의학석사 학위논문

Effect of pharmacological inhibition of protein phosphatase Shp2 on hippocampal plasticity

탈인산화효소 Shp2의 약리학적 억제가 해마 시냅스 가소성에 미치는 영향

2022 년 8 월

서울대학교 대학원

의과학과 의과학 전공

장 한 별

A Thesis of Master's Degree

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August 2022

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지도교수 이 용 석

이 논문을 의학석사 학위논문으로 제출함

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장한별의 의학석사 학위논문을 인준함

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Effect of pharmacological inhibition of protein phosphatase Shp2 on hippocampal plasticity

by
Hanbyul Jang

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

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Abstract

Protein phosphorylation is required for numerous biological processes. Especially, it participates in synaptic plasticity which is basis of learning and memory. Although it has been well known that protein tyrosine phosphatases are involved in mGluR-LTD, which specific phosphatases are critical for this type of synaptic plasticity is not clear yet. In this study, I discovered that protein tyrosine phosphatase Shp2 is critically involved in mGluR-LTD in mouse hippocampus by using extracellular field recording. Interestingly, Shp2 inhibitor did not block the NMDAR-LTD. Interestingly, Shp2 inhibitor blocked NMDAR-LTP in an induction protocol dependent manner: Inhibition of Shp2 did not block TBS-induced LTP, while it blocks HFS-induced LTP. In all, my data demonstrate that Shp2 is a key molecule regulating mGluR-LTD and HFS-induced LTP in mouse hippocampus, albeit the detailed mechanism remains to be investigated.

Keyword: Synaptic plasticity, protein tyrosine phosphatase, Shp2, electrophysiology, mGluR-LTD, NMDAR-LTD, NMDAR-LTP

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Introduction

1.1. Study Background

Protein phosphorylation, a type of post-translational modification, is a remarkable process that controls cellular signal transduction and even synaptic plasticity. In terms of long-term potentiation (LTP), protein kinase such as CaMKII react to strong and fast Ca^{2+} influx through N-Methyl-D-Aspartate (NMDA) receptor and phosphorylate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, GluA) receptor to induce an exocytosis of AMPA receptor (Barria, Derkach, & Soderling, 1997; Yang, Tang, & Zucker, 1999). In long-term depression (LTD), protein phosphatase such as Calcineurin perform dephosphorylation on AMPA receptor for its endocytosis(Yan et al., 1999; Yang et al., 1999).

Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (Shp2) is a non-receptor protein tyrosine phosphatase which is involved in several cell signaling such as RAS/MAPK pathway, PI3K/AKT pathway, and JAK/STAT pathway. Although *Ptpn11* (gene name of Shp2) is the first identified proto-oncogene that encodes protein tyrosine phosphatase, it acts not only as a factor of cancer but also as a factor of neurodevelopmental disease (Chan & Feng, 2007; Tartaglia et al., 2001). Hyperactivation of Shp2 results in Noonan syndrome (Tartaglia et al., 2001).

Shp2 consists of three characteristic domains which are N-SH2, C-SH2, PTP domain (Hof, Pluskey, Dhe-Paganon, Eck, & Shoelson, 1998). In normal state, N-SH2 blocks PTP domain to block phosphatase activity (Hof et al., 1998). After combining p-Y motif with N-SH2 and C-SH2, their autoinhibition within Shp2 is disrupted and PTP is freely released (Hof et al., 1998; Qiu et al., 2014). Shp2 also has two tyrosine sites (Y542 and Y580). Phosphorylation of those tyrosine site indicates active state of Shp2 and even can be provided as the p-Y motif to N-SH2 and C-SH2 (Neel, Gu, & Pao, 2003).

There are two distinctive types of LTD – NMDAR-LTD and metabotropic glutamate receptor (mGluR)-LTD. As the receptors managing those two types of LTD are disparate, the phosphatases involved in each LTD are different. For instance, serine/threonine phosphatases are involved in NMDAR-LTD and tyrosine phosphatases are involved in mGluR-LTD (Gladding et al., 2009).

Although it has been reported that nonspecific protein tyrosine phosphatase (PTP) inhibitors (phenylarsine oxide and orthovanadate) reverse mGluR-LTD by (S)-3, 5-dihydroxyphenylglycine (DHPG), discerning the identity of a specific protein tyrosine phosphatase involved in mGluR-LTD (Huang & Hsu, 2006). So far, only two PTPs are reported – a striatal-enriched protein tyrosine phosphatase (STEP) in hippocampus and a megakaryocyte protein tyrosine phosphatase (PTPMEG) in cerebellum (Kohda et al., 2013; Y. Zhang et al., 2008).

STEP is the first identified PTP which is involved in mGluR-LTD. Translation of STEP is facilitated by dose-dependent DHPG stimulation (Y. Zhang et al., 2008). In addition, STEP substrate-trapping construct blocks an internalization of GluA1 and GluA2 (Y. Zhang et al., 2008). In STEP knock-out mice, DHPG-induced GluA1 and GluA2 internalization is absent (Y. Zhang et al., 2008). Similarly, it has been reported that PTPMEG-induced GluA2 dephosphorylation is important in mGluR-LTD (Kohda et al., 2013). PTPMEG binds to C-terminal of GluD2 and dephosphorylate Y876 of GluA2 (Kohda et al., 2013). Dephosphorylation of Y876 enables phosphorylation of GluA2 S880 and it changes S880 anchoring protein GRIP to PICK1 allowing AMPAR endocytosis (Kohda et al., 2013).

Recently, it has been reported that Shp2 takes part in LTP via AMPA receptor trafficking (B. Zhang et al., 2016). While LTP, Shp2 moves to PSD with GluA1, leading the increased number of activated Shp2. Additionally, inhibition or knock out of Shp2 suppresses the trafficking of GluA1 to membrane and disrupts LTP (B. Zhang et al., 2016). However, it remains unclear whether Shp2 regulates other synaptic plasticity except LTP.

1.2. Purpose of Research

In this study, I investigated the role of Shp2 in synaptic plasticity using extracellular field recording. Treatment of Shp2 inhibitor blocks DHPG and PP-LFS induced mGluR-LTD. In terms of

synaptic plasticity, mutation form of Shp2 was well studied, but wild-type form was not well understood. Therefore, I aimed to find the new role of wild-type shp2 in synaptic plasticity

Materials and Methods

Mice

Male wild-type mice (C57Bl/6J) were purchased from Orient Bio Inc. (South Korea). 7~8 weeks-old mice were used for mGluR-LTD, NMDAR-LTP and 3~4 weeks-old mice were used for NMDAR-LTD. Mice were housed under Specific pathogen free (SPF) condition with controlling humidity (40~60 %) and temperature (23 ℃ ± 3 ℃). Around 4 mice share one cage which is applied with individually ventilated cage systems (IVC systems) and each room undergoes 12 h light/dark cycle. Food and water were provided to mice *ad libitum*. All animal experiments in this study were approved by Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Registration number: SNU-191203-3-4).

Materials

(S)-3,5-DHPG, D-AP5, MPEP were obtained from Hello bio (Bristol, UK). The Shp2 inhibitor NSC87877 were purchased from Tocris Bioscience (Bristol, UK).

Hippocampal slice preparation

Mouse was quickly decapacitated following isoflurane anesthesia. Dorsal hippocampal sagittal slices were obtained by a vibratome (Campden, 7000 smz-2) in ice-cold artificial cerebrospinal fluid

(ACSF; 120 mM NaCl, 3.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 10 mM D-glucose, and 26 mM $NaHCO_3$, oxygenated with mix gas containing 95% oxygen and 5% carbon dioxide). Slices were incubated in room temperature ACSF for more than 1 hour.

Extracellular field recording

Field excitatory postsynaptic potential (fEPSP) was recorded in the CA1 region with platinum-iridium microelectrode (FHC, UEPMECSEDN3M). Schaffer collaterals were targeted with bipolar stimulating electrode (FHC, CE2C55). Recorded signal was amplified (WPI, DAM80), filtered at 1 kHz and acquired using WinLTP software (WinLTP Ltd., Bristol, UK).

For basal synaptic transmission, Input-output ratio was measured by increasing the stimulation intensity (0 to 100 μA). Paired pulse facilitation (PPF) ratio was measured by calculating the ratio of P2/P1 with 10, 25, 50, 100, 200, 400 ms interpulse interval (IPI) and identified presynaptic function.

All recordings are conducted in stratum radiatum (SR) layer of CA1 region in hippocampus.

In mGluR-LTD, DHPG-LTD was induced by 100 μM DHPG and PP-LFS was induced by 900 paired pulse with 50 ms IPI at 1Hz.

For NMDAR-LTD, another ACSF (124 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 10 mM D-glucose, and 26 mM $NaHCO_3$) and sucrose-based dissection buffer (5 mM

KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM D-glucose, 0.5 mM CaCl2, 10 mM MgCl2, and 212.5 mM sucrose) were used. CA3 region was removed shortly after obtaining the slices to eliminate the CA3-CA3 recurrent synapse. In recording chamber, another ACSF but containing 4 mM $CaCl_2$ was circulated at 25~26 ℃. NMDAR-LTD protocol was modified to 3 trains of 300 pulse at 1 Hz and each train was separated by 5 minutes.

In high frequency stimulation (HFS), the protocol is made up with 100 pulses at 100 Hz. For 4x theta burst stimulation (4XTBS), the protocol is made up with 4 theta bursts in 100 Hz separated by 200 ms.

All LTD protocols were executed at specific stimulation intensity which can induce the 60 % of maximum response at 100 μA. On the other hand, all LTP protocols are stimulated at the intensity that can induce 40% of maximum response.

Statistical analysis

Unpaired two-tailed t test was used in comparing average of last 10 minutes and paired pulse ratio after LTD induction.

Statistical analyses were implemented using GraphPad Prism 7.0 (GraphPad software). All data are represented as the mean ± SEM.

Results

Role of Shp2 in basal synaptic transmission and presynaptic function

I first treated Shp2 inhibitor while input/output (I-O) and paired-pulse facilitation (PPF) are recorded. I-O relationship was obtained by increasing stimulation intensity gradually. PPF was calculated by dividing second pulse (P2) to first pulse (P1). The ratio is increased until 50 ms IPI and decreased after it. NSC87877, a Shp2 inhibitor, does not affect the input-output relationship or paired-pulse ratio (Fig. 1A and B). This result represents Shp2 does not affect the basal synaptic transmission and presynaptic function.

Inhibiting Shp2 activity impairs mGluR-LTD

Prior to recording mGluR-LTD with Shp2 inhibitor, I first confirmed a solid PP-LFS protocol to induce mGluR-dependent LTD when treated with the mGluR antagonist, MPEP (Fig. 2). mGluR-dependent LTD was demonstrated by blocked PP-LFS using MPEP (Fig. 2).

Next, I treated NSC87877, the Shp2 inhibitor, and it blocks PP-LFS (Fig. 3). DHPG-LTD, another form of mGluR-LTD which is induced by application of DHPG, was also blocked by NSC87877 (Fig. 4).

Increase in PPR is associated with presence of mGluR-LTD. The concomitant change is observed in normal conditions, but not in Shp2 inhibitor conditions (Fig. 5).

Shp2 is not required for NMDAR-LTD

Because NMDAR-LTD is difficult to induce, instead of delivering consecutive 900 pulses (1 Hz), I delivered 3 blocks of 300 pulses (1 Hz) with 5 min interval. I then confirmed that the new protocol induces NMDAR dependent LTD by treating NMDAR antagonist AP5 (Fig. 6). Unlike in mGluR-LTD (Fig. 3-4), NSC87877 does not block the NMDAR-LTD (Fig. 7).

Induction protocol-dependent effect of Shp2 inhibition on NMDAR-LTP

Lastly, I test the effect of Shp2 on NMDAR-LTP. Two different protocols were used: 4XTBS-LTP and HFS-LTP. NSC87877 treatment had no effect on 4XTBS-LTP (Fig. 8), whereas it impaired HFS-LTP (p = 0.0556) (Fig. 9). It is also consistent with a previous study (B. Zhang et al., 2016).

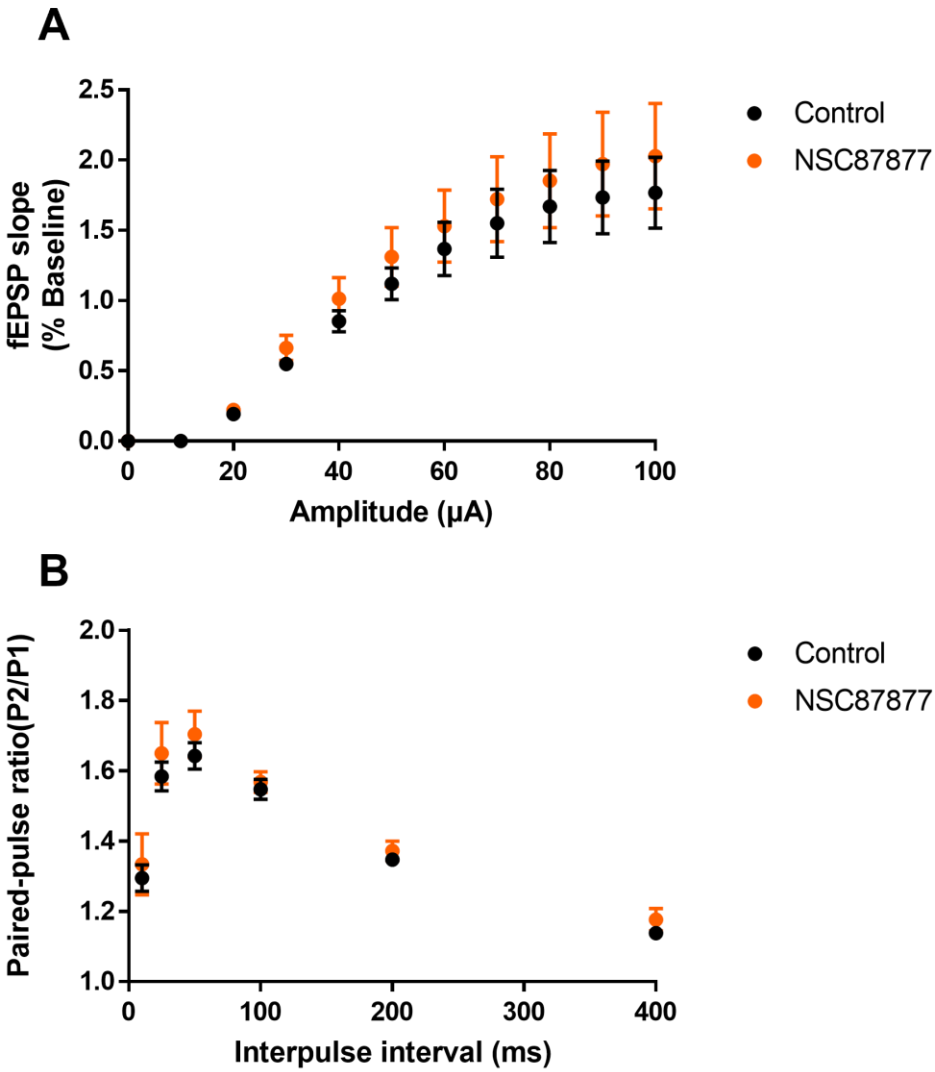

Figure 1. NSC87877 does not affect input/output relationship and paired pulse facilitation.

(A) I-O relationship is unchanged when NSC87877 is treated. Stimulation intensity was 0~100 μA with 10 μA interval. Pulse was injected every 20s. (B) Paired pulse ratio was observed with 10~400 ms of interpulse interval. Control group and treated group show almost same ratio in the graph. (Control, n = 7; NSC87877, n = 6)

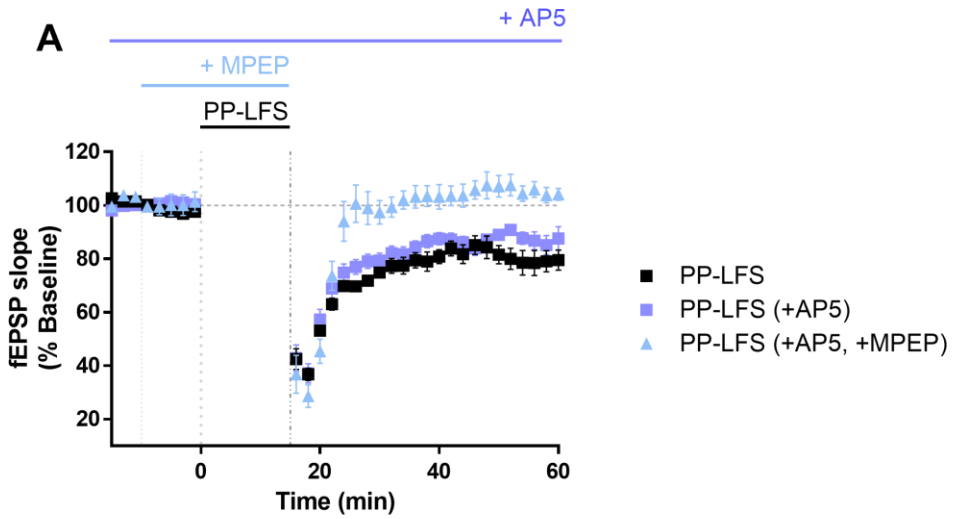

Figure 2. MPEP blocks PP-LFS induced LTD.

(A) PP-LFS was blocked by mGluR antagonist MPEP, while it was not blocked by NMDAR antagonist AP5. (PP-LFS, n = 6; PP-LFS (+AP5), n = 8; PP-LFS (+AP5, +MPEP), n = 4) (Collaboration with Dr. Hyun-Hee Ryu)

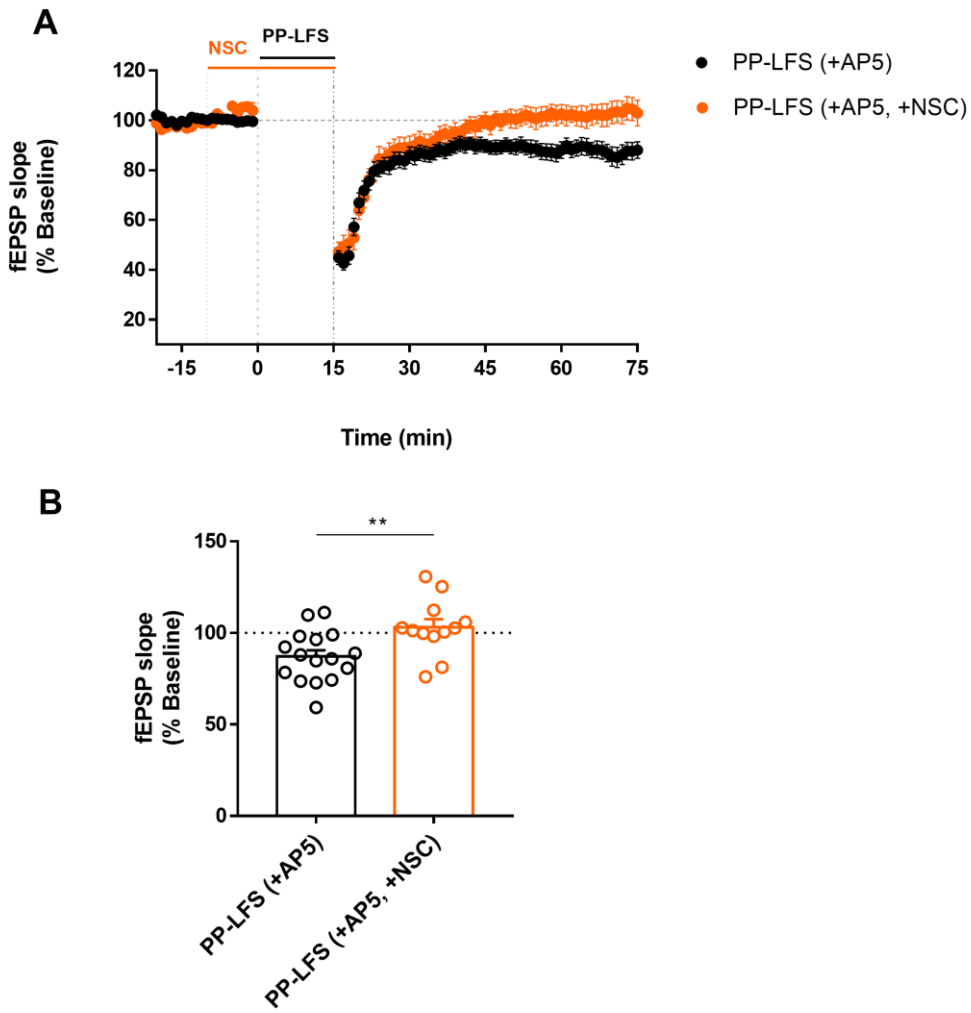

Figure 3. NSC87877 treatment impairs PP-LFS.

(A) NSC87877 (10 μM) blocks PP-LFS (900 paired pulse, 1Hz). **(B)** Average of last 10 minutes of fEPSP slope (% Baseline) is significantly decreased in NSC87877 treated group. (PP-LFS (+AP5), 87.13 ± 3.488, n=16; PP-LFS (+AP5, +NSC), 103.1 ± 4.443, n=12; unpaired t test, $^{**}P$ = 0.0081) (Collaboration with Dr. Hyun-Hee Ryu)

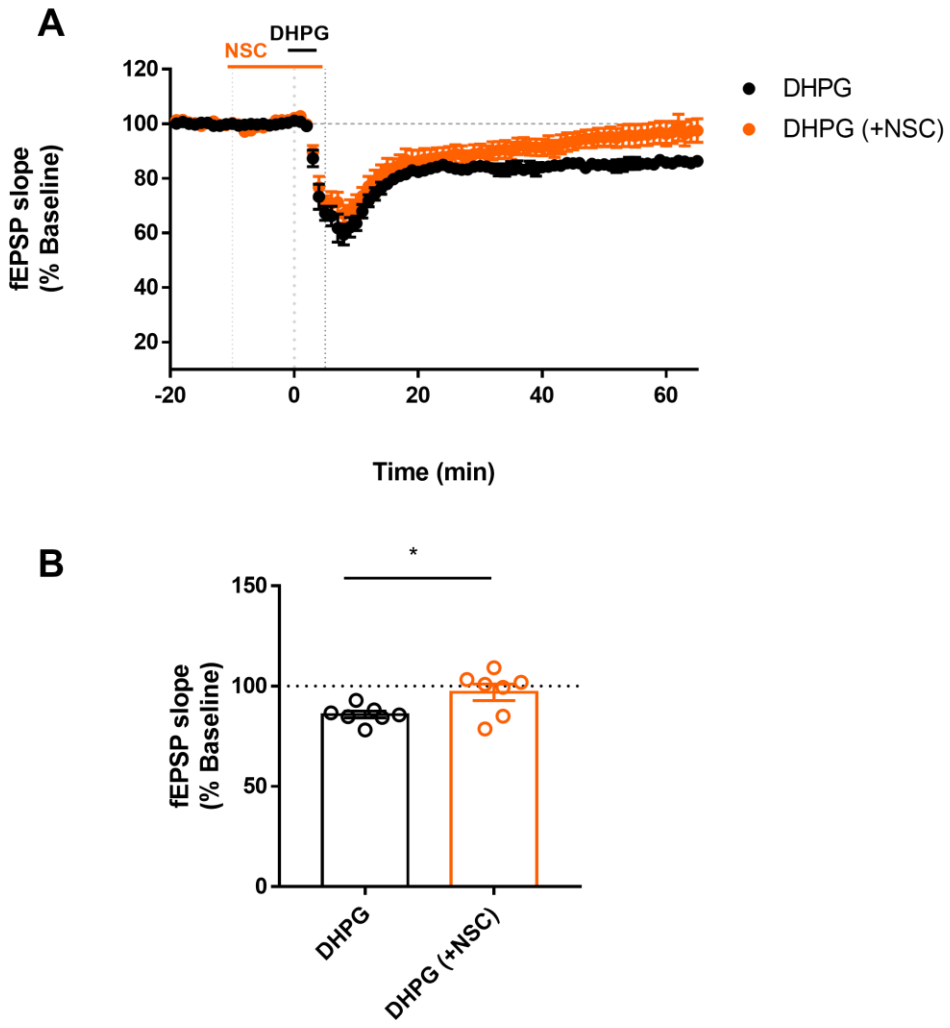

Figure 4. NSC87877 treatment impairs DHPG-LTD.

(A) NSC87877 (10 μM) blocks DHPG-LTD (100 μM, 5 min). **(B)** Average of last 10 minutes of fEPSP slope (% Baseline) is significantly decreased in NSC87877 treated group. (DHPG, 85.85 ± 1.679, n=7; DHPG (+NSC), 96.93 ± 4.123, n=7; unpaired t test, $*P = 0.0285$)

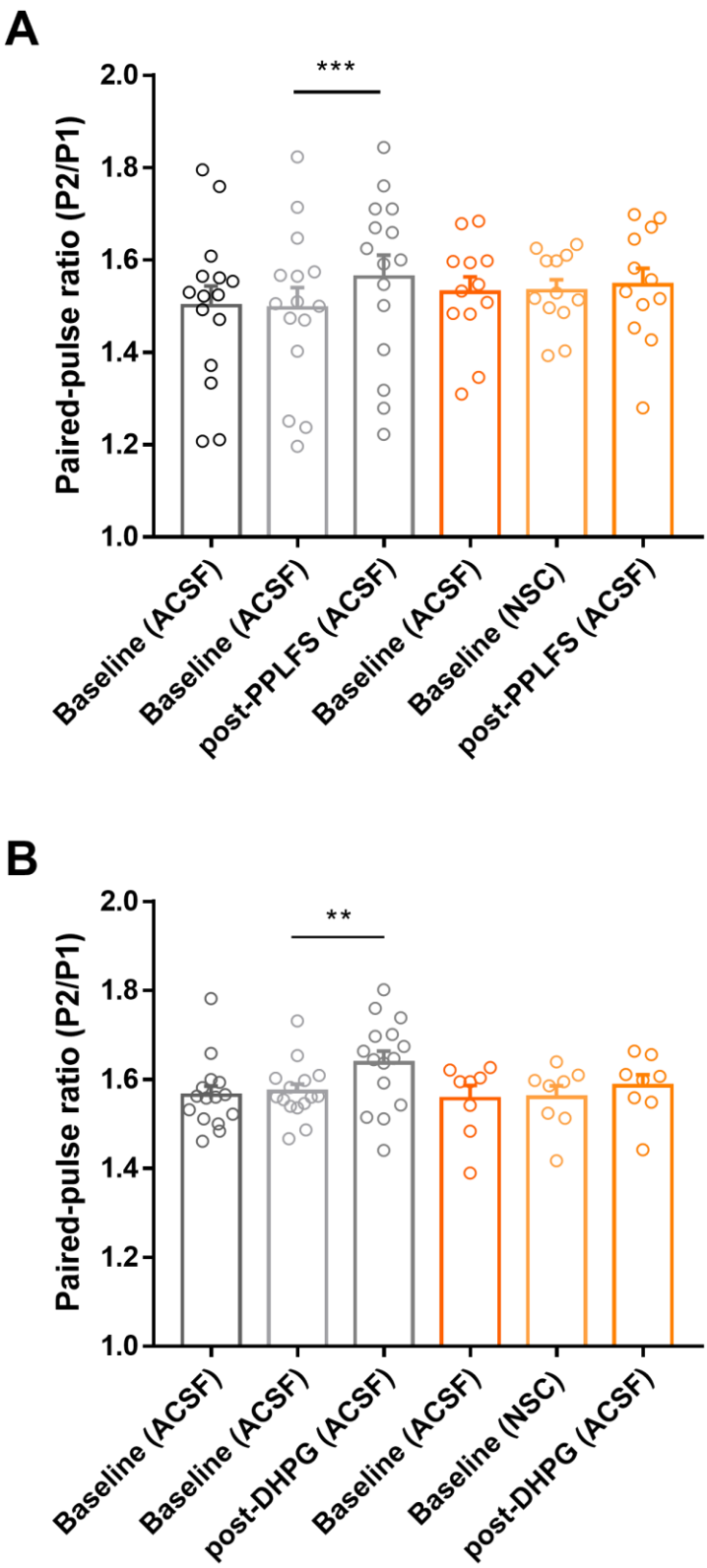
A
***
Paired-pulse ratio (P2/P1)
2.0
1.8
1.6
1.4
1.2
1.0
Baseline (ACSF)
Baseline (ACSF)
post-PPLFS (ACSF)
Baseline (ACSF)
Baseline (NSC)
post-PPLFS (ACSF)
B
**
Paired-pulse ratio (P2/P1)
2.0
1.8
1.6
1.4
1.2
1.0
Baseline (ACSF)
Baseline (ACSF)
post-DHPG (ACSF)
Baseline (ACSF)
Baseline (NSC)
post-DHPG (ACSF)

Figure 5. Treating NSC87877 does not alter PPR associated with mGluR-LTD.

(A) PPR is increased after PP-LFS induction in control group, but not in NSC87877 treated group. (***P = 0.0006) **(B)** PPR is increased after DHPG-LTD induction in control group, but not in NSC87877 treated group. (**P = 0.0011)

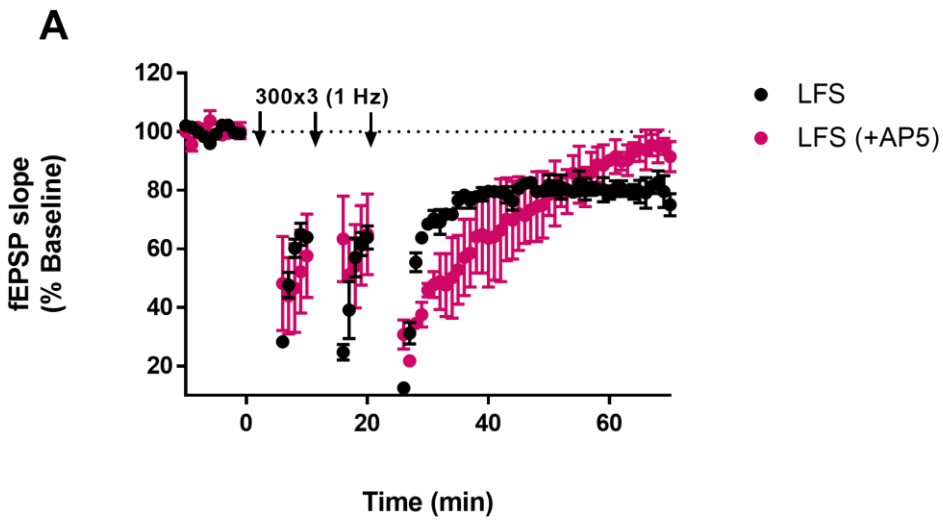

Figure 6. AP5 blocks NMDAR-LTD.

(A) LFS was blocked by NMDAR antagonist AP5. (LFS, n = 2; LFS (+AP5), n = 2)

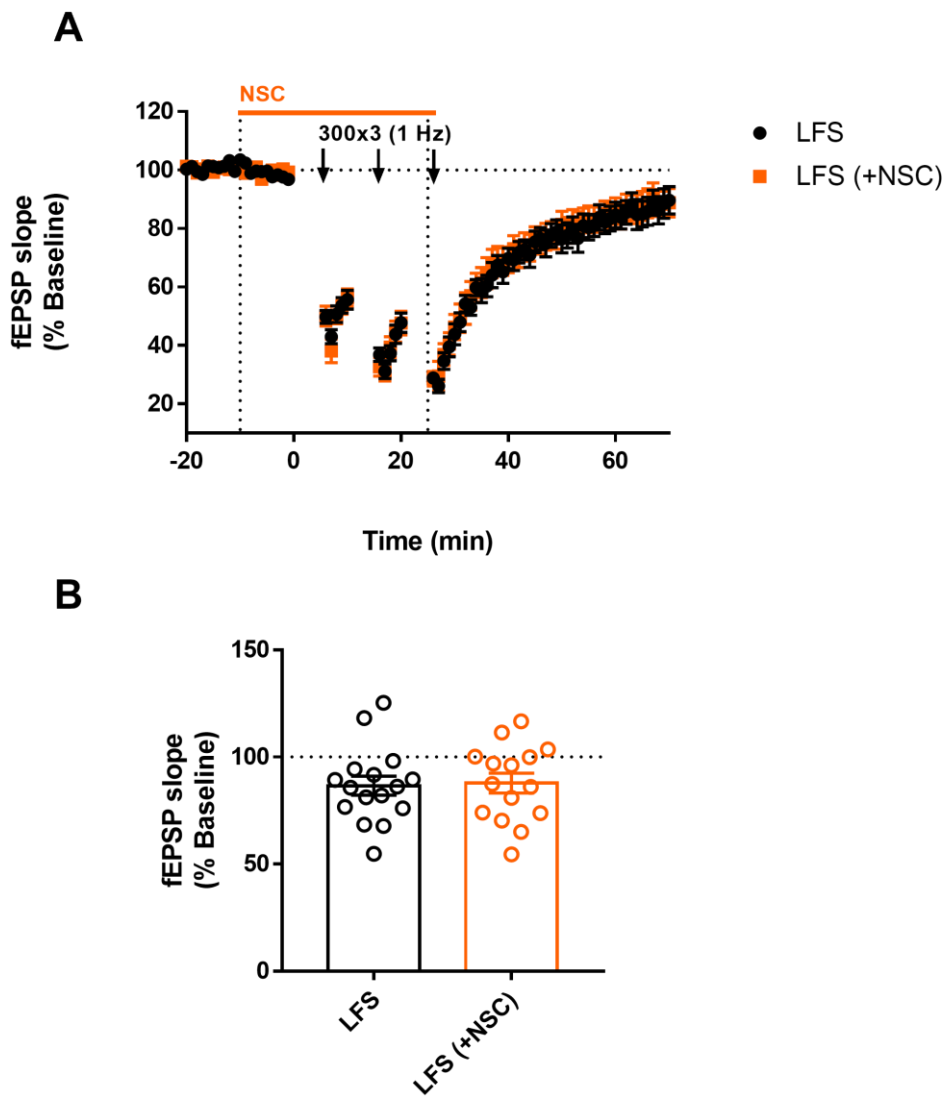

Figure 7. NSC87877 treatment does not block NMDAR-LTD.

(A) NSC87877 (10 μM) has no effect on LFS (900 pulse, 1 Hz). (B) Average of last 10 minutes of fEPSP slope (% Baseline) is roughly same between control group and NSC87877 treated group. (LFS, 86.61 ± 4.418, n=16; LFS (+NSC), 87.81 ± 4.611, n=15)

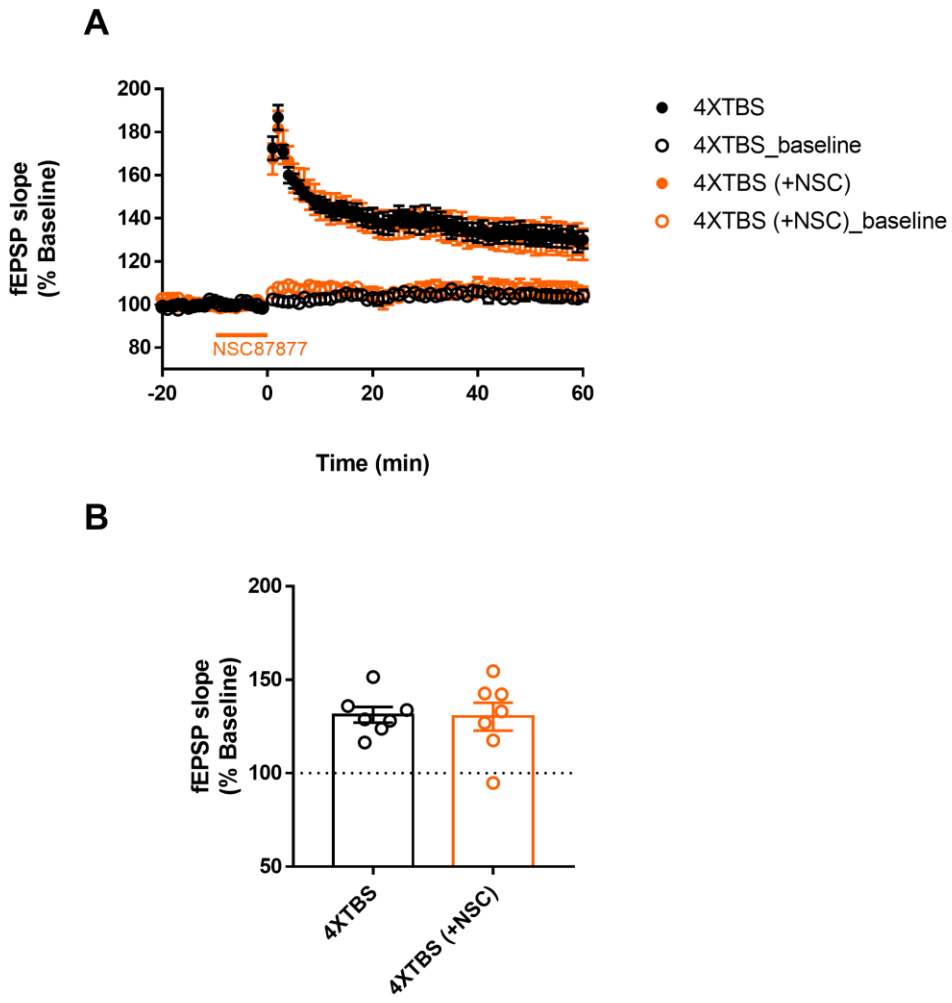

Figure 8. NSC87877 treatment does not block 4XTBS-LTP.

(A) NSC87877 (10 μM) has no effect on 4XTBS-LTP. **(B)** Average of last 10 minutes of fEPSP slope (% Baseline) is roughly same between control group and NSC87877 treated group (4XTBS, 131.2 ± 4.16, =7; 4XTBS (+NSC), 130.3 ± 7.437, n=7)

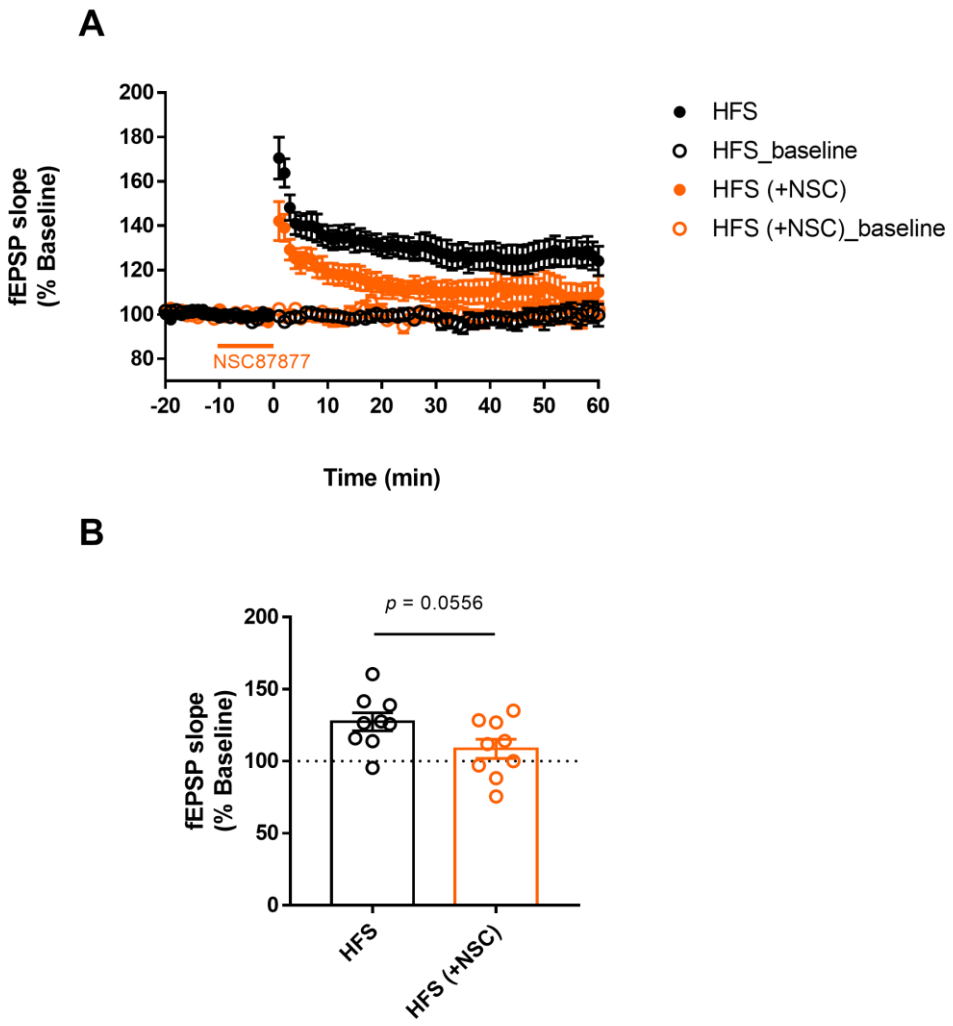

Figure 9. NSC87877 treatment impairs HFS-LTP.

(A) NSC87877 (10 μM) blocks HFS-LTP. (B) Average of last 10 minutes of fEPSP slope (% Baseline) is decreased in NSC87877 treated group (HFS, 127.3 ± 6.197, n=9; HFS (+NSC), 108.6 ± 6.651, n=9; unpaired t test, P = 0.0556)

Discussion

Recent studies reported that protein tyrosine phosphatases participate in mGluR-LTD, but specific molecules are not fully discovered. Here, I found that: (1) protein tyrosine phosphatase Shp2 is participated in mGluR-LTD, especially maintenance of the mGluR-LTD. (2) However, Shp2 does not affect to basal synaptic transmission and presynaptic function. (3) As Shp2 is not required for NMDAR-LTD, Shp2 is selectively involved in LTD according to type of LTD. (4) Similar to the result of NDMAR-LTD, Shp2 has not involved in NMDAR-LTP.

Functional studies of Shp2

Shp2 is well known in field of cancer and neurodevelopmental disease (Nussinov, Tsai, & Jang, 2022). In tumor cell, Shp2 binds with many molecules such as GRB2-associated-binding protein 1 (GAB1), Growth factor receptor-bound protein 2 (GRB2), Son of Sevenless (SOS) and promote cell proliferation or metastasis (J. Zhang, Zhang, & Niu, 2015). For suppressing the tumor growth, Shp2 inhibitors are actively investigated (L. Chen et al., 2006; Y. N. Chen et al., 2016). In addition to small molecule inhibitors, Proteolysis-targeting chimeras (PROTACs) targeting Shp2 are also discovered (Zheng et al., 2021). In field of neurodevelopmental

disease, mutations in Shp2 are well researched. It is well known that Noonan syndrome which is a kind of RASophathies is caused by hyperactivation of Shp2 (Tartaglia et al., 2001). Mutation in Shp2 shows impaired synaptic plasticity(Lee et al., 2014). However, functions of wild-type Shp2 in synaptic plasticity are unclear. There are two reports that explain the role of Shp2 in synaptic plasticity (B. Zhang et al., 2016; B. Zhang & Lu, 2017). Shp2 is activated while LTP induction and it phosphorylate GluA1 to promote the exocytic trafficking (B. Zhang et al., 2016). In addition to LTP, Shp2 is also involved in synaptic homeostasis (B. Zhang & Lu, 2017). Recently, there is a report that Shp2 is involved in NMDAR-LTD (Zhou et al., 2022). It is incompatible with my data, but there are some differences in methods and approaches.

Mechanism for regulating synaptic plasticity of Shp2

According to the report that Shp2 is involved in LTP (B. Zhang et al., 2016), Shp2 ultimately controls two different types of synaptic plasticity. As I mentioned in introduction, calcium can be an example that is involved in two types of synaptic plasticity(Yang et al., 1999). Calcium has different kinetics according to LTP and LTD(Yang et al., 1999). Shp2 also has several kinetics, not only open and closed form. There is a report that Shp2 acts like "multiple gear" and it suggests that Shp2 have three conformational forms (Tao et al., 2021). So, Shp2 can participate in bi-directional

synaptic plasticity. Different kinetics of Shp2 can be measured by Fluorescence Resonance Energy Transfer (FRET) system(Sun et al., 2013). In this report (Sun et al., 2013), engineered Shp2 reporter represents activity of Shp2 and there would be differences between LTP and LTD situations. Protein tyrosine phosphatases commonly regulate mGluR-LTD by dephosphorylate GluA2 AMPAR subunit, while protein serine/threonine phosphatases dephosphorylate GluA1. Likewise, shp2 may also dephosphorylate GluA2 to trigger AMPAR endocytosis. Several kinases would phosphorylate the Shp2 for activation in these processes. They can be Src-family protein tyrosine kinases (SFKs) because SFKs are highly expressed in nervous system and related with glutamate receptors (Hayashi & Huganir, 2004; Hayashi, Umemori, Mishina, & Yamamoto, 1999; Salter & Kalia, 2004; Wagner, Mei, & Huganir, 1991). Therefore, it is possible that Shp2 phosphorylate GluA2 by means of SFKs.

Importance of mGluR-LTD in hippocampus

There are numerous mGluR subtypes, but mGluR5 is abundantly expressed in hippocampus (Ferraguti & Shigemoto, 2006). In hippocampus, excessive mGluR-LTD indicates fragile X syndrome (FXS) (Huber, Gallagher, Warren, & Bear, 2002). Fragile X mental retardation protein (FMRP) suppresses mRNA translation which is associated with mGluR signaling. In the FXS, translation of FMRP is

absent and mGluR-LTD is increased which is the result of exaggerated protein synthesis. Therefore, chemical (Levenga et al., 2011; Michalon et al., 2012; Stoppel, McCamphill, Senter, Heynen, & Bear, 2021) or genetic (Dölen et al., 2007) inhibition of mGluR reverses the phenotype of FXS such as increased dendritic spine density (Levenga et al., 2011), impaired learning and memory (Stoppel et al., 2021), exaggerated mGluR-LTD (Michalon et al., 2012). Also, there are some evidence that mGluR-LTD encodes spatial memory. For example, mice lacking mGluR5 show impaired performance in Morris water maze task or radial arm maze (Lu et al., 1997; Manahan-Vaughan & Braunewell, 2005). Likewise, mGluR-LTD has an important role for disease and learning in hippocampus.

Mechanism of PPR change in DHPG-LTD

It is controversial that PPR change in DHPG-LTD is presynaptic mechanism or postsynaptic mechanism. The paper that discovered PTPs are involved in mGluR-LTD also tested PPR change (Moult et al., 2006). By PTPs inhibitors, PPR change which is caused by DHPG-LTD was also blocked. They tried to figure out expression mechanism of DHPG-LTD by treating actin stabilizing drug jasplakinolide to postsynaptic neuron. As a result, PPR change produeced by DHPG-LTD was also blocked. In other word, PPR change caused by DHPG-LTD is postsynaptic mechanism. However,

there is issue of DHPG concentration that high (100 μM) concentration DHPG induces postsynaptic LTD, whereas low (30 μM) DHPG induces presynaptic LTD with NMDAR activation (Sanderson et al., 2022).

Determining the concentration of chemicals

The concentration of NSC87877 was determined by cell culture study (L. Chen et al., 2006). At least concentration that fully reduces pERK protein level which is increased by EGF stimulation was 10 μM. (S)-3,5-DHPG concentration was determined by two conditions. One is the condition that LTD is well performed until after induction 60 minutes, the other is the condition that can be blocked by mGluR antagonist MPEP.

Concluding remarks

All the results account for the role of wild-type Shp2 in synaptic plasticity, especially in mGluR-LTD. Inhibition of Shp2 by NSC87877 had effects on mGluR-LTD, but not on NMDAR-LTD. It is important that Shp2 is the protein tyrosine phosphatase that involves in mGluR-LTD. When it comes to NMDAR-LTP, inhibition of Shp2 had no effect on 4XTBS-LTP but had blocking effect on HFS-LTP. Almost kinds of synaptic plasticity were screened and it

is discovered that Shp2 selectively involves in synaptic plasticity. However, research on underlying mechanisms is not conducted in this paper. Further studies are required for explaining how Shp2 is involved in mGluR-LTD.

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국 문 초 록

단백질 인산화는 수많은 생물학적 과정에 필요하다. 특히, 단백질 인산화는 학습과 기억의 기반이 되는 시냅스 가소성에도 또한 관여한다. 티로신 탈인산화효소는 mGluR-LTD에 관여하는 후보로 잘 알려져 있다. 그러나, 그것에 관여하는 특정한 분자가 무엇인지는 아직까지 완전히 밝혀지지 않았다. 이 연구에서 세포 외 기록 및 Shp2 저해제를 사용함으로써 티로신 탈인산화효소 Shp2가 mGluR-LTD에 관여한다는 것을 밝혀냈다. 반면에, Shp2 저해제는 NMDAR-LTD를 억제할 수 없었다. 결론적으로 Shp2는 특히 mGluR-LTD에 필요하며 NMDAR-LTD, 기본적인 시냅스 전달 및 시냅스 전 기능에는 필요하지 않았다. Shp2는 단순히 mGluR-LTD만을 억제하지 않고 그것에 수반되는 변화인 PPR의 증가 또한 억제하였다. 종합적으로, Shp2 가 mGluR-LTD에 중요한 분자라는 사실을 확립하였다.

주요어: 시냅스 가소성, Shp2, 전기생리학, 대사성 글루타메이트 수용체 의존적 장기 시냅스 약화, NMDA 수용체 의존적 장기 시냅스 약화, NMDA 수용체 의존적 장기 시냅스 강화

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