

# Optogenetic control of mGluR1 signaling modulates synaptic plasticity and cerebellum driven learning

**Tatjana Surdin**

Ruhr University Bochum

**Michelle Grömmke**

Ruhr-University Bochum

**Hanna Böke**

Ruhr University Bochum

**Maike Barcik**

University Hospital Duesseldorf

**Zohre Azimi**

Ruhr University Bochum

**Dirk Jancke**

Ruhr University Bochum <https://orcid.org/0000-0001-8440-6259>

**Stefan Herlitze**

Ruhr University Bochum

**Melanie Mark**

Ruhr University Bochum <https://orcid.org/0000-0002-2788-6003>

**Ida Siveke** (✉ [ida.siveke@rub.de](mailto:ida.siveke@rub.de))

Ruhr University Bochum

---

## Article

**Keywords:** optogenetics, synaptic plasticity, cerebellum, mGluR1, motor learning, LTD

**Posted Date:** February 17th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1344254/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

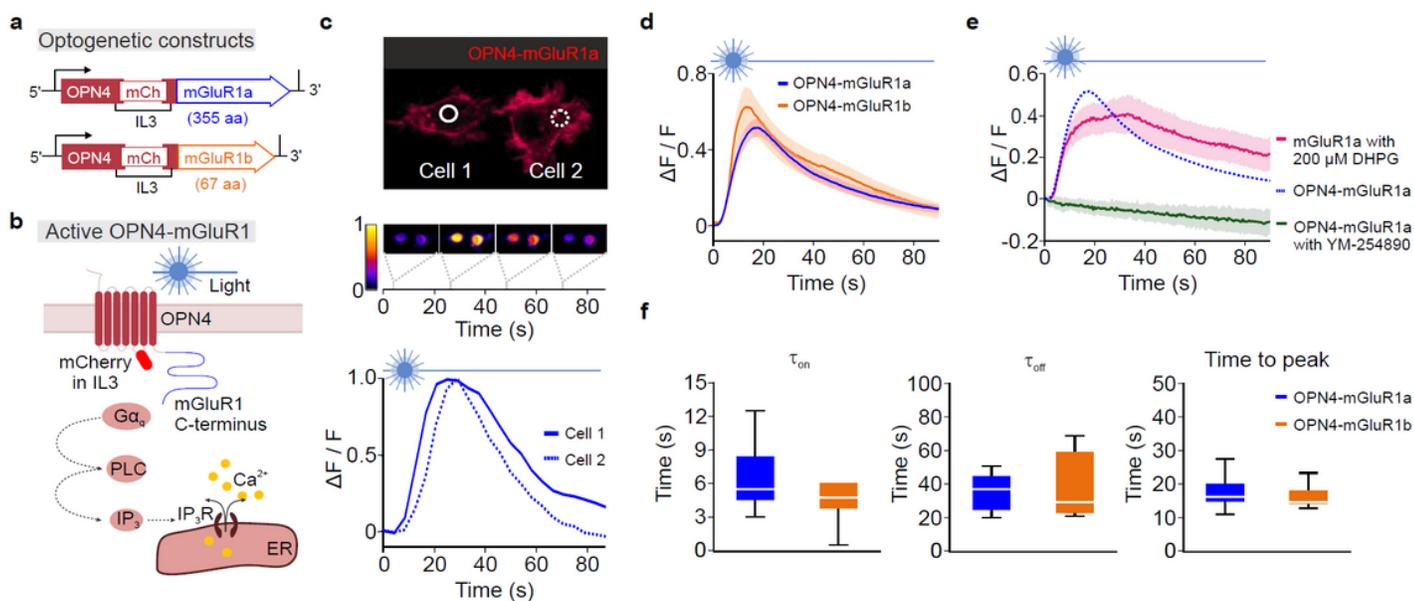
# Abstract

Neuronal plasticity underlying cerebellar learning behavior is strongly associated with type 1 metabotropic glutamate receptor (mGluR1) signaling. This receptor is located at perisynaptic sites at cerebellar Purkinje cells (PCs) and detects glutamate spill over. Activation of mGluR1 leads to activation of the Gq/11 pathway, inducing synaptic plasticity at the parallel fiber-Purkinje cell synapse (PF-PC) in form of long-term depression (LTD). To optogenetically modulate mGluR1 signaling we fused mouse melanopsin (OPN4) that activates the Gq/11 pathway to the C-termini of mGluR1 splice variants (OPN4-mGluR1a and OPN4-mGluR1b). We provide the prove-of-concept approach to modulate synaptic plasticity via optogenetic activation of OPN4-mGluR1a inducing LTD at the PF-PC synapse *in vitro*. Moreover, we demonstrate that light activation of mGluR1a signaling pathway by OPN4-mGluR1a in PCs leads to an increase in intrinsic activity of PCs *in vivo* and improved cerebellum driven learning behavior.

## Full Text

This preprint is available for [download as a PDF](#).

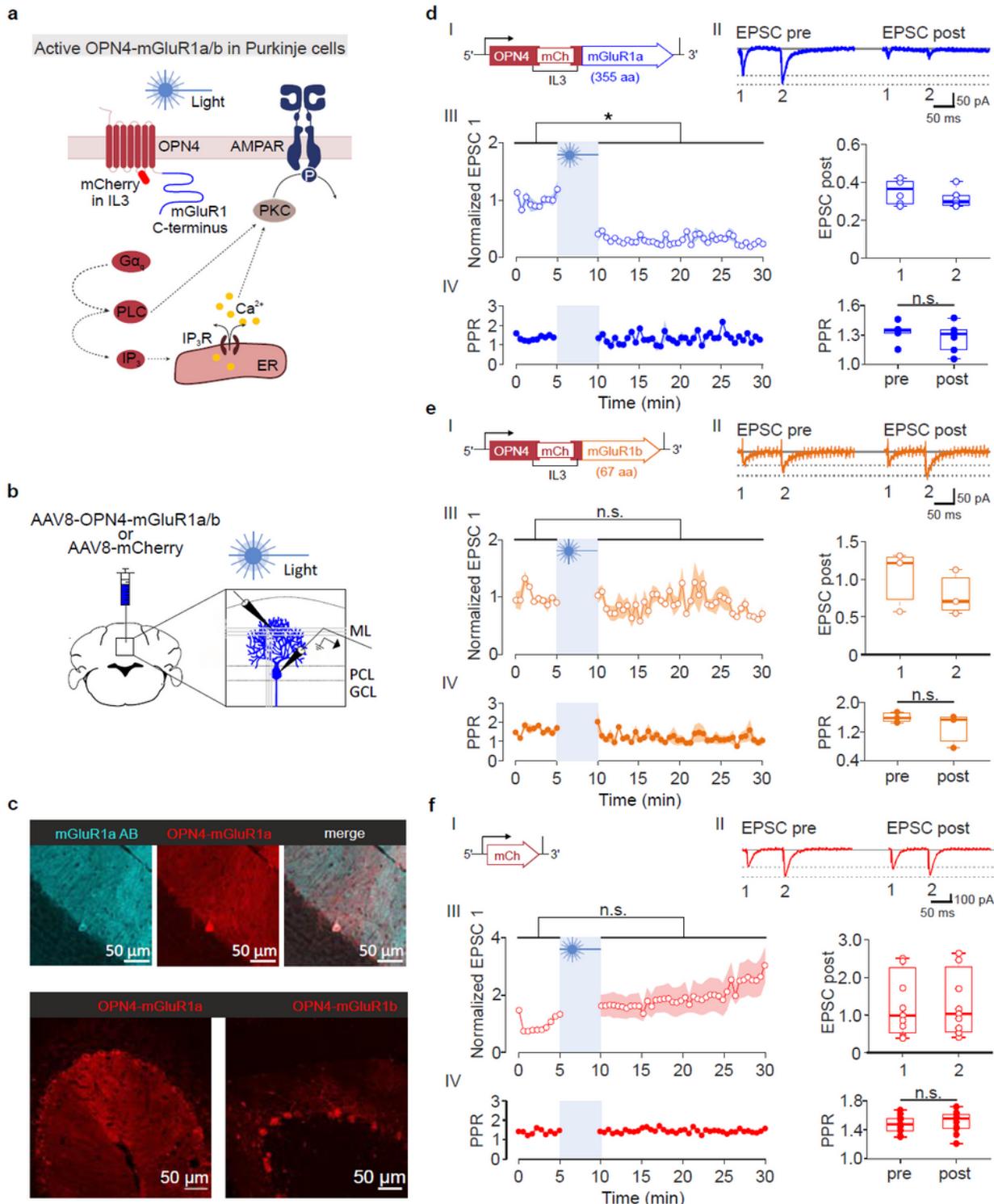
## Figures



**Figure 1**

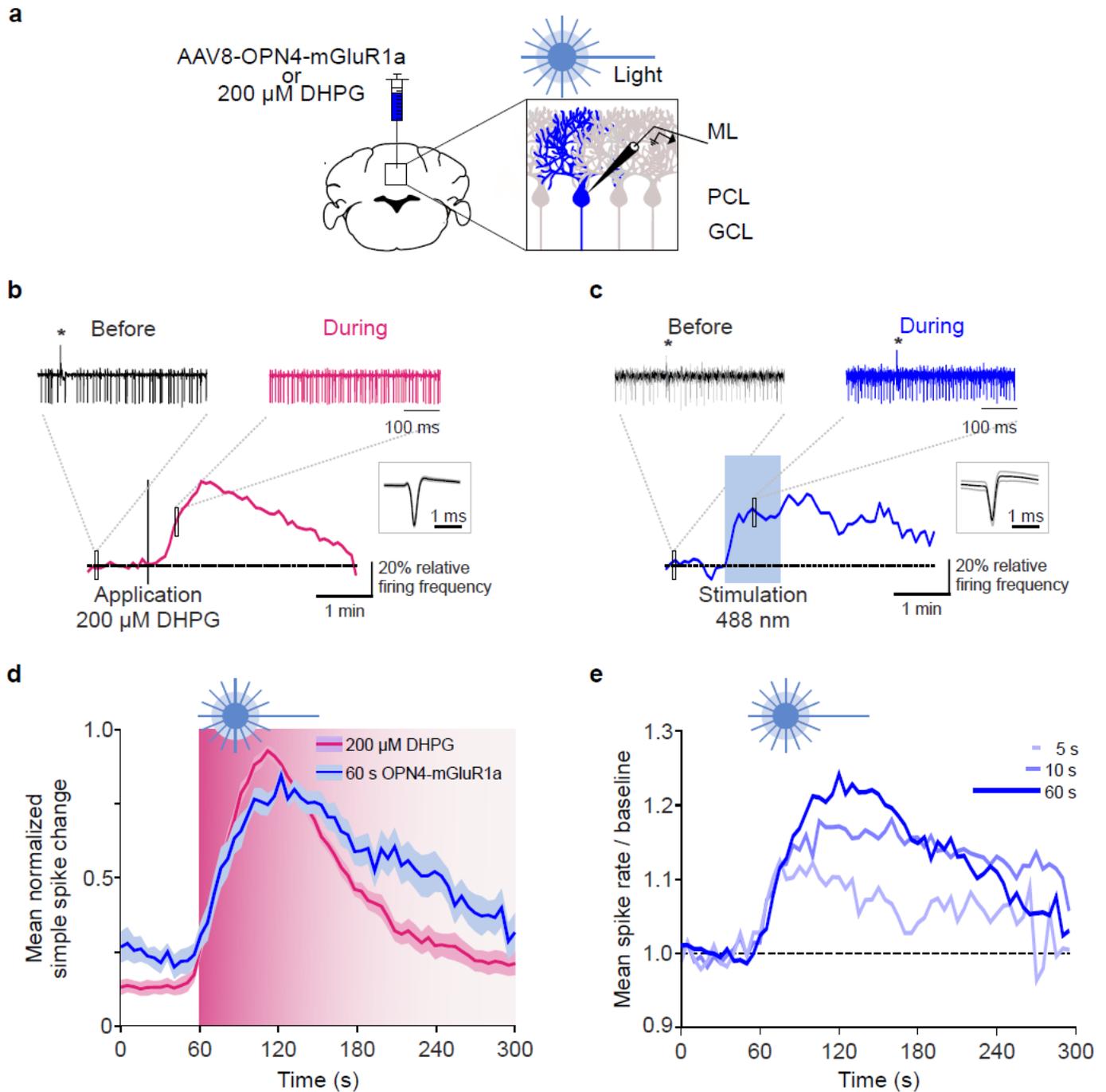
Design and characterization of OPN4-mGluR1 variants. (a) Design of OPN4-mGluR1a (blue) and OPN4-mGluR1b (orange) constructs. Chimeras comprise OPN4, mCherry in the third intracellular loop and mGluR1a or mGluR1b fused c-terminally. (b) Scheme of OPN4-mGluR1 design and downstream signaling cascade following photostimulation. (c) Top, exemplary OPN4-mGluR1a expression in two HEK293 cells. The construct is localized in the cell membrane. Bottom, transient calcium signal of both cells during light

stimulation. (d) Time-course of light-activated Ca<sup>2+</sup> signaling of OPN4-mGluR1a (blue, n=15 dishes) and OPN4-mGluR1b (orange, n=8 dishes) over a stimulation period of 90 seconds. Shading indicates  $\pm$  SEM. (e) Pharmacological activation of mGluR1a in HEK293 cells shows comparable increase post and delayed peak of Ca<sup>2+</sup> signaling (red, n=13 dishes). Pharmacological block of Gq/11 protein signaling abolishes OPN4-mGluR1a Ca<sup>2+</sup> signals (green). (f) Calcium signal components ton, toff and time to peak were compared between optogenetic chimeras and showed no statistical difference. Population data of the responses shown in d are shown as boxplot (mean  $\pm$  SEM).



## Figure 2

Activation of OPN4-mGluR1a but not OPN4-mGluR1b induced LTD in Purkinje cell recordings *in vitro*. (a) Schematic drawing of downstream Gq/11-protein coupled signaling cascade after optogenetic stimulation of OPN4-mGluR1 variants. Optimal signaling results in phosphorylation and internalization of AMPA-receptors inducing LTD of excitatory postsynaptic currents (EPSCs) evoked by parallel fiber stimulation in Purkinje cells. (b) Scheme of experimental procedure for cerebellar slice recordings. OPN4-mGluR1a, OPN4-mGluR1b or mCherry were expressed in Purkinje cells using AAVs. (c) Expression pattern of OPN4-mGluR1a and OPN4-mGluR1b in Purkinje cells; mGluR1a antibody (cyan), OPN4-mGluR1a and OPN4-mGluR1b (red), colocalization (white). OPN4-mGluR1b is sparsely expressed in dendrites compared to OPN4-mGluR1a. (d-f) EPSCs were evoked by double pulse stimulation of parallel fibers combined with 470 blue light stimulation of OPN4-mGluR1a (d; n = 5), OPN4-mGluR1b (e; n = 3) and mCherry-control (f; n = 9). I Design of OPN4-mGluR1a (blue), OPN4-mGluR1b (orange) and mCherry (red) constructs. Chimeras comprise OPN4, mCherry in the third intracellular loop and mGluR1a or mGluR1b fused c-terminally. II Example traces of EPSCs are shown on the top, III shows the normalized EPSC1 and IV the paired pulse ratio (PPR) over stimulation time. EPSCs were normalized to the average pre EPSC (0-5min). Response of the post EPSCs and PPRs before and after blue light stimulation are given as mean response over time (pre: 0-5 min; post: 10-30 min). Significant LTD of post EPSCs ( $p = 0.031$ ; two-sided Wilcoxon signed-rank test) was only induced by optical stimulation of OPN4-mGluR1a. PPR showed no significant change.

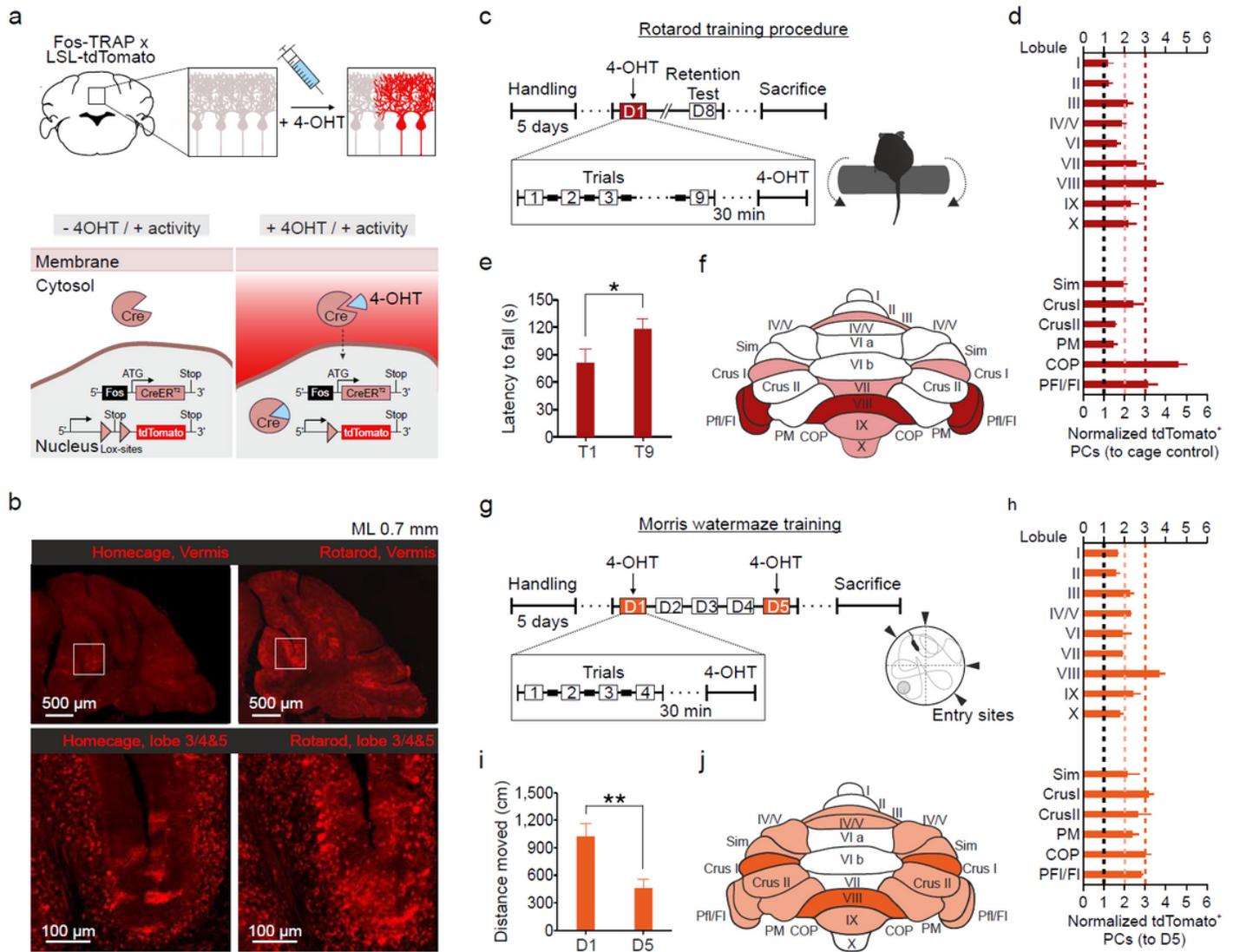


**Figure 3**

OPN4-mGluR1a elevates Purkinje cell simple spike firing rate in vivo.

(a) Scheme of experimental procedure for single-cell recordings in cerebellum in anaesthetized mice. Recordings were made two weeks after AAV8-OPN4-mGluR1a injection, DHPG was locally applied during recordings. (b, c) Exemplary Purkinje cell recordings (identified by characteristic simple and complex spike firing pattern, marked with \*) over 5 min with 60 s baseline recoding and subsequent (b) pharmacological (DHPG - mGluR1 agonist) or (c) optogenetic activation using 488nm wavelength. (d) Pharmacological activation of mGluR1 (DHPG - mGluR1 agonist, pink trace) or blue light activation

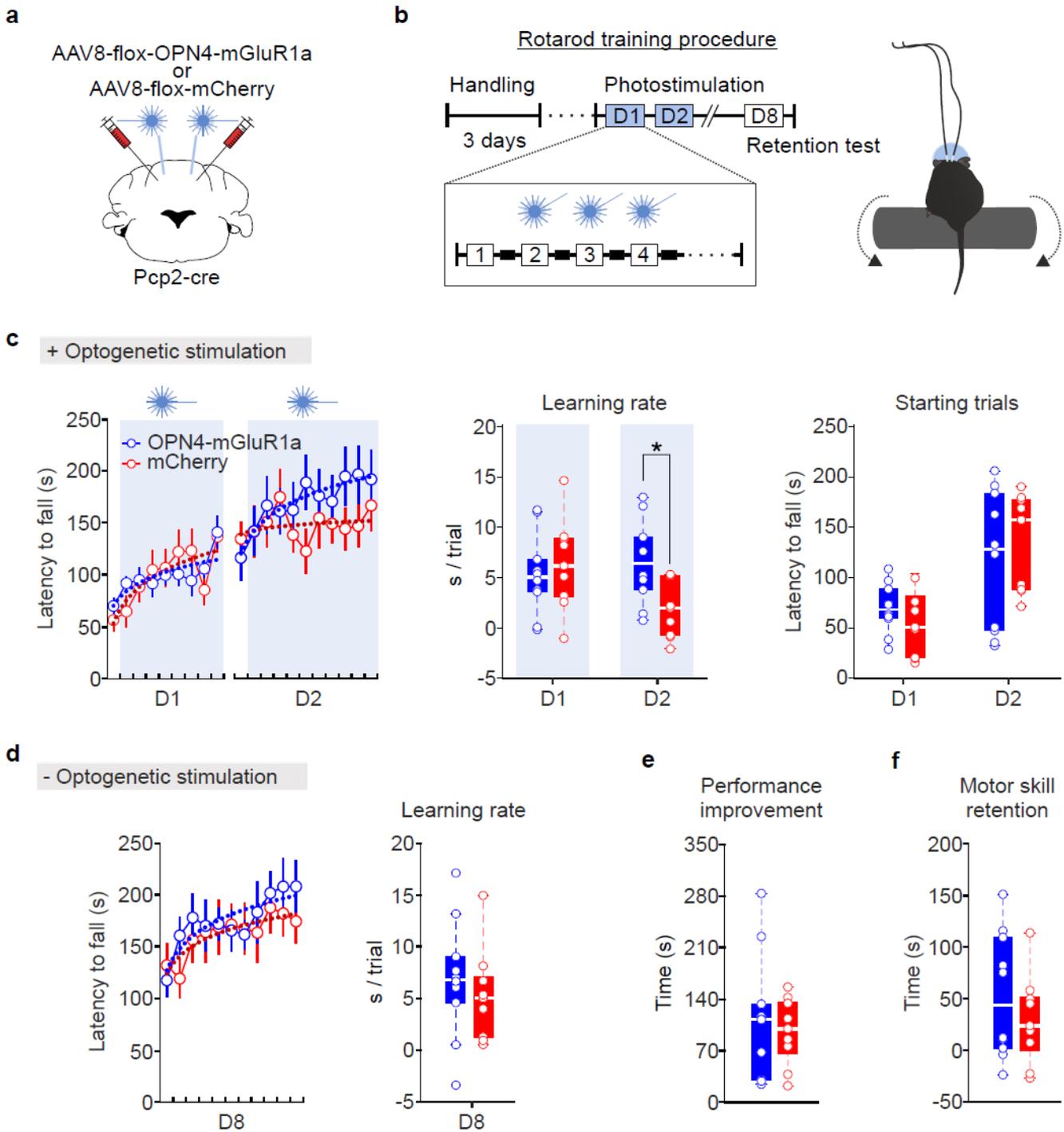
OPN4-mGluR1a (488nm; blue trace) lead to a rise in simple spike firing frequency (DHPG: n=30 PCs in n=3 mice; OPN4-mGluR1a: n=12 PCs in n=4 mice). Shading indicates  $\pm$  SEM. (e) Firing rate depends on pulse length and increases with light stimulation duration of 5 s (lightest blue, n=2 PCs in n = 1 mice), 10 s (blue, n = 8 PCs in n = 3 mice) or 60 s (dark blue, n=12 PCs in n = 4 mice), respectively.



**Figure 4**

Distribution of c-Fos activity of Purkinje cells during motor learning on the accelerated rotarod and Morris watermaze. (a) Schematic drawing showing induction of tdTomato expression by active cells in Fos-TRAP x LSL-tdTomato mouse line. Neuronal activation induces expression of immediate early gene c-Fos. CreERT2 is expressed under c-Fos promoter but is retained in the cytoplasm when 4-OHT is absent. Upon injection of 4-OHT, CreERT2 enters the nucleus, excises the STOP-cassette and enables tdTomato expression. (b) Exemplanary expression patterns of control (left; n = 3) and rotarod trained mice (right; n = 3). Bottom, cerebellar lobes 3, 4 and 5 are shown magnified. (c) Protocol for rotarod training and 4-OHT injection 30 min after completion of the last trial. (d) Active cerebellar areas expressed as tdTomato+ Purkinje cells in each lobe of rotarod trained mice, normalized to homecage controls (rotarod-trained, n=3;

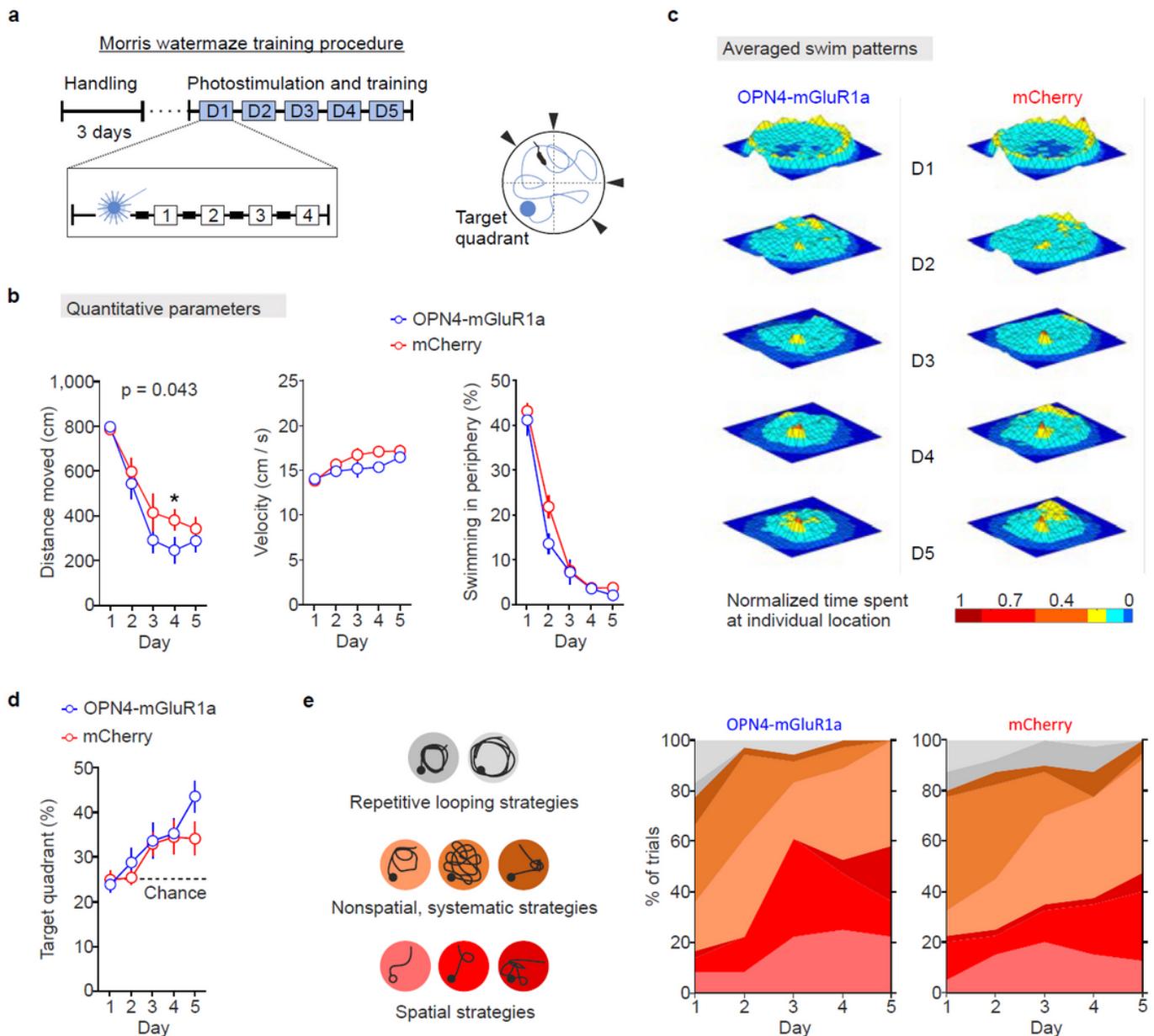
homecage, n=4 mice). (e) Motor learning indicated by elevated latency to fall on accelerating rotarod (mean + SEM,  $t_2 = -6.672$ ,  $p = 0.022$ ; n = 3 trained mice). (f) Summary of active zones of the cerebellum after rotarod training. Light red stippled line corresponds to areas showing twofold increase, dark red indicates threefold increase in tdTomato positive Purkinje cells. (g) Protocol for Morris watermaze training and 4-OHT injection 30 min after last trial is completed. Mice were divided into two groups, receiving 4-OHT injections on D1 or D5, respectively. (h) Active cerebellar areas expressed as tdTomato+ Purkinje cells in each lobe of Morris watermaze trained mice injected on D1, normalized to mice injected on D5 (labeled on D1, n=2; labeled on D5, n=2). (i) Spatial learning indicated by decreasing distance moved in the Morris watermaze test (mean + SEM,  $t_3 = 6.071$ ,  $p = 0.009$ , n = 4). (j) Active zones of the cerebellum after spatial navigation training. Light orange corresponds to areas showing twofold increase, bright orange indicates threefold increase in tdTomato+ Purkinje cells.



**Figure 5**

Enhanced motor learning performance in the accelerated rotarod induced by OPN4-mGluR1a. (a) OPN4-mGluR1a ( $n = 9$ ) or mCherry ( $n = 9$ ) were injected into cerebellar vermis and light guides were implanted. (b) Rotarod training protocol consisted of two days (D1 & D2) with optogenetic stimulation during rotarod sessions followed by a retention test session on day 8 (D8) without optogenetic stimulation. One training session consisted of 9 trials on D1 and 11 trials on D2. Optical stimulation started at trial 2 on both days. (c) Left, performance (measured as latency to fall) increases with training on D1 and D2 (Mean  $\pm$  SEM.).

Middle, learning rates of OPN4- mGluR1a expressing mice on D2 are significantly higher after photostimulation ( $p = 0.028$ , Mann-Whitney U test). Right, performance did not differ between groups on starting trials of both days (without stimulation). (d) In the retention test session in absence of optogenetic stimulation, performance and learning rate did not differ between groups. (e) Performance improvement and motor skill retention (f) were not affected by tool activation or light stimulation.



**Figure 6**

OPN4-mGluR1a activation facilitates navigation efficiency in the Morris watermaze. (a) Training protocol to test spatial learning performance in Morris watermaze test. During training, mice had to search for a submerged platform in 4 trials over 5 consecutive days. On each day, mice were optically stimulated using the same optical stimulation protocol established for LTD induction in cerebellar slices (see figure 2; 70 pulses, 400ms duration, 0.5 Hz) before training. (b) Left, both groups learned the task within 5 days

(within subjects effect,  $F_{4,68}=27.346$ ,  $p < 0.0001$ , repeated measures ANOVA). OPN4-mGluR1a expressing mice ( $n = 9$ ) swam significantly shorter distances to find the hidden platform than mCherry expressing mice ( $n = 10$ ) (between subjects effect,  $F_{1,17} = 4.79$ ,  $p = 0.043$ , repeated measures ANOVA) while velocity (middle) and thigmotaxis (defined as time swimming in periphery) did not differ between groups (mean  $\pm$  SEM). (c) Averaged swim patterns of OPN4- mGluR1a (left) and mCherry (right) expressing mice. The search radius of the mice decreases with training days. This pattern is also detected by relative time spent in the target quadrant (d; mean  $\pm$  SEM). (e) Platform searching strategies were divided into three searching categories consisting of 2-3 sub strategies. Searching strategies of OPN4-mGluR1a expressing mice exhibited a shift towards enhanced spatial strategies (red) starting on D3 and show lower percentage of nonspatial (ochre) and repetitive looping strategies (grey) compared to controls.