SUMMARY OF SUPPLEMENTARY MATERIAL

Eriksson Supplementary.doc. Supplementary Materials and Methods, Table S1, Supplementary References and Supplementary Figure Captions.

Fig. S1.pdf. Schematic setup of the passive avoidance (PA) procedure, emotional memory performance of female WT, p11HET and p11KO mice and responses to 5-HT$_4$R agonist stimulation.

Fig. S2.pdf. Schematic setup of the novel object recognition (NOR) procedure, NOR performance of WT, p11HET and p11KO mice and responses to 5-HT$_4$R agonist stimulation.

Fig. S3.pdf. Open field behaviors of WT, HET and p11KO mice and responses to 5-HT$_4$R agonist stimulation.

Fig. S4.pdf. NOR performance of WT and p11KO mice following hippocampal gene transfer with control AAV.YFP or overexpressing AAV.p11.

Fig. S5.pdf. Open field behaviors of WT and p11KO mice following hippocampal gene transfer with control AAV.YFP or overexpressing AAV.p11.

Fig. S6.pdf. Complementary data on a microelectrode array measurements of glutamate dynamics in the hippocampus.

SUPPLEMENTARY MATERIALS AND METHODS

Animals

Mice were group housed in TypeIII Macrolon cages, with ad libitum access to food pellets and water, controlled room temperature and relative humidity (45–55%) under a constant 12 h light/dark cycle (lights on at 0700 hours).

Passive avoidance emotional memory

The passive avoidance (PA) task was chosen to assess emotional memory processing. This task is an one-trial associative learning paradigm based on contextual fear conditioning combined with the requirement of the animal to express instrumental learning. Instead of generating fear-induced freezing, PA memory may circumvent ceiling effects on memory performance, which enables investigations of facilitatory effects on contextual long-term memory. [1] The passive avoidance emotional memory procedure is illustrated in Fig. 1A and Fig. S1A. Animal handling and experimental procedures were performed as previously described in mice [2-3] (UgoBasile, Comerio, Italy) and were similar to procedures used in rats. [4] The experimental design of the PA procedure has been implemented to reduce variability of the animals and induce an intermediate memory response in control groups. These aspects are critical, as it is possible to assess both impairing and enhancing effects of treatment or genotype on memory performance based on an adaptive response. The memory performance is assessed by the return of the animal to the naturally preferred context, or PA by suppressed entry with into the aversive context by learning to associate the context with an electrical stimulus, the unconditioned stimulus (US). Emotional memory involves the storage and recall of emotionally arousing events that are mediated by the acquisition or learning of an emotional state. [1]

Novel object recognition memory

The novel object recognition (NOR) memory procedure is shown and described in Fig. S2A, in accordance with previously described experimental procedures. [5-7] During training (T1), the mice explored an open-field arena containing two identical objects and returned to the arena for a memory test (T2), performed 1 or 24 hours after training. The testing arena contained one familiar (F) and one novel (N) object, and the relative discrimination index was calculated as $(Time_N\text{-}Time_F)/(Time_N\text{+}Time_F)$. Positive scores indicate preference for the novel object, negative scores derive from familiar object preference and scores close to “0” indicate no preference for either object. In addition, general open field behaviors were recorded to assess genotype- and treatment-dependent effects (ANY-maze, UgoBasile, Comerio, Italy). The delay between training and the memory test was either 1 h (short-term memory used in baseline and 5-HT_{1B}R agonism studies) or 24 h (long-term memory used in studies of 5-HT_4R agonism). Object recognition memory performance of mice was tested once per mouse.

The involvement of brain regions in the NOR task remains controversial, especially the specific roles of different subregions within the medial temporal lobe. Data derived from rats and mice have shown that the perirhinal cortex, in the parahippocampal region, is particularly important for novel object recognition memory. [6,8-11] The role of the hippocampus in novelty recognition has been debated and the available data indicate that the hippocampus is involved when novelty recognition involves spatial and/or contextual components or long-term memory formation. [10,12-13] A recent study also showed that novelty-evoked exploration was affected in mice with reductions of adult-born neurons by focal hippocampal x-irradiation,

genetic ablation of hippocampal neuronal progenitor cells as well as in aged mice with reductions of hippocampal neurogenesis. [5] Notably, the level of novel object exploration was increased after hippocampal manipulations and masked by pre-exposure to the experimental arena with allowance to use extra-maze spatial cues. These data provide additional evidence that contextual input may either dominate or interfere with encoding processing in interconnected brain circuits and illustrate complex interactions of the hippocampus in novelty exploration under different experimental conditions.

Focal AAV gene transfer of p11 in the hippocampus

Authorization of the use of genetically modified microorganisms (AAV) in laboratory animals was granted from the Swedish Work Environment Authority, with the application process, risk assessment and establishment of safety regulations overseen by the Biosafety Committee at the Karolinska Institute. Each step of the surgical and behavioral procedures with AAV gene transfer was performed under supervision by the designated veterinarian and conducted in a Biosafety Level P2 classified laboratory.

Locally applied adeno-associated virus (AAV) vectors in which a p11 overexpression viral vector (AAV.p11), containing a p11 complementary DNA (cDNA) or a control AAV vector containing YFP (AAV.YFP) which were prepared and utilized as previously described. [14] WT and p11KO mice were anesthetized with isoflurane gas (3.0% isoflurane for induction and 0.9-2.0% for maintenance) (Univentor 400, AgnTho's, Stockholm, Sweden). The depth of anesthesia was constantly monitored and body temperature maintained (World Precision Instruments, Stevenage, UK). Buprenorphine (Temgesic®) was used as analgesic agent and bupivacain (Marcaine®) was used as a local anesthetic. The mice were placed in a stereotaxic frame (David Kopf Instruments) fitted with a mouse adaptor, Cunningham™ Mouse Adaptor (Stoelting Co., Wood Dale, IL, USA), similar to the stereotaxic procedure previously described. [3] AAV.YFP or AAV.p11 vector was bilaterally infused using stereotaxic coordinates according to the mouse brain atlas [15] (Fig. 2I). For targeting the hippocampal CA1 and DG regions: the anterior–posterior (AP) coordinate referring to bregma was AP: -1.7 mm. Medial-lateral (ML) coordinates referring to the midsagittal suture line were ML: ±1.0 mm with bilateral infusions in both hemispheres. The dorsal-ventral (DV) coordinates from the surface of the brain were DV: -1.5 to -2.0, i.e. referring to the DG and the more superficial CA1 region (Fig. 2I). The syringe was lowered to DV: -2.0 for the start of infusion of AAV.YFP or AAV.p11 in the DG region. During the infusion, the syringe was gradually lifted to DV: -1.5 mm for the CA1 region. This procedure aimed at achieving a more widespread distribution of the vector applied in a pocket centered in the CA1 and DG regions, avoiding pressure leakage of vector applied to adjacent brain regions and relieving local tissue damage associated with administration in a single location. The speed of infusion was adjusted to further adapt to the ability of the brain to adjust to the local infusion, at a speed of <0.1 µl/min for 2.0 µL. After infusion, the guide was kept in the CA1 region for an additional 10 minutes before being slowly withdrawn. The surface was sealed with bone wax (Lukens™, Reading, PA, USA).

Behavioral testing of mice started 6 weeks after infusion of AAV vectors. During this delay phase, all mice were group-housed in the same littermate groups kept from weaning the mice at age 3-4 weeks. The mice were kept under daily observations for general health conditions. Stereotaxic coordinates used for the hippocampus were the same as used for the enzyme-based microelectrode array measurements (Fig. 3A-B, Fig.S6C-D). Correct infusion sites in control groups of mice receiving bilateral infusions of AAV.YFP were determined by *in situ*

hybridization against YFP mRNA (Fig. 2A-B). In groups of mice infused with AAV.p11 vector, p11 mRNA expression was instead assessed (Fig. 2E-F). To confirm that the restored p11 was functional, we took advantage of the fact that p11 upregulates 5-HT_{1B}R at the cell surface and used quantitative [^{125}I]cyanopindolol autoradiography (see below) as readout of cell surface 5-HT_{1B}Rs (Fig. 2J).

In situ hybridization

For *in situ* experiments, fresh frozen (14 µm) coronal cryostat sections were postfixed in 4% PFA, prepared and hybridized with ^{35}S-radiolabeled antisense riboprobes against p11 and YFP mRNA as previously described in detail. [16-17] Sections were exposed to Kodak MR films in room temperature for 5-21 days prior to development of films. Densitometric measurements were performed with the NIH ImageJ 1.40 (National Institute of Mental Health, Bethesda, MD, USA).

Radioligand binding

For quantitative receptor autoradiography, [^{125}I]cyanopindolol was used to assess binding to 5-HT_{1B}Rs as previously described. [4,14,17] Coronal cryostat (14 µm) sections were preincubated for 20 min at room temperature in assay buffer (170mM Tris/150mM NaCl, adjusted to pH 7.4). 5-HT_{1B}R binding was detected by incubating the sections for 2 hours at room temperature in assay buffer containing 12 pM [^{125}I]cyanopindolol (2200 Ci/mmol) (PerkinElmer, Waltham, MA, USA), 100 nM 8-OH-DPAT (Sigma-Aldrich), to block 5-HT_{1A}Rs, and 30 µM isoproterenol (Sigma-Aldrich), to block β-adrenergic receptors. Non-specific binding was determined by incubation in the presence of 10 µM serotonin (Sigma-Aldrich). Following incubation, the sections were washed twice for 5 min in cold assay buffer at 4 °C, dipped in distilled water at 4 °C and dried under air. Sections were exposed together to Kodak MR films for 2-16 days in cassettes kept at -20 °C. Sections were co-exposed with a high activity [^{125}I] microscale (range 1500-2.8 nCi/mg) (American Radiolabeled Chemicals Inc., Saint Louis, MO, USA) to express [^{125}I] in nCi/mg of radioactivity concentrations in the defined regions of interest.

Enzyme-based microelectrode array measurements of glutamate release and reuptake kinetics

Ceramic microelectrode arrays (MEAs) containing four platinum (Pt) recording surfaces, as illustrated in Fig. S6B, were acquired from Quanteon, L.L.C., Nicholasville, KY, USA. The size of an electrode site was 15x333 µm, with a distance of 100 µm between the pairs of electrodes, each pair being 30 µm apart. The MEAs were step-wise coated in order to measure L-glutamate as previously described [18-19] with in depth description and illustration in a previously published book chapter. [20] All four sites of the MEAs were electroplated with meta-phenylenediamine (mPD, 1,3-phenylenediamine dihydrochloride, Fisher Scientific, Gothenburg, Sweden), to form a size-exclusion matrix over the sites, preventing monoamines and electroactive compounds including 5-HT, dopamine and ascorbic acid from reaching the recording surface. The mPD layer was applied using a 5 mM mPD solution prepared in deoxygenated 0.05 M phosphate-buffered saline (PBS, pH 7.4), by applying a potential of +0.5V to the Pt sites versus a silver/silver chloride (Ag/AgCl) reference electrode (Pronexus Analytical, Stockholm, Sweden). Two of the MEA Pt sites were coated with L-glutamate oxidase (L-Glu-Ox, Yamasa Corporation, Tokyo, Japan) within bovine serum albumin (BSA, Sigma) and glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA), while the other pair of sites were only coated with the inert protein matrix of BSA and glutaraldehyde serving as sentinel

sites. L-glutamate is broken down into α-ketoglutarate and peroxide (H_2O_2), in the presence of Glu-Ox, enabling detection of L-glutamate levels at the enzyme coated sites and self-referencing minimization of background signals or interferents by subtraction using the electrochemical measures from the sentinel sites. H_2O_2 is small enough to go through the mPD layer, resulting in oxidation with loss of electrons when the Pt surfaces are polarized at +0.7 volts verus a Ag/AgCl reference electrode. These processes enable recordings of currents by use of constant voltage amperometry, using the FAST-16 equipment (Fast Analytical Sensor Technology) (Quanteon, L.L.C., Nicholasville, KY, USA). Each MEA was calibrated for sensitivity for glutamate concentrations and for electroactive interferents. MEAs were selected on the basis of results of *in vitro* calibration prior to each experiment as shown in Fig. S6A, in accordance with a previous protocol. [18-19] Additions of 250 µM ascorbic acid (Sigma-Aldrich); and 2 µM dopamine (Sigma-Aldrich) were used to assess the size-exclusion of the mPD coating by testing for blockade of electroactive molecules that are abundant within the brain regions where the *in vivo* recordings were performed. Stepwise increases in concentration of 20, 40, and 60 µM of L-glutamate (Sigma-Aldrich) were applied during the calibration to calculate the slope for the MEA sensitivity to L-glutamate, selectivity ratio for recording of L-glutamate over ascorbic acid and limit of detection for L-glutamate. A 8.8 µM addition of H_2O_2 (Sigma-Aldrich) was used to ensure that all four channels of the MEA were working properly, with equal responses at the two Glu-Ox coated sites compared to responses at the two sentinel sites (Fig. S6A). [20]

WT and p11KO mice were anesthetized with isoflurane gas (3.0% isoflurane for induction and 0.9-2.0% for maintenance) (Univentor 400, AgnTho's, Stockholm, Sweden) via spontaneous breathing, with constant monitoring of the depth of anesthesia. Mice were placed in a stereotaxic frame (David Kopf Instruments) fitted with a Cunningham™ Mouse Adaptor (Stoelting Co., Wood Dale, IL, USA), similar to our stereotaxic procedure previously described. [3] Body temperature was constantly maintained, using electrophysiology compatible control systems (World Precision Instruments, Stevenage, UK). The MEA assemblies were inserted into regions of interest using stereotaxic coordinates according to the mouse brain atlas [15] (Fig. S6C-D). For hippocampal CA1 and DG: anterior–posterior (AP) coordinates referring to bregma AP: -1.7 mm, medial-lateral (ML) coordinates to the midsagittal suture line ML: ±1.0 mm. The dorsal-ventral (DV) coordinates from the surface of the brain DV: -1.5 mm for CA1 and further lowering the MEA to DV: -2.0 for recordings in the DG. For histological verification of MEA recording sites, mice received an injection of 0.25 µl methylene blue solution under isoflurane anesthesia. Correct MEA position was determined by microscopic evaluation of 50 µm coronal brain slices sectioned in a cryostat and counterstained with nuclear fast red (NFP) [21] (see Fig. S6C-D).

Constant voltage amperometry was performed by applying a potential of +0.7V versus an Ag/AgCl reference electrode (Pronexus Analytical, Stockholm, Sweden), implanted into the cerebellum, a site remote from the recording areas. [18-20] An isotonic solution of 120 mM KCl (120 mM KCl, 29 mM NaCl, and 2.5 µM $CaCl_2$, pH 7.4, Sigma) or 10 µM CP94253 in 120 mM KCl solution was filled into a single-barrel glass micropipette positioned at a distance of 50-100 µm from the MEA recording sites. The depolarizing solutions were pressure ejected for one second using a Picospritzer III (Parker Hannifin, Cleveland, OH, USA). Amperometric data were recorded at a frequency of 2 Hz by the FAST system. After reaching a stable baseline signal, 3-5 distinctive peaks were averaged for generation of one data point for each position and mouse as shown in Fig. 3B. The Quanteon FAST 2.3 software (Quanteon, L.L.C., Nicholasville, KY, USA) was used for assessment of the peaks, in conjunction with the MEA calibration constants for analysis of the responses.

Immunoblotting of total proteins and their phosphorylation state

Mice used for western blotting were treated pharmacologically as described at specific time points and sacrificed by focused microwave irradiation (1.4s at 4.5–5kW) using a small animal microwave (Muromachi Kikai, Tokyo, Japan) to inhibit phosphatase activity. This procedure enables accurate measurements of protein phosphorylation. Regions of interest were dissected, frozen on dry ice and stored at -80°C until processed as previously described [4,17,22] Tissue from hippocampi were sonicated and boiled in 1% sodium dodecyl sulfate (SDS). Protein concentration was determined in each sample using a bicinchoninic acid protein assay (BCA-kit, Pierce, Rockford, IL, USA). Equal amounts of protein (10 or 20µg) were separated by SDS–polyacrylamide gel electrophoresis using 7.5-9% lower running gels. Proteins were transferred to Immobilon-P (Polyvinylidene Difluoride) membranes (Sigma-Aldrich, St. Louis, MO, USA). Membranes were blocked by incubation in 5% (w/v) dry milk in TBS-Tween20 for 1 h at room temperature. Primary antibodies used for detection of the levels of total proteins and their phosphorylation-states are shown in supplementary Table S1. Following incubation with primary antibodies for 2 hours or overnight, the membranes were washed three times with TBS-Tween20 and incubated for 1 hour with secondary horseradish peroxidase (HRP)-linked Anti-Rabbit IgG(H+L) (Thermo Scientific; 1:6000 dilution). Immunoreactive bands were detected by enhanced chemiluminescence (Perkin Elmer, Waltham, MA, USA) and quantified by densitometry with the National Institutes of Health ImageJ 1.40 software. All data are presented as values normalized to the levels of β-actin (Fig. 3C-D). The level of the phosphorylated form of a protein was normalized to the total level of the same protein.

Table S1. Antibodies used for measurements of phosphorylation states and total protein levels in hippocampal tissue samples from WT and p11KO mice

Primary antibody	Company	Dilution
Phosphorylation-state specific antibodies		
P-Ser^{831}-GluR1	Millipore	1:500
P-Ser^{845}-GluR1	Upstate Biotechnology Inc.	1:500
P-Ser^{896}-NR1	Upstate Biotechnology Inc.	1:500
P-Ser^{897}-NR1	Cell Signaling Technology	1:500
P-Ser^{1232}-NR2A	Tocris	1:500
P-Ser^{1303}-NR2B	Upstate Biotechnology Inc.	1:500
P-Tyr^{1472}-NR2B	Calbiochem	1:500
Specific antibodies against total protein		
T-GluR1	Upstate Biotechnology Inc.	1:1000
T-NR1	Upstate Biotechnology Inc.	1:1000
T-NR2A	Calbiochem	1:500
T-NR2B	Calbiochem	1:1000
β-Actin	Sigma-Aldrich	1:4000

Calbiochem, Merck, Darmstadt, Germany; Cell Signaling Technology Inc., Danvers, MA, USA; Millipore Corporation, Billerica, MA, USA; Sigma-Aldrich, St. Louis, MO, USA; Tocris Bioscience, Bristol, UK; Upstate Biotechnology Inc., MA, USA.

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

The technical progress of MR-based imaging methods for mice has enabled an increased use of backtranslational studies to identify subtle abnormalities of neurochemical alterations, structural abnormalities, neuronal connectivity and functions found in depressed patients and healthy subjects. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive imaging tool to measure global metabolism of multiple neurochemicals in selected brain areas.

Quantification of brain metabolites by ^{1}H-MRS was carried out at the Experimental MRI Research Center at the Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden. [23] WT and p11KO mice were anesthetized with 3.0% isoflurane for induction and kept at 0.9-2.0% isoflurane with 50% oxygen by spontaneous breathing through a mask for maintenance of anesthesia. Mice were gently placed in a supine position in a plastic holder and secured to the acrylic rig. Heart rate and respiration were monitored with the body temperature adjusted to 36 ±1.0 °C by a warm air stream, using a MRI compatible control system (Small Animal Instruments Inc., Stony Brook, NY, USA). The amount of isoflurane was adjusted to maintain 80±30 respirations/min throughout the imaging. ^{1}H-MRS determination of metabolites was performed with a 4.7 T/ 40-cm magnet with a horizontal bore (Bruker BioSpec Avance 47/40; Bruker, Karlsruhe, Germany), equipped with a 12-cm inner diameter self-shielded gradient system (maximum gradient strength = 200 mTm^{-1}). A volume coil with inner diameter of 25 mm was used for excitation and detection signal [23-24] The positioning of the volume of interest (VOI), i.e. the voxel, was based on axial, sagittal, and coronal multislice high-resolution anatomical images [fast spin echo/rapid acquisition with relaxation enhancement (RARE)]. The voxel was unilaterally positioned in one hippocampus per mouse as illustrated in Fig. 4A. Assessment of the water peak in the VOI was performed before each ^{1}H-MRS spectroscopy. Spectra were obtained from WT and p11KO mice with VOI of 3.0 x 1.8 x 1.8 (representing a volume of 9.72 uL) (Fig. 4B-C). Each spectrum represents an average of 512 scans, with an acquisition duration of 29 min. [24]

The software package LCModel (Linear Combination of Model spectra) (http://s-provencher.com) was used for analysis of the spectra and assessment of metabolites. [23] LCModel applies a quantification algorithm to linear combinations of model spectra to calculate the best fit of the experimental spectra. Calibration of the model spectra are performed to match magnetic field strength, sequence type, and sequence parameters used for data acquisition. Criteria set to select reliable metabolic quantification were based on error estimates (%SD) of the fit for each metabolite (Cramér–Rao lower bounds, CRLB). Metabolites which had an estimated concentration of zero were considered unreliable. Resulting metabolite concentrations are not absolute but given as estimated ratios relative to total creatine concentrations (Cr + PCr) as shown in Fig. 4D.

SUPPLEMENTARY REFERENCES

1. Ögren SO, Eriksson TM, Elvander-Tottie E, D'Addario C, Ekström JC, Svenningsson P, Meister B, Kehr J, Stiedl O. The role of 5-HT(1A) receptors in learning and memory. *Behav Brain Res* 2008; **195**(1): 54-77.

2. Eriksson TM, Madjid N, Elvander-Tottie E, Stiedl O, Svenningsson P, Ogren SO. Blockade of 5-HT 1B receptors facilitates contextual aversive learning in mice by disinhibition of cholinergic and glutamatergic neurotransmission. *Neuropharmacology* 2008; **54**(7): 1041-1050.

3. Baarendse PJ, van Grootheest G, Jansen RF, Pieneman AW, Ogren SO, Verhage M, Stiedl O. Differential involvement of the dorsal hippocampus in passive avoidance in C57bl/6J and DBA/2J mice. *Hippocampus* 2008; **18**(1): 11-19.

4. Eriksson TM, Delagrange P, Spedding M, Popoli M, Mathé AA, Ogren SO, Svenningsson P. Emotional memory impairments in a genetic rat model of depression: involvement of 5-HT/MEK/Arc signaling in restoration. *Mol Psychiatry* 2012; **2**: 173-184.

5. Denny CA, Burghardt NS, Schachter DM, Hen R, Drew MR. 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. *Hippocampus* 2011; Jul 7, doi: 10.1002/hipo.20964.

6. Ennaceur A. One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* 2010; **215**(2): 244-254.

7. McDowell KA, Hutchinson AN, Wong-Goodrich SJ, Presby MM, Su D, Rodriguiz RM, Law KC, Williams CL, Wetsel WC, West AE. Reduced cortical BDNF expression and aberrant memory in Carf knock-out mice. *J Neurosci* 2010; **30**(22): 7453-7465.

8. Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp Brain Res* 1997; **113**: 509–519.

9. Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci* 2004; **24**: 5901–5908.

10. Goodman T, Trouche S, Massou I, Verret L, Zerwas M, Roullet P, Rampon C. Young hippocampal neurons are critical for recent and remote spatial memory in adult mice. *Neuroscience* 2010; **171**(3): 769-778.

11. Barker GR, Warburton EC. When is the hippocampus involved in recognition memory? *J Neurosci* 2011; **31**(29): 10721-10731.

12. Goodrich-Hunsaker, N, Hunsaker M, Kesner R. Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information processing. *Behav Neurosci* 2005; **119**: 1307–1315.

13. Hammond RS, Tull LE, Stackman RW. On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* 2004; **82**: 26–34.

14. Alexander B, Warner-Schmidt J, Eriksson T, Tamminga C, Arango-Lievano M, Ghose S, Vernov M, Stavarache M, Musatov S, Flajolet M, Svenningsson P, Greengard P, Kaplitt MG. Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens. *Sci Transl Med* 2010; **2**(54): 54ra76.

15. Franklin KBJ, Paxinos G. *The Mouse Brain in Stereotaxic coordinates*, Academic, San Diego, 1997.

16. Svenningsson P, Le Moine C, Aubert I, Burbaud P, Fredholm BB, Bloch B. Cellular distribution of adenosine A2A receptor mRNA in the primate striatum. *J Comp Neurol* 1998; **399**: 229–240.

17. Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M, Vaugeois JM, Nomikos GG, Greengard P. Alterations in 5-HT1B receptor function by p11 in depression-like states. *Science* 2006; **311**(5757): 77-80.

18. Bao X, Pal R, Hascup KN, Wang Y, Wang WT, Xu W, Hui D, Agbas A, Wang X, Michaelis ML, Choi IY, Belousov AB, Gerhardt GA, Michaelis EK. Transgenic expression of Glud1 (glutamate dehydrogenase 1) in neurons: in vivo model of enhanced glutamate release, altered synaptic plasticity, and selective neuronal vulnerability. *J Neurosci* 2009; **29**(44): 13929-13944.

19. Thomas TC, Grandy DK, Gerhardt GA, Glaser PE. Decreased dopamine D4 receptor expression increases extracellular glutamate and alters its regulation in mouse striatum. *Neuropsychopharmacology* 2009; **34**(2): 436-445.

20. Hascup KN, Rutherford EC, Quintero JE, Day BK, Nickell JR, Pomerleau F, Huettl P, Burmeister JJ, Gerhardt GA. Second-by-Second Measures of L-Glutamate and Other Neurotransmitters Using Enzyme-Based Microelectrode Arrays. *Electrochemical Methods for Neuroscience.* Boca Raton (FL): CRC Press; 2007; Chapter 19.

21. Stiedl O, Birkenfeld K, Palve M, Spiess J. Impairment of conditioned contextual fear of C57BL/6J mice by intracerebral injections of the NMDA receptor antagonist APV. *Behav Brain Res* 2000; **116**(2): 157-168.

22. Svenningsson P, Lindskog M, Ledent C, Parmentier M, Greengard P, Fredholm BB et al. Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D1, dopamine D2, and adenosine A2A receptors. *Proc. Natl. Acad. Sci. U S A*. 2000; **97**: 1856–1860.

23. Ross JM, Öberg J, Brené S, Coppotelli G, Terzioglu M, Pernold K, Goiny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, Olson L. High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proc Natl Acad Sci U S A* 2010; **107**(46): 20087-20092.

24. Westman E, Spenger C, Oberg J, Reyer H, Pahnke J, Wahlund LO. In vivo 1H-magnetic resonance spectroscopy can detect metabolic changes in APP/PS1 mice after donepezil treatment. *BMC Neurosci* 2009; **10**: 33.

SUPPLEMENTARY FIGURE CAPTIONS

Fig. S1. Schematic illustration of the passive avoidance procedure (A) and emotional memory performance of female mice (B-C) and responses to 5-HT_4R agonist stimulation (D-E). During training, a mouse is allowed to explore a bright compartment for 60 s, without access to the adjacent compartment in the automated passive avoidance arena. After opening the door, time is measured for the mouse to enter the dark compartment (training latency). Upon step-though, the door is automatically closed and a weak electrical stimulus (0.3 mA, 2 s scrambled current) is delivered through the grid floor, *i.e.* the unconditioned stimulus (US). After 30 s, the mouse is removed and following a 5 min or 24 h delay, the mouse is placed back into the bright compartment. The door is first closed to the dark compartment to prevent premature step-through responses of the mouse when gently lifted into the arena. The door to the dark compartment is opened and voluntary step-through latency to return to the dark compartment is automatically measured (retention latency). Basal emotional memory performance of female mice in the PA task. PA step-through latencies at training and short-term memory test after 5 min, or long-term memory test 24 h after learning in female WT, H and p11KO mice. Step-through latencies at training and testing in female mice passed normality test and were analyzed by parametric statistics. Basal training latencies were not statistically different in untreated female mice on a two-way ANOVA (B). For retention latencies, a two-way ANOVA showed a main effect of genotype (G: $*P < 0.05$) ($F_{2,37} = 3.300$; $P = 0.048$), with no main effect of test delay ($F_{1,37} = 0.007$; $P = 0.934$) or interaction ($F_{2,37} = 3.173$; $P = 0.054$) (C). Analyses of training versus retention performances were also performed with a two-way ANOVA with repeated measures and subject matching, showing an overall effect of delay ($F_{1,37} = 110.665$; $P < 0.001$) and an interaction between delay and group ($F_{5,37} = 2.543$; $P = 0.045$), but no effect of group ($F_{5,37} = 2.288$; $P = 0.066$). Learning-related avoidance to return to the aversive context were induced in all genotypes when tested after 5 min, (WT; KO: $+++P < 0.001$; H: $++P < 0.01$) (C). In comparison, significantly increased avoidance latencies were present only in WT and H mice when instead tested after the longer delay of 24 h ($+++P < 0.001$) (C). A two-way ANOVA for training latencies showed an overall effect of the partial 5-HT_4R agonist RS67333 in male mice (1 mg/kg, 6 days subchronic) ($F_{1,54} = 5.762$; $P = 0.020$), genotype ($F_{1,54} = 5.283$; $P = 0.025$), but no interaction ($F_{1,54} = 1.738$; $P = 0.193$) (D). When analyzing performance at the memory retention test, a two-way ANOVA showed an interaction between RS67333 and genotype ($F_{1,54} = 6.114$; $P = 0.017$), main effect of RS67333 ($F_{1,54} = 23.895$; $P < 0.001$) and not genotype ($F_{1,54} = 0.472$; $P = 0.495$) (E). Post hoc analysis with Newman-Keuls multiple comparison test indicated that p11KO mice treated with vehicle performed significantly lower than WT mice with vehicle ($P < 0.05$). However, subchronic treatment of p11KO mice with RS67333 induced a significant improvement compared to p11KO mice with vehicle ($P < 0.001$). Repeated measures two-way ANOVA of training versus retention latencies showed an overall difference between training and testing days ($F_{1,54} = 109.646$; $P < 0.001$), group ($F_{3,54} = 7.500$; $P < 0.001$) and interaction ($F_{3,54} = 10.818$; $P < 0.001$) (E). Pairwise Bonferroni test indicated significant increase of latencies in WT groups given either vehicle ($P < 0.001$) or RS67333 ($P < 0.001$) and p11KO mice given RS67333 ($P < 0.001$) (E). Thus, while p11 KO mice given vehicle did not display significant passive avoidance behavior at the memory test, the 5-HT_4R agonist improved emotional memory performance in p11KO mice. Data are presented as means ±S.E.M. n= 6-13 (B-C), 10-19 (D-E). p11KO: p11 knock-out mice, H: p11 heterozygous mice, WT: wild type mice, RS: RS67333 (partial 5-HT_4R agonist).

Fig. S2. Schematic setup of the Novel object recognition (NOR) procedure (A) and NOR performance of WT, p11HET and p11KO mice (B-C) and responses to 5-HT_4R agonist stimulation (D-E). During training (T1), a mouse explores an open-field arena containing two identical objects. Following a delay of either 1 h or 24 h, the mouse is once again placed in the open-field arena for a memory test (T2). One of the two objects previously presented is replaced with another object, *i.e.* the arena contains one familiar (F) and one novel (N) object at T2. The relative level of exploration of each object is calculated as a discrimination index: $(Time_N - Time_F)/(Time_N + Time_F)$. (B) shows novel object recognition memory performance of female mice. Positive scores indicate preference for the novel object, negative values derive from preference for the familiar object, and scores close to "0" indicate no preference for either object. One-way ANOVA showed no main effect of genotype. When assessing whether the level of preference was significantly different from chance, WT (+P <0.05) (one-sample *t* test, t_{12} =2.777, P =0.017) and p11HET mice (++*P* <0.01) (t_{11} =3.328, *P* =0.007) showed a significant preference for the novel object, while p11KO mice did not discriminate (B). To control for individual, genotype- and treatment-dependent differences in exploration, the total level of object exploration was measured. One-way ANOVA showed an overall difference in object exploration ($F_{2,42}$ =9.096; *P* <0.001), with post hoc Newman-Keuls multiple comparison test indicating that p11HET (**P* <0.05) and p11KO (****P* <0.001) mice spent less time exploring the objects (C). Since RS67333 may mediate procognitive effects, the novel object recognition task was made more difficult by prolonging the delay between object exposure and testing from 1 to 24 h. The longer delay of 24 h between training and testing resulted in a low basal memory performance in the study with RS67333 (D). A two-way ANOVA on discrimination index showed a significant effect of genotype (G: **P* <0.05) ($F_{1,44}$ =6.704; *P* =0.013), with no main effect of RS67333 ($F_{1,44}$ =1.102; *P* =0.300) or interaction ($F_{1,44}$ =0.459; P =0.502) (D). WT mice showed no significant preference for the novel object, by vehicle or RS67333. P11KO mice treated with vehicle did not discriminate, while RS67333 significantly increased discrimination in p11KO mice, supporting recognition of the familiar (one-sample *t* test, t_{11}=2.526, *P* =0.028) (D). Object exploration differed on a two-way ANOVA, with an effect of genotype (G: **P* <0.05) ($F_{1,44}$ =6.899; *P* =0.012), with no effect of treatment ($F_{1,44}$ =2.208; *P* =0.144) or interaction ($F_{1,44}$ =2.627; *P* =0.112) (E). Post hoc analysis with Newman-Keuls multiple comparison test, showed a significant reduction of exploration in p11KO mice by RS67333 (**P* <0.05) and that the effect of RS67333 differed in WT and p11KO mice (**P* <0.05) (E). Data are presented as means ±S.E.M. p11KO: p11 knock-out mice, p11HET: p11 heterozygous mice, WT: wild type mice, RS: RS67333 (partial 5-HT_4R agonist).

Fig. S3. Open field performance of WT, p11HET and p11KO mice (A-B) and responsivity to 5-HT_4R agonist stimulation (C-D). The total distance moved in the open-field arena did not differ (A). The level of thigmotaxis differed in one-way ANOVA ($F_{2,37}$ =9.483; *P* <0.001). Newman-Keuls test indicated that p11KO (****P* <0.001) and p11HET (**P* <0.05) mice spent more time within 5 cm from the walls in the open-field arena (thigmotaxis) (B). For RS67333, there were no statistical differences on total distance or the level of thigmotaxis (C-D). Data are presented as means ±S.E.M. p11KO: p11 knock-out mice, p11HET: p11 heterozygous mice, WT: wild type mice, RS: RS67333 (partial 5-HT_4R agonist).

Fig. S4. Object recognition of WT and p11KO mice following hippocampal gene transfer with control AAV.YFP or overexpression AAV.p11. NOR data support a role of hippocampal p11 in a bidirectional response to 5-HT_{1B}R agonist stimulation with CP94253 in WT versus p11KO mice. In mice given the control AAV.YFP, a two-way ANOVA showed no overall effect between the groups on discrimination index (A). Treatment with CP94253 in p11KO mice induced an enhanced preference for the novel object (+P <0.05) (one-sample *t* test, t_9=2.825, P =0.020) (A), while WT mice treated with either vehicle or CP94253 as well as vehicle-treated p11KO mice did not show significant object recognition for the novel object (A). Object exploration did not significantly differ (B). In groups that were given the AAV.p11 vector, a two-way ANOVA showed no overall effect on discrimination index. WT groups of mice and p11KO mice treated with vehicle did not show significant preference for the novel object following AAV.p11. However, after hippocampal overexpression of p11, p11KO mice no longer displayed the abnormal facilitatory effect of CP94253 (C). Thus, the enhancing effect of CP94253 was reversed after hippocampal AAV.p11, reminiscent of the normalization of passive avoidance performance (Fig. 2M-N). There was a significant effects on object exploration on a two-way ANOVA by CP94253 (CP: ***P <0.001) ($F_{1,28}$ =15.630; P <0.001), genotype (G: *P <0.05) ($F_{1,28}$ =6.529; P =0.016) with no interaction ($F_{1,28}$ =1.766; P <0.195) (D). Post hoc Newman-Keuls test indicated that object exploration was significantly higher in WT mice given CP94253 compared to WT mice treated with vehicle, while there was no difference between p11KO mice treated vehicle or CP94253 (D). Data are presented as means ±S.E.M. p11KO: p11 knock-out mice, WT: wild type mice, CP: CP94253 (10 mg/kg, acute injection).

Fig. S5. Open field behaviors of p11KO mice following hippocampal gene transfer with control AAV.YFP or overexpressing AAV.p11. None of the hippocampal AAV treatments altered open-field behaviors, supporting that effects of hippocampal overexpression of p11 were restricted to behavioral functions largely involving hippocampal neurotransmission (A-D). A two-way ANOVA showed effect on distance by genotype (G: *P <0.05) ($F_{1,30}$ =5.460; P =0.026) and CP94253 (CP: **P <0.01) ($F_{1,30}$ =8.774; P =0.006) with no interaction ($F_{1,30}$ =1.724; P =0.199). Newman-Keuls test indicated that CP94253 induced a stimulation of mobility in WT mice (*P <0.05), and that this stimulatory effect of CP94253 was significantly lower in p11KO mice (#P <0.05) (A). There were no statistical differences in thigmotaxis in any of the groups (B). Open-field activity differed in mice given AAV.p11 (C), similar to groups given AAV.YFP (A), as shown by effects on distance in a two-way ANOVA by genotype (G: ***P <0.001) ($F_{1,28}$ =14.640; P <0.001), and CP-94253 (CP: **P <0.01) ($F_{1,28}$ =8.774; P =0.008) with no interaction ($F_{1,28}$ =2.484; P =0.126) (C). Newman-Keuls test demonstrated an increased distance by CP94253 in WT mice (**P <0.01), significantly different from the effect of CP94253 in p11KO mice (##P <0.01) (C), thus regardless whether mice had been injected with AAV.YFP (A) or AAV.p11 (C). Thigmotaxis did not differ between the studied groups (D). Data are presented as means ±S.E.M. p11KO: p11 knock-out mice, WT: wild type mice, CP: CP94253 (10 mg/kg, acute injection).

Fig. S6. Complementary data on MEA measurements of glutamate dynamics in the hippocampus. Schematic drawing of the principle for amperometric recordings with glutamate-oxidase enzyme coated recording sites (channels 1 and 2) and reference sites (channels 3 and 4) coated only with inert protein matrix, as described in Materials and Methods (A). Representative recordings from an *in vitro* calibration of an MEA, showing responses on glutamate recording sites (corresponding to responses in red and black), reference sites (corresponding to responses in blue and green) and event markers (B). The 250 µM ascorbic acid (AA) and 2 µM dopamine (DA) additions were used to evaluate the size-exclusion of the mPD layer. The 20-60 µM additions of L-glutamate (Glu) were applied to calculate the slope, selectivity ratio and limit of detection for L-glutamate. The 8.8 µM of H_2O_2 addition was used to test for similar responsivity at all four channels of the MEA. Histological verification of DG and CA1 hippocampal recording sites (C-D) in the mouse hippocampus. The same stereotaxic coordinates were used as for focal AAV gene transfer of p11. Photomicrograph of 50 µm coronal brain section counterstained with NFR for contrast enhancement illustrating representative location of unilateral methylene blue solution in the CA1 region of the mouse (C). Atlas diagram of corresponding brain regions (D) as defined and adopted from the mouse brain atlas. [15] Stereotaxic coordinates refer to bregma. Infused methylene blue in the hippocampal CA1 region at a depth of DV -1.5 mm, illustrated by the blue box for CA1 (D). The stereotaxic coordinates used for DG were identical to those for CA1, except for deeper recordings at DV -2.0, illustrated by the green box for DG (D). The T_{80} clearance duration in seconds between peak maximum amplitude and 80% reduction, with no significant differences in hippocampal DG and CA1 subregions are seen in part E. Average volumes of depolarizing 120 mM KCL solution used for evoking glutamate release did not differ in DG and CA1 (F). Data are presented as means ±SEM for n = DG:10-14 and CA1:8-10 recordings per group. CA1: cornu ammonis 1 of hippocampus, DG: dentate gyrus of hippocampus, CP: CP94253 (5-HT_{1B}R agonist), K: KCL (potassium chloride, 120 mM), MEA: microelectrode array, NFR: nuclear fast red counterstaining.