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Supplemental Data

Reversible Silencing of Neuronal Excitability

in Freely Behaving Mice by Expression of a Cl^-

Channel Gated by a Systemically Administered Ligand

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Supplemental Experimental Procedures

Stereotaxic Surgery

Mice were anaesthetized using IP injection of a mixture of 10 mg/kg Xylazine (Xylaject, Phoenix Pharmacytical, Inc.) and 100 mg/kg Ketamine (Ketaject, Phoenix Pharmacytical, Inc.). After whisker movement had ceased, the animals were placed into a stereotaxis apparatus (Kopf). Approximately 15 min before virus delivery the mice received an intraperitoneal injection of 1 ml 20% Manitol to increase virus spread (Burger 2005). A small incision was made to expose the skull and a 28 gauge Hamilton syringe was inserted into the brain at bregma level, 2 mm lateral to the midline. Injection flow was controlled with a micro-injector (Micro 4 ™, World Precission Instruments), by pushing down the plunger at controlled speed. At 4.2 mm dorsal from bregma, a 1 s pulse injection was given at 80 nl/sec, then the needle was pulled up 0.2 mm and injection was continued at 80 nl/min to inject a total of 2 µl. After each 400 - 600 µl the needle was pulled up 0.5 mm to cover a ventral to dorsal range of 4 mm to 2.5 mm within the striatum. The needle was left for another minute at the 2.5 mm position, pulled up for 0.5 mm and left at this position for 1 min without injection, before it was removed. The skin incision was closed with Vetbond ™ glue (3M) and locally treated with Marcaine ® (Hospira) for pain relief. The animal received a 150 µl subcutaneous injection of 3 µg/ml ketoprofen for additional pain relief and help with dehydration, and was kept on a heating pad until waking up.

Open Field Tests

Animals were transferred into the open field apparatus directly from their home cage. The movement of up to four mice was recorded with a top-mounted camera and captured with the Mediacruise program (Canopus ®). The movie files were then analyzed using the Ethovision ™ software (Noldus). Traces for each mouse were recorded for 15 min, starting within 1 min after the mouse was placed into the openfield setup. The center zone of the open field box was defined as a square area of 18.33 cm x 18.33 cm (1/3 of the length of the total with) centered and aligned with the sides of the box.

Rotarod Behavior

The rotarod consisted of 5 grooved plastic rollers with 4 cm diameter, separated by plastic barriers. Animals were trained and tested on a roller at a fixed speed of 30 turns/min. Up to 4 mice were tested simultaneously. Each session consisted of three runs, spaced 15 min apart. If animals stayed on the roller for less than 15 s, they had to repeat the run. Latency to fall was recorded with an automated switch. Animals were removed from the rotarod if they stayed for the maximum of 180 s. Latency to fall for each session was averaged over all animals and all three runs.

Tests of IVM Influences on Behavior in Control Mice

In order to determine whether the highest concentration of IVM used in our experiments (10 mg/kg) causes any changes in exploratory behavior, motor learning or motor performance, we subjected control (i.e., uninjected) IVM-treated mice to various tests (Supplementary Figure 1A-F)). 16 C57B6 mice that had not undergone any surgical procedure were injected either with 10mg/kg IVM (n = 8) or vehicle (propylene

glycol; n=8). Twelve hours after injection, the animals were tested for various exploratory behaviors in the open field apparatus. During the 15 min test period, we did not find any significant differences between IVM- or vehicle-injected animals in the total distance moved (Supplementary Fig. 1A; 7.59 ± 0.94 m vs. 6.41 ± 0.43 m, $p > 0.3$), latency to enter into the center zone (Supplementary Fig 1B; 37.2 ± 11.5 s vs. 48.3 ± 15.0 s, $p > 0.5$), total duration in the center zone (Supplementary Fig. 1C; 53.9 ± 15.9 s vs. 49.8 ± 11.5 s) or in the number of entries into the center zone (27.8 ± 3.9 vs. 35.4 ± 6.3, $p > 0.3$). Thus neither exploratory behavior nor anxiety (which is indirectly measured by the parameters in this test) are affected by 10 mg/kg IVM.

We next tested the effect of IVM on the ability to learn a motor task. The same mice tested in the open field apparatus were trained on a fixed speed rotarod (30 rotations per minute). Between 24 hr and 60 hr post IVM- or vehicle-injection, mice received 4 sessions of training, each consisting of 3 runs (Supplementary Fig. 1E). The latency to fall off the rod was recorded at each run. The maximum latency was 180 s, after which the mice were removed from the rotarod. The minimum latency was 15 s. Mice that fell off the rotarod before 15 s were re-tested. We did not find any significant difference between IVM and vehicle injected animals during any of the sessions ($p > 0.5$ for all time points). All of the time points tested fell within the window of maximal effect of 10 mg IVM, as measured using the AMP-induced rotation test (Table 1).

We also tested the mice 14 days after IVM or vehicle injection, when the effect of IVM should have subsided (Table 1) and again found no significant difference ($p > 0.5$). Twenty-four hr later we injected the 8 animals previously injected with vehicle, with 10 mg/kg IVM and tested their rotarod performance 24h later. No statistical difference was found (Supplementary Fig. 1F; 69.2 ± 6.5 % vs. 64.6 ± 6.3 % of maximal latency, $p > 0.5$). Finally, 8 mice injected with (AAV2-α+AAV2-β) were trained on the rotarod for 4 sessions, spaced 12 hr apart, injected with 10mg/kg IVM 24 hr after the last training session and tested for latency to fall (Supplementary Fig. 1G). There was no statistical difference between these animals and controls in the latency to fall at these two time points (65.6 ± 5.8 % vs 65.7 ± 8.6 %, $p > 0.5$).

Slice Preparation for Electrophysiology

Mice, aged 14 – 18 weeks, were deeply anesthetized with sodium pentobarbital (40 mg/kg). Then, cardiac perfusion was performed using ice-cold glycerol-based artificial cerebrospinal fluid (gACSF), containing 252 mM glycerol, 1.6 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 18 mM $NaHCO_3$, and 11 mM glucose, and oxygenated with 95%O_2/5%CO_2. After decapitation, the brain was removed and kept in gACSF (0 – 4 ºC), and was cut into 250 µm thick slices in the coronal plane using Microslicer DTK-1000 (TED PELLA Inc., CA). The slices were allowed to recover for at least 1 hr in a holding chamber in regular ACSF, containing: 126 mM NaCl, 1.6 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 18 mM $NaHCO_3$, and 11 mM glucose, and oxygenated with 95%O_2/5%CO_2. On the basis of the expression region of GluClαβ channel in injected mouse, the slice through bregma and one to two slices before or after bregma, were selected for electrophysiological recordings.

Histology

Animals were anaesthetized using intraperitoneal injection of a mixture of 10 mg/kg Xylazine (Xylaject, Phoenix Pharmacytical, Inc.) and 100 mg/kg Ketamine (Ketaject, Phoenix Pharmacytical, Inc.). After exposing the heart and making an incision into the right atrium, 5 ml filtered PBS was perfused via gravity flow (1 m) into the left ventricle, followed by perfusion via gravity flow (1 m) with 35 ml 4% paraformaldehyde. For histology, brains were dissected, equilibrated with 30% sucrose overnight and were then embedded in OCT, frozen on dry ice and stored at -80º C. 30 µm frozen sections were cut on a cryostat (Bright), dried for 30 min at room temperature stored at -20º C under dry conditions. Before antibody staining or visualization of native YFP or CFP the sections were rehydrated with PBS, washed twice with PBST (PBS +0.1% Triton X100), and blocked for 30 min with PBSTS (PBST + 10% goat serum). For visualization of native YFP or CFP, sections were washed twice with PBST and mounted with 70% glycerol. For anti-GFP, antiNeuN double staining, the sections were incubated in 2 µg/ml anti-GFP rabbit IgG (Molecular Probes A11122) and 1 µg/ml anti-NeuN mouse IgG1 (Chemicon MAB377) in PBSTS overnight at 4º C. The next morning the sections were washed four times with PBST, blocked with PBSTS and incubated with anti-mouse Alexa 564 and anti-rabbit-Alexa 488 for 3 hours at room temperature. Sections were then washed twice with PBST and mounted in 70% glycerol. Cells were

visualized on a Zeiss confocal microscope. For double color staining of 120 µm floating sections, dissected brains were post-fixed in PFA for 2 hr and stored in PBS at 4º C. 120 µm sections were cut on a cooled vibratome (Technical Products International, series 300) and collected in a 12 well dish with mesh inserts, up to three sections per insert. Sections were washed in Tris buffered saline + 0.1% Triton X100 (TBST), blocked for 30 min with TBST +2% goat serum (TBSTS) and incubated in 2 µg/ml anti-GFP rabbit IgG (Molecular Probes A11122) and 1 µg/ml anti-NeuN mouse IgG1 (Chemicon MAB377) in TBSTS overnight at 4º C. After several 30 min washes in TBST the sections were blocked again for 30 min, incubated with anti-mouse IgG1-horse-radish peroxidase (HRP) secondary antibody (Southern Biotech 1070, 1:1000) for 3 hr at room temperature, and washed 3 times 30 min with TBST. After equilibration with Acetate-Imidazole buffer (175mM Na acetate, 10mM Imidazole, pH 7.2) sections were stained black for NeuN in 0.3 mM DAB, 0.05 mM NiS, 0.0015% H_2O_2 in Acetate-Imidazole buffer. The staining was stopped by several washes with PBS and the remaining HRP activity was killed by incubation in PBS/0.02% NaN3 overnight at 4º C. Red detection of YFP and CFP was achieved by washing several times for 30 min in TBST, blocking for 30 min in TBSTS, incubating with anti-rabbit-HRP antibody (Vector PI-1000, 1:1000) for 3 hr at RT, followed by several 30 min washes in TBST and staining using the Vector AEC substrate kit (SK-4200). Following staining, sections were washed several times in PBS, equilibrated in 30% glycerol and mounted in 70% glycerol.

Quantification of CFP and YFP Fluorescence after Electrophysiology

Slices with a single Alexa647 filled cell were fixed in 4% PFA for 30 min, equilibrated in 15% sucrose for at least two hours and frozen in OCT on dry ice. 30 µm sections were cut on a cryostat, mounted in glycerol and coverslipped.

For quantification of GluClαβ subunit fluorescence, images were collected from a Nikon C1si microscope equipped with a spectrally resolved multiple detector system. Each pixel of the X-Y image has complete spectral emission data, comprising the lambda stack. With a Plan Apochromat 60x 1.4 NA oil immersion objective(Dickinson et al., 2001; Lansford et al., 2001; Nashmi et al., 2005), Z-stack of images were collected at wavelengths between 460 and 620 nm with bandwidths of 5 nm during excitation of CFP with the 439 nm line of a diode laser (1% of full power, 5 mW) or during excitation of YFP with the 488 nm line of an argon laser (2% of full power, 40 mW). Pinhole was set at 60 µm. Images were collected at a 12-bit intensity resolution over 512 x 512 pixels at a pixel dwell time of 10 µsec, over a 75 x 75 µm zoom area. Alexa 647 was imaged with a 638 nm diode laser (15% of full power, 10 mW) at 20 ms pixel dwell time at wavelengths between 650 and 750 nm with 5 nm bandwidths.

In all cases, detectors gains and laser attenuators were adjusted to lie within the linear range of the detector. The output of the appropriate laser was calibrated at each session with a power meter. CFP and YFP fluorescence were spectrally deconvolved from background autofluorescence of the tissue and CFP and YFP fluorescence was quantified from one optical slice through the center of each neuron that was filled with Alexa 647 dye after electrophysiological recording.

Supplemental References

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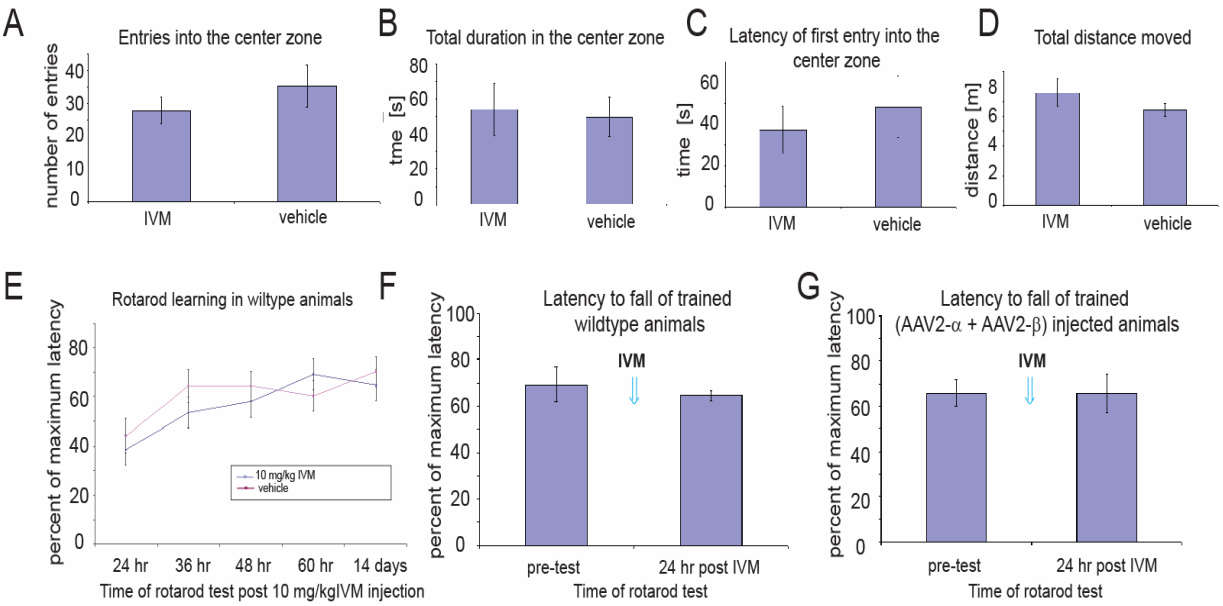

Figure S1. Animals Injected with 10 mg/kg Do Not Show Deficits in Motor Coordination and Motor Learning

(A-D) Open field test 12 hr post 10mg/kg IVM injection, n = 8 per group. $p > 0.3$ for all measures
(A) Number of entries into the center zone.
(B) Total duration in the center zone.
(C) Latency of first entry into the center zone.
(D) Total distance moved in the openfield.
(E) Rotatod learning in wildtype animals injected with IVM or vehicle. There was no statistical difference at any time point post IVM ($n = 8$, $p > 0.5$).
(F) Latency to fall 12 hr post 10 mg/kg IVM injection of previously trained control animals ($n = 8$, $p > 0.5$).
(G) Latency to fall 12 hr post 10 mg/kg IVM injection of previously trained (AAV2-α +AAV2-β) injected animals ($n = 8$, $p > 0.5$). P-values for A-E were determined by a two-tailed unpaired t-test for unequal variance.
P-values for F-G were determined by a two-tailed paired t-test.

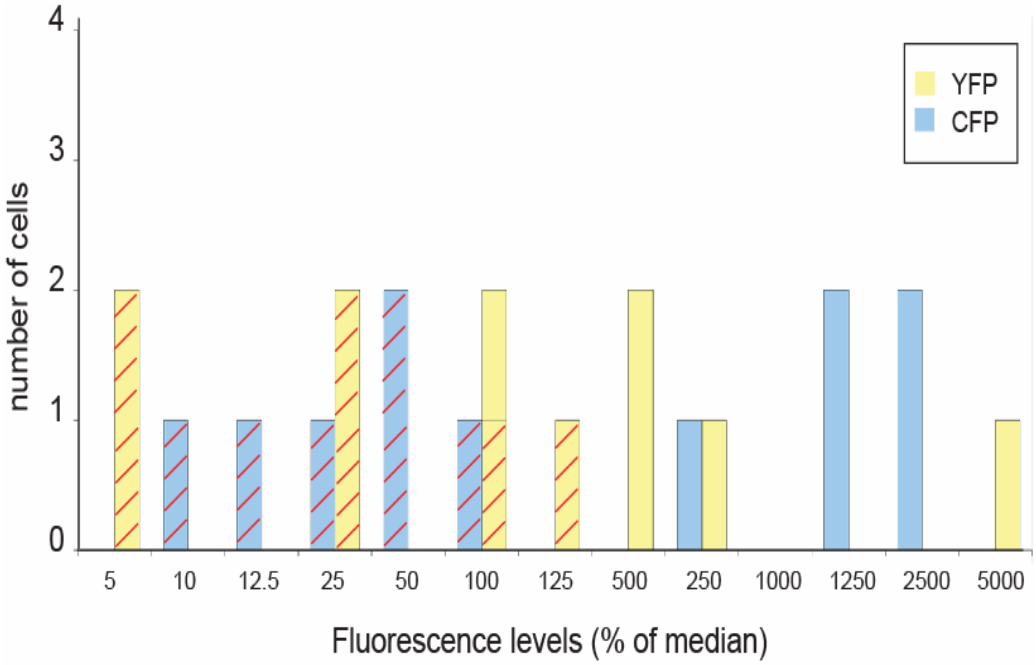

Figure S2. Histogram of Relative CFP (Cyan Shading) and YFP (Yellow Shading) Levels from 11 Cells

Hatched bars represent the six cells filled with Alexa 647 after recording, open bars 5 additional cells selected for strong XFP expression in a randomly selected region from the GluClαβ –expressing area of the same section. Percentages of CFP/YFP fluorescence levels were normalized to the median CFP/YFP fluorescence levels in the 11 cells.