary 7e | single factor ANOVA | legend | WT: 38/3, KO: 33/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mIPSC rise: p = 0.939; decay: p = 0.943 | legend | dF: 2<br>rise<br>F = 0.656<br>F-crit = 7.7<br>decay<br>F = 0.0056<br>F-crit = 7.7 | |
| + - | supplementary 7f | K-S test | legend | Nrxn3SS4+: 31/3, Nrxn3SS4-: 29/3 | # of experiments | legend | error bars are mean +/- SEM | legend | p = 0.671 | legend | | |
| + - | supplementary 7g | single factor ANOVA | legend | Nrxn3SS4+: 31/3, Nrxn3SS4-: 29/3 | # of experiments | legend | error bars are mean +/- SEM | legend | mIPSC rise: p = 0.520; decay: p = 0.713 | legend | dF: 2<br>rise<br>F = 0.497<br>F-crit = 7.7<br>decay<br>F = 0.155<br>F-crit = 7.7 | |

## Representative figures

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

   If so, what figure(s)?

   Yes. ICC for Figures 3a, 3e and 6b. Western blot for Supplementary figure 1b.

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 #)?

   yes, each ICC experiment was repeated at least 3 times independently with at least 10-15 cells per condition analyzed. Western blots were performed from whole cell lysates harvested from at least 3 animals. These numbers are reported in the bars for the representative bar graphs.

## 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. Our sample sizes were determined based on historical requirements for each experiment - at least 3 independent cultures for electrophysiology and ICC and at least 3 animals for behavior and acute slice electrophysiology.
   This justification is found in the methods section under "statistics."

| | |
|---|---|
| 2. Are statistical tests justified as appropriate for every figure?<br>Where (section, paragraph #)? | Yes. The statistics used are found in each figure legend and the justification is found in the methods section under "statistics." |
| a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined? | yes. |
| 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 (section, paragraph #)? | From a historical standpoint, we assume normality in all of our experiments and our statistics make the assumption. For all 2 bar bar-graphs, we have plotted individual data points. We have stated our assumption in the "statistics" paragraph in the methods section. |
| 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 (section, paragraph #)? | |
| d. Are tests specified as one- or two-sided? | yes - see statistics |
| e. Are there adjustments for multiple comparisons? | no |
| 3. Are criteria for excluding data points reported?<br>Was this criterion established prior to data collection?<br>Where is this described (section, paragraph #)? | no - we follow the traditional methods of data point exclusion that are based on changes in cell health at the time of recording and are thus never analyzed. |
| 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>Where does this appear (section, paragraph #)? | yes, statistics |
| 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, state so.<br>Where (section, paragraph #)? | yes. statistics |
| 6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?<br>Where (section, paragraph #)? | yes, under animals. |
| 7. Is the species of the animals used reported?<br>Where (section, paragraph #)? | yes, animals |
| 8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?<br>Where (section, paragraph #)? | yes, animals. |

9. Is the sex of the animals/subjects used reported?

   Where (section, paragraph #)?

   yes, figure legends, main figures and methods - "buried food finding"

10. Is the age of the animals/subjects reported?

    Where (section, paragraph #)?

    yes - main text, main figures, figure legends

11. For animals housed in a vivarium, is the light/dark cycle reported?

    Where (section, paragraph #)?

    yes, mouse husbandry

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?

    Where (section, paragraph #)?

    no

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

    Where (section, paragraph #)?

    We did not perform a circadian rhythm experiments so all experiments are performed during the light cycle.

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 #)?

       n/a

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

    Where (section, paragraph #)?

    none

    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

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

Where does this appear (section, paragraph #)?

citation - routinely used by multiple laboratories in the field.

2. Cell line identity

a. Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by ICLAC and NCBI Biosample?

Where (section, paragraph #)?

N/A

b. If yes, include in the Methods section a scientific justification of their use--indicate here in which section and paragraph the justification can be found.

c. For each cell line, include in the Methods section a statement that specifies:

- the source of the cell lines
- have the cell lines been authenticated? If so, by which method?
- have the cell lines been tested for mycoplasma contamination?

Where (section, paragraph #)?

## 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 #)?

N/A

## 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.

N/A

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.

n/a

## 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