의학석사 학위논문

Induction of Long-Term Depression at Flocculus Purkinje Cells in Juvenile Mice

청소년기 생쥐의 소뇌 편엽 퍼킨지 세포에서의 장기 저하 유도

2013 년 02 월

서울대학교 대학원

의과학과 석사과정

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February 2013

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by

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A thesis submitted to the Department of Biomedical Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Medicine at Seoul National University College of Medicine

February 2013

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ABSTRACT

Cerebellum is a brain region important in motor control. Cerebellum can be subdivided into cerebrocerebellum, spinocerebellum and vestibulocerebellum. Spinocerebellum receives inputs from spinal cord and auditory system, while vestibulocerebellum receives vestibular inputs from semicircular canals and vestibular nuclei. Vestibular ocular reflex (VOR) learning is usually used to test cerebellar functions. Flocculus, a part of vestibulocerebellum, is supposed to be the initial place of VOR learning and long-term depression (LTD) is the underlying mechanism for it. However, LTD had not been observed directly in floccular slices.

Here whole-cell recordings were made at lobules III to V of cerebellar vermis and lobule X and flocculus, which are known to be the spinocerebellum and vestibulocerebellum, respectively. Data were analyzed them for LTD and other electrophysiological properties. First, AMPA receptor-mediated fast excitatory post-synaptic current (EPSC) showed no difference in input-output curve and paired pulse ratio among three groups, meaning they have similar presynaptic properties. Second, Second, flocculus showed metabotropic

glutamate receptor (mGluR)-mediated slow EPSC measured in the presence of NBQX and its amplitude was not significantly different from that in lobule III to V. However, lobule X which is also a part of vestibulocerebellum as flocculus, showed much smaller amplitude in slow EPSC. Third, it was possible to record LTD at flocculus with similar amplitude of LTD, while much smaller LTD was recorded from lobule X. The result suggests that LTD is normally induced at flocculus and it can be underlying cellular mechanisms for VOR learning.

Keywords: Cerebellum, Vermis, Flocculus, Synaptic Plasticity, Long-term Depression
Student number: 2011-21952

CONTENTS

Abstract i
Contents iii
List of Figures iv
List of Abbreviations v

Introduction 1
Material and Methods 5
Results 10
Discussion 24
References 28
Abstract in Korean 32

LIST OF FIGURES

Figure 1 PF−PC synaptic transmission shows no significant difference among three groups of Purkinje cells. 3

Figure 2 Short−term plasticity at PF−PC synapse shows no significant difference among three groups of Purkinje cells 6

Figure 3 Whole−cell Recordings, at vermis lobules III to V (n=20), lobule X (n=19) and flocculus (n=12), with bath application of 2.5μM NBQX and aCSF 7

Figure 4 Long−term Depression induced by CF/PF pairing stimulation 9

LIST OF ABBREVIATIONS

AMPA: 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid

AMPAR: AMPA receptor

CF: climbing fiber

DISC: depolarization-induced slow current

EPSC: excitatory post synaptic current

EAAT: excitatory amino-acid transporter

I/O curve: input-output curve

LTD: long-term depression

mGluR: metabotropic glutamate receptor

NMDA: N-Methyl-D-aspartic acid

NMDAR: N-Methyl-D-aspartic acid receptor

PC: Purkinje cell

PF: parallel fiber

PPR: paired pulse ratio

INTRODUCTION

Cerebellum is well-known for regulation of balance and movements. It can be subdivided into three regions, spinocerebellum, vestibulocerebellum, and cerebrocerebellum (1). These three groups receive inputs from different parts of brain and spinal cord. Spinocerebellum consists of vermis and intermediate parts of hemispheres, receives inputs from spinal cord, and regulates body and limb movements. Vestibulocerebellum consists of flocculus, paraflocculus, and vermis of lobules IX and X, receives inputs from vestibular organs, and regulates balance and eye movements. Cerebrocerebellum consists of lateral parts of hemisphere, receives inputs from cerebrum, plans movements and evaluates sensory information. Since cerebellum can be subdivided into distinct regions with distinct function growing number of studies have been conducted to investigate differences between lobules.

Along with regions, cerebellar lobules have differences in molecular expression, intrinsic excitability, and current characteristics. First, expression of glutamate transporter excitatory amino-acid transporter 4(EAAT4) differs among

regions of the cerebellum (2). The density of glutamate transporter EAAT4 is lower in lobule III whereas it is higher in lobule X. In lobule X, glutamate concentration is low and thus mGluR1-mediated slow EPSC is low and LTD is not induced. Second, intrinsic excitability is different among lobules. Only two types of firing patterns were observed in lobules III-V whereas four types of firing patterns were observed in lobule X (3). Third, depolarization-induced slow current (DISC) was different across lobules (4). DISC is a slow inward cation current following strong depolarization of cerebellar Purkinje cells. Due to different expression of dopamine receptors among regions, DISC is weak in lobule II, V, and VI, whereas it is strong in lobules IX and X. These previous studies show that there are differences between vestibulocerebellar PCs and spinocerebellar PCs.

Cellular mechanisms of learning and memory in many brain regions have been reported. In hippocampus, NMDA-mediated long-term potentiation and long-term depression are well known learning mechanisms (14). One of the underlying cellular mechanism of learning in cerebellum is mGluR1-mediated long-term depression (LTD) (5, 11, 15). Cerebellar

LTD can be induced by repetitive excitatory synaptic inputs from both parallel fibers (PFs) and climbing fibers (CFs) at low frequencies (12). When mGluR1-mediated LTD observed the mGluR1-dependent slow EPSC was evoked (15).

For the test of test cerebellar behavior, eyeblink test and vestibulo-ocular reflex (VOR) are usually performed (13, 6). During VOR experiment, mice are restrained from head movement and they are placed on the rotating table in the dark. While rotating the table, mice eye movement is recorded and later analyzed with frequency of table rotation. This allows researchers to study cerebellar function while excluding ocular input. Masao Ito hypothesized that the flocculus is the initial place of VOR learning (5). After this, many experiments have shown that VOR learning is related to flocculus and mGluR LTD may mediate the memory consolidation.

Both flocculus and lobule X are parts of vestibulocerebellum, thus they may have similar physiological properties. If LTD cannot be induced in flocculus as in it cannot be in lobule X, then Ito's flocculus hypothesis may not be right. To test the hypothesis, basal synaptic properties such as input-output relationship of stimulus and AMPAR-mediated

EPSC and paired-pulse ratio, mGluR1-mediated slow EPSC, and presence of LTD in mice cerebellar slice will be compared between the flocculus and lobule X, for the first time.

MATERIALS AND METHODS

1. Animals

All experiments were undertaken using protocols approved by the Experimental Animal Care and Ethics Committee of Seoul National University. 7 to 9 weeks old C57BL6 mice, provided by KOATECH, were used.

2. Slice preparation

Animals were anesthetized with isoflurane and decapitated. Cerebellum was removed and placed in artificial cerebrospinal fluid (aCSF) consisting of following (in mM): NaCl, 124; KCl, 2.5; $MgCl_2 \cdot 6H_2O$, 1.3; $CaCl_2 \cdot 2H_2O$, 2.5; NaH_2PO_4, 1; $NaHCO_3$, 26.2; Glucose, 20, were bubbled with 95 % O_2 and 5 % CO_2. Cerebellum was placed in chamber containing 2 to 6 ℃ aCSF and sliced by vibratome (Microm HM 650V, Germany). Parasagittal slices (220μm-thick) were cut for vermis recordings and coronal slices (220μm-thick) were cut for flocculus recordings. The slices were recovered at 32 ℃ for 30 minutes in the same solution and kept at room temperature before recording.

3. Electrophysiological Recordings

After recovery period, cerebellar slices were placed in a recording chamber under a microscope (Nikon, FN-S2N, Japan). Slices were perfused with the same aCSF containing 100 μM picrotoxin, which blocks $GABA_A$ receptors at the rate of 100 mL/min at 32 ℃. For vermis recordings, parasagittal slices were used and recordings were taken at Purkinje cells (PC) of lobules III to V and lobule X. For flocculus recordings, coronal slices were used and recordings were taken at PC of flocculus.

Patch electrodes were made by pulling glass capillaries (GC150t-7.5; Harvard Apparatus, KENT) on a microelectrode puller (Narishige, Japan). For slow EPSC experiment, patch electrode (2.8 to 3.6 MΩ) was filled with Cs-internal solution consisting of following (in mM): CsMS, 135; CsCl, 10; HEPES, 10; EGTA, 0.2; Na_2-ATP, 4; Na_3-GTP, 0.4. For all other experiments, patch electrode was filled with K-gluconate internal solution consisting of following (in mM) : K-gluconate, 130; NaCl, 10; HEPES, 10;

EGTA, 0.3; $MgCl_2$, 2; Na_2-ATP, 4; Na_3-GTP, 0.4; Tri-phosphocreatine, 10. Whole-cell patch-clamp recordings were obtained using EPC-9 amplifier (HEKA elektronik, Germany).

To investigate basal synaptic transmission function, input-output (I/O) relationship was measured. Stimulating electrodes were filled with aCSF and placed in the molecular layer to activate PF. Recordings were taken at voltage clamp mode and the membrane potential was held at -70 mV. 0.1 ms-long square pulses were applied every 15 seconds. Stimulating pulse was increased by 5 μA from 5 to 50 μA.

To investigate short-term plasticity, paired-pulse ratio (PPR) was measured. Stimulating electrodes were filled with aCSF and placed in the molecular layer to activate PF inputs. Recordings were taken at voltage clamp mode and the membrane potential was held at -70mV. 9 different interspike intervals (ISI) were used: 10, 20, 30, 50, 70, 100, 150, 200, 300 ms.

To investigate slow EPSC, tetanic stimulation was applied and stimulus intensities were increased. 2.5 μM NBQX and 100 μM picrotoxin containing aCSF was perfused. Stimulating electrodes were filled with aCSF and placed in the molecular layer to activate PF inputs. Slow EPSCs were evoked by 10 PF stimuli at 100Hz every 1 min. Stimulating pulse was increased by 5 μA from 5 to 50 μA. When analyzing data, if difference in slow EPSCs evoked at 5 μA and 50 μA was smaller than 10pA, the cell was defined as “not having slow current” and was not included in the graph. Number of cells having slow current and not having slow current was written in the result part.

To investigate LTD, PF/CF pairing protocol was used. aCSF-filled stimulating electrodes were placed in the molecular layer to activate PF input and in the granule layer to activate CF. Test responses at PF were stimulated 4 times every 1 minute in voltage clamp mode and averaged every 4 responses. After recording 10 minutes

for baseline recording, recordings were switched to current clamp mode for LTD induction. LTD was induced by 30 times of pairing stimulation for 5 minutes. Pairing stimulation consisted of 7 PF stimuli at 100 Hz with 1 CF stimulus delayed 150 ms.

4. Drugs and Statistical analysis

2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline-7sulfonamide (NBQX) was obtained from Tocris Bioscience. All other drugs were purchased from Sigma-Aldrich .

All data were shown as mean ± SEM. Statistical significance was evaluated by using Origin (OriginLab, USA) and the Student's t-test and ANOVA were used.

RESULTS

Basal Synaptic Transmission

First, the basal synaptic transmission was investigated to see if there is any difference between regions. Basal synaptic transmission is related to I/O relationship. Whole-cell voltage clamp recordings on cerebellar Purkinje cells were performed at lobule III to V in the parasagittal slices for vermis recording and at flocculus in the coronal slices. aCSF was complemented with 100 μM picrotoxin to block GABA inputs. Holding membrane potential at -70 mV, parallel fiber was stimulated with stimulation intensities 5 to 50 μA with increment of 5 μA. Then, input-output relationship curve was plotted.

Floccular PCs did not show any significant difference in I/O curve from PCs in other regions (Figure 1). At stimulation intensity of 50 μA, the amplitude of PF-EPSC at the Purkinje cells in lobules III to V was 458 ± 44.5 pA (n=36), that in lobule X was 435 ± 51.1 (n=26), and that in

flocculus was 493 ± 64.7 pA (n=22). At the significance level of 0.05, they were not significantly different. (p-value: 0.767; one-way ANOVA). PCs in three different parts of cerebellum showed similar trend in basal synaptic transmission.

Short-term plasticity

Next short-term plasticity was investigated. Changes in PPR indicate synaptic changes in neurotransmitter release. To investigate PPR, a pair of stimulation pulses was given at stimulus intensity which gives response about 200 to 300 pA and changed interspike interval (ISI): 10, 20, 30, 50, 70, 100, 150, 200, 300 ms. Then the ratio of the second EPSC versus the first EPSC as a function of ISI was plotted (Figure 2). PPR at PF-PC synapse is always larger than 100 %. Overall, the two groups of PCs did not show any significant difference. At ISI = 20 ms, PPR at lobules III to V was 248 ± 9.94 % (n=36), PPR at lobule X was 252 ± 8.10 % (n=26), and PPR at flocculus was 245 ± 15. 4 %

(n=22). At significant level of 0.05, PPR in floccular PC did not show any significant difference between other two groups, meaning that the short-term plasticity at PF-PC is similar between flocculus and other two groups (p-value: 0.770; one-way ANOVA). PCs in all three regions of cerebellum showed similar trend in short-term plasticity.

mGluR1-mediated Slow EPSC

Then mGluR1-mediated slow EPSC was investigated. The slice was perfused with aCSF containing 2.5 μM NBQX, AMPA and Kainate receptors antagonist. While holding membrane potential at -70 mV, stimulating electrode was placed on the molecular layer to activate PF and tetanic stimuli, 10 pulses at 100 Hz, were applied with it. Stimulus intensities were varied from 5 to 50 μA. In Figure 3 (A) fast EPSC, (B) slow EPSC, and (C) ratio of two against stimulus intensity were plotted (Figure 3).

When tetanus stimulus was applied, the fast EPSC at PCs in lobules III to V was 209 ± 30.9 pA (n=20), that in

lobule X was 188 ± 42.5 pA (n=19), and that in flocculus was 137 ± 22.8 pA (n=12). The PCs in lobule III to V, those in lobule X and those in flocculus were not significantly different in NBQX reduced fast EPSC. (p value: 0.420; one-way ANOVA) In other words, NBQX reduced the fast EPSC in PCs in all three regions at similar degree.

PCs were distinguished as showing slow EPSC if the difference in slow EPSC evoked by 5 μA and 50 μA was less than 10 pA. In lobule III to V, all of the 20 PCs showed slow EPSC, whereas 9 out of 12 PCs were excluded in flocculus and 7 out of 19 PCs showed slow EPSC in lobule X. There is a certain difference in occurrence of slow EPSC among groups.

At the stimulation intensity of 50μA, slow EPSC at PCs in lobules III to V was 271 ± 35.5 pA, slow EPSC at PCs in lobule X was 128 ± 47.2 pA and slow EPSC at PCs in flocculus was 192 ± 63.7 pA. Tetanus-induced slow EPSCs were significantly different among groups of PCs. (p-value: 0.120; one-way ANOVA). P-value is large

enough to conclude that three groups are not significantly different using one-way ANOVA. However, lobules III to V and lobule X are significantly different (p-value: 0.041; two sample t-test). Lobules III to V and flocculus are not significantly different (p-value: 0.250; two sample t-test). Lobule X and flocculus are not significantly different (p-value: 0.460; two sample t-test). It can be concluded that lobules III to V and lobule X are significantly different, lobule X shows very smaller slow EPSC than lobule III to V does. Flocculus also shows smaller slow EPSC than lobule III to V, but it is not significantly different. In general, vestibulocerebellum shows smaller slow EPSC than spinocerebellum.

Ratio of fast EPSC over slow EPSC was also calculated and plotted against stimulation intensity. At the stimulation intensity of 50 μA, the ratio at PCs in lobules III to V was 2.60 ± 0.574, that in lobule X was 0.711 ± 0.290 and that in flocculus was 3.11 ± 1.06. The ratio was significantly different among three groups (p-value: 0.153,

one-way ANOVA).

Flocculus shows similar trend as lobules III to V but different from lobule X, another part of vestibulocerebellum.

Long-term plasticity

Then mGluR1-mediated long-term depression induction was investigated. If memory consolidation at flocculus is via mGluR-mediated LTD, as many papers have hypothesized, LTD would be induced. If flocculus has similar property as lobule X of vermis, which is another part of vestibulocerebellum, then LTD would not be induced.

Baseline EPSCs were recorded every 15 s for 10 minutes. After baseline recording, LTD was induced using pairing stimulation, which consists of 7 PF stimuli and 1 delayed CF stimulus. Then, EPSCs were recorded every 15 s for 40 minutes (Figure 4).

In three groups of PCs, LTD was induced at similar degree. 25 minutes after the induction, the percentage at PCs in

lobules 3 to 5 was 62.6 ± 3.35 (n=11), and that at flocculus was 60.3 ± 3.47 (n=6). The change in fast EPSC was not significantly different between lobule III-V and flocculus (p-value: 0.689).

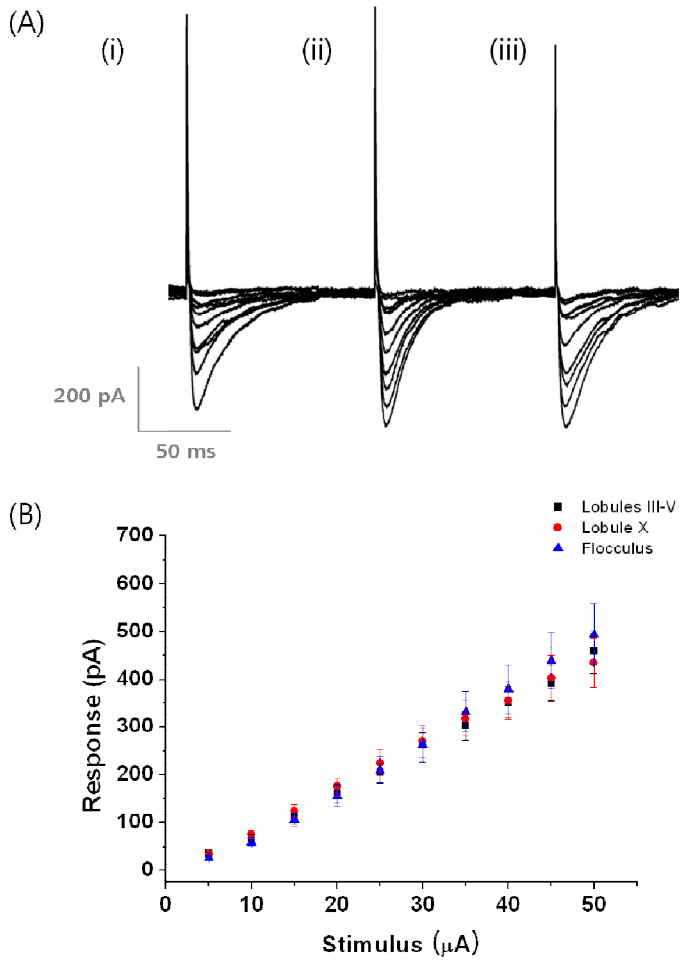


Figure 1. PF−PC synaptic transmission shows no significant difference among three groups of Purkinje cells. (A) Raw trace of EPSC at stimulus intensity increased by 5 ㎂ from 5 to 50 ㎂ (i) vermis lobule III−V, (ii) vermis lobule X and (iii) flocculus (B) Mean peak amplitudes of PF−EPSCs of Purkinje cells at lobules III to V (black, n=36), lobule X (red, n=26) and flocculus (blue, n=22)

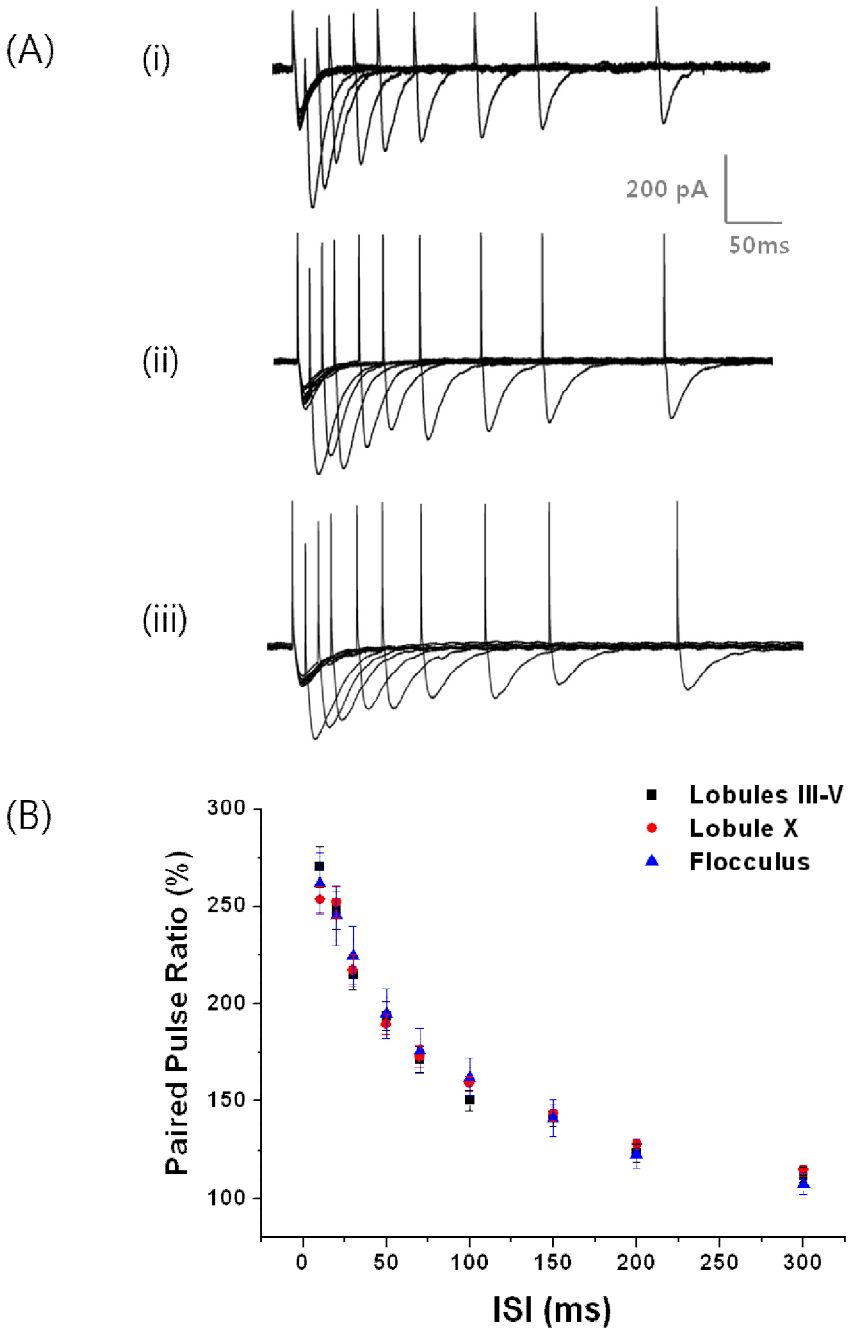
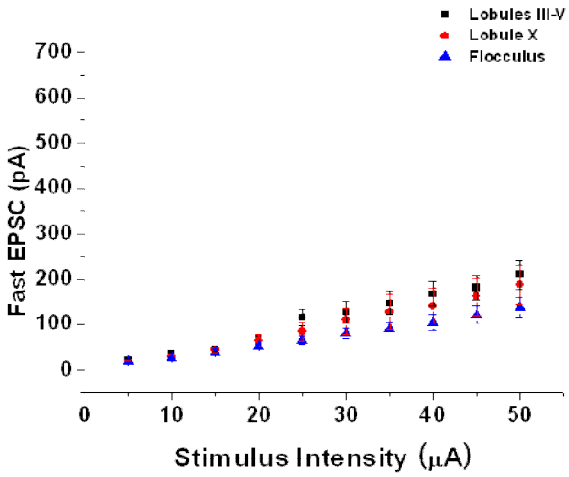
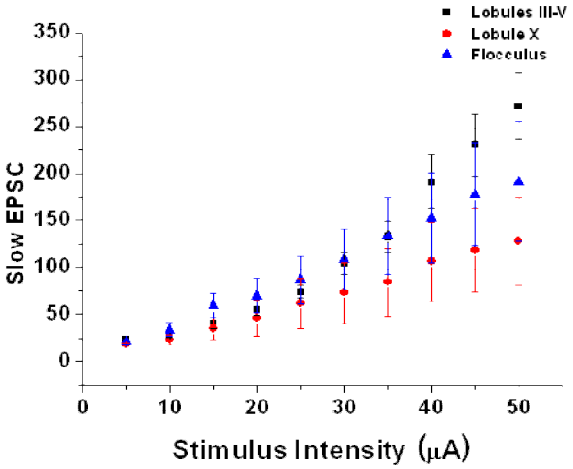

(A)
(i)
200 pA
50ms
(ii)
(iii)
(B)
Lobules III-V
Lobule X
Flocculus
Paired Pulse Ratio (%)
300
250
200
150
100
0
50
100
150
200
250
300
ISI (ms)

Figure 2. Short-term plasticity at PF-PC synapse shows no difference among three groups of Purkinje cells. Paired-pulse facilitation of PF-EPSCs of Purkinje cells at lobules III to V (black, n=36), lobule X (red, n=26) and flocculus (blue, n=22) at different interspike interval (ISI) showed no significant difference among three groups of Purkinje cells. (A) Raw traces of PPR recordings at ISI = 10, 20, 30, 50, 70, 100, 150, 200, and 300ms. (B) The second response is expressed as a percentage of the response to the first pulse and plotted as a function of ISI (10, 20, 30, 50, 70, 100, 150, 200, and 300ms). Holding potential was −70mV.

(A)


Lobules III-V
Lobule X
Flocculus
Fast EPSC (pA)
0
100
200
300
400
500
600
700
0
10
20
30
40
50
Stimulus Intensity (μA)

(B)


Lobules III-V
Lobule X
Flocculus
Slow EPSC
0
50
100
150
200
250
300
350
0
10
20
30
40
50
Stimulus Intensity (μA)

(C)

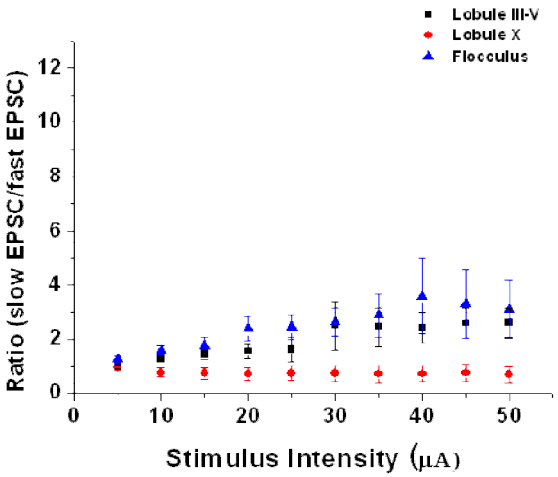

Lobule III-V
Lobule X
Flocculus
Ratio (slow EPSC/fast EPSC)
0
2
4
6
8
10
12
0
10
20
30
40
50
Stimulus Intensity (μA)

Figure 3. Whole-cell Recordings, at vermis lobules III to V (n=20), lobule X (n=19) and flocculus (n=12), with bath application of 2.5μM NBQX and aCSF. Stimulating electrode was placed on PF and gave titanic stimulus. AMPAR-mediated fast EPSC and mGluR-mediated slow EPSC were measured.

(A) Fast EPSC in presence of NBQX was the same for all three groups of PCs. (B) Slow EPSC at PCs in lobule X was significantly different from that in lobule III to V. Slow EPSC at PCs in flocculus was not significantly from that in lobules III to V or that in flocculus.

(C) Ratio of Slow EPSC over Fast EPSC was compared. Ratio of PCs in lobule X was significantly different from that in lobules III to V and that in flocculus.

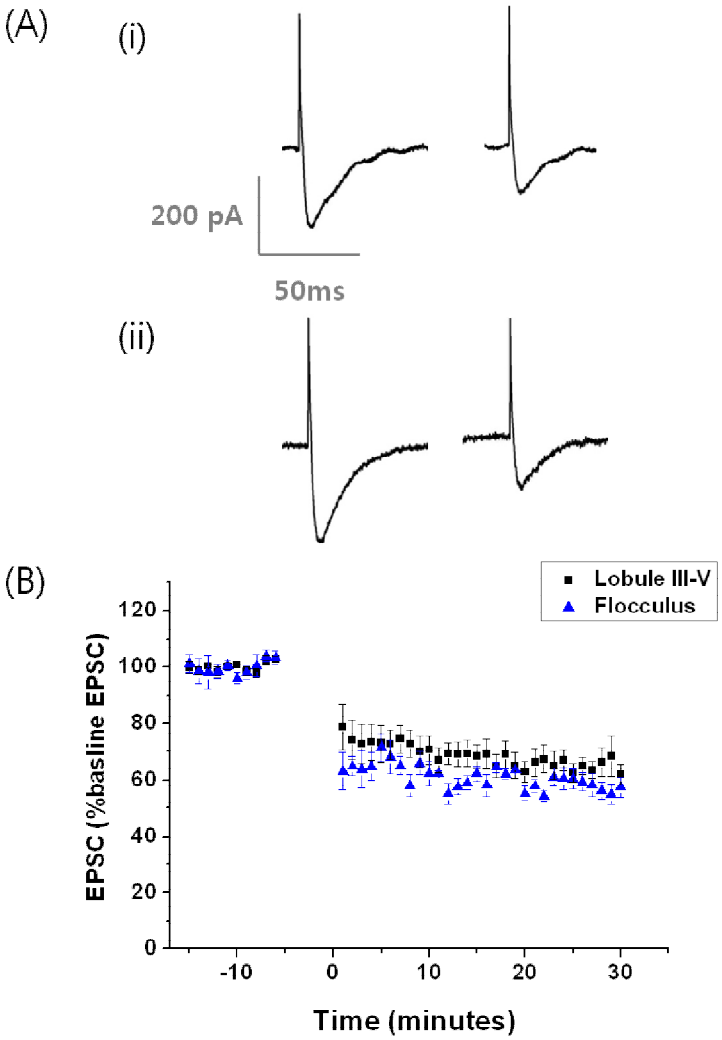

(A)
(i)
200 pA
50ms
(ii)
(B)
Lobule III-V
Flocculus
EPSC (%basline EPSC)
120
100
80
60
40
20
0
-10
0
10
20
30
Time (minutes)

Figure 4. Long-term Depression induced by CF/PF pairing stimulation was not significantly different between vermis lobules III to V and flocculus. (A) Raw traces of EPSC before and 20 minutes after LTD induction (i) vermis lobules III to V (black, n=11) and (ii) flocculus (blue, n=6) (B) Time course graph indicates percentage change of fast EPSC amplitude before and after LTD induction.

DISCUSSION

Flocculus is a part of cerebellum of which function is to regulate balance and movements. It is supposed to be the initial site of VOR learning and mGluR1-mediated long-term depression is supposed to be the mechanism for it. However, many studies on VOR conducted LTD induction in vermis. However, different parts of cerebellum receive different inputs. Thus LTD can be different depending on the region.

In order to study the electrophysiological properties in flocculus and compare them with those in other parts of cerebellum, juvenile mice were sacrificed and cerebellums were separated. Then, the cerebellum was sliced either in parasagittal plane, for vermis recordings, or in coronal plane, for flocculus recordings. Basal synaptic transmission, short-term plasticity, and long-term plasticity were analyzed.

I/O relationship curve shows basal synaptic transmission in neurons. Changes in PPR indicate presynaptic changes in neurotransmitter release (9, 10) and are related to the short-term plasticity. Here, PCs at flocculus had the same I/O curve and PPR as PCs at lobules III to V and PCs at lobule X

did. Hence flocculus has the same basal synaptic transmission and short term plasticity as lobule III to V and lobule X.

PF bursts activate mGluR1-mediated slow EPSC (8). Slow EPSC is related to calcium transient and induction of mGluR1-mediated LTD (7). Slow EPSC was not induced in Lobule X whereas slow EPSC was induced in Lobule III (2). It was questionable that flocculus would act like lobule X, another part of vestibulocerebellum, or act like Lobule III, where LTD can be induced. First, fast EPSCs were compared in order to exclude any chance of differences in NBQX effect. In figure 1, fast EPSC in absence of NBQX was the same for all three regions. In figure 3 (A), fast EPSC in presence of NBQX was the same for all three regions as well. Effect of NBQX was the same for all three regions. Slow EPSC induced in lobules X was smaller than that in lobule X. Slow EPSC induced in flocculus was not significantly different from that in lobules III to V or that in lobule X. Since slow EPSC was induced enough in flocculus and it is related to long-term depression induction, it may be possible that LTD can be induced in flocculus.

Long-term Depression is thought to be the underlying mechanism of learning of regulation of movement at cellular

level. Also, when mGluR1 is inhibited in flocculus, VOR learning is impaired (6). Thus, it has been assumed that LTD in flocculus governs the VOR learning and regulation of balance via conveying vestibular information. LTD can be induced by repetitively stimulating climbing fiber and parallel fiber. When such protocol was used, fast EPSC was reduced in lobule III to V and flocculus at similar degree; but in lobule X, reduction in fast EPSC was smaller. Even though both lobule X and flocculus are part of vestibulocerebellum, they have different electrophysiological properties. LTD was not induced in lobule X PF-PC synapse but it was induced in flocculus PF-PC synapse. This is consistent with the many previous reports suggesting that VOR learning and mGluR1-mediated LTD at flocculus is related. Thus this may also support Ito's hypothesis that memory forms at flocculus by LTD.

Lobules III to V of vermis and lobule X are physically close, and lobule X and flocculus receive the same inputs. All three regions have the same or similar presynaptic function. However, lobules III to V and flocculus have similar postsynaptic electrophysiological properties and differ from lobule X. Further studies can be done to investigate underlying

mechanisms such as differential expression of gene or molecular distribution.

From this study, one may conclude that there is reasoning for recording and evaluating LTD at flocculus for mice. Hence further study will be conducted on mice having impaired VOR learning and compare LTD in flocculus and lobules III to V.

REFERENCES

1. Kandel ER, Schwartz JH and Jessell, TM. Principles of Neural Science, 4th ed. McGraw-Hill . 2000 : 257.

2. Wadiche et al. Gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? Nat Rev Neurosci. 2002 Aug; 3(10): 281-290.

3. Kim CH et al. Lobule-specific membrane excitability of cerebellar Purkinje cells. J Physiol. 2012 Jan; 590 : 273-288

4. Kim YS et al. Dopamine signaling is required for depolarization-induced slow current in cerebellar Purkinje cells. J Neurosci. 2009 July 1;29(26): 8530-8538

5. Ito M. Cerbellar Long-Term Depression : Characterization, Signal Transduction, and Functional Roles. Physiol Rev. 2001 July; 81(3) 1143-1195.

6. Broussard et al. Motor Learning in VOR : the Cellular Compononent. Exp Brain Research. 2010 ; 210(3-4): 451-463.

7. Canepari M, Auger C et al. Ca 2+ Ion Pearmeability and Single-Channel Properties of the Metabotropic Slow EPSC of Rat Purkinje Neurons. J Neurosci. 2004 ; 24(14): 3563-3573

8. Kim SJ and Linden D. Ubiquitous Plasticity and Memory Storage. Neuron. 2007 ; 56: 582-592

9. McNaughton BL. Long-Term Synaptic Enhancement and Short-Term Potentiation in Rat Fascia Dentata Act

through Different Mechanisms. J Physiol. 1982 ; 324: 249-262

10. Schulz PE, Cook EP, Johnston D. Changes in Paired-Pulse Facilitation Suggest Presynapatic Involvement in Long-term Potentiation. J Neurosci. 1994; 14: 5325-2337

11. Ito M. Long-term depression. Annual Review of Neuroscience. 1989; 12 : 85-102

12. Ito M, Sakurai M et al. Climbing Fiber Induced Depression of Both Mossy Fiber Responsiveness and Glutamate Sensitivity of Cerebellar Purkinje Cells. J Physiol-London. 1982 Mar; 324 : 113-134

13. Kim JJ and Thompson RF. Cerebellar Circuits and Synaptic Mechanisms involved in Classical Eyeblink Conditioning. Trends in Neuroscience. 1997; 20(4) : 177-181

14. Larkman AU and Jack JJB. Synaptic Plasticity : Hippocampal LTP. Current Opinion in Neurobiology. 1995; 5 : 324-334

15. Jin Y, Kim SJ, Kim J, Worley PF, and Linden D. Long-term depression of mGluR1 signaling. Neuron. 2007 July; 55(2) : 277-87

국문 초록

소뇌는 운동 조절에 중요한 뇌의 한 부분이다. 소뇌의 기능을 확인하기 위해 전정안구반사 (vestibulo-ocular reflex, VOR) 학습을 주로 사용한다. 소뇌는 입력 받는 정보에 따라 크게 세 부분, 즉 cerebrocerebellum, spinocerebellum, 그리고 vestibulocerebellum 으로 나눠질 수 있다. Spinocerebellum 은 척수와 청각 기관에서 정보를 받는 반면, vestibulocerebellum 은 반고리관과 전정핵에서 정보를 받는다. Vestibulocerebellum 과 spinocerebellum 이 다른 정보를 받고 vestibulocerebellum 이 평형과 안구운동을 조절하는 곳임에도 불구하고, spinocerebellum 에서 주로 기록이 행해진다. 소뇌 편엽이 전정안구반사 학습의 첫 장소로 여겨지며 장기 저하가 그 기작으로 추축된다. 아직 소뇌 편엽 절편에서 장기 저하가 보고되지 않고 있다.

여기서는 whole-cell 기록이 소뇌 충부(vermis)의 III 번부터 V 번까지의 lobule, X 번 lobule 과 편엽에서 이뤄졌으며, III 번부터 V 번까지의 lobule 은 spinocerebellum 에 해당하고, 나머지 두 부위는 vestibulocerebellum 에 해당한다. 기록은 장기 저하와 다른 전기생리학적 특성으로 분석되었다. 첫째로, fast EPSC 가 기록되었

고, input-output curve 와 paired pulse ratio 에서 유의한 차이를 보이지 않았으므로, 이들은 시냅스 전 기능이 비슷하다고 말할 수 있다. 둘째로, NBQX 처리시의 slow EPSC 를 기록하였다. Vestibulocerebellum 으로 알려진 소뇌 충부 lobule X 에서는 slow EPSC 가 나오지 않는다고 알려져 있고, 여기에서는 상대적으로 적게 나오는 것으로 보여졌다. 반면, 다른 vesetibulocerebellum 인 편엽에서는 III 번부터 V 번까지의 lobules 에서와 비슷한 크기의 slow EPSC 가 나왔다. 셋째로, 편엽에서 장기저하를 기록하는 것이 가능하며, lobules III 번부터 V 번에서와 같은 경향성이 보여진다. 위치적으로는 소뇌 충부 내 부분들이 가깝지만, 시냅스 후 전기생리학적 성질은 III 번부터 V 번까지의 lobule 과 편엽은 비슷한 성질을 가지고, X 번 lobule 은 다른 성질을 가지는 것으로 보여진다. VOR 에 장애가 있는 knock-out 생쥐를 사용하여 추후 실험을 진행할 것이다.

주요어 : 소뇌, 충부, 편엽, 신경가소성, 장기 저하

학 번 : 2011-21952