Acquisition phase-specific contribution of climbing fiber transmission to cerebellum-dependent motor memory

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Abstract

Climbing fiber (CF) transmission from the inferior olivary triggers complex spikes (Cs) in Purkinje cells (PCs) driven by a burst of calcium spikes. In the context of motor learning, especially the compensatory optic response, CF transmission serves as instructive signals selectively conveyed to PCs. While the significance of CF input in motor memory formation is widely acknowledged, a comprehensive understanding of its distinct contribution across different temporal windows, spanning from the initial phase of learning to the retrieval period, remains incomplete. Therefore, we aimed to investigate the necessity of CF-induced instructive signals in motor learning by assessing their roles in memory acquisition, consolidation, and retrieval. We employed optogenetics to selectively inhibit CF transmission during targeted time windows. Consequently, the absence of CF-induced instructive signals during motor learning impairs memory acquisition. However, when these signals were suppressed during the consolidation and retrieval period, there was neither a loss of long-term memory nor prevention of memory retrieval. Our results highlight that CF transmission plays a specialized and critical role primarily in memory acquisition, rather than in subsequent processes.

Introduction

The cerebellar architecture, characterized by the presence of multiple synapses connecting various types of neurons, enables precise adjustments within intricate neural networks (Boyden et al., 2004). Within this framework, the climbing fiber (CF) transmission derived from the inferior olive (IO) projecting to the cerebellar cortex is generally recognized as the primary pathway for relaying instructive signals to correct errors and enhance motor performance, including oculomotor behaviors (Albus, 1971; Frens et al., 2001; Ito, 1972; Marr, 1969).

The optokinetic reflex (OKR), a compensatory optic response evoked by an unstable visual field, is a representative example of cerebellum-dependent motor learning (Cahill & Nathans, 2008; Schweigart et al., 1997). Successful adaptation to stabilize retinal images in the presence of mismatched visual input requires the engagement of multiple neural substrates. Complex spikes (Cs) in floccular Purkinje cells (PCs) accompanied by excitatory inputs of CF are known to appear in response to unpredicted errors in motor performance (Clopath et al., 2014; Eccles et al., 1966; Goossens et al., 2004; Palmer et al., 2010). Rotational visual stimulation induces Cs discharge in floccular PCs, reflecting CF-induced Cs generation in response to error signals generated from errors in oculomotor performance (Frens et al., 2001; Graf et al., 1988). Thus, such fine-tuning of Cs in PCs is expected to control the amount of learning (Yang & Lisberger, 2014).

A previous study employing a modified behavioral paradigm that intentionally suppresses the involvement of Cs has suggested that simple spike (Ss) alone is sufficient to drive cerebellar motor learning (Ke et al., 2009). In contrast, others have demonstrated that pharmacological lesion and optogenetic inactivation of the IO impair oculomotor learning (Pham et al., 2020) and prevent eye blink conditioning (Kim et al., 2020; Medina et al., 2002; Welsh & Harvey, 1998). However, due to limitations in spatial and temporal specificity, these findings have not

conclusively determined the necessity of CF in learning. Moreover, while the general perspective of the contribution of CF is primarily focused on the acquisition of memory during the active learning period, it remains unclear whether CF is dispensable in the consolidation phase, despite its continuous role in shaping PC output, which consists of CF-driven Cs with a rate of approximately 1 Hz, reaching the medial vestibular nucleus. To address this knowledge gap, we investigated the necessity of CF-induced instructive signals carried for motor learning by evaluating their contributions to memory acquisition, consolidation, and retrieval. Using optogenetics, we selectively inhibited CF signals in the flocculus, a lobe-like cerebellar structure that is mainly responsible for ocular reflex, during different phases of motor learning. Our findings demonstrate compelling evidence for the acquisition phase-specific contribution of climbing fiber transmission in motor learning.

Results

Optogenetic inhibition of climbing fiber transmission derived from the inferior olive

We first sought to specifically manipulate CF signals in the cerebellar cortex, generally known to be derived from the IO, by injecting AAV1-CaMKIIα-eNpHR 3.0-EYFP or AAV1-CaMKIIα-EGFP into the IO of wild-type mice (Figure 1A, B). This allowed for the visualization of the expression of IO neurons and CF terminals in the brainstem nucleus and flocculus. Furthermore, selectively segregated expression of these regions enabled us to manipulate the terminals without interrupting the soma of IO neurons, which, when damaged, can bring critical malfunction of motor performance and ocular reflexes, because the IO is the prominent center of sensory integration (Pham et al., 2020; Shaikh et al., 2017; Van Der Giessen et al., 2008).

To validate the optogenetic inhibition of CF transmission, we adopted whole-cell patch-clamp recordings of PCs in acute cerebellar slices using a 593 nm-yellow laser. The stimulation electrode was placed on the granule cell layer near the PC (Figure 1C). The stimulation intensity was calibrated to minimally evoke CF excitatory postsynaptic current (EPSC). Once the stimulation intensity was determined, CF ESPC was induced under a laser turned on or off to investigate whether optogenetic inhibition could suppress synaptic transmission. When the laser illumination was applied, the amplitude of the EPSC was robustly decreased. In contrast, when the laser was turned off, suppression was disengaged. Laser illumination did not yield inhibition of CF EPSC in the GFP group (Figure 1D and 1E). Furthermore, we verified the potential reduction of Cs firing rate in PCs of awake mice in vivo by inhibiting CF signals. In summary, these findings indicate the feasibility of optogenetic interventions in blocking CF transmission.

Inhibition of climbing fiber transmission during memory acquisition

An evaluation of CF's direct contribution in a region-specific manner has not been performed. Thus, after confirming the viability of optogenetic inhibition of CF, we investigated whether the elimination of CF transmission in the cerebellar flocculus affects cerebellar motor learning. When images on the retina are unstable due to mismatched visual inputs, CF signals are conventionally perceived to be responsible for sending error signals to the cerebellar cortex to improve ocular reflexes (Bloedel & Bracha, 1998; Frens et al., 2001; Zang & De Schutter, 2019).

Using the same mouse model shown in Figure 1, we surgically implanted an optical fiber in flocculus to suppress CF transmission in vivo during OKR (Figure 2A). Head-fixed mice were placed in a rotating drum painted with a black striped pattern to provide visual input, and its rotating motion elicited an unstable retinal image. Hence, in the beginning, the trace of the mouse's eye movement represented in a sine curve was only partially overlapping with the trace of the drum's kinetics, but as the mouse underwent more sessions of OKR learning, the trace of eye movement became almost identical (Figure 2Bi). Notably, the group with inhibited CF transmission exhibited only slight changes or no improved trace of eye movements (Figure 2Bii). Overall, the results demonstrated that CF transmission is required for motor memory acquisition.

This indicates that CF transmission is essential for cerebellar motor learning by presenting two contrasting learning curves. The control group exhibited an increasing trend in the learning curve, whereas the NpHR group's learning curve was almost flat, which reflects no change of gain (Figure 2C). The gain value through the learning sessions was distinctly increased in the control group, whereas the NpHR group showed no significant change of gain (Figure 2D). Furthermore, from 0 to 50 min period of learning, the control group demonstrated almost a doubling in gain, whereas the NpHR group showed little or almost no

change of gain (Figure 2E). These results indicate that CF transmission is required for the proper acquisition of motor memory.

Inhibition of climbing fiber transmission during memory consolidation and retrieval phase

When memories are initially acquired, they are generally unstable; hence, it requires some time to shape a stabilized long-term memory (Attwell et al., 2002). Therefore, memories undergo multiple processes that allow distinct shifts from short-term to long-term memory, leading to the construction of adapted behavior (Shutoh et al., 2006). Having confirmed the active involvement of CF transmission during memory acquisition, we sought to understand if its role is limited to the acquisition period or extends to memory transfer during the consolidation phase for long-term storage. We implemented identical surgical and behavioral procedures as previously conducted. However, instead of optogenetically inhibiting the transmission during memory acquisition, we suppressed CF activity in two distinct optogenetic manipulation schemes: one targeting short-term inhibition (30 min) and the other for long-term inhibition (6 hr) (Figure 3A and 3D). This approach was based on findings that long-term memories associated with cerebellum-dependent behaviors are vulnerable to disruption within specific time windows of consolidation, spanning from minutes to hours (Cooke et al., 2004; Titley et al., 2007). Learning curves confirmed the successful gain enhancement, validating the integrity of memory acquisition (Figure 3B and 3E). Once the gain was robustly enhanced, optogenetic inhibition of CF transmission during consolidation did not yield any change in the maintenance of memory, irrespective of the duration of manipulation. Consequently, long-term memory was well-established in both NpHR and GFP groups (Figure 3C and 3F). In addition, we verified whether CF signaling is required during the retrieval phase (Figure 3G). When mice, fully trained in the optokinetic reflex, displayed

increased gain, disrupting CF signaling did not affect the retrieval of motor memory (Figure 3H). Thus, our findings suggest that CF activity is less likely to be essential for memory consolidation and retrieval. Its predominant effectiveness during the learning phase suggests a more concentrated role in memory acquisition.

Discussion

We examined the role of CF activity in different temporal phases of motor learning by employing optogenetic tools to specifically disrupt cortical CF transmission. Our results suggest that CF transmission is important for enhancing gain during memory acquisition but serves no critical role in conducting memory transfer during the consolidation period and recalling a maintained memory. In this study, we deliberated on these implications to expand the perspective of CF transmission in cerebellar learning. Cs is formed in response to excitatory synaptic input from the CF and is known to carry instructive signals that eventually reduce behavioral errors and enhance cerebellum-dependent motor performance (Albus, 1971; Hull, 2020; Ito, 1972; Marr, 1969). Classic theories emphasize that CF-driven Cs activity is strongly associated with motor learning; however, for various reasons, including the uncertainty of regional and temporal specificity, incomplete understanding of CF signaling remains a concern.

Once the memory is properly acquired following cerebellum-dependent learning, it is suggested to be transferred to the vestibular nuclei for long-term storage (Jang et al., 2020; Shutoh et al., 2006). By providing optogenetic stimulation to inhibit CF activity at different time points, we were able to distinctly characterize the contrasting effects of CF inhibition in different phases of memory. Our results indicated that CF transmission was pivotal to establishing strong and functional learning outcomes. However, during the process of memory transfer, it was not as essential as it was during the active learning period to acquire an increase of gain. Therefore, it is suggested that CF signaling is one of the mechanisms required for memory acquisition, but not for the transfer or retrieval of memory.

The process of cerebellum-dependent motor learning culminates in systems consolidation involving the medial vestibular nuclei (MVN), which contains post-circuitry of floccular PCs, for long-term memory formation (Shutoh et al., 2006). Because CF signaling is one of the

critical factors for shaping floccular PC firing, we expected that it would indirectly modulate the activity of MVN, and thus, it would eventually affect the consolidation and retrieval of memory. However, our results found no evident influence on long-term memory formation and retrieval, which also implies the absence of a direct impact of CF signaling on MVN activity. Previous studies have suggested that intrinsic plasticity in PCs is essential for memory consolidation (Jang et al., 2020) and that the functional role of granule cells is pivotal for retrieving motor memory (Wada et al., 2014). Consequently, interfering with CF activity during consolidation did not induce significant alterations, indicating a more pronounced role of CF in memory acquisition. Accordingly, we believe there are distinct and segregated functions of substructures in the cerebellar circuit for memory processing.

The underlying mechanism of disrupted motor memory through robust suppression of CF signaling may be described as long-term depression (LTD) of CF-PC synapse, characterized by a distinct reduction in the depolarization peak of Cs (Hansel & Linden, 2000). CF firing typically supplements the induction of parallel fiber-Purkinje cell (PF-PC) LTD, facilitated by calcium influx through voltage-gated channels, when errors like retinal slip trigger Cs (Coesmans et al., 2004). However, the presence of CF-LTD alters the waveform of Cs and attenuates CF-evoked large dendritic calcium transients (Weber et al., 2003). Therefore, in the present study, the attempt at optogenetic manipulation to abolish CF activity during the learning period reproduced CF-LTD, impacting PF-PC LTD, susceptible to changes of CF-evoked calcium transients (Han et al., 2007), potentially disrupting PF-PC plasticity and subsequently impairing gain enhancement.

In conclusion, this study revealed that mice with suppressed CF signaling demonstrated a highly specific impairment of gain increase for memory acquisition. Our evidence provides a clear view of the role of CF in cerebellum-dependent motor learning; however, the underlying mechanisms remain to be fully elucidated. Future investigations on the dynamics

of CF transmission in live neurons using fluorescent probes may reveal detailed mechanisms including transient calcium dynamics.

Materials and Methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Virus	AAV9.CaMKIIα.EGFP	Addgene	RRID: Addgene_50469	Plasmid #50469
Virus	AAV1.CaMKIIα.eNpHR3.0.EYFP	Addgene	RRID: Addgene_26971	Plasmid #26971
Chemical compound, drug	Zoletil	Virvac		
Chemical compound, drug	Rompun	Bayer		
Chemical compound, drug	Dexamethasone	Samyang Pharmaceutical		
Chemical compound, drug	Meloxicam	Boehringer Ingelheim		
Software, algorithm	ZEN	Zeiss	RRID: SCR_013672	
Software, algorithm	Fiji	http://fiji.sc	RRID: SCR_002285	
Software, algorithm	Patchmaster	HEKA	RRID: SCR_000034	
Software, algorithm	Igor Pro	WaveMetrics	RRID: SCR_000325	
Software, algorithm	GraphPad Prism	GraphPad	RRID: SCR_002798	

Animals and Stereotaxic Surgery for Virus Injection

All experimental procedures were approved by the Seoul National University Institutional Animal Care and Use Committee. First, wild-type mice aged 7–10 weeks were anesthetized using intraperitoneal injections of a Zoletil/Rompun mixture (30 mg / 10 mg/kg). For optogenetic expression in CF, the virus was injected into the IO 2–3 weeks before the

behavioral test, as previously described (Kimpo et al., 2014; Roh et al., 2020). Briefly, bilateral injections were made at the midpoint between the edge of the occipital bone and the C1 cervical vertebra. The glass pipette was set at a 55° angle from the vertical and 7° from the midline. After approaching a 3 mm depth, a virus solution containing 100–200 nL of AAV1-CaMkIIα-eNpHR3.0-EYFP or AAV9.CaMkIIα-EGFP was injected with a Picopump at 5 nL/s. The pipettes were left in place for 10 min before they were removed to minimize backflow.

Stereotaxic Surgery for Optic Cannula Implantation

After the mice were fully recovered (a few days to a week) from the first surgery of virus injection, a second operation of optic cannula implantation (Ø1.25 mm Ceramic Ferrule, L=3.0 mm) near cerebellar flocculus and head fixation was conducted. Unlike the fiber optic cannula, head fixation parts were hand-made. Before the surgery, mice were anesthetized by intraperitoneal injection of Zoletil 50 (Virbac, 15 mg/kg) and xylazine (Rompun, Bayer, 15 mg/kg) mixture. Mice were given 48 hours to recover after surgery.

Confirmation Viral Expression.

After completion of all the experiments, sampling using cardiac perfusion is performed. The brain was then extracted and coronal sections were made at 30 μm intervals. Images were acquired and processed using a confocal microscope (Zeiss LSM 7 MP, Carl Zeiss, Jena, Germany) and Zen software (Zeiss). The location of virus expression was confirmed by comparison with the brain atlas (Paxinos and Franklin's The Mouse Brain in Stereotaxic Coordinates 4th Edition by George Paxinos).

Optical suppression of CF terminals in the cerebellar cortex in vivo

To manipulate CF transmission-induced Cs in PCs, AAV1.CaMKIIα.eNpHR3.0.eYFP or AAV8.CaMKIIa.eGFP was bilaterally injected into the IO. A glass micro-pipette was used to inject 200 nL per site. To optically suppress the CF terminals and record CF-PC synaptic response, a small cranial window was made above the cerebellar cortex lobule IV/V a week after the viral injection. After 2 weeks from the viral injection, mice underwent single-unit recordings. The recordings were performed as described in Neural data acquisition and analysis. Cs responses were acquired during 10 seconds of yellow on and off sessions 20 times to test the effect of CF terminal suppression on CF-PC synaptic transmission.

Neural data acquisition and analysis

Single-unit recordings were performed while mice were head-fixed and awake in the recording rack. A Digital Lynx system (Neuralynx, Bozeman, MT) was used to amplify, band pass filter (0.1–8000 Hz for simple spikes, 10–200 Hz for complex spikes), and digitize the electrode recordings. A silicon neural probe (Cambridge NeuroTech) was used to probe and acquire Cs signals. Cs signals were collected at a 32 kHz sampling rate. Cs waveforms were manually selected based on visual inspection of the averaged appearance of characteristic negative Ca^{2+} peaks.

Slice preparation, electrophysiology, and optogenetic validation

An acute brain slice preparation and electrophysiological experiments were carried out as previously described (Lee et al., 2023). First, mice aged 9–10 weeks with AAV1.CaMKIIα.eNpHR 3.0.WPRE-EYFP injection into their inferior olivary neuron 2–3 weeks before the preparation were anesthetized by isoflurane and briefly decapitated. Then, 250 μm-thick sagittal slices of the cerebellar vermis were obtained from the mice using a

vibratome (VT1200S, Leica). The ice-cold cutting solution contained 75 mM sucrose, 75 mM NaCl, 2.5 mM KCl, 7 mM MgCl2, 0.5 mM CaCl2, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 25 mM glucose with bubbled 95% O2 and 5% CO2. The slices were immediately transferred into the artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 10 mM glucose with bubbled 95% O2 and 5% CO2. They were allowed to recover at 32 °C for 30 min and at room temperature for 1 hour. All recordings were performed within 8 hr of recovery.

Brain slices were placed in a submerged chamber with perfusion of ACSF for at least 10 min before recording. We used OptoPatcher (HEKA Elektronik) holding recording pipette (2–3 MΩ) filled with internal solution containing 9 mM KCl, 10 mM KOH, 120 mM K-gluconate, 3.48 mM MgCl2, 10 mM HEPES, 4 mM NaCl, 4 mM Na2ATP, 0.4 mM Na3GTP, and 17.5 mM sucrose (pH 7.25). The stimulation electrode was placed on the granule cell layer near the PC. The stimulation isolator injected a brief current pulse to the stimulation electrode and was controlled using PatchMaster software (HEKA Elektronik). Stimulation intensity (6–30 μA) was calibrated to minimally evoke CF EPSC. Regarding the laser on trial, a 593.5 nm irradiation from a yellow laser was applied 5 s before CF stimulation. The eEPSC amplitude was averaged over three lasers on and off trials. Electrophysiological data were acquired using an EPC10 patch-clamp amplifier (HEKA Elektronik) and PatchMaster software (HEKA Elektronik) with a sampling frequency of 20 kHz, and the signals were filtered at 2 kHz. All electrophysiological recordings were acquired from the central cerebellar vermis. The amplitude of EPSC was analyzed using Igor Pro (WaveMetrics).

Optokinetic reflex behavior test

First, two sessions of acclimation were conducted. During each session, the mouse was

restrained using a custom-made restrainer for 20 min both with and without light for habituation to the recording environment. Following the procedure reported by Stahl et al. (Stahl, 2002), a calibration was performed. This allowed for the conversion of the dynamics of pupil to eye rotation. Next, three basal oculomotor performances including (OKR), vestibulo-ocular reflex in the dark (dVOR), and vestibulo-ocular reflex in the light (lVOR) were measured. During the recording of eye movements, it was necessary to control pupil dilation that was induced by an absence of light in dark condition. Thus, physostigmine salicylate solution (Eserine; Sigma Millipore) was given to mice under brief isoflurane anesthesia. The concentration of eserine solution was increased from 0.1%, 0.15%, and 0.2% based on the pupil size. Basal oculomotor performances were described in gains. For the actual learning protocol, we adopted OKR and it consisted of five training sessions (10 min per session), six checkup points, and 24 hr of consolidation period. During the training sessions, the mice were visually stimulated with a sinusoidally rotating drum (±5 degrees). Completely trained mice were placed back into their home cage, which was stored in the dark condition until the last gain checkup points.

Gain Analysis

Gains acquired from basal oculomotor performances and OKR learning were calculated as the ratio of evoked eye movements to the movement of the screen or turn table as visual or vestibular stimuli, respectively. A custom-built LabView (National Instrument) analysis tool was used for all the calculations

Statistical analysis

Graph plotting and statistical analysis were performed using GraphPad Prism (GraphPad Software Inc, CA, USA). The hypothesis was tested by using paired and unpaired t-tests

between sample pairs using GraphPad Prism. Statistical significance was set at $p < 0.05$. Asterisks denoted in the graph indicate the statistical significance. * indicates $p < 0.05$, ** < 0.01, *** < 0.001, and **** < 0.0001. The test name and statistical values are presented in each figure legend.

Acknowledgments

This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean Government (MSIT) (NRF-2018R1A5A2025964 and 2022M3E5E8017970 to Sang Jeong Kim).

Competing interest

The authors declare that they have no conflicts of interest.

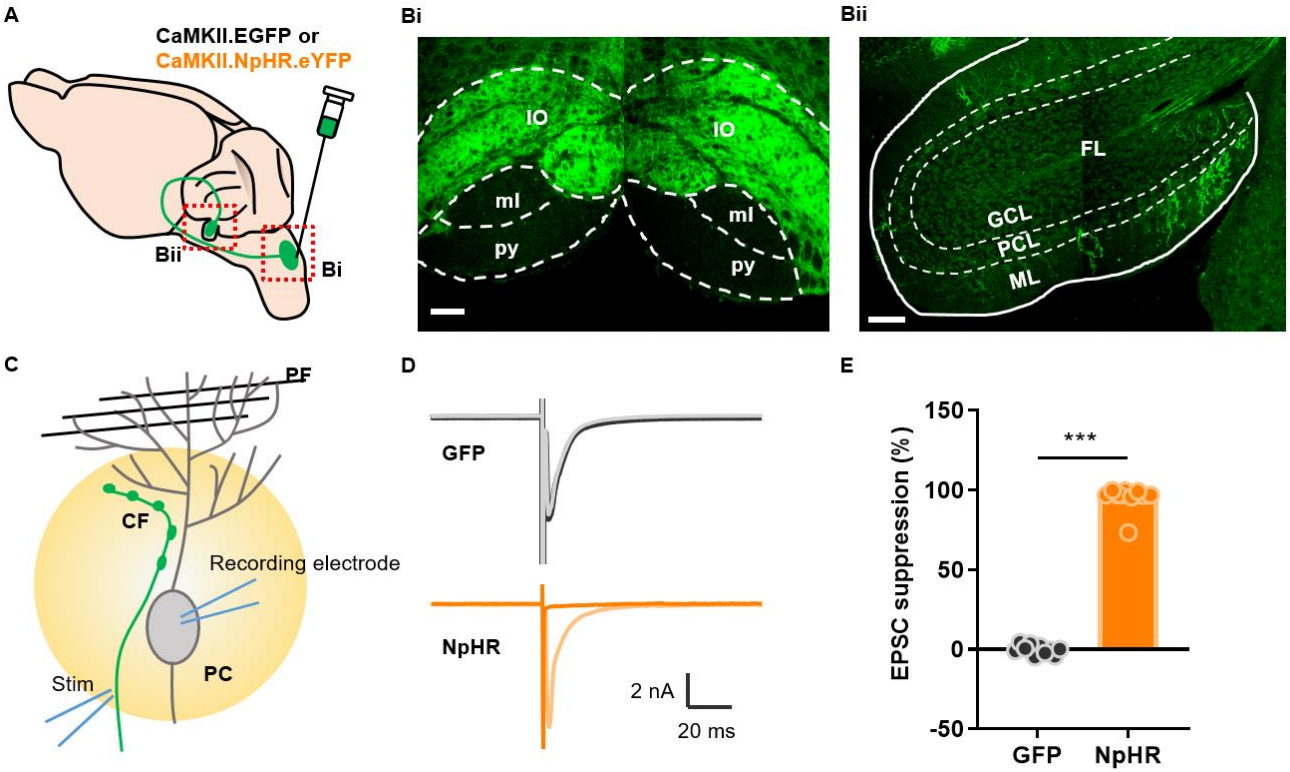


Figure 1. Optogenetic inhibition of climbing fiber transmission derived from the inferior olive.

A. Schematic diagram showing virus injection for optogenetic modulation of CF.

B. Bi. A virus is expressed specifically only in the IO region (IO, inferior olive; py, pyramidal tract; ml, medial lemniscus). Scale bar, 100 μm. Bii. Virus expression along CF in the cerebellar flocculus region. (GCL, granule cell layer; PCL, Purkinje cell layer; ML, molecular layer; FL, flocculus). Scale bar, 100 μm.

C. A scheme of optogenetic suppression using a yellow laser (593 nm, 3–5 mW) while recording CF EPSCs.

D. Representative traces of CF EPSCs of GFP (top) and NpHR group (bottom) with (dark line) and without (light line) optostimulation.

E. Quantitative analysis of suppression of CF EPSCs (n = 9 cells for each group, *** $p < 0.001$; Unpaired t test).

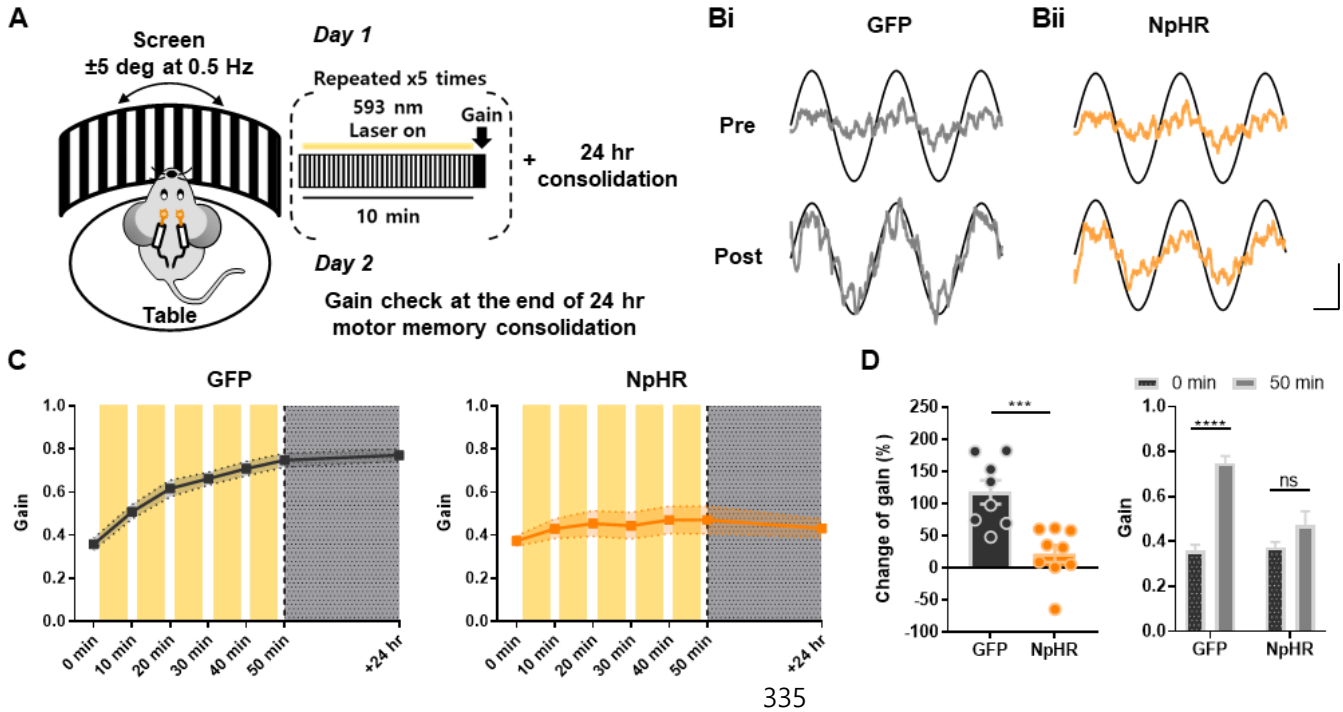


Figure 2. Inhibition of climbing fiber transmission during memory acquisition of OKR.

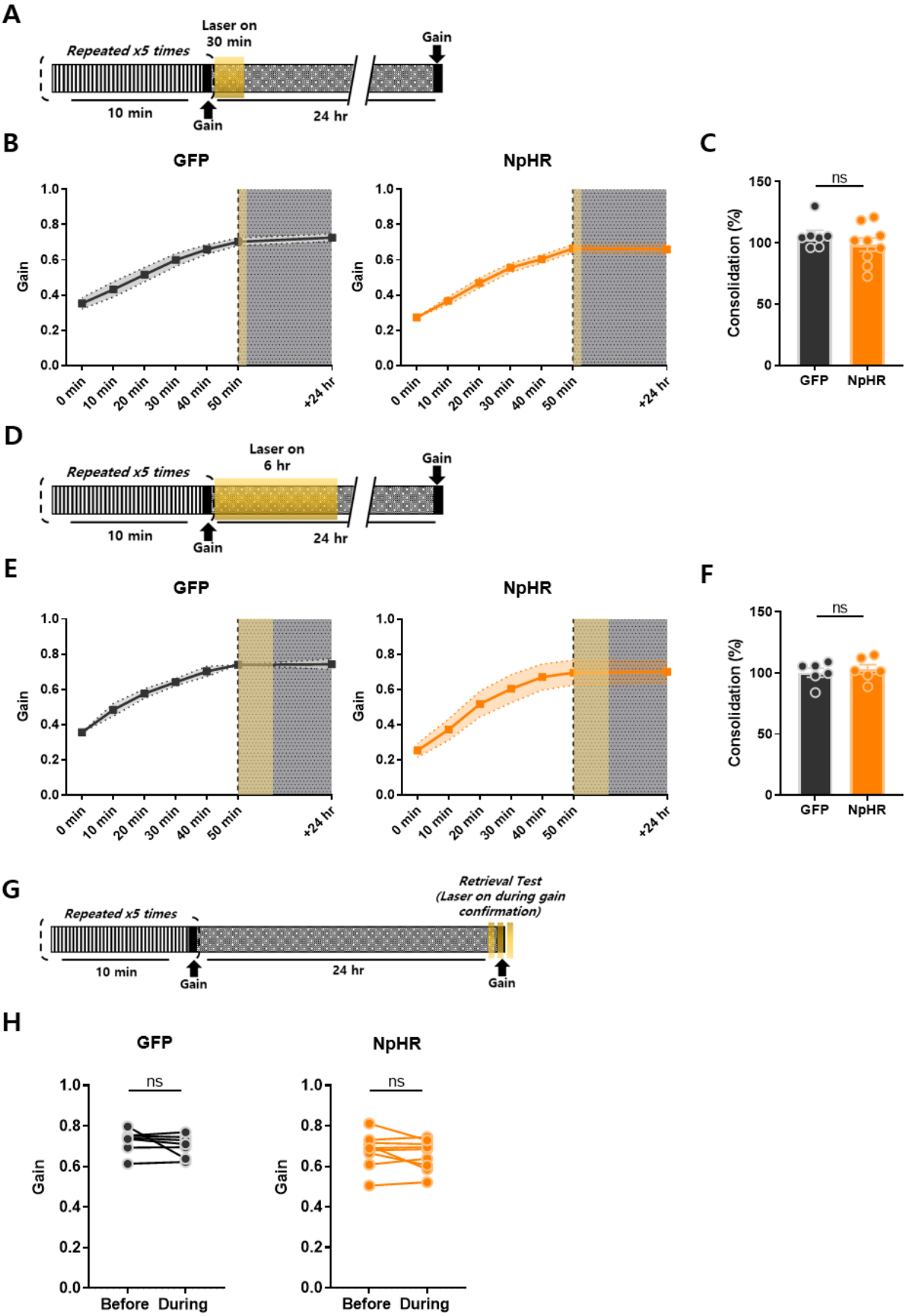
A. Illustration of the optokinetic reflex behavioral test (left) and experimental scheme (right). For training, visual stimulation was given by constant rotation of the screen, while the table, where a mouse's head is fixed, remained stationary. For day 1, a mouse was trained for a total of 50 minutes, and on day 2, an amount of sustained memory was validated via gain check.

B. Representative traces of the screen (black sinusoidal curve) and eye movements (grey sinusoidal curve) divided into GFP (i) and NpHR (ii) groups. Top traces (pre) were acquired before learning, and bottom traces (post) were obtained after 50 min of learning. The vertical scale bar represents 10 degrees per second and the horizontal scale bar represents 0.5 seconds.

C. The learning curve from 0 min to 50 min, and +24 hr period. Change of gain is indicated at each time point (+10 min increment). Yellow boxes indicate opto-stimulation (12.5

mW for 10 min/session, total of 5 sessions). The left graph represents GFP (n = 8) and the right graph indicates the NpHR (n = 9) group.

D. Comparison of gain changes between GFP and NpHR groups (GFP, n = 8 mice; NpHR, n = 9 mice). Percentages of gain increment from 0 min to 50 min were calculated. The change of gain of the GFP group was significantly larger than that of the NpHR group (left; Unpaired t test, $p = 0.0007$). The gain of the GFP group was significantly increased from 0 min to 50 min (right; Paired t test, **** $p < 0.0001$), while the NpHR group showed no significant improvement of gain from 0 min to 50 min (right; Paired t test, $p = 0.1046$).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30 min
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20 min
30 min
40 min
50 min
+24 hr
C
ns
Consolidation (%)
GFP
NpHR
D
Repeated x5 times
Laser on
6 hr
Gain
10 min
Gain
24 hr
E
GFP
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10 min
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24 hr
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GFP
NpHR
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Gain
Before
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Figure 3. Inhibition of climbing fiber transmission during memory consolidation and retrieval phase.

A. Illustration of the experimental scheme. 10 min of optokinetic reflex training was repeated 5 times, and then opto-stimulation (12.5 mW) was given at 0 min post-learning for 30 min.

B. The learning curve from 0 min to 50 min, and +24 hr period. Change of gain is indicated at each time point (+10 min increment). A yellow box indicates opto-stimulation at 0 min post-learning. The left graph represents GFP and the right graph indicates the NpHR group.

C. Comparison of consolidation percentage between GFP and NpHR groups (GFP group, n = 7 mice; NpHR group, n = 9 mice). The percentage of sustained memory after 24 hr was calculated. No difference was observed between the groups ($p = 0.3173$; Unpaired t test).

D. Illustration of the experimental scheme. 10 min of optokinetic reflex training was repeated 5 times, and then opto-stimulation (12.5 mW) was given at 0 min post-learning for 6 hr.

E. The learning curve from 0 min to 50 min, and +24 hr period. Change of gain is indicated at each time point (+10 min increment). A yellow box indicates opto-stimulation at 0 min post-learning. The left graph represents the GFP group, and the right graph indicates the NpHR group.

F. Comparison of gain changes between GFP and NpHR groups (GFP group, n = 6 mice; NpHR group, n = 6 mice). Percentages of gain increment from 0 min to 50 min were calculated. No difference was observed between the groups (Unpaired t test, $p = 0.6405$).

G. Illustration of the experimental scheme. Following proper motor learning on day 1, the remaining gain value was checked on day 2, after testing 1st gain retrieval without opto-stimulation, 2nd gain retrieval was tested with opto-stimulation to validate the effect of CF

inhibition on gain retrieval (opto-stimulation was given 3 times for 24 seconds each at 12.5 mW).

H. Gain retrievals before and during opto-stimulation were compared. The left graph represents the GFP group, and the right shows the NpHR group (GFP group, n = 6 mice; NpHR group, n = 8 mice). There was no significant variation between the groups (NpHR group, p = 0.2439; GFP group, p = 0.3180; Paired t test).

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