Supporting Information

Sasaki et al. 10.1073/pnas.1213458109

SI Materials and Methods

Generation of ChR2(C128S)-EYFP-Expressing Mice. Details for generating transgenic mice are described elsewhere (1). In short, the transgene coding ChR2(C128S)-EYFP under tet operator (teto) promoter was inserted as knockin just downstream of the β-actin gene polyadenylation signal by ES cell homologous recombination to enhance ChR2(C128S)-EYFP expression. This protein would only be expressed in the presence of tetracycline transactivator (tTA); thus, bigenic mice were prepared by crossing lines expressing tTA under Mlc1 promoter, which is selective for astrocytes, with the tetO line. Although transgene induction can be turned off by antibiotic selection, only when the cRNA probes (3) were used.

Acute Cerebellar Slice Electrophysiology. Parasagittal cerebellar slices were prepared from young mice [postnatal day 17 (P17) to P24, unless otherwise specified]. The animals were anesthetized by inhalation of halothane before decapitation, and the brain was sliced in ice-cold solution containing the following (in mM): 119.0 NaCl, 2.5 KCl, 0.1 CaCl2, 3.2 MgCl2, 1.0 NaH2PO4, 26.2 NaHCO3, 11.0 glucose, 3.0 myo-inositol, 0.5 ascobic acid, and 2.0 Na-pyruvate [saturated with 95% (vol/vol) O2 and 5% (vol/vol) CO2]. Slices were cut at 250-μm sections using a microslicer (PRO7; Dosaka EM). The slices were then incubated in the above solution with CaCl2 and MgCl2 concentrations substituted to 2.0 and 1.3 mM, respectively, at 34 °C for 30 min and then stored at room temperature. The slice was transferred to a submerged-type recording chamber and continuously superfused. All recordings were performed at room temperature (22–25 °C). Cells were visualized using a chamber 60x water-immersion objective on an upright microscope equipped with infrared-DIC (BX61WI; Olympus). Whole-cell recordings were made with a patch-clamp amplifier (Axopatch 200B; Molecular Devices). Patch electrodes with resistances of 2–4 MΩ were used. Pipette solution (PS) with one of the following compositions was used: PS1 (in mM): 35 CsF, 100 CsCl, 10 Hepes, 10 EGTA; PS2: 127 K-glucanate, 2 KCl, 10 Hepes, 0.5 EGTA, 4 MgCl2, 4 Na2-ATP, 0.5 NaGTP, 10 K-phosphothreonate; PS3: 142 K-glucanate, 2 KCl, 10 Hepes, 0.5 EGTA, 4 MgCl2, 4 Na2-ATP, 0.5 NaGTP (pH 7.2 with CsOH titration for PS1 and with KOH titration for PS2 and PS3). PS1 was used for most of the voltage-clamp experiments (Vh = −70 mV), PS2 for the LTD experiment, and PS3 for current-clamp experiment. Series resistance was compensated ~80%. For cell-attached recordings, pipettes were filled with artificial cerebrospinal fluid (ACSF). Signals were filtered at 2 kHz and digitized at 10 kHz.

Photostimulation was delivered to the slices from the epifluorescence port and through the 60x objective. Lumenor Light Engine was used as the light source with colors, blue (center: 475 nm; width: 28 nm; power below the objective lens, 0.9 mW), yellow (575 nm, 25 nm, 1.4 mW), and UV (390 nm, 18 nm, 0.6 mW). Photostimulation consisting of a sequence of 10-s blue and 200-ms yellow light was typically applied every 2 min during the recording. To electrically stimulate the parallel fibers, a theta glass pipette filled with bath solution was used and brief electrical pulses were given using a constant voltage-isolated stimulator (DS2A; Digimeter). Paired-pulse stimuli with interstimulus interval of 100 ms was applied every 20 s. For the parallel fiber stimulation, stimulus strength was adjusted so that the first EPSCs recorded in Purkinje cells did not exceed 400 pA. The sources of the chemicals were as follows: tetrodotoxin (TTX), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoxazine-7-sulfonamide (NBOX), GYK153655, and 4-aminopyridine (4-AP) were from Ascent Scientific; CdCl2, picrotoxin (PIC), 4,4′-disothiocyanostilbene-2,2′-disulfonic acid (DIDS), 1-naphthylacetyl spermine (NASPM), and carbenoxolone (CBX) were from Sigma; threo-β-benzoyloxyaspartic acid (TBOA), cyclothiazide (CTZ), and LY367385 were from Tocris Cookson; BaCl2 was from Nacala; 5-nitro-2-(3-phenylpropyl amino)benzoic acid (NPPB) was from BioMol; and Lucifer Yellow and Alexa 594-dextran were from Invitrogen.

In Situ Hybridization and Immunohistochemistry. To confirm the cell type-specific expression of tTA, in situ hybridization for tTA was combined with immunohistochemistry for brain lipid-binding protein (BLBP), calbindin-D, and NeuN. Detailed methods for in situ hybridization are described elsewhere (2). In brief, cryosections from 4% paraformaldehyde-perfused brains were hybridized to digoxigenin-labeled tTA cRNA probes. Individual marker proteins were labeled with anti-BLBP antibody (rabbit monoclonal antibody; gift from M. Watanabe, Department of Anatomy, Hokkaido University Graduate School of Medicine, Sapporo, Japan), anti-GFAP (rabbit polyclonal antibody; DakoCytomation), anti-Calbindin-D antibody (mouse monoclonal antibody; DBS), and anti-NeuN antibody (mouse monoclonal antibody; Chemicon). Following the incubation of biotin-tagged secondary antibody, VECTASTAIN Elite Kit (Vector Lab) was used for the color development with 3,3′-diaminobenzidine (DAB).

In Vivo Optical Stimulation of Freely Moving Mice and in Situ Hybridization. Mice over 12 wk were used for the in vivo experiments. Blue (445 nm) and yellow (589 nm) lights were generated by laser diodes and applied through a plastic optical fiber (0.75-mm diameter). An optical swivel (Lucir) was used for unrestrained in vivo photostimulation. Blue and yellow light power intensities at the tip of the plastic fiber were 1.3 and 0.9 mW, respectively. Under anesthesia with 50 mg/kg pentobarbital, the plastic optical fiber was placed on the skull just above the cerebellum, 6.0 mm posterior to the bregma and fixed using dental cement. After at least a couple of days from the surgery, the photostimulation experiment was executed. The mice were killed after 10 min from the photostimulation and in situ hybridization for c-fos and c-fms was performed as described previously (2). In brief, digoxigenin-labeled c-fos, c-fms, and GAD67 cRNA probes (3) were hybridized to sections, nitro blue tetrazolium chloride (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) compounds (Roche) were used for color development, and Nuclear Fast Red (Vector Lab) was used for counterstaining.

Horizontal Optokinetic Reflex Experiments. For the surgery, the mice were anesthetized with 1.5% isoflurane. Two holes (∼1.0-mm diameter) were drilled bilaterally in the cranium, 6.0 mm posterior, 2.7 mm lateral to the bregma. Plastic optical fibers (0.5-mm diameter) with the ends entering the brain cut at a 45° angle were inserted 1.5-mm deep (Fig. 5 A). The fiber was fixed onto the skull with dental cement. A flat head screw (6-mm head, 7-mm length) was also fixed onto the skull with dental cement on the midline between the bregma and lambda. After a couple of days from the surgery, the head of the unanesthetized mice was fixed to a home-made device using the above screw and nuts. Lights from blue and yellow LEDs (470 and 590 nm) were delivered to the open ends of the optical fibers (power intensity at the output tip, ∼0.6 and ∼0.6 mW, respectively).

A sheet of paper with checkered pattern was placed semi-circularly (radius, 32 cm) around the head-fixed mouse and oscillated horizontally and sinusoidally. The screen movement was 17° and the cycle was set at 3.0–5.0 s. The eye image was captured using a CCD camera sensitive to infrared light. The pupil location and size were calculated with the method described previously (4) and with the FIJI software (GPL).
Statistical Analysis. Electrophysiological and horizontal optokinetic reflex (HOKR) data were analyzed with AxoGraphX. Statistical analysis was conducted by Excel and SPSS. Data are shown as mean ± SEM.


Fig. 51. Schematics of glia-to-neuron communication. Activation of Bergmann glial cells (BGs) in the cerebellum triggers the release of glutamate (red) through DIDS-sensitive anion channels (Fig. 2C). The released glutamate reaches the low-affinity AMPA receptors (AMPAR) (yellow) on Purkinje cells (PCs) at a sufficient concentration to activate them (Fig. 2). Metabotropic glutamate receptor 1 (mGluR1) (magenta) is expressed on the perisynaptic region of the spine and on the dendrites on PCs and is generally assumed to be activated by glutamate spilled over from the synaptic cleft. However, to activate the mGluR1, the released glutamate has to overcome the glutamate buffering provided by both neuronal and glial glutamate transporters (1). BG processes are situated directly adjacent to the mGluR1s, which seem to be an ideal location for providing the mGluR1 with glutamate. In accordance with this idea, glia-photo-stimulated release of glutamate was shown to act also on the mGluR1, which by itself was sufficient in inducing mGluR1-dependent long-term plasticity of the parallel fiber (PF)-to-PC synapses (Fig. 3). Such glial activation of neurons was also demonstrated in vivo. We show that cerebellar-modulated motor behavior is modified as a consequence of neuronal activation by glial stimulation during the horizontal optokinetic response (HOKR) paradigm (Fig. 5). Previous studies have shown that BGs could be rapidly activated by ectopic release of glutamate from PF and climbing fiber terminals (2) and more slowly by neuronal ATP release (3). Our results show that signals can be lead from glial cells to neurons. These results suggest a potential role of glial cells in actively participating in the information processing in the brain. In the schematics, the size of the presynaptic PF-bouton, the PC-spine, and the BG-encasement of a synaptic contact is exaggerated and not up to scale.

Fig. S2. In situ hybridization and immunohistochemistry of the cerebellum of Mlc1-tTA::tetO-ChR2(C128S)-EYFP mice. (A) Simultaneous in situ hybridization (ISH) (blue) for tTA and immunohistochemistry (IHC) (brown) for BLBP, calbindin-D, and NeuN. Images show tTA mRNA strongly colocalized with astrocyte specific marker, BLBP, but not with Purkinje cell marker, calbindin-D, or neuronal marker, NeuN. The arrows in the left panel indicate tTA signals overlapping with BLBP-positive BGs, and the arrowheads in the right panel represent stellate/basket cells (SC/BCs) in the molecular layer devoid of tTA signals. Note that it is known that PCs are not labeled with NeuN (1). These results show the selective expression of the transgene in the astrocytic population. (B) Simultaneous ISH for tTA and IHC for GFAP, an astrocyte-specific marker. Strong tTA signal was detected in BGs in the PC layer and weaker tTA signal was detected in the granule cell layer (GL). EYFP fluorescent signal intensity was 3.3-fold higher in the molecular layer (ML) compared with the GL (Fig. 1A). (C) (Upper) ISH for c-fos. (Lower) ISH for GAD67 in a nearby slice from the same animal. The population of PCs and SC/BCs that were photoactivated sufficiently to express c-fos within the region directly beneath the optical fiber was 45.5 and 50.4%, respectively. The population of granule cells (GCs) that were affected by photostimulation was difficult to quantify as the cells were not as sparsely distributed as the SC/BCs. (D and E) Strong expression of c-fos mRNA was not detected in ChR2(+) mice when no light was delivered through the placed optical fibers (D) or when the light was delivered to ChR2(C128S) nonexpressing ChR2(−) mice (E). (F) c-fms mRNA expression, which gets increased in activated microglial cells, was not specifically increased surrounding the operated and illuminated area in mice with optical fiber placed on top of the skull. c-fms expression was similar in photostimulated and nonphotostimulated Mlc1-tTA::tetO-ChR2-EYFP cerebellum as well as in photostimulated cerebellum from ChR2 nonexpressing mice. This shows that the manipulations applied were not damaging to the brain tissue.

**Fig. S3.** Photocurrent of ChR2-expressing BGs. (A and B) Animals were prepared in which Mlc1-tTA or PV-tTA drove ChR2(C128S)-EYFP expression. Epi-fluorescence images of cerebellar slices and expanded confocal images are shown in the middle panels. The bottom panels show photocurrent recorded from a BG in a Mlc1-tTA::tetO-ChR2(C128S)-EYFP mouse and from a PC in a PV-tTA::tetO-ChR2(C128S)-EYFP mouse. UV light was similarly effective in terminating the direct ChR2(C128S) photocurrent as the yellow light. A slowly developing current in addition to the photocurrent was observed in BGs (similar results from four and four cells for BG and PC recordings, respectively). (C) Slowly developing component of the current in a ChR2-expressing BG was largely blocked in the presence of 5 mM Ba²⁺ and 5 mM 4-AP. (D) The effect of K⁺ channel blockade on the ratio of the slow (9.5–10 s after blue light) to the fast component (40–60 ms) of the photocurrent in BGs (n = 4; *P < 0.05, paired t test). Recordings were performed in the presence of TTX, Cd²⁺, PIC, and NBQX to avoid possible additional transmitter mediated current in BGs. (E) Representative ChR2(C128S) photocurrents recorded from ChR2(+)–BGs in P7, P14, and P23 mice in the presence of TTX, Cd²⁺, PIC, and NBQX. (F) Summary of the amplitudes of the photocurrent (P7, n = 5; P14, n = 5; P18–P23, n = 10 cells). (G) Summary of the amplitudes of the fast and slow components at P14 and P18–P23 animals. Results from Fig. S3D indicate that the fast component is likely attributable to ChR2 opening itself and the slow component to extracellular K⁺ change. The size of both of these components developed in an age-dependent manner with less than 10% of BGs exhibiting any photocurrent in P7 mice and with both components progressively increasing from P14 to P18–P23 mice.
Fig. S4. Glia photostimulation triggers glutamate release which activates AMPARs. (A) Cell-attached recordings from PCs revealed that glia photostimulation induced transient PC firings in the absence of any pharmacological agents in slice preparations. The region indicated by the gray bar (asterisk) is magnified in the right panel. To elevate neuronal excitability, the ionic composition of ACSF was slightly modified (1 mM Ca$^{2+}$, 1 mM Mg$^{2+}$). Similar results were obtained from five recordings. (B–D) Glia-photostimulated PC currents (Left) and time course of the amplitude change (Right) before and after bath application of drugs (drug application from time = 0). Amplitudes of the current are plotted against time with the amplitude normalized to account for the rundown as described in Fig. S5C. (B) Addition of PIC had no effect on the slow inward current, suggesting that GABA is not released in response to glia photostimulation ($n$ = 5; $P > 0.05$ 0–2 min before versus 10–14 min after drug application, Student $t$ test). Notice that fast current noise does decrease with PIC application, but this is likely due to the inhibition of GABA$_A$-mediated current in response to spontaneous quantal release of GABA from inhibitory SC/BCs. (C) The PC current was completely eliminated in the presence of an AMPAR blocker, NBQX ($n$ = 4, * $P < 0.05$). (D) Inhibition of AMPAR desensitization by CTZ augmented the PC current ($n$ = 6). As unblocking AMPAR desensitization by CTZ often resulted in overexcitation of the whole tissue, shorter duration of photostimulation was used in this experiment (2-s blue, 200-ms yellow). (E) A current trace in a PC in response to puff application (10 s, 10 psi) of glutamate (100 $\mu$M). The kinetics of the current response was slow and similar to that observed for the glia-photostimulated PC current (similar results from four cells), which is consistent with the idea that the glutamate released from photostimulation diffuses through the tissue and produces the slow inward current in PCs. (F) Photocurrent in BG was decreased by application of NBQX ($n$ = 5; * $P < 0.05$, paired $t$ test), which indicates that the released glutamate can act directly on the AMPARs expressed on BGs (1). These recordings were performed in the presence of CTZ and TBOA to maximally increase the sensitivity of BG-AMPARs to glutamate. TTX, Cd$^{2+}$, and PIC were also included to avoid neuronal transmitter release and possible contamination of GABA$_A$ current in the BG recordings (2).

Fig. S5. Rundown of photocurrents and glia-photostimulated PC currents. (A) Photocurrents recorded from a PC in a PV-tTA::tetO-ChR2(C128S)-EYFP mouse at 4–10, 14–20, and 22–28 min from the start of the recordings. Photostimulation was consecutively applied every 2 min. Normalized amplitudes are shown in the right histogram (n = 4 cells). On average, 22–28 min after the initial stimulation, the amplitude of photocurrent was decreased by 40.7 ± 11.1%. Recordings were performed in the presence of TTX, Cd\textsuperscript{2+}, PIC, and NBQX. This result indicates that activation of ChR2(C128S) runs down with repetitive stimulation. (B) Photocurrent recorded from a BG in a Mlc1-tTA::tetO-ChR2(C128S)-EYFP mouse. The amplitude of photocurrent was decreased by 34.1 ± 4.8% 22–28 min after the initial stimulation (n = 5 cells). Recordings were performed in the presence of TTX, Cd\textsuperscript{2+}, PIC, and NBQX. This shows that the rundown of ChR2(C128S) photocurrent was observed also in the case where the channel was expressed on BGs. (C) Glia-photostimulated PC currents in Mlc1-tTA::tetO-ChR2(C128S)-EYFP mice are due to AMPAR activation in response to glutamate release. The amplitude of this PC current was also decreased by 32.0 ± 23.1% 22–28 min after the initial stimulation (n = 5 cells). Recordings were performed in the presence of only TTX and Cd\textsuperscript{2+}. These results imply that the decay of the amplitude of glia-photostimulated PC current during repetitive photostimulation is, at least in part, due to the rundown of ChR2 activation in BGs; although the role of rundown of glutamate release itself cannot be denied. To take this rundown effect into account, each data were normalized to the average amplitude of currents recorded in the presence of TTX and Cd\textsuperscript{2+} at each corresponding period after establishment of the whole-cell recordings.
Fig. S6. Assessment of alternative pathways leading from glia photostimulation to PC current. (A) Glia photostimulation could cause K\(^{+}\) efflux from glia and lead to depolarization of PCs. This could result in glutamate release from PCs and produce AMPAR-mediated inward current in the recorded PC. To assess this possibility, photocurrents were recorded from a PC in a PV-tTA::tetO-ChR2(C128S)-EYFP mouse before and after bath application of NBQX. Blocking AMPARs had no effect on the photocurrent amplitude (\(n=5\); \(P>0.05\), Student t test), indicating that PC depolarization does not produce detectable glutamate release in this condition. (B) Glial release of glutamate by photostimulation could activate cells other than PCs, and K\(^{+}\) efflux from these cells could be detected as currents in PCs. BGs and SC/BCs express Ca\(^{2+}\)-permeable (GluR2-lacking) AMPARs but blocking these AMPARs with 100 \(\mu\)M NASPM had no detectable effect on glia-photostimulated PC currents in Mlc1-tTA::tetO-ChR2(C128S)-EYFP mouse (\(n=5\); \(P>0.05\)). Therefore, it is unlikely that excitation of BGs and SC/BCs via Ca\(^{2+}\)-permeable AMPARs underlies the inward current recorded in PCs.
Glutamate release by glia photostimulation is not mediated by the opening of gap junctional hemichannels or NPPB-sensitive anion channels. (A) Blocking gap junctional hemichannels with CBX had no detectable effect on the amplitude of the glia-photostimulated PC currents (Upper; n = 5; P > 0.05, Student t test). The effect of CBX on gap junction inhibition was confirmed by blockade of dye coupling of Lucifer Yellow which is permeable to gap junctions between astrocytes (1). A BG was loaded with intracellular solution containing 1 mg/mL Lucifer Yellow (yellow) and 100 μM Alexa 594-dextran (red) by whole-cell recording, and dye coupling of neighboring BGs stained by Lucifer Yellow was monitored 45 min after establishing the whole-cell configuration (Left). For clarity, the recorded cell is shown as red, and the dye-coupled cells are shown as yellow. Addition of CBX significantly decreased the number of dye-coupled BGs (Right; n = 3 and 3; *P < 0.01, Student t test). (B) Application of NPPB had no effect on the PC currents (n = 5; P > 0.05). Tetanus toxin or Baflomycin A pretreatment, which is reported to disrupt vesicle fusion or vesicular filing of glutamate, respectively, failed to abolish neuronal synaptic release in our condition, possibly due to the rather developed age of animals that we used, which may preclude the penetration of these molecules; therefore, we were unable to assess the role of vesicle exocytosis mediating gliotransmitter release. (C) PC currents in response to puff application (10 s, 10 psi) of glutamate (100 μM) were not significantly inhibited by DIDS treatment (n = 7; P > 0.05). This shows that inhibition of glia-photostimulated PC current by DIDS is not due to AMPAR inhibition by DIDS but due to inhibition of glutamate release by DIDS.


Fig. S8. Assessment of alternative pathways leading from glia photostimulation to LTD at PF–PC synapse. (A) PFs were electrically stimulated every 20 s, and extracellular fiber volley was recorded. For this experiment, coronal cerebellar slices with a thickness of 350 μm from P20 mice (1) were prepared and 2.5-MΩ resistance recording electrodes were used. The average amplitude of the fiber volley was not changed with glia photostimulation with 10-s blue followed by 200-ms yellow (Right; n = 4; P > 0.05 0–5 min before versus 20–25 min after photostimulation, Student t test). This suggests that LTD induced by glia photostimulation (Fig. 3 C and D) is not due to the reduction of the number of stimulated fibers by the photostimulation. (B) PCs were selectively depolarized by photostimulation in PV-tTA::tetO-ChR2(C128S)-EYFP mice. No changes in the EPSC amplitudes were evoked by the PC-photostimulation (n = 3; P > 0.05, Student t test). This result suggests that activation of postsynaptic PCs alone is not sufficient to trigger long-term changes in PF–PC EPSC. All recordings were done in the presence of PIC.
Fig. S9. Comparison of glial membrane voltage changes evoked by glia photostimulation and by brief bursts of neuronal afferent fibers. (A) Voltage response to glia photostimulation in a ChR2(C128S)-expressing BG. Summary data are shown in the right panel (n = 6, 6, 9 cells for 100 ms, 2 s, and 10 s, respectively). These recordings were done in the presence of TTX, Cd^{2+}, and PIC. (B) Typical PF-to-PC excitatory postsynaptic currents (EPSCs) evoked by paired-pulse electrical stimulation (100-ms interval). A theta glass pipette filled with bath solution was used for the stimulation, and brief electrical pulses (~100 µs) of 60 V in amplitude were created across the two compartments of the theta pipette with a constant voltage-isolated stimulator. The average amplitudes of the first EPSC with this stimulus were ~300 pA, which were comparable to the amplitude obtained in Fig. 3 C and D. The right panel shows summary of the average amplitudes of current response in PCs (n = 5 cells). (C) Voltage changes of a BG triggered by paired-pulse stimulation (60V ×2), burst stimulation (100 Hz) of 10 pulses at 60 V (60V ×10), 20 pulses at 60 V (60V ×20), and 20 pulses at 100 V (100V ×20). Even the largest response came back to baseline, and similar response could be created repeatedly, indicating that the recorded cell was not directly damaged with the stimulation. The voltage response amplitude would vary depending on the exact location of the stimulation electrode from the recorded cell, but what is evident from this experiment is that a population of BGs would experience this amount of voltage change when applying typical stimulation protocol to induce synaptic plasticity. Another thing to point out is that, as ChR2 (C128S) is likely to be expressed throughout the cell, ~10-mV voltage change is likely experienced throughout the whole cell in response to photostimulation (A). However, in the case of electrical stimulation, local depolarization could be much higher than what is recorded at the soma and a simulation study estimates as much as ~40 mV change in response to synaptic stimuli (1). (D) Summary of the average amplitudes of voltage response in BGs in response to electrical stimuli (n = 4 cells each). Moderate electrical stimuli of just two pulses produced depolarization of ~1 mV and 100-ms blue light was required to produce similar amount of depolarization (A). Such 100-ms blue light stimulation was sufficient to evoke recordable amount of glutamate by PCs (Fig. 4B). Recordings in B–D were done in the presence of PIC.

Fig. S10. Effect of the glia photostimulation on the HOKR gain increase after 1-h visual training was not apparent. By moving the visual stimulus horizontally and sinusoidally, horizontal optokinetic reflex (HOKR) could be evoked, and the amplitude of the eye movement has been shown to increase with repetitive visual trials. (A) HOKR learning paradigm used. A checkered pattern was presented with the horizontal and sinusoidal movement for 1 h, and photostimulation was delivered three times through the inserted optical fibers at the time indicated. HOKR gain was measured during the first 5 min (Initial) and the last 5 min (After) of the 1 h session. (B) The amount of HOKR gain increase was observed both in the ChR2(C128S)-nonexpressing mice [ChR2(−); n = 5 animals] and the ChR2(C128S)-expressing mice [ChR2(+); n = 4 animals; P > 0.05]. This shows that, even though transient increase in the HOKR gain was observed in ChR2(+)-expressing mice upon glia photostimulation (Fig. 5G), this effect did not produce a net increase in the HOKR gain nor did it interfere significantly with the HOKR learning using the current protocol of visual training and glia photostimulation.

Movie S1. Effect of glia photostimulation on the eye movement and the pupil size during an HOKR experiment. Plastic optical fibers were inserted into the cerebellum of the Mlc1-tTA::teto-Chr2(C128S)-EYFP mouse close to the flocculus region a couple of days before the experiment. The mouse was head-fixed but not anesthetized, and HOKR was induced by moving the screen surrounding the mouse. A single sequence of glia photostimulation through the optic fibers evoked transient pupil dilation and perturbation of the smooth eye pursuit of the visual stimuli. The timing of blue and yellow light pulses is indicated by the bottom left squares. Traces of horizontal eye position (scaled by two times in amplitude) before glia photostimulation (blue trace) and after pupil dilation triggered by the glia photostimulation has settled (green trace) are shown. The increase in HOKR amplitude was relatively short lasting and came back close to the baseline after a couple of minutes. This close-up movie of the eye is shown fivefold faster than real time.