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Intrinsic plasticity of Purkinje cells in cerebellum-dependent motor learning

소뇌 담당 학습의 과정 중 퍼킨지 세포에서 발생하는 내재적 가소성의 역할

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Intrinsic plasticity of Purkinje cells in cerebellum-dependent motor learning

A Dissertation Submitted to the
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by

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Abstract

Intrinsic plasticity of cerebellar Purkinje cells (PCs) is recently highlighted in the cerebellar local circuits, however, its physiological impact on the cerebellar learning and memory remains elusive. Knocking out one of endoplasmic reticulum membrane-bounded protein, which is stromal interaction molecule type 1 (STIM1), in PC-specific manner causes interesting behavior phenotype. These mice showed normal acquisition of memory, but a day after, they lost almost all memory. Since this memory consolidation deficit was found in every learning paradigm, I suspect that the deficit is based on a common pathway among cerebellar learning circuit. Intriguing results from electrophysiological recording were that these mice showed normal synaptic plasticity but no intrinsic plasticity in the PCs. Through the electrophysiological recordings after gain-up training of the VOR, I found that this learning protocol induces a decrease of both synaptic weight and intrinsic excitability in PCs. The synaptic plasticity was found in both the wild-type and knockout groups. However, intrinsic plasticity was impaired only in the knockout mice. Furthermore, the observed defects in the intrinsic plasticity of PCs led to the formation of improper neural plasticity in the vestibular nucleus (VN) neurons. These results suggest that the synergistic modulation of intrinsic and synaptic plasticity in PCs is required for the changes in the local connectivity between the cerebellum and VN that contribute to the long-term storage of motor memory.

Keyword : Cerebellum, Vestibulo-ocular reflex (VOR), Memory consolidation, Purkinje cell, Intrinsic plasticity, Vestibular nucleus (VN) neurons

Student Number : 2013-22457

Preface

This dissertation consists of several works that I involved as a co-first author. Some parts have been published already, and some parts are in preparation. The introduction is a part of my review paper which is under review, and early part of behavior results is a part of the published paper (Ryu et al., 2017). Most of the results including ex-vivo recordings and behavior test are from the work which is currently under review. Thus, figures and text are partially modified from the publication and unpublished works:

<Research Article>

Ryu, C.*, Jang, D.C.*, Jung, D.*, Kim, Y.G., Shim, H.G., Ryu, H.-H., Lee, Y.-S., Linden, D.J., Worley, P.F., Kim, S.J., 2017. STIM1 Regulates Somatic Ca^{2+}Signals and Intrinsic Firing Properties of Cerebellar Purkinje Neurons. J. Neurosci. 37, 8876–8894. doi:10.1523/JNEUROSCI.3973-16.2017 *Equal contribution

Jang, D.C.*, Shim, H.G.*, Kim, S.J., Intrinsic plasticity of cerebellar Purkinje cells in motor learning circuits. bioRxiv doi:10.1101/513283 *Equal contribution

<Review Article>

Jang, D.C., Kim, S.J., The plasticity followed by the cerebellum-dependent eye movement learning: In two different regions and two different types. (Under review)

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1. Introduction

The vestibulo-ocular reflex (VOR) is a representative cerebellum-dependent behavior. It is a compensatory eye movement toward the opposite direction of vestibular input to stabilize the objects on the retina. The gain of this reflex can be modulated by repetitive visual-vestibular coupling, and the newly acquired information is stored in the cerebellar circuit. For decades, several significant hypotheses have been proposed to understand the mechanism of cerebellum-dependent motor learning. Many studies have attempted to determine the brain region in the cerebellar circuit that primarily contributes to learning. The Marr-Albus-Ito hypothesis suggested the synapse between the parallel fiber (PF) and the cerebellar Purkinje cell (PC) (Albus, 1971; Ito, 1982; Marr, 1969). (Miles and Lisberger, 1981) proposed plasticity in the vestibular nucleus (VN) neurons. The cerebellar circuit for VOR adaptation is simple (Figure 1.1). Vestibular input is delivered to both granule cells (GCs) and VN neurons through Mossy fibers (MFs). PCs gather many inputs from GCs through PFs and convey this information to VN neurons. Finally, the VN transmits the command to ocular motor neurons (OMNs) to make appropriate eye movement. When visuo-vestibular stimulation is applied

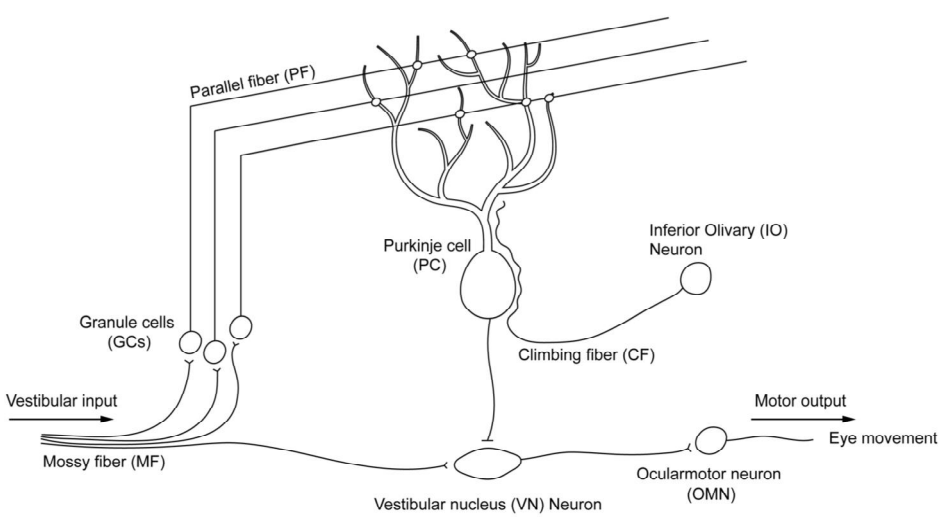


Figure 1.1. Neural circuit for VOR learning.

for learning, an error between visual feedback and vestibular input is known to be delivered back to PCs via climbing fibers from the inferior olive (IO).

1.1. Previously suggested hypotheses

1.1.1. Classical hypotheses

The two major components of successful learning include PCs in the cerebellar cortex and the VN neurons, and there are two influential hypotheses based on these two components. As described above, the Marr-Albus-Ito hypothesis focused on the cerebellar cortex, especially the plasticity of PF-PC synapses, while the Miles-Lisberger hypothesis concentrated on the plasticity of VN neurons. Although these hypotheses had different perspectives, both shared a general background, such as the cerebellar flocculus has important role in motor learning (Ito et al., 1982; Lisberger et al., 1984; McElligott et al., 1998; Nagao, 1983; Robinson, 1976).

Marr (1969) and Albus (1971) proposed that the changes in synaptic strength between PFs and PCs stores the associative motor memory. In this model, CF inputs deliver the instructive signal to regulate the synaptic strength of PF-PC. Ito (1982; 1972) applied the Marr-Albus theory to VOR adaptation. In his hypothesis, Ito suggested that VOR is rapidly corrected by retinal error signals through CF (Ito, 1972) and progressive changes by this correction is stored in the cerebellar cortex. In this process, synaptic plasticity of PF-PC synapses, especially long-term depression (LTD), could be induced by coupling vestibular-driven PF input and visually-driven CF input. Because the Marr-Albus-Ito hypothesis proposed that PCs are the computational center of the learning process, the alteration in eye movement would be interpreted as the consequence of PF-PC synaptic strength change. Thus, the Marr-Albus-Ito hypothesis suggested that PF-PC synaptic plasticity may encode the VOR memory. As a supportive results, modified activity of PCs was found after VOR adaptation (Ito et al., 1974; E. Watanabe, 1985; 1984). Most of LTD-deficit mouse models demonstrate a defect in VOR adaptation (De Zeeuw et al., 1998; Feil et al., 2003; Galliano et al., 2013; Hansel et al., 2006; Shutoh et al., 2002; 2003). The importance of LTD in eye movement adaptation strongly supported by recent works, inhibition of LTD at PF-PC

significantly suppress the adaptation (Kakegawa et al., 2018), and PF-PC LTD was occluded after the adaptation (Inoshita and Hirano, 2018). Additionally, training the monkey at high-stimulation frequency (e.g., 5Hz), the CF—but not PC—activity is able to discriminate the direction of VOR training. These results are supportive for the notion that the CF contains instructive signal to the PC (Raymond and Lisberger, 1998).

Although Marr-Albus-Ito hypothesis is supported by numerous studies, there are some controversial results. Representatively, mice without PICK1, a kinase responsible for the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, showed LTD deficit, but demonstrated normal motor learning (Schonewille et al., 2011). In this paper, the authors used two more different transgenic mice, which has mutation and deletion at the target site of PICK1, these mice also showed normal VOR adaptation without PF-PC LTD. In addition, the idea that instructive signal through CF input has conflict in a monkey study (Ke et al., 2009). In this study, VOR adaptation was successfully induced under inactivation of CF activity.

Miles-Lisberger had a different perspective on the VOR adaptation circuit (Miles and Lisberger, 1981). In this hypothesis, the VN neurons are regarded as the center of the learning process; therefore, the site of memory storage is the VN. The role of PCs in learning is conveying instructive signals to induce synaptic plasticity between MF and VN neurons, rather than storing memory. Furthermore, this hypothesis suggested that the changes in PC activity followed by VOR adaptation would be based on the altered inputs from MF, which conveys modified efference copies of eye movement command generated by the VN. Thus, synaptic changes in the cerebellar cortex is not necessary for VOR adaptation. VOR adaptation alters the activity of both floccular PCs and flocculus target neurons (FTNs) in the VN; however, the latency of firing change in PCs was too late to explain changes in VOR (Lisberger et al., 1994a; 1994b). Additionally, the observed change in PC activity was insufficient to describe the learning (Hirata and Highstein, 2001; Lisberger et al., 1994b). The altered responses of FTNs after VOR adaptation were not totally lost when the flocculus was chemically inactivated (Kassardjian et al., 2005; Nagao and Kitazawa, 2003; Okamoto et al., 2011a; 2011b; Partsalis et al., 1995), supporting the view that memory is stored outside

of the cerebellum.

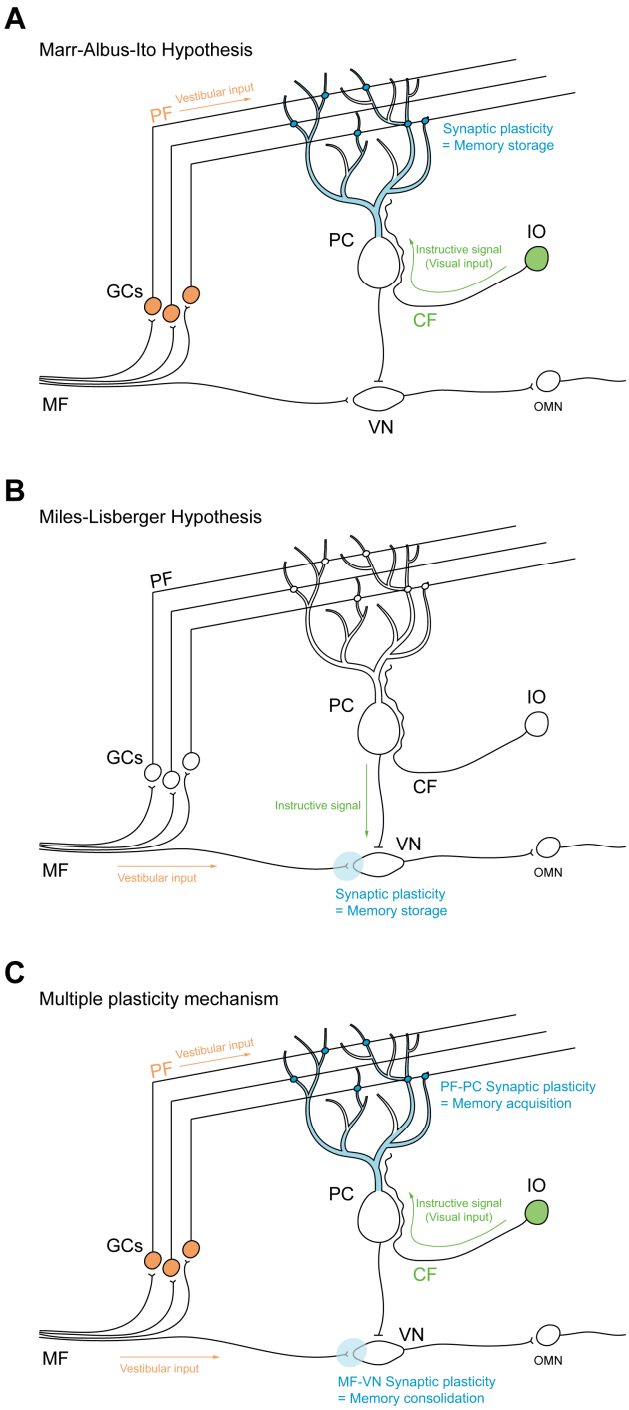


Figure 1.2. Neural circuit suggested by each hypothesis. (A) Marr-Albus-Ito hypothesis suggested that PF-PC plasticity is important for memory storage. (B) Miles-Lisberger hypothesis proposed that MF-VN plasticity as mechanism for memory storage. (C) Multiple plasticity mechanism reconcile both hypotheses above.

1.1.2. Multiple plasticity mechanism

Although both classical theories have been supported by numerous results, neither of models could completely explain the learning mechanism. To compensate the theories, the involvement of multiple-plasticity has been suggested (Hansel et al., 2001). Few years later, a theory named multiple plasticity mechanism reconciled both classic hypotheses about VOR adaptation, and suggested that interaction between two brain regions can account for acquisition and consolidation (Boyden et al., 2004). (Figure 1.2C). In this suggestion, VOR memory is initially acquired in the cerebellar cortex and transferred to the VN for consolidation. Most of PF-PC synaptic plasticity deficit mice models showed learning deficit, which means that these mice could not acquire novel information (De Zeeuw et al., 1998; Feil et al., 2003; Galliano et al., 2013; Hansel et al., 2006; Shutoh et al., 2003; 2002). Chemical inactivation of the flocculus after VOR training reveals that acquired memory must be transferred to the outside of the cerebellum for long-term memory (Kassardjian et al., 2005; Okamoto et al., 2011b). This hypothesis is strongly supported by the computational modeling studies (Clopath et al., 2014; Porrill and Dean, 2007; Yamazaki et al., 2015), which have suggested that single synaptic plasticity between the PF and the PC is not sufficient to account for the VOR learning and the memory process (Porrill and Dean, 2007). Including the plasticity in VN neurons enabled the model to reproduce previous experimental results (Clopath et al., 2014; Yamazaki et al., 2015). In addition to this, Gao et al., (2012) reviewed that several more types of synaptic plasticity in the cerebellar cortex are also considered to be important for motor learning. Since there are several cell-types, including GCs, Golgi cells, and molecular layer interneurons, synaptic plasticity between these-cells-types must also contribute to learning.

1.2. Plasticity in VOR circuits

1.2.1. Synaptic plasticity and intrinsic plasticity in the VOR circuit

Synaptic plasticity is regarded to be the principle mechanism of learning and memory

(Bliss and Collingridge, 1993; Kandel et al., 2014; Martin et al., 2000). As described above, hypotheses of motor learning are based on the premise that synaptic plasticity is the primary mechanism of learning and memory (Boyden et al., 2004; Gao et al., 2012; Ito, 1989; 1982; Miles and Lisberger, 1981). Therefore, those previous studies in the past were designed to verify the importance of synaptic plasticity in the circuit. Most VOR studies strongly supports the premise that synaptic plasticity is a critical component for successful learning and memory (De Zeeuw et al., 1998; Feil et al., 2003; Galliano et al., 2013; Gao et al., 2012; Hansel et al., 2006; Schonewille et al., 2010; Shutoh et al., 2002; Wulff et al., 2009). However, there are several controversial results which could not be understood by synaptic plasticity alone. For instance, some transgenic mice exhibited a memory deficit even though synaptic plasticity was successfully induced (Ryu et al., 2017; Wulff et al., 2009). In contrast, some transgenic mice showed normal learning and memory without PF synaptic plasticity (Schonewille et al., 2011). These conflicting results indicate that other components are necessary to fully understand motor learning.

Emerging evidence suggests that different types of plasticity, such as intrinsic plasticity, contributes to the memory process (Daoudal and Debanne, 2003; Shim et al., 2018; Zhang and Linden, 2003). The bi-directional intrinsic plasticity of PCs, which is accompanied by synaptic plasticity, has been described (Belmeguenai et al., 2010; Shim et al., 2017).

1.2.2. Excitability and intrinsic plasticity in the cerebellum-dependent motor learning

Excitability is an important factor in VOR adaptation and synaptic plasticity. In eye-blink conditioning, another type of cerebellum-dependent learning, it is well-known that the excitability of PCs contributes to learning and memory (Attwell et al., 2002; Cooke et al., 2004; Kellett et al., 2010). Similarly, the VOR training changes the activity of both PCs and VN neurons (Carcaud et al., 2017; Lisberger et al., 1994b; 1994a; E. Watanabe, 1985). Immediate shut-down of floccular cortical activity after training significantly affects learned memory (Kassardjian et al., 2005; Nagao, 1983;

Okamoto et al., 2011b; 2011a). Several transgenic mice with abnormal PC activity showed memory deficits (Ryu et al., 2017; Wulff et al., 2009). These results indicate that the activity of PCs is critical for acquired memory. However, although previous studies have described changes in the excitability of PCs and VN neurons after learning (Carcaud et al., 2017; Lisberger et al., 1994b; 1994a; E. Watanabe, 1985), basal excitability itself may not highly contribute to the VOR adaptation process (Ryu et al., 2017).

Rather than basal excitability of neurons, changes in intrinsic excitability, known as intrinsic plasticity, has been suggested to be one of the critical factors for successful learning and memory (Daoudal and Debanne, 2003; Zhang and Linden, 2003). According to previous studies, VOR memory is acquired in the cerebellar cortex through PF-PC synaptic plasticity and transferred to the VN for storage (Boyden et al., 2004; Ito, 2013). Based on this concept, there are several interesting points to focus on excitability and intrinsic plasticity. First, the PC is a sole output of the cerebellar cortex. With regard to transfer, the output of PCs should be considered for signal transduction to the VN. Second, the change in PC excitability could be a consequence and a cause of learning (Lisberger et al., 1994b; 1994a; E. Watanabe, 1985), because shut-down of PC after learning caused the loss of trained VOR memory (Kassardjian et al., 2005; Okamoto et al., 2011b; 2011a). Third, both cerebellar PCs and VN neurons exhibit bi-directional intrinsic plasticity (Belmeguenai et al., 2010; McElvain et al., 2010; Shim et al., 2017), and intrinsic plasticity was accompanied by synaptic plasticity, occurring in both PCs and VN neurons (Belmeguenai et al., 2010; Daoudal et al., 2002; Li et al., 2004; McElvain et al., 2010; Shim et al., 2017). Considering these points collectively, it is possible to expect that the intrinsic plasticity is induced together with synaptic plasticity in both sites during VOR adaptation. Moreover, given this expectation, the intrinsic plasticity was considered to be induced by the VOR adaptation (Carcaud et al., 2017), and mice exhibiting a deficit in PC intrinsic plasticity showed memory deficits (Ryu et al., 2017; Schonewille et al., 2010).

In this dissertation, I provide insight into the circuit mechanism through which the intrinsic plasticity of cerebellar PCs is required for long-term memory storage. I

found that knocking out stromal interaction molecule 1 (STIM1), which is one of endoplasmic reticulum (ER) membrane-bounded protein, in PC specifically ($STIM1^{PKO}$) causes severe VOR memory consolidation deficit. Using these mice as a memory consolidation deficit model, I investigated that the role of both synaptic and intrinsic plasticity during the VOR memory process. The VOR learning is concomitant with synaptic and intrinsic plasticity in the cerebellar PCs in the wild-type mice. Interestingly, the $STIM1^{PKO}$ mice showed deficient intrinsic plasticity, although synaptic plasticity was induced after learning. To investigate the impact of intrinsic plasticity within the motor learning circuits, I assessed neural activity in the VN neurons following the gain-increase learning. Firing rate potentiation was gradually developed over time, and excitatory synaptic transmission was also increased after learning. Notably, neither synaptic plasticity nor intrinsic plasticity of the VN neurons was observed in $STIM^{PKO}$ mice. This implies that the subsequent increases in the neural activity in the VN neurons may be derived from changes in the cortical output, as determined by the intrinsic plasticity of the cerebellar PCs.

2. Results

2.1. Behavior test in $STIM1^{PKO}$

2.1.1. Consolidation deficit was found in $STIM1^{PKO}$ mice

Before the learning, to verify that the mice have normal oculomotor performance, three different basal oculomotor responses were tested; optokinetic response (OKR), VOR in dark (dVOR), and VOR under light (lVOR) (Figure 2.1). $STIM1^{PKO}$ mice showed a similar level of wild-type in these parameters. These results indicate that $STIM1^{PKO}$ mice have normal visual and vestibular functions comparable to wild-type littermate (wild-type, n=67, $STIM1^{PKO}$, n=56).

Visual stimuli in the opposite direction of vestibular stimuli induce an increase of

VOR gain, which is the ratio of eye velocities to head velocities (gain-up learning; Figure 4.3A), whereas visual stimuli in the same direction of vestibular stimuli induce a decrease of VOR gain (gain-down learning; Figure 4.3B). Firstly, I applied both gain-up and -down protocol to two different genotypes (Figure 2.2). Both genotypes showed a successful increase and decrease during three times of learning sessions.

Acquired memory was fully retained until 1hr after learning, but, interestingly, $STIM1^{PKO}$ showed significant memory loss at 24hrs after learning (24Hr, Figure 2.2). These results indicate that $STIM1^{PKO}$ might have a deficit in common factor among the VOR learning circuit, since previous studies classify that gain-up and -down learning are based on different synaptic mechanism (Boyden et al., 2006).

To investigate whether observed long-term memory deficit in $STIM1^{PKO}$ continues through multiple day training, I trained mice for three days and checked the gain value (Figure 2.3). As I observed in 1 day of learning, $STIM1^{PKO}$ showed significant loss of acquired memory at 24hrs after learning. Interestingly, even though $STIM1^{PKO}$ started the second day learning from considerably low gain value, they caught up the level of wild-type littermate (Figure 2.3A). Learn and loss pattern in $STIM1^{PKO}$ continued to day 4. Furthermore, this pattern repeated even in different learning paradigm (Figure 2.3B). Next, since the adaptation of the VOR also occurs in the phase difference between the eye and head velocities as well as VOR gain, I adopted one more different learning protocol, which is phase reversal learning (Figure 4.3C) (Wulff et al., 2009). Through the phase reversal learning, I could verify whether the deficit in $STIM1^{PKO}$ is limited to gain value or not. Both groups were tested in phase reversal learning, which reduces the gain on day 1 and afterwards shifts the phase of VOR on day 2 and 3 (Figure 2.3C). The protocol of the first-day learning was similar to that of gain-down learning but with two more learning sessions. During the learning in day 1, both groups performed the gain-down learning equally well without differences in both gain and phase values, but after 24hrs, the level of memory consolidation in gain was significantly

different between two groups. These results confirmed the defect of STIM1^{PKO} mice, by showing that the same phenomenon occurs even in more intensive learning. The phase was shifted from the second day of learning, and memory consolidation deficiency of STIM1^{PKO} mice was also reproduced in the phase learning. On the third day of learning, the direction of learning in wild-type mice was changed from gain-down-bound to gain-up-bound, as previously reported (Wulff et al., 2009), since the phase of VOR was completely reversed as a consequence of accrued memory by repetitive phase shifting. However, STIM1^{PKO} mice were not able to change the direction of alterations in gain due to loss of the memory. Thus, since STIM1^{PKO} showed global consolidation deficit without learning defect, we could speculate that PC-specific STIM1 deletion may impair the common pathway for consolidation, such as the memory transfer process, in the VOR circuit.

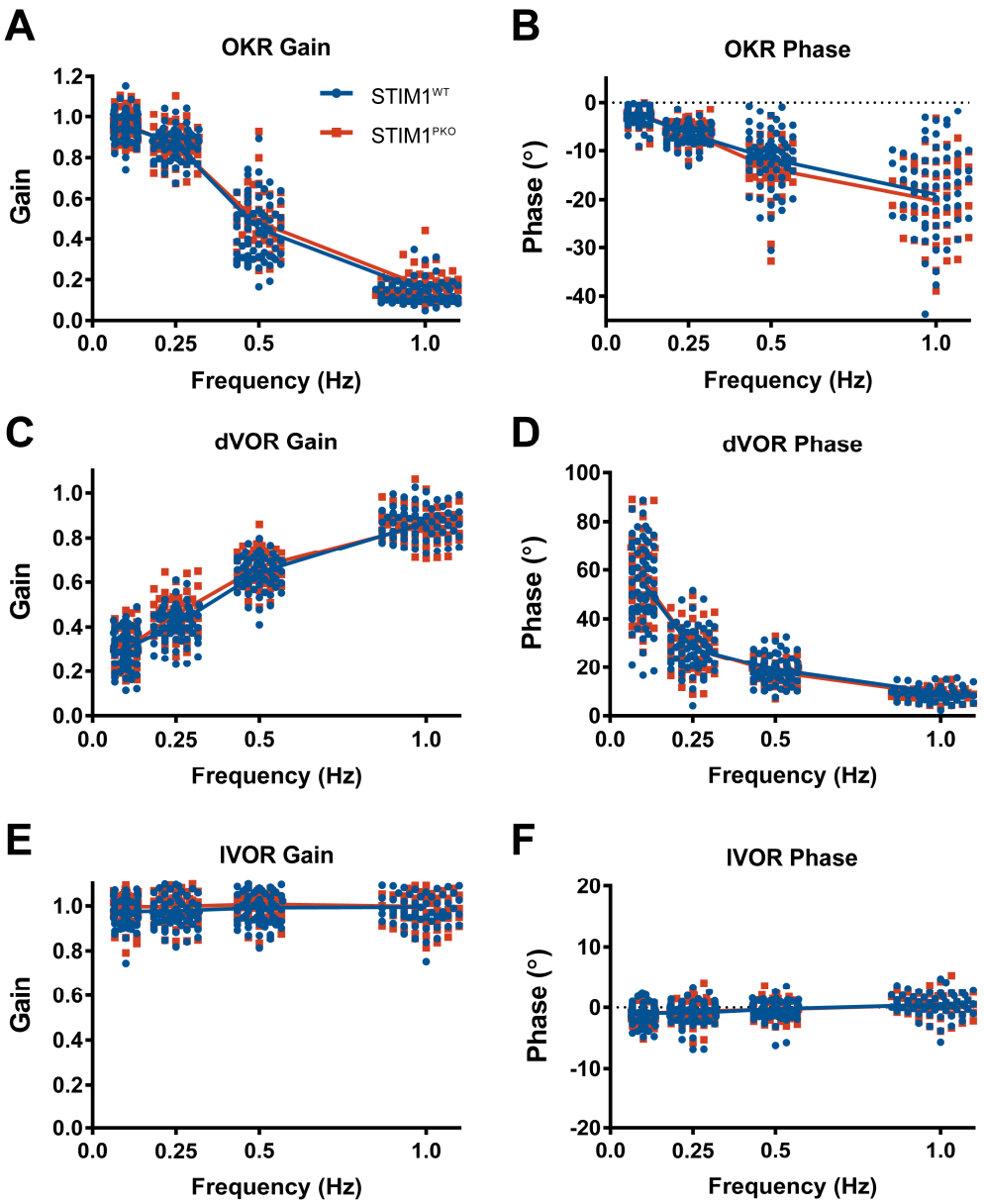


Figure 2.1. The basal ocular-motor performance of STIM1^{PKO} mice was not altered compared to wild-type littermates. (A) OKR responses in various frequency of drum rotating. OKR gain and phase values were not different between wild-type littermates (n=67) and STIM1^{PKO} (n=56). (B) VOR responses under dark (dVOR) in various frequency of drum rotating. dVOR gain and phase values were not different between wild-type littermates and STIM1^{PKO}. (C) VOR responses under light (lVOR) in various frequency of drum rotating. lVOR gain and phase values were not different between wild-type

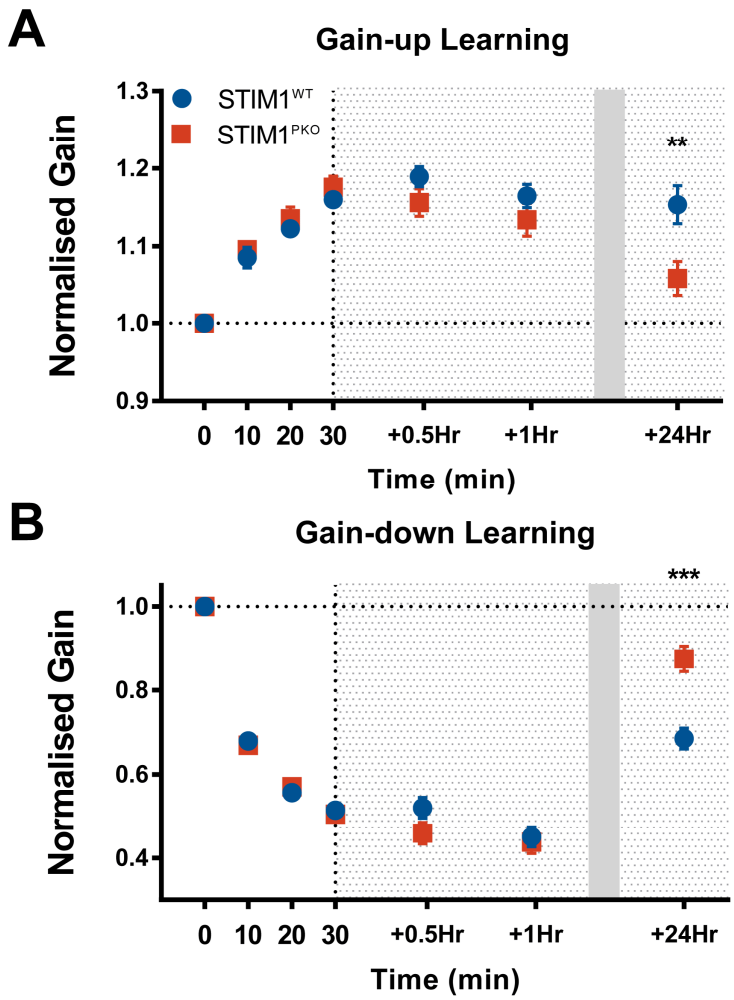


Figure 2.2. Long-term not short-term memory deficit showed in STIM1^{PKO}. (A) Gain-up learning in both genotypes. Both genotype showed successful learning during the training session (wild-type littermate, n=36; STIM1^{PKO}, n=39), and the memory was retained for 1hr after learning (wild-type littermate, n=22; STIM1^{PKO}, n=21). At 24hrs after learning, STIM1^{PKO} (n=17) showed significant reduction of gain value, whereas wild-type littermate (n=13) retained almost memory (p=0.002). (B) Gain-down learning in both genotypes. As gain-up training, both mice showed normal learning (wild-type littermate, n=24; STIM1^{PKO}, n=24), and retained memory until 1hr after (wild-type littermate, n=11; STIM1^{PKO}, n=10). At 24hrs after, gain of STIM1^{PKO} (n=14) significantly recovered as baseline, while wild-type littermates (n=13) retained reduced gain value (p<0.001). Two-way repeated measure ANOVA was used, and asterisks were marked by post-hoc Sidak's multiple comparison test. Error bars denote SEM. **p<0.01, ***p<0.001.

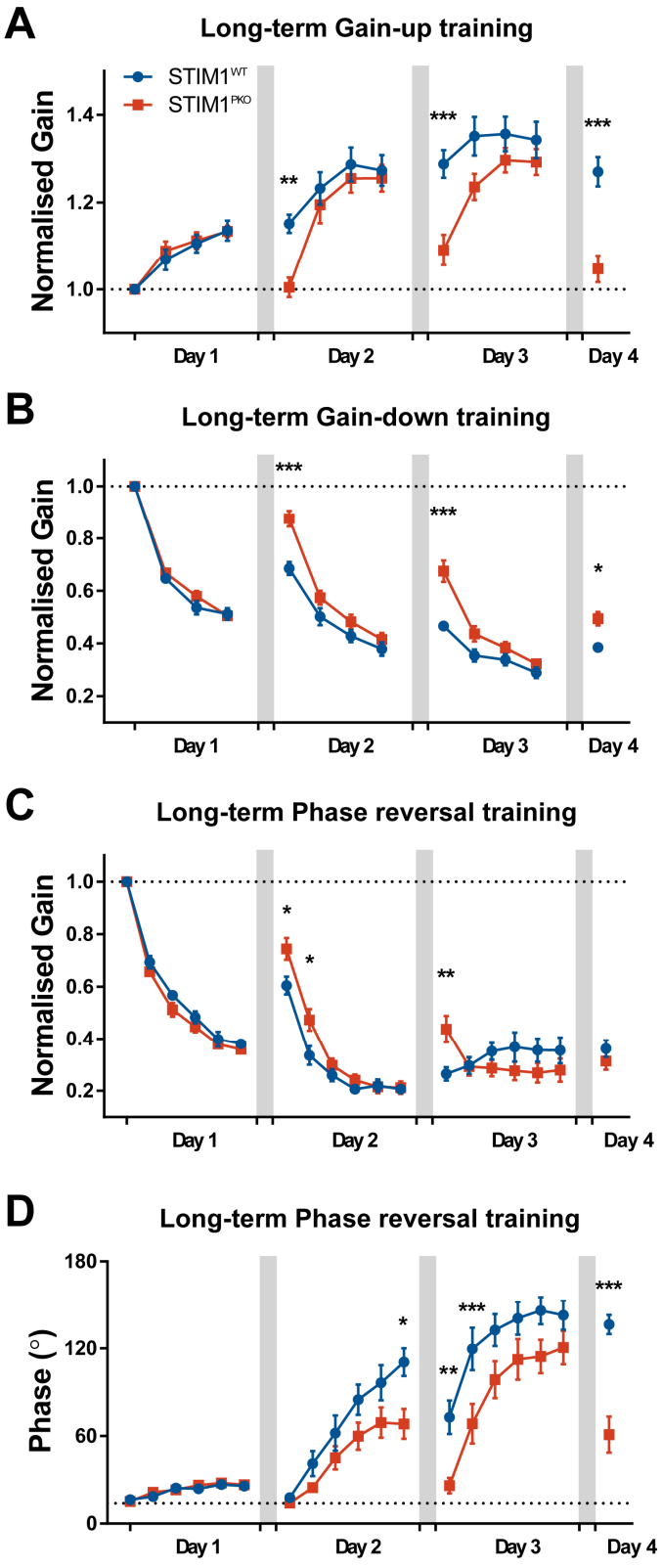

A
Long-term Gain-up training
STIM1WT
STIM1PKO
Normalised Gain
1.4
1.2
1.0
**
***
***
Day 1
Day 2
Day 3
Day 4
B
Long-term Gain-down training
Normalised Gain
1.0
0.8
0.6
0.4
0.2
***
***
*
Day 1
Day 2
Day 3
Day 4
C
Long-term Phase reversal training
Normalised Gain
1.0
0.8
0.6
0.4
0.2
*
*
**
Day 1
Day 2
Day 3
Day 4
D
Long-term Phase reversal training
Phase (°)
180
120
60
0
*
**
***
***
Day 1
Day 2
Day 3
Day 4

Figure 2.3. Extended learning protocol in both genotypes. (A) Long-term Gain-up learning. In day 1 training, both genotype successfully learned (wild-type littermate, n=11; $STIM1^{PKO}$, n=11). Initial point in day 2, $STIM1^{PKO}$ showed consolidation deficit ($p=0.009$), and the gap between wild-type and $STIM1^{PKO}$ was narrowed at the end of day 2 training. Gain values of $STIM1^{PKO}$ at the beginning of each day were significantly lower than wild-type littermates (Day 3, $p<0.001$; Day 4, $p<0.001$). (B) Long-term Gain-down learning. As gain-up training, gain of both genotypes was successfully trained (wild-type littermate, n=14; $STIM1^{PKO}$, n=14). Except the gain values at the beginning of each day (Day 2, $p<0.001$; Day 3, $p<0.001$; Day 4, $p=0.017$), there were no significant differences between wild-type and $STIM1^{PKO}$ in other points. (C) Gain values during long-term phase reversal learning (wild-type littermate n=11; $STIM1^{PKO}$ n=11). At the beginning of day 2 and 3, the gain of $STIM1^{PKO}$ mice was significantly higher than wild-type littermate (Day 2, $p=0.030$; Day 3, $p=0.002$). (D) Phase values during long-term phase reversal learning. There were no differences in the end of entire learning (Last point of the third day, $p=0.728$). Same as the gain graph, there were significant differences in the beginning point (Day 3, $p=0.003$; Day 4, $p<0.001$). Two-way repeated measure ANOVA was used, and asterisks were marked by post-hoc Sidak's multiple comparison test. Error bars denote SEM. *$p<0.05$, **$p<0.01$,

2.1.2. The consolidation deficit may occur between 1 to 4 hours after learning.

Accumulating evidence supports the theory that eye movement memory is firstly formed in the cerebellar cortex and is then transferred to the sub-cortical regions (Ito, 2013; Kassardjian et al., 2005; Matsuno et al., 2016; Okamoto et al., 2011a). This sequential memory acquisition within the cortical and sub-cortical areas has been implicated in long-term memory storage. Interestingly, (Okamoto et al., 2011a) observed that memory transfer occurs between 2.5 and 4hrs after learning in mice. This implies that this period of time is the critical period for communication between the cerebellar cortex and sub-cortical areas (in this circumstance, the VN). To determine whether VOR memory was attenuated in the $STIM1^{PKO}$ mice over the memory transfer period, I set one more checking point at 4hrs after learning. Thus, if 0.5 and 1hr two points are regarded as short-term period, and 24hrs as the long-term period, 4hrs could be understood as the mid-term period. Interestingly, at this mid-term period, the memory retention level was significantly lower in the

STIM1^{PKO} group than in the wild-type group (Figure 2.4A). This temporal alteration in the memory retention level was re-calculated as the ratio of the remaining memory at the test session to the acquired memory at the training session (Figure 2.4B). This revealed a showing gradual reduction in memory retention over the studied periods. Given that the slight decline in the level of memory retention began 1hr after the learning task and developed further, we speculated that the impaired motor memory consolidation that was observed in the STIM1^{PKO} mice was based on defect of the memory transfer process, leading to inappropriate communication between the cerebellar cortex and the VN.

2.2. *ex vivo* recordings from PCs after VOR learning.

2.2.1. Learning generally induces PF-PC synaptic plasticity in both groups

Many previous studies, using various LTD-deficient animal models, have reported that synaptic plasticity at the PF-PC synapse is strongly correlated with motor learning (Boyden et al., 2006; De Zeeuw et al., 1998; Hansel et al., 2006). Late-phase LTD has also been implicated in VOR memory consolidation (Ahn et al., 1999; Boyden et al., 2006). This suggests that a learning-induced long-lasting reduction of cerebellar cortical activity drives the transduction of memory to the sub-cortical region. Interestingly, because the time course of late-phase LTD is similar to that of the mid-term period of study, I firstly investigated whether PF-PC LTD is involved in memory consolidation. To verify this in detail, electrophysiological *ex vivo* recordings were made from floccular PCs to investigate the signs of synaptic plasticity at the short-, mid- and long-term periods after the learning task. This approach enabled me to monitor neuronal activity for periods of over an hour, overcoming the experimental limitation of the whole-cell patch clamp technique. Due to the location of microzone of the flocculus that regulates horizontal VOR behavior, all the recordings were made in the medial part of the flocculus (Schonewille et al., 2006).

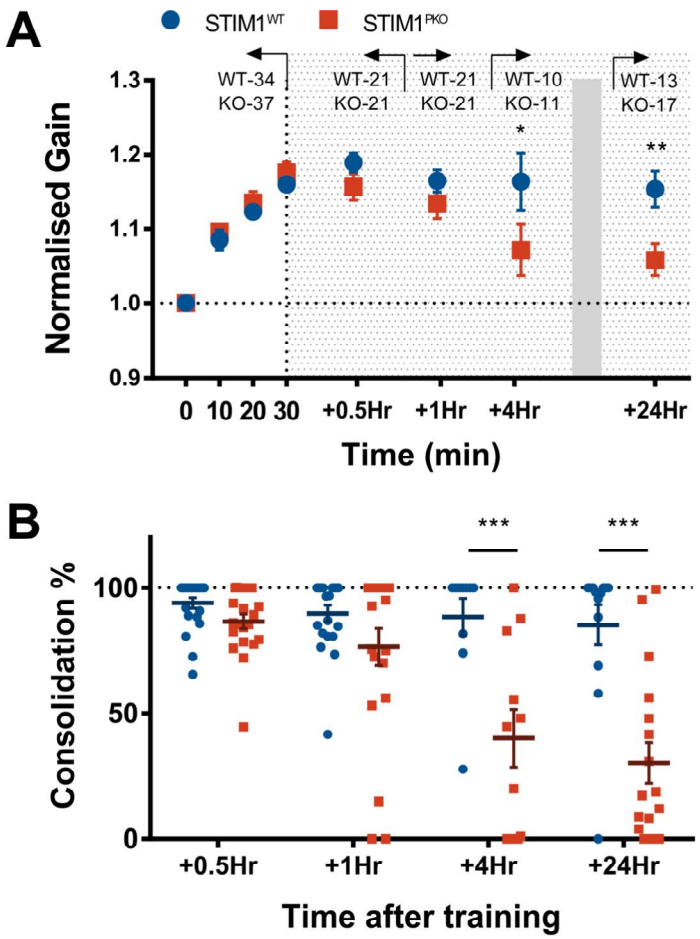


Figure 2.4. Long-term memory storage was impaired in STIM^{PKO} mice. (A) Normalized gain of the eye movement in learning. Note there is no significant differences between wild-type and STIM1^{PKO} mice in learning (points on white-colored background; wild-type n=36, STIM1^{PKO} n=39). We measured memory retention level at 0.5 and 1hr (described as short-term; wild-type n=21, STIM1^{PKO} n=21), 4hrs (mid-term; wild-type n=10, STIM1^{PKO} n=11) and 24hrs (long-term period; wild-type n=13, STIM1^{PKO} n=17) after training (points on grey-dotted background). STIM1^{PKO} showed significantly lower memory retention level from the mid-term period compared to the wild-type littermates (4hr, p=0.037; 24hr; p=0.004). (B) Calculated consolidation level. Memory retention level was obtained by calculating the ratio remained to learned memory (4hr, p <0.001; 24hr, p<0.001). Two-way repeated measure ANOVA was used for panel B, and asterisks in the graph were marked by post-hoc Sidak test for pairwise comparison. Unpaired *t*-test was used for panel C. Error bars denote SEM, *p<0.05, **p<0.01, ***p<0.001.

As a control group, I used sham animals that had undergone surgery and restraint without the learning task. Firstly, I measured the PF-stimuli-evoked synaptic response (eEPSC) following the injection of ranges of electrical stimuli intensities (Figure 2.6A-C). In these experiments, the amplitude of the eEPSC was decreased at the short- (1hr) and mid-term (4hr) period than sham group. However, this alteration was recovered, with the eEPSC returning to a level that was not significantly different to that of sham control before the long-term phase in both groups (Figure 2.6B-C). In addition to the PF-evoked synaptic events, the spontaneous excitatory postsynaptic current (sEPSC) was recorded after the learning task. Between the sham groups of both genotypes, there was no significant difference in the spontaneous glutamatergic synaptic transmission (Figure 2.5). The distribution of the inter-event intervals (IEIs) of synaptic events was shifted to the right after the learning task and was restored at the long-term period in both groups (Figure 2.6E-H). However, the mean frequency of sEPSC in the $STIM1^{PKO}$ mice was transiently reduced until 1hr after the learning task and was recovered 4hrs later, while in the change of wild-type littermates, the change was maintained throughout all of the time periods after the learning task (insets of Figure 2.6E-H). Conversely, the aspect of changes in the sEPSC amplitude in the $STIM1^{PKO}$ mice was comparable to that of the wild-type littermates (Figure 2.6F-I). In both groups, the distribution of the sEPSC amplitude was left-shifted after the learning task and maintained until the long-term period. Collectively, these results, as well as the results of a previous study (Boyden et al., 2006), indicate that VOR gain-up training elicited synaptic weakening of the PF-PC synapses. Although the alterations of sEPSC frequency that were observed in the $STIM1^{PKO}$ mice were relatively transient compared to the results observed in the wild-type group, overall, the aspects of plasticity that were induced by VOR learning were similar between the groups. Therefore, I suggest that the learning-induced PF-PC LTD presumably did not contribute to the long-term memory deficit observed in the $STIM1^{PKO}$ mice.

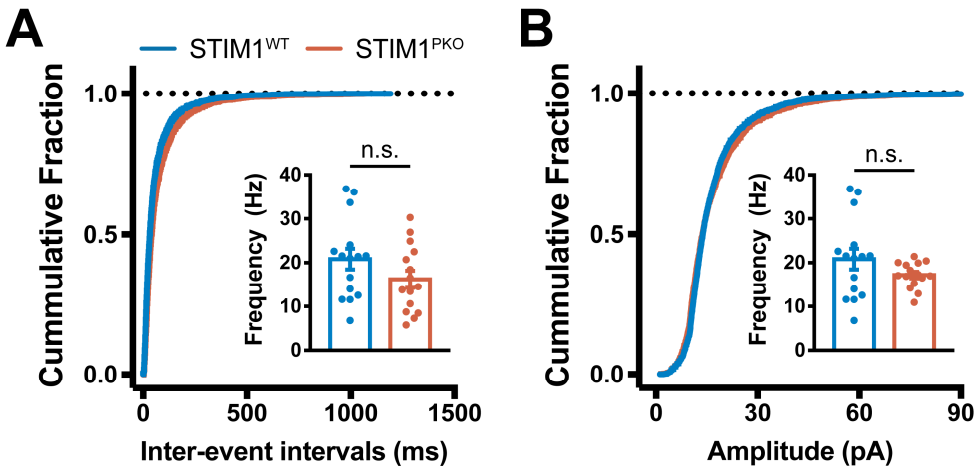


Figure 2.5. Synaptic transmission in STIM1^{WT} and STIM1^{PKO} was not significantly different. (A) Cumulative plots of IEI of sEPSC in wild-type (blue) and STIM1^{PKO} mice (red). The cumulative fraction of IEI and bar graph (inset) of sEPSC frequency indicated that frequency of sEPSC was not changed in STIM1^{PKO} compared to wild-type littermates (wild-type, n=15; STIM1^{PKO}, n=15, p=0.145). (B) Cumulative plots of amplitude of sEPSC. The cumulative fraction of amplitude and bar graph (inset) of sEPSC frequency indicated that amplitude of sEPSC was not changed in STIM1^{PKO} compared to wild-type littermates (p=0.587). Unpaired *t*-test was used for bar graphs. Error bar denotes SEM.

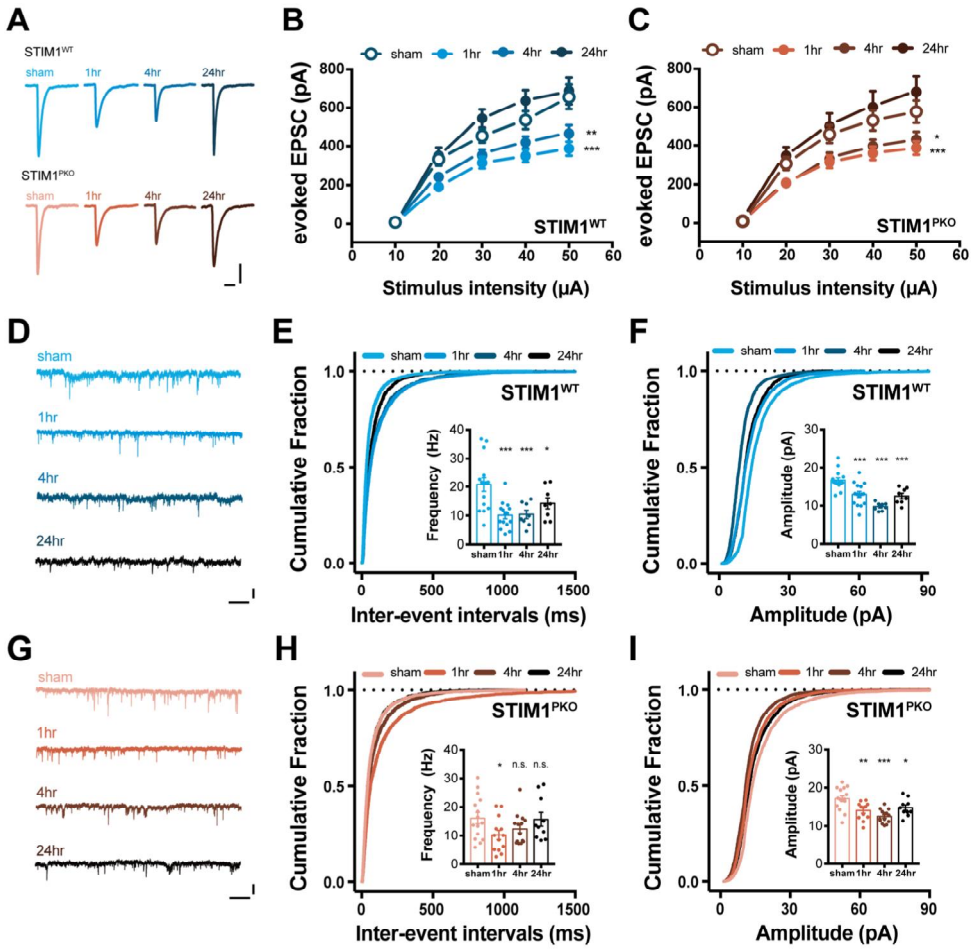


Figure 2.6. Long-term depression at the PF-PC synapses after gain-up learning. (A) Representative eEPSCs traces of both genotype groups in each time point. Scale bars, 200pA (vertical) and 30ms (horizontal). (B) Amplitude of eEPSC by serial PF stimulation in wild-type littermates. In comparison to sham group (n=9), the amplitude was significantly reduced at 1h and 4hrs after learning (at 50 μA injection; 1hr, n=19, $p<0.001$; 4hr, n=9, p=0.002), and depressed amplitude was recovered 24hrs after (at 50μA injection; 24hr, n=8, p=0.922). (C) Amplitude of eEPSC by serial PF stimulation in $STIM1^{PKO}$. Same as wild-type littermates, the amplitude was considerably decreased at 1 and 4hrs after, and restored at 24hrs after learning (at 50 μA injection; sham, n=14; 1hr, n=14, $p<0.001$; 4hr, n=14, p=0.013; 24hr, n=7, p=0.232). (D) Representative sEPSC traces of wild-type group in each time point. Scale bars, 25pA (vertical) and 1s (horizontal). (E) Cumulative plots of IEI of sEPSC in wild-type littermates. The cumulative plots of IEI of sEPSC in wild-type littermates. The cumulative fraction was right-shifted after learning, implying reduction of frequency (sham n=15, 1hr n=15, 4hr n=8, 24hr n=8).

Inset bar graph is mean frequencies of sEPSC indicating depression of frequency was maintained until 24hrs after learning in comparison to sham group (1hr, $p<0.001$; 4hr, $p<0.001$; 24hr, $p=0.029$). (F) Cumulative plots of amplitude of sEPSC in wild-type littermates. The cumulative fraction was left-shifted after learning, implying reduction of amplitude. Inset bar graph is mean amplitudes of sEPSC indicating that depression of amplitude was maintained for 24hrs after learning (1hr, $p <0.001$; 4hr, $p<0.001$; 24hr, $p<0.001$). (G) Representative sEPSC traces of $STIM^{PKO}$ group in each time point. Scale bars, 25pA (vertical) and 1s (horizontal). (H) Cumulative plots of IEI of sEPSC in $STIM^{PKO}$ mice. The cumulative fraction was right-shifted 1hr after learning, but most of changes returned to sham level from 4hrs after training (sham n=15, 1hr n=13, 4hr n=14, 24hr n=10). Inset shows summarizing bar graph of sEPSC frequency, implying that frequency of sEPSC was transiently depressed at 1hr and recovered from 4hrs after learning (1hr, $p <0.001$; 4hr, $p<0.001$; 24hr, $p<0.001$). (I) Cumulative plots of amplitude of sEPSC in $STIM^{PKO}$ mice. The cumulative fraction was left-shifted after learning. Inset bar graph shows that amplitude of sEPSC was depressed and maintained for 24hrs after learning (1hr, $p =0.001$; 4hr, $p<0.001$; 24hr, $p=0.016$). Two-way repeated measure ANOVA was used for panel A and B, and asterisks in the graph were marked by post-hoc Sidak test for pairwise comparison. One-way ANOVA with Fisher LSD test was used for insets in panel C, D, E and F. Asterisks in each time points were calculated by comparing to sham groups. Error bars denote SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

2.2.2. Learning-induced intrinsic plasticity shows relevance in memory consolidation

Experience-dependent neural plasticity includes not only synaptic plasticity but also alterations in the intrinsic excitability (Daoudal and Debanne, 2003; Zhang and Linden, 2003). Because PF-PC LTD was not sufficient to account memory consolidation, I investigated whether if intrinsic plasticity, the other form of neural plasticity, could be a considerable factor in memory consolidation. To investigate whether excitability changes of cerebellar PCs are required for memory consolidation, I firstly performed whole-cell patch clamp recordings to compare the long-term depression of intrinsic excitability (LTD-IE) in the $STIM1^{PKO}$ group and the wild-type littermates. A PF burst protocol (7 of 100 Hz PF burst followed by a single CF stimulation; (Shim et al., 2017)) was introduced to induce PC

synaptic and intrinsic plasticity in the presence of an inhibitory synaptic transmission inhibitor, picrotoxin. As shown in Figure 2.6, both $STIM1^{PKO}$ and the wild-type groups showed normal induction of PF-PC LTD (Figure 2.7A). In intrinsic plasticity, while the wild-type showed the long-lasting decrease of excitability (>55 min), $STIM1^{PKO}$ showed a transient reduction of excitability only in 20 min after induction protocol, and rapidly recovered 40 min after (Figure 2.7B). These intrinsic plasticity defects were not limited to LTD-IE but also observed in LTP-IE (Figure 2.7C). Given the notion that the intrinsic plasticity of cerebellar PCs amplifies the modification of synaptic weight to properly project the learned signal from the PCs to their relay neurons, our observation may imply that the impairment of intrinsic plasticity results in dysfunctional memory transfer to the VN neurons. Because intrinsic plasticity was either not actually induced or restored 40 min after plasticity induction in the $STIM1^{PKO}$ group (Figure 2.7B-C), I expect that the learning-induced intrinsic plasticity may be already abolished within 1hr after. Taken together, we conclude that the intrinsic plasticity of the cerebellar PCs would be involved in the long-lasting reduction of the cerebellar cortical activity, and thereby, may contribute to VOR memory consolidation.

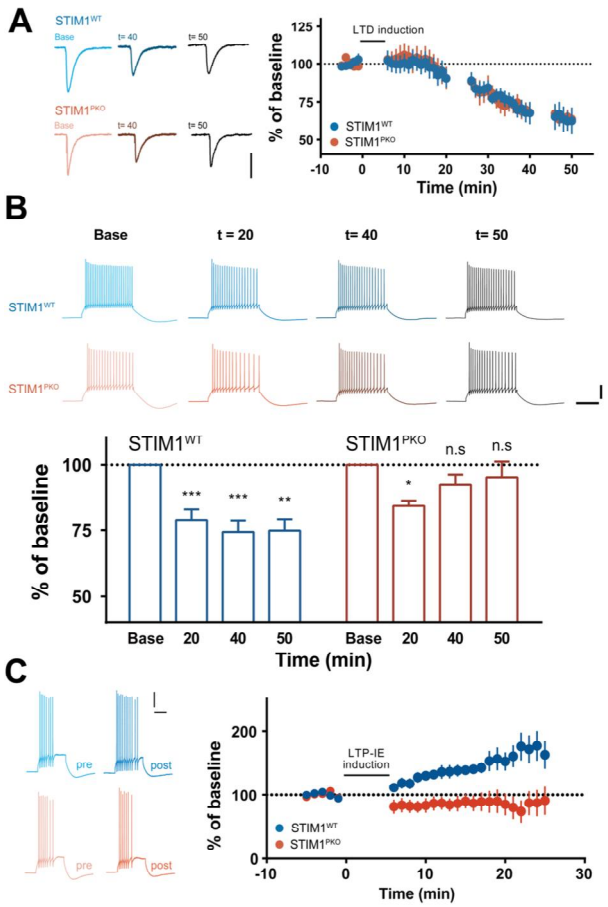


Figure 2.7. Not synaptic but intrinsic plasticity was impaired in STIM1^{PKO}. (A) Induction of synaptic LTD. Plots of the normalized EPSC change after application of LTD induction protocol in wild-type littermates (n=8, n=4 after 40min, blue) and STIM1^{PKO} mice (n=7, n=6 after 40min, red). Synaptic plasticity was normally induced in STIM1^{PKO} mice. Scale bar, 200pA. (B) LTD-IE during the synaptic LTD. Bar graphs showed the comparison of excitability changes after LTD induction. In STIM1^{PKO} mice, down-regulation of excitability in PCs was shown 20 min after induction (n=7, p=0.010), however, fully recovered 40 min and 50 min after induction (40 min, n=7, p=0.331; 50 min, n=6, p=0.748). On the contrary, the intrinsic plasticity was induced and slightly further developed in time from wild- type littermates (20 min, n=8, p<0.001; 40 min, n=8, p<0.001; 50 min, n=4, p=0.001). Scale bars (upper), 20mV (vertical) and 200ms (horizontal). (C) Induction of LTP-IE. Introducing a tetanus stimulation for LTP-IE causes gradual increase of excitability in wild-type littermates, but no changes were found in STIM1^{PKO}. Scale bars (upper), 20mV (vertical) and 100ms (horizontal). One-way repeated measure ANOVA with post hoc Sidak correction was used for panel B. Error bars denote

Next, I examined the temporal alteration of PC excitability through *ex vivo* recordings after the learning task; at short-, mid- and long-term time periods. In agreement with the results of *in vitro* experiments ((Shim et al., 2017); Figure 2.7A), the firing frequency was decreased 1hr after training in the wild-type littermates (Figure 2.8A-B). The AP firing frequency of PCs was measured in current clamp mode through the injection of brief current steps from the membrane potential of approximately -70 mV (500 ms, from +100 pA to +500 pA with an increment of 100 pA, step interval 4.5 s). The learning-induced intrinsic plasticity was partially recovered at the mid-term time period and fully recovered to the value from the sham control at the long-term period. However, the $STIM1^{PKO}$ group showed a deficiency in the learning-induced intrinsic plasticity throughout the studied periods (Figure 2.8C-D). Comparing the results from the different genotypes over the same time period, wild-type littermates has significantly higher firing frequency in sham control, but the frequency reversed in the short-term period and gradually recovered over the time period studied (Figure 2.9A-D). Given the notion that intrinsic training. Taken together, I conclude that the intrinsic plasticity of the cerebellar PCs would be involved in the long-lasting reduction of the cerebellar cortical activity, and thereby, may contribute to VOR memory consolidation.

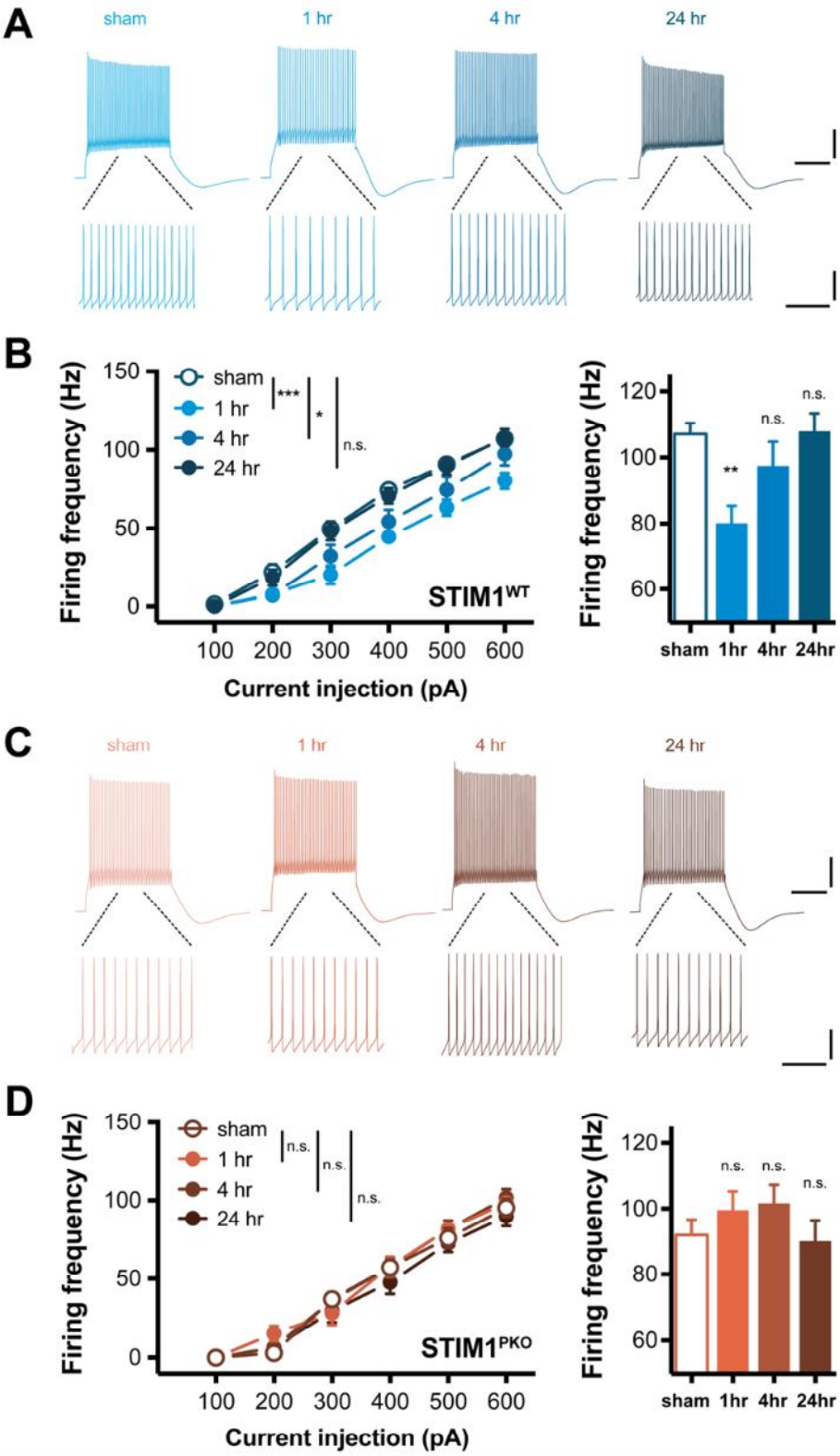

A
sham
1 hr
4 hr
24 hr
B
Firing frequency (Hz)
sham
1 hr
4 hr
24 hr
***
*
n.s.
STIM1WT
Current injection (pA)
**
n.s.
n.s.
sham 1hr 4hr 24hr
C
D
STIM1PKO

Figure 2.8. VOR learning also induces plastic change of intrinsic excitability in PCs. (A) Representative traces from whole-cell recording in wild-type group. Scale bars (upper), 20mV (vertical) and 200ms (horizontal). Scale bars (lower), 20mV (vertical) and 50ms (horizontal). (B) PC excitability of wild-type littermates over several time points. VOR learning decreased gain responses of the cerebellar PCs in response to square-wised current injection ranging from 100 pA to 600 pA for 500 ms (sham vs 1hr, $p<0.001$; sham vs 4hr, $p=0.024$; sham vs 24hr, $p=0.717$, left; sham, n=20; 1hr, n=11; 4hr, n=16; 24hr, n=20). Excitability in 600 pA injection was significantly decreased at short-term (1hr, $p=0.002$) and mostly recovered at mid-term (4hr, $p=0.196$) and fully recovered at long-term (24hr, $p=0.914$). (C) Representative traces from whole-cell recording in $STIM1^{PKO}$ group. Scale bars (upper), 20mV (vertical) and 200ms (horizontal). Scale bars (lower), 20mV (vertical) and 50ms (horizontal). (D) Excitability of PC in $STIM1^{PKO}$. Different from wild-type littermates, $STIM1^{PKO}$ showed no alteration of excitability after learning (sham vs 1hr, $p=370$; sham vs 4hr, $p=0.343$; sham vs 24hr, $p=0.768$, left; sham, n=17; 1hr, n=13; 4hr, n=17; 24hr, n=13). There were no significant changes in 600 pA injection (1hr, p=0.371.; 4hr, $p=0.184$; 24hr, $p=0.814$). Two-way repeated measure ANOVA was used for injected current-frequency graphs. One-way ANOVA with Fisher LSD test was used for bar graphs. Asterisks in each time points were calculated by comparing to sham groups. Error bars denote SEM. **$p<0.01$.

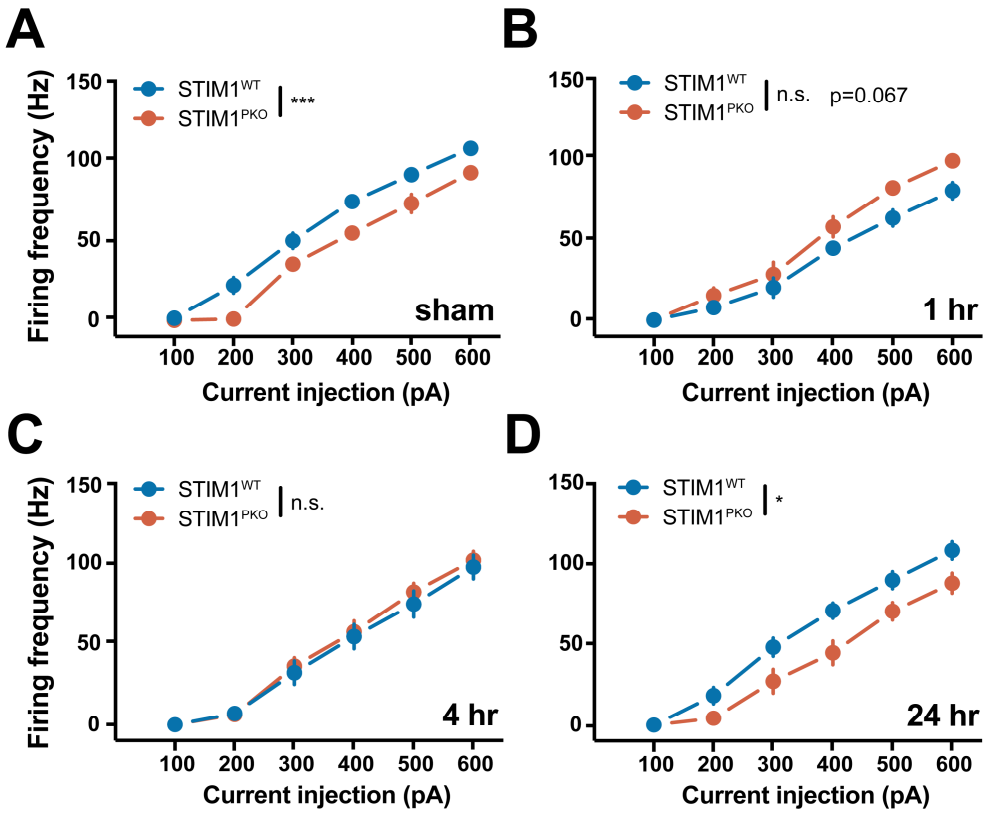


Figure 2.9. Comparing PC excitability of wild-type littermates and STIM1^{PKO} mice in each time points. (A) STIM1^{PKO} group (red, n=17) showed significantly lower excitability than wild-type littermates (blue, n=20, $p<0.001$). (B) While the excitability of STIM1^{PKO} (n=13) was unchanged, excitability of wild-type littermates (n=11) was plunged ($p=0.067$). (C) At 4hrs after learning, the altered excitability of wild-type littermates (n=16) partly restored, and overlapped to excitability of STIM1^{PKO} (n=17), which was still unchanged ($p=0.644$). (D) As the excitability of wild-type littermates (n=20) was fully recovered, significant difference from STIM1^{PKO} group (n=13) was also restored ($p=0.022$). Two-way repeated measure ANOVA was used for panels. Error bars denote SEM. *$p<0.05$, ***$p<0.001$.

2.3. Computational analysis of AP properties in PCs.

Changes of action potential active properties from PCs connote learning.

The absence of PC intrinsic plasticity seems strong candidate of consolidation deficit in STIM1^{PKO}. Even though small-conductance Ca^{2+}-activated K^{+} channel (SK channel) is one of the known candidates for intrinsic plasticity (Belmeguenai et al., 2010), the cellular mechanism of this plasticity is remaining ambiguous. Using intrinsic plasticity deficit mouse model is a huge advantage to investigate the underlying mechanism. I analysed ten active properties from the action potentials (APs) of both genotypes, including spike number, actual frequency (Hz), first spike latency, medium after hyperpolarization (mAHP; mV), fast after hyperpolarization (fAHP; mV), Voltage threshold (mV), Full spike width at half maximum (FWHM; ms), Action potential amplitude of AP (mV), Ratio 1st to last AP, Instantaneous frequency (Hz). To address the changes in the properties from the sham group, I adopted Euclidian distance in 10-dimensional space. Firstly, data at each time point was normalized by the mean values of sham group. Because the data is scattered in the space, I calculated the center of the cluster and then estimated the Euclidean distance between these centers. In wild-type littermate, distances to 1hr after learning group were especially far from sham group than other points (Figure 2.10A). Interestingly, 1hr group is not only far from sham group but also from 4hrs and 24hrs group. Except 1hr group, distances between other 3 groups were much closer than the distances from 1hr group. Different from wild-type littermate, there was no considerable change from 1hr group in STIM1^{PKO}, which indicates that no much alteration happened in cellular scale at this time point (Figure 2.10B). Rather than 1hr, distances from 4hr after learning to other time points were far, but these were not much as wild-type littermate. From these results, I could speculate that

intrinsic plasticity alters many active properties of APs, and especially in gain-up learning, the alteration peaks at 1hr after training.

Measuring Mahalanobis distance or using support vector machine could give another information from Euclidean distance. For further investigation to find the most important channel or property related to learning-induced intrinsic plasticity, several machine learning methods could be applied to the obtained data sets.

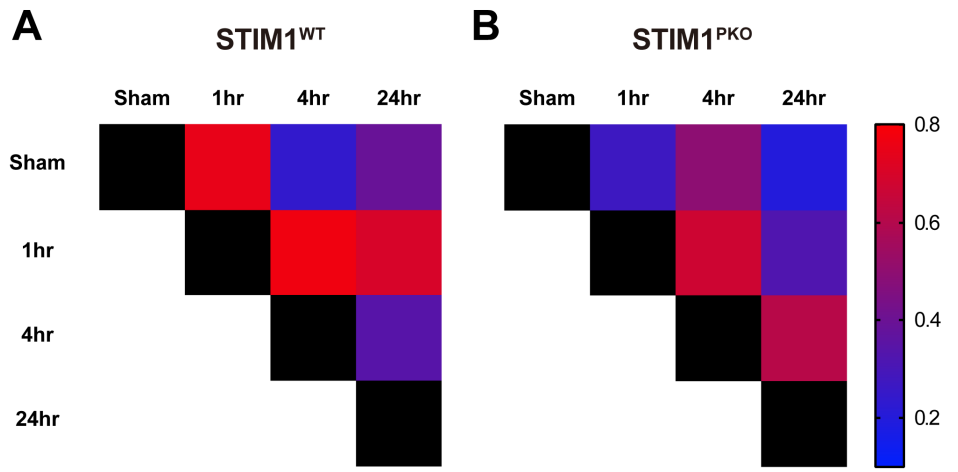


Figure 2.10. Euclidean distance from sham group to each time points within genotype. (A) Euclidean distance between time points in wild-type littermate. The distance to 1hr after group is far from other groups (1hr vs sham, 0.748; 1hr vs 4hr, 0.771; 1hr vs 24hr 0.700). Distance between other groups are relatively closer than distance from 1hr group (sham vs 4hr, 0.239; sham vs 24hr, 0.391; 4hr vs 24hr, 0.341). (B) Euclidean distance between time points in $STIM1^{PKO}$. Distances from sham group were not much far as wild-type littermate (sham vs 1hr, 0.270; sham vs 4hr, 0.493; sham vs 24hr, 0.197). Distance between 4hr groups and other groups are relatively far (1hr vs 4hr, 0.673; 4hr vs 24hr, 0.611). The distance between 1hr and 24hrs was 0.322.

Representatively, random forest or gradient-boosted tree give us the most influence factor for classification.

2.4. *ex vivo* recordings from VN neurons after VOR learning.

Appropriate synaptic and intrinsic plasticity of VN neurons require intrinsic plasticity of cerebellar PC

I observed an impairment of intrinsic plasticity in the cerebellar PCs of the

STIM1^{PKO} group through *in vitro* and *ex vivo* recordings. This suggests that memory consolidation requires the transduction of a memory from the cerebellar cortex into the sub-cortical area through the intrinsic plasticity of PCs. A large population of VN neurons receive information from floccular PCs (Matsuno et al., 2016; Shin et al., 2011). Furthermore, the output of the cerebellar cortex serves as an instructive signal in the control the aspect of neuronal plasticity between mossy fibers (MFs) and VN neurons (Clopath et al., 2014; Dean et al., 2010; McElvain et al., 2010; Medina, 2010; Porrill and Dean, 2007; Shutoh et al., 2006; Yamazaki et al., 2015). Thus, I hypothesized that the impairment of the intrinsic plasticity of the cerebellar cortex that was observed in STIM1^{PKO} mice would lead to an inadequate alteration of the VN neuron activity following VOR learning. Because major excitatory inputs to VN is from MFs, I blocked all inhibitory inputs through picrotoxin and strychnine to measure the synaptic strength between the MFs and the VN. Under this condition, I performed *ex vivo* recordings to investigate spontaneous synaptic transmission after the learning task during three distinct time periods, short-, mid- and long-term by *ex vivo* recordings. In the sham groups, the frequency of sEPSC in the STIM1^{PKO} group showed remarkable augmentation compared to the wild-type group (Figure 2.11A). However, the sEPSC amplitude was not significantly different between the STIM1^{PKO} group and wild-type group (Figure 2.11B). These results imply that the homeostatic scaling in the VN neurons is due to the reduction of PC excitability in the STIM1^{PKO} group (Figure 2.14A). Intriguingly, synaptic transmission was found to be potentiated after VOR learning in the wild-type littermates throughout the periods of study (Figure 2.12A-C). Although the increase in the mean frequency of sEPSC after training seemed to be restored at the long-term period, the cumulative distribution of the IEIs was found to be left-shifted throughout the periods of study (Figure 2.12B). The cumulative fraction of the sEPSC amplitude was especially right shifted at the long-term period, indicating that the proportion of increased glutamatergic synaptic events was enhanced during this period (Figure 2.12C). However, the mean value was not

significantly altered compared to that of the sham control (Figure 4B, inset). These results indicate that VOR gain-up learning induces LTP at the MF-VN synapse, in line with the previous expectation (Boyden et al., 2006). In contrast to the results presented from the wild-type littermates, the $STIM1^{PKO}$ group showed a slight depression of sEPSC frequency in cumulative distribution in the short- and mid-term time periods that continuously recovered to baseline (Figure 2.12E). However, the mean frequency was not significantly altered among the periods of study (Figure 2.12E, inset). The amplitude of sEPSC was slightly left-shifted in the short- and mid-term time periods, and the mean amplitude in the mid-term time period was significantly lower than that of the sham group (Figure 2.12F). There is a limitation to estimate the synaptic plasticity through sEPSC recordings, but this synaptic events also partially reflect the change of synaptic strength. Since the change of sEPSC has been shown only in wild-type littermate, I could interpret

there was no synaptic changes found in STIM1^{PKO}. In light of previous reports, which have suggested that cerebellar PC activity contribute to MF-VN plasticity (Dean et al., 2010; Matsuno et al., 2016; McElvain et al., 2010; Medina, 2010), I speculated that the synaptic plasticity at the MF-VN synapse is inappropriately induced in the STIM1^{PKO} group due to the absence of PC intrinsic plasticity.

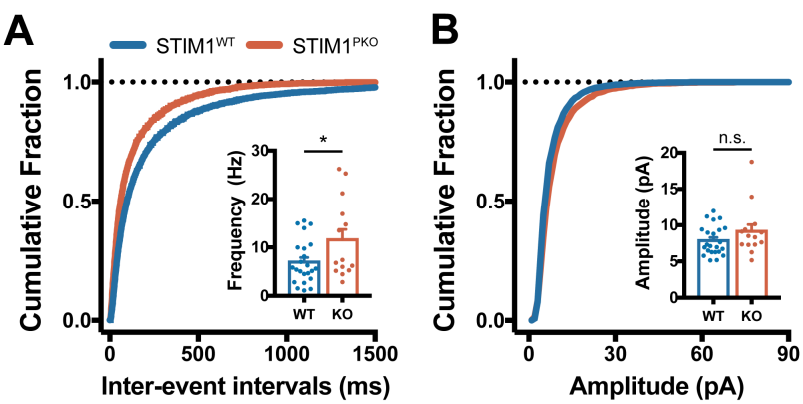


Figure 2.11. Basal synaptic transmission in VN neurons. (A) Frequency of synaptic transmission in VN neurons from wild-type littermates (n=23, blue) and STIM1^{PKO} mice (n=14, red). The cumulative fraction of IEI and bar graph (inset) of sEPSC frequency indicated that frequency of sEPSC was higher in STIM1^{PKO} compared to wild-type littermates (p=0.032). (B) Amplitude of sEPSC in VN neurons from wild-type littermates (blue) and STIM1^{PKO} mice (red). The cumulative fraction of amplitude and bar graph (inset) of sEPSC frequency indicated that amplitude of sEPSC was not changed in STIM1^{PKO} compared to wild-type littermates (p=0.161). Unpaired *t*-test was used for bar graphs in panel A and B. Error bars denote SEM. *p<0.05.

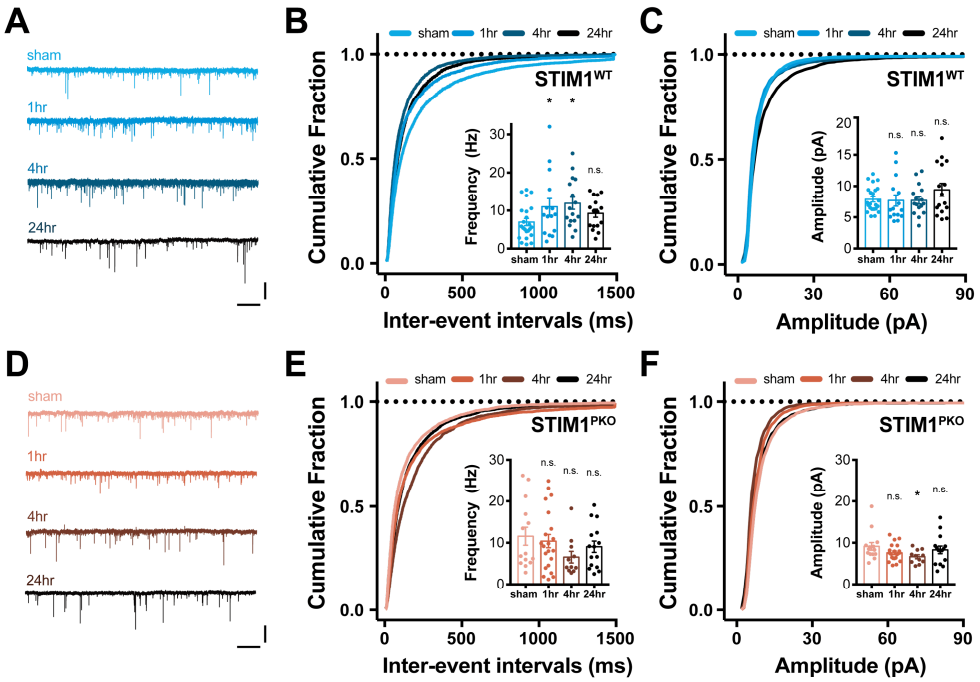
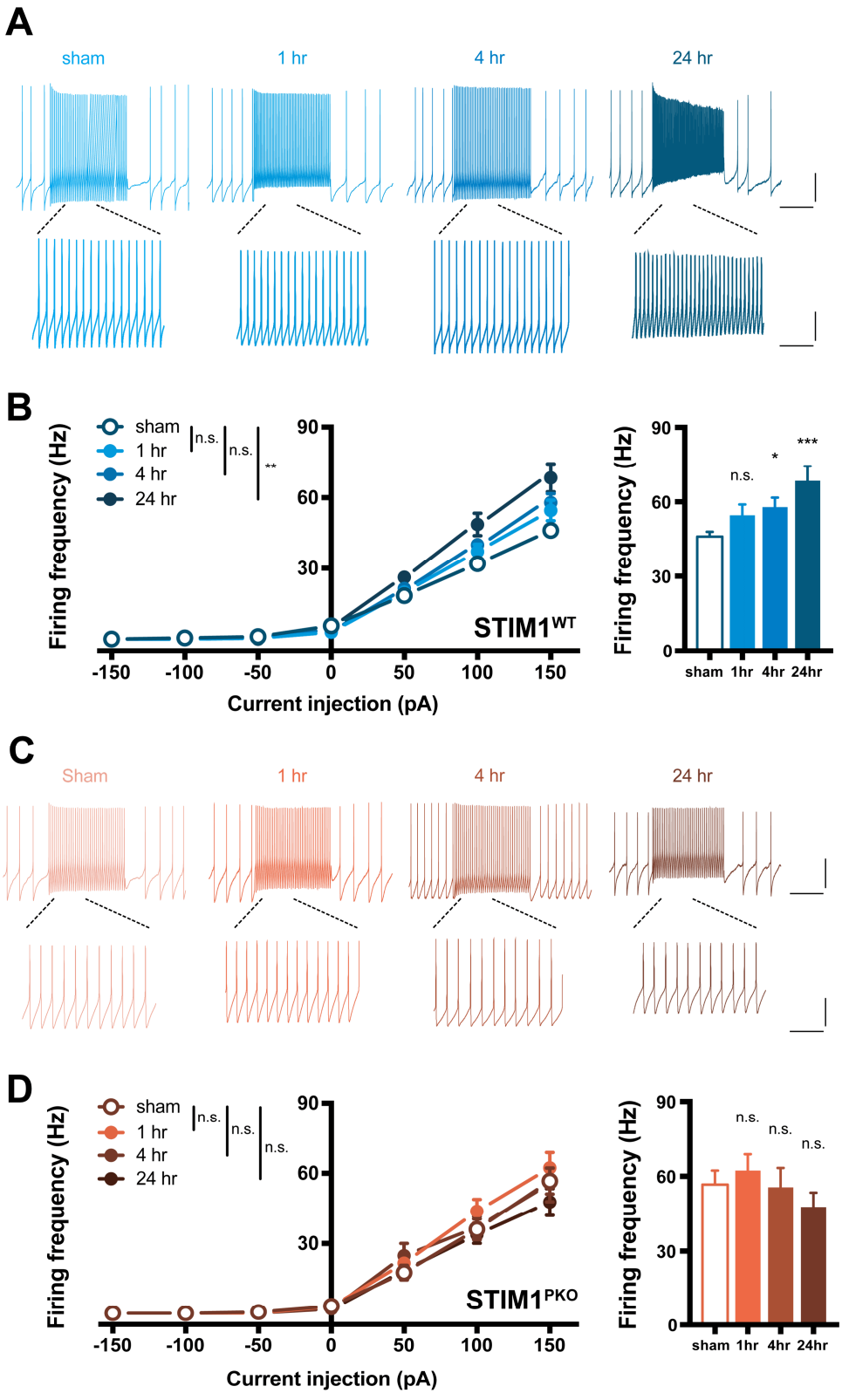


Figure 2.12. VOR gain-up learning induced long-term depression of the excitatory input in VN neurons. (A) Representative sEPSC traces of wild-type group in each time point. Scale bars, 25pA (vertical) and 1s (horizontal). (B) IEI of sEPSC in wild-type mice. The cumulative distributions of IEI were left-shifted after learning (sham, n=23; 1hr, n=15; 4hr, n=16; 24hr, n=16). The frequency of sEPSC was potentiated at short- (1hr) and mid-term (4hr) after training (inset bar graph; 1hr, p=0.046; 4hr, p=0.013; 24hr, p=0.242). (C) Amplitude of sEPSC in wild-type mice. The cumulative distribution was right-shifted at 24hrs after learning. There was trend of potentiation at long-term (24hr) after training, but overall, the amplitude of sEPSC was not significantly affected by learning (inset bar graph; 1hr, p=0.850; 4hr, p=0.874; 24hr, p=0.122). (D) Representative sEPSC traces of $STIM1^{PKO}$ group in each time point. Scale bars, 25pA (vertical) and 1s (horizontal). (E) IEI of sEPSC in $STIM^{PKO}$ mice. In contrast to wild-type littermates, the cumulative distribution was slightly right-shifted after learning (sham, n=14; 1hr, n=21; 4hr, n=111; 24hr, n=15). The mean frequency of sEPSC was not significantly altered after learning (inset bar graph; 1hr, p=0.613; 4hr, p=0.066; 24hr, p=0.314). (F) Cumulative plots of amplitude of sEPSC in $STIM^{PKO}$ mice. The cumulative distribution was slightly left-shifted at 4hrs after learning. The amplitude of sEPSC was significantly reduced at mid-term (4hr) after learning (inset bar graph; 1hr, p=0.111; 4hr, p=0.030; 24hr, p=0.420). One-way ANOVA with Fisher LSD test was used for bar graphs in all panels. Asterisks in each time points were calculated by comparing to sham groups. Error bars denote SEM. *p<0.01

Furthermore, I asked whether the intrinsic plasticity of cerebellar PCs is also required for the adequate induction of intrinsic plasticity in the VN neurons, because VOR training involves a change in the excitability as well as synaptic transmission (Carcaud et al., 2017; Shutoh et al., 2006). To answer this, the gain responses were measured through the injection of square-wised somatic depolarizing current into the VN neurons at the three time periods after the learning task. The VN neurons of the STIM1^{PKO} group showed higher firing frequency in response to the current injection than were observed in the wild-type littermates in the sham group (Figure 2.14A). Interestingly, VOR training elicited the intrinsic plasticity of the VN neurons in the wild-type littermates (Figure 2.13A-B), whereas, there was no alteration of the gain responses in the STIM1^{PKO} group (Figure 2.13C-D). The excitability of the MVN neurons gradually increased over the studied time periods and the intrinsic plasticity was maintained 24hrs after training (Figure 2.13B, right). The difference between the sham groups of both genotypes was faded during the short- and the mid-term time period, and finally reversed with statistical significance at the long-term time period (Figure 2.14). To clarify whether the neural plasticity in the VN neurons is affected by knockout of STIM1 in the PC, we delivered the conventional protocol for the induction of LTP in VN neurons. The VN neurons from the wild-type and STIM1^{PKO} groups exerted potentiation of the synaptic weight and excitability (Figure 2.15). Taken together with modification of synaptic weight and intrinsic properties in VN neurons, I suggest that the intrinsic plasticity of the cerebellar PCs following VOR learning could enable to induction of the proper forms of neuronal plasticity in VN neurons, corresponding to the specific behavior, such as consolidation of memory.


A
sham
1 hr
4 hr
24 hr
B
sham
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n.s.
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**
Firing frequency (Hz)
90
60
30
STIM1WT
-150
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Current injection (pA)
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Firing frequency (Hz)
STIM1PKO
Current injection (pA)
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Figure 2.13. VOR gain-up learning induced potentiation of intrinsic excitability in VN neurons. (A) Representative traces from whole-cell recordings of wild-type group in each time point. Scale bars (upper), 20mV (vertical) and 500ms (horizontal). Scale bars (lower), 20mV (vertical) and 125ms (horizontal). (B) The excitability of VN neurons in wild-type littermates. VOR learning significantly potentiated gain responses of the VN neurons in response to square-wised current injection ranging from -150 pA to 150 pA for 1 s (sham vs 1hr, $p=0.319$; sham vs 4hr, $p=0.072$; sham vs 24hr, $p=0.002$, left; sham, n=23; 1hr, n=16; 4hr, n=38; 24hr, n=19). Excitability in 150 pA injection was significantly increased at mid- (4hr, $p=0.032$) and long-term (24hr, $p<0.001$) after training. (C) Representative traces from whole-cell recordings of wild-type group in each time point. Scale bars (upper), 20mV (vertical) and 500ms (horizontal). Scale bars (lower), 20mV (vertical) and 125ms (horizontal). (D) Excitability of VN neurons in $STIM1^{PKO}$. There was no alteration of excitability after learning (sham vs 1hr, $p=0.422$; sham vs 4hr, $p=0.801$; sham vs 24hr, $p=0.493$, left; sham, n=17; 1hr, n=25; 4hr, n=15; 24hr, n=15), and no significant changes in 150 pA injection as well (1hr, $p=0.530$.; 4hr, $p=0.908$; 24hr, $p=0.371$) Two-way repeated measure ANOVA was used for injected current-frequency graphs. One-way ANOVA with Fisher LSD test was used for bar graphs. Asterisks in each time points were calculated by comparing to sham groups. Error bars denote SEM. $*p<0.05$ $***p<0.001$.

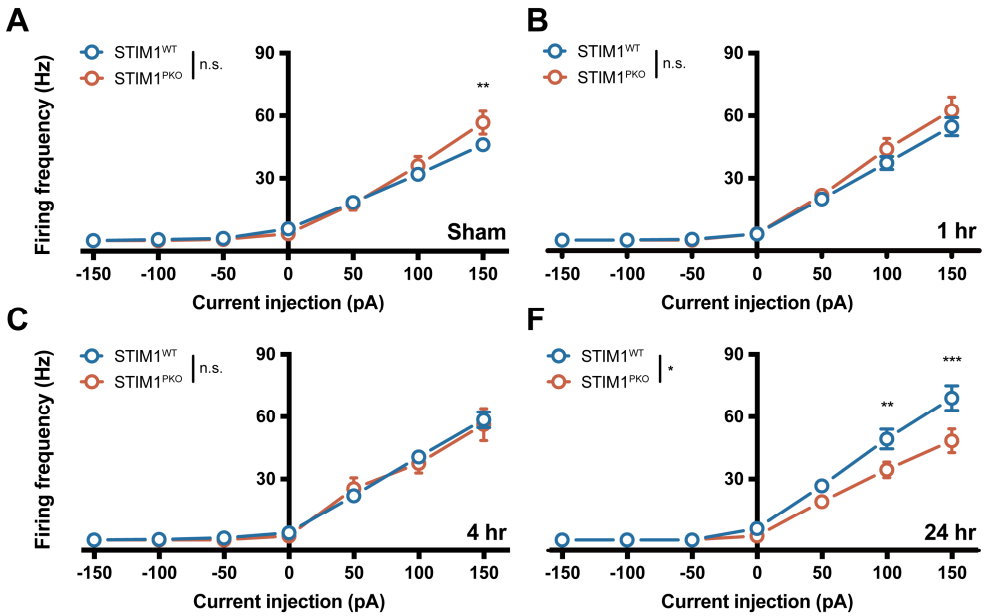

Figure 2.14. Excitability in VN neurons from wild-type littermates and STIM1^{PKO} mice in each time points. (A) Square-wised somatic current steps were injected from membrane potential with various ranges from -150 pA to 150 pA with increment of 50 pA for 1 s. (C) Overall, the gain responses of VN neurons from STIM1^{PKO} (red, n=16) were not significantly different from wild-type littermates (blue, n=23, p=0.423), but at 150 pA injection, STIM1^{PKO} showed higher excitability than wild-type littermates (p=0.003). (D) At the short-term period, the excitability of VN neurons of the wild-type (n=16) has slightly increased, while the excitability of STIM1^{PKO} (n=25) was unchanged. Statistical difference has disappeared (p=0.428). (E) At 4hrs after learning, the excitability of VN neurons of the wild-type (n=38) increased more, and that of STIM1^{PKO} (n=15) was unchanged again. IO curve of both groups are overlapped (p=0.759). (F) As the excitability of wild-type littermates (n=19) became much higher at the long-term period, the wild-type group showed significant higher frequency than STIM1^{PKO} group (n=15) (p=0.016). At 100pA and 150pA injection, statistical significance was 0.005 (100pA) and <0.001 (150pA) by post-hoc test. Two-way repeated measure ANOVA with Sidak test was used for panels. Error bars denote SEM. *p<0.05, **p<0.01, ***p<0.001

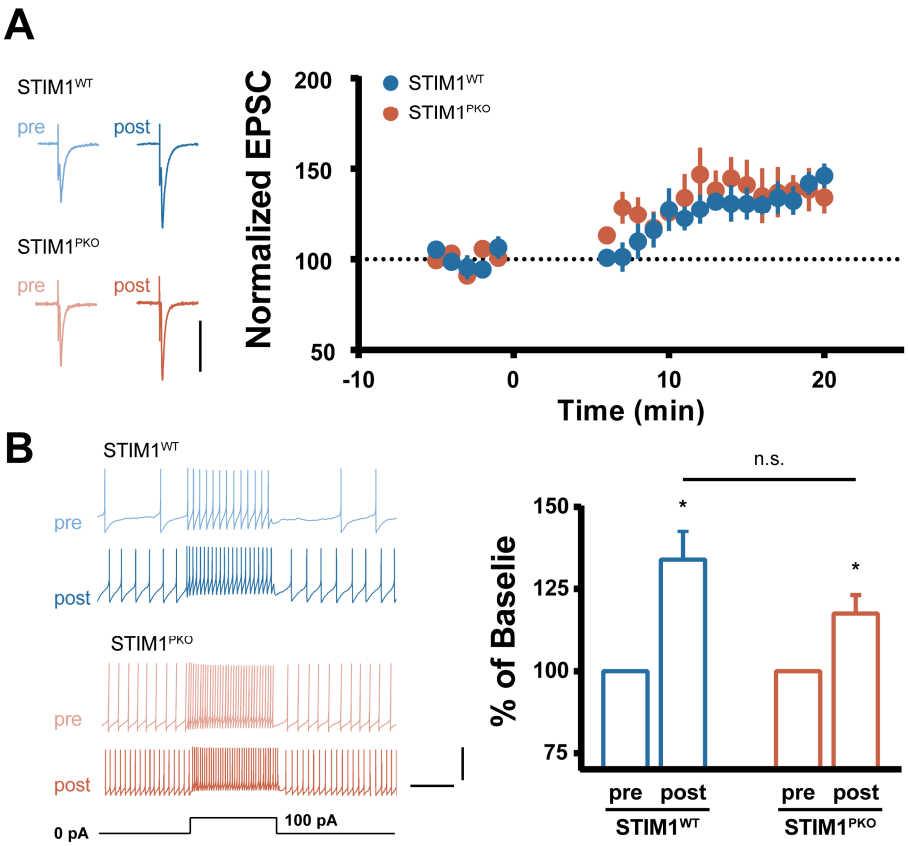

Figure 2.15. Both synaptic and intrinsic plasticity in VN neurons were able to be induced in STIM1^{PKO}. (A) Synaptic LTP of VN neuron. Both wild-type (n=6) and STIM1^{PKO} (n=7) groups showed intact synaptic plasticity through *in vitro* induction protocol. (B) Intrinsic plasticity of VN neuron was intact in both genotypes. Through LTP induction protocol, the excitability of VN neurons was significantly increased in both genotypes (wild-type, n=6, p=0.031; STIM1^{PKO}, n=6, p=0.031). There was no significant difference between post-induction groups of both genotypes (p=0.386) Mann-Whitney U test was used for panel B. Error bars denote SEM. *p<0.05.

3. Discussion

In this dissertation, I demonstrate a role of the intrinsic plasticity of cerebellar Purkinje cells in motor learning through which the VOR adaptive memory is transferred to the sub-cerebellar cortical region for long-term memory storage. After gain-up training, the synaptic strength at PF-PC synapses is decreased and learning-induced PF-PC LTD occurs concomitantly with a reduction of intrinsic excitability in PCs. Furthermore, VOR learning causes potentiation of the synaptic weight and intrinsic excitability in VN neurons, as well as the plasticity in PCs. Mice that were subjected to impaired memory consolidation, the $STIM1^{PKO}$ group, show a normal learning curve and synaptic plasticity, whereas the PC intrinsic plasticity is declined within an hour. In addition to the unstable induction of intrinsic plasticity, there were no appropriate learning-induced alterations of synaptic transmission and excitability in the VN neurons. These observations indicate that experience-dependent modulation of the neuronal excitability is required for long-term memory consolidation, in terms of the cerebellum-dependent motor learning.

There have been two long-lasting hypotheses for VOR learning that hold many different points of views. Marr-Albus-Ito proposed that plasticity in the cerebellar cortex may be the key player in the adaptive eye-movement motor learning (Albus, 1971; Ito, 1982; Marr, 1969). During the VOR training, PF-PC LTD has established through the conjunction of head movement-driven PF activation with visuo-vestibular mismatch-driven instructive CF activation. For decades, the Marr-Albus-Ito theory has been confirmed and expanded by abundant experimental evidence supporting the contribution of the PF-PC LTD to the adjustment of the VOR gain (De Zeeuw et al., 1998; Hansel et al., 2006; Schonewille et al., 2010). In spite of several literatures corroborating this hypothesis, there has been an alternative perspective for VOR behavior. (Miles and Lisberger, 1981) suggested that the cellular basis for the adaptive motor learning is activity-dependent neural

plasticity of the VN neurons guided by an instructive signal from the cerebellar cortex. In parallel with this perspective, several experimental observations have insisted that cerebellar LTD might be not sufficient to be a central dogma of the cellular mechanism for the VOR learning (Ke et al., 2009; Schonewille et al., 2011; van Alphen and De Zeeuw, 2002). There is, however, accumulating evidence supporting a reconciliatory learning model. (Boyden et al., 2004) proposed multiple plasticity mechanisms, insisting that plasticity at the MF-VN synapse, as well as PF-PC synapses require the motor memory formation. In addition, VOR gain-up learning has been found to be selectively engaged in the PF-PC LTD and thereby continue to MF-VN LTP (Boyden et al., 2006). The authors pointed out that the output of PCs may be responsible for the VN plasticity as Miles and Lisberger suggested. More recently, numerous computational modeling studies have supported the theory that synaptic plasticity in both regions is required for successful memory storage (Clopath et al., 2014; Dean et al., 2010; Porrill and Dean, 2007; Yamazaki et al., 2015). This theory highlights the importance of communication between these brain regions. Despite many implications suggesting that long-term storage of motor memory requires the memory transfer process from cerebellar cortex to nuclei, the detailed mechanisms of this memory transfer have yet to be elucidated. The data we presented here provide experimental evidence that VOR training results in the alteration of synaptic weight and excitability at multiple sites, the cerebellar cortex and the VN. Moreover, this study elucidates an unrevealed role of the intrinsic plasticity of cerebellar PCs in the VOR memory circuit by using the memory consolidation deficit mice model (Figure 2.2). Learning-induced synaptic plasticity at the PF-PC synapse is observed in wild-type littermates, however, intrinsic plasticity is abolished within an hour of the learning task in the memory consolidation deficient mouse model (Figure 2.7 and 2.8). Furthermore, this impairment of the intrinsic plasticity of PCs is concomitant with the failure of VOR training-induced plasticity induction in the VN neurons, although the VN neurons in the $STIM1^{PKO}$ mice are endowed with neural plasticity

in vitro, implying that the learning-induced alteration of excitability in the PCs might serve as an instructive signal to induce the appropriate plasticity induction in VN neurons (Figure 3.1). These results support the previous expectations in which suggested that PC activity can affect the synaptic and intrinsic plasticity induction in VN neurons (McElvain et al., 2010; Medina, 2010). Collectively, our results reconcile two long-standing hypotheses by providing experimental evidence for the induction of multiple forms of plasticity through VOR learning in both the cerebellar cortex and sub-cortical regions.

It has been assumed that motor memory is firstly formed in the cerebellar cortex and that neurons in the VN are involved in late phase adaption for VOR gain (Ito, 2013; Shutoh et al., 2006). This assumption implies that the temporal order between PC and VN plasticity has to be considered in memory processing. In my results, the VN plasticity is induced at a relatively later period than the plasticity in the PCs, and it indicates two major aspects. One is that PF-PC LTD contributes to memory acquisition, and the other is that the consequent induction of plasticity in VN neurons encodes long-term memory storage. Our data indicate that the impaired intrinsic plasticity of the cerebellar PCs would impair memory transfer and disrupt long-term memory storage. This supports the theory that the intrinsic plasticity of PCs connects two distinct brain regions and shapes the flow of information flow from the cerebellar cortex to the sub-cortical area. The temporal order of plasticity at multiple sites may reflect the loci of memory storage. The *ex-vivo* recordings we presented here were executed at distinct time points: short- (~1hr), mid- (~4hrs) and long-term (~24hrs) periods after learning. At the short-term period, the VOR learning curve and synaptic plasticity were not impaired in the memory consolidation deficient mouse model, although intrinsic plasticity was abolished (Figure 3.1). These results indicate that the memory acquisition may require synaptic plasticity in the cerebellar cortex, but not intrinsic plasticity. Rather, the aspects of the memory retention and deficiency of intrinsic plasticity in $STIM1^{PKO}$ lead us to assume that the learning-

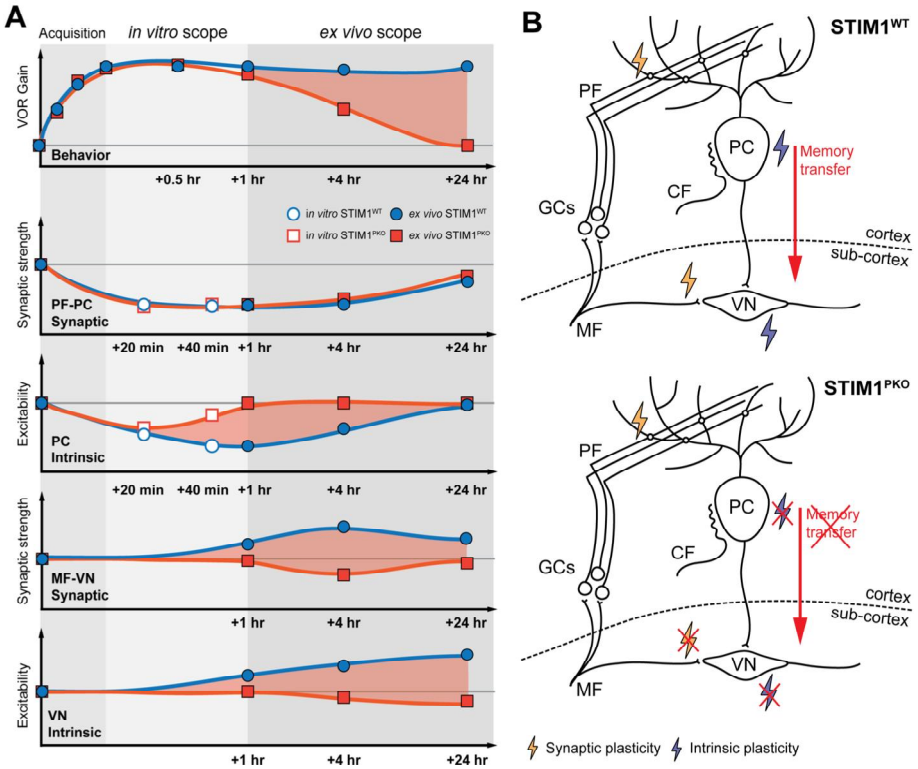

Figure 3.1. Schematic illustration for memory trace of VOR motor memory. (A) Summary of behavioral test and *in vitro*, *ex vivo* recording showing Temporal order of the memory retention and neural plasticity in PCs and VN neurons. Differences between wild-type and STIM1^{PKO} mice are indicated as red shade of each plots). VOR memory retention level is maintained over a day whereas the motor memory is declined at mid-term period (+1h - +4h) in STIM1^{PKO}. Alterations of the neural activity corresponding to each period are presented below. There is no difference in PF-PC synaptic plasticity between wild-type and STIM1^{PKO} mice. However, learning-induced LTD-IE is abolished within an hour in STIM1^{PKO} and the difference in PC intrinsic plasticity between groups may lead to MF-VN synaptic plasticity and intrinsic plasticity of VN neurons (see the red shade). Furthermore, peak difference of each plot seems to move from 1 to 24 hrs after learning, indicating that the plasticity in cerebellar PCs and VN neurons is connected in order. (B) Schematic illustration of neural circuit for VOR memory storage shown in wild-type (top) and STIM1^{PKO} mice (bottom). For successful memory acquisition and storage, four different types of neural plasticity are necessary (both plasticity in the PCs and VN neurons). Especially, intrinsic plasticity of PC has important role in transfer acquired memory to sub-cortical area in this circuitry (top). When PC intrinsic plasticity is abolished, synaptic and intrinsic plasticity in VN neurons are impaired thereby failure to long-term memory storage

induced alteration in PC excitability might be involved in the memory transfer process. Consistent with previous implications, our results suggest that the memory transfer occurs within 4hrs after learning (Kassardjian et al., 2005; Okamoto et al., 2011a; Shutoh et al., 2006). Synergies between synaptic and intrinsic plasticity may provide an instructive signal to convey the learned information into the sub-cortical area, the VN, at the mid-term (~4hrs) period. Interestingly, the synaptic plasticity in the VN neurons is observed slightly later than the intrinsic plasticity of the PC. Additionally, there is another slight delay in the VN intrinsic plasticity to reach a peak (Figure 3.1). These results indicate that the sequential flow of information from the cerebellar cortex to the sub-cortical region is responsible for memory processing. Taken together, we conclude that the acquired VOR memory might be located in the cerebellar cortex and the VN at the short- and long-term period, respectively, and a guiding instructive signal, driven by the intrinsic plasticity of the PCs may take part in the transfer of memory from the cortical area to the sub-cortical area during the mid-term time period.

It is widely believed that the plasticity of neuronal excitability is involved in the cellular mechanism for memory storage. In particular, the intrinsic plasticity of cerebellar PCs shows features in the cerebellar memory circuits that are distinct from other types of neurons. In the neurons in the amygdala and hippocampal, learning-related neurons show higher excitability (Zhou et al., 2009), and the depolarization of the membrane potential of these cells enables the promotion of further synaptic plasticity (Ramakers and Storm, 2002; S. Watanabe et al., 2002). Thus, these excitable neurons form a stable connection by strengthening the synaptic weight the given neural network, thereby consolidating the memory. In contrast, one previous study suggested that the intrinsic plasticity of PCs occludes the subsequent induction of PF-PC synaptic plasticity (Belmeguenai et al., 2010). Hence, the plasticity of excitability may ensure that synaptic activity remains within a physiological limit by restricting further synaptic plasticity and adjusting the impact of PF activation on the output of PCs. In addition, the data show that

there is no significant difference in the magnitude of synaptic plasticity at the PF-PC synapses between the wild-type littermate group and $STIM1^{PKO}$ group, although the excitability is lower in the $STIM1^{PKO}$ group than the wild-type group (Figure 2.7A and 2.9A). This suggests that the basal membrane excitability in PCs is not correlated with the synaptic plasticity induction or the magnitude of synaptic plasticity.

In the previous report, interestingly, there were no developmental differences between wild-type littermates and $STIM1^{PKO}$, such as the morphology of PCs, expression level of Ca^{2+}-related channels and CF-induced complex spike. (Ryu et al., 2017). One big difference found in this mice model was basal excitability of PCs and Ca^{2+} dynamics. Given that potentiation of the spontaneous firing rates in cerebellar PCs is not sufficient for affecting the firing rates in the DCN neurons (Belmeguenai et al., 2010), this implies that potentiation and/or reduction of PC excitability alone would be unable to significantly influence the neuronal activity in the sub-cortical area. Addition to this, another study suggest that The most important factor to deliver the PC output to DCN neuron is temporal synchrony of PCs output rather than the individual activity of PC (Person and Raman, 2011). Thus, in $STIM1^{PKO}$ case, the plasticity of excitability may count for the defect rather than reduced basal excitability. Because the intrinsic plasticity of PCs is modulated with the same polarity of the PF-PC synaptic plasticity, concurrence of synaptic and intrinsic plasticity may synergistically produce an appropriate PC output in response to external inputs, such as vestibular stimuli (Belmeguenai et al., 2010; Shim et al., 2017). The *ex-vivo* recordings in this study reveal that VOR gain-up learning induces PF-PC LTD and LTD-IE in PCs, indicating that the intrinsic plasticity is accompanied by synaptic plasticity, which corresponds to the activity-pattern (Figure 2.6 and 2.8). Collectively, I suggest that learning-induced intrinsic plasticity may amplify the alteration of the synaptic transmission, resulting in the synergistic modulation of the net output of PCs in order to maximize information storage.

4. Materials & Methods

Animals

$STIM1^{PKO}$ mice were generated by crossing the homozygous PCP2-Cre line (B6.129-Tg(Pcp2-cre)2Mpin/J line from the Jackson Laboratory) with the STIM1-floxed line (C57BL/6 background). The first filial generation (F1) was crossed again with the STIM1-floxed line. Among the second filial generation (F2), male mice which were homozygous for floxed-STIM1 alleles were used for this study. I used male mice in all the experiments. All procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University College of Medicine.

Behavioral tests

Surgical procedure. Mice at the age of 7- to 12- weeks were used. Head fixation pedestal was formed with two nuts (M2) and four screws (M1.2 X 5.5). Nuts were placed on Bregma and Lambda of skull and screws were implanted between the nuts (Figure 4.1A). Mice were under isoflurane anesthesia during surgery. After surgery, at least 24hrs of recovery time were given to mice.

Instrumentation. The image of the eye of the mouse was taken by CCD camera (IPX-VGA210, IMPERX) with; a lens (VS-LD 35, VST) and an Infrared filter (LP830) and was processed into a PC through camera link grabber board (PCI-1426, National Instruments). IR lighting was generated by IR-LED (DR4-56R-IR85, LVS) and additional single IR-LED was placed around the camera to generate reference cornea reflex (CR) for calibration, which is described below. Optokinetic stimulation was applied by the drum, 50 cm in diameter, mounted on a motor (AKM22E-VBBNR-00, Kollmorgen). Custom-made turn table was also mounted on another motor (D061M-12-1310, Kollmorgen) for vestibular

stimulation. Since both stimuli were generated by independent motors, visuo-vestibular mismatch stimulation could be applied. Data acquisition (DAQ) PCI board (PCI-6230, National Instruments) was responsible for the I/O between PC and motion. The acquired image data were processed by several virtual instruments written by LabView (National Instruments) (Figure 4.1B)

Recording preparation. Before every recording, physostigmine salicylate solution (Eserine; Sigma Aldrich) was treated for pupil dilatation control with brief isoflurane anesthetization. The concentration of eserine solution was constantly increased from 0.1%, 0.15% and 0.2% because of drug resistance. To effectively washout the side effect of anesthetics, mice were given a recovery phase for at least 20 minutes after eserine treatment. After recovery, mice were restrained in a custom-built animal holder. The holder was placed in the center of machinery

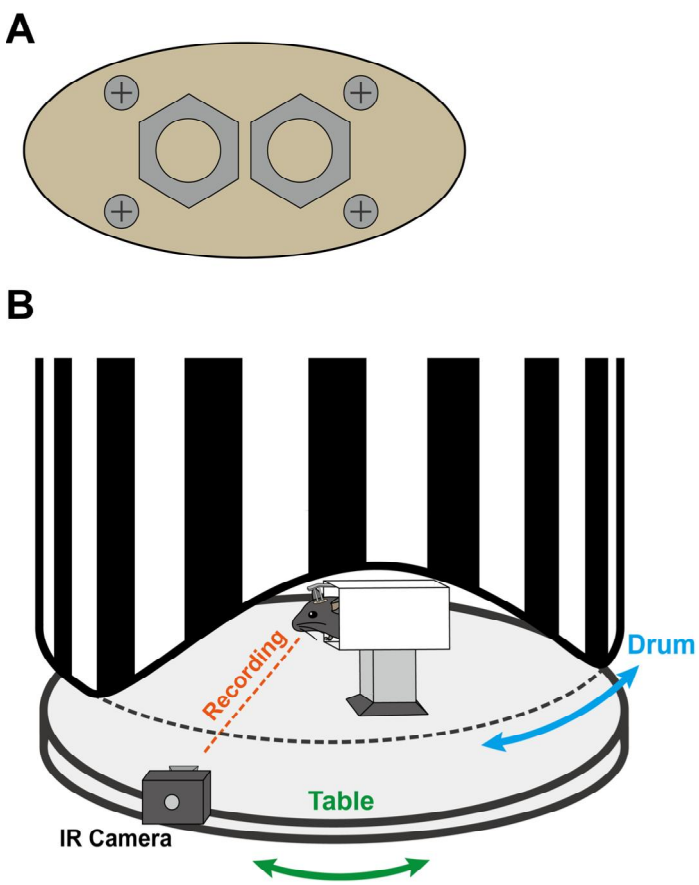

Figure 4.1. Schematic figure of behavior test. (A) Surgery plan on mice skull. Two nut are placed on bregma and lambda of skull and four bolts are implanted around the nuts. (B) Mice placed on the center of turn table, and drum is covered from the top. Both table and drum are able to rotate. Infrared(IR) camera possess in front of the eye of mouse to take clear eye image.

turntable.

Acclimation and calibration. Acclimation began at least 24hrs after surgery. Two sessions of acclimation were performed. During acclimation, the mouse was fixed onto a custom-made restrainer for 15mins without any stimulation. Calibration was performed in the day after 2 days of acclimation. Briefly, the purpose of calibration was to convert linear eye position to angular eye positions. As the results of calibration, I could calculate the radius of the pupil (R_P) which is an important value for calculating the gain and phase values of eye movement. Equation and procedure for calibration were performed according to (Stahl et al., 2000). At the recording after calibration, the mouse and holder were placed in the position where calibration was performed.

Eye movement recordings. Three basal ocular-motor responses, which are Optokinetic response (OKR), VOR in dark condition (dVOR) and VOR under light (lVOR), were measured (Figure 4.2). For OKR, drum stimulation was provided in sinusoidal rotation with ±5° of rotation amplitude. For dVOR and lVOR, turn table stimulation was applied in sinusoidal rotation with ±5° of rotation amplitude. The only different between dVOR and lVOR was under light off and on, respectively. Each response was recorded at four different rotating frequencies 0.1, 0.25, 0.5, 1.0Hz.

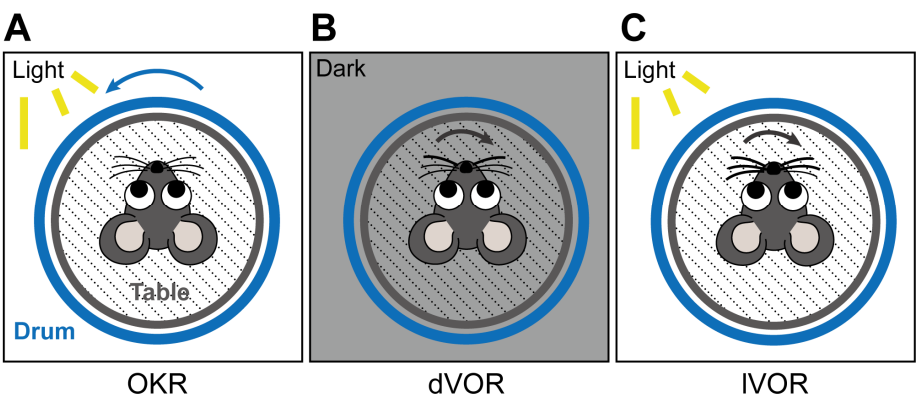

Figure 4.2. Recording procedures. OKR, VOR in dark and VOR in light.

Learning Protocols. Associative visuo-vestibular stimulation was applied to induce dVOR learning at 0.5Hz frequency. Drum and table simultaneously rotated with ±5° of amplitude in and out of phase. For gain-up and down learning, the protocols contained three 10min training sessions and four check points (Figure 4.3A-B). After daily learning, mice were placed in complete dark condition for 24hrs until next learning. Afterward, dVOR was measured again as pre-learning check point and another daily learning began. For phase reversal learning, the dVOR was recorded six times, before learning started and after finishing five

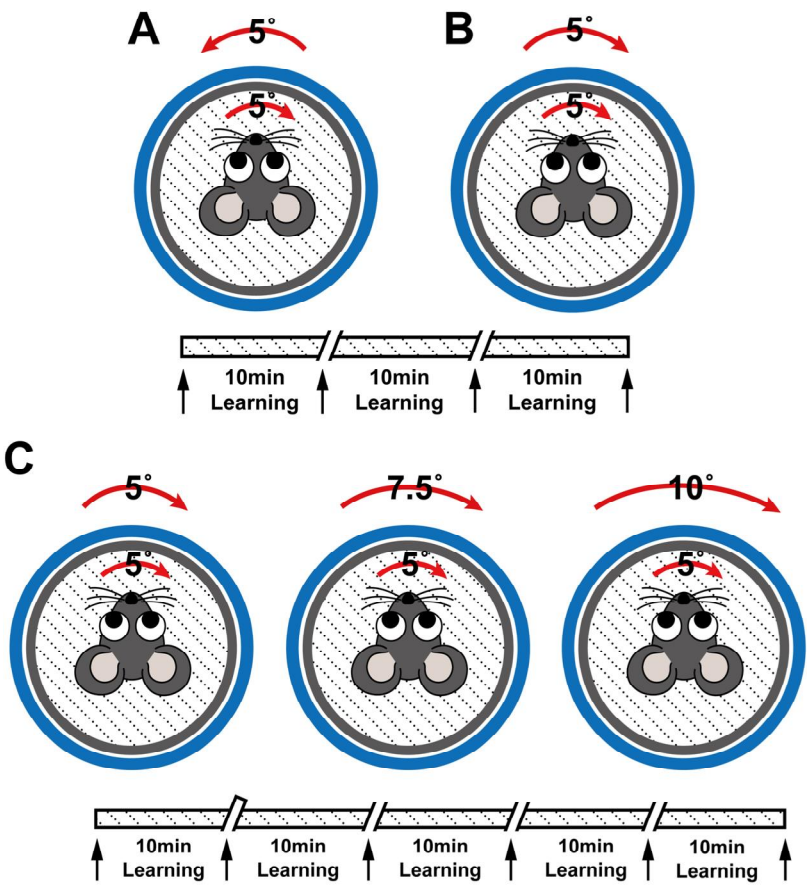

Figure 4.3. Learning protocols. (A) Gain-up learning protocol. Same as gain-down protocol but out-phase paired stimulation. (B) Gain-down learning protocol. 5° of in-phase paired table and drum stimulation were applied 3 times and 10 minutes each. 0.5Hz dVOR was recorded in pre- and post-learning and every interval between learning. (C) Phase reversal learning protocol. Five learning sessions for each day. From day 1 to 3, rotating amplitude of drum increases 2.5° each day from 5°. Table rotating amplitude was fixed to

learning sessions (Figure 4.3C). While gain-up and down keep the protocol till the end of learning, the protocol of phase reversal contained daily different drum stimulation. On the first day, table and drum rotated in phase with 5°. Next day, the drum rotated 2.5° more than the table. On the third day, the final learning day, drum rotated 5° than table totally. Caging in complete dark condition between learning was the same as gain-up and down. Each mouse was trained by only one protocol, not learned multiple visuo-vestibular stimulations.

Data Analysis. The given stimulus and the response were fitted to sine curves. In the fitted curves, gain value was obtained by calculating the ratio of the response amplitude to stimulus amplitude. The time lag and the lead of response (Phase) were determined by calculating the phase difference between the two sine curves. For all these procedures, I used custom built Labview data analysis tool. To measure the level of memory consolidation, percent ratio of remained memory to learned memory was calculated.

Electrophysiology

Slice preparation. Coronal cerebellar (flocculus) and brainstem slices of 270 - 320 μm were dissected by vibratome (Leica, VT1200) from behaviour tested 9 to 11 weeks old male mice in ice-cold NMDG cutting solution contained with the following (in mM): 93 NMDG, 93 HCl, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 Glucose, 5 sodium ascorbate, 2 Thiourea, 3 Sodium pyruvate, 10 $MgSO_4 \cdot 7H_2O$, 0.5 $CaCl_2 \cdot 2H_2O$ (pH 7.3). The brainstem slices containing VN were obtained from more rostral part in which the brainstem was attached to the cerebellum. The coronal plane of the cerebellar and brainstem slices were transferred into recovery chamber containing NMDG-cutting solution at 32 ˚C for 10 minutes, and then incubated in standard artificial cerebrospinal fluid (aCSF) contained with the following (in mM): 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose at room temperature for an hour. NMDG-cutting solution and aCSF were oxygenated with 95% O2-5% CO2 (pH 7.4).

Whole cell recording. Brain slices were put onto a submerged recording chamber on the stage of Olympus microscope (BX50WI, Japan) and perfused with standard aCSF. I used EPC9 amplifier with PatchMaster software (HEKA Elektronik) and multiclamp 700B amplifier with pClamp 10 (Molecular Device). Sampling frequency of 20 kHz and signals were filtered at 2 kHz (1 kHz filter for sEPSC). Inhibitory synaptic inputs were totally blocked by 100 μM picrotoxin (Sigma) in PC recording, and strychnine (1 μM) was added to block glycinergic input in VN recording. Patch pipettes (3-4 MΩ) were borosilicate glass and filled with internal solution containing the following: 9 KCl, 10 KOH, 120 K-gluconate, 3.48 $MgCl_2$, 10 HEPES, 4 NaCl, 4 Na_2ATP, 0.4 Na_3GTP and 17.5 sucrose (pH 7.25) for testing *in vitro* recordings, *ex vivo* PC excitability and *ex vivo* VN recordings; 140 CsCl, 4 NaCl, 0.5 $CaCl_2$, 10 HEPES, 2 MgATP and 5 EGTA (pH 7.3) for *ex vivo* sEPSC recording from PCs in the medial part of the flocculus. Only tonic firing PC was selected for the data and PCs showing other firing patterns were discarded. One of obstacles that I have to overcome was sorting the Flocculus targeting neurons (FTN), because not all VN neurons receives the input from the floccular PCs. According to previous study, there are two types of neurons in the VN and only type A neurons are related to VOR learning (Carcaud et al., 2017). Since the type of neurons is distinguishable by the firing patterns, I sorted out the type A neurons from the recordings. All patch clamp data, except for sEPSC recordings, were imported and analyzed by Igor Pro (Wave Metrics). The sEPSC data were analyzed using Mini Analysis (Synaptosoft). Other recording and analysis details including plasticity induction protocol were similar to published paper (Ryu et al., 2017; Shim et al., 2017).

Quantification and statistical analysis.

As I described above, behavior data was analyzed by custom built LabView (National Instrument) tool, and electrophysiology data was analyzed by custom-built python code, Igor Pro (Wave Matrics) and Mini Analysis (Synaptosoft). All

statistical analysis was performed using Graphpad Prism 7 and Microsoft Excel. One-way ANOVA with Fisher's LSD test, One- or Two-way repeated measure ANOVA with Sidak's post-hoc test was used for several time groups analysis, and unpaired *t*-test was performed to compare wild-type and knockout group. All graphs are shown as mean ± SEM, and asterisks *, ** and *** indicates $p<0.05$, $p<0.01$ and $p<0.001$, respectively. *n* for each experiments are written in the figure legends.

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국문 초록

소뇌 퍼킨지 세포에서 발생하는 내재적 가소성(intrinsic plasticity)은 근래에 그 방향성이 밝혀지며, 소뇌 신경회로에서의 그 역할에 대한 궁금증이 많아졌다. 유전자 조작을 통해서 소포체(endoplasmic reticulum)의 막단백질 중의 하나인 STIM1 (Stromal interaction molecule type 1)을 퍼킨지 세포에서만 특별하게 제거한 생쥐에서는 아주 흥미로운 행동 표현형을 확인 할 수 있었다. 이 생쥐는 전정안구반사 (Vestibulo-ocular reflex; VOR) 훈련을 하였을 때, 학습은 잘 하지만 기억의 공고화 (consolidation) 과정에 문제가 있어 장기 기억의 결핍을 발견할 수 있었다. 이러한 현상은 모든 학습 방법에서도 확인 할 수 있었기 때문에, 이를 담당하는 신경회로에서 공통적으로 지나가야하는 부분에 문제가 있을 것이라 추측하였고, 그 대상을 퍼킨지 세포의 내재적 흥분성으로 정하였다. 흥미로운 점은 이 생쥐의 퍼킨지 세포에서 시냅스 가소성(synaptic plasticity)은 정상이지만, 내재적 가소성은 결핍 되어있었다는 점이다. 전정안구반사 훈련을 수행한 생쥐의 뇌 절편을 만들어 전기생리학적 기록을 해본 결과, 정상쥐와 STIM1 이 제거된 쥐 모두에서 퍼킨지 세포의 시냅스 가소성은 발견되었지만, 내재적 가소성은 정상쥐에서만 발견되었다. 또한, 정상쥐의 퍼킨지 세포에서 발생한 가소성은 퍼킨지 세포의 신호를 받는 전정핵 (Vestibular nucleus; VN)에서의 시냅스, 내재적 가소성을 발생시키도록 유도하였지만, STIM1 이 제거된 쥐는 전정핵에서 발생하는 가소성 또한 망가져 있었다. 이러한 결과를 통해 퍼킨지 세포에서 발생하는 내재적 가소성이 전정핵에서 발생하는 가소성에 중요하게 작용하여 장기기억을 위한 기억의 공고화 과정에 필요한 요소임을 확인 할 수 있었다.

핵심어 : 소뇌, 퍼킨지 세포, 전정안구반사, 기억의 공고화, 내재적 가소성, 전정핵
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감사의 글

먼저 모든 일을 주관하시는 하나님 아버지께 감사드립니다.

박사과정을 돌아보면 감사한 분들이 아주 많습니다.

대학원에 들어오기 전부터 지금까지 언제나, 모든 면에서, 힘이 되어준 아내에게 고맙다는 말을 전하고 싶습니다. 박사과정을 저보다 한 발 먼저 걸어가고 있었던 것도 크게 의지가 되고 또 서로 위로 할 수 있었기 때문에 아내가 없었다면 학위과정이 내내 많이 힘들었을 것입니다. 제가 아주 어렸을 때부터 아낌없는 조언으로 제 의견을 지지해주시는 아버지와 어머니께 감사의 말씀을 전하고 싶고, 분야도 학위과정을 보내고 있는 나라도 다르지만 같이 논문을 쓰고 공부를 이어가고 있다는 것에 서로 의지 할 수 있는 형에게도 고맙다는 말을 하고 싶습니다. 가족들의 전폭적인 지지 덕에 모든 과정을 즐겁고 감사하게 마무리 할 수 있었습니다.

학위기간동안 자유롭게 연구주제를 설정하고, 이끌 수 있도록 지켜봐 주시고, 지원해주신 김상정 교수님과 김전 교수님, 이용석 교수님께 감사의 말씀을 드리고 싶습니다. 또한 이 기간 동안에 랩에서 같이 생활한 여러 선배님들과 후배들의 물리적, 정신적 도움이 없었다면 모든 실험과 논문은 아마도 출판되지 못했을 것입니다. 특별히 논문의 작성과 실험에서 많은 도움을 주었고, 학위과정이라는 시간을 즐겁게 보낼 수 있도록 해준 정지훈, 심현근, 유창현, 김승하, 이재건 선생님에게 감사의 말을 전하고 싶습니다.

그러나 내가 나 된 것은 하나님의 은혜로 된 것이니 내게 주신 그의 은혜가 헛되지 아니하여 내가 모든 사도보다 더 많이 수고하였으나 내가 한 것이 아니요 오직 나와 함께 하신 하나님의 은혜로라 -고린도전서 15:10