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Systemic pharmacological suppression of neural activity reverses learning impairment in a mouse model of Fragile X syndrome

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Abstract

The enhancement of associative synaptic plasticity often results in impaired rather than enhanced learning. Previously, we proposed that such learning impairments can result from saturation of the plasticity mechanism (Nguyen-Vu et al., 2017), or, more generally, from a history-dependent change in the threshold for plasticity. This hypothesis was based on experimental results from mice lacking two class I major histocompatibility molecules, MHCI H2-K^b and H2- D^b (MHCI $K^bD^{b-/-}$), which have enhanced associative long-term depression at the parallel fiber-Purkinje cell synapses in the cerebellum (PF-Purkinje cell LTD). Here, we extend this work by testing predictions of the threshold metaplasticity hypothesis in a second mouse line with enhanced PF-Purkinje cell LTD, the *Fmr1* knockout mouse model of Fragile X syndrome (FXS). Mice lacking *Fmr1* gene expression in cerebellar Purkinje cells (L7-*Fmr1* KO) were selectively impaired on two oculomotor learning tasks in which PF-Purkinje cell LTD has been implicated, with no impairment on LTD-independent oculomotor learning tasks. Consistent with the threshold metaplasticity hypothesis, behavioral pre-training designed to reverse LTD at the PF-Purkinje cell synapses eliminated the oculomotor learning deficit in the L7-*Fmr1* KO mice, as previously reported in MHCI $K^bD^{b-/-}$mice. In addition, diazepam treatment to suppress neural activity and thereby limit the induction of associative LTD during the pre- training period also eliminated the learning deficits in L7-*Fmr1* KO mice. These results support the hypothesis that cerebellar LTD-dependent learning is governed by an experience-dependent sliding threshold for plasticity. An increased threshold for LTD in response to elevated neural activity would tend to oppose firing rate stability, but could serve to stabilize synaptic weights and recently acquired memories. The metaplasticity perspective could inform the development of new clinical approaches for addressing learning impairments in autism and other disorders of the nervous system.

eLife assessment

This **important** manuscript follows up on previous findings from the same lab supporting the idea that deficits in learning due to enhanced synaptic plasticity are due to saturation effects. **Compelling** evidence is presented that behavioral learning deficits associated with enhanced synaptic plasticity in a transgenic mouse model can be rescued by manipulations designed to reverse the saturation of synaptic plasticity. In particular, the finding that a previously FDA-approved therapeutic can rescue learning could provide new insights for biologists, psychologists, and others studying learning and neurodevelopment.

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Introduction

Since its discovery, long term synaptic plasticity has been of great interest to neuroscientists as a therapeutic target for brain disorders, especially disorders affecting learning and memory. Scientific and technological advances have provided an array of tools for enhancing synaptic plasticity. In some cases, experimental manipulations that augment plasticity have succeeded in augmenting learning (Tang et al., 1999; Van Praag et al., 1999; Lee and Silva, 2009). However, in many cases, manipulations that augment plasticity have impaired learning (Migaud et al., 1998; Uetani et al., 2000; Gu et al., 2002; Cox et al., 2003; Rutten et al., 2008; Navakkode et al., 2022). Surprisingly, there have been few attempts to reconcile these conflicting findings with a mechanistic explanation for why enhancing synaptic plasticity can have opposite effects on learning. Such mechanistic insight about how enhanced synaptic plasticity functions *in vivo* could facilitate the development of this approach as a viable clinical intervention for learning disorders, recovery from stroke or brain injury, dementia, addiction, and other neurological and psychiatric disorders.

Recently, we proposed a testable hypothesis about what can go wrong with augmented plasticity *in vivo*, based on experimental and theoretical analysis of learning in mice with enhanced associative synaptic plasticity in the cerebellum (Nguyen-Vu et al., 2017). Associative LTD at the cerebellar PF-Purkinje cell synapses (PF-Purkinje cell LTD) has been implicated in certain cerebellum-dependent learning tasks and not others, based in part on the observation of selective learning impairments in mouse lines with impaired PF-Purkinje cell LTD (reviewed in Raymond and Medina, 2018; De Zeeuw et al., 2021). Initially, we expected that mice with enhanced PF-Purkinje cell LTD would exhibit the exact opposite behavioral phenotype as mice with impaired PF-Purkinje LTD, i.e., enhancement of learning on the same behavioral tasks in which mice with impaired PF-Purkinje cell LTD exhibit impaired learning. Contrary to this expectation, double knockout of the major histocompatibility class I molecules MHCI H2-K^b and H2-D^b (MHCI $K^bD^{b-/-}$), which enhances PF-Purkinje cell LTD (McConnell et al., 2009), results in the very same, specific oculomotor learning impairment as observed in mice with impaired PF-Purkinje cell LTD (Nguyen-Vu et al., 2017). To explain the puzzling observation that the enhancement of a plasticity mechanism could yield the same behavioral phenotype as its impairment, we hypothesized that enhanced LTD prevents learning by allowing spontaneous activity in the circuit to aberrantly recruit and saturate this form of plasticity, making it unavailable at the specific synapses where it is needed to support learning. Two key predictions of this hypothesis were confirmed experimentally by previous work: optogenetic stimulation of the circuit designed to induce PF- Purkinje cell LTD before training recapitulated in WT mice the same, specific

oculomotor learning deficit observed in the MHCI $K^bD^{b-/-}$ mice with enhanced LTD; and a behavioral manipulation designed to reverse PF-Purkinje cell LTD before oculomotor training reversed the learning deficit in MHCI $K^bD^{b-/-}$mice (Nguyen-Vu et al., 2017).

Here we further tested the hypothesis that the enhancement of PF-Purkinje cell LTD can result in its aberrant recruitment by ongoing neural activity and consequent increased threshold for its induction and reduced availability to support learning. First, we replicated key behavioral findings in a different line of mice with enhanced PF-Purkinje cell LTD. Purkinje cell-specific knock out of the Fragile X gene *Fmr1* enhances PF-Purkinje cell LTD (Koekkoek et al., 2005). We show that these L7-*Fmr1* KO mice are selectively impaired on LTD-dependent oculomotor learning tasks, and that their learning deficit can be reversed with behavioral pre-training designed to reverse PF-Purkinje cell LTD, as previously reported in the MHCI $K^bD^{b-/-}$ mice with enhanced PF-Purkinje cell LTD. We then test a new prediction about a pharmacological treatment to reverse the learning deficit in mice with enhanced associative synaptic plasticity. The experimental results support and extend the influential concept of a history-dependent sliding threshold for synaptic plasticity (Bienenstock, Cooper, and Munro, 1982) as a regulator of learning.

Results

Selective learning impairment in mice with enhanced associative long-term depression in the cerebellum

We assessed oculomotor learning in mice lacking expression of the Fragile X gene *Fmr1* in cerebellar Purkinje cells, which have been shown to have enhanced PF-Purkinje cell LTD *in vitro* (Koekkoek et al., 2005). Purkinje cell-specific *Fmr1* knock out mice were generated by crossing conditional *Fmr1* knockout mice (Mientjes et al., 2006) with mice expressing Cre under the control of the L7/Pcp2 promoter (Zhang et al., 2004; see Methods). We tested the ability of these L7-*Fmr1* KO mice to adaptively modify their eye movement responses to vestibular and visual stimuli, and compared their performance on different oculomotor learning tasks that have previously been shown to have different sensitivity to perturbations of PF-Purkinje cell LTD.

We first assessed adaptive modification of the vestibulo-ocular reflex (VOR). The VOR stabilizes images on the retina by using the vestibular sensory input caused by a head movement to drive an oppositely directed eye movement response. Learning can adjust the amplitude of this oculomotor reflex to improve the stabilization of visual images on the retina for successful navigation in the world (Gonshor and Melvill Jones, 1973; Ito et al., 1974; Miles and Fuller, 1974; Broussard and Kassardjian, 2004; Gittis and du Lac, 2006; Cullen, 2023). Mice were trained to adaptively increase or decrease their VOR amplitude using two types of vestibular-visual stimulus pairings (**Fig. 1**; Boyden and Raymond 2003; Boyden et al., 2004). When a vestibular stimulus (1 Hz sinusoidal rotation about an earth-vertical axis with peak velocity of ±10°/s) was paired with oppositely directed motion of a large-field visual stimulus for 30 min (**Fig. 1A**, *left*; see Methods), this induced an adaptive learned increase in the eye movement responses of wild type (WT) mice to the vestibular stimulus alone (VOR-increase learning; **Fig. 1A**, *right, black*; p=7.37x10^{-4}, 0 vs. 30 min, Tukey). When the vestibular stimulus was instead paired with motion of a visual stimulus in the same direction as the head (**Fig. 1B**, *left*), this induced an adaptive learned decrease in the eye movement responses of WT mice to the vestibular stimulus alone (VOR-decrease learning; **Fig. 1B**, *right, black;* p=0.001, 0 vs. 30 min, Tukey).

Both VOR-increase and VOR-decrease learning are cerebellum dependent (Ito et al., 1974; Robinson, 1976; Lisberger et al., 1984; Nagao, 1983; Michnovicz and Bennett, 1987; Pastor et al., 1994; Koekkoek et al., 1997; McElligott et al., 1998). However, manipulations that impair or enhance PF-Purkinje cell LTD have previously been found to selectively alter VOR-increase learning, with less or no effect on VOR-decrease learning (Li et al., 1995; Boyden et al.,

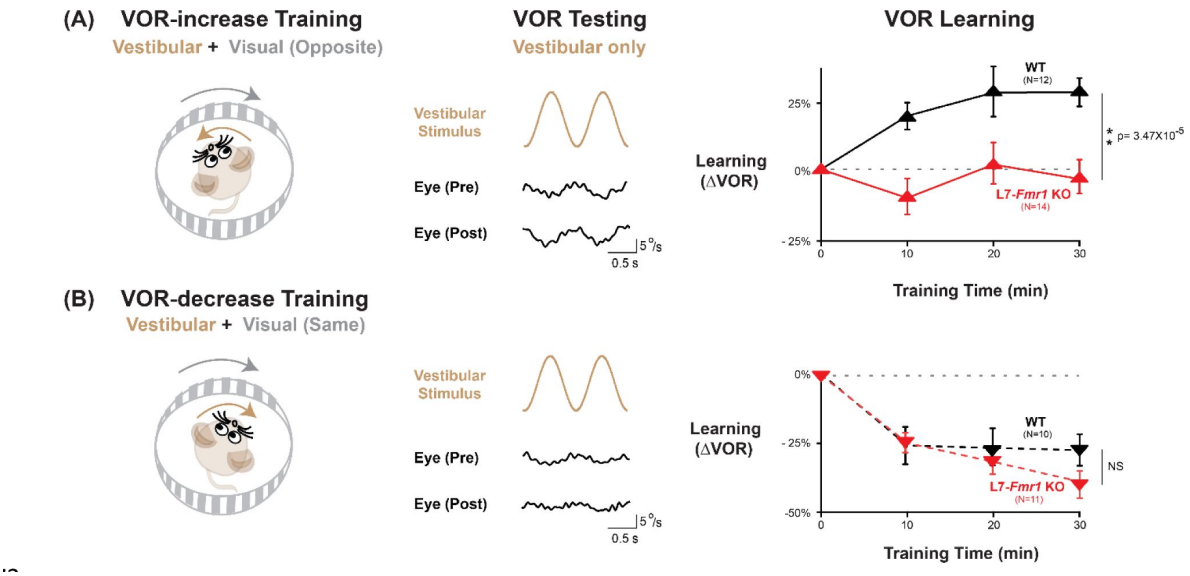

Figure 1.

VOR-increase learning is impaired in L7-*Fmr1* KO mice with enhanced cerebellar LTD.

(A) Training to increase the VOR. ***Left***, VOR-increase training paired a vestibular stimulus (1 Hz sinusoidal rotation about an earth-vertical axis, *brown*) with oppositely directed visual stimulus motion (*grey*). ***Middle***, Example raw eye velocity responses (*black*) to the vestibular stimulus alone in the dark, i.e., the VOR, measured Pre and Post VOR-increase training. ***Right***, Average learned change in the amplitude of the VOR relative to pre-training (*upward triangles*), measured in the dark after each 10-min VOR-increase training block in the L7-*Fmr1* KO (*red*) and WT mice (*black*). **(B)** Training to decrease the VOR. ***Left***, VOR-decrease training paired a vestibular stimulus (1 Hz sinusoidal rotation) with visual stimulus motion in the same direction. ***Middle***, Example VOR responses in the dark, measured Pre and Post VOR-decrease training. ***Right***, VOR-decrease learning (*downward triangles*). NS= not significant. In this and all figures, values plotted are mean ± SEM.

2006; Hansel et al., 2006; Guo et al., 2014; Kimpo et al., 2014; Nguyen-Vu et al., 2017; Kakegawa et al, 2018; Zhang et al., 2023). Accordingly, the L7-*Fmr1* KO mice with enhanced PF-Purkinje cell LTD were selectively and profoundly impaired on VOR-increase learning. Unlike the WT control group, L7-*Fmr1* KO mice exhibited no significant change in the amplitude of their VOR after 30 min of VOR-increase training (**Fig. 1A**, *red*; p=0.97, L7-*Fmr1* KO, 0 vs. 30 min; $p=3.47x10^{-5}$, L7-*Fmr1* KO vs. WT, 30 min; Tukey). In contrast, VOR-decrease learning in the L7-*Fmr1* KO mice was robust and indistinguishable from that of their WT littermates (**Fig. 1B**, *red*; $p=1.10x10^{-5}$, L7-*Fmr1* KO, 0 vs. 30 min; p= 0.091, L7-*Fmr1* KO vs. WT, 30 min; Tukey). The VOR-increase learning deficit was observed in both male and female L7-*Fmr1* KO mice (**Fig. 1 - figure supplement 1**). Baseline oculomotor performance of L7-*Fmr1* KO mice was normal, as were the eye movement responses to the paired presentation of visual and vestibular stimuli used for both types of training (**Fig. 1 - figure supplement 2**), suggesting that there was no deficit in the vestibular, visual or oculomotor functions required to perform the learning tasks; rather the L7-*Fmr1* KO mice have a selective deficit in learning. These results support previous findings that manipulations of PF-Purkinje cell LTD selectively affect VOR-increase learning, and that the enhancement of PF-Purkinje cell LTD impairs rather than enhances this form of learning.

Behavioral pre-training eliminates learning impairment in L7-*Fmr1* KO mice with enhanced LTD

A key question is why the enhancement of PF-Purkinje cell LTD would impair LTD-dependent learning. One potential explanation is that the enhancement of LTD allows the spontaneous activity in the cerebellar circuit to aberrantly recruit this mechanism and increase the threshold for its further recruitment, reducing its availability to support new LTD-dependent learning. If this is the case, then manipulations that prevent or reverse excessive PF-Purkinje cell LTD before training should reset the circuit to a state compatible with new LTD-dependent learning, and thereby improve VOR-increase learning in the L7-*Fmr1* KO mice. To test this prediction, we first employed a behavioral approach designed to reverse PF-Purkinje cell LTD in the oculomotor cerebellum before training.

In wild-type mice, VOR-decrease training can rapidly reverse any behavioral evidence of prior VOR-increase learning, which suggests that VOR-decrease training can reverse any plasticity induced during VOR-increase learning, including any PF-Purkinje cell LTD (Boyden and Raymond, 2003), presumably through the induction of PF-Purkinje cell LTP (Lev-Ram et al, 2003; Shim et al., 2022). Accordingly, VOR-decrease pre-training was previously found to reverse the oculomotor learning deficit in *MHCI* $K^bD^{b-/-}$ mice with enhanced PF-Purkinje cell LTD (Nguyen-Vu et al., 2017). We tested whether the same behavioral pre-training intervention could also eliminate the learning deficit in L7-*Fmr1* KO mice **(Fig. 2A-C)**.

L7-*Fmr1* KO and WT mice were subjected to 30 min of VOR-decrease pre-training followed by 30 min of VOR- increase training. In WT mice, there were adaptive changes in the amplitude of the VOR during both the pre-training and training periods—first a decrease and then an increase in the eye movement response to the vestibular stimulus alone (**Fig. 2B**, *black;* VOR-decrease, *dotted lines*, p=0.02, WT -30 vs. 0 min; VOR-increase, *solid lines,* p=0.001, WT 0 vs. 30 min; Tukey). The L7-*Fmr1* KO mice exhibited adaptive changes in the VOR during both the pre-training and training periods that were statistically indistinguishable from WT (**Fig. 2B**, *red;* VOR-decrease, *dotted lines*, p=0.18, L7-*Fmr1* KO vs. WT, 0 min, Tukey; **Fig. 2B**, *bar graphs*, p=0.17, L7-*Fmr1* KO vs. WT, VOR-increase from 0 to 30 min, t test; **Fig. 2—figure supplement 1**). Although in the absence of pre-training, VOR-increase training failed to induce any significant change in the VOR of the L7-*Fmr1* KO mice (**Fig. 2A**, *red, solid lines and bar graph;* p= 0.99, L7-*Fmr1* KO, 0 vs. 30 min, Tukey), the same VOR-increase training procedure did induce a significant increase in VOR amplitude when delivered to the same cohort of mice after VOR-decrease pre-training (**Fig. 2B**, *red, solid lines and bar graph*, p=0.001, 0 vs. 30 min, Tukey). In other words, the ability of the L7-*Fmr1* KO mice to learn in response to the VOR-increase training varied with the recent history of

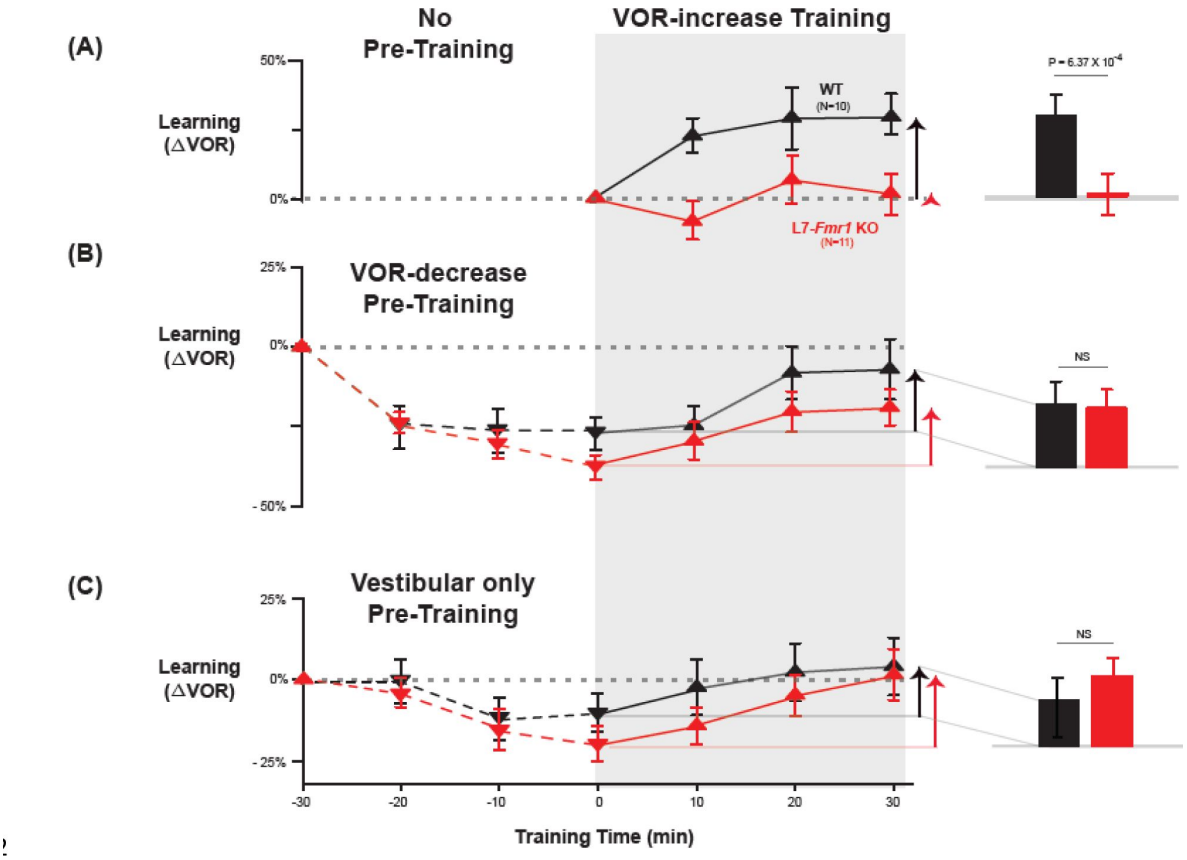

Figure 2.

Behavioral pre-training rescued learning impairment of L7-*Fmr1* KO mice with enhanced associative LTD.

Associative VOR-increase learning (*shaded area* and *bar graphs*), without pre-training (***A***), after VOR-decrease pre-training (***B***), and after Vestibular only pre-training (***C***). ***A,*** learned change in the VOR response measured in the dark after each 10-min block of VOR-increase training in the subset of L7-*Fmr1* KO (*red*) and WT (*black*) mice from **Figure 1A** that were also tested after pre-training. ***B,*** Changes in the VOR measured in the dark after each block of VOR-decrease pre-training *(downward triangles, dashed lines)* and then subsequent VOR-increase training *(upward triangles, solid lines)*. ***C,*** Changes in the VOR measured in the dark after each block of Vestibular only pre-training *(downward triangles, dashed lines)* and then VOR-increase training *(upward triangles, solid lines)*. ***Right,*** *Arrows* and *bars graphs* show the total change in the VOR induced by 30 min of VOR-increase training (training time = 30) compared with just before VOR-increase training (training time = 0).

experience (**Fig. 2**, *compare red bars in **A*** vs. ***B**;* p= 0.01, VOR-increase learning of L7-*Fmr1* KO without vs. with pre-training, paired sample t-test). Pre- training experience did not have the same effect in WT mice. The amount of learning exhibited by WT mice in response to VOR-increase training was not enhanced after VOR-decrease pre-training (**Fig. 2**, *compare black bars in **A*** vs. ***B***; p= 0.41, paired sample t-test). Thus, VOR-decrease pre-training had different effects on the L7-*Fmr1* KO and WT mice, putting the L7-*Fmr1* KO mice, but not the WT mice, into a state more compatible with VOR-increase learning.

A second behavioral pre-training procedure, habituation of the VOR, induced by presentation of the vestibular stimulus alone in complete darkness (Vestibular only pre-training), had effects similar to those of VOR-decrease pre- training on subsequent VOR-increase learning. After thirty minutes of Vestibular only pre-training, subsequent VOR- increase learning in the L7-*Fmr1* KO mice was comparable to that of their WT littermates (**Fig. 2C**, *red* vs. *black bars;* p=0.84, L7-*Fmr1* KO vs. WT, VOR-increase from 0 to 30 min, paired sample t-test).

Pharmacological suppression of neural activity the day before training eliminates learning impairment of L7-*Fmr1* KO mice with enhanced LTD

The preceding results are consistent with the hypothesis (Nguyen-Vu et al., 2017) that in mice with enhanced PF- Purkinje cell LTD, spontaneous activity in the circuit can induce LTD and thereby increase the threshold for its subsequent induction, and that behavioral pre-training can alter neural activity in a manner that prevents or reverses this increased threshold for LTD *in vivo*, thereby reversing the learning impairment. Since PF-Purkinje cell LTD is driven by co-activation of cerebellar parallel fibers and climbing fibers (Ito and Kano, 1982; Ito et al., 1982; Linden and Connor, 1995), pharmacological suppression of neural activity should also prevent the induction and increase in the threshold for LTD during the pre-training period, and restore the capacity for subsequent LTD-dependent learning in mice with enhanced LTD. We tested this prediction by administering the benzodiazepine diazepam, a positive allosteric modulator of $GABA^A$ receptors, to enhance inhibition and suppress neural activity in the L7-*Fmr1* KO mice during the period preceding VOR-increase training. Diazepam has been shown to reduce neural firing in cerebellar neurons and neural responses to vestibular stimuli (Ryu and McCabe, 1974; Barmack, N. H. & Pettorossi, 1980). We assessed VOR learning 2 hours after a single, systemic dose of diazepam, immediately after recovery from diazepam (18-24 hours after administration), and 1 week later.

The acute effect of diazepam administration was to impair learning. There was no effect of diazepam on the baseline amplitude of the VOR response measured in the dark 2 hours after diazepam (**Fig. 3 – figure supplement 1**), contrary to what has been reported in rabbit (Barmack, N. H. & Pettorossi, 1980). However, when VOR-increase training was delivered 2 hours after systemic administration of diazepam, VOR-increase learning was profoundly impaired in WT as well as L7-*Fmr1* KO mice (**Fig. 3 – figure supplement 2**).

It is not surprising that the acute effect of suppressing neural activity was to impair learning. The key question was whether this suppression of activity could reset the circuit to a state compatible with subsequent LTD-dependent learning. Therefore, VOR learning was tested after recovery from the acute effects of diazepam. Diazepam has a long half-life of ~24 hours (Riss et al., 2008), therefore mice were allowed to recover in their home cage for 18-24 hours after diazepam administration, and then VOR learning was tested after recovery from this prolonged period of pharmacological suppression of neural activity **(Fig. 3A)**. Remarkably, the L7-*Fmr1* KO mice exhibited robust VOR- increase learning, comparable to their WT littermates (**Fig. 3B**, *top*, red vs. *black*; p=0.86, L7-*Fmr1* KO vs. WT, 30 min, Tukey). Although the same individual L7-*Fmr1* KO had exhibited no significant learning in response to VOR- increase training in the absence of the pharmacological pre-treatment (p= 0.99, 0 vs. 30 min, data for subset of mice in **Fig. 1A** used in **Fig. 3** experiments, Tukey), diazepam pre-treatment eliminated this learning deficit.

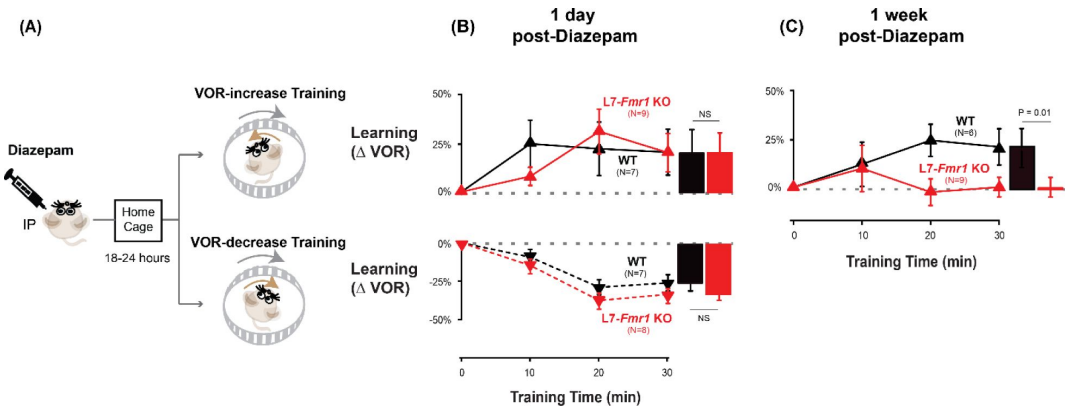

Figure 3.

Diazepam pre-treatment rescued learning impairment of L7-*Fmr1* KO mice with enhanced associative LTD.

(A) Mice were given an intraperitoneal (IP) injection of diazepam (0.5 mg/kg) and then returned to the home cage for 18-24 hours, followed by VOR-increase (***top***) or VOR-decrease (***bottom***) training. **(B)** ***Top,*** VOR-increase learning 1 day (18-24 hours) after diazepam administration in L7-*Fmr1* KO (*red upward triangles*) and WT mice (*black upward triangles).* ***Bottom,*** VOR-decrease learning (*downward triangles)* 1 day after diazepam. **(C)** VOR-increase learning in the same mice as in **B**, 1 week after diazepam treatment, and 18-24 hours after IP saline injection.

The enhancement of learning by diazepam pre-treatment was temporary. When the same mice were re-tested one week after diazepam administration, the L7-*Fmr1* KO mice again failed to learn in response to VOR-increase training (**Fig. 3C**, *red;* p=0.12, 0 vs. 30 min, Tukey). Thus, diazepam pre-treatment could restore the VOR circuit of L7-*Fmr1* KO mice to a state compatible with VOR-increase learning, but this effect was transient.

Specificity of the effects of pre-training treatments on learning

The ability of behavioral and pharmacological pre-training interventions to enhance learning was specific to mice with enhanced PF-Purkinje cell LTD and to the type of VOR learning task. Wild type mice did not exhibit enhanced VOR- increase learning after diazepam pre-treatment (**Fig. 3B** *top* **vs. 2A**, *black*, p= 0.55; **Fig. 3B vs. 3C**, *top, black*, p= 0.99; paired sample t test). Moreover, there was no effect of diazepam pre-treatment on VOR-decrease learning in either the WT or L7-*Fmr1* KO mice (*compare* **Fig. 3B**, *bottom* vs. **1B**; p= 0.91, WT, 1-day post-diazepam vs. control, VOR-decrease at 30 min, paired sample t-test; p= 0.37, L7-*Fmr1* KO, 1-day post-diazepam vs. control, VOR-decrease at 30 min, paired sample t-test; p=0.11, L7-*Fmr1* KO vs. WT 1-day post-diazepam, VOR-decrease at 30 min, Tukey). Thus, both the learning impairment in the L7-*Fmr1* KO mice and the effects of diazepam pre-treatment were selective for VOR-increase learning, consistent with previous evidence that this form of VOR learning is more dependent on

PF-Purkinje cell LTD than VOR-decrease learning (Li et al., 1995; Boyden et al., 2006; Hansel et al., 2006; Guo et al., 2014; Kimpo et al., 2014; Nguyen-Vu et al., 2017; Kakegawa et al, 2018; Jang et al., 2020; Shim et al., 2022; Zhang et al., 2023).

Previous work has also suggested a selective contribution of PF-Purkinje cell LTD to VOR learning induced with high-frequency (≥ 1 Hz) vestibular and visual stimuli, with less contribution of LTD when VOR learning is induced with low-frequency (≤0.66 Hz) vestibular and visual stimuli (Boyden et al., 2006; Nguyen-Vu et al., 2017). We found a trend for low-frequency (0.5 Hz, **Fig. 4**) as well as high-frequency (1 Hz, **Figs. 1-3**) VOR-increase learning to be impaired in the L7-*Fmr1* KO mice (**Fig. 4A**, *left, red* vs. *black;* p= 0.06, L7-*Fmr1* KO vs. WT, 30 min, Tukey). However, the low-frequency learning impairment was not reversed by the pre-training procedures that reversed the high-frequency learning impairment. Neither behavioral pre-training (**Fig. 4A**, *red, middle,* p= 0.47, L7-*Fmr1* KO, VOR-decrease Pre-training vs. no Pre-training; *right,* p= 0.35, L7-*Fmr1* KO, Vestibular only Pre-training vs. no Pre- training; paired sample t test), nor treatment with diazepam 18-24 hours before training (**Fig. 4B**, *compare red bars;* p= 0.66, L7-*Fmr1* KO, saline vs. diazepam, paired sample t test), reversed the impairment of low-frequency VOR- increase learning in the L7-*Fmr1* KO mice, in contrast to their effectiveness at reversing the impairment of high- frequency VOR-increase learning (**Figs. 2, 3**). This is consistent with the hypothesis that the behavioral and pharmacological pre-training manipulations selectively restore the capacity for learning tasks that depend on PF- Purkinje cell LTD.

To further test the specificity of the effects of *Fmr1* knockout and diazepam pre-treatment for learning tasks that depend on PF-Purkinje cell LTD, we tested optokinetic reflex (OKR) adaption, which is the oculomotor learning task for which there is arguably the strongest evidence for a critical role of PF-Purkinje cell LTD (Takeuchi et al., 2008; Wang et al., 2013; Inoshita and Hirano, 2018; Kakegawa et al, 2018). The OKR was elicited by rotating a striped drum about an earth-vertical axis centered on the head of the mouse, using a sinusoidal velocity profile with frequency of 1 Hz and peak velocity of ±10°/s (**Fig. 5**), with the head restrained and stationary. Baseline OKR gain was not significantly different in the L7-*Fmr1* KO and WT mice (**Fig. 5 – figure supplement 1**). In WT mice, 60 min of training with the optokinetic stimulus induced a learned increase in the amplitude of the OKR response, as the mice learned to more closely match their eye movements to the motion of the optokinetic stimulus and thus improve stability of its image on the retina (**Fig. 5A**, *black,* p=0.001, 0 vs. 60 min, Tukey). The L7-*Fmr1* KO mice with enhanced PF-Purkinje cell LTD had significantly impaired OKR adaptation (**Fig. 5A**, *red* vs. *black;* p=1.27x10^{-4},

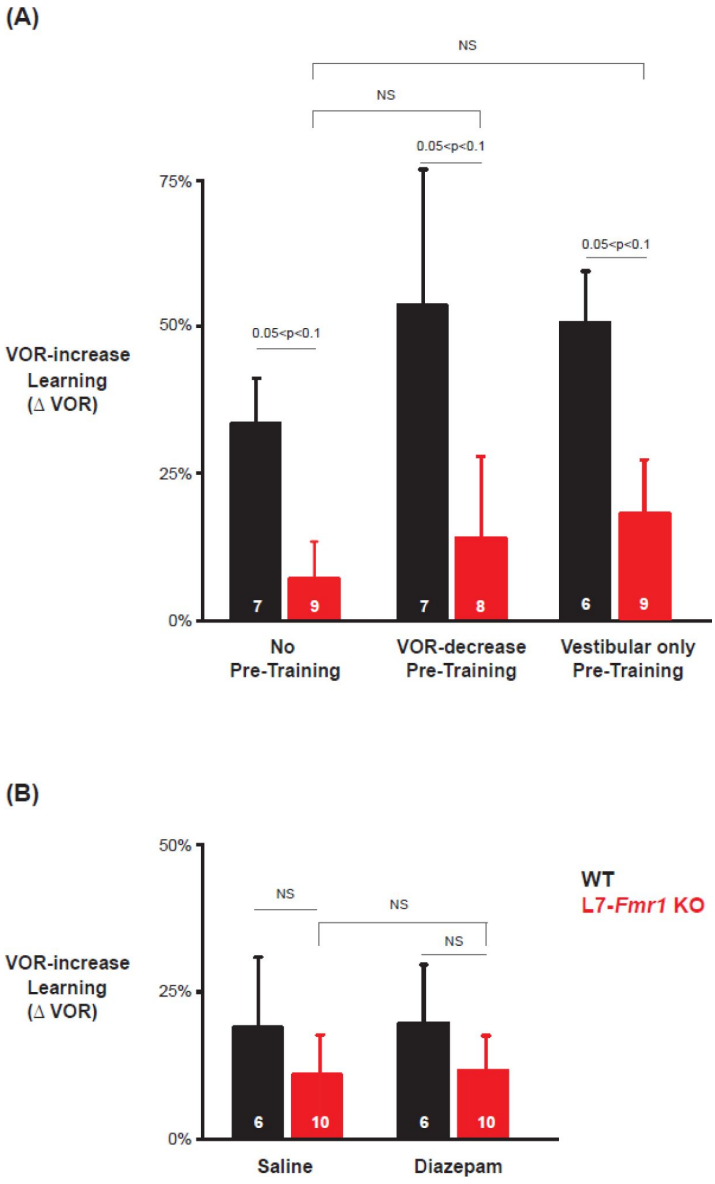

Figure 4.

Low frequency (0.5 Hz) VOR-increase learning impairment was not rescued by behavioral pre-training or diazepam pre-treatment.

(A) Low-frequency VOR-increase learning of L7-*Fmr1* KO mice (*red*) and WT mice (*black*), without pre-training (*left)*, after 0.5 Hz VOR-decrease pre-training (*middle*), and after 0.5 Hz Vestibular only pre-training (*right*). **(B)** Low frequency (0.5 Hz) VOR-increase learning 18-24 hours after IP injection of saline (*left*) or 0.5 mg/kg diazepam (*right*).

L7-*Fmr1* KO vs. WT, 60 min; p=0.87, L7-*Fmr1* KO, 0 vs. 60 min; Tukey). This learning deficit was completely eliminated by pre- treatment with diazepam 18-24 hours before training (**Fig. 5B**). Indeed, the L7-*Fmr1* KO mice not only learned better 18-24 hours after diazepam than they did without this pre-treatment (Compare **Fig. 5A** **vs. B**, *red*; p=0.0001, saline vs. diazepam pre-treatment, 60 min, Tukey), but their learning after diazepam pre-treatment was even enhanced relative to WT (**Fig. 5B**, *red* vs. *black;* p=0.02, L7-*Fmr1* KO vs. WT, 60 min, Tukey). Thus, the OKR adaptation results support the findings from high-frequency VOR-increase learning suggesting a deficit in PF-Purkinje cell LTD- dependent learning in the mice with enhanced LTD that varies dramatically with the recent history of activity in the circuit.

Discussion

A question of central scientific and clinical importance is why the enhancement of synaptic plasticity can impair rather than enhance learning. One hypothesis is that a lower threshold for the induction of plasticity might cause it to be over-recruited during training, at synapses that should not have undergone plasticity in addition to synapses where it would support adaptive behavioral changes, thereby corrupting the memory trace (Migaud et al., 1998; Koekkoek et al., 2005). An alternative hypothesis is that the enhancement of plasticity might allow spontaneous activity in the circuit to aberrantly recruit the plasticity mechanism even before training begins, and thereby reduce its availability during training to support new learning (**Fig. 5**). Nguyen-Vu and colleagues (2017) described this reduced availability as saturation of plasticity, but more generally, the reduced availability could reflect an increased threshold for the induction plasticity (Bienenstock, Cooper, and Munro, 1982; Leet, Bear, and Gaier, 2022). This threshold metaplasticity hypothesis differs from the over-recruitment hypothesis by suggesting that the enhanced plasticity mechanism is under- rather than over- recruited during training. It also differs by suggesting that the problem with enhanced plasticity arises because of what it does to the circuit before training, rather than how it functions during training, and therefore more readily accounts for effects of pre-training manipulations on learning in mice with enhanced plasticity. The current findings thus bolster the evidence from Nguyen-Vu and colleagues (2017) suggesting threshold metaplasticity rather than over-recruitment as the cause of impaired learning in mice with enhanced associative LTD at the cerebellar parallel fiber-Purkinje cell synapses.

We found that L7-*Fmr1* KO mice with enhanced PF-Purkinje cell LTD exhibit impaired rather than enhanced learning on oculomotor learning tasks in which PF-Purkinje cell LTD has been implicated, as previously shown for MHCI $K^bD^{b-/-}$ mice. In both mouse lines, behavioral manipulations designed to prevent or reverse the induction of PF- Purkinje cell LTD during the period before training reversed the learning impairment. The additional finding that pharmacological suppression of neural activity with diazepam can enhance subsequent learning in mice with enhanced associative LTD provides new evidence that these mice are not incapable of LTD-dependent learning. Rather, the interaction of the ongoing neural activity in the circuit with the enhanced plasticity appears to create a state in which LTD is unavailable to support learning. However, this state of reduced availability can be reversed when the patterns of neural activity that create it are eliminated. The capacity for PF-Purkinje cell LTD-dependent learning is dynamically regulated by the recent history of activity.

Comparison across closely related cerebellum-dependent learning tasks reveals the specific behavioral consequences of enhanced PF-Purkinje cell LTD. Although it was the main candidate mechanism of cerebellum-dependent learning for many decades, there is growing evidence that PF-Purkinje cell LTD contributes selectively to certain cerebellum- dependent learning tasks, and not others (Shibuki et al., 1996; Boyden et al., 2006; De Zeeuw et al., 2021). Oculomotor learning is particularly advantageous for analyzing the function of PF-Purkinje cell LTD during learning because this plasticity mechanism is thought to contribute differentially to a set of closely

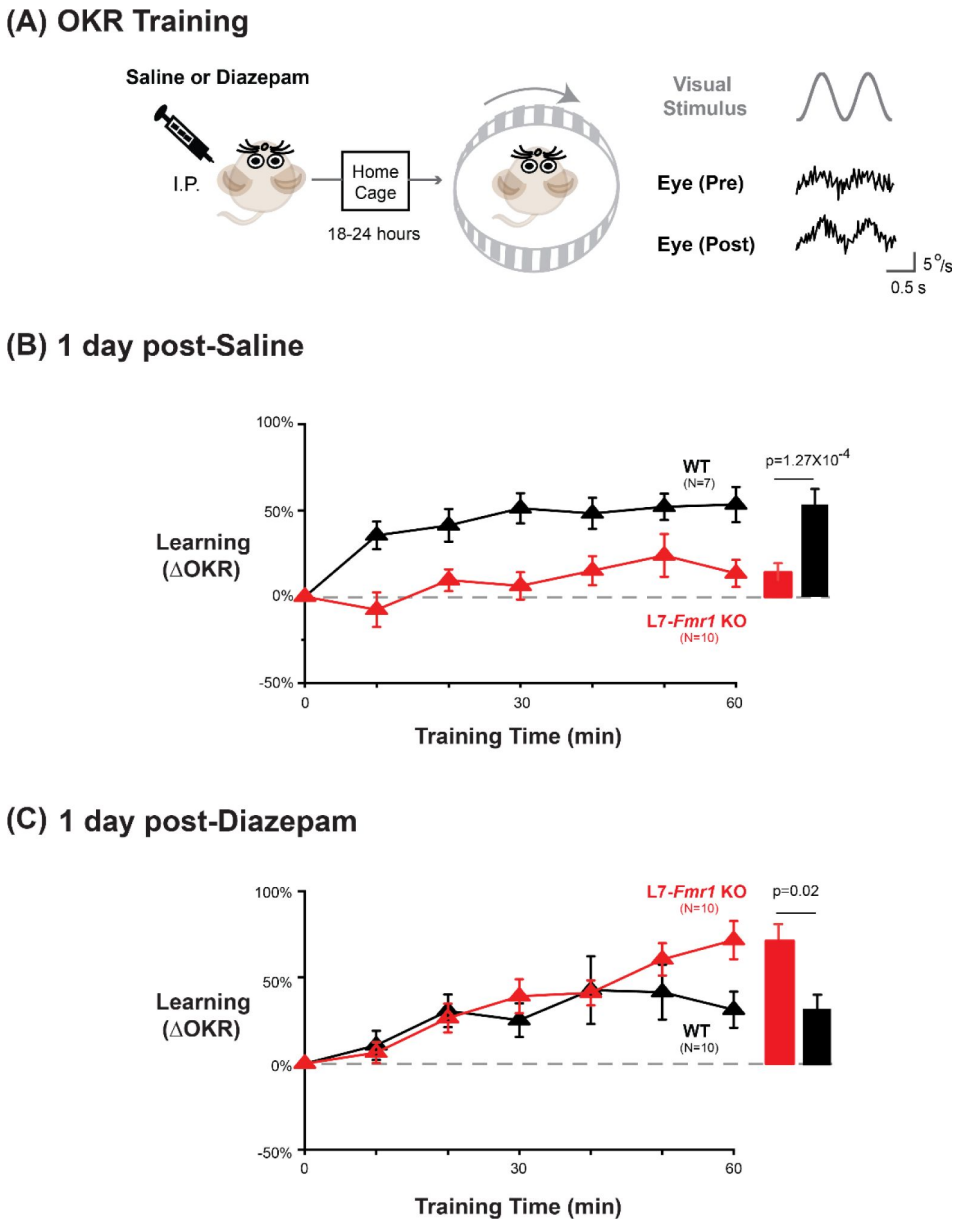

Figure 5.

Diazepam pre-treatment rescued OKR learning impairment of L7-*Fmr1* KO mice with enhanced associative LTD.

(A) *Left,* OKR adaptation was assessed 18-24 hours after a single injection of saline or diazepam. *Middle* OKR adaptation was induced by rotating a striped optokinetic drum about an earth-vertical axis centered on the head of the mouse with a 1 Hz sinusoidal velocity profile and peak velocity of ±10°/s. *Right,* Example raw eye velocity responses (*black*) to the optokinetic visual stimulus (*gray*), measured at the beginning (Pre) and end (Post) of 60 min of OKR adaptation training. (B) Average learned change in the amplitude of the OKR relative to pre-training, after each 10-min OKR training block in the L7-*Fmr1* KO (*red*) and WT mice (*black*), 1 day after saline injection. (C) Average learned change in the amplitude of the OKR relative to pre-training in L7-*Fmr1* KO (*red*) and WT (*black)* mice that received diazepam the previous day.

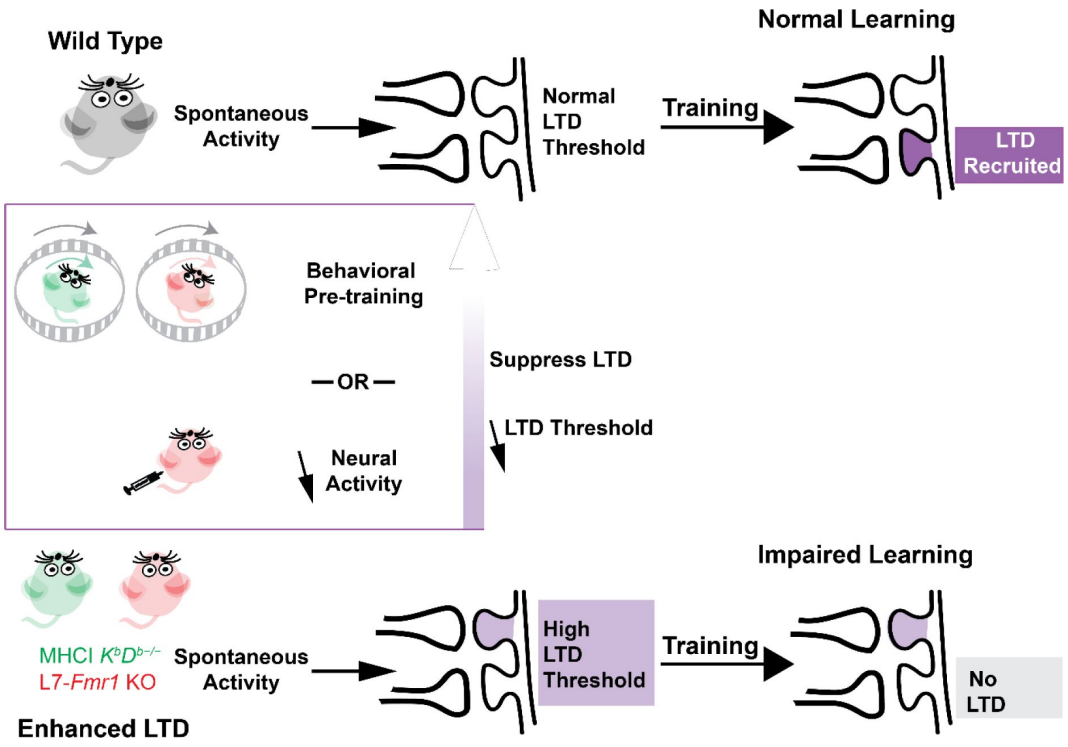

Figure 6.

Metaplasticity hypothesis for how enhanced plasticity could impair learning.

Top, In naïve wild type mice, synapses have a normal threshold for associative LTD, and undergo LTD (*dark violet*) in response to training, thereby supporting normal learning. ***Bottom,*** In mice with enhanced LTD, such as L7-*Fmr1* KO (*pink*) and MHCI $K^bD^{b-/-}$ (*green*), the lower threshold for induction of LTD allows it to be aberrantly recruited by spontaneous activity in the circuit (*light violet*), thereby increasing the threshold for additional LTD induction. This prevents the recruitment of LTD during training at the synapses where it is needed to support learning (*grey box*), which impairs learning. Behavioral pre-training or drugs that reduce neural activity can suppress LTD induction and reset the threshold for LTD to normal (*upward arrow*), restoring the capacity for LTD-dependent learning.

related oculomotor learning tasks that all depend on the same vestibular, visual and motor signaling pathways through the cerebellar flocculus. Despite the shared cerebellar and extra-cerebellar circuitry, a number of experimental approaches, including *ex vivo* slice physiology (Inoshita and Hirano, 2018; Jang et al., 2020; Shim et al., 2022), optogenetic stimulation (Kimpo et al., 2014; Nguyen-Vu et al., 2017; Zhang et al., 2023) and studies of oculomotor learning in mice with impaired LTD (Boyden et al., 2006; Hansel et al., 2006; Kakegawa et al., 2018) have suggested a selective contribution of PF- Purkinje cell LTD to OKR adaptation and VOR-increase learning induced by high-frequency (≥ 1 Hz) vestibular and visual stimuli, with less or no contribution to VOR-decrease learning or VOR-increase learning induced with lower frequency vestibular and visual stimuli.

In both the L7-*Fmr1* KO and MHCI $K^bD^{b-/-}$ mice with enhanced PF-Purkinje cell LTD, the learning impairments and the enhancement of learning by behavioral or pharmacological pre-training manipulations were remarkably selective for the oculomotor learning tasks in which PF-Purkinje cell LTD has been most strongly implicated. Both lines of mice were profoundly impaired on high-frequency VOR-increase learning and OKR adaptation. The effects of behavioral and pharmacological pre-training were strikingly specific for the same oculomotor learning tasks. Moreover, pre-training only enhanced learning in the L7-*Fmr1* KO and MHCI $K^bD^{b-/-}$ mice, and not WT mice, consistent with the pre-training selectively reversing limitations caused by enhanced LTD, rather than generally enhancing cerebellum-dependent learning. In the L7-*Fmr1* KO mice, low-frequency as well as high-frequency VOR- increase learning was impaired. However, the behavioral and diazepam pre-treatments designed to prevent or reverse LTD during the period before training only improved the high-frequency and not the low-frequency VOR-increase learning in the L7-*Fmr1* KO mice, suggesting different mechanistic underpinnings of the low- and high-frequency impairments. In other words, the deletion of *Fmr1* from Purkinje cells may have two distinct effects: enhancement of PF-Purkinje cell LTD, which recapitulates the high-frequency VOR-increase learning phenotypes observed in the MHCI $K^bD^{b-/-}$ mice with enhanced LTD, plus disruption of an additional cellular mechanism that contributes to low- frequency VOR-increase learning. Overall, in the two lines of mice with enhanced PF-Purkinje cell LTD, both the learning impairments and the effects of manipulations designed to reverse or prevent LTD before training were remarkably selective for the specific oculomotor learning tasks with the strongest evidence for a contribution of PF- Purkinje cell LTD. This oculomotor learning task specificity strengthens the evidence that the behavioral phenotypes in the L7-*Fmr1* KO and MHCI $K^bD^{b-/-}$ mice are a reflection of the enhanced LTD at PF-Purkinje cell synapses rather than other functional properties of the Purkinje cells, their synapses, or other cells or plasticity mechanisms within the same circuit.

The very similar behavioral phenotypes observed when PF-Purkinje cell LTD is enhanced by manipulating different molecular cascades further strengthens the evidence that their shared effect of enhancing LTD is responsible for their shared learning impairments, rather than other, off-target effects of the molecular manipulations. MHCI H2- D^b acts on MAP kinase and integrin via interaction with immune receptors such as PirB (Shatz, 2009), whereas *Fmr1* acts by inhibiting mGluR-dependent dendritic protein translation (Huber et al., 2002). Cell-type specific manipulations localize the site where loss of expression of MHCI H2-D^b or *Fmr1* yields the oculomotor learning deficits to the Purkinje cells. Each yields enhancement of PF-Purkinje cell LTD, as measured by the ability of protocols that fail to induce LTD in slices from WT mice to induce LTD in slices from MHCI $K^bD^{b-/-}$(McConnell et al., 2009) and L7- *Fmr1* KO mice (Koekkoek et al, 2005). Along with this shared effect of enhancing PF-Purkinje cell LTD, loss of MHCI H2-D^b or *Fmr1* expression from the Purkinje cells may each have additional effects on the intrinsic and synaptic properties of Purkinje cells, which are likely to be different for the two molecules. These latter, 'off-target' effects may contribute to the impairment of L7-*Fmr1* KO mice on low-frequency VOR-increase learning (**Fig. 4**), which was not observed in MHCI $K^bD^{b-/-}$ mice (**Fig. 1 - figure supplement 1**, Ngyuen-Vu et al., 2017). Off-target effects also might have contributed to the different cerebellar learning phenotypes reported previously in the two lines of mice – enhanced

rotorod learning in the MHCI $K^bD^{b-/-}$ mice (McConnell et al., 2009), but impaired eyeblink conditioning in the L7-*Fmr1* KO mice, global *Fmr1* KO mice and Fragile X patients (Koekkoek et al., 2005). However, the behavioral tasks used in those previous studies were also different, and our current results highlight the importance of the specific choice of behavioral task for assessing cerebellar learning, and the differential dependence of different cerebellar learning tasks on specific molecular and cellular processes within the cerebellum.

The current findings and the metaplasticity conceptual framework could guide the development of new clinical approaches for Fragile X syndrome and a range of other neurological and psychiatric conditions with enhanced associative plasticity. Pre-treatment with the FDA-approved drug diazepam restored the capacity for high-frequency VOR-increase learning and OKR adaptation in the L7-*Fmr1* KO mice without compromising other forms of oculomotor learning or baseline oculomotor performance. This was true even for VOR-decrease learning, which may depend on LTP of the same population of PF-Purkinje cell synapses that undergo LTD during VOR-increase learning (Shim et al., 2022). In other words, diazepam pre-treatment fully rescued the learning impairments with no apparent side effects on other, closely related functions of the same neural circuitry. This specificity enhances its therapeutic potential. At the same time, this approach of suppressing neural activity during a pre-training period may be generally applicable to any learning task, motor or cognitive (Rochefort et al., 2013; Badura et al., 2018; Ashburn et al., 2020; Frontera et al., 2020; Stoodley and Tsai, 2021; Hwang et al., 2022), that is impaired by enhanced associative LTD. Pharmacological suppression of neural activity should suppress PF-Purkinje cell LTD throughout the cerebellum, and hence may have the general effect of restoring all regions of the cerebellum to a state compatible with new LTD- dependent learning. This generality of the pharmacological approach stands in contrast to the behavioral pre-training approach, which would require extensive additional knowledge and experimentation to design the appropriate behavioral pre-treatment to reset each functional region of the cerebellum to a state compatible with LTD-dependent learning, and different behavioral pre-treatments would be required to target each of the many functional regions supporting the myriad motor and cognitive functions of the cerebellum. Thus, from a practical standpoint, pharmacological pre-treatment to prevent or reverse the recruitment of LTD before training and thereby lower the threshold for its subsequent recruitment during training is a more feasible and general approach to restoring the capacity for PF-Purkinje cell LTD-dependent learning. Such an approach could be tested even when the specific plasticity mechanism responsible for a learning deficit has not been identified, because lower activity should reduce induction of most associative plasticity mechanisms. Thus, the approach of limiting neural activity during a period before training to reset elevated thresholds for plasticity may be broadly applicable throughout the brain for resetting neural circuits to a state compatible with adaptive plasticity and new learning. Consistent with this, suppression of neural activity in the retina has been successfully employed to reset the visual circuitry and enable recovery from amblyopia in adult mice and cats (Fong et al., 2021). The suppression of neural activity may be an especially useful approach if plasticity is pathologically enhanced in areas like the cerebellum or basal ganglia, with a high level of spontaneous spiking activity.

The results predict history-dependent changes in the availability of PF-Purkinje cell LTD to support learning due to activity-dependent changes in the threshold for LTD. Threshold metaplasticity has not been directly documented at these synapses, however several factors that influence climbing fiber-induced calcium influx or the probability of LTD induction have been identified (reviewed in Zang and De Schutter, 2019), which could provide the mechanistic substrate for threshold metaplasticity. These include plasticity of the climbing fiber synapse onto the Purkinje cell (Hansel and Linden 2000), plasticity of Purkinje cell dendritic excitability (Ohtsuki et al, 2012), changes in the number of spikes in a climbing fiber burst (Mathy et al., 2009; Medina and Lisberger 2009), changes in short-term plasticity mechanisms at the PF-Purkinje cell synapses (Hunley et al., 2023), and plasticity of inhibitory synapses onto the Purkinje cells (Kano et al., 1992; Kawaguchi and Hirano 2007; Rowan et al., 2018).

The concept of experience-dependent changes in the threshold for synaptic plasticity has been highly influential in theoretical and computational neuroscience (Abraham and Bear, 1996; Benusková et al., 1999; Cooper and Bear, 2012; Hulme et al., 2012; Lee, 2022), since the foundational work of Bienenstock, Cooper and Munro decades ago (1982; BCM model). However experimental evidence for whether and how such threshold metaplasticity supports the function of neural circuits has been limited, and derived largely from studies of the effects of sensory deprivation on the functional connectivity of circuits (Mioche and Singer., 1989; Kirkwood et al., 1996; Philpot et al., 2003; He et al., 2007; Yee et al., 2016) rather than studies of learning *per se*. Analysis of threshold metaplasticity in the context of cerebellum-dependent learning and associative LTD offers a new perspective on the BCM model. Most fundamentally, the present results predict threshold metaplasticity at synapses where the plasticity is not Hebbian. A sliding threshold for plasticity has been conceived as a mechanism for countering an instability inherent in Hebbian LTP whereby correlated pre- and post-synaptic activity strengthens a synapse, which leads to an increase in correlated activity, which in turn leads to further strengthening. An increased threshold for LTP in response to an increase in neural activity would counter this instability and provide a mechanism to stabilize firing rates and synaptic weights within a desired range (Van Rossum et. al., 2000; Toyoizumi et al., 2014; Yger and Gilson, 2015; Zenke et al., 2017). In contrast, plasticity at the cerebellar PF-Purkinje cell synapse is described as "anti-Hebbian" because the associative form of plasticity is LTD. Associative LTD lacks the instability inherent in Hebbian LTP. Moreover, an increased threshold for LTD in response to an increase in neural activity or a decreased threshold for LTD in response to decreased neural activity would tend to oppose rather than support the stability of firing rates of the postsynaptic Purkinje cells. Yet the present results provide evidence for these activity-dependent changes in the threshold for cerebellar LTD. Thus, rather than supporting homeostatic control of firing rates, the central function of threshold metaplasticity at these synapses may be to limit the amount of plasticity. In addition, the finding that manipulations of neural activity during the pre-training period had different effects in the mice with enhanced LTD than in WT mice suggests that changes in the threshold for plasticity may be driven, not directly by firing rates, but by the recent history of activity-dependent induction of plasticity (Montgomery and Madison, 2002; Lev-Ram et al, 2003; Hunley et al., 2023 Abraham, 2008; Martin and Kosik, 2002; Redondo and Morris, 2011) A plasticity-driven increase in the threshold for further plasticity could serve to protect newly acquired memories from being overwritten (Fusi et al., 2005; Benna and Fusi, 2016). A second potential function would be to separate memories acquired in close succession onto the synapses of different Purkinje cells, in contrast to findings in the amygdala, where there is evidence that a plasticity-driven decrease in the threshold for further plasticity supports the allocation of memories acquired in close succession to the same neurons (Han et al.2007; Benna and Fusi, 2016; Cai et al., 2016; Rashid et al., 2016 Lau et al., 2020).

Conclusion

We leveraged the relatively simple and well understood physiology and function of the cerebellum and oculomotor system to develop and test a new hypothesis to explain why enhanced plasticity often impairs rather than enhances learning. The current results, along with the previous work by Nguyen-Vu and colleagues (2017) provide convergent evidence that a lower threshold for synaptic plasticity can result in its aberrant recruitment by ongoing activity in a circuit, resulting in an increased threshold for its subsequent induction and hence the impairment of learning. This threshold metaplasticity perspective may be useful in considering the impact of enhanced plasticity not only in the cerebellum, but in other brain areas as well, and for developing new clinical approaches for reversing maladaptive plasticity and resetting neural circuits to a state compatible with adaptive plasticity and new learning. More generally, the present results highlight the principle that synaptic properties do not control learning in isolation but interact with the patterns of neural activity in the corresponding circuits to control the capacity for new learning. The implication is that learning deficits associated with abnormal plasticity are not necessarily

permanent, but in some cases can be remedied with appropriate reset of the circuit, opening up the possibility for therapeutic approaches targeting neural activity as well as the plasticity mechanisms themselves.

Materials and Methods

All experimental procedures were approved by the Administrative Panel on Laboratory Animal Care at Stanford University.

Mice

Mice with the *Fmr1* gene knocked out selectively from cerebellar Purkinje cells were generated through the following breeding strategy. First, homozygous female mice whose *Fmr1* gene, located on the X-chromosome, was floxed (*Fmr1* conditional knockout, cKO; Mientjes et al., 2006) were crossed with male mice expressing L7/Pcp2-Cre on an autosome (L7/Pcp2-Cre *Jdhu*; The Jackson Laboratory, Stock No. 010536; Zhang et al., 2004;). The L7/Pcp2-Cre *Jdhu* line expresses Cre-recombinase in a manner that is highly selective for Purkinje cells. Male offspring from this first cross were mated with females homozygous for the *Fmr1 c*KO allele to generate offspring homozygous for *Fmr1* cKO, with some mice L7/Pcp2-Cre-positive and some L7/Pcp2-Cre-negative. Cre-positive offspring of this second cross are referred to as L7-*Fmr1* KO, and their Cre-negative littermates were used as controls and referred to as wild type (WT). Genotyping was performed by Transnetyx Inc on ear-clipped samples to confirm the presence of the floxed *Fmr1* allele in all mice and the presence or absence of Cre using RT-qPCR.

Mice were kept on a reversed 12-h light/12-h dark cycle, and behavioral experiments were conducted during the dark cycle of the mice. After surgical implantation (see below), mice were housed individually in standard cages and provided food and water *ad libidum.* Male and female mice 8-22 weeks old were used in the behavioral experiments. Similar learning deficits were observed in male and female L7-*Fmr1* KO mice (**Fig. 1-figure supplement 1**), therefore results were pooled across sex.

Surgery

Mice underwent surgery between 8-12 weeks of age to implant hardware for restraining the head and measuring eye movements, as described previously (Payne and Raymond, 2017; Nguyen-Vu et al., 2017). Mice were anesthetized with 1.5-2.5% isoflurane. An incision was made in the scalp and a custom-made head post (Shapeways Inc) was attached to the top of the skull using dental acrylic (Relyx Unicem Self-Adhesive Universal Resin Cement, Aplicap Capsule Refills-3M). Two stacked neodymium magnets with a total size of 0.75 x 2 mm (grade N50, axially magnetized, *SuperMagnetMan.com*) were implanted beneath the conjunctiva on the temporal side of the left eye. An angular magnetic field sensor (HMC1512, Honeywell Inc.) was soldered to an 8-pin connector and attached to the skull above the eye using dental acrylic, in a plane parallel to horizontal (nasal-temporal) eye movements. Eye movements were measured by detecting changes in the magnetic field created by movements of the magnet implanted on the eye (Payne and Raymond, 2017). Mice recovered from surgery for at least five days before experiments were performed.

Behavioral experiments

Mice were acclimatized to the laboratory for at least 20 min after transport from the animal care facility before the start of an experiment. Experiments were conducted in a light-proof, sound-attenuated chamber (IAC acoustics). The head of the mouse was secured by attaching its head post to a restrainer, which was then attached to a vestibular turntable controlled by a Carco Model 823 rate table and Model 405D controller. The turntable delivered vestibular stimuli to the mouse by

rotation about a yaw (earth-vertical) axis centered on the head of the mouse. An optokinetic drum controlled by a Yaskawa AC-Servo SGMCS-02B3B11 motor provided visual stimulation by rotation about an earth-vertical axis aligned with that of the vestibular turntable. The drum was made of translucent white plastic, and had alternating black and white stripes, with each stripe subtending approximately 7.5° of the visual field, illuminated by an LED light strip attached to the rim of the drum. Eye movements were recorded using the method described in Payne & Raymond (2017).

Experiments to assess VOR learning consisted of testing blocks and training blocks. Testing blocks consisted of three 45 second tests of the eye movement response to the vestibular stimulus delivered alone in complete darkness, i.e., the VOR. The vestibular stimulus was sinusoidal vestibular turntable rotation at 1 Hz or 0.5 Hz with a peak velocity of ±10°/s. The three 45 s VOR tests in a block were separated by 10 s with the turntable stationary. Training blocks were ten minutes long, and were repeated three times for a total of 30 min training, with a testing block following each training block. For VOR-increase training, the vestibular stimulus used for testing the VOR (1 Hz or 0.5 Hz) was paired with oppositely directed motion of the illuminated optokinetic drum with the same peak velocity (±10°/s). For VOR-decrease training, the vestibular stimulus used for testing was paired with motion of the optokinetic drum in the same direction with the same velocity, so that the drum was stationary relative to the head of the mouse. In behavioral pre-training experiments, the pre-training consisted of three 10-min blocks of either VOR-decrease training or delivery of the vestibular stimulus alone in the dark (Vestibular only), with a testing block before each training block. Calibration of the signals from the magnetic sensor used to record eye movements was performed after the experiment, as described in Payne & Raymond (2017).

Experiments to OKR adaptation consisted of sixty 50-second long blocks of training with 1 Hz sinusoidal rotation of the optokinetic drum with peak velocity of ±10°/s, with the vestibular turntable stationary. Each 50-s block of training was followed by 10 seconds in darkness.

Prior to some experiments (**Figures 3**, **3-figure supplement 1** and 2, 4B, 5 and **5-figure supplement 1**), mice received a single IP injection of 0.4, 0.5, or 2.5 mg/kg diazepam (in saline) or saline control. After diazepam or saline administration, mice were returned to the home cage, and then behavioral testing was performed either 2 hours, 18- 24 hours, and/or 1 week later.

Each mouse underwent multiple behavioral experiments, with at least two days between successive experiments. The same cohort of mice was used for the experiments shown in **Figures 1** and **2**, with the order of the experiments randomized. A subset of the same cohort was then used for the diazepam experiments shown in **Figure 3**. A separate cohort of mice was used for the low-frequency training experiments shown in **Figure 4**, with the order of randomized for the behavioral pre-training conditions shown in **Fig. 4A** (no pretraining, VOR-decrease pre-training and Vestibular only pre-training) followed by the diazepam pre-treatment experiments in **Fig. 4B**, with randomized order for drug and saline conditions. Another separate cohort of mice was used for the OKR adaptation experiments shown in **Fig.5**. The order of experiments with diazepam and saline treatment was pseudorandomized.

Analysis of eye movement measurements

Signals from the magnetic sensor related to eye position were fourth-order low-pass (15 Hz) Butterworth filtered and then digitally differentiated to obtain eye velocity using a windowed (30 ms) Savitzky-Golay filter. Eye velocity data from each VOR test or OKR block were fit with a 1 Hz or 0.5 Hz sinusoid. Values deviating from the sinusoidal fit by more than 31°/s were identified as saccades or movement artifacts and excluded, along with data from 50 ms before and after. Segments of data less than 10 ms in duration were also excluded. The entire 45 s VOR or 50 s OKR test was excluded if more than 45% of the data points were excluded, Subsequently, the remaining eye velocity data underwent a second round of fitting using sinusoids at frequencies of either 1 Hz or 0.5 Hz. The amplitude of this second sinusoidal fit provided the measure of the amplitude of the

eye movement response, Values from the three VOR tests in a block were averaged. VOR learning (ΔVOR) was calculated as the percentage change in the VOR amplitude following each 10 min block of training relative to the baseline VOR amplitude measured before training. For OKR, values from the first three 50 s OKR blocks were averaged to obtain the pre-training baseline, and values from the last three blocks (58-60 min) were averaged to obtain the post-training OKR measure. OKR learning (ΔOKR)

was calculated as the percentage change in the OKR amplitude post- vs. pre-training. Learned changes in the OKR relative to pre-training are are also reported for blocks 10, 20, 30, 40 and 50. Eye movement gain was calculated at the ratio of eye movement amplitude to either vestibular (VOR) or visual (OKR) stimulus amplitude.

Statistical analysis

Data were analyzed with a Shapiro-Wilk test of normality, followed by a two-factor repeated measures ANOVA with posthoc Tukey or by a two-sample or paired sample t-test, executed in OriginPro 2022 software. A value of p less than 0.05 was considered significant. Data are plotted as mean ± SEM.

Code

All code used for data acquisition (*https://github.com/RaymondLab/Code/tree/Master/Experiment%20Protocols*) and analysis (*https://github.com/RaymondLab/Code/tree/Master/Tools/VOR_Analysis*) is available at github.com/RaymondLab/Code.

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In preparing this manuscript, we made a conscious effort to address citation bias. Following the approach outlined in Dworkin et al., (2020), we used open source code to assess the gender balance of our citations based on the first names of the first and last authors (Zhou et al., 2020). Excluding self-citations, our article includes citations as follows: 56.98% man/man, 9.30% man/woman, 23.26% woman/man, and 10.47% woman/woman citations. For comparison, the proportions obtained from articles in the top five neuroscience journals (Dworkin et al., 2020) are as follows: 58.4% man/man, 9.4% man/woman, 25.5% woman/man, and 6.7% woman/woman. Our references also contain 21.96% author of color (first)/author of color(last), 18.55% white author/author of color, 21.03% author of color/white author, and 38.46% white author/white author.

Declaration of interest

The authors declare no competing interests.

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Author contributions

A.S. designed the study, performed the experiments, analyzed, and interpreted experimental data, and wrote the manuscript. J.G.F. and A.B.N. conducted experiments. J.B. conducted experiments and wrote the first draft of the methods section. J.L.R. designed experiments, interpreted data, and wrote the manuscript.

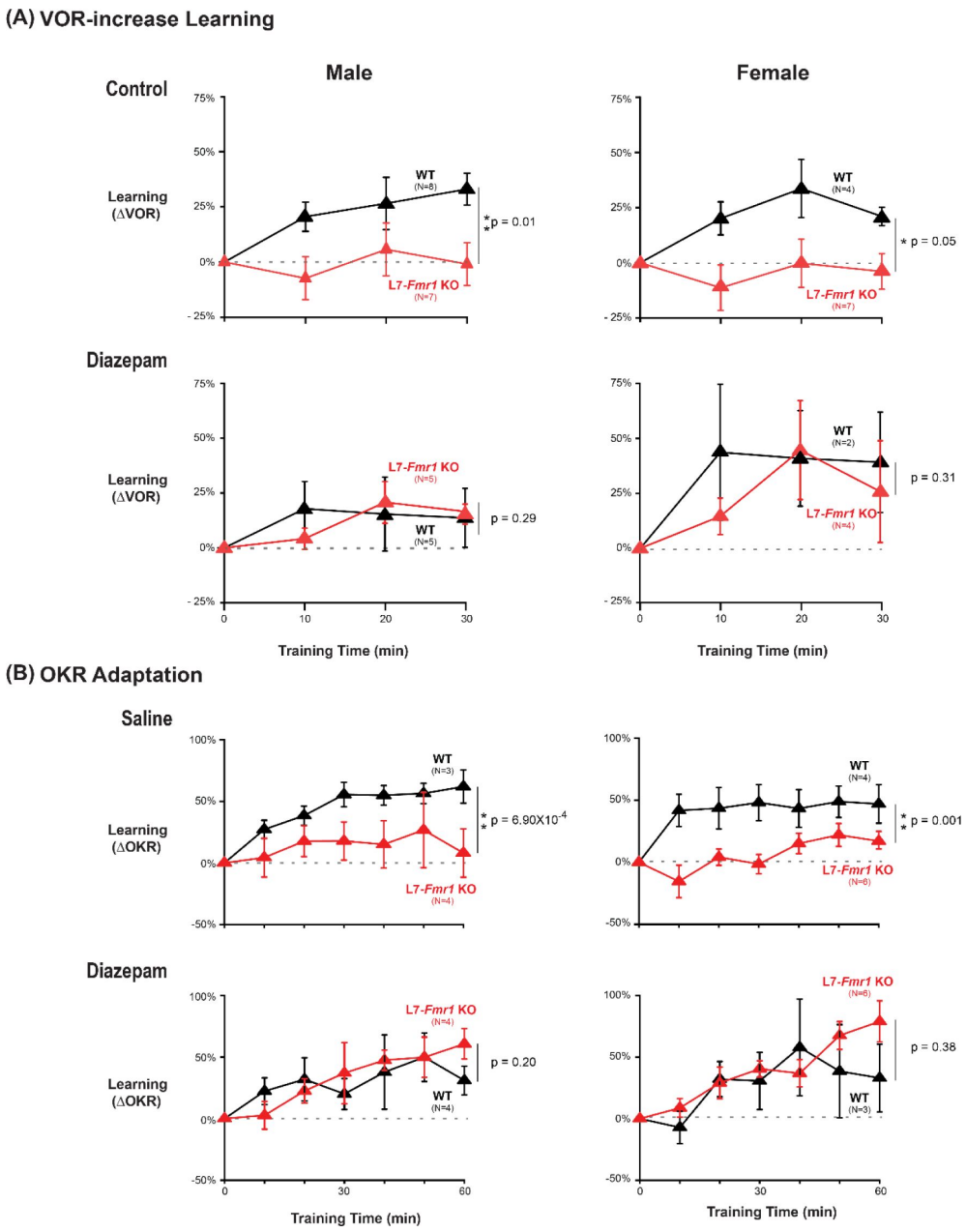

Figure 1 - figure supplement 1.

Similar oculomotor learning impairments and efficacy of diazepam pretreatment in male and female L7-*Fmr1* KO mice.

(A) VOR-increase training. *Top Left,* In male L7-*Fmr1* KO mice (*red*), VOR-increase learning was impaired relative to male WT (*black*) (p=0.01, 30 min, Tukey). *Top Right,* In female L7-*Fmr1* KO mice (*red*), VOR- increase learning was impaired relative to female WT (*black*) (p=0.05, 30 min, Tukey). *Bottom Left,* 18-24 hours after diazepam administration, male L7-*Fmr1* KO mice exhibited VOR-increase learning indistinguishable from that of male WT (p=0.290, 30 min, Tukey). *Bottom Right*, 18-24 hours after diazepam administration, female L7-*Fmr1* KO mice exhibited VOR-increase learning indistinguishable from that of female WT (p=0.31, 30 min, Tukey). **(B)** OKR adaptation training. *Top Left,* In male L7- *Fmr1* KO mice, OKR adaptation was impaired relative to male WT ($p=6.90x10^{-4}$, 30 min, Tukey). *Top Right,* In female L7-*Fmr1* KO mice, OKR adaptation was impaired relative to female WT (p=0.001, 30 min, Tukey). *Bottom Left,* 18-24 hours after diazepam administration, male L7-*Fmr1* KO mice exhibited OKR adaptation indistinguishable from that of male WT (p=0.20, 30 min, Tukey). *Bottom Right*, 18-24 hours after diazepam administration, female L7-*Fmr1* KO mice exhibited OKR adaptation indistinguishable from that of female WT (p=0.38, 30 min, Tukey).

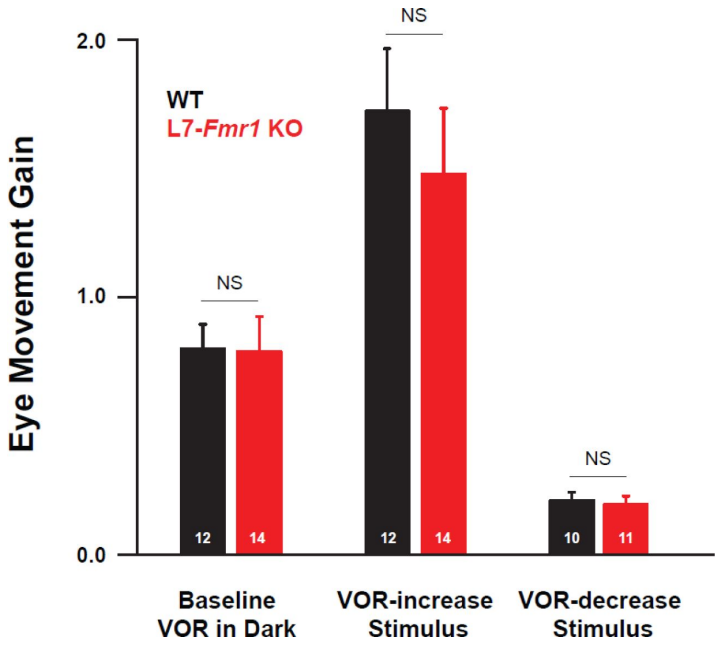

Figure 1-figure supplement 2.

Baseline oculomotor performance of L7-*Fmr1* KO mice was indistinguishable from WT.

The gain of the eye movement responses (ratio of eye movement amplitude to vestibular stimulus amplitude; see Methods) of L7-*Fmr1* KO mice (*red*) was not significantly different from that of WT mice (*black*) during baseline tests of the VOR in the dark before training (***left***; p= 0.95, two sample t-test) or during the first 45 sec of the paired presentation of visual and vestibular stimuli used for VOR-increase training (***middle***; p= 0.50, two sample t-test) or for VOR-decrease training (***right***; p= 0.76, two sample t-test). Number of mice tested is indicated in each bar.

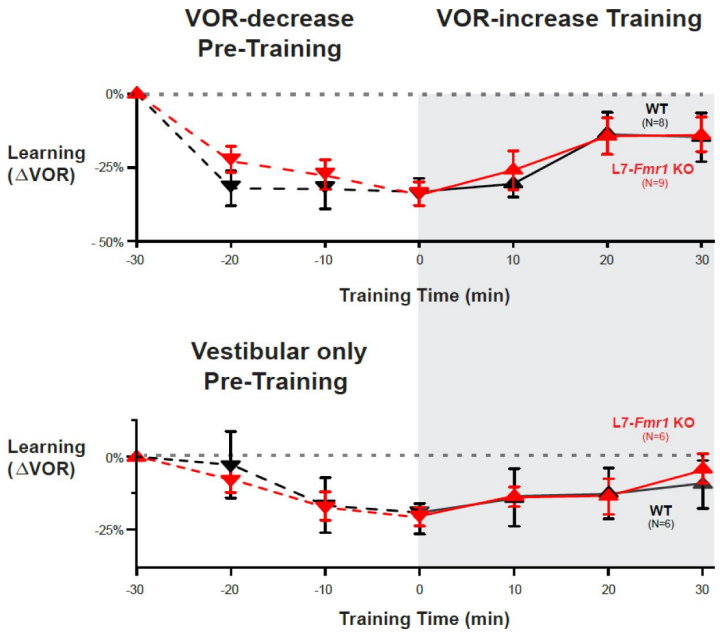

Figure 2-figure supplement 1. Data from Figure 2

were subsampled to compare VOR-increase learning in subpopulations of mice matched for the mean learned decrease in the VOR during pre-training.

Subsampling was done by eliminating the WT mice (*black*) with the smallest decrease and L7-*Fmr1* KO mice (*red*) with the largest decrease in the VOR measured after 30 min of pre-training (just before the start of VOR-increase training), until the mean values in the two populations were within 2%. In these sub-sampled populations, the amount of VOR-increase learning was not significantly different between the L7-*Fmr1* KO and WT mice after VOR-decrease pre-training (*top;* p=0.74, L7-*Fmr1* KO mice vs. WT, 30 min, Tukey) or after Vestibular only pre-training (*bottom;* p=0.40, L7-*Fmr1* KO mice vs. WT, 30 min, Tukey), as also observed in the full samples.

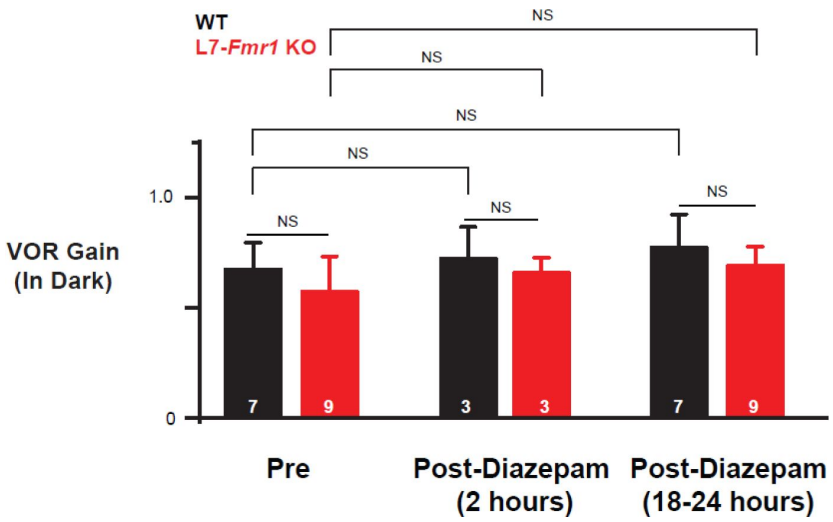

Figure 3 - figure supplement 1.

Diazepam did not affect baseline VOR performance.

The gain of the VOR (ratio of eye velocity to vestibular stimulus velocity) was measured in the dark in L7-*Fmr1* KO (*red*) and WT (*black*) mice before (*Pre*), 2 hours after (*Post-diazepam (2 hours)*) and 18-24 hours after (*Post-diazepam (18-24 hours)*) an IP injection of diazepam (0.5 mg/kg). There was no effect of diazepam on the gain of the VOR in L7-*Fmr1* KO mice (*red;* p=0.72, Pre vs. 2 hours Post Diazepam; p= 0.77, Pre vs. 18-24 hours Post-diazepam; Tukey) or WT mice (*black*; p=0.99, Pre vs. 2 hours Post diazepam; p= 0.36, Pre vs. 18- 24 hours Post-diazepam; Tukey). Moreover, the gain of the VOR of L7-*Fmr1* KO mice was not significantly different from that of WT mice during baseline tests of the VOR in the dark before diazepam administration Pre (***left***; p= 0.79, Tukey), Post-diazepam (2 hours) (***middle***; p= 0.77, Tukey) and Post-diazepam (18-24 hours) (***right***; p= 0.97, Tukey). The 2-hour and 18-24-hour VOR performance measurements were made just before the VOR-increase training sessions (training time = 0) shown in **Fig. 3-figure supplement 2B**, and **Fig. 3B *top*,** respectively. The Pre VOR-performance measurements were made just before the VOR-increase training sessions shown in **Fig. 1A*, right*** for the subset of mice that were also tested 1 day after diazepam administration.

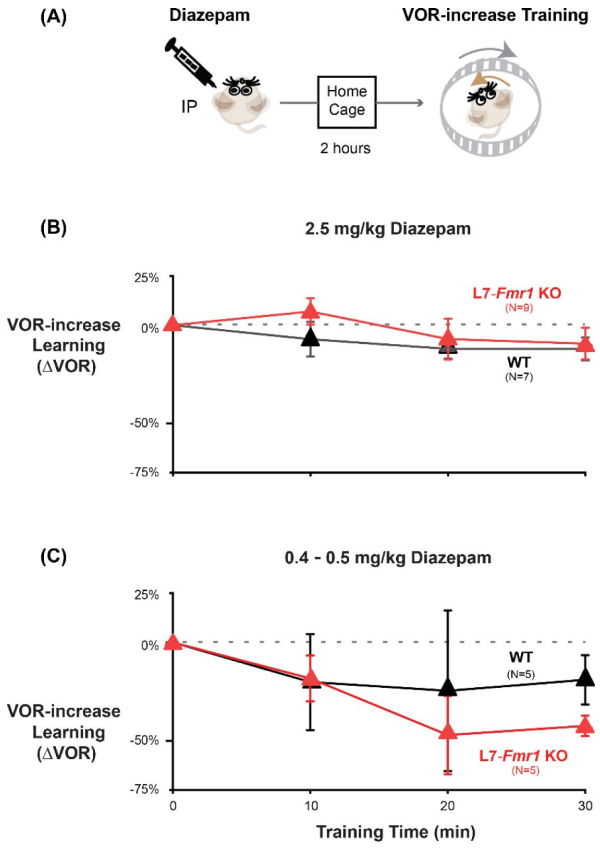

Figure 3 - figure supplement 2.

The acute effect of diazepam was inhibition of VOR-increase learning.

(A) Mice were given an intraperitoneal (IP) injection of diazepam (2.5 mg/kg or 0.4-0.5 mg/kg) and then returned to the home cage for 2 hours before VOR-increase training. When VOR-increase training was delivered two hours after IP injection of 2.5 mg/kg diazepam **(B)**, 0.4-0.5 mg/kg diazepam **(C)**, no learned increase in VOR amplitude was observed in L7-*Fmr1* KO (*red*) or WT (*black*) mice.

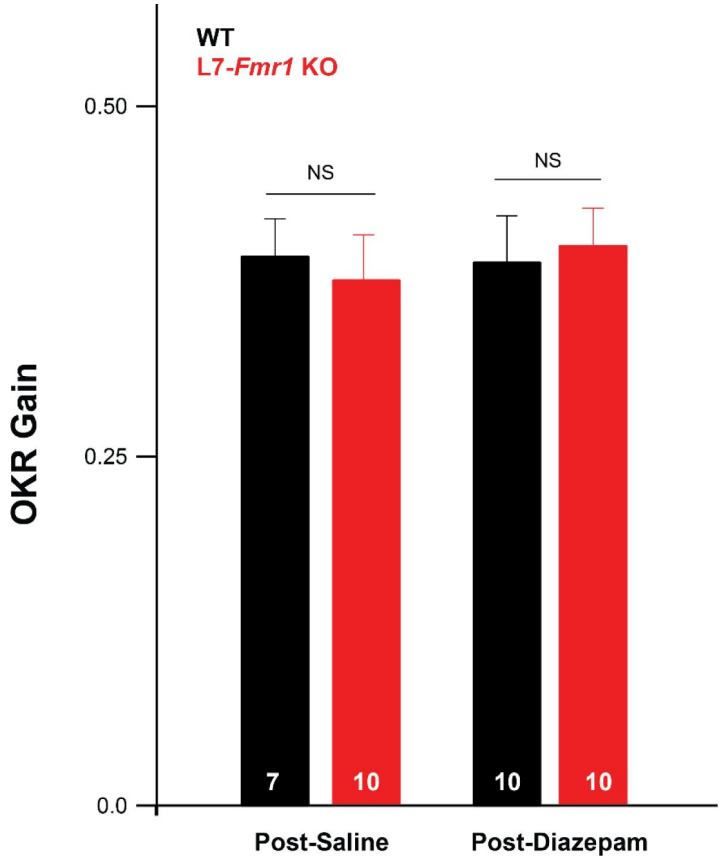

Figure 5 - figure supplement 1.

Baseline optokinetic reflex (OKR) performance normal in L7-*Fmr1* KO mice and after diazepam pre-treatment. The baseline OKR was measured during the first three minutes of OKR adaptation training in L7-*Fmr1* KO (*red*) and WT (*black*) mice, 18-24 hours after an IP injection of saline or diazepam (0.5 mg/kg). There was no difference in the baseline OKR gain (ratio of eye velocity to optokinetic drum velocity) of L7-*Fmr1* KO vs. WT mice (p=0.690 Pre) and no effect of diazepam pre-treatment on the baseline OKR performance (L7-*Fmr1* KO, p= 0.690, post-saline vs. post-diazepam; WT, p= 0.55, post-saline vs. post-diazepam; Tukey).

References

1. Abraham W. C (2008) **Metaplasticity: tuning synapses and networks for plasticity** *Nature Reviews. Neuroscience* **9** https://doi.org/10.1038/nrn2356

2. Abraham W. C., Bear M. F (1996) **Metaplasticity: the plasticity of synaptic plasticity** *Trends in Neurosciences* **19**:126–130 https://doi.org/10.1016/s0166-2236(96)80018-x

3. Ashburn S. M., Flowers D. L., Napoliello E. M., Eden G. F (2020) **Cerebellar function in children with and without dyslexia during single word processing** *Human Brain Mapping* **41**:120–138 https://doi.org/10.1002/hbm.24792

4. Badura A., Verpeut J. L., Metzger J. W., Pereira T. D., Pisano T. J., Deverett B., Bakshinskaya D. E., Wang S. S.-H (2018) **Normal cognitive and social development require posterior cerebellar activity** *ELife* **7** https://doi.org/10.7554/eLife.36401

5. Barmack N. H., Pettorossi V. E (1980) **Influence of a GABA agonist, diazepam, on the vestibuloocular reflexes of the rabbit** *Brain Research Bulletin* **5**:705–712 https://doi.org/10.1016/0361-9230(80)90116-1

6. Benna M. K., Fusi S (2016) **Computational principles of synaptic memory consolidation** *Nature Neuroscience* **19**:1697–1706 https://doi.org/10.1038/nn.4401

7. Benusková L., Ebner F. F., Diamond M. E., Armstrong-James M (1999) **Computational study of experience- dependent plasticity in adult rat cortical barrel-column. Network (Bristol** *England* **10**:303–323

8. Bienenstock E., Cooper L., Munro P (1982) **Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex** *The Journal of Neuroscience* **2**:32–48 https://doi.org/10.1523/JNEUROSCI.02-01-00032.1982

9. Boyden E. S., Katoh A., Pyle J. L., Chatila T. A., Tsien R. W., Raymond J. L (2006) **Selective engagement of plasticity mechanisms for motor memory storage** *Neuron* **51**:823–834 https://doi.org/10.1016/j.neuron.2006.08.026

10. Boyden E. S., Katoh A., Raymond J. L (2004) **Cerebellum-dependent learning: the role of multiple plasticity mechanisms** *Annual Review of Neuroscience* **27**:581–609 https://doi.org/10.1146/annurev.neuro.27.070203.144238

11. Boyden E. S., Raymond J. L (2003) **Active Reversal of Motor Memories Reveals Rules Governing Memory Encoding** *Neuron* **39**:1031–1042 https://doi.org/10.1016/S0896-6273(03)00562-2

12. Broussard D. M., Kassardjian C. D (2004) **Learning in a Simple Motor System** *Learning & Memory* **11**:127–136 https://doi.org/10.1101/lm.65804

13. Cai D. J. *et al.* (2016) **A shared neural ensemble links distinct contextual memories encoded close in time** *Nature* **534**:115–118 https://doi.org/10.1038/nature17955

14. Cooper L. N., Bear M. F (2012) **The BCM theory of synapse modification at 30: interaction of theory with experiment** *Nature Reviews. Neuroscience* **13**:798–810 https://doi.org/10.1038/nrn3353

15. Cox P. R., Fowler V., Xu B., Sweatt J. D., Paylor R., Zoghbi H. Y (2003) **Mice lacking tropomodulin-2 show enhanced long-term potentiation, hyperactivity, and deficits in learning and memory** *Molecular and Cellular Neuroscience* **23**:1–12 https://doi.org/10.1016/S1044-7431(03)00025-3

16. Cullen K. E (2023) **Vestibular motor control** *Handbook of Clinical Neurology* :31–54 https://doi.org/10.1016/B978-0-323-98818-6.00022-4

17. De Zeeuw C. I., Lisberger S. G., Raymond J. L. (2021) **Diversity and dynamism in the cerebellum** *Nature Neuroscience* **24**:160–167 https://doi.org/10.1038/s41593-020-00754-9

18. Fong M., Duffy K. R., Leet M. P., Candler C. T., Bear M. F (2021) **Correction of amblyopia in cats and mice after the critical period** *ELife* **10** https://doi.org/10.7554/eLife.70023

19. Frontera J. L., Baba Aissa H., Sala R. W., Mailhes-Hamon C., Georgescu I. A., Léna C., Popa D (2020) **Bidirectional control of fear memories by cerebellar neurons projecting to the ventrolateral periaqueductal grey** *Nature Communications* **11** https://doi.org/10.1038/s41467-020-18953-0

20. Fusi S., Drew P. J., Abbott L. F (2005) **Cascade models of synaptically stored memories** *Neuron* **45**:599–611 https://doi.org/10.1016/j.neuron.2005.02.001

21. Gittis A. H., du Lac S. (2006) **Intrinsic and synaptic plasticity in the vestibular system** *Current Opinion in Neurobiology* **16**:385–390 https://doi.org/10.1016/j.conb.2006.06.012

22. Gonshor A., Jones G. M (1973) **Proceedings: Changes of human vestibulo-ocular response induced by vision- reversal during head rotation** *The Journal of Physiology* **234**:102–103

23. Guo C. C., Ke M. C., Raymond J. L (2014) **Cerebellar Encoding of Multiple Candidate Error Cues in the Service of Motor Learning** *Journal of Neuroscience* **34**:9880–9890 https://doi.org/10.1523/JNEUROSCI.5114-13.2014

24. Gu Y. *et al.* (2002) **Impaired Conditioned Fear and Enhanced Long-Term Potentiation in Fmr2 Knock-Out Mice** *The Journal of Neuroscience* **22**:2753–2763 https://doi.org/10.1523/JNEUROSCI.22-07-02753.2002

25. Han J.-H., Kushner S. A., Yiu A. P., Cole C. J., Matynia A., Brown R. A., Neve R. L., Guzowski J. F., Silva A. J., Josselyn S. A (2007) **Neuronal competition and selection during memory formation. Science (New York** *N.Y* **316**:457–460 https://doi.org/10.1126/science.1139438

26. Hansel C., de Jeu M., Belmeguenai A., Houtman S. H., Buitendijk G. H. S., Andreev D., De Zeeuw C. I., Elgersma Y. (2006) **αCaMKII Is Essential for Cerebellar LTD and Motor Learning** *Neuron* **51**:835–843 https://doi.org/10.1016/j.neuron.2006.08.013

27. Hansel C., Linden D. J. (2000) **Long-Term Depression of the Cerebellar Climbing Fiber–Purkinje Neuron Synapse** *Neuron* **26**:473–482

28. Han X. *et al.* (2013) **Forebrain Engraftment by Human Glial Progenitor Cells Enhances Synaptic Plasticity and Learning in Adult Mice** *Cell Stem Cell* **12**:342–353 https://doi.org/10.1016/j.stem.2012.12.015

29. He H.-Y., Ray B., Dennis K., Quinlan E. M (2007) **Experience-dependent recovery of vision following chronic deprivation amblyopia** *Nature Neuroscience* **10**:1134–1136 https://doi.org/10.1038/nn1965

30. Huber K. M., Gallagher S. M., Warren S. T., Bear M. F (2002) **Altered synaptic plasticity in a mouse model of fragile X mental retardation** *Proceedings of the National Academy of Sciences* **99**:7746–7750 https://doi.org/10.1073/pnas.122205699

31. Hulme S. R., Jones O. D., Ireland D. R., Abraham W. C (2012) **Calcium-dependent but action potential- independent BCM-like metaplasticity in the hippocampus** *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **32**:6785–6794 https://doi.org/10.1523/JNEUROSCI.0634-12.2012

32. Hunley C., Mitra S., Pugh JR. (2023) **Parallel fiber metaplasticity mediated by cannabinoid type 1 receptors . Program No. 213.05. 2023 Neuroscience Meeting Planner**

33. Hwang K.-D., Kim S. J., Lee Y.-S (2022) **Cerebellar Circuits for Classical Fear Conditioning** *Frontiers in Cellular Neuroscience* **16** https://doi.org/10.3389/fncel.2022.836948

34. Inoshita T., Hirano T (2018) **Occurrence of long-term depression in the cerebellar flocculus during adaptation of optokinetic response** *ELife* **7** https://doi.org/10.7554/eLife.36209

35. Ito M (1982) **Cerebellar control of the vestibulo-ocular reflex--around the flocculus hypothesis** *Annual Review of Neuroscience* **5**:275–296 https://doi.org/10.1146/annurev.ne.05.030182.001423

36. Ito M., Kano M (1982) **Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex** *Neuroscience Letters* **33**:253–258 https://doi.org/10.1016/0304-3940(82)90380-9

37. Ito M., Shiida T., Yagi N., Yamamoto M (1974) **The Cerebellar Modification of Rabbit's Horizontal Vestibulo-Ocular Reflex Induced by Sustained Head Rotation Combined with Visual Stimulation** *Proceedings of the Japan Academy* **50**:85–89 https://doi.org/10.2183/pjab1945.50.85

38. Ito M., Shiida T., Yagi N., Yamamoto M (1974) **Visual influence on rabbit horizontal vestibulo-ocular reflex presumably effected via the cerebellar flocculus** *Brain Research* **65**:170–174 https://doi.org/10.1016/0006-8993(74)90344-8

39. Jang D. C., Shim H. G., Kim S. J (2020) **Intrinsic plasticity of cerebellar Purkinje cells contributes to motor memory consolidation** *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* https://doi.org/10.1523/JNEUROSCI.1651-19.2020

40. Kakegawa W., Katoh A., Narumi S., Miura E., Motohashi J., Takahashi A., Kohda K., Fukazawa Y., Yuzaki M., Matsuda S (2018) **Optogenetic Control of Synaptic AMPA Receptor Endocytosis Reveals Roles of LTD in Motor Learning** *Neuron* **99**:985–998 https://doi.org/10.1016/j.neuron.2018.07.034

41. Kano M., Rexhausen U., Dreessen J., Konnerth A (1992) **Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells** *Nature* **356**:601–604 https://doi.org/10.1038/356601a0

42. Kawaguchi S., Hirano T (2007) **Sustained structural change of GABA(A) receptor-associated protein underlies long-term potentiation at inhibitory synapses on a cerebellar Purkinje neuron** *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **27**:6788–6799 https://doi.org/10.1523/JNEUROSCI.1981-07.2007

43. Kimpo R. R., Rinaldi J. M., Kim C. K., Payne H. L., Raymond J. L (2014) **Gating of neural error signals during motor learning** *ELife* **3** https://doi.org/10.7554/eLife.02076

44. Kirkwood A., Rioult M. G., Bear M. F (1996) **Experience-dependent modification of synaptic plasticity in visual cortex** *Nature* **381**:526–528 https://doi.org/10.1038/381526a0

45. Koekkoek S. K. E. *et al.* (2005) **Deletion of FMR1 in Purkinje Cells Enhances Parallel Fiber LTD, Enlarges Spines, and Attenuates Cerebellar Eyelid Conditioning in Fragile X Syndrome** *Neuron* **47**:339–352 https://doi.org/10.1016/j.neuron.2005.07.005

46. Koekkoek S., Alphen V., Burg A.V.d., Grosveld J., Galjart F., De Zeeuw C (1997) **Gain adaptation and phase dynamics of compensatory eye movements in mice** *Genes and Function* **1**:175–190 https://doi.org/10.1046/j.1365-4624.1997.00018.x

47. Lau J. M. H., Rashid A. J., Jacob A. D., Frankland P. W., Schacter D. L., Josselyn S. A (2020) **The role of neuronal excitability, allocation to an engram and memory linking in the behavioral generation of a false memory in mice** *Neurobiology of Learning and Memory* **174** https://doi.org/10.1016/j.nlm.2020.107284

48. Lee H.-K (2022) **Metaplasticity framework for cross-modal synaptic plasticity in adults** *Frontiers in Synaptic Neuroscience* **14** https://doi.org/10.3389/fnsyn.2022.1087042

49. Leet M. P., Bear M. F., Gaier E. D (2022) **Metaplasticity: a key to visual recovery from amblyopia in adulthood?** *Current Opinion in Ophthalmology* **33**:512–518 https://doi.org/10.1097/ICU.0000000000000901

50. Lee Y.-S., Silva A. J (2009) **The molecular and cellular biology of enhanced cognition** *Nature Reviews Neuroscience* **10**:126–140 https://doi.org/10.1038/nrn2572

51. Lev-Ram V., Mehta S. B., Kleinfeld D., Tsien R. Y (2003) **Reversing cerebellar long-term depression** *Proceedings of the National Academy of Sciences of the United States of America* **100**:15989–15993 https://doi.org/10.1073/pnas.2636935100

52. Li J., Smith S. S., McElligott J. G (1995) **Cerebellar nitric oxide is necessary for vestibulo-ocular reflex adaptation, a sensorimotor model of learning** *Journal of Neurophysiology* **74**:489–494 https://doi.org/10.1152/jn.1995.74.1.489

53. Linden D. J., Connor J. A (1995) **Long-Term Synaptic Depression** *Annual Review of Neuroscience* **18**:319–357 https://doi.org/10.1146/annurev.ne.18.030195.001535

54. Lisberger S. G., Miles F. A., Zee D. S (1984) **Signals used to compute errors in monkey vestibuloocular reflex: possible role of flocculus** *Journal of Neurophysiology* **52**:1140–1153 https://doi.org/10.1152/jn.1984.52.6.1140

55. Martin K. C., Kosik K. S (2002) **Synaptic tagging -- who's it?** *Nature Reviews. Neuroscience* **3**:813–820 https://doi.org/10.1038/nrn942

56. Mathy A., Ho S. S. N., Davie J. T., Duguid I. C., Clark B. A., Häusser M (2009) **Encoding of oscillations by axonal bursts in inferior olive neurons** *Neuron* **62**:388–399 https://doi.org/10.1016/j.neuron.2009.03.023

57. McConnell M. J., Huang Y. H., Datwani A., Shatz C. J (2009) **H2-Kb and H2-Db regulate cerebellar long- term depression and limit motor learning** *Proceedings of the National Academy of Sciences* **106**:6784–6789 https://doi.org/10.1073/pnas.0902018106

58. McElligott J. G., Beeton P., Polk J (1998) **Effect of Cerebellar Inactivation by Lidocaine Microdialysis on the Vestibuloocular Reflex in Goldfish** *Journal of Neurophysiology* **79**:1286–1294 https://doi.org/10.1152/jn.1998.79.3.1286

59. Medina J. F., Lisberger S. G (2008) **Links from complex spikes to local plasticity and motor learning in the cerebellum of awake-behaving monkeys** *Nature Neuroscience* **11**:1185–1192 https://doi.org/10.1038/nn.2197

60. Michnovicz J. J., Bennett M. V. L (1987) **Effects of rapid cerebellectomy on adaptive gain control of the vestibulo-ocular reflex in alert goldfish** *Experimental Brain Research* **66** https://doi.org/10.1007/BF00243305

61. Mientjes E. J., Nieuwenhuizen I., Kirkpatrick L., Zu T., Hoogeveen-Westerveld M., Severijnen L., Rifé M., Willemsen R., Nelson D. L., Oostra B. A (2006) **The generation of a conditional Fmr1 knock out mouse model to study Fmrp function in vivo** *Neurobiology of Disease* **21**:549–555 https://doi.org/10.1016/j.nbd.2005.08.019

62. Migaud M. *et al.* (1998) **Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein** *Nature* **396**:433–439 https://doi.org/10.1038/24790

63. Miles F. A., Fuller J. H (1974) **Adaptive plasticity in the vestibulo-ocular responses of the rhesus monkey** *Brain Research* **80**:512–516 https://doi.org/10.1016/0006-8993(74)91035-X

64. Mioche L., Singer W (1989) **Chronic recordings from single sites of kitten striate cortex during experience- dependent modifications of receptive-field properties** *Journal of Neurophysiology* **62**:185–197 https://doi.org/10.1152/jn.1989.62.1.185

65. Montgomery J. M., Madison D. V (2002) **State-dependent heterogeneity in synaptic depression between pyramidal cell pairs** *Neuron* **33**:765–777 https://doi.org/10.1016/s0896-6273(02)00606-2

66. Nagao S (1983) **Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo- ocular and optokinetic responses in pigmented rabbits** *Experimental Brain Research* **53** https://doi.org/10.1007/BF00239396

67. Navakkode S., Zhai J., Wong Y. P., Li G., Soong T. W (2022) **Enhanced long-term potentiation and impaired learning in mice lacking alternative exon 33 of CaV1.2 calcium channel** *Translational Psychiatry* **12** https://doi.org/10.1038/s41398-021-01683-2

68. Nguyen-Vu T. D. B., Zhao G. Q., Lahiri S., Kimpo R. R., Lee H., Ganguli S., Shatz C. J., Raymond J. L (2017) **A saturation hypothesis to explain both enhanced and impaired learning with enhanced plasticity** *ELife* **6** https://doi.org/10.7554/eLife.20147

69. Ohtsuki G., Piochon C., Adelman J. P., Hansel C (2012) **SK2 channel modulation contributes to compartment-specific dendritic plasticity in cerebellar Purkinje cells** *Neuron* **75**:108–120 https://doi.org/10.1016/j.neuron.2012.05.025

70. Pastor A. M., de la Cruz R. R., Baker R. (1994) **Cerebellar role in adaptation of the goldfish vestibuloocular reflex** *Journal of Neurophysiology* **72**:1383–1394 https://doi.org/10.1152/jn.1994.72.3.1383

71. Payne H. L., Raymond J. L (2017) **Magnetic eye tracking in mice** *ELife* **6** https://doi.org/10.7554/eLife.29222

72. Philpot B. D., Espinosa J. S., Bear M. F (2003) **Evidence for Altered NMDA Receptor Function as a Basis for Metaplasticity in Visual Cortex** *The Journal of Neuroscience* **23**:5583–5588 https://doi.org/10.1523/JNEUROSCI.23-13-05583.2003

73. Rashid A. J. *et al.* (2016) **Competition between engrams influences fear memory formation and recall. Science (New York** *N.Y* **353**:383–387 https://doi.org/10.1126/science.aaf0594

74. Raymond J. L., Medina J. F (2018) **Computational Principles of Supervised Learning in the Cerebellum** *Annual Review of Neuroscience* **41**:233–253 https://doi.org/10.1146/annurev-neuro-080317-061948

75. Riss J., Cloyd J., Gates J., Collins S (2008) **Benzodiazepines in epilepsy: pharmacology and pharmacokinetics** *Acta Neurologica Scandinavica* **118**:69–86 https://doi.org/10.1111/j.1600-0404.2008.01004.x

76. Robinson D. A (1976) **Adaptive gain control of vestibuloocular reflex by the cerebellum** *Journal of Neurophysiology* **39**:954–969 https://doi.org/10.1152/jn.1976.39.5.954

77. Rochefort C., Lefort J., Rondi-Reig L (2013) **The cerebellum: a new key structure in the navigation system** *Frontiers in Neural Circuits* **7** https://doi.org/10.3389/fncir.2013.00035

78. Rowan M. J. M., Bonnan A., Zhang K., Amat S. B., Kikuchi C., Taniguchi H., Augustine G. J., Christie J. M (2018) **Graded Control of Climbing-Fiber-Mediated Plasticity and Learning by Inhibition in the Cerebellum** *Neuron* **99**:999–1015 https://doi.org/10.1016/j.neuron.2018.07.024

79. Rutten K., Misner D. L., Works M., Blokland A., Novak T. J., Santarelli L., Wallace T. L (2008) **Enhanced long-term potentiation and impaired learning in phosphodiesterase 4D-knockout (PDE4D −/−) mice** *European Journal of Neuroscience* **28**:625–632 https://doi.org/10.1111/j.1460-9568.2008.06349.x

80. Ryu J. H., McCabe B. F (1974) **effects of diazepam and dimenhydrinate on the resting activity of the vestibular neuron** *Aerospace Medicine* **45**:1177–1179

81. Shatz C. J (2009) **MHC Class I: An Unexpected Role in Neuronal Plasticity** *Neuron* **64**:40–45 https://doi.org/10.1016/j.neuron.2009.09.044

82. Shibuki K. *et al.* (1996) **Deficient Cerebellar Long-Term Depression, Impaired Eyeblink Conditioning, and Normal Motor Coordination in GFAP Mutant Mice** *Neuron* **16**:587–599 https://doi.org/10.1016/S0896-6273(00)80078-1

83. Shim H., Fanning A., Raymond J. (2022) **Plasticity mechanisms for bidirectional oculomotor learning. Program No. 213.05. 2022 Neuroscience Meeting Planner**

84. Stoodley C. J., Tsai P. T (2021) **Adaptive Prediction for Social Contexts: The Cerebellar Contribution to Typical and Atypical Social Behaviors** *Annual Review of Neuroscience* **44**:475–493 https://doi.org/10.1146/annurev-neuro-100120-092143

85. Takeuchi T. *et al.* (2008) **Enhancement of Both Long-Term Depression Induction and Optokinetic Response Adaptation in Mice Lacking Delphilin** *PLoS ONE* **3** https://doi.org/10.1371/journal.pone.0002297

86. Tang Y.-P., Shimizu E., Dube G. R., Rampon C., Kerchner G. A., Zhuo M., Liu G., Tsien J. Z (1999) **Genetic enhancement of learning and memory in mice** *Nature* **401**:63–69 https://doi.org/10.1038/43432

87. Toyoizumi T., Kaneko M., Stryker M. P., Miller K. D (2014) **Modeling the dynamic interaction of Hebbian and homeostatic plasticity** *Neuron* **84**:497–510 https://doi.org/10.1016/j.neuron.2014.09.036

88. Uetani N (2000) **Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice** *The EMBO Journal* **19**:2775–2785 https://doi.org/10.1093/emboj/19.12.2775

89. van Praag H., Christie B. R., Sejnowski T. J., Gage F. H. (1999) **Running enhances neurogenesis, learning, and long-term potentiation in mice** *Proceedings of the National Academy of Sciences* **96**:13427–13431 https://doi.org/10.1073/pnas.96.23.13427

90. Yee A. X., Hsu Y.-T., Chen L (2017) **A metaplasticity view of the interaction between homeostatic and Hebbian plasticity** *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **372** https://doi.org/10.1098/rstb.2016.0155

91. Yger P., Gilson M (2015) **Models of Metaplasticity: A Review of Concepts** *Frontiers in Computational Neuroscience* **9** https://doi.org/10.3389/fncom.2015.00138

92. Zang Y., De Schutter E. (2019) **Climbing Fibers Provide Graded Error Signals in Cerebellar Learning** *Frontiers in Systems Neuroscience* **13** https://doi.org/10.3389/fnsys.2019.00046

93. Zenke F., Gerstner W., Ganguli S (2017) **The temporal paradox of Hebbian learning and homeostatic plasticity** *Current Opinion in Neurobiology* **43**:166–176 https://doi.org/10.1016/j.conb.2017.03.015

94. Zhang X.-M., Ng A. H.-L., Tanner J. A., Wu W.-T., Copeland N. G., Jenkins N. A., Huang J.-D (2004) **Highly restricted expression of Cre recombinase in cerebellar Purkinje cells** *Genesis* **40**:45–51 https://doi.org/10.1002/gene.20062

95. Zhang K, Yang Z, Gaffield MA, Gross GG, Arnold DB, Christie JM (2023) **Molecular layer disinhibition unlocks climbing-fiber-instructed motor learning in the cerebellum** *bioRxiv* :2023–8

Editors

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Megan Carey
Champalimaud Foundation, Lisbon, Portugal

Senior Editor
Kate Wassum
University of California, Los Angeles, Los Angeles, United States of America

Reviewer #1 (Public Review):

Summary:

Shakhawat et al., investigated how enhancement of plasticity and impairment could result in the same behavioral phenotype. The authors tested the hypothesis that learning impairments result from saturation of plasticity mechanisms and had previously tested this hypothesis using mice lacking two class I major histocompatibility molecules. The current study extends this work by testing the saturation hypothesis in a Purkinje-cell (L7) specific Fmr1 knockout mouse mice, which have enhanced parallel fiber-Purkinje cell LTD. The authors found that L7-Fmr1 knockout mice are impaired on an oculomotor learning task and both pre-training, to reverse LTD, and diazepam, to suppress neural activity, eliminated the deficit when compared to controls.

Strengths:

This study tests the "saturation hypothesis" to understand plasticity in learning using a well-known behavior task, VOR, and an additional genetic mouse line with a cerebellar cell-specific target, L7-Fmr1 KO. This hypothesis is of interest to the community as it evokes novel inquisition into LTD that has not been examined previously.

Utilizing a cell-specific mouse line that has been previously used as a genetic model to study Fragile X syndrome is a unique way to study the role of Purkinje cells and the Fmr1 gene. This increases the understanding in the field in regards to Fragile X syndrome and LTD.

The VOR task is a classic behavior task that is well understood, therefore using this metric is very reliable for testing new animal models and treatment strategies. The effects of pretraining are clearly robust and this analysis technique could be applied across different behavior data sets.

The rescue shown using diazepam is very interesting as this is a therapeutic that could be used in clinical populations as it is already approved.

All previous comments have been addressed with additional studies, explanations, or analyses. These additions strengthen a very impactful study.

The authors achieved their study objectives and the results strongly support their conclusion and proposed hypothesis. This work will be impactful on the field as it uses a new Purkinje-cell specific mouse model to study a classic cerebellar task. The use of diazepam could be further analyzed in other genetic models of neurodevelopmental disorders to understand if effects on LTD can rescue other pathways and behavior outcomes.

https://doi.org/10.7554/eLife.92543.2.sa1

Reviewer #2 (Public Review):

This manuscript explores the seemingly paradoxical observation that enhanced synaptic plasticity impairs (rather than enhances) certain forms of learning and memory. The central hypothesis is that such impairments arise due to saturation of synaptic plasticity, such that the synaptic plasticity required for learning can no longer be induced. A prior study provided evidence for this hypothesis using transgenic mice that lack major histocompatibility class 1 molecules and show enhanced long-term depression (LTD) at synapses between granule cells

and Purkinje cells of the cerebellum. The study found that a form of LTD-dependent motor learning-increasing the gain of the vestibulo-ocular reflex (VOR)-is impaired in these mice and can be rescued by manipulations designed to "unsaturate" LTD. The present study extends this line of investigation to another transgenic mouse line with enhanced LTD, namely, mice with the Fragile X gene knocked out. The main findings are that VOR gain increase learning is selectively impaired in these mice but can be rescued by specific manipulations of visuomotor experience known to reverse cerebellar LTD. Additionally, the authors show that a transient global enhancement of neuronal inhibition also selectively rescues gain increase learning. This latter finding has potential clinical relevance since the drug used to boost inhibition, diazepam, is FDA-approved and commonly used in the clinic. The evidence provided for the saturation is somewhat indirect because directly measuring synaptic strength in vivo is technically difficult. Nevertheless, the experimental results are solid. In particular, the specificity of the effects to forms of plasticity previously shown to require LTD is remarkable.

https://doi.org/10.7554/eLife.92543.2.sa0

Author response:

The following is the authors' response to the original reviews.

> ***eLife assessment***
> *This important manuscript follows up on previous findings from the same lab supporting the idea that deficits in learning due to enhanced synaptic plasticity are due to saturation effects. Compelling evidence is presented that behavioral learning deficits associated with enhanced synaptic plasticity in a transgenic mouse model can be rescued by manipulations designed to reverse the saturation of synaptic plasticity. In particular, the finding that a previously FDA-approved therapeutic can rescue learning could provide new insights for biologists, psychologists, and others studying learning and neurodevelopment.*

eLife assessment, Significance of findings

This valuable manuscript follows up on previous findings from the same lab supporting the idea that deficits in learning due to enhanced synaptic plasticity are due to saturation effects.

According to the eLife criteria for assessing significance, the "valuable" assessment indicates "findings that have theoretical or practical implications for a subfield." We have revised the manuscript to emphasize the "theoretical and practical implications beyond a single subfield" which "substantially advance our understanding of major research questions", with "profound implications" and the potential for "widespread influence," the eLife criteria for a designation of "landmark" significance.

The most immediate implications of our results are for the two major neuroscience subfields of cerebellar research and autism research. However, as recognized by Reviewer 2, the implications are much broader than that: "the finding that a previously FDA-approved therapeutic can rescue learning could provide important new insights for biologists, psychologists, and others studying learning and neurodevelopment." We have substantially revised the Discussion section of the manuscript to more explicitly lay out how the central idea of our manuscript-- that the capacity for learning at any given moment is powerfully influenced by dynamic, activity- and plasticity-dependent changes in the threshold for synaptic plasticity over short timescales of tens of minutes to hours --has implications for scientific thinking and experiments on plasticity and learning throughout the brain, as well as clinical practice for a wide array of brain disorders associated with altered plasticity and learning impairment.

To emphasize the broad conceptual implications of our research, we have reframed our conclusions in terms of metaplasticity rather than saturation of plasticity throughout the revised manuscript. In our previous submission, we had used the "saturation " terminology for continuity with our previous NguyenVu et al 2017 eLife paper, and mentioned the related idea of threshold metaplasticity in a single sentence: "Similarly, the aberrant recruitment of LTD before training may lead, not to its saturation per se, but to some other kind of reduced availability, such as an increased threshold for its induction (Bienenstock, Cooper, and Munro, 1982; Leet, Bear, and Gaier, 2022)." However, we now appreciate that metaplasticity is a more general conceptual framework for our findings, and therefore emphasize this concept in the revised manuscript, while still making the conceptual link with the "saturation" idea presented in NguyenVu et al 2017 (lines 236-238).

The concept of a sliding threshold for synaptic plasticity (threshold metaplasticity) was proposed four decades ago by Bienenstock, Cooper and Munro (1982) as a mechanism for countering an instability inherent in Hebbian plasticity whereby correlated pre- and post-synaptic activity strengthens a synapse, which leads to an increase in correlated activity, which in turn leads to further strengthening. To counter this, BCM proposed a sliding threshold whereby increases in neural activity increase the threshold for LTP and decreases in activity decrease the threshold for LTP, thereby providing a mechanism for stabilizing firing rates and synaptic weights. This BCM sliding threshold model has been highly influential in theoretical and computational neuroscience, but experimental evidence for whether and how such a mechanism functions in vivo has been quite limited.

Our work extends the previous, limited experimental evidence for a BCM-like sliding threshold in vivo in several significant ways, which we now discuss in the revised manuscript:

First, we analyze threshold metaplasticity at synapses where the plasticity is not Hebbian and lacks the inherent instability that inspired the BCM model. The synapses onto cerebellar Purkinje cells have been described as "anti-Hebbian" because the associative form of plasticity is synaptic LTD of excitatory inputs. This anti-Hebbian associative plasticity lacks the instability inherent in Hebbian plasticity. Moreover, a BCM-like sliding threshold that increases the threshold for associative LTD with increased firing rates and decreases threshold for LTD with decreased firing rates would tend to oppose rather than support the stability of firing rates, nevertheless we find evidence for this in our experimental results. Thus, for cerebellar LTD, the central function of the sliding threshold may not be the stabilization of firing rates, but rather to limit plasticity in order to suppress the overwrite of new memories or to allocate different memories to the synapses of different Purkinje cells.

Second, we analyze the influence of a BCM-like sliding threshold for plasticity on behavioral learning. Most previous evidence for the BCM model in vivo has derived from studies of the effects of sensory deprivation (e.g., monocular occlusion) on the functional connectivity of sensory circuits (Kirkwood et al., 1996; Desai et al. 2002; Fong et al., 2021) rather than on learning per se.

Third, our results provide evidence for major changes in the threshold for plasticity over short time scales and with more subtle manipulations of neural activity than used in previous studies, with practical implications for clinical application. Previously, metaplasticity has been demonstrated with sensory deprivation over multiple days (Kirkwood et al., 1996; Desai et al. 2002) or with drastic changes in neural activity, such as with TTX in the retina (Fong et al, 2021), TMS (Hamada et al 2008), or high frequency electrical stimulation in vitro (Holland & Wagner 1998; Montgomery & Madison 2002) or in vivo (Abraham et al 2001). In contrast, we provide evidence for metaplasticity induced by 30 min of behavioral manipulation (pre-training) and by the relatively subtle pharmacological manipulation of activity with systemic administration of diazepam, a drug approved for humans. Thus, our work contributes not

only conceptually to understanding the function of threshold metaplasticity in vivo, but also offers practical observations that could pave the way for novel therapeutic interventions.

Fourth, whereas efforts to enhance plasticity and learning have largely focused on increasing the excitability of neurons during learning to help cross the threshold for plasticity (e.g., Albergaria et al., 2018; Yamaguchi et al., 2020; Le Friec et al., 2017), we take the opposite, somewhat counterintuitive approach of inhibiting the excitability of neurons during a period before learning to reset the threshold for plasticity to a state compatible with new learning. To our knowledge, the only other application of such an approach in an animal model of a brain disorder has been inhibiting peripheral (retinal) activity with TTX for treatment of amblyopia (Fong et al, 2021). Our findings from CNS inhibition with a single systemic dose of diazepam greatly expands the potential applications, which could readily be tested in other mouse models of human disorders, and other learning deficits. Even in cases where the specific synaptic impairments and circuitry are less fully understood, the impact of suppressing neural activity during a period before training to reduce the threshold for plasticity could be empirically tested.

Fifth, our work extends the consideration of a BCM-like sliding threshold for plasticity to the cerebellum, whereas previous work has focused on models and experimental studies of forebrain circuits. Currently there is a surge of interest in the contribution of the cerebellum to functions and brain disorders previously ascribed to forebrain, hence we anticipate broad interest in this work.

Sixth, our results suggest that the history of plasticity rather than the history of firing rates may be the homeostat controlling the threshold for plasticity, at least at the synapses under consideration. Diazepam pre-treatment only enhanced learning in the L7-Fmr1 KO mice with a low “baseline” threshold for plasticity, as measured in vitro, and not WT mice. This suggests it is not the neural activity per se that drives the change in threshold for plasticity, but the interaction of activity with the plasticity mechanism.

In the revised Discussion, we make all of the above points, to make the implications more clear to readers.

The broad interest in this topic is illustrated by two concrete examples. First, an abstract of this work was honored with selection for oral presentation at the November 2023 Symposium of the Molecular and Cellular Cognition Society, a conceptually wide-ranging organization with thousands of members worldwide. Second, the most closely related published work on activity-dependent metaplasticity in vivo, the Fong et al 2021 eLife paper demonstrating reversal of amblyopia by suppression of activity in the retina by TTX, attracted such broad interest, not just of professional scientists, but also the general public, as to be reported on National Public Radio’s All Things Considered, with an audience of 11.9 million people worldwide.

In considering the potential of this work for widespread influence, it is important to note that activitydriven changes in the threshold for plasticity could very well be a general property of most if not all synapses, yet very little is known about its function in vivo, especially during learning. Therefore, the seminal conceptual and practical advances described above have the potential for profound implications throughout neuroscience, psychiatry, neurology and computer science/AI, the eLife criterion for designation as “landmark” in significance. We respectfully request that the reviewers and editor reassess the significance of our findings in light of our much-improved discussion of the broad significance of the work.

eLife assessment, Strength of support

Convincing evidence is presented that behavioral learning deficits associated with enhanced synaptic plasticity in a transgenic mouse model can be rescued by manipulations designed to reverse the saturation of synaptic plasticity. In particular, the finding that a previously FDA-

approved therapeutic can rescue learning could provide important new insights for biologists, psychologists, and others studying learning and neurodevelopment.

The designation of “Convincing” indicates “methodology in line with current state-of the-art.” In the revised Discussion, we more clearly highlight that our evidence is “more rigorous than current state-ofthe-art” in several respects, thereby meeting the eLife criterion for “Compelling”:

(1) Comparison of learning deficits and effects of behavioral and pharmacological pretreatment across five closely related oculomotor learning tasks, which all depend on the same region of the cerebellum (the flocculus), but which previous work has found to vary in their dependence on LTD at the cerebellar parallel fiber-to-Purkinje cell synapses.

The “state-of-the-art” behavioral standard in the field of learning is assessment of a single learning task that depends on a given brain area, with the implicit or explicit assumption that the task chosen is representative of “cerebellum-dependent learning” or hippocampus-, amygdala-, basal ganglia-, cortex- dependent learning, etc. Sometimes there is a no-learning behavioral control.

Our study exceeds this standard by comparing across many different closely related learning tasks, which all depend on the cerebellar flocculus and other shared vestibular, visual, and oculomotor circuitry, but vary in their dependence on LTD at the cerebellar parallel fiber-to-Purkinje cell synapses. In the original submission, we reported results for high-frequency VOR-increase learning that were dramatically different than for three other VOR learning tasks for which there is less evidence for a role of LTD. Reviewer 2 noted, “the specificity of the effects to forms of plasticity previously shown to require LTD is remarkable.” In the revised manuscript, we provide new data for a second oculomotor learning task in which LTD has been implicated, OKR adaptation, with very similar results as for high-frequency VORincrease learning. The remarkable specificity of both the learning deficits and the effects of pre-training manipulations, in two different lines of mice, for the two specific learning tasks in which LTD has been most strongly implicated, and not the other three oculomotor learning tasks, substantially strengthens the evidence for the conclusion that the learning deficits and effects of pre-training are related specifically to the lower threshold for LTD, rather than the result of some other effect of the gene KO or pre-treatment on the cerebellar or oculomotor circuitry (discussed on lines 270-290 of revised manuscript).

(2) Replication of findings in more than one line of mice, targeting distinct signaling pathways, with a common impact of enhancing LTD at the cerebellar PF-Purkinje cell synapses.

State-of-the-art is to report the effects of one specific molecular signaling pathway on behavior.

In the first part of this Research Advance, we replicate the findings of Nguyen-Vu et al 2017 for a completely different line of mice with enhanced LTD at the parallel fiber-to-Purkinje cell synapses. Like the comparison across LTD-dependent and LTD-independent oculomotor learning tasks, the comparison across completely different lines of mice with enhanced LTD strengthens the evidence that the shared behavioral phenotypes are a reflection of the state of LTD rather than other “off-target” effects of each mutation (discussed on lines 291-309 of revised manuscript).

(3) Reversal of learning impairments with more than one type of treatment.

State-of-the-art is to be able to reverse a learning deficit or other functional impairment in an animal model of a brain disorder with a single treatment; indeed, success in this respect is viewed as wildly exciting, as evidenced by the reception by the scientific and lay communities of the Fong et al, 2021 eLife report of reversal of amblyopia by TTX treatment of the retina.

In the current work, we demonstrate reversal of learning deficits with two different types of treatment during the period before training, one behavioral and one pharmacological. The current diazepam pretreatment results provide a fundamentally new type of evidence for the hypothesis that the threshold for LTD and LTD-dependent learning varies with the recent history of activity in the circuit, complementing the evidence from behavioral and optogenetic pre-training approaches used previously in Nguyen-Vu et al, 2017 (discussed on lines 151-158 and 246-255 of revised manuscript).

> ***Public Reviews:***
>
> ***Reviewer #1 (Public Review):***
>
> *Summary:*
>
> *Shakhawat et al., investigated how enhancement of plasticity and impairment could result in the same behavioral phenotype. The authors tested the hypothesis that learning impairments result from saturation of plasticity mechanisms and had previously tested this hypothesis using mice lacking two class I major histocompatibility molecules. The current study extends this work by testing the saturation hypothesis in a Purkinje-cell (L7) specific Fmr1 knockout mouse mice, which have enhanced parallel fiber-Purkinje cell LTD. The authors found that L7-Fmr1 knockout mice are impaired on an oculomotor learning task and both pre-training, to reverse LTD, and diazepam, to suppress neural activity, eliminated the deficit when compared to controls.*
>
> *Strengths:*
>
> *This study tests the "saturation hypothesis" to understand plasticity in learning using a well-known behavior task, VOR, and an additional genetic mouse line with a cerebellar cell-specific target, L7-Fmr1 KO. This hypothesis is of interest to the community as it evokes a novel inquisition into LTD that has not been examined previously.*
>
> *Utilizing a cell-specific mouse line that has been previously used as a genetic model to study Fragile X syndrome is a unique way to study the role of Purkinje cells and the Fmr1 gene. This increases the understanding in the field in regards to Fragile X syndrome and LTD.*
>
> *The VOR task is a classic behavior task that is well understood, therefore using this metric is very reliable for testing new animal models and treatment strategies. The effects of pretraining are clearly robust and this analysis technique could be applied across different behavior data sets.*
>
> *The rescue shown using diazepam is very interesting as this is a therapeutic that could be used in clinical populations as it is already approved.*
>
> *There was a proper use of controls and all animal information was described. The statistical analysis and figures are clear and well describe the results.*

We thank the reviewer for summarizing the main strengths of our original submission. We have further strengthened the revised submission by

(1) more fully discussing the broad conceptual implications, as outlined above;

(2) adding additional new data (Fig. 5) showing that another LTD-dependent oculomotor learning task, optokinetic reflex (OKR) adaptation, is impaired in the L7-Fmr1 KO mice and rescued by pre-treatment with diazepam, as we had already shown for high-frequency VOR increase learning; 3) responding to the specific points raised by the reviewers, as detailed below.

> *Weaknesses:*
>
> *While the proposed hypothesis is tested using genetic animal models and the VOR task, LTD itself is not measured. This study would have benefited from a direct analysis of LTD in the cerebellar cortex in the proposed circuits.*

Our current experiments were motivated by the direct analysis of cerebellar LTD in Fmr1 knock out mice that was already published (Koekkoek et al., 2005). In that previous work, LTD was analyzed in both Purkinje cell selective L7-Fmr1 KO mice (Koekkoek et al., 2005; Fig. 4D), as used in our study, and global Fmr1 knock out mice (Koekkoek et al., 2005; Fig. 4B). Both lines were found to have enhanced LTD, as cited in the Introduction of our manuscript (lines 48-51, 63-64). The goal of our current study was to build on this previous work by analyzing the behavioral correlates of the findings from this previous, direct analysis of LTD.

> *Diazepam was shown to rescue learning in L7-Fmr1 KO mice, but this drug is a benzodiazepine and can cause a physical dependence. While the concentrations used in this study were quite low and animals were dosed acutely, potential side-effects of the drug were not examined, including any possible withdrawal.*

In humans, diazepam (valium) is one of the most frequently prescribed drugs in the world, and the side effects and withdrawal symptoms have been extensively studied and documented.1 Withdrawal symptoms are generally not observed with treatments of less than 2 weeks (Brett and Murnion, 2015). After longterm treatments tapering of the dosage is recommended to mitigate withdrawal (Brett and Murnion, 2015 and https://americanaddictioncenters.org/valium-treatment/withdrawal-duration). The extensive data on the safety of diazepam in humans lowers the barrier to potential clinical translation of our basic science findings, although we emphasize that our own expertise is scientific, and translation to Fragile X patients or other patient groups will require additional development of the research by clinicians.

Given the extensive history of research on this drug, we focused on looking for side effects that would reflect an adverse effect of diazepam on the function of the same oculomotor neural circuitry whose ability to support certain oculomotor learning tasks was improved after diazepam. In other words, we assessed whether the pharmacological manipulation was enhancing certain functions of a given circuit at the expense of others. As we note (line 164), “The acute effect of diazepam administration [measured 2 hours after administration] was to impair learning” in both WT and L7-Fmr1 KO mice. One could consider this a side effect. More importantly, we also tested extensively for oculomotor side-effects during the therapeutic period when learning impairments were eliminated in the L7-Fmr1 KOs, 18-24 hours post-administration, and have a full section of the Results describing our findings about this, titled “Specificity of pre-training effects on learning.” As described in the Results and Discussion (lines 184195, 312-318, Figure 3, figure 3-supplement1; figure 4B; figure 5-supplement 1), we found no such adverse side-effects, which is again encouraging with respect to the translational potential of our findings.

> *This drug is not specific to Purkinje cells or cerebellar circuits, so the action of the drug on cerebellar circuitry is not well understood for the study presented.*

The effects of diazepam are indeed not specific to Purkinje cells, but rather are known to be widespread. Diazepam is a positive allosteric modulator of GABAA receptors, which are found throughout the brain, including the cerebellum. When delivered systemically, as we did in our experiments, diazepam will suppress neural activity throughout the brain by facilitating inhibition, as documented by decades of previous research with this and related benzodiazepines, including dozens of studies of the effects of diazepam in the cerebellum.

To our knowledge, there is currently no drug that can specifically inhibit Purkinje cells, especially one that can be given systemically to cross the blood-brain barrier. Moreover, if such a drug did exist, we would not predict it to have the same effect as diazepam in reversing the learning deficits of the L7-Fmr1 KO mice, because the latter presumably depends on suppression of activity in the cerebellar granule cells and neurons of the inferior olive, whose axons form the parallel fibers and climbing fibers, and whose correlated activity controls LTD at the parallel fiber-Purkinje cell synapses.

We have revised the text to clarify the key point that despite its widespread action on the brain, the effects of diazepam on cerebellum-dependent learning were remarkably specific (lines 184-195, 210-228, 312318). During the period 18-24 hours after a single dose of diazepam, the learning deficits of L7-Fmr1 KO mice on two LTD-dependent oculomotor learning tasks were completely reversed, with no effects on the same tasks in WT mice, and no effects ("side-effects") in L7-Fmr1 KO mice or WT mice on other, LTDindependent oculomotor learning tasks that depend on the same region of the cerebellum, and no effects on baseline performance of visually or vestibularly driven eye movements.

As described in the revised Discussion (lines 318-323), the non-specific mild suppression of neural activity throughout the brain by diazepam makes it a potentially generalizable approach for inducing BCM-like shifts in the threshold for associative plasticity to facilitate subsequent learning. More specifically, diazepam-mediated reduction of activity throughout the brain has the potential to lower any aberrantly high thresholds for associative plasticity at synapses throughout the brain, and thereby reverse any learning deficits associated with such aberrantly high plasticity thresholds. This approach might even be useful in cases where the neural circuitry supporting a given behavior is not well characterized and the specific synapses responsible for the learning deficit are unknown. On lines 323-327 we compare this generalizable approach with the challenges of designing task- and circuit-specific approaches to reset the threshold for plasticity, particularly in circuits that are less well characterized than the oculomotor circuit.

> *It was not mentioned if L7-Fmr1 KO mice have behavior impairments that worsen with age or if Purkinje cells and the cerebellar microcircuit are intact throughout the lifespan.*

At the adult ages used in our study (8-22 weeks), the oculomotor circuitry, including the Fmr1-deficient Purkinje cells, appears to be functionally intact because all of the oculomotor performance and learning tasks we tested were either normal, or could be restored to normal with brief behavioral and/or pharmacological pre-treatment.

Any degeneration of the Fmr1-deficient Purkinje cells or cerebellar microcircuit or additional behavioral impairments at older ages, if they should exist, would not alter our interpretation of the results from 8-22 week old adults regarding history- and activity-dependent changes in the capacity for LTD-dependent learning. Therefore, we leave the question of changes throughout the lifespan to investigators with an interest and expertise in development and/or aging.

Only a small handful of the scores of previous studies of the Fmr1 KO mouse model have investigated age-dependent effects; the reviewer may be interested in papers such as Tang et al., 2015 (doi: 10.1073/pnas.1502258112) or Martin et al., 2016 (doi: 10.1093/cercor/bhv031).

> *Connections between Purkinje cells and interneurons could also influence the behavior results found.*

This comment is repeated below in a more general form (Reviewer 1, second to last comment)—please see our response there and lines 270-309 of the revised manuscript for a discussion of how concerns about "off-target" effects are mitigated by the high degree of

specificity of the learning deficits and effects of pre-training for the specific learning tasks in which LTD has been previously implicated, and the very similar findings in two different lines of mice with enhanced LTD.

> *While males and females were both used for the current study, only 7 of each sex were analyzed, which could be underpowered. While it might be justified to combine sexes for this particular study, it would be worth understanding this model in more detail.*

We performed additional analyses to address the question of whether there might be sex differences that were not detected because of the sample size.

(1) In a new figure, Fig. 1-figure supplement 1, we break out the results for male and female mice in separate plots, and show that all of the effects of both the KO of Fmr1 from the Purkinje cells and of pretreatment with diazepam that are observed in the full cohort are also statistically significant in just the subset of male mice, and just the subset of female mice (see Fig. 1-figure supplement 1 legend for statistics). In other words, qualitatively, there are no sex differences, and all of the conclusions of our manuscript are statistically valid in both male and female mice. This strengthens the justification for combining sexes for the specific scientific purposes of our study.

(2) We performed a power analysis to determine how many mice would be needed to determine whether the very, very small quantitative differences between male and female mice are significant. The analysis indicates that this would require upwards of 70 mice of each sex for WT mice (Cohen's d, 0.6162; power

0.95) and upwards of 2500 mice of each sex for L7-Fmr1 KO mice (Cohen's d, 0.0989; power 0.95). Since the very small quantitative sex differences observed in our cohorts would not alter our scientific conclusions or the possibility for clinical application to patients of both sexes, even if the small quantitative differences turned out to be significant, the very large number of animals needed did not seem warranted for the current scientific purposes. Researchers focused on sex differences may find a motivation to pursue this issue further.

> *Training was only shown up to 30 minutes and learning did not seem to plateau in most cases. What would happen if training continued beyond the 30 minutes? Would L7-Fmr1 KO mice catch-up to WT littermates? Nguyen-Vu*

(1) For VOR learning, we used a 30 min training time because in our past (e.g., Boyden et al., 2003; Kimpo and Raymond, 2007; Nguyen-Vu et al., 2013; Nguyen-Vu et al., 2017) and current results, we find that VOR learning does plateau quite rapidly, with little or no additional adaptive change in the VOR observed between the tests of learning after 30 min vs 20 min of VOR-increase training, in WT or L7Fmr1 KO mice (Fig. 1A; WT, p=0.917; L7-Fmr1 KO, p=0.861; 20 vs. 30 min; Tukey). In the L7-Fmr1 KO mice, there is no significant high-frequency VOR-increase learning after 30 min training, and the mean VOR gain is even slightly lower on average (not significant) than before training (Fig. 1A, red). Therefore, we have no reason to expect that the L7-Fmr1 KO mice would catch up to WT after additional VOR-increase training.

(2) We have added new data on OKR adaptation, induced with 60 min of training (Fig. 5). The L7-Fmr1 KO mice exhibited impaired OKR adaptation, even with 60 min of training (p= 1.27x10-4, Tukey). In our experience, restraint for longer than 60 min produces a behavioral state that is not conducive to learning, as also reported by (Katoh and Yamagiwa, 2018), therefore longer training times were not attempted.

> *The pathway discussed as the main focus for VOR in this learning paradigm was connections between parallel fibers (PF) and Purkinje cells, but the possibility of other*

> *local or downstream circuitry being involved was not discussed. PF-Purkinje cell circuits were not directly analyzed, which makes this claim difficult to assess.*

In the revised manuscript (lines 299-309), we have expanded our discussion of the possibility that loss of expression of Fmr1 from Purkinje cells in the Purkinje cell-specific L7-Fmr1 KO mice might influence other synapses or intrinsic properties of the Purkinje cells (including synapses from interneurons, as raised in this reviewer's comment above), in addition to enhancing associative LTD at the parallel fiberPurkinje cell synapses.

It is a very general limitation of all perturbation studies, even cell-type specific perturbation studies as in the current case, that it is never possible to completely rule out "off-target" effects of the manipulation. Because of this, causality cannot be definitively concluded from correlations (e.g., between the effects of a perturbation observed at the cellular and behavioral level), and therefore we make no such claim in our manuscript. Rather, we conclude that our results "provide evidence for," "support," "predict," or "are consistent with" the hypothesis of a history- and activity-dependent change in the threshold for associative LTD at the parallel fiber-Purkinje cells.

That said, perturbation is still one of the major tools in the experimental toolbox, and there are approaches for mitigating concern about off-target effects. We highlight three aspects of our experimental design that accomplish this (lines 184-228, 256-309). First, we show nearly identical learning impairments and effects of behavioral pretreatment in lines of mice with two completely different molecular manipulations that have the common effect of enhancing PF-Purkinje cell LTD, but are likely to have different off-target cellular effects on the Purkinje cells and their synapses. Second, we show that the learning impairments were highly specific to oculomotor learning tasks in which PF-Purkinje cell LTD was previously implicated, with no such effects on three other oculomotor learning tasks that depend on the same region of the cerebellum and oculomotor circuitry. In the original submission, we provided data for one LTDdependent oculomotor learning task, high-frequency VOR-increase learning; in the revised manuscript we provide new data for a second LTD-dependent oculomotor learning task, optokinetic reflex adaptation, with nearly identical results (Fig. 5). Third, we show that the effects of diazepam pre-treatment were highly specific to the same two LTD-dependent oculomotor learning tasks and also highly specific to the L7-Fmr1 KO mice with enhanced LTD and not WT mice. These three features of the experimental design are not common in studies of learning, especially in combination. On lines 256-309, we provide an expanded discussion of how together, these three features of the design strengthen the evidence that the learning impairments and effects of diazepam pre-treatment on learning are related to LTD at the PF-Pk synapses, while acknowledging the possibility of other effects on the circuit.

> *The authors mostly achieved their aim and the results support their conclusion and proposed hypothesis. This work will be impactful on the field as it uses a new Purkinje-cell specific mouse model to study a classic cerebellar task. The use of diazepam could be further analyzed in other genetic models of neurodevelopmental disorders to understand if effects on LTD can rescue other pathways and behavior outcomes.*

We agree that the present findings are potentially relevant for a very wide array of behavioral tasks, disease models, and brain areas beyond the specific ones in our study, and we make this point on lines 310-338 of the revised manuscript.

> ***Reviewer #2 (Public Review):***
>
> *This manuscript explores the seemingly paradoxical observation that enhanced synaptic plasticity impairs (rather than enhances) certain forms of learning and memory. The central hypothesis is that such impairments arise due to saturation of synaptic plasticity, such that the synaptic plasticity required for learning can no longer be induced. A prior*

> *study provided evidence for this hypothesis using transgenic mice that lack major histocompatibility class 1 molecules and show enhanced long-term depression (LTD) at synapses between granule cells and Purkinje cells of the cerebellum. The study found that a form of LTD-dependent motor learning-increasing the gain of the vestibulo-ocular reflex (VOR)-is impaired in these mice and can be rescued by manipulations designed to "unsaturate" LTD. The present study extends this line of investigation to another transgenic mouse line with enhanced LTD, namely, mice with the Fragile X gene knocked out. The main findings are that VOR gain increased learning is selectively impaired in these mice but can be rescued by specific manipulations of visuomotor experience known to reverse cerebellar LTD. Additionally, the authors show that a transient global enhancement of neuronal inhibition also selectively rescues gain increases learning. This latter finding has potential clinical relevance since the drug used to boost inhibition, diazepam, is FDA-approved and commonly used in the clinic. The evidence provided for the saturation is somewhat indirect because directly measuring synaptic strength in vivo is technically difficult. Nevertheless, the experimental results are solid. In particular, the specificity of the effects to forms of plasticity previously shown to require LTD is remarkable. The authors should consider including a brief discussion of some of the important untested assumptions of the saturation hypothesis, including the requirement that cerebellar LTD depends not only on pre- and postsynaptic activity (as is typically assumed) but also on the prior history of synaptic activation.*

We thank the reviewer for this exceptionally clear and concise assessment of the findings and strengths of the manuscript.

We agree that one of the most “remarkable” aspects of our findings is the specificity of the effects for oculomotor learning tasks for which there is the strongest previous evidence for a role of PF-Purkinje cell LTD. In the original manuscript, we tested just one LTD-dependent oculomotor learning task, highfrequency VOR increase learning; in the revised manuscript, we strengthen the case for LTD-dependent task specificity by adding new data (Fig. 5) showing the same effects for OKR adaptation, an additional LTD-dependent oculomotor learning task.

The reviewer’s suggestion to include discussion of “untested assumptions”, “including the requirement that cerebellar LTD depends not only on pre- and postsynaptic activity (as is typically assumed) but also on the prior history of synaptic activation” prompted us to more deeply consider the broader implications of our results, and extensively revise the Discussion accordingly. We clarify that we consider historydependent changes in the threshold for LTD to be a prediction of the behavioral and pharmacological findings (lines 339-347, 356) rather than an assumption. In addition, we highlight the broader implications of the results by putting them in the context of work in other brain areas on historydependent changes in the threshold for plasticity, i.e., metaplasticity, going back to the seminal Bienenstock-Cooper-Munro (BCM; year) theory (lines 348-378).

> ***Reviewer #1 (Recommendations for The Authors):***
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> *The text and figures are very clear to read, but there are a couple of questions that remain:*
>
> *The concentrations chosen for diazepam are not well described and it is unclear why the concentrations jump from 2.5 mg/kg to 0.5 mg/kg. Please add an explanation for these concentrations and if any additional behavior outcomes were observed.*

Our choice of diazepam concentrations was guided by the concentrations reported in the literature to be effective in mice, which suggest that a higher dose (2 mg/kg) can have additional effects not observed with a lower effective dose (0.5 mg/kg) (Pádua-Reis et al,

2021). Since we did not know how much enhancement of inhibition/suppression of activity might be necessary to substantially reduce the induction of PF-Purkinje cell LTD, we did pilot experiments to test concentrations at the low and high ends of the doses typically used in mice. These pilot experiments revealed that a lower dose of 0.4 or 0.5 mg/kg was comparable to the higher dose of 2.5 mg/kg in suppressing VOR-increase learning 2 hours after administration (Fig. 3 – figure supplement 2). Anecdotally, we observed higher levels of locomotor activity and other abnormal cage behavior during the period immediately after administration of the higher compared to the lower dose. To limit these side effects and any possibility of dependence, we used only the lower dose in all subsequent experiments. We clarify this rationale for using a lower dose in the legend of Fig. 3 – figure supplement 2.

> *Figure 4 describes low-frequency VOR, but the paragraph discussing these results (line 191) mentions high-frequency VOR-increase learning. It is unclear where the results are for the high-frequency data. Please include or rephrase for clearer understanding.*

In the revised manuscript, we clarify that the 1 Hz vestibular and visual stimuli used in Figs. 1-3 is the

“high” frequency, which yields different results than the “low” frequency of 0.5 Hz (Fig. 4), as also observed in Boyden et al 2006, and Nguyen-Vu et al, 2017.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *The authors should consider including a brief discussion of some of the important untested assumptions of the saturation hypothesis, including the requirement that cerebellar LTD depends not only on pre- and postsynaptic activity (as is typically assumed) but also on the prior history of synaptic activation.*

We thank the reviewer for this comment, which, along with your public comments, inspired us to thoroughly reconsider and revise our Discussion. We think this has greatly improved the manuscript, and will substantially increase its appeal to a broad segment of the neuroscience research community, including computational neuroscientists as well as those interested in synaptic physiology, learning and memory, or plasticity-related brain disorders including autism.

Note that we consider the idea that ”LTD depends not only on pre- and post- synaptic activity but also on the prior history of synaptic activation” to be the central prediction of the threshold metaplasticity hypothesis rather than an assumption, and in the revised manuscript we explicitly refer to this as a prediction (line 339, 356). We also added a discussion of multiple known cellular phenomena in the Purkinje cells and their synapses that can regulate LTD and thus represent candidate mechanisms for LTD threshold metaplasticity (lines 339-347). Again, sincere thanks for prompting us to write a vastly improved Discussion section.

> ***Editor's note**:*
>
> *Should you choose to revise your manuscript, please include full statistical reporting including exact pvalues wherever possible alongside the summary statistics (test statistic and df) and 95% confidence intervals. These should be reported in the main text for all key questions and not only when the p-value is less than 0.05.*

We have added exact p-values throughout the manuscript.

References

Albergaria C, Silva NT, Pritchett DL, Carey MR. (2018). Locomotor activity modulates associative learning in mouse cerebellum. Nat Neurosci.21:725-735. doi: 10.1038/s41593-018-0129-x.

Abraham WC, Mason-Parker SE, Bear MF, Tate WT. (2001). Heterosynaptic metaplasticity in the hippocampus in vivo: A BCM-like modifiable threshold for LTP. Proc Natl Acad Sci USA. 98:1092410929.

Bienenstock E, Cooper L, Munro P. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci. 2:32-48. https://doi.org/10.1523/JNEUROSCI.02-01-00032.1982

Brett J, Murnion B. (2015). Management of benzodiazepine misuse and dependence. Aust Prescr.38:152155. doi: 10.18773/austprescr.055.

Boyden ES, Raymond JL. (2003). Active Reversal of Motor Memories Reveals Rules Governing Memory Encoding. Neuron.39:1031-1042. https://doi.org/10.1016/S0896-6273(03)00562-2

Boyden ES, Katoh A, Pyle JL, Chatila TA, Tsien RW, Raymond JL. (2006). Selective engagement of plasticity mechanisms for motor memory storage. Neuron. 51:823-834. https://doi.org/10.1016/j.neuron.2006.08.026

Desai NS, Cudmore RH, Nelson SB, Turrigiano GG. (2002). Critical periods for experience-dependent synaptic scaling in visual cortex. Nat Neurosci. 5:783-789. doi: 10.1038/nn878.

Fong M, Duffy KR, Leet MP, Candler CT, Bear MF. (2021). Correction of amblyopia in cats and mice after the critical period. ELife.10:e70023. https://doi.org/10.7554/eLife.70023

Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, Matsumoto H, Ugawa Y. (2008). Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol. 586:3927-3947. doi: 10.1113/jphysiol.2008.152793.

Katoh A, Yamagiwa A. (2018). Inhibition of PVN neurons influences stress-induced changes of motor learning in the VOR. Society for Neuroscience. Online Program No. 067.14.

Kimpo RR, Raymond JL. (2007). Impaired motor learning in the vestibulo-ocular reflex in mice with multiple climbing fiber input to cerebellar Purkinje cells. J Neurosci. 27:5672-5682. doi:

10.1523/JNEUROSCI.0801-07.2007.

Kirkwood A, Rioult MG, Bear MF. (1996). Experience-dependent modification of synaptic plasticity in visual cortex. Nature. 381:526–528. https://doi.org/10.1038/381526a0

Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, De Graaf W, Smit AE, VanderWerf F, Bakker CE, Willemsen R, Ikeda T, Kakizawa S, Onodera K, Nelson DL, Mientjes E, Joosten M, De Schutter E, Oostra BA, Ito M, De Zeeuw CI. (2005). Deletion of FMR1 in Purkinje Cells Enhances Parallel Fiber LTD, Enlarges Spines, and Attenuates Cerebellar Eyelid Conditioning in Fragile X Syndrome. Neuron. 47:339–352. https://doi.org/10.1016/j.neuron.2005.07.005

Le Friec A, Salabert AS, Davoust C, Demain B, Vieu C, Vaysse L, Payoux P, Loubinoux I. (2017). Enhancing Plasticity of the Central Nervous System: Drugs, Stem Cell Therapy, and Neuro-Implants. Neural Plast. 2017:2545736. doi: 10.1155/2017/2545736.

Leet MP, Bear MF, Gaier ED. (2022). Metaplasticity: a key to visual recovery from amblyopia in adulthood? Curr Opin Ophthalmol. 33:512–518. https://doi.org/10.1097/ICU.0000000000000901

Martin HGS, Lassalle O, Brown JT, Manzoni OJ. (2016). Age-Dependent Long-Term Potentiation Deficits in the Prefrontal Cortex of the Fmr1 Knockout Mouse Model of Fragile X Syndrome. Cereb Cortex. 26:2084–2092. doi: 10.1093/cercor/bhv031.

Montgomery JM, Madison DV. (2002). State-dependent heterogeneity in synaptic depression between pyramidal cell pairs. Neuron. 33:765-777. doi: 10.1016/s0896-6273(02)00606-2.

Nguyen-Vu TDB, Kimpo RR, Rinaldi JM, Kohli A, Zeng H, Deisseroth K, Raymond JL. (2013). Cerebellar Purkinje cell activity drives motor learning. Nat Neurosci. 16:1734-1736. doi:

10.1038/nn.3576.

Nguyen-Vu TB, Zhao GQ, Lahiri S, Kimpo RR, Lee H, Ganguli S, Shatz CJ, Raymond JL. (2017). A saturation hypothesis to explain both enhanced and impaired learning with enhanced plasticity. ELife. 6:e20147. https://doi.org/10.7554/eLife.20147

Pádua-Reis M, Nôga DA, Tort ABL, Blunder M. (2021). Diazepam causes sedative rather than anxiolytic effects in C57BL/6J mice. Sci Rep. 2021;11:9335.

Singh A, Nagpal R, Mittal SK, Bahuguna C, Kumar P. (2017). Pharmacological therapy for amblyopia. Taiwan J Ophthalmol. 7:62-69. doi: 10.4103/tjo.tjo_8_17.

Tang B, Wang T, Wan H, Han L, Qin X, Zhang Y, Wang J, Yu C, Berton F, Francesconi W, Yates JR 3rd, Vanderklish PW, Liao L. (2015). Fmr1 deficiency promotes age-dependent alterations in the cortical synaptic proteome. Proc Natl Acad Sci USA. 112:E4697-E4706. doi: 10.1073/pnas.1502258112.

Yamaguchi T, Moriya K, Tanabe S, Kondo K, Otaka Y, Tanaka S. (2020). Transcranial direct-current stimulation combined with attention increases cortical excitability and improves motor learning in healthy volunteers. J Neuroeng Rehabil. 17:23. doi: 10.1186/s12984-020-00665-7.

https://doi.org/10.7554/eLife.92543.2.sa3