Supplementary Information

*Imbalanced post- and extrasynaptic SHANK2A functions during development affect social behavior in SHANK2-mediated neuropsychiatric disorders*

Ahmed Eltokhi et al., 2021

Supplemental Figures
Figures S1 – S11

Supplemental Tables
S1) Proteomics data for (SH-WT, SH-RX andCtrls.)
S2) Proteomics data for (SH-RX andCtrls.)
S3) Proteomics data (Tg\(^{CaMK2A-tTA}\) vs. wild-typeCtrls.)
S4) List of used antibodies
S5) nCounter probes

Proteomics data are available in the Supplementary Data 1 (Dataset 1)

Supplemental Methods
Supplemental References
Fig. S1. Expression pattern of the SHANK2A(R462X) transgene as monitored by the co-regulated nuclear-localized β-galactosidase. The nuclear β-galactosidase activity monitored by X-Gal staining is shown in a coronal brain section of an adult SH-RX mouse (C57Bl6/N; Tg(PtetO-nlacZ-SHANK2A(R462X))/TgCamk2a-tTA). The intensity of the X-Gal identifies the CA1 pyramidal, DG granular cells and cells in the piriform cortex as neuronal cell types with the strongest expression of TgCamk2a-tTA driven PtetO-nlacZ-SHANK2A(R462X). Lower expression was found in other forebrain regions including the amygdala and striatum. Scale bars are given in mm.
**Fig. S2. Immunoblot expression analysis.**

**A.** Expression of the transgenic SHANK2A (left) and SHANK2A(R462X) (right) in the cortex, OB and CB of SH-WT and SH-RX mice, respectively.

**B.** Expression of the transgenic SHANK2A and SHANK2A(R462X) in the hippocampus of two animals per genotype. The proteins were visualized by immunoblotting using antibodies specific to the human SHANK2, β-galactosidase and Venus. β3-tubulin was used as a loading control. SHANK2A (SH-WT); SHANK2A(R462X) (SH-RX).

**C.** Validation of SHANK2, β-galactosidase, Venus and β3-Tubulin antibodies in different brain regions of SH-WT mice in immunoblots. Immunoreactive proteins on nitrocellulose membranes detected by human SHANK2, β-galactosidase, β3-tubulin, and Venus were visualized and recorded by the Fujifilm LAS-3000 in the sampling mode with 2 – 5 sec intervals. Images that were out of the linear recording mode were excluded from the quantitative analysis. HPC: Hippocampus, OB: Olfactory bulb, CB: Cerebellum.
**Fig. S3.** Prenatal and early postnatal expression of the transgene **TgαCaMKII-tTA** as monitored by tTA reporter transgenic expression units. **A.** In situ hybridization (ISH) on sagittal sections of double transgenic **TgCamk2a-tTA/NR1(R)tetO7lacZ** mice revealed the expression of tTA (top left), LacZ (top right) and NR1(R) (bottom left) in Str. and OB at P0. The expression was confirmed using X-gal staining to visualize β-galactosidase activity (bottom right). **B.** Double transgenic **TgCamk2a-tTA/GFPtetO7lacZ** mice (indicated by the green spots) revealed an autofluorescence of GFP in the ON/OB and FB at P3. In this system, the milk in the stomach of the pups can be detected by a false positive signal (for details, see Krestel et al., 2001\(^1\)). Str.: Striatum, ON: Olfactory nucleus, OB: Olfactory bulb, FB: Forebrain. The genetic mouse model used in **A** was described in detail in\(^1,2\).
Fig. S4. Expression analysis of SHANK and glutamate receptors in SH-WT and SH-RX mice. A. Immunoblot analysis of hippocampal extracts from 5 SH-WT and 5 control littermates and 6 SH-RX and 6 control littermates. B. Expression analysis of SH-WT P10-on hippocampi by nCounter revealed a significant down-regulation of Gria2, Gria3 and Grm1 indicated by asterisks (n=7 SH-WT and 8 control mice, 3 – 5 months). C. Expression analysis of hippocampi by nCounter revealed a significant downregulation of the Gria2,3 and Grm5 mRNA for SH-WT Ad-off mice. For SH-RX Ad-off mice, Gria2 expression was downregulated (n = 7 SH-WT Ad-off and 8 Ctrl. Ad-off mice at 5 – 8 months of age; n = 7 SH-RX Ad-off and Ctrl. Ad-off mice at 5 – 8 months of age). Unpaired two-tailed Student’s t-test followed by the Benjamini-Hochberg test, *p ≤ 0.05. Error bars indicate the standard error of the mean (SEM).
**Fig. S5. Peptide and protein quality control for the SWATH analysis.** Boxplots showing the coefficient of variation (CoV) at **A.** peptide level; **B.** protein level and **C.** protein level of the proteins quantified with one and with 2 or more peptides in each experimental condition, as a quality metric of the reproducibility of replicate measurements. The number (n) of peptides or proteins analyzed is given together with the median CoV (black line across the box). Replicas were for controls 14, for SH-WT 7 and for SH-RX 7 biologically independent samples (animals) of the synaptically enriched protein fraction. The box length indicates the interquartile range. e.g in (B) The control group showed 11% median CoV for the 2466 proteins quantified in 14 biological replicates.
Fig. S6. SYNGO enrichment analysis A. Enrichment analysis of the significantly regulated proteins (excluding Shank proteins) using brain expressed genes as background identified the most prominent enrichment of altered proteins in the presynaptic component. B. Enrichment analysis of the proteins specifically regulated in SH-WT, strengthens further the prominent enrichment of altered proteins in the presynaptic versus postsynaptic proteins in both lines. Terms are color-coded according to enrichment Q-value.
Fig. S7. Body weight and anxiety in mouse lines. Body weight of A. SH-WT, B. SH-WT<sup>P10-on</sup> and C. SH-WT<sup>Ad-off</sup> was 30%, 20% and 10% lower, respectively, compared to their respective control mice. Body weight of D. SH-RX, but not E. SH-RX<sup>Ad-off</sup> mice were 10% lower than their respective control. For anxiety analysis, A. SH-WT, B. SH-WT<sup>P10-on</sup>, C. SH-WT<sup>Ad-off</sup>, D. SH-RX and E. SH-RX<sup>Ad-off</sup> were analyzed in the open field, dark/light box and by the latency to explore or drink novel food. All mouse cohorts showed some sort of anxiety. However, anxiety was not consistently apparent in the three tests. In the 10 min exploration of the open field test, mild hyperactivity could only be detected in SH-RX mice.
**Fig. S8. Locomotion and burrowing test.** Hyperactivity was observed in A. SH-WT, B. SH-WT<sub>P10-on</sub>, C. SH-WT<sub>Ad-off</sub>, D. SH-RX and E. SH-RX<sub>Ad-off</sub> mice with longer distance traveled, duration and number of locomotion and decreased immobility, but was more pronounced in SH-WT and SH-WT<sub>Ad-off</sub> mice. In the burrowing test, the amount of food left in a tube was significantly increased in all 5 cohorts after 2h compared to controls. After 12 h, only the SH-WT<sub>P10-on</sub> mice showed regular burrowing.
Fig. S9. Cognitive and emotional behavioral tests. A. In the puzzle box test, less ability to solve the puzzle was shown for SH-WT in sessions 1, 8 and 10 with no impairment in either the context or cued memory in the fear conditioning test (n=12 SH-WT and 15 littermate control mice, 6-8 months). B. SH-RX mice showed less ability to solve the puzzle in the sawdust trials (5, 6 and 7) in the puzzle box test with a reduced context memory in the fear conditioning test (n =10 SH-RX and 16 littermate control mice, 6-8 months).
Fig. S10. Effect of amphetamine on SH-WT and SH-RX mice. A. In the open field, i.p. injection of amphetamine but not of saline attenuated the hyperactivity of SH-WT and SH-RX mice. B. In the trace maps, SH-WT and SH-RX travel distances were significantly less compared to the 60 min time window trace maps before amphetamine injection. Of note, the increased jumping activity in the four corners of the open field in SH-RX traces before amphetamine injection.
Fig. S11. Unit responses in the olfactory cortex of SH-RX, SH-WT and controls. 

**A.** Plot of the mean spike width (from peak to trough) and the mean baseline firing rate for each unit in the three genotypes. **B.** The number of significant (left) excitatory and (right) inhibitory responses (-1.96 > z-score > 1.96) per unit, which showed any significant response was comparable across genotypes. **C.** Heat plots of the z-score of the firing rate of all units recorded in (top) control (middle) SH-WT and (bottom) SH-RX mice. Units were sorted according to their z-score in the response to the CHO3 odorant in a window of 1 s from inhalation onset. For all other odorants, the unit order of CHO3 was kept. **D.** Raster plot of spikes (upper part) and peri-stimulus histogram of the mean firing rate (lower part) of a unit from (top) control (middle) SH-WT and (bottom) SH-RX mice. For each genotype, units were chosen with broad responses to all odorants with increasing c-
chain length (CHO3-9) applied for 0.5 s. E. Mean firing rate (without baseline subtraction) of all cell-odor pairs with an excitatory or inhibitory response to the odorants for each genotype. **F. top.** Time to peak response from odor onset of all cell-odor pairs with an excitatory (ANOVA: p=0.76) or inhibitory (ANOVA: p<0.0001) odor response; **bottom.** and half-width of the odor response of all cell-odor pairs with an excitatory (ANOVA: p<0.0001) or inhibitory (ANOVA: P=0.003) odor response for the three genotypes. Only significant posttests are indicated in the figure bar graphs.
Table S1: Alteration in synapse-enriched protein levels from the hippocampi of SH-WT and SH-RX mice. Mutations in genes that are highlighted in red are associated with neuropsychiatric or neurodegenerative disorders. Genes that are highlighted in green are also differentially expressed in Camk2a-tTA mice. Differently expressed genes related to postsynaptic functions are highlighted in yellow. Proteins that were found in SYNGO, and which were annotated in SYNGO are labeled by (++; Syngoportal.Org (Gene list using "ID convert tool" followed by “start gene set analysis” and finally by “annotations”. P values ≤ 0.05 are highlighted in gray.

| Gene        | # Peptides | SH-WT vs controls | SH-RX vs controls | Function/localization in neurons |
|-------------|------------|-------------------|-------------------|----------------------------------|
|             |            | Fold-change | FDR adjusted p-values | Fold-change | FDR adjusted p-values |                          |
| Kif1a       | 7          | 2.18       | 1.14E-09            | 1.83       | 4.63E-09              | Axonal transport               |
| Eva1a       | 1          | 1.55       | 2.18E-07            | 0.98       | 0.7665                | Neuronal differentiation        |
| Rae1        | 1          | 1.51       | 4.16E-07            | 1.41       | 0.0003                | Synapse formation               |
| Shank3      | 8          | 0.66       | 4.16E-07            | 0.95       | 0.4375                | ++ Postsynapse                   |
| Pels        | 1          | 1.60       | 6.77E-07            | 1.19       | 0.0102                | Metabolic enzyme                |
| Mvenp2      | 1          | 1.76       | 1.92E-05            | 1.61       | 0.0007                | Both                           |
| Map7d2      | 1          | 1.39       | 6.17E-05            | 1.24       | 0.0279                | Proximal axon                   |
| Shank2/SHANK2A | 13    | 2.39       | 7.34E-05            | 0.87       | 0.5450                | ++ Postsynapse                   |
| Ndr52       | 4          | 1.24       | 0.001              | 1.24       | 3.41E-05              | Gener. of new neurons           |
| Apba2       | 2          | 1.32       | 0.001              | 1.22       | 0.0005                | Vesicle release                 |
| Shank1      | 14         | 0.74       | 0.0003             | 0.97       | 0.7490                | ++ Postsynapse                   |
| C1qc        | 2          | 0.67       | 0.0003             | 1.09       | 0.4087                | Pruning of synapse              |
| Slc6a7      | 4          | 0.79       | 0.0005             | 0.87       | 0.0987                | ++ Presynapse                    |
| Serpinb1a   | 1          | 0.56       | 0.0008             | 0.73       | 0.0136                | Neurotrophic                     |
| Cadm3       | 4          | 0.76       | 0.0013             | 0.82       | 0.0342                | ++ Contact axon-glia             |
| Rgs14       | 3          | 0.71       | 0.0014             | 0.72       | 0.0022                | Both, G-protein regulator        |
| Cd44        | 1          | 0.79       | 0.0016             | 0.96       | 0.6913                | Both                           |
| Homer2      | 1          | 1.30       | 0.0019             | 0.99       | 0.9010                | ++ Postsynapse                   |
| Mapk10      | 1          | 1.17       | 0.0029             | 1.09       | 0.0681                | ++ Postsynapse                   |
| Rasgrp1     | 1          | 0.78       | 0.0031             | 0.87       | 0.1001                | Parkinson                       |
| Npxr        | 2          | 0.82       | 0.0032             | 0.97       | 0.6410                | ++ Postsynapse                   |
| Grm3        | 7          | 0.73       | 0.0035             | 0.88       | 0.2845                | Postsynapse                      |
| Cyb5r1      | 4          | 0.78       | 0.0045             | 0.91       | 0.3434                | ++ Both                         |
| Dkk3        | 1          | 0.81       | 0.0057             | 0.88       | 0.0790                | Membranes of synapses            |
| Pom21l      | 7          | 1.48       | 0.0062             | 1.51       | 0.0022                | Neuroprotection                 |
| Sod2        | 2          | 0.63       | 0.0077             | 0.68       | 0.0330                | Binds t-Snare                    |
| Sla18a2/VMAT2 | 1       | 0.85       | 0.0079             | 0.93       | 0.2111                | ++ Presynaptic vesicles          |
| Snph        | 2          | 0.82       | 0.0098             | 0.93       | 0.3092                | Docking axonal mitochondria      |
| Idh2        | 7          | 1.25       | 0.0098             | 1.01       | 0.9234                | Neuronal Apoptosis               |
| Slc25a22    | 2          | 0.79       | 0.0104             | 0.94       | 0.4890                | Neuronal Mitochondria            |
| Scapdh      | 1          | 0.83       | 0.0104             | 1.02       | 0.7705                | ++ Both                         |
| Epha4       | 6          | 0.83       | 0.0105             | 0.87       | 0.0136                |                           |
| Rasal1      | 8          | 0.83       | 0.0125             | 0.96       | 0.5676                |                           |
| Snx2        | 3          | 1.20       | 0.0126             | 1.11       | 0.1479                |                           |
| Slc16a1     | 1          | 0.75       | 0.0126             | 0.99       | 0.9538                |                           |
| Pip3Ras2    | 1          | 0.62       | 0.0133             | 0.63       | 0.0301                | ++ Synaptic vesicle Pro.         |
| Rmd1        | 3          | 0.83       | 0.0133             | 0.92       | 0.1530                |                           |
| Nwd2        | 6          | 0.80       | 0.0133             | 0.78       | 0.0136                |                           |
| St100a13     | 2          | 0.81       | 0.0133             | 0.93       | 0.4032                |                           |
| Tmem132a     | 1          | 0.75       | 0.0136             | 0.83       | 0.1573                |                           |
| Fam171a2     | 2          | 0.81       | 0.0136             | 0.97       | 0.7397                |                           |
| Cntnap1p     | 12         | 0.86       | 0.0140             | 0.87       | 0.0415                | ++ Presynapse                   |
| Traf3       | 1          | 0.65       | 0.0148             | 0.98       | 0.8337                |                           |
| 2010300c02rik | 4         | 1.39       | 0.0154             | 1.08       | 0.6367                |                           |
| Rac1        | 1          | 0.82       | 0.0154             | 0.94       | 0.4809                |                           |
| gene         | fold | size   | se   | Comments               |
|--------------|------|--------|------|------------------------|
| Stxbp5       | 5    | 0.82   | 0.0169 | 0.94 | 0.4868 | ++ Presynapse     |
| Vti1a        | 1    | 0.84   | 0.0170 | 0.97 | 0.6489 | ++ Presynapse     |
| Rab15        | 1    | 0.51   | 0.0170 | 1.05 | 0.7714 |                |
| Lyps         | 9    | 0.82   | 0.0173 | 0.89 | 0.1001 | ++ Presynapse     |
| Dgkb         | 2    | 0.79   | 0.0180 | 0.90 | 0.3057 | ++ Presynapse     |
| Atp8a1       | 14   | 0.86   | 0.0181 | 0.93 | 0.3421 | ++ Presynapse     |
| Glnp         | 1    | 0.79   | 0.0195 | 0.86 | 0.1700 |                |
| Lman1L       | 1    | 1.19   | 0.0199 | 1.00 | 0.9873 |                |
| Basap        | 12   | 0.86   | 0.0203 | 0.94 | 0.3932 | ++ Both          |
| Abhd3        | 1    | 0.78   | 0.0203 | 0.92 | 0.4542 |                |
| Ssamp4       | 1    | 0.81   | 0.0203 | 0.99 | 0.9254 |                |
| Stx16        | 1    | 0.82   | 0.0215 | 0.94 | 0.5026 | ++ Presynapse     |
| Pip4k2c      | 4    | 1.37   | 0.0215 | 1.11 | 0.5325 |                |
| Rimbp2       | 3    | 0.86   | 0.0244 | 0.83 | 0.0136 | ++ Presynapse     |
| Synl7        | 1    | 0.71   | 0.0244 | 0.74 | 0.1001 |                |
| Mif2         | 1    | 0.80   | 0.0246 | 0.87 | 0.1352 |                |
| Cacng8       | 3    | 0.83   | 0.0246 | 1.00 | 0.9697 | ++ Postsynapse    |
| Gria2        | 11   | 0.81   | 0.0262 | 0.82 | 0.0294 | ++ Postsynapse    |
| 231006j104rik| 1    | 0.79   | 0.0277 | 0.92 | 0.2981 |                |
| Not1         | 2    | 0.84   | 0.0282 | 0.88 | 0.0296 | ++ Postsynapse    |
| Them4        | 4    | 0.88   | 0.0287 | 0.92 | 0.0904 |                |
| Unc5c        | 1    | 0.73   | 0.0291 | 0.83 | 0.1611 |                |
| Ppap2b       | 4    | 0.82   | 0.0291 | 0.94 | 0.4399 |                |
| Coro2b       | 3    | 0.82   | 0.0292 | 0.92 | 0.4154 |                |
| Calu         | 3    | 1.22   | 0.0295 | 1.18 | 0.1716 |                |
| Itm26b       | 1    | 0.49   | 0.0295 | 1.02 | 0.9538 |                |
| Myadm        | 1    | 0.89   | 0.0298 | 0.95 | 0.4052 |                |
| Rtn4g1f5'    | 1    | 0.79   | 0.0299 | 0.79 | 0.0342 | Mitochondria     |
| Cpe257.14    | 4    | 0.83   | 0.0304 | 0.82 | 0.0415 | Neurite outgrowth|
| Smpd3        | 1    | 0.78   | 0.0304 | 1.02 | 0.9199 |                |
| Kcnn2        | 2    | 0.83   | 0.0311 | 0.92 | 0.3454 | ++ Postsynapse    |
| Rab11fip2    | 2    | 0.84   | 0.0317 | 0.91 | 0.3434 |                |
| Htrap        | 1    | 0.83   | 0.0321 | 0.87 | 0.1001 |                |
| Plxna1       | 8    | 0.83   | 0.0327 | 0.80 | 0.0277 | Axon guidance    |
| Synpr        | 2    | 0.78   | 0.0327 | 0.84 | 0.2184 | ++ Synaptic vesicle protein |
| Rmdn3        | 3    | 0.81   | 0.0327 | 0.98 | 0.8659 | ++ Both          |
| Rpl13a       | 1    | 1.25   | 0.0371 | 0.91 | 0.3155 | ++ Postsynapse, Ribosome |
| Bsg          | 4    | 0.87   | 0.0372 | 0.90 | 0.1144 |                |
| Atp6v0d1     | 5    | 0.86   | 0.0372 | 0.98 | 0.8508 | ++ Synaptic vesicle |
| Plxna2       | 3    | 0.76   | 0.0424 | 0.79 | 0.1001 | Axon guidance    |
| Txl1         | 1    | 1.32   | 0.0424 | 1.14 | 0.1827 |                |
| Pycr1        | 1    | 0.81   | 0.0424 | 1.05 | 0.6754 |                |
| Gm996        | 4    | 0.81   | 0.0438 | 0.95 | 0.5439 |                |
| Samm50       | 6    | 0.86   | 0.0438 | 0.97 | 0.7049 | ++ Presynapse    |
| Svp9         | 1    | 0.74   | 0.0443 | 0.96 | 0.7871 | ++ Presynapse    |
| Gm22         | 1    | 0.81   | 0.0464 | 0.83 | 0.0819 |                |
| Myo18a       | 6    | 1.46   | 0.0464 | 1.15 | 0.2845 |                |
| E90q6c7      | 1    | 0.79   | 0.0464 | 0.90 | 0.3956 |                |
| Sept2        | 2    | 0.89   | 0.0479 | 0.99 | 0.9086 |                |
| Svt12        | 2    | 0.85   | 0.0485 | 0.94 | 0.4154 | ++ Presynapse    |
| Mark4        | 1    | 0.86   | 0.0485 | 0.96 | 0.6549 |                |
| Vdac1        | 6    | 0.88   | 0.0485 | 0.98 | 0.7490 | ++ Both          |
| Mch2         | 3    | 0.82   | 0.0485 | 0.98 | 0.8166 |                |
| Scl6a11      | 3    | 0.76   | 0.0485 | 1.03 | 0.8608 | ++ Both          |
| Ip55         | 5    | 1.18   | 0.0486 | 1.03 | 0.6489 |                |
| Map11c3b     | 1    | 0.89   | 0.0488 | 0.97 | 0.6369 |                |
| Pdel         | 1    | 0.84   | 0.0489 | 0.96 | 0.5938 |                |
### Genes differentially expressed

**Genes associated with psychiatric disorders**

| Gene         | Log2FC | adj.P.Val | fold.Changed | P-value       |
|--------------|--------|-----------|--------------|---------------|
| Pmm1         | 2      | 1.36      | 0.0489       | 1.03          | 0.8221       |
| Ca4          | 2      | 2.42      | 0.0585       | 3.11          | 0.0277       |
| Plxna4       | 20     | 0.87      | 0.0617       | 0.84          | 0.0321       |
| Lancl1       | 3      | 1.14      | 0.0808       | 1.17          | 0.0136       |
| Cryz         | 2      | 0.90      | 0.0962       | 0.85          | 0.0368       |
| Cpe4         | 3      | 0.90      | 0.0970       | 0.82          | 0.0028       |
| Slc7a14      | 4      | 0.86      | 0.1059       | 0.84          | 0.0489       |
| Pde1a        | 5      | 1.20      | 0.1296       | 1.25          | 0.0454       |
| Hsd17b8      | 2      | 0.92      | 0.1500       | 0.87          | 0.0446       |
| Slc35a1      | 1      | 1.29      | 0.1928       | 1.55          | 0.0406       |
| Ccdc177      | 1      | 0.93      | 0.2111       | 0.88          | 0.0375       |
| Gpr56        | 1      | 1.14      | 0.2205       | 1.26          | 0.0406       |
| Uchl1        | 3      | 1.18      | 0.2270       | 1.32          | 0.0342       |
| Ermp1        | 2      | 0.93      | 0.2414       | 0.84          | 0.0084       |
| Kit          | 2      | 0.87      | 0.2476       | 0.70          | 0.0321       |
| Ech1         | 1      | 1.13      | 0.2488       | 0.79          | 0.0294       |
| Pld3         | 3      | 1.07      | 0.2764       | 1.15          | 0.0279       |
| Anov1        | 4      | 0.91      | 0.3161       | 0.76          | 0.0224       |
| Scn8a        | 3      | 0.91      | 0.3885       | 0.86          | 0.0303       |
| Gm20671;Pisd | 2      | 0.95      | 0.3937       | 0.79          | 0.0415       |
| Ppm1f        | 1      | 0.97      | 0.6217       | 0.87          | 0.0303       |
| Pgbd5        | 1      | 0.96      | 0.6378       | 0.86          | 0.0406       |
| 6330430j02rik| 2      | 0.96      | 0.6446       | 0.86          | 0.0415       |
| Pex11b       | 1      | 0.97      | 0.6508       | 0.87          | 0.0321       |
| Tnks1bp1     | 2      | 0.97      | 0.7521       | 0.86          | 0.0489       |
| Rhoa;Rhoc    | 2      | 0.97      | 0.7753       | 1.22          | 0.0303       |
| Wbcr17       | 3      | 1.01      | 0.9113       | 0.83          | 0.0330       |
| Ypsl6        | 3      | 1.01      | 0.9408       | 1.10          | 0.0489       |
| Acot13       | 2      | 1.00      | 0.9649       | 0.86          | 0.0303       |
| Them6        | 1      | 1.00      | 0.9654       | 0.82          | 0.0415       |
| Clcn6        | 3      | 1.00      | 0.9949       | 1.13          | 0.0337       |

**Axon guidance**

**Postsynaptic localization**

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**Genes differentially expressed in Camk2a-tTA mice (KT1)** see [Table S5](#)

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Table S2: Differentially expressed hippocampal genes in SH-RX mice. Mutations in genes are as in Table S3 but now sorted according to FDR adjusted P values. Differentially expressed genes related to postsynaptic functions are highlighted in yellow (see Table S3). For color code in the first column, see Table S3.

| Gene     | SH-RX vs controls fold change | SH-RX vs controls FDR adjusted p-values | Function/localization          |
|----------|-------------------------------|----------------------------------------|--------------------------------|
| Kif11a   | 1.83                          | 4.63E-09                               | Axonal transport              |
| Ndrg3    | 1.24                          | 3.41E-05                               | Nuclear; Cell growth          |
| Rae1     | 1.41                          | 0.000274634                            | Synapse formation             |
| Abha2    | 1.22                          | 0.000499013                            | Axon trafficking               |
| Mvcbp2   | 1.61                          | 0.000722405                            |                               |
| Pgm2l1   | 1.51                          | 0.002230564                            | Metabolic enzyme              |
| Rgs14    | 0.72                          | 0.002230564                            | Both                          |
| Cpm4     | 0.82                          | 0.002830756                            | Outgrowth of processes        |
| Ernp1    | 0.84                          | 0.008358106                            | ER                            |
| Pgd1     | 1.19                          | 0.010176294                            | Metabolic enzyme              |
| Lancl1   | 1.17                          | 0.013645122                            | Neuron survival               |
| Rimb2    | 0.83                          | 0.013645122                            | Presynapse                    |
| Epha4    | 0.87                          | 0.013645122                            | Both                          |
| Nwd2     | 0.78                          | 0.013645122                            | Both/actin regulation         |
| Serpinb1a| 0.73                          | 0.013645122                            | Neurotrophic                  |
| Loxa14   | 0.76                          | 0.022434897                            | Ca²⁺ Homeostasis              |
| Cad4     | 3.11                          | 0.027671                               | Carbonic anhydrase            |
| Plxna1   | 0.80                          | 0.027671                               | Axon guidance                 |
| Map7d2   | 1.24                          | 0.027864551                            | Proximal axon                 |
| Pld3     | 1.15                          | 0.027864551                            | Phospholipase D               |
| Lgr4     | 1.28                          | 0.029387066                            | Tumor suppressor              |
| Ech1     | 0.79                          | 0.029387066                            | Soma, Dendrites               |
| Gria1    | 0.82                          | 0.029387066                            | Postsynapse/membrane          |
| Nos1A    | 0.88                          | 0.029595798                            | Postsynapse                   |
| Ppm2    | 0.63                          | 0.030111267                            | Vesicle secretion             |
| Scn8a    | 0.86                          | 0.030268813                            | Node of Ranvier               |
| Acot13   | 0.86                          | 0.030289017                            | Acyl-CoA hydrolysis           |
| Rhoa;rhoc| 1.22                          | 0.030289017                            | Postsynapse                   |
| Ppm1f    | 0.87                          | 0.030289017                            | Presynapse                    |
| Pex11b   | 0.87                          | 0.032101342                            | Neuronal migration            |
| Ap3     | 0.70                          | 0.032101342                            | Growth factor receptor        |
| Plxna4   | 0.84                          | 0.032101342                            | Axon guidance                 |
| Wbscr17  | 0.83                          | 0.033011016                            | Lamellipodium formation       |
| Sh3bp6   | 0.68                          | 0.033011016                            | Transporter in vesicles       |
| Clcn6    | 1.13                          | 0.033737627                            | Lysosomal storage             |
| Uchi1    | 1.32                          | 0.034195896                            | Neuronal injury               |
| Rtn4ip1  | 0.79                          | 0.034195896                            | Dendritic growth              |
| Cadm3    | 0.82                          | 0.034195896                            | Contact axon-glia            |
| Cryz     | 0.85                          | 0.036849927                            | Quinone oxidoreductase        |
| Ccde177  | 0.88                          | 0.037525782                            | Coiled-coiled domain protein  |
| Slc35f1  | 1.55                          | 0.040580386                            | Transmembrane transporter     |
| Gpr56    | 1.26                          | 0.040580386                            | Neuronal migration            |
| Pgbd5    | 0.86                          | 0.040580386                            | Transposon protein            |
| Them6    | 0.82                          | 0.041542566                            | Thioesterase                  |
| 6330403a02rik | 0.86 | 0.041542566 | Neuronal marker |}

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| Gene   | Fold Change | p-value        | Function                           |
|--------|-------------|----------------|------------------------------------|
| Cpne5  | 0.82        | 0.041542566    | Neurite outgrowth                  |
| Hsd17b8| 0.87        | 0.044621876    | Mitochondria                       |
| Pde1a  | 1.25        | 0.04541138     | Cyc. Nuc. Phosphodiest.            |
| Vps16  | 1.10        | 0.048906198    | Presynaptic                        |
| Tnks1bp1| 0.86        | 0.048906198    | Tankyrase-1-bind. protein          |
| Slc7a14| 0.84        | 0.048906198    | Lysosomal localized                |
| Cpne6  | 0.82        | 0.049821894    | Ca²⁺ sensor                        |

Genes differentially expressed in SH-WT and SH-RX (see Table S3).
For the color codes in the first column see Table S3.
Table S3: Alteration in synapse-enriched protein levels from the hippocampi of transgenic KT1 (TgCamk2a-tTA) and wild-type control mice. Genes highlighted in green were listed in Table S3 in SH-WT and/or SH-RX mice as differentially expressed. Cut off of FDR adjusted P values ≤ 0.05.

| Gene   | Fold change | p-value        | FDR adjusted p-values |
|--------|-------------|----------------|-----------------------|
| Stxbp6 | 0.584111467 | 1.4287845E-09  | 3.4590872E-06         |
| Cad7   | 4.009301583 | 1.0676870E-08  | 1.2924352E-05         |
| Slc35f1| 1.85793191  | 2.7592248E-08  | 2.2266944E-05         |
| Ofnm1  | 0.766127951 | 1.1867941E-06  | 0.0007183             |
| Sgce3  | 0.762382231 | 2.9637421E-06  | 0.0014350             |
| Cpmc2  | 0.703610436 | 2.7592248E-08  | 2.2266944E-05         |
| Aldh1b1| 1.353040824 | 2.6867273E-05  | 0.0092922             |
| P2ry2  | 0.595345824 | 3.1996665E-05  | 0.0096830             |
| Cpne5  | 0.822226982 | 6.2159861E-05  | 0.0150489             |
| Tpbg   | 0.666049461 | 7.1904545E-05  | 0.0158255             |
| Mal2   | 0.834569888 | 9.6085728E-05  | 0.0178941             |
| Slc2a12| 0.808782733 | 9.0513329E-05  | 0.0178941             |
| Lrrtm1 | 0.784899168 | 0.0001084      | 0.0187436             |
| Lstx   | 1.301927373 | 0.0001180      | 0.0190526             |
| Cdh13  | 0.681560583 | 0.0001728      | 0.0246026             |
| Mtd2   | 3.511112672 | 0.0001694      | 0.0246026             |
| Gabrb2 | 0.819076622 | 0.0002022      | 0.0271969             |
| Gprin1 | 0.755129595 | 0.0002276      | 0.0279656             |
| Mife2  | 0.815984152 | 0.0002131      | 0.0279656             |
| Tpp1   | 0.826847404 | 0.0002426      | 0.0279656             |
| Ntrk3  | 0.800467473 | 0.0002704      | 0.0297581             |
| Gabra2 | 0.736327141 | 0.0003559      | 0.0309522             |
| Gas7   | 0.821378174 | 0.0003350      | 0.0309522             |
| Gm20503;Gng10 | 1.230576882 | 0.0003580 | 0.0309522 |
| Ngef   | 0.764237944 | 0.0003540      | 0.0309522             |
| Rap2b  | 0.821467914 | 0.0003227      | 0.0309522             |
| Tfr2   | 0.833245843 | 0.0003193      | 0.0309522             |
| Aicam  | 0.768304076 | 0.0003758      | 0.0313715             |
| Rgs12  | 0.813187957 | 0.0004152      | 0.0335085             |
| Lypd1  | 0.692205352 | 0.0004321      | 0.0337439             |
| Ptprg  | 0.762074305 | 0.0004474      | 0.0338506             |
| Negr1  | 0.778066008 | 0.0004665      | 0.0342211             |
| Pfn1   | 1.419474624 | 0.0006345      | 0.0436435             |
| Rras2  | 0.82012858  | 0.0006670      | 0.0436435             |
| Vgf    | 0.818633406 | 0.0006564      | 0.0436435             |
| Kr     | 0.733114719 | 0.0007311      | 0.0462119             |
| Rap2c  | 0.837069453 | 0.0007444      | 0.0462119             |
| Anxa1A | 0.778999913 | 0.0008429      | 0.0467966             |
| Cpmc2  | 0.753871507 | 0.0008636      | 0.0467966             |
| Fam81A | 0.775109678 | 0.0008698      | 0.0467966             |
### Protein functions:

**Stxbp6** encodes AMYSIN and is involved in the secretion of large dense-core vesicles by interacting with Syntaxin.

**Ca4** encodes the carbonic anhydrase CA4. A dominant-negative point mutation in **Ca4** is involved in Retinitis pigmentosa.

**Slc35f1** encodes the drug, metabolite transmembrane transporter called ‘solute carrier family 35 member F1’. The function is unknown.

**Ptprn2** encodes the receptor type tyrosine-protease N28 resides within the deletion of the Camk2a-tTA transgene insertion site in Chr18. Ptprn2 shows the highest expression in the brain (https://www.ncbi.nlm.nih.gov/gene/?term=Ptpn2) and is heterozygous in SH-WT, SH-RX and KT1 mice.

**Cpne5** encodes CopinV, which is Ca^{2+}-dependent membrane-binding protein. Cpne5 is involved in anxiety, alcohol dependence and obesity.

**Lnx** encodes the E3 ubiquitin-protein ligase LNX1.

**MLF** encodes the myeloid leukemia factor 2.

**Gng7** encodes the guanine nucleotide-binding protein subunit gamma.

| Gene   | Log2 Fold Change | p-value | q-value |
|--------|------------------|---------|---------|
| Gabbr1 | 0.858401519      | 0.0007791| 0.0467966|
| Prdx6  | 1.261447637      | 0.0008282| 0.0467966|
| Rab3c  | 0.844168945      | 0.0008140| 0.0467966|
| Synpo  | 0.830685523      | 0.0009039| 0.0475735|
| Ocrl   | 0.784630039      | 0.0009453| 0.0486949|
Table S4: Antibodies for immunohistochemistry and western blot

| Antibody                        | Host species | Concentration used | Reference number | Company         | RRID            |
|---------------------------------|--------------|--------------------|------------------|-----------------|-----------------|
| monoclonal anti-GFP            | mouse        | 1:10000            | ab38689          | Abcam           | AB_732715       |
| monoclonal anti-Shank2         | mouse        | 1:1000             | 75-088           | Neuromab        | AB_2254586      |
| anti-β-galactosidase           | mouse        | 1:20000            | Z3783            | Promega         | AB_430878       |
| monoclonal anti-β3-tubulin     | mouse        | 1:20000            | G7121            | Promega         | AB_430874       |
| anti-mGlur1                     | rabbit       | 1:200              | PA1-4516         | Thermo Fischer  | AB_2294910      |
| anti-mGlur5                     | mouse        | 1:1000             | MABN540          | Merck Millipore | not available   |
| anti-GluN2A                     | rabbit       | 1:1000             | 07-632           | Merck Millipore | AB_11213002     |
| anti-GluN2B                     | rabbit       | 1:1000             | NB300-106        | Novus Biological| AB_10000537     |
| anti-GluA1                      | rabbit       | 1:1000             | AB1768-25UG      | Chemicon        | AB_2113602      |
| anti-GluA2                      | rabbit       | 1:1000             | AB1504           | Chemicon        | AB_2247874      |
| anti-GluA3                      | rabbit       | 1:1000             | ab40845          | Abcam           | AB_776310       |
| anti-GluN1                      | rabbit       | 1:1000             | AB9864R          | Millipore „Merck“| AB_10807557     |
| anti-Shank1                     | rabbit       | 1:1000             | 162013           | Synaptic system | AB_2619859      |
| anti-Shank3                     | mouse        | 1:1000             | ab93607          | Abcam           | AB_10563849     |
Table S5: Oligonucleotide sequences of the nCounter probes for selected genes in SH-WT, SH-RX and control mice

| Gene  | Sequence | Probe |
|-------|----------|-------|
| **Gapdh** | A  | ATGGGGCTCCCTAGGGATGCAAGCATGTCCTCCATCCAAAGCCCGAACGAGGCGTGACCTGTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Hspd1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Sdha** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Hprt1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Pgk1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Gpi1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Shank1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Shank2** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Shank3** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Venus** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Grm5** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Grin1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Grin2a** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Grin2b** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Gria1** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Gria2** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
| **Gria3** | A  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
|       | B  | CAGAAGCGATGCCCTCGACACTGAGCGATGCCGGCAAGCGACTCCCTCACCTGAGCTAGCAAGCTTCCCATTCTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA |
Supplemental Methods

The open field test
The mouse was placed in the corner of a white acrylic open-field box (40 × 40 × 40 cm) and allowed to explore the arena freely for 10 min while its path was monitored and tracked by a video camera, placed 1 m above the center of the arena. The automatic detection of the mouse’s traveled distance and the time spent in the central zone (15 cm apart from the walls) was recorded.

The dark-light box test
The dark-light box is an open white rectangle (30 x 20 x 20 cm) attached to a 3 x 3 cm opening to a dark chamber (with a lid and painted in black) (15 x 20 x 20 cm). The light chamber was illuminated at 600 lux. Each mouse was put in the dark chamber, and the latency, as well as the number of visits to the light chamber within 10 min, were measured. Only when all four limbs of the subject crossed the entrance, it was considered as an entry to the light chamber.

The neophobia test
Each subject was placed in an arena with an unfamiliar drink (100 μl sweetened condensed milk) in the center. The mouse was allowed to roam the arena for 10 min and the latency, as well as the number of contacts with the drink, was manually assessed17.

The burrowing test
The burrowing test is based on the mouse’s behavior towards the displacement of items from the tube within its home cage18. The tube was filled with 200 g of food pellets covered with 60 g of bedding. The test was performed at 5 p.m. and the pellets remaining in the tube were weighed after 2 h. Then the tube was placed in the cage again. After 12 h, the weight of the remaining pellets in the tube was finally assessed.

The puzzle box test
The puzzle box test was slightly modified from the one described in20. The puzzle box consisted of two compartments (a brightly-lit start zone and a smaller covered goal zone) separated by a barrier that had a narrow underpass (about 4 cm wide). Each mouse was introduced into the start zone and the task was to enter the goal zone where it could find some bedding from its home cage. Mice underwent a total of 11 trials over 4 consecutive days, with three trials per day on the first three days, and two trials on the last day. On day 1, during trial 1, the underpass was unblocked and the barrier had an open door above the underpass. In trial 2 and trial 3, the barrier had no doorway and the animals had to enter the goal zone via a small underpass. On day 2, trial 4 was identical to trial 2 and trial 3. In trial 5 and trial 6, however, the underpass was filled with sawdust and the animals had to dig through the sawdust. On day 3, the animals had to repeat trial 6 first as well as in trial 7. In trial 8 and trial 9, the animals were presented with the underpass being blocked by a cardboard plug that the mice had to pull out with their teeth and paws to enter the goal zone. Trial 10 on day 4 was again a repetition of trial 9. At the end of the test, in trial 11, the task was to repeat trial 1, like on the first day. After each trial, mice were left for 1 min inside the goal zone.
The fear conditioning test

The fear conditioning test evaluates natural fear learning as described before. For the acquisition session, at first, each mouse had to spend 180 s as habituation in the new arena. Then, an auditory tone was presented for 30 s at a level of 90 dB and frequency of 5,000 Hz with a rise time of 50 ms. A mild foot shock (0.5 mA) was administered during the last 2 s of the tone presentation and co-terminated with the tone. After the shock presentation, an inter-trial interval of 90 s preceded the second and third identical trials. Following the third shock presentation, the mouse remained in the arena for an additional 90 s. On the following day, the contextual testing was conducted similar to the acquisition session including lighting and odor, but without the tone and the foot shock. The experiments lasted for 300 s. On the third day, the cued memory was tested by placing each mouse in a new chamber with different odors, allowing it to habituate for 180 s. The same tone cue as in the acquisition session was then activated for 30 s. Then, an inter-trial interval of 90 s proceeded the second and third trials. The third tone was activated for 300 s until the end of the experiment. The video freeze software was used to record and measure freezing time and numbers.

Amphetamine injection in the open field

Baseline activity in the open field was measured by placing the mouse in the corner of a wooden arena measuring 40 x 40 x 40 cm and allowing it to explore freely for 60 min, while its path was monitored and tracked by a video camera placed 1 m above the center of the arena. Automatic detection of the distance traveled by the mouse as well as its speed was recorded with the SYGNIS tracker software (Sygnis AG). After 1 h, mice were removed from the open field, i.p. injected with 5 mg/kg amphetamine and immediately returned to the open field for another 60 min.
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