Supplementary Materials and Methods

Animals.

Experiments were conducted in compliance with the standard ethical guidelines (European community guidelines and French Agriculture and Forestry Ministry guidelines for handling animals-decree 87849). The mouse lines were maintained on a C57Bl/6 background and locally bred. For *Pet1KO* mouse line (gift of Evan Deneris, Case Western Reserve University, Cleveland, USA), heterozygote $Pet1^{+/-}$ females were mated with $Pet1^{+/-}$ or $Pet1^{-/-}$ males to produce mixed litters. Littermates were used in all experiments and both $Pet1^{+/+}$ and $Pet1^{+/-}$ served as controls since no differences in 5-HT levels were found between the two genotypes (Hendricks *et al*, 2003; Kiyasova *et al*, 2011). 5-HT transporter (*Sert*, Slc6a4) Cre mouse line is a knock-in of the nls-Cre in the 5'UTR region of the *Sert* gene; experiments were done on *Sert* Cre/+ mice obtained by crossing *Sert* Cre/Cre male mice with C57Bl/6 mice (Narboux-Nême *et al*, 2008). The floxed *VMAT2* mouse line was previously described (Narboux-Nême *et al*, 2011). In all cases genotyping of the progeny was done by PCR analysis of tail DNA.

Three-chamber social recognition

Sociability and preference for social novelty were measured in a three-chamber box (30 × 15 × 15 cm per compartment) with an opening (5 × 5cm) in each delimiting wall. The test is divided in 4 phases, each lasting 5 min. During the habituation phase, the test mouse was restrained to the central chamber, with the two doorways closed. Secondly, the doors were opened allowing the mouse to visit either the compartment containing an unfamiliar adult male mouse (C57BL/6J) enclosed in a wired cage or the other compartment containing an empty wired cage (sociability phase). The amount of time mice spent in interaction with each wired cage was scored. During the third phase, the mouse was restrained to the chamber containing the mouse and a new social counterpart was place in the wired cage in the opposite compartment. For the preference for social novelty, the mouse was allowed to investigate the familiar mouse or the unfamiliar mouse. The time spent interacting was scored as described for the sociability phase (Moy *et al*, 2004).

Operant conditioning

Seven days prior to the experience mice were food-restricted to 80% of food weigh respective to their normal food consumption initially measured. This food intake protocol was maintained during the test. Mice were trained for operant conditioning using polymodal cages with two nose-poke holes on either side of a central food magazine (Imétronic, France). The active hole was cued by a white light above it, and was paired with a delivery of a 20mg sucrose pellet in the food magazine (reward). Poking in the "inactive hole", with no light above, had no consequence (sham). After poking in an active hole and delivery of a pellet, the cue light was switched off for 10 s, during which poking was without effect. During training, the numbers of pokes in the reinforced hole, non-reinforced hole, and food cup (full or empty) were recorded every 5 min, in addition to the number of obtained pellets. Mice were trained in 1-h daily sessions in the same operant boxes for a total of 15 days. The training was comprised of three session blocks of 5 days in which increasing fixed ratio (FR) schedules were applied: FR1 (days 1–5), FR5 (days 6–10) and FR10 (days 11–15). Only the results from FR5 sessions are shown here (Maroteaux *et al*, 2014).

Rotarod

Motor learning was measured in a rotating rod across daily sessions. The cylinder was 3cm in diameter and rotated from 4 rpm to 40 rpm in 5 min. Latency to fall in seconds was scored with a 5-min cut off limit. Animals were given 5 trials each day.

Cued Fear extinction

Fear extinction was adapted from (Herry *et al*, 2006). In brief, animals were submitted to a fear conditioning protocol where a tone (5 KHz, 80 dB, 29 s) was paired with a footshock (0.6 mA, 1 s). The onset of the footshock concurred with the offset of the tone. After a 2-min habituation period, five pairings were delivered to each mouse with a semi-randomized inter-trial interval of 30-50 s. Fear extinction occurred 4 h after in a context different in shape, smell, texture and light intensity. After a 2-min period, 16 tone presentations (30 s each) were delivered with a semi-randomized inter-trial interval of 30-45 s (total length of the session app. 20 min). In all cases, sessions were recorded and freezing was manually scored by an experienced observer. Freezing was defined as complete absence of movement except for respiration. Data were expressed as % time freezing.

In vitro fEPSP recordings

Coronal hippocampal slices (350 µm) were prepared from 6-8 weeks old male C57Bl/6 mice using a vibratome (Microm HM650V, Thermo Scientific) and placed in aCSF containing (in mM): 11 glucose, 2.5 KCl, 26.2 NaHCO3, 1 NaH2PO4, 124 NaCl, 2 CaCl2, 2 MgCl2 bubbled with a mixture of 95% O2/5% CO2 and allow 1 h to recover. fEPSPs were recorded in the stratum radiatum of the CA1 region with a borosilicate glass pipettes filled with aCSF and stimuli delivered to the Schaeffer Collateral Pathway by a bipolar tungsten electrode (WPI, Germany). Slices were visualized in a chamber on an Olympus BX51WI upright microscope and were perfused with the oxygenated aCSF at 31°C in the continuous presence of 100 µM picrotoxin to block GABAergic function. Stimuli were delivered at 0.1Hz, and an input-output curve was created at the beginning of the recordings and stimulus was kept at 30-40% of maximal response. The amplitude of the fEPSP was measured and averaged baseline values were normalized to 100%; subsequent values of the experiment were normalized to this baseline average (one minute bins). 8-OH-DPAT was dissolved in water and added to the bath perfusate at a final concentration of 100nM.

The following replication-defective adeno-associated viruses (AAV) were used: AAV8-hSyn-DIO-hM4D(Gi)-mCherry (UNC Vector Core, University of North Carolina, USA); AAV2/9-hSyn-eGFP-Cre-WPRE and AAV2/9-hSyn-eGFP-rBG (Penn Vector Core, University of Pennsylvania, USA). The titration of these AAVs was within 10^{12}-10^{13} viral molecules/ml.

For stereotaxic virus delivery, mice were injected with ketamine/xylazine solution (150mg/kg and 10mg/kg, respectively) and placed in a small-animal stereotaxic frame (David Kopf Instrument, CA, USA). The skin was incised and retracted to expose the skull and a small hole was drilled using a drill bit with a 0.5mm round tip (Hager and Meisinger, Germany). Viral suspensions were pressure-injected using a pulled glass capillary (30 to 50 µm tip diameter; PCR micropipette, Drummond Scientific Company) fixed to an adapter specially designed to be mounted on the oil hydraulic micromanipulator MO-10 (Narishige, Japan). After injecting 300nl of viral suspension, the pipette was left in place for 5min to prevent back-flow. The coordinates used to target the MnR and the DR were adjusted from (Paxinos and Franklin, 2004), and were

as follows: -4.0 anterior/posterior from bregma, -1.2 medio/lateral and -5.0 dorso/ventral for the MnR; +0.5 anterior/posterior from lambda, -0.8 medio/lateral and -3.2 dorso/ventral for the DR. Injections were done adding a 10° angle to the pipette-holding arm in order to enter the brain diagonally and avoid damaging the ventricle. Animals were given a 3-weeks recovery period to allow maximal expression.

The efficiency of the inhibitory DREADD hM4D to silence neuronal activity in 5-HT cells was tested by in vitro electrophysiological recordings. Briefly, 3 weeks after injection of AAV-DIO-hM4D-mCherry in *Sert*-Cre/+ mice, animals were killed and brain dissected out and used to produce 250µm coronal brain slices containing the raphe nuclei. Slices were kept in aCSF as described above during 1 h before recordings. Slices transferred to a chamber on an Olympus BX51WI upright microscope, and neurons were visualized by combined epifluorescent and infrared/differential interference contrast visualization with 5x and 40x objectives. Expression of mCherry was verified using a TRITC (Rhodamine)/DiI/Cy3 filter (Chroma Technology Corp, USA, #31002). Borosilicate glass pipettes (3-5 MΩ) were made in a HEKA PIP5 puller and filled with 2M NaCl solution. Single mCherry-positive neurons were approached with a pipette and cell-attached recordings were obtained using a Multiclamp 700B (Molecular Devices, Sunnyvale, CA). Signals were collected and stored using a Digidata 1440A converter and pCLAMP 10.2 software (Molecular Devices, CA). Because most 5-HT neurons are silent in slices, phenylephrine 3µM was added to the bath to induce regular firing (Vandermaelen and Aghajanian, 1983). Once a stable discharge frequency was observed, clozapine-N-oxide (CNO) 10 µM was added to the bath to activate hM4D receptors, and changes in firing were monitored.

AAV-injected mice were used to test for object recognition as described before, except that each mouse was subjected to two independent sessions of NORT. During the first session, control and hM4D-expressing mice were divided into saline and CNO groups and a first NORT was conducted. 10 days later, the same animals were tested again but groups were inverted, e.g. mice that received saline were injected with CNO, and vice versa. With this protocol the second NORT was conducted using a complete new set of objects. Object recognition performance was thus compared for the same animal under the two different treatments. After completion of behavioral experiments, brains were processed to confirm the correct injection sites. For this, mice were anesthetized (Pentobarbital 0.5 mg/g) and perfused transcardially with 4% paraformaldehyde in 0.12 M phosphate buffer (PB), pH 7.4. Brains were removed from the skull

and post-fixed overnight in the same fixative. After cryoprotection (PB with 30% sucrose for 48 h) serial 50-µm-thick coronal sections were made on a freezing microtome and collected in PB. Sections containing the raphe nuclei were mounted and cover-slipped in Mowiol (10%, Calbiochem, Germany)-Dabco (2.5%, Sigma-Aldrich Co., MO, USA). Sections were observed and photos taken using a Leica DM6000 fluorescence microscope equipped with a CCD Coolsnap camera, and controlled by Metamorph (Molecular Devices, France). To verify specificity of mCherry expression in 5-HT cell bodies, double immunofluorescent labeling was carried out using primary antibodies against Ds-Red (rabbit, Clontech, France) and TPH2 (sheep, Millipore, France) antibodies. Co-localization was evaluated using a compact confocal microscope FV10i (Olympus), and Z series stacks of confocal images were acquired at 1024 x 1024 pixel resolution, with a pinhole set to one unit. Images were adjusted for optimal brightness and contrast using ImageJ software; additional indications and/or anatomical landmarks were incorporated using Adobe Illustrator v14.0 (Adobe Systems, Mountain View, CA).

Drugs

CNO (Enzo Life Sciences, Lyon, France), (±)-8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT) from Tocris (R&D systems, Lille, France) and benserazide hydrochloride (Sigma-Aldrich) were dissolved in saline solution. 5-Hydroxytryptophan, 5-HTP (Sigma-Aldrich) was dissolved in 0.1N HCl. All the drugs were injected intraperitoneally (ip) in a volume of 0.1 ml/10 g body weight of the animals.

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Supplementary Figures

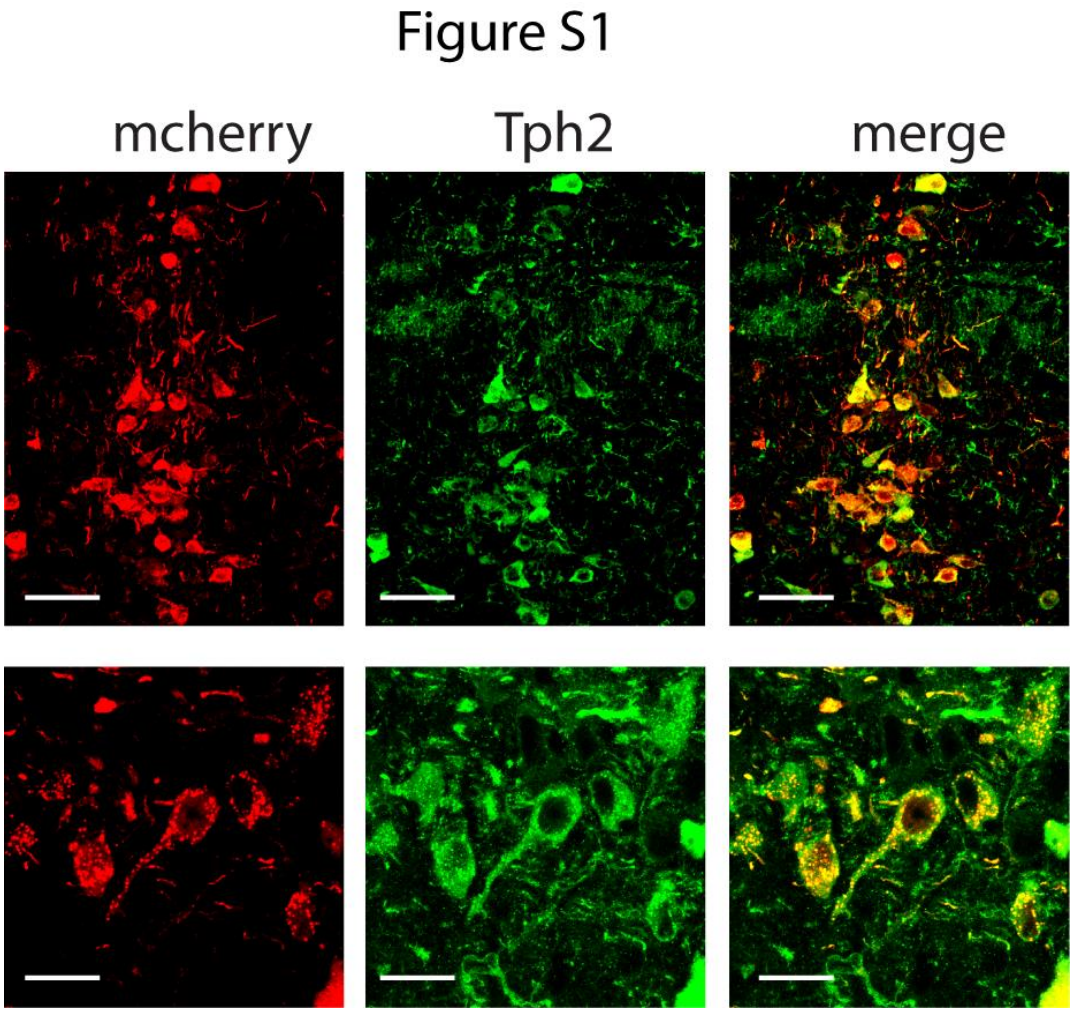

Figure 1. Stereotactic injection of AAV8-hSyn-DIO-hM4D(Gi)-mCherry into the MnR of SertCre mice results in the expression of hM4-mCherry in 5-HT neurons exclusively. Immunohistochemistry confocal micrographs showing expression of mCherry (red) co-expressed with TPH2 (green). Scale bar = 100 µm (top) and 20µm (bottom).

Figure S2

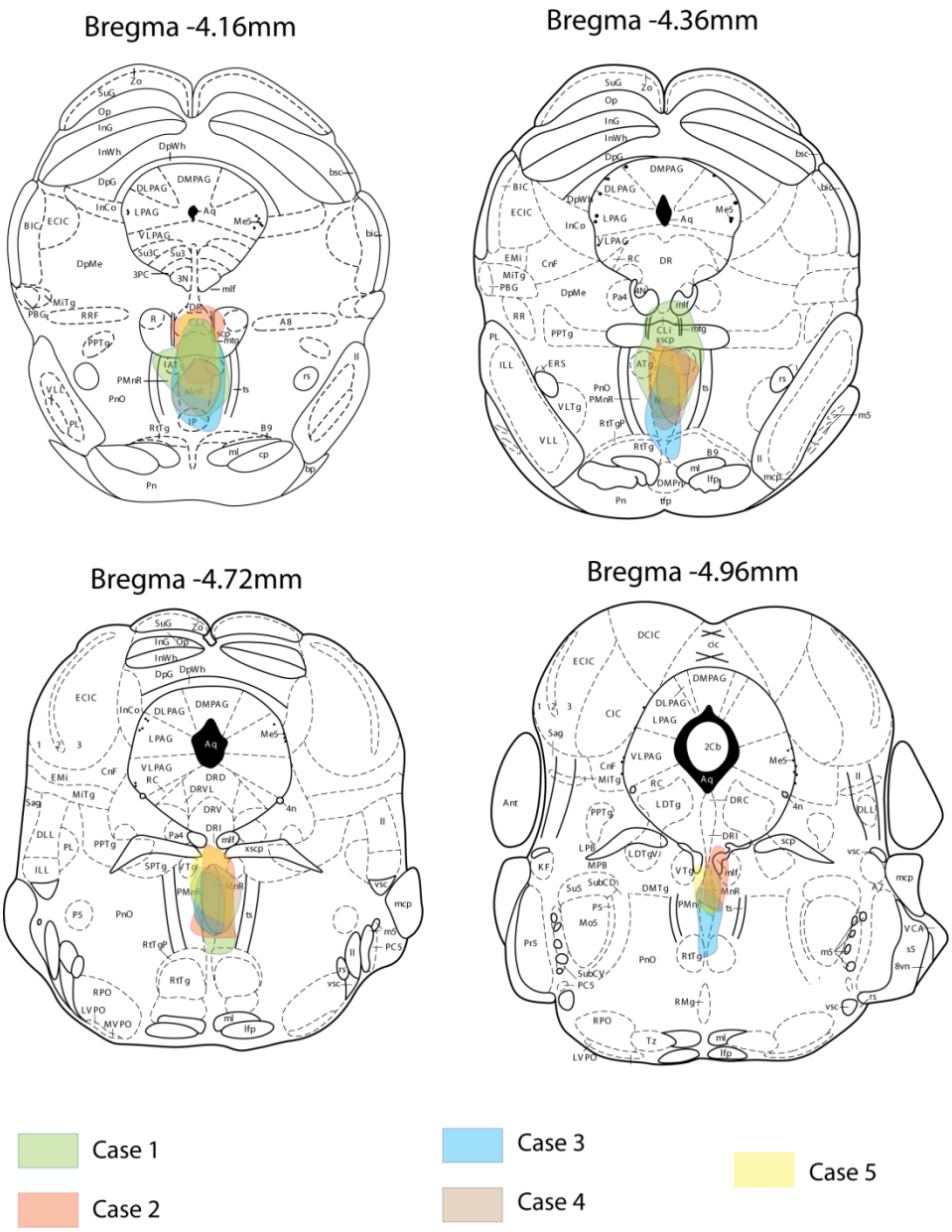

Figure 2. Schematic representation of the localization and extension of viral transfected areas at different rostro-caudal levels in the MnR. Only five independent cases are shown for clarity. Rostro-caudal levels have been matched to corresponding planes on the adult brain mouse atlas (Paxinos and Franklin 2001) using common anatomical landmarks.

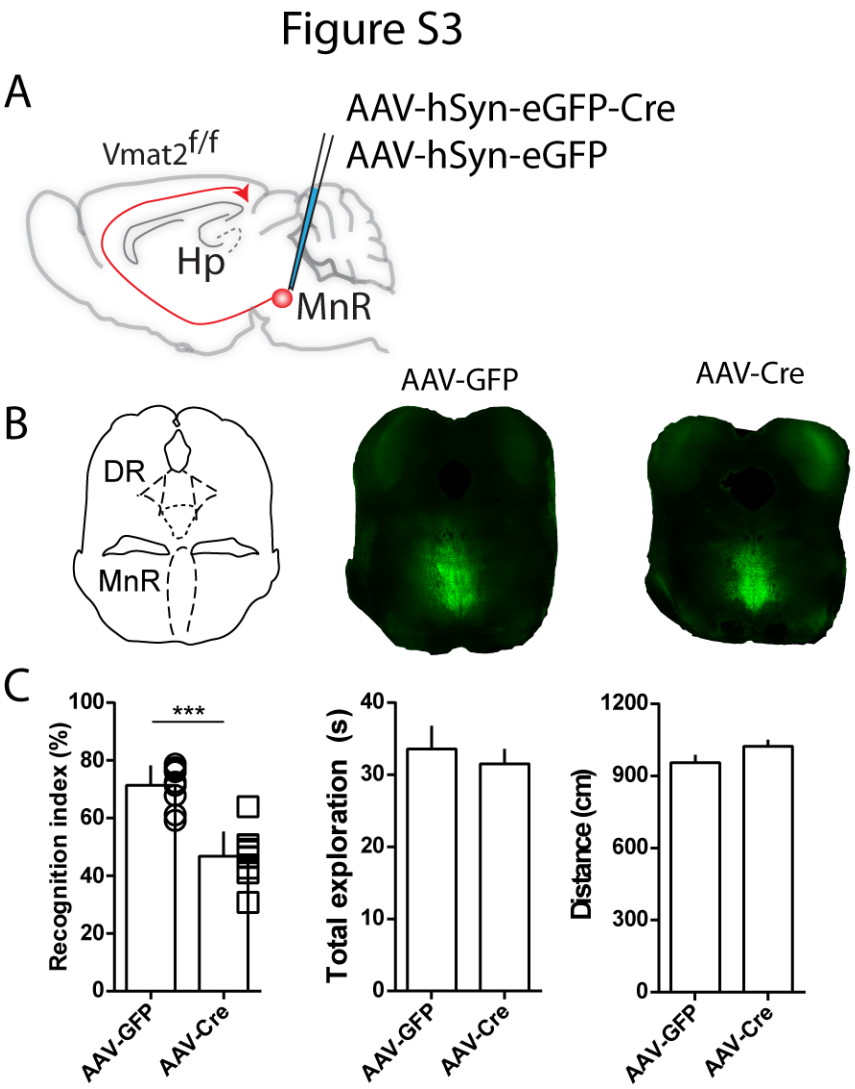

Figure 3. Chronic depletion of brain 5-HT in adulthood impairs object memory. A) Vmat2 floxed mice were injected with a AAV-hSyn-eGFP-CRE or AAV-hSyn-eGFP in the MnR using an stereotaxic apparatus. B) The MnR was successfully targeted without affecting other raphe regions. C) Four weeks after surgery, mice were subjected to the object recognition task as described before using a 2 h interval between training and retention sessions. Recognition index was significantly lower in AAV-Cre injected mice compared to control mice injected with an AAV-eGFP (***P<0.001, unpaired t test, n = 10/group). No changes in locomotor activity or total time exploring the objects were observed. All plots depict mean ± SEM.

Figure S4

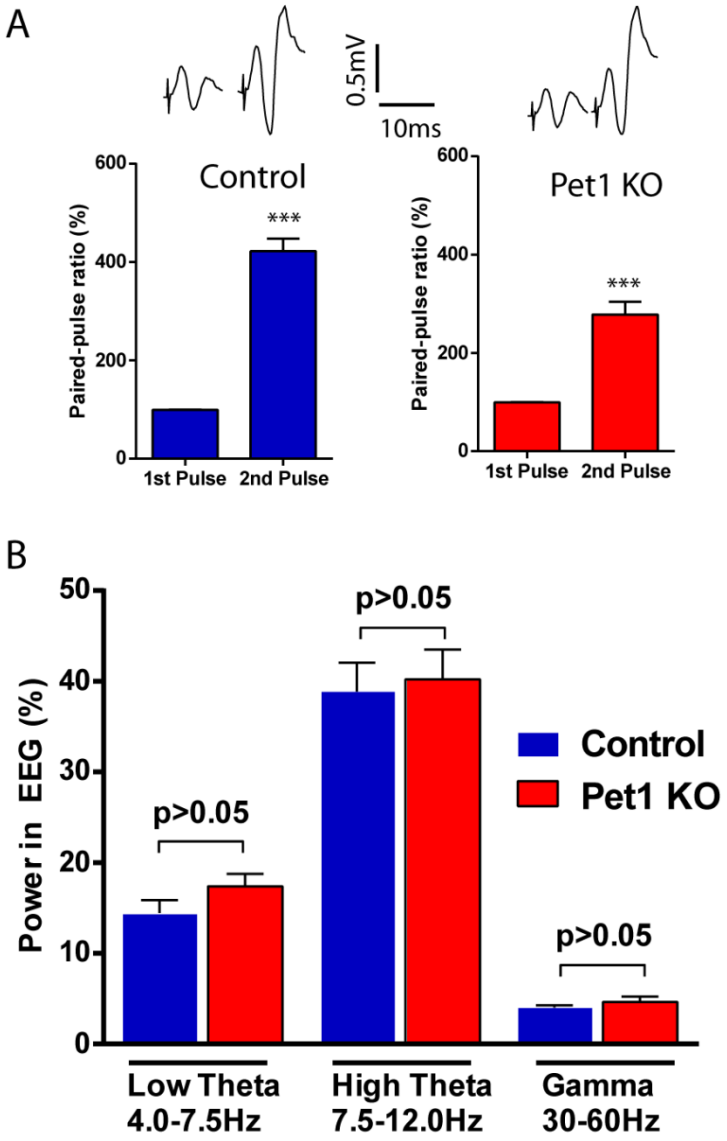

Figure 4. A) Paired-pulse facilitation of the CA3→CA1 synapse in vivo. fEPSPs were evoked at the CA1 with two stimulation pulses (100ms inter-pulse interval). ***P<0.001, paired t test, n = 10 (control) and 9 (*Pet1KO*). B) Electroencephalogram recordings in the CA1 region of the hippocampus of control and *Pet1KO* mice. Theta and gamma frequency oscillations were not different between control and *Pet1KO* mice.