**Supplementary Information**

*Extended Materials and Methods*

*Generation and characterization of Pv-mG5$^{-/-}$ mice*

All animal procedures were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and were approved by The Salk Institute for Biological Studies and University of California San Diego Institutional Animal Care and Use Committees. mGluR5-floxed (control) animals[1] were backcrossed to C57BL/6 for at least six generations, and then crossed to a Pv-Cre line to generate mGluR5-deficient animals carrying the deletion only in Pv-neurons. Cre recombination in the cortex of the Pv-Cre line begins at approximately postnatal day 10 when parvalbumin expression starts and remains stable once it reaches a plateau at approximately 4 weeks of age[2]. Effective Cre-mediated recombination in Pv$^{+}$ neurons was assessed by crossing the Pv-Cre line with mice carrying Cre-dependent tdTomato fluorescence (Ai14 line, Jackson Laboratories; Supplementary Fig. 1B).

*Determination of Pv$^{+}$ neuron numbers.*

Determination of Pv immunoreactive neurons was performed on 50 μm coronal sections using anti-parvalbumin antibody (Swant, Bellizona, Switzerland) and Vectastain as described[3]. Briefly, sections (4 PFC and 6 for hippocampus per animal) were incubated in a 1:2000 dilution of anti-Pv antibody for 24h in PBS containing 2% normal horse serum and 0.1% Triton, followed by Vectastain. Coordinates for regions analyzed were as follows: Prelimbic (bregma 1.9 to 2.4), dorsal caudate putamen (bregma 0.5 to 1.5), and dorsal hippocampus (bregma -1.6 to -2.2). Cell counts across all slices for each region per animal were corrected using Abercrombie's

algorithm[4], and expressed as mean ± SEM.

*In situ hybridization (ISH)*

Postnatal brains were perfused with PFA, washed with PBS and then cryopreserved in 30% sucrose in PBS. Tissues were embedded in Tissue-Tek OCT (Sakura Finetek) and sectioned on a Cryostat (Leica). Antisense RNA probes for ISH were labeled using a DIG-RNA labeling kit (Roche). After ISH, sections were incubated with anti-parvalbumin antibody (Swant, Bellizona, Switzerland) and incubated over night at 4°C in blocking solution containing 3% bovine serum albumin (Fisher Scientific) and 0.1% Tween20 (Sigma) in PBS. Biotinylated (Jackson Immuno Research) secondary antibodies were incubated for 45 minutes at room temperature and then washed with PBS. Immunostaining was developed using the DAB (di-amino-benzidine) colorimetric reaction and the Vectastain kit (Vector) or the AEC substrate kit (Vector).

*Fluorescence immunodetection and confocal imaging.*

The time course of Cre-mediated recombination in $Pv^+$ neurons was assessed by crossing the Pv-Cre line with mice carrying Cre-dependent tdTomato fluorescence (Ai14 line, Jackson Laboratories). Mice were perfusion-fixed and brains were sliced into 50 μm sections. To assess the co-localization of tdTomato and Pv expression, the slices were stained with anti-Pv and Alexa-Fluor488-conjugated secondary antibody. Co-localization studies across five animals showed that 95 ± 2% of tdTomato-labeled cells were also positive for parvalbumin. Determination of Pv and GAD67 immunoreactivity in coronal slices was performed as previously described[5] using a Zeiss LSM780 confocal microscope and a 63× oil immersion objective. Images were taken across 1.2 μm at 0.2 μm Z-steps. To determine levels of

parvalbumin and GAD67 in synaptic contacts, all $Pv^+$ synaptic boutons in the image were selected, and mean fluorescence levels for parvalbumin and GAD67 were determined using ImageJ.

*Electrophysiological recordings in vitro*

Following anesthesia by isofluorane inhalation and euthanasia, slices (350-400 μm thick) that contained the hippocampus were cut from (I) 7-9 week old mice for mIPSC recordings or (II) 4-11 week old mice for fEPSPs studies in (I) a sucrose-based ice-cold solution containing (in mM) 110 sucrose, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 $NaHCO_3$, 1.25 $NaH_2PO_4$, and 10 glucose or (II) in standard aCSF composed of (in mM) 130 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4$, 24 $NaHCO_3$, 1.3 $MgCl_2$, 2 $CaCl_2$, and 10 glucose. After preparation, all slices were transferred to standard aCSF equilibrated with 95% $O_2$/5% $CO_2$. The tissue was then incubated at 35°C for 30 min (skipped for fEPSP experiments) and afterward kept at room temperature (24°C) for ≥1 h before use. For recording, slices were kept minimally submerged at room temperature under superfusion with aCSF bubbled with 95% $O_2$/5% $CO_2$ at ~2 ml/min.

Field-excitatory postsynaptic potentials (fEPSPs) were evoked at 0.067 Hz with 0.2 ms rectangular pulses applied to the Schaffer collaterals, except for PPF, in which pairs of stimuli (with 25-300 ms intervals) were delivered at 0.33 Hz to avoid interference with slower forms of short-term potentiation (augmentation). fEPSPs were recorded with glass pipettes filled with aCSF (2-6 MΩ) and reduced to approximately 40-60% or 70-80% for LTP and LTD experiments, respectively, and stable baselines of at least 20 min were collected. LTP was induced by high-frequency stimulation (100 Hz for 1 s repeated five times at 10 s intervals) or a theta-burst protocol (4 stimuli at 100 Hz repeated 10 times at 200 ms intervals); LTD was

induced by low-frequency stimulation (1000 stimuli at 1 Hz) in low-$MgCl^{2+}$ aCSF (0.65 mM). Synaptic fatigue was accessed by 12 stimuli at 40 Hz.

Miniature synaptic currents (mIPSCs) were recorded in voltage-clamp mode (Vh=-60 mV) in standard aCSF (as above) supplemented with TTX (0.5-1 μM), CNQX (25 μM), and APV (10 μM). The data were digitized at 5 kHz and filtered at 2 kHz. Pipette tip resistance was 4-8 MΩ when filled with internal solution composed of (in mM): CsCl 120, 15 KCl, 4 $MgCl_2$, 0.1 EGTA, 10 HEPES, 4 MgATP, 0.3 NaGTP, and 10 Naphosphocreatine (pH 7.3 adjusted with CsOH). Access resistance (typically 15-35 MΩ) was monitored to ensure stable recordings. mIPSCs larger than 6 pA were detected off-line using Minianalysis software (Synaptosoft). All data were acquired using a Multiclamp 700B amplifier and pCLAMP 9 software (Axon Instruments) for recording mIPSCs or the WinLTP program[6] for fEPSP studies.

*Auditory Event-Related Potentials*

*Headstage construction and implantation:* Two epidural electrodes and one ground, made of insulated stainless steel (diameter: 1/8 mm), were soldered into an electrode interface board (EIB-8, Neuralynx). A 12-mm-long electromyographic (EMG) wire made of stranded stainless steel and coated with nylon (diameter: 0.36 mm, PlasticsOne) was also soldered into the electrode interface board. In mice, aged 4-7 months, under isoflurane anesthesia, the EMG wire was positioned under the skin to record from the neck muscles, and recording electrodes were placed in craniotomies in the right hemisphere at (1, 1) and (1.5, -1) mm to bregma and midline, respectively. The ground electrode was positioned at (0, -6). Electrodes were placed in the craniotomies at a depth of 0.75 mm to collect electrocorticographic (ECoG) data. Three jewelry screws and dental acrylic fixed the implant onto the skull.

After 1 week recovery, mice were connected to a pre-amplifier (HS-8, Neuralynx) with an interface cable to a slip-ring commutator (SL-88-10, Dragonfly) for recordings. ECoG and EMG signals were amplified and digitized (sampled at 1 kHz with 16-bit precision) by the Digital Lynx system (Neuralynx) and electronically stored using Cheetah software (Neuralynx). For each recording session, animals moved freely within a 25 × 25 cm chamber. All animals were habituated to the chamber for 15 min before their first recording session. No sedatives were applied before or during recordings.

*Event-related potentials:* For the recording of event-related potentials (ERPs), passive field speakers mounted on the ceiling of the recording chamber produced 10 ms Gaussian white-noise "clicks" 25 dB above a 65-dB white-noise background every 2-3 s. Nine hundred sixty stimuli were presented in the 40-min protocol stimulation.

Recorded ECoG signals were analyzed offline in Matlab using custom-written software and the Chronux toolbox. Trials were event-locked to the stimulus, with the stimulus centered at 0 ms. Trials were baseline-corrected by subtracting the median amplitude in the 100 ms before the stimulus from the post-stimulus amplitude. Individual trials with artifacts (>1 mV) and channels whose average showed no evoked response were rejected. A band pass filter from 0.5 to 200 Hz was applied on all the data.

*Event-related oscillatory activity*: Spectral decomposition was performed on the amplitude data using Matlab scripts developed in-house in conjunction with the Chronux toolbox. The evoked power gain from baseline, accounting for the phase-locked changes in oscillatory activity pre- and post-stimulus, was determined by dividing the power of the averaged ERPs in a 500 ms window post-stimulus by the baseline power in a 500 ms window immediately

preceding the stimulus. This ratio was converted to decibels by taking the base-10 logarithm and multiplying by 10. The baseline power per trial was averaged across trials before taking the ratio, as the corresponding evoked baseline power approaches zero as the number of trials increases. The induced oscillatory activity was also calculated, referring to those oscillations not phase-locked to the stimulus, by subtracting the evoked power from each trial of the post-stimulus power before converting again into a baseline power gain for each trial.

Principal component analysis (PCA) was performed on these two power gain measures following channel concatenation. Leave-one-out cross-validation (LOOCV) was implemented on the resulting principal components by leaving out a single animal in each loop of the cross-validation and averaging the resulting success across all animals. This LOOCV used the first principal component (PC) and successively through each additional PC such that the final LOOCV included all possible PCs. The optimal number of PCs separating the genotypes was then determined along with the corresponding cross-validated success rate. LOOCV was also performed for each 2-Hz frequency band to determine the power gain frequency bands that best separated the genotypes per channel.

*Behavioral procedures*

The procedures were conducted between 8 AM and 6 PM during the animals' night cycle (reverse 12-h light cycle, lights off at 7 AM), under red or dim lighting (unless otherwise stated) and blind of genotype. Mice were aged 8-12 weeks at start of behavioral testing and housed 2-4 per cage. Experimental designs were counterbalanced, ensuring comparable numbers of each group were exposed to each experimental variable (e.g., side, box, novel object, treatment group, etc.). Animals were tested in a pseudorandom order controlling for potential sources of bias (e.g.,

sounds, smells, time of day, etc). All equipment was cleaned at the start and between trials with 50% ethanol to remove olfactory cues/distracters.

Behavioral data were analyzed using mixed analyses of variance (ANOVAs), depending on the experimental design and associated variables, followed by Fisher's LSD *post hoc* test when appropriate. Unless otherwise stated, behaviors were videotaped and scored offline using ODLog (Macropod Software) by an observer blind to genotype. Behavioral data are presented as mean ± SEM, graphically displayed using GraphPad Prism (v5.0), and analyzed using Statsoft Statistica (v8.0). Sphericity was assumed but not formally tested. Outliers, providing data 2 SD from the mean, were excluded from the analyses.

*Light/dark box:* Assessment of anxiety levels took place in a two-chambered box, with one chamber smaller (14.5 × 27 × 26.5 cm) than the other (28.5 × 27 × 26.5 cm). The larger compartment was illuminated with white light to a brightness of ~600 Lux, and the smaller compartment was maintained at 30-40 Lux. On the dividing wall, a small opening (10 × 10 cm) allowed transitions between the two compartments. Animals were placed in the dark compartment and able to explore the apparatus for 5 min.

*Barnes maze:* A circular, opaque Plexiglas disc (75 cm diameter) with 20 holes (5 cm diameter) located around its perimeter was used. One hole provided a route to an escape tunnel (19 × 8 × 7 cm). The maze was brightly lit (~600-700 Lux) to encourage mice to locate the escape tunnel. Large, distinctive shapes surrounded the maze to provide spatial cues. On day 1, the mouse was placed in the escape tunnel for 1 min. Immediately afterward, the mouse was placed into a cylindrical start chamber in the center of the maze for 30 s. The chamber was then raised, beginning the trial. The trial was terminated after 5 min or when the mouse entered the

escape tunnel, where it remained for 30 s. If the mouse failed to locate the escape tunnel, it was gently guided to the tunnel where it remained for 30 s. Relative to spatial cues, the location of the escape tunnel remained constant for each mouse but was varied between mice. The Barnes maze was rotated 90º each day, ensuring that local maze cues were not guiding performance. Mice were tested once daily for 9 days (acquisition period). The latency to discover the escape tunnel, the number of incorrect hole visits, and perseverative errors (two or more sequential hole visits to an incorrect hole) were recorded. The probe trial occurred on day 10 and consisted of a 5 min trial without the escape tunnel. Time spent in each quadrant was recorded. After a 14-day break, a retention trial was conducted with the escape tunnel in its original position.

*Social preference and social recognition:* A three-chambered, clear Perspex (63 × 42.5 × 22 cm) box, similar to one used previous[7], was used. White, removable dividing panels were used to confine mice in the middle compartment between trials. Mice were first confined to the middle chamber for 1 min. The dividers were then removed enabling mice to habituate to the empty arena for 10 min. Mice were then confined to the middle chamber, and an empty cup (chrome Galaxy pencil cup, Spectrum Diversified) and a cup containing a novel stranger mouse (S1) was introduced to the lateral compartments. The dividers were removed, and sociability was assessed. After 10 min, mice were confined in the middle compartment for an inter-trial interval (ITI; 1, 5, or 10 min). During the ITI, a new stranger mouse (S2) was introduced to the side that previously contained an empty cup. After the ITI, the dividers were removed for a 10 min trial to assess social recognition. The stranger mice were age-, sex-, and weight-matched control mice and never cage-mates. Prior to testing, stranger mice were habituated to the wire cups for 30 min. Stranger mice that displayed unusual behavior (e.g., gnawing on cup, climbing, etc.) were excluded. Test mice were transferred to a holding cage after testing to prevent scent carryover to

cage-mates still to be tested. Surgical screens enclosed the testing chamber, and upward-pointing lights provided low-level illumination (~10 Lux) which was even throughout the apparatus (<1-2 Lux variation). The duration of direct interactions with the cup and duration of self-grooming were recorded. Using the duration of interactions, a discrimination index [(S2-S1)/(S2+S1)] was calculated to give an index of discriminability for social recognition trials.

*Novel object and novel place recognition:* Novel object recognition (NOR) was conducted in an identical manner to the social preference/recognition procedure, described above. Each trial lasted 10 min. The session began with an habituation trial. After habituation, mice were able to interact with two identical cups, either chrome with vertical bars (Galaxy pencil cup, Spectrum Diversified) or black with mesh grating (Nestable jumbo mesh pencil cup, WebOfficeMart). After a 1, 5, or 10 min ITI, mice could then interact with a familiar cup or a novel cup, assessing novel object recognition. Novel place recognition (NPR) was conducted in the same animals 24 h after NOR testing in an identical manner. During the ITI, one of the identical cups from the initial trial was moved to a novel location, assessing novel place recognition.

*Marble burying test:* The procedure, as described previously[8], involved a clean home cage (30 × 18 × 12 cm) filled with clean bedding (depth of 5 cm), patted to give a smooth, firm surface. Twenty black marbles (12 mm diameter) were placed in a 5 × 4 equidistant arrangement. Mice were individually placed in the test chamber and allowed to investigate the marbles. After the 30 min session, the mice were removed, and the number of marbles buried (>50% covered in sawdust) was counted.

*Prepulse inhibition of acoustic startle response:* Startle testing was conducted in one of

three startle chambers (San Diego Instruments). The inner chamber consisted of a speaker mounted to the wall and piezoelectric sensing platform on the floor. The background noise consisted of 70 dB white noise. The session began with a 5 min habituation of background noise followed by the testing session[9]. The PPI testing session began with the presentation of six pulse-alone trials (120 dB, 40 ms). Then, a series of pulse-alone trials and prepulse trials (74, 78, or 82 dB; 20 ms followed by 100 ms pulse trial; 120 dB) were each presented 12 times in a pseudorandom order. The session concluded with the presentation of six pulse-alone trials. The startle amplitude was calculated in Newtons (N), and the acoustic startle response was the average startle amplitude of pulse-alone trials. The percent PPI was calculated as follows: [100-(mean prepulse response/mean pulse alone response) × 100].

*EMG assessment of startle response and prepulse inhibition*: For the recording of the startle response and PPI via EMG, a startle stimulus, a prepulse stimulus, and a combination of the two (prepulse + startle) were each presented 333 times in pseudo-random order during a single 50-min recording session and overlaid a continuous 64 dB white-noise background. Startle trials consisted of a 50-ms 94-dB startle pulse of uniform white noise. Prepulse trials consisted of a 50-ms 70-dB noise prepulse. Prepulse + startle trials consisted of the prepulse followed by the startle pulse separated by 100 ms onset-to-onset. Latency between trials was 2.5-3.5 s.

Recorded EMG signals were analyzed offline in Matlab. The EMG amplitude data were rectified then smoothed with a 10 ms span using a zero-phase digital filter. Individual trials were defined as a 4 s time window, with the startle onset beginning at 0 ms and the prepulse at -100 ms. Any startle trials with activity before the startle pulse were rejected, where activity is defined as the mean EMG data from -50 to 0 ms of a startle trial 2 SD from this mean baseline of all startle trials per recording. Responses in startle trials and prepulse + startle trials were determined by

the peak value within a 20-ms time window 10 ms following the onset of the startle pulse for each trial. Recordings were rejected if the animal's average startle response was 2 SD from the mean of all startle responses within its genotype. Baseline activity was determined as the median of the prepulse trials for each recording, whereas response to the prepulse was measured by the median of a 120-ms time window beginning at the presentation of the prepulse. Recordings were rejected if the prepulse response was significantly ($p<0.05$) greater than baseline activity as determined by the Wilcoxon rank-sum test. Percent PPI was calculated as stated previously.

*Pharmacology*

*Open field:* Activity in a novel open field arena (42 × 42 × 30 cm) was measured with a VersaMax Animal Activity Monitoring System (AccuScan Instruments). Each arena was equipped with sets of 16 photobeam arrays, spaced 2.5 cm apart. Photobeam breaks and software (VersaMax, AccuScan Instruments) recorded performance. Mice were administered PCP (0-15 mg/kg) or amphetamine (0-4 mg/kg) and immediately placed into the center of the open field arena for 90 min. Behavioral parameters were collected in 5 min time bins, and the total distance traveled was determined.

*PPI:* Assessment of the effect PCP or amphetamine on sensorimotor gating was conducted in an acoustic startle session identical to the one described above. After the mice were administered PCP (0-10 mg/kg, i.p.) or amphetamine (0-4 mg/kg i.p.), they were kept in individual holding cages for the predetermined pretreatment time (10 min, PCP[10]; 15 min, amphetamine[11]). The PPI session was then initiated.

*Supplementary References*


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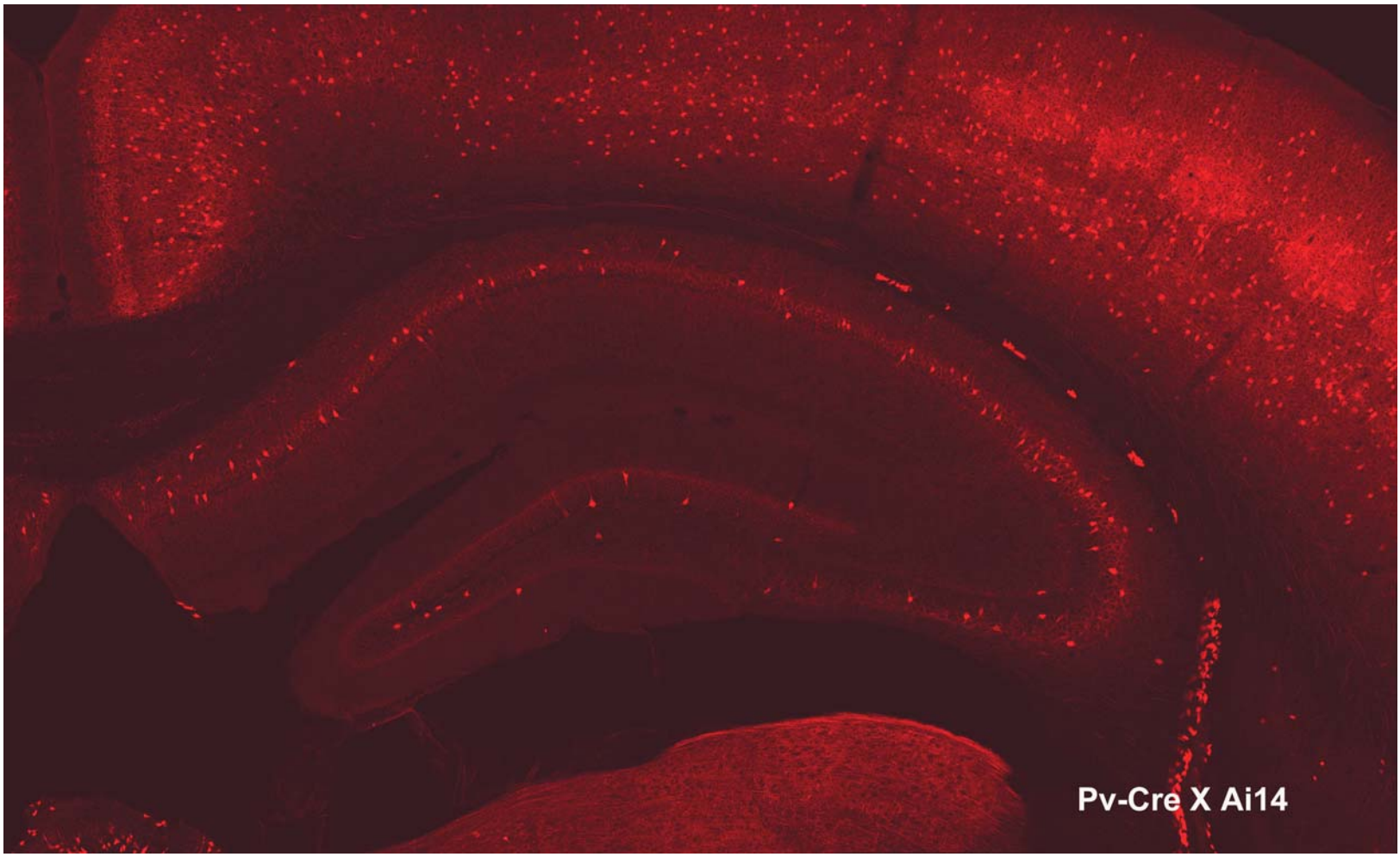
Supplementary Figure 1-Behrens



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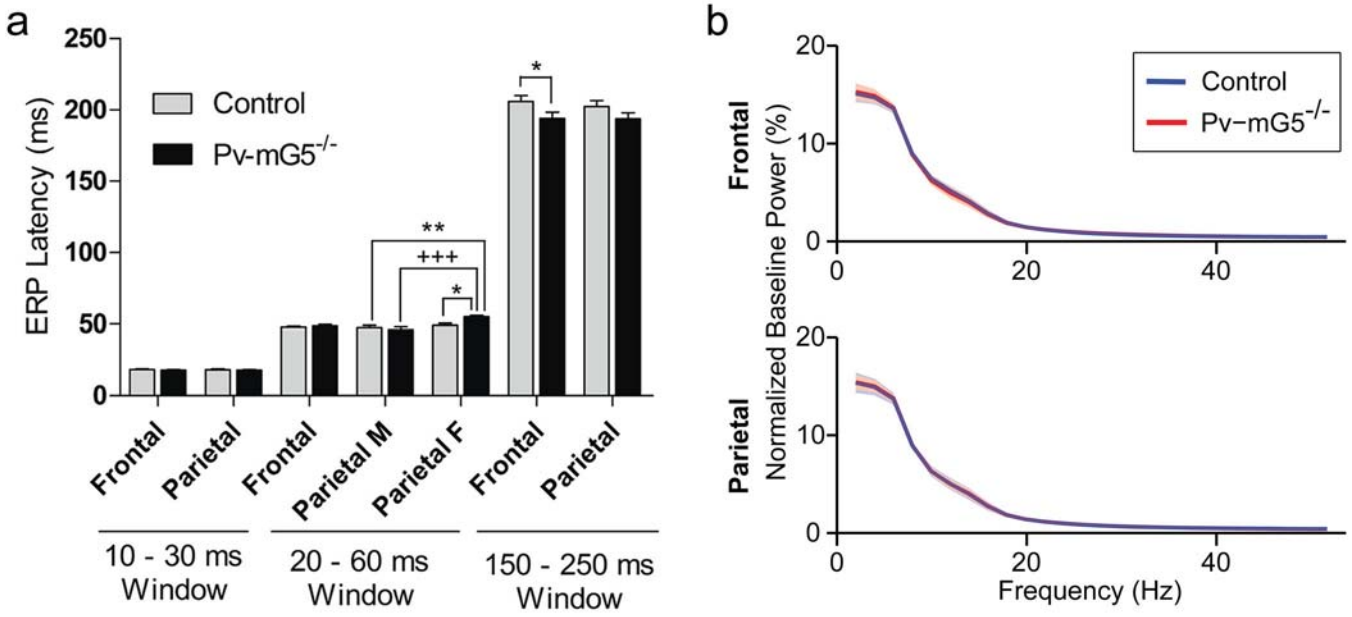

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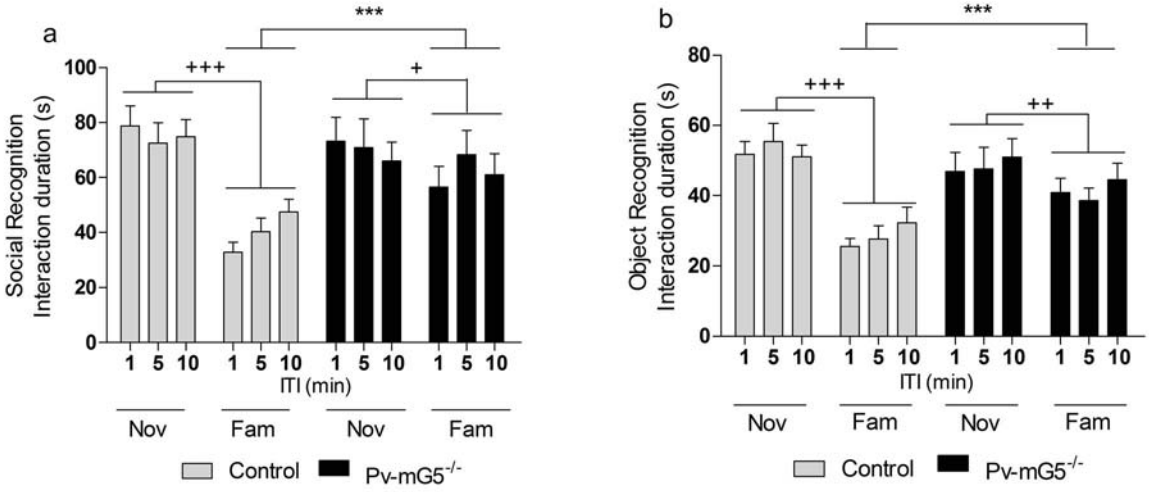

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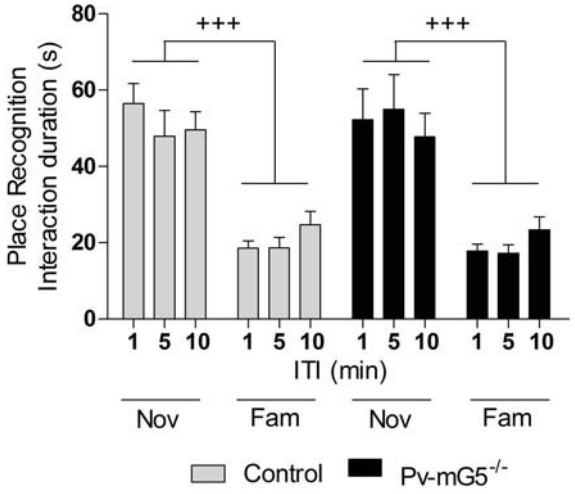

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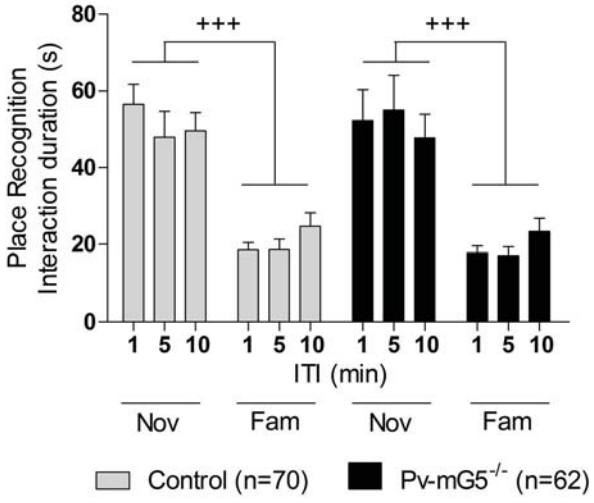

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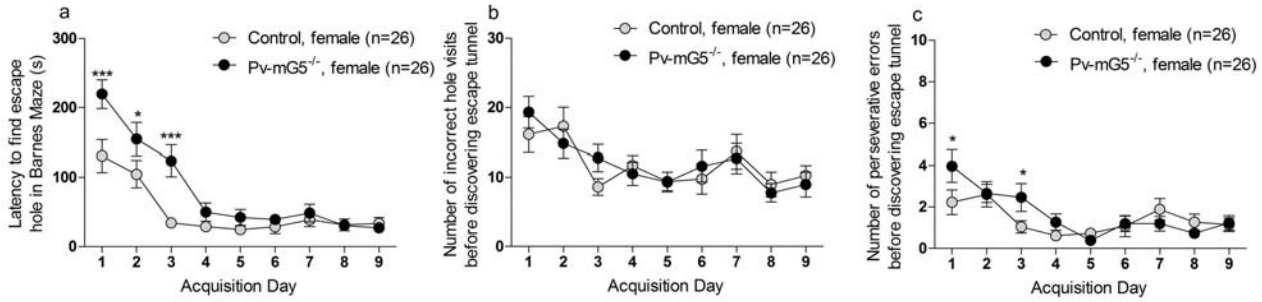

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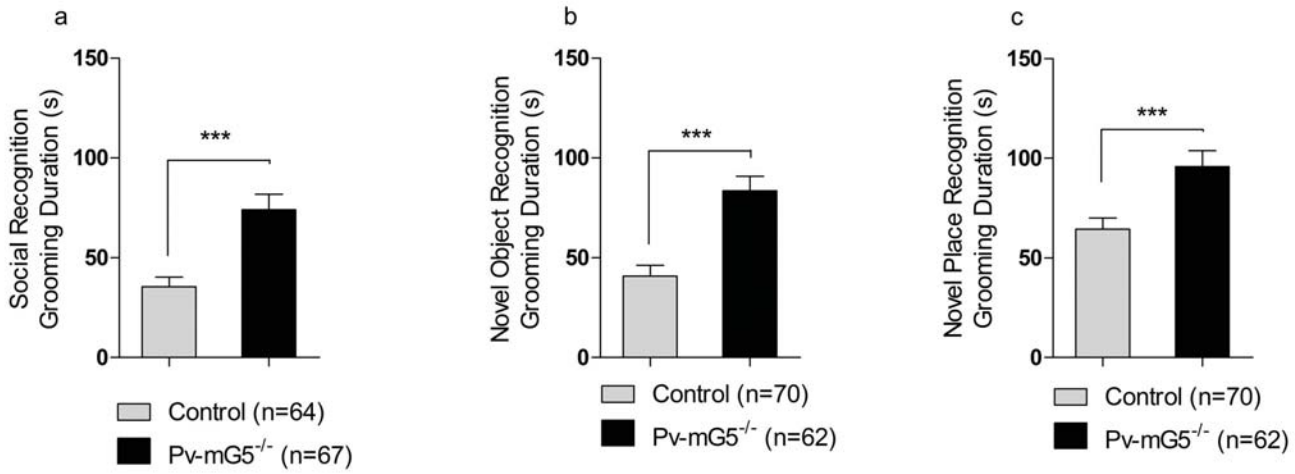

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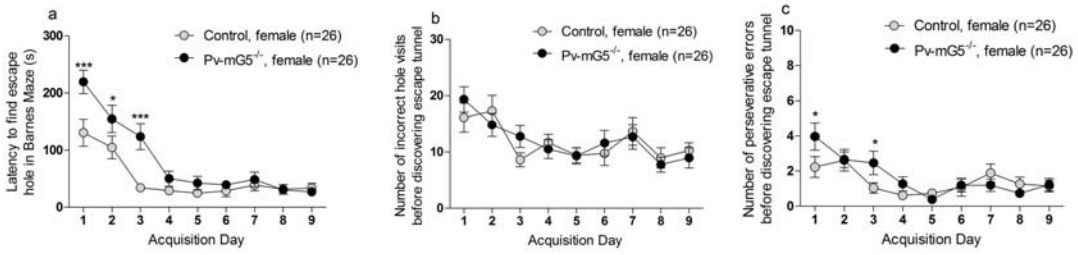
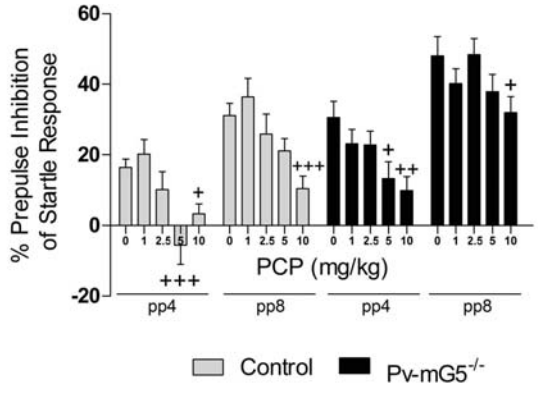


Supplementary Figure 9-Behrens



Supplementary Figure 10-Behrens