Supplementary materials

Amygdala GluN2B-NMDAR dysfunction is critical in abnormal aggression of neurodevelopmental origin induced by St8sia2 deficiency

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Supplementary Materials

**Genotyping.** All mice were genotyped before and after behavioral testing by polymerase chain reaction (PCR). DNA extracted from ear punches or phalanges with Accustart II protocol and submitted to PCR using the Supermix enzyme of QuantaBio (MA, USA), with use of the following primers: Forward primers for WT and KO: 5′-GGATAGTTACTGGGGTCAAGG-3’, and 5′-GCAGGTCGAGGGACCTAATA-3’, respectively; Reverse primer for both: 5’-AGACAGGAGGGTTTCGGAAT-3’.

**Behavioral analyses.** Behavioral characterization was performed in five cohorts of animals, (see Results section for more details). For the pERK experiments, cannulated and silenced animals, the behavioral schemes are detailed in Results section. Key behavioral experiments (resident-intruder and cue fear conditioning) were performed twice, and all attempts of replication were successful.

**Auditory capacity.** Startle response to acoustic stimuli was measured by a movement sensor with the SR-LAB™ system (San Diego SA). After 3 days of habituation to the recording chamber, consisting of a tubular enclosure, with a background noise of 65 dB, animals were exposed to tones of different intensity, from 70 dB to 120 dB, for a duration of 40 ms.

**Sensitivity to foot shock.** This test was performed according to a previously published method. Mice were individually placed in the conditioning chamber to receive 1-s shocks of gradually increasing current intensity by an increment of 0.01 mA, which typically elicited progressively increasing behavioral responses (flinching, 0.05–0.1 mA; jumping, 0.1–0.3 mA). The interval
between shocks was 20 s. The minimum current intensities required to elicit flinching and jumping in mice were measured.

_Elevated plus maze._ The maze consisted of two opposite open arms and two opposite closed arms (30 × 5 × 14 cm) arranged at right angles, and with a common central platform (5 × 5 cm) that gave access to all arms. Lighting was maintained at 15-16 lux on the open arms and 5-7 lux in the closed. The mouse was gently placed in a closed arm facing the wall and allowed to move undisturbed for 5 min. After each trial, the arms were cleaned with 10% ethanol and dried. A digital camera was mounted above the maze. Images were captured at a rate of 5 Hz and transmitted to a PC running the Ethovision (v. 3.1; Noldus Technology, Wageningen, The Netherlands) tracking system. The percentage of time spent in the open arms was taken as an indicator of anxiety. The total distance traveled in the entire surface (arms and central platform) of the maze provided a measure of general locomotor activity.

_Open Field._ Animals were placed in a rectangular arena (50 x 50 cm) and left to freely explore for 20 min. The light was adjusted to a level of 8-10 lux in the center of the arena. Video tracking of the animal's location was performed by a camera fixed above the arena, and images were transmitted at 5 Hz to a PC running Ethovision tracking system for further processing. The percentage of time spent in the center was taken as an indicator of anxiety.

_Marble-burying test._ To test the spontaneous burying behavior as an indication of anxiety-like behavior, mice were placed individually in a cage measuring 35 × 17 × 12 cm (L x W x H), containing bedding of 5 cm depth, with 12 glass marbles (2-3 cm diameter) evenly spaced on the
surface of the bedding. Testing was conducted for 20 min, and buried marbles (i.e. at least one-half covered with bedding) were counted at 1 min bins.

**Locomotor activity.** Animals were placed in individual new cages for a test duration of 24 hours. After 2 hours of habituation, locomotor activity was assessed by the number of laser breaks in PhenoMaster system (TSE Systems GmbH, Germany).

**Social preference test.** The sociability test was carried out in a three-chambered box (the center compartment was $20 \times 35 \times 35$ cm and the left and right compartments were $30 \times 35 \times 35$ cm). The dividing walls had retractable doorways that allowed access to each chamber. The test mouse was habituated to explore the entire apparatus for 10 min during 2 days. Each of the two side chambers contained an empty wire cage. The wire cages were 10 cm in height, with a bottom diameter of 9 cm and each bar spaced 1 mm apart. Juvenile mice (23 day-old C57BL/6J male mice) were habituated for 10 min to the wire cage for 2 days. In the third day (after habituation sessions), a test mouse was placed in the center compartment and allowed to explore the entire apparatus for 10 min. A juvenile mouse was enclosed in one of the wire cages, which was placed in one of the two sides of the social test box during the 10-min session. A dummy black mouse was placed in the other wire cage on the other side of the box. The time spent sniffing each wire cage was video-recorded and manually scored to evaluate the level of preference for the unfamiliar mouse compared with the object.

**Immunohistochemistry**

*GABAergic and glutamatergic immunostaining.* Sections were labeled with a goat anti-GAD67 (abcam ab80589, 1/500) and Rabbit anti-CamkII (abcam ab52476, 1/1000) to stain GABAergic
and glutamatergic neurons, respectively. Secondary antibodies were anti-Rabbit-alexa-488 and anti-goat-alexa-568 (abcam ab150073 and ab175474 respectively, 1/1000).

**Quantification.** Images were taken with confocal microscope (Zeiss LSM-700) using a 20X objective. Sample images were captured from different areas at the same coordinates for each animal using the mouse stereotaxic atlas as a reference. Quantification was performed on original, unenhanced images only. Quantification of immunofluorescence LSM images were stitched together using the grid stitching plug-in for FIJI. The background intensity of each channel was measured at five different random areas and averaged to generate a mean background that was subtracted from each channel. Cells were delineated using a Triangle threshold to label only those stained with NeuN within 200-1000 pixels. The number of labeled cells that were co-labeled with phospho-ERK and the antibody of interest was counted and converted to a percentage of the total number of NeuN-stained cells for each section. Analyses were made blind to experimental conditions. GluN2A or GluN2B fluorescence intensities were measured in NeuN-positive cells.

**Electrophysiological recordings.**

The experiments described here were performed in 5-8 week-old mice. Current-clamp recordings were performed with pipettes (2-3 MΩ) filled with an intracellular solution containing (in mM): 130 KGlucanate, 10 KCl, 10 HEPES, 10 phosphocreatine, 0.2 EGTA, 4 Mg-ATP, 0.2 Na-GTP, (290-300 mOsm, pH 7.2-7.3). Resting membrane potential ($V_{rmp}$) was measured with no current injection, within 1 min from the establishment of the whole-cell configuration. Neuronal firing was induced by 2-s long depolarizing current steps (25 pA increments) from a membrane potential of -60 mV.
Miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of tetrodotoxin (1 µM), DNQX (10 µM) and D,L-APV (100 µM). Pipettes (2-3 MΩ) were filled with (in mM): 120 CsCl, 10 HEPES, 8 NaCl, 10 phosphocreatine, 0.2 MgCl₂, 0.2 EGTA, 2 Mg-ATP, 0.2 Na-GTP (290-300 mOsm, pH 7.2-7.3). Recordings were conducted at room temperature. Spontaneous events were acquired for 5 min at -60 mV, starting from >5 min after the establishment of the whole-cell configuration, to allow the diffusion of the intracellular solution. The contribution of perisomatic inhibition was evaluated by selecting the events with fast rise time (< 3 ms; this limit was chosen based on the cumulative distributions of all events, that display a relative peak below this value).³,⁴

To elicit asynchronous EPSCs (aEPSCs) at cortical inputs, extracellular CaCl₂ was replaced by equimolar SrCl₂. Cells were patched with the CsGluconate-based solution and held at -80 mV to amplify AMPAR-mediated currents. Only aEPSCs occurring between 20-500 ms after the onset of the first evoked EPSC were considered.

For detection of both mIPSCs and aEPSCs, traces were filtered at 1 kHz and analysed using the MiniAnalysis Program with a threshold corresponding to 2 times the baseline noise (Synaptosoft Inc., Decatur, USA).

**Statistics.** The number of animals/recordings per group was in agreement with the resource equation method to determine the sample size⁵ and was guided by previous work of the lab with the same animal model. Power analysis was not performed, as animals typically underwent different experimental series (see Supplementary Figure 2), yielding multiple parameters with a group difference that was not known a priori. All groups consisted of males and were always matched per age among compared groups. For data exclusion, Grubbs' test for outliers was performed in GraphPad with an alpha level of 0.05, resulting in the exclusion of 1 animal from
the cue fear learning series, 1 animal from mRNA analysis, and of 1 datapoint in the LTP control series in WT animals. Electrophysiological data are presented with n numbers representing recordings pooled from at least 3 animals per series, to take into account inter-animal variability. Data from key experiments (such as AMPA/NMDA ratio, input-output curves, and LTP) were obtained from at least 5 animals. The animals used in this study were in general randomly distributed. Before any experiment, a number was allocated to each animal, and after all behavioral characterization and analyses, the genotype was uncovered. For the experiment involving treatment, trait anxiety of animals was measured as described in the methods, and subjects were assigned to control and drug groups in order to obtain groups with comparable mean anxiety. Data analysis was performed either by a researcher that was blind to genotype and treatment group or by an automated software.

Sample sizes are indicated in each Figure. The choice of parametric or nonparametric tests was based on normal distribution of the data (Shapiro-Wilk normality test). Unpaired two-tailed t test or Mann-Whitney test were used to compare sets of data obtained from independent groups of animals (WT vs. KO; Ct vs. sh; Ct vs stress). For two factors comparison, two-way analysis of variance (ANOVA) was used to analyze the effect of genotype, as well as treatment and interaction when applicable. Additional within-subject factors (e.g. CS-US, CS) were also included as determined by the nature of the dependent variables under consideration. Supplementary restricted analyses were also conducted to assist data interpretation whenever appropriate. Bonferroni and Fisher’s LSD post hoc tests were used. All statistical analyses were performed with Prism 7 (Graphpad software Inc., San Diego, CA), except for MANOVA which were performed with SPSS 11 (IBM, San Francisco). Electrophysiological data were analysed with Clampfit 10 (Molecular Devices), Igor 6 (Wavemetrics) and MiniAnalysis (Synaptosoft Inc.). Detailed parameters from statistical tests are reported in Figure legends or in the
Supplementary Table 2, with the second decimal rounded to the nearest value. Statistical significance was set at $\alpha < 0.05$.

References

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Supplemental Figures

Supplementary Figure 1. Postnatal expression of Polysialic acid and its regulators in wild-type and St8sia2/- mice. (a) Quantification of mRNA levels of St8sia2, St8sia4 and NCAM at postnatal day 5 (P5), P15 and adulthood (P90) in wild-type mouse brain regions (n = 7 for P5, n = 6 for P15 and n = 7 for P90). (b) Quantification of Polysialic acid (PolySia) at different time points of development and adulthood in amygdala (AMY), hippocampus (HPC) and prefrontal cortex (PFC) (n=6 for P5, 7 for P15 and 8 for P90). (c) Quantification of mRNA levels of St8sia2 (left) and St8sia4 (right) in the amygdala of P4 St8sia2/- (KO) mice compared to control (WT) littermates (unpaired t test, t8=4.095, p=0.0035 for St8sia2; t8=2.001, p=0.081 for St8sia4). **p<0.01.
Supplementary Figure 2. Timelines of different experiments aiming at a behavioral characterization of St8sia2-/- mice. (a) Cohort 1 underwent tests for anxiety-related behavior (EPM: Elevated Plus Maze, OF: Open Field), resident-intruder (RI), Fear conditioning and Startle response. (b) Cohort 2 was tested for anxiety and social interaction with female. (c-d) Cohort 3 & 4 were tested for social interaction with juvenile and for pain sensitivity to foot shock, respectively. (e) Cohort 5 was tested for locomotor activity and gene expression.
Supplementary Figure 3. Behavioral and immunohistochemical characterization of St8sia2-/mice that complements Figure 1. (a) Social investigation of the intruder in the resident-intruder test. (b) Social investigation of juvenile or female conspecifics. (c) Localization and quantification of pERK activation after resident-intruder test in medial amygdala (pd: posterodorsal, pv: posteroventral) (unpaired t test, t_{14}=3.151, p=0.0062 for pd; t_{14}=1.576, p=0.13 for pv). (d-e) Characterization of abnormal aggression phenotype in the cohort in which pERK was quantified (d), including (in e) latency to attack (unpaired t test: t_{14}=3.089, p=0.009), duration of attacks (t_{14}=2.478, p=0.03), bites in vulnerable body parts (Mann-Whitney test: U=4, p=0.007) and number of attacks while the intruder is displaying submissive postures (Mann-Whitney test: U=5, p=0.012). (f) Localization and quantification of pERK activation after resident-intruder test in prefrontal cortex (PFC, IL: infralimbic, PL: prelimbic, Cg: cingulate). (g) Localization and quantification of pERK activation after resident-intruder test in ventromedial hypothalamus (VMH; dm: dorsomedial nucleus; vl: ventrolateral nucleus) (unpaired t-test: t_{14}=2.034, p=0.069 for dm; t_{14}=0.359, p=0.72 for vl). (h) Corticosterone levels measured just after resident-intruder, and 30 min later. (i) Auditory capacity assessed by startle responses to acoustic stimuli. (j) Pain sensitivity upon foot shock measured by flinching (left) and jumping (right). (k) Acoustic fear conditioning protocol (top) and corresponding freezing levels (bottom; two-way ANOVA: main effect of CS-US, F_{2,22}=96.58, p<0.0001; no effect of genotype and no interaction) used to investigate pERK activation. CS: conditioned stimulus (tone); US: unconditioned stimulus (foot shock); Hab.: habituation. (l-m) Assessment of pERK activation in the PFC (l) and in the auditory cortex (m) following training in the acoustic fear conditioning task. (n-p) Anxiety-related behaviors assessed in the elevated plus maze (n; unpaired t test, t_{24}=3.631, p=0.0006), open field (o; unpaired t test, t_{24}=3.11, p=0.0048) and marble burying test (p; two-way ANOVA: main effect of genotype, F_{1,15}=8.25, p=0.0018, Bonferroni post hoc tests). (q) Locomotor activity measured in the home cage (two-way ANOVA, genotype factor: F_{1,11}=3.3, p=0.72). Results are given as mean ± s.e.m. $p<0.07; *p<0.05; **p<0.01; ***p<0.001 vs WT.
Supplementary Figure 4. Cellular composition and synaptic structure in the lateral amygdala that complement Figure 2. (a) Quantification of the GABAergic and glutamatergic neurons in the basolateral amygdala (right), with representative images (left) of GABAergic immunostaining in the amygdala of St8sia2-/− (KO) and wild-type (WT) mice (LA: lateral, BA: basal, CeA: central amygdala). (b) Morphological analyses of GFP-labeled neurons, with location of the injection site of GFP-lentivirus, with zoom in the lateral amygdala part analyzed. (c) Morphological analyses of electron microscopy, with location of the region analyzed (yellow rectangle). Results are given as mean ± s.e.m.
Supplementary Figure 5. Electrophysiological analyses in St8sia2/- and wild-type mice that complement Figure 3 and 4. (a) Frequency of firing elicited in LA pyramidal neurons by increasing somatic depolarizing currents did not differ between genotypes (two-way ANOVA, genotype: F_{1,20}=0.229, p=0.64). Color-coded traces on the right are representative voltage responses to steps of 25, 75 and 125 pA. (b) LA neurons from wild-type (WT) and St8sia2/- (KO) mice did not differ for resting membrane potential (V_{rm}; unpaired t test: t_{20}=0.2, p = 0.84), firing threshold (t_{20}=0.64, p=0.53), and rheobase (Mann-Whitney test, U=46.5, p=0.35). (c) Cumulative distributions of peak amplitude, rise time and inter-event interval of mIPSCs, showing no difference between genotypes (Kolmogorov-Smirnov test of cumulative distributions: peak, D=0.055, p=0.59; rise time, D=0.042, p=0.99; inter-event interval, D=0.017, p=0.34). Color-coded example traces are shown at the top. Bar graphs underneath the distributions represent mean values of the three parameters calculated from all events (all) and from events with fast rise time (<3 ms), reflecting mainly perisomatic inputs (peris.)(unpaired t test: p>0.05 for all parameters). (d-e) Paired-pulse ratio (PPR) of synaptic currents were measured throughout development at cortical (d) and subcortical (e) inputs (p>0.05 at all age windows). (f) Left, examples (superimposition of three single traces) of asynchronous release evoked at cortical inputs in the presence of Sr^{2+} (2 mM). Shaded area indicates the time window in which asynchronous excitatory postsynaptic currents (aEPSCs) were analysed. Right, cumulative distributions and mean peak amplitude (insets) of aEPSCs assessed in both genotypes (Kolmogorov-Smirnov test of cumulative distributions, D=0.029, p=0.73; Mann-Whitney test on mean peak values, U=14, p=0.36). (g) Hebbian LTP in WT mice was prevented by NMDAR blockade with 100 µM D,L-APV. (h) Summary of LTP recordings in WT and St8sia2/- mice (One-way ANOVA: F_{4,33}=6.488, p=0.0006, followed by Fisher’s LSD test). Results are given as mean ± s.e.m. *p<0.05; **p<0.01.
**Supplementary Figure 6.** Behavioral effects of DCS injection intra-amygdala and in the lateral ventricle that complement Figure 5. (a) Protocol for both intracerebroventricular (icv) and intra-amygdala infusion of DCS (OF: Open-Field, EPM: Elevated Plus Maze, SP: Social preference, RI: resident-intruder). (b) Effect of intra-amygdala DCS infusion (20 min before acquisition of acoustic fear conditioning) on freezing behavior during training (left panel) and during the memory test (two-way ANOVA: main effect of genotype, F$_{1,24}=13.75$, p=0.001). (c) Representative images of pERK (red) and NeuN (green) colabeling in vehicle (veh) and DCS-infused animals (insets show pERK labeling of lateral amygdala, with activated neurons indicated by yellow arrows). Scale bars, 100 µm. (d) Quantification of pERK activation in LA (two-way ANOVA: effect of treatment: F$_{1,26}=11.94$, p=0.003). (e-f, h-i) Prior to DCS experiments, anxiety-related behavior was assessed after recovery from cannulation (e, h) in the OF test to balance groups assigned to vehicle (veh) and DCS with equivalent *a priori* levels of anxiety. (e, two-way ANOVA, main effect of genotype for intra-amygdala: F$_{1,26}=5.42$, p=0.029). After DCS or veh infusion, anxiety was tested in the elevated plus maze (EPM) (f, two-way ANOVA, main effect of genotype: F$_{1,26}=7.84$, p=0.011; effect for DCS: F$_{1,26}=2.81$, p=0.098; i, two-way ANOVA: interaction factor “treatment x genotype”: F$_{1,27}=3.51$, p=0.05). (g, j) Social Preference (SP, g, for intra-amygdala infusion, two-way ANOVA reveals a main effect of genotype only: F$_{1,26}=9.65$, p=0.0056). Results are given as mean ± s.e.m. *p=0.098, *p<0.05, **p<0.01, ***p<0.001.
Supplementary Figure 7. Amygdala-restricted silencing of St8sia2 during early development that complements Figure 6. (a) Design of the plasmid that was transfected in vivo through intra-amygdala infusion on postnatal day 2 (P2) in WT mice. (b) Quantification of mRNA levels of St8sia4 in amygdala 2 days after injection of sh-St8sia2 plasmid (unpaired t-test: $t_8=0.024$, $p=0.98$). (c) Quantification of mRNA levels of St8sia2 and Polysialic acid at P4 in the prefrontal cortex (PFC) of control (Ct) and silenced (sh) animals. (d) Body weight of adult animals (3 months old). (e) Anxiety-related behaviors assessed by the elevated plus maze. (f) Social preference test. Results are given as mean ± s.e.m. *$p<0.05$; **$p<0.001$. 
**Supplementary Table 1.** Sequence of primers used for RT-qPCR. In green, the reference genes.

| Name      | RefSeq    | Primer Forward       | Primer Reverse       |
|-----------|-----------|----------------------|----------------------|
| TBP       | NM_013684.3 | CTGGAATTGTACCGCAGCTT | CAGTTGTCCCGTGCTCTCTT |
| EEF1α1    | NM_010106.2 | TTCACCTTGACCCGCTCTGGC | CTTCTTGTACCCAGCTTTGATGA |
| GAD67     | NM_008077.4 | CTTCCTTGACCCGCTCTGGC | CAGTTGTCCCGTGCTCTCTT |
| PV        | NM_013645.3 | TTTGCTGTTCAGAAGCTCTTG | AAGCCCTTCAGAATGGGACCC |
| SST       | NM_009215.1 | GAGATATGGCTGCTGAGCTGC | AAGTACTGAGCAGGTCTCTGG |
| vGAT      | NM_009508.2 | CACTTATTATACGGCAAGCC | GCAAGCATATGCCCCTGCTAT |
| GABAα1    | NM_010250.5 | ACACCAGTAGGCTGTGGGCT | TGGCTAGACAGAGCACGGG |
| GABAα2    | NM_008066.3 | GGAAGCTACGTACGTAACACG | TGGCTAGACAGAGCACGGG |
| GABAγ2    | NM_009508.2 | ACATTCACCTTGCTCCTGGT | GTGGCTAGACAGAGCACGGG |
| Nlgn2     | NM_198862.2 | CCAAGTGGGCTGTGACCCA | CCAAGTGGGCTGTGACCCA |
| vGlut1    | NM_182993.2 | TTGTCGCTGCTGACGCTCT | CCAAGTGGGCTGTGACCCA |
| vGlut2    | NM_008053.3 | GACTATGCGCAATCCGCTG | CCAAGTGGGCTGTGACCCA |
| GluN1     | NM_008169.2 | TGGTACCCATGTCACTCGA | CCAAGTGGGCTGTGACCCA |
| GluN2A    | NM_008170.2 | AAGATGCTACGTTACGATGT | CCAAGTGGGCTGTGACCCA |
| GluN2B    | NM_008171.3 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| GluN2C    | NM_010350.2 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| GluN2D    | NM_008172.2 | GCTACCTACGTGACGCTG | CCAAGTGGGCTGTGACCCA |
| GluN3A    | NM_001033351.2 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| GluA1     | NM_008165.4 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| GluA2     | NM_001083806.1 | CCAAGTGGGCTGTGAGCTT | CCAAGTGGGCTGTGACCCA |
| GluA3     | NM_016886.4 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| mGlur1    | NM_016976.3 | AAGATGCTACGTTACGATGT | CCAAGTGGGCTGTGACCCA |
| mGlur2    | NM_001160353.1 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| mGlur5    | NM_001143834.1 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| mGlur7    | NM_177328.3 | AAGATGCTACGTTACGCTG | CCAAGTGGGCTGTGACCCA |
| Nlgn1     | NM_001163387.1 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| St8sia2   | NM_009181.2 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| St8sia4   | NM_009183.2 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
| NCAM      | NM_001081445.1 | TGGTACCGCTGCTGAGCTT | CCAAGTGGGCTGTGACCCA |
### Supplementary Table 2. Extensive statistical analyses.

| Paradigm               | Measurement | Statistical Test                                      | Comparison                          | Statistics   | p   | Figure |
|------------------------|-------------|-------------------------------------------------------|-------------------------------------|--------------|-----|--------|
| Fear conditioning      | Freezing in training | 2-way ANOVA, with repeated measures for CS-US    | Factor 1 genotype interaction       | $F_{1,24}=0.27$ | 0.60 | 1e     |
|                        | Freezing in tone test | 2-way ANOVA, with repeated measures for CS       | Factor 2 CS interaction           | $F_{2,48}=0.61$ | 0.54 |        |
| pERK activation after Fear | CeA    | unpaired t test                                 | WT vs KO                           | $t_{11}=0.24$ | 0.81 | 1g     |
| mRNA level             | GAD67      | unpaired t test                                   | WT vs KO                           | $t_{11}=0.97$ | 0.35 |        |
|                        | PV         | unpaired t test                                   | WT vs KO                           | $t_{11}=1.54$ | 0.15 |        |
|                        | SST        | unpaired t test                                   | WT vs KO                           | $t_{11}=1.37$ | 0.19 |        |
|                        | vGAT       | unpaired t test                                   | WT vs KO                           | $t_{11}=0.72$ | 0.5  |        |
|                        | GABAα1     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.12$ | 0.91 |        |
|                        | GABAα2     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.76$ | 0.46 |        |
|                        | GABAγ2     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.19$ | 0.84 |        |
|                        | Nlgn2      | unpaired t test                                   | WT vs KO                           | $t_{11}=0.23$ | 0.83 |        |
|                        | vGlut1     | unpaired t test                                   | WT vs KO                           | $t_{11}=1.07$ | 0.31 |        |
|                        | vGlut2     | unpaired t test                                   | WT vs KO                           | $t_{11}=1.79$ | 0.26 |        |
|                        | GluN1      | unpaired t test                                   | WT vs KO                           | $t_{11}=1.39$ | 0.19 |        |
|                        | GluN2C     | unpaired t test                                   | WT vs KO                           | $t_{11}=1.16$ | 0.27 |        |
|                        | GluN2D     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.16$ | 0.88 |        |
|                        | GluN3A     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.45$ | 0.42 |        |
|                        | GluA1      | unpaired t test                                   | WT vs KO                           | $t_{11}=0.17$ | 0.87 |        |
|                        | GluA2      | unpaired t test                                   | WT vs KO                           | $t_{11}=0.31$ | 0.76 |        |
|                        | GluA3      | unpaired t test                                   | WT vs KO                           | $t_{11}=0.03$ | 0.97 |        |
|                        | mGluR1     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.68$ | 0.51 |        |
|                        | mGluR2     | unpaired t test                                   | WT vs KO                           | $t_{11}=1.34$ | 0.21 |        |
|                        | mGluR5     | unpaired t test                                   | WT vs KO                           | $t_{11}=1.4$  | 0.19 |        |
|                        | mGluR7     | unpaired t test                                   | WT vs KO                           | $t_{11}=0.08$ | 0.93 |        |
| Protein expression     | GluN2A - LA | unpaired t test                                  | WT vs KO                           | $t_{22}=0.81$ | 0.45 | 2b     |
|                        | GluN2A - BA | unpaired t test                                  | WT vs KO                           | $t_{22}=0.79$ | 0.46 |        |
|                        | GluN2A - CeA | unpaired t test                              | WT vs KO                           | $t_{22}=0.075$ | 0.94 |        |
|                        | GluN2B - BA | unpaired t test                                  | WT vs KO                           | $t_{22}=1.67$ | 0.11 |        |
|                        | GluN2B - CeA | unpaired t test                             | WT vs KO                           | $t_{22}=2.11$ | 0.12 |        |
| AMPA/NMDA ratio at subcortical inputs | 1-2 weeks | unpaired t test | WT vs KO | t_{23}=0.427 | 0.67 |
|--------------------------------------|-----------|----------------|---------|-------------|-----|
|                                      | 4-5 weeks | Mann-Whitney test | WT vs KO | U=18 | 0.7 |
|                                      | 8-11 weeks | Mann-Whitney test | WT vs KO | U=13 | 0.18 |
|                                      | 11-18 weeks | unpaired t test | WT vs KO | t_{15}=0.39 | 0.69 |

| Input-output curves | NMDA-currents at cortical inputs | 2-way ANOVA | Factor 2 stimulation | F_{7,98}=135.7 | <0.0001 |
|---------------------|----------------------------------|-------------|----------------------|----------------|----------|
|                     | AMPA-currents at cortical inputs | 2-way ANOVA | Factor 1 genotype | F_{1,14}=0.092 | 0.76 |
|                     |                                   |             | Factor 2 stimulation | F_{7,98}=65.8 | <0.0001 |
|                     |                                   |             | interaction | F_{7,98}=0.064 | 0.999 |
|                     | NMDA-current at subcortical inputs | 2-way ANOVA | Factor 1 genotype | F_{1,14}=0.096 | 0.76 |
|                     |                                   |             | Factor 2 stimulation | F_{6,84}=54.16 | <0.0001 |
|                     |                                   |             | interaction | F_{6,84}=0.33 | 0.917 |
|                     | AMPA-currents at subcortical inputs | 2-way ANOVA | Factor 1 genotype | F_{1,14}=0.386 | 0.54 |
|                     |                                   |             | Factor 2 stimulation | F_{6,84}=90.2 | <0.0001 |
|                     |                                   |             | interaction | F_{6,84}=0.176 | 0.982 |

| Resident-intruder | latency to attack | 2-way ANOVA | Factor 1 genotype | F_{1,24}=3.67 | 0.067 |
|                   |                   |             | Factor 2 treatment | F_{1,24}=0.72 | 0.41 |
|                   |                   |             | interaction | F_{1,24}=1.15 | 0.29 |
|                   | Number of vulnerable bites | 2-way ANOVA | Factor 1 genotype | F_{1,24}=9.39 | 0.0064 |
|                   |                   |             | interaction | F_{1,24}=2.84 | 0.11 |
|                   | Number of attacks while intruder submissive | 2-way ANOVA | Factor 1 genotype | F_{1,24}=6.17 | 0.023 |
|                   |                   |             | interaction | F_{1,24}=3.45 | 0.079 |

| Fear conditioning | Freezing in training | MANOVA | CS | F_{2,23}=43.5 | p<0.0001 |
|                   | MANOVA, Between Subjects effect | Factor 1 genotype | F_{1,24}=1.31 | 0.26 |
|                   |                               | Factor 2 treatment | F_{1,24}=0.56 | 0.46 |
|                   |                               | interaction | F_{1,24}=1.85 | 0.19 |
|                   | Freezing in tone test | MANOVA | CS | F_{2,23}=0.31 | 0.74 |
|                   | MANOVA, Between Subjects effect | Factor 1 genotype | F_{1,24}=6.23 | 0.02 |
|                   |                               | Factor 2 treatment | F_{1,24}=2.39 | 0.14 |

| Resident-intruder | latency to attack | 2-way ANOVA | Factor 1 genotype | F_{1,26}=0.51 | 0.48 |
|                   |                   |             | Factor 2 treatment | F_{1,26}=1.96 | 0.18 |
|                   |                   |             | interaction | F_{1,26}=1.76 | 0.2 |
|                   | Number of vulnerable bites | 2-way ANOVA | Factor 1 genotype | F_{1,26}=4.5 | 0.051 |
|                   |                   |             | Factor 2 treatment | F_{1,26}=11.54 | 0.004 |
| Fear conditioning | Number of attacks while intruder submissive | 2-way ANOVA | Factor 1 genotype | F<sub>1,26</sub>=0.68 | 0.42 | interaction | F<sub>1,26</sub>=1.22 | 0.29 |
|---|---|---|---|---|---|---|---|---|
| Freezing in training | MANOVA | CS | Factor 1 genotype | F<sub>1,26</sub>=0.22 | 0.64 | Factor 2 treatment | F<sub>1,26</sub>=0.096 | 0.76 |
| | MANOVA, Between Subjects effect | Factor 1 genotype | F<sub>1,26</sub>=0.123 | 0.73 |
| Freezing in tone test | MANOVA | CS | Factor 1 genotype | F<sub>1,26</sub>=2.78 | 0.15 | Factor 2 treatment | F<sub>1,26</sub>=1.38 | 0.25 |
| | MANOVA, Between Subjects effect | Factor 1 genotype | F<sub>1,26</sub>=2.78 | 0.15 |
| Gene expression | St8sia4 | unpaired t test | Ct vs stress | t<sub>17</sub>=1.772 | 0.136 |
| Resident-intruder latency - trial 1 | unpaired t test | Ct vs sh | t<sub>15</sub>=1.06 | 0.35 |
| attacks while submissive | Mann-Whitney test | Ct vs sh | U=12 | 0.26 |
| Fear conditioning | Freezing in training | 2-way ANOVA, with repeated measures for CS-US | Factor 1 genotype | F<sub>1,15</sub>=0.34 | 0.56 | interaction | F<sub>2,30</sub>=0.61 | 0.55 |
| Freezing in tone test | 2-way ANOVA, with repeated measures for CS | Factor 2 tones | F<sub>2,30</sub>=1.42 | 0.26 | interaction | F<sub>2,30</sub>=0.42 | 0.69 |
| Gene expression | St8sia4 | unpaired t test | Ct vs stress | t<sub>11</sub>=0.019 | 0.98 |
| NCAM | unpaired t test | Ct vs stress | t<sub>11</sub>=0.12 | 0.9 |
| GluN2A | unpaired t test | Ct vs stress | t<sub>10</sub>=0.71 | 0.5 |

### Supplementary Figures

| RI | sniffing time | unpaired t test | WT vs KO | t<sub>24</sub>=0.04 | 0.97 | S3a |
|---|---|---|---|---|---|---|
| Social interaction sniffing juvenile | unpaired t test | WT vs KO | t<sub>15</sub>=0.1 | 0.91 | S3b |
| sniffing female | unpaired t test | WT vs KO | t<sub>15</sub>=0.69 | 0.49 |
| pERK activation after RI | unpaired t test | WT vs KO | t<sub>14</sub>=1.42 | 0.18 | S3f |
| IL | | | t<sub>14</sub>=0.036 | 0.97 |
| PL | | | t<sub>14</sub>=0.036 | 0.97 |
| Cg | | | t<sub>14</sub>=0.61 | 0.55 |
| IL - layer II | | | t<sub>14</sub>=1.55 | 0.14 |
| PL - Layer II | | | t<sub>14</sub>=1.04 | 0.31 |
| Cg - Layer II | | | t<sub>14</sub>=0.48 | 0.64 |
| CORT after RI | unpaired t test | WT vs KO | t<sub>14</sub>=0.61 | 0.547 | S3h |
| +0 min | | | t<sub>14</sub>=0.23 | 0.81 |
| +30 min | | | t<sub>14</sub>=0.49 | 0.48 |
| Audition Startle response | 2-way ANOVA, with repeated measures for dB | Factor 1 genotype | F<sub>1,24</sub>=0.49 | 0.48 | S3i |
| | | Factor 2 dB | F<sub>10,24</sub>=48.83 | p<0.0001 |
|                |                        |                      | interaction | F_{10,240}=0.94 | 0.49 | S3j |
|----------------|------------------------|----------------------|-------------|-----------------|------|-----|
| Pain sensitivity | Flinching unpaired t test |                     | WT vs KO   | t_{11}=0.72     | 0.48 | S3k |
|                | Jumping unpaired t test |                     | WT vs KO   | t_{11}=0.30     | 0.76 |     |
| Fear conditioning | Freezing in training 2-way ANOVA, with repeated measures for CS-US | Factor 1 genotype interaction | F_{1,1}=0.71 | 0.42 | S3l |
| pERK activation after fear | CeA unpaired t test |                     | WT vs KO   | t_{11}=0.24     | 0.81 |     |
|                | IL unpaired t test     |                     | WT vs KO   | t_{11}=1.1      | 0.31 |     |
|                | PL unpaired t test     |                     | WT vs KO   | t_{11}=0.14     | 0.89 |     |
|                | Cg unpaired t test     |                     | WT vs KO   | t_{11}=0.51     | 0.62 |     |
|                | IL - layer II unpaired t test |                     | WT vs KO   | t_{11}=0.39     | 0.71 |     |
|                | PL - Layer II unpaired t test |                     | WT vs KO   | t_{11}=0.53     | 0.61 |     |
|                | Cg- Layer II unpaired t test |                     | WT vs KO   | t_{11}=0.36     | 0.73 |     |
| pERK activation after Fear | AuCx unpaired t test |                     | WT vs KO   | t_{11}=0.85     | 0.42 | S3m |
| marble burying test | Marble buried 2-way ANOVA, with repeated measures for time | Factor 2 Time interaction | F_{1,16}=116 | p<0.0001 |     |
| Locomotor activity | Total distance traveled unpaired t test |                     | WT vs KO   | t_{11}=0.31     | 0.76 | S3p |
| Locomotor activity | distance traveled 2-way ANOVA, with repeated measures for Time | Factor 2 Time interaction | F_{43,473}=7.92 | p<0.0001 |     |
| Number of neurons | GABAergic unpaired t test |                     | WT vs KO   | t_{16}=0.12     | 0.91 | S3q |
|                | Glutamatergic unpaired t test |                     | WT vs KO   | t_{16}=0.079    | 0.94 |     |
| Morphological composition of LA, with GFP-neurons | Total synapse unpaired t test |                     | WT vs KO   | t_{2}=2.819     | 0.11 | S4a |
| Morphological composition of LA, with EM | asymmetric unpaired t test |                     | WT vs KO   | t_{6}=0.19      | 0.85 | S4b |
|                | total unpaired t test  |                     | WT vs KO   | t_{6}=0.61      | 0.56 |     |
| Firing | Firing 2-way ANOVA |                      | Factor 2 current interaction | F_{10,20}=139.9 | p<0.0001 | S4c |
| mIPSCs amplitude | All unpaired t test |                     | WT vs KO   | t_{16}=0.27     | 0.78 | S5a |
|                | peris unpaired t test |                     | WT vs KO   | t_{16}=0.014    | 0.99 |     |
| mIPSCs rise time | All unpaired t test |                     | WT vs KO   | t_{16}=0.64     | 0.53 | S5b |
|                | peris unpaired t test |                     | WT vs KO   | t_{16}=0.94     | 0.36 |     |
| mIPSCs inter event interval | All unpaired t test |                     | WT vs KO   | t_{16}=0.18     | 0.85 | S5c |
|                | peris unpaired t test |                     | WT vs KO   | t_{16}=0.023    | 0.98 |     |
| Paired Pulse ratio at cortical inputs | 1-2 weeks unpaired t test |                     | WT vs KO   | t_{23}=1.26     | 0.22 | S5d |
|                | 4-5 weeks Mann-Whitney test |                     | WT vs KO   | U=33.5          | 0.83 |     |
|                          | 8-11 weeks | Paired Pulse ratio at subcortical inputs | 1-2 weeks | Mann-Whitney test | WT vs KO | U=22 | 0.83 | S5e  |
|--------------------------|------------|------------------------------------------|-----------|------------------|---------|------|------|------|
|                          | 4-5 weeks  | Mann-Whitney test                         | 8-11 weeks| Mann-Whitney test | WT vs KO | U=18.5| 0.51 |      |
|                          |            | Frezing in training                       |           | MANOVA           | CS-US   | F_{2,22}=55.72 | p=0.0001  | S6b  |
|                          |            | MANOVA, Between Subjects effect           |           | Factor 1 genotype| F_{1,24}=0.039 | 0.84 |      |      |
|                          |            |                                           |           | Factor 2 treatment| F_{1,24}=2.97 | 0.098|      |      |
|                          |            |                                           |           | interaction      | F_{1,24}=0.033 | 0.86 |      |      |
|                          |            | Frezing in tone test                      |           | MANOVA           | CS       | F_{2,23}=0.79 | 0.46  |      |
|                          |            | MANOVA, Between Subjects effect           |           | Factor 2 treatment| F_{1,24}=0.94 | 0.34 |      |      |
|                          |            |                                           |           | interaction      | F_{1,24}=0.14 | 0.71 |      |      |
|                          |            | pERK activation                           | LA        | 2-way ANOVA      | Factor 1 genotype| F_{1,26}=1.76 | 0.20  | S6d  |
|                          |            |                                           |           | interaction      | F_{1,26}=0.64 | 0.44 |      |      |
|                          |            | Open field intra-AMY                      | Time in center | 2-way ANOVA | Factor 2 group | F_{1,26}=0.0009 | 0.98  | S6e  |
|                          |            |                                           |           | interaction      | F_{1,26}=0.079 | 0.78 |      |      |
|                          |            | elevated plus maze intra-AMY              | time in open arms | 2-way ANOVA | interaction | F_{1,26}=0.0003 | 0.99  | S6f  |
|                          |            | social preference intra-AMY               | social preference | 2-way ANOVA | Factor 2 treatment | F_{1,26}=0.24 | 0.63  | S6g  |
|                          |            |                                           |           | interaction      | F_{1,26}=0.44 | 0.51 |      |      |
|                          |            | Open field icv                            | Time in center | 2-way ANOVA | Factor 1 genotype| F_{1,27}=1.59 | 0.22  | S6h  |
|                          |            |                                           |           | Factor 2 group | F_{1,27}=0.67 | 0.42 |      |      |
|                          |            |                                           |           | interaction      | F_{1,27}=0.015 | 0.90 |      |      |
|                          |            | Elevated plus maze icv                    | Time of interaction | 2-way ANOVA | Factor 1 genotype| F_{1,27}=3.76 | 0.063 | S6i  |
|                          |            |                                           |           | Factor 2 treatment| F_{1,27}=0.37 | 0.55 |      |      |
|                          |            | social preference icv                     | social preference | 2-way ANOVA | Factor 1 genotype| F_{1,27}=0.94 | 0.34  | S6j  |
|                          |            |                                           |           | Factor 2 treatment| F_{1,27}=0.021 | 0.88 |      |      |
|                          |            |                                           |           | interaction      | F_{1,27}=0.077 | 0.78 |      |      |
|                          |            | St8sia2 mRNA levels                      | PFC       | unpaired t test  | Ct vs sh | t_{8}=0.28 | 0.79  | S7c  |
|                          |            | PolySia level                             | PFC       | unpaired t test  | Ct vs sh | t_{8}=0.35 | 0.73  |      |
|                          |            | Body weight                               | distance moved | unpaired t test | Ct vs sh | t_{15}=0.14 | 0.88  | S7d  |
|                          |            | elevated plus maze                        | time in open arms | unpaired t test | Ct vs sh | t_{15}=0.73 | 0.44  | S7e  |
|                          |            | social preference                         | Time of interaction | 2-way ANOVA | Factor 1 genotype| F_{1,30}=0.0048 | 0.95 | S7f  |
|                          |            |                                           |           | Factor 2 juvenile-object | F_{1,30}=144.5 | p=0.0001 |      |      |
|                          |            |                                           |           | interaction      | F_{1,30}=0.09 | 0.76 |      |      |