

Intracellular Ca²⁺ thresholds for induction of excitatory long-term depression and inhibitory long-term potentiation in a cerebellar Purkinje neuron

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Abbreviations

LTD, long-term depression

RP, rebound potentiation

cAMP, cyclic adenosine monophosphate

mGluR, metabotropic glutamate receptor

EGTA, O,O bis(2-aminoethyl)ethyleneglycol-N,N,N',N tetraacetic acid

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

ATP, adenosine triphosphate

GTP, guanosine triphosphate

GABA, γ -aminobutyric acid

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

SEM, standard error of measurement

Abstract

Synaptic plasticity in the cerebellar cortex contributes to motor learning. In particular, long-term depression at excitatory parallel fiber – Purkinje neuron synapses has been intensively studied as a primary cellular mechanism for motor learning. Recent studies showed that synaptic plasticity other than long-term depression such as long-term potentiation at inhibitory interneuron – Purkinje neuron synapses called rebound potentiation is also involved in motor learning. It was suggested that long-term depression and rebound potentiation might synergistically support motor learning. Here, we have examined induction conditions of long-term depression and rebound potentiation in cultured rat Purkinje neurons, and found that both of them were induced simultaneously by certain patterns of depolarization of a Purkinje neuron. Further, we found that long-term depression was induced by shorter depolarizing pulses causing a smaller intracellular Ca^{2+} increase than rebound potentiation. These results support an idea that long-term depression and rebound potentiation synergistically contribute to motor learning, and suggest that long-term depression may

play a primary role in wider variety of motor learning paradigms than rebound potentiation.

Key words

Long-term depression, rebound potentiation, Ca^{2+} , Purkinje neuron, Cerebellum

Introduction

Synaptic plasticity in a cerebellar Purkinje neuron, in particular long-term depression (LTD) at excitatory parallel fiber synapses has been considered as a primary cellular mechanism for motor learning [1-3]. LTD is the long-lasting decrease in responsiveness to glutamate of a Purkinje neuron, which is induced by co-activation of presynaptic parallel fibers and a climbing fiber. Several LTD-deficient mice show failures in motor learning [3, 4-7], supporting roles of LTD in motor learning. However, the necessity of LTD in motor learning was challenged by demonstration of normal motor learning in LTD-suppressed animals [8, 9]. These results lead to an idea that synaptic plasticity mechanisms other than LTD in the cerebellum might contribute to motor learning [2, 10, 11]. One candidate mechanism is rebound potentiation (RP) at inhibitory interneuron – Purkinje neuron synapses [12, 13]. RP is the long-lasting increase in GABA responsiveness of a Purkinje neuron induced by activation of the climbing fiber [14, 15]. Indeed, RP-deficient transgenic mice show defects in adaptation of vestibulo-ocular reflex, a motor learning paradigm [16].

There are some similarities between LTD and RP. LTD is the depression

of excitatory synaptic response, whereas RP is the potentiation of inhibitory synaptic response. Thus, both work to suppress the activity of a Purkinje neuron. It was also reported that both LTD and RP are induced by large increases in the intracellular Ca^{2+} concentration of a Purkinje neuron [17-19], which can be caused by climbing fiber activation [20]. Further, there are some common molecules in the intracellular molecular signaling cascades regulating LTD and RP such as cAMP, Ca^{2+} /calmodulin-dependent kinase II, metabotropic glutamate receptor mGluR1 etc. [4, 21-24]. Therefore, some mutant mice deficient in LTD such as mGluR1 knockout mice might also have defects in RP, and might show motor learning failure due to defects in not only LTD but also RP [13]. Taken together, we thought that LTD and RP might occur at the same time in certain conditions, work synergistically and compensate the defects of the other during motor learning. However, whether LTD and RP are induced simultaneously in any conditions has not been reported. In this study, we have addressed this issue, demonstrated that RP and LTD were induced at the same time by certain patterns of depolarization of a Purkinje neuron, and then clarified the respective intracellular Ca^{2+} thresholds for inducing LTD or RP.

Materials and Methods

Culture

The methods for preparing primary culture of rat cerebellar Purkinje neurons were similar to previous studies [25]. Whole-cell patch clamp recordings and fluorescent Ca^{2+} imaging were performed 3-4 weeks after preparation of culture. All experiments were carried out in accordance with the guideline regarding care and use of animals for experimental procedures of National Institute of Health, U.S.A. and Kyoto University, and approved by the local committee for handling experimental animals in the Graduate School of Science, Kyoto University.

Whole-cell voltage-clamp experiments

Methods of electrophysiological experiments were similar to previous studies [15, 24, 25]. Whole-cell voltage-clamp recording from a cultured Purkinje neuron was performed in the extracellular solution containing (in mM) 145 NaCl, 5 KOH, 2 CaCl_2 , 1 MgCl_2 , 10 HEPES, and 10 glucose (pH 7.3) at room temperature (20~24 °C). The solution contained tetrodotoxin (1 μM , Wako, Osaka, Japan) and SCH50911 (10 μM , Tocris, Bristol, UK) to

inhibit action potential generation and GABA_B receptor, respectively. A glass patch pipette used for whole-cell recording was filled with the Cs⁺ internal solution containing (in mM) 155 CsCl, 10.5 CsOH, 0.5 EGTA, 10 HEPES, 2 Mg-ATP (Sigma, St. Louis, MO) and 0.2 Na-GTP (Sigma), or with the mixed K⁺ and Cs⁺ solution containing (in mM) 116 CsCl, 39 KCl, 9 CsOH, 3 KOH, 1 EGTA, 10 HEPES, 2 Mg-ATP and 0.2 Na-GTP (pH 7.3). Mg-ATP and Na-GTP were used to minimize rundown of ion channels. The membrane potential of a Purkinje neuron was corrected for the liquid junction potential (-3 mV) and was held at -70 mV. To minimize the series resistance error, the amplitude of glutamate or GABA response at the beginning of experiments was set around 300 pA or 200 pA, respectively. Only recordings with an input resistance of more than 100 MΩ and series resistance of less than 25 MΩ were accepted for analyses. Series resistance and input resistance were monitored every 2 min, and experiments were terminated when a change of more than 20 % in one or both of them was detected.

The methods for iontophoretic application of glutamate or GABA were similar to previous studies [15, 24-26], except that we used basic GABA

containing solution in this study. Glass pipette containing 10 mM glutamate solution titrated to pH 7.3 with HEPES and NaOH, and 10 mM GABA solution titrated to pH 12.6 with NaOH were aimed at two different primary or secondary dendrites, which were about 100 μm apart. Then, the 20 msec negative voltage pulses were applied to the glutamate or GABA containing pipette alternately every 20 seconds. We used the negative voltage pulses for application of both transmitters by controlling pH of solutions, because independent control of drug applications was difficult when the directions of voltage were opposite in the two pipettes. Data are presented as mean \pm SEM. Statistical significance was assessed by paired Student's t-test.

Ca²⁺ imaging

Intracellular Ca²⁺ concentration was monitored with a Ca²⁺ imaging system (Aquacosmos, Hamamatsu Photonics, Japan) mounted on an upright microscope (BX50WI, Olympus, Japan) using fura-4F (50 μM , Invitrogen, USA). Fura-4F was introduced into a Purkinje neuron through a whole-cell patch pipette, and excited alternately with 340 and 380 nm light for 310

msec. Each fluorescence image was recorded at 1 Hz, and the fluorescence ratio (the fluorescence excited at 340 nm divided by that at 380 nm, F340/F380) was calculated. The Ca^{2+} concentration was estimated from F340/F380 as in previous studies [18, 27]. F340/F380 depends on not only the intracellular Ca^{2+} concentration but also pH and various intracellular proteins etc. Thus, the estimated values should be regarded as rough approximations.

Results

LTD and RP were examined in cultured Purkinje neurons prepared from rat neonatal cerebella. A Purkinje neuron was whole-cell voltage-clamped at -70 mV with the intracellular Cs^+ solution, and glutamate and GABA were applied to different dendritic branches by electrophoresis (Fig. 1A). The decrease in amplitude of glutamate-induced current and the increase in amplitude of GABA-induced current 30 minutes after the onset of conditioning depolarization were regarded as LTD and RP, respectively, as in previous studies [15, 24-26]. Depolarization of a Purkinje neuron to 0 mV

(500 msec, 0.5 Hz, 5 times) decreased the amplitude of glutamate-induced current ($54.6 \pm 12.3\%$, $n = 5$, $p = 0.016$), and increased that of GABA-induced current ($128 \pm 11\%$, $n = 5$, $p = 0.019$) (Fig. 1A, B). Thus, LTD and RP were induced simultaneously by a certain pattern of conditioning depolarization.

Next, we aimed to find the thresholds for inducing LTD or RP in order to clarify which occurs more easily. Previous studies showed that both LTD and RP induction depend on the leaky time integration of the intracellular Ca^{2+} concentration [17-19]. We tested various conditions in which intracellular Ca^{2+} concentration could be relatively well controlled. Use of K^+ and Cs^+ mixed (1 : 3) intracellular solution and 50 depolarization pulses at 5 Hz enabled us to regulate the intracellular Ca^{2+} concentration monitored as F340/F380 of fura-4F in a desirable range by changing the duration of each depolarization pulse (Fig. 2). The depolarization pulses of 5, 10, 50, 150 msec duration increased the peak intra-dendritic Ca^{2+} concentration to $0.60 \pm 0.09 \mu\text{M}$ ($n = 8$), $1.07 \pm 0.25 \mu\text{M}$ ($n = 7$), $1.93 \pm 0.36 \mu\text{M}$ ($n = 7$), $2.52 \pm 0.48 \mu\text{M}$ ($n = 6$) from the base line value of $0.08 \pm 0.01 \mu\text{M}$ ($n = 28$), and made the time integration of intracellular Ca^{2+} increase to $6.20 \pm 1.06 \mu\text{M}\cdot\text{sec}$, $10.4 \pm 1.8 \mu\text{M}\cdot\text{sec}$, $22.0 \pm 2.0 \mu\text{M}\cdot\text{sec}$, 33.0 ± 4.6

$\mu\text{M}\cdot\text{sec}$, respectively.

Next, the threshold duration of conditioning depolarization pulse in LTD or RP induction was examined. By the 5 msec depolarization pulses, neither LTD nor RP was induced (Fig. 3). The 10 msec depolarization pulses depressed the glutamate-induced current ($84.9 \pm 7.4\%$, $n = 8$, $p = 0.037$), but did not affect GABA-induced current ($99.5 \pm 4.2\%$, $n = 7$, $p = 0.44$). The 50 msec depolarization pulses also induced LTD ($87.8 \pm 3.1\%$, $n = 7$, $p = 0.049$), but did not induce RP ($112 \pm 6.9\%$, $n = 7$, $p = 0.084$), although transient increase in the amplitude of GABA-induced current occurred 5 min after the depolarization pulses ($146 \pm 10.9\%$, $n = 7$, $p = 0.003$). By the 150 msec depolarization pulses, both LTD ($76.2 \pm 6.1\%$, $n = 8$, $p = 0.002$) and RP ($124 \pm 4.6\%$, $n = 8$, $p = 0.001$) were induced. Thus, LTD was induced by shorter depolarization pulses than RP, suggesting that the threshold of intracellular Ca^{2+} concentration and its time integration for LTD induction are lower than those for RP induction (Figure 4). In addition, we found that the transient increase in GABA responsiveness was induced by slightly weak depolarizing stimulations (50 msec pulses) than the long-lasting RP (150 msec pulses).

Discussion

We demonstrate that LTD and RP were induced by certain patterns of depolarization of a Purkinje cell, and that LTD was more easily induced than RP. Although previous studies showed that certain patterns of increase in the intracellular Ca^{2+} concentration are sufficient to induce either LTD or RP [17-19], direct comparison of induction conditions between them has not been reported. Here, we show that a smaller intracellular Ca^{2+} increase could induce LTD but not RP, suggesting that LTD might occur more often during in vivo learning processes.

LTD is induced by co-activation of parallel and climbing fibers [1-3, 28-30]. However, climbing fiber or parallel fiber stimulation can be replaced by direct depolarization of a Purkinje neuron or glutamate application to induce LTD, respectively [26, 31]. More recently, it was shown that a large increase in intracellular Ca^{2+} concentration by itself can induce LTD [17]. It was proposed that the Ca^{2+} influx caused by climbing fiber activation or direct depolarization of a Purkinje neuron, and Ca^{2+} release from intracellular endoplasmic reticulum through inositol trisphosphate receptor [32, 33] synergistically causes a large enough increase in the intracellular

Ca^{2+} concentration to induce LTD [34]. On the other hand, climbing fiber stimulation or direct depolarization of a Purkinje neuron by itself is known to induce RP [14, 15]. Here, we used depolarization of a Purkinje neuron at a fixed frequency and number to induce both LTD and RP, so that comparison of their induction conditions was possible. Our results indicate that LTD occurs more easily than RP, and that the transient increase in GABA response takes place by a smaller intracellular Ca^{2+} increase than long-lasting RP. The transient potentiation and RP might be regulated differently. We note that this study used a simple model experimental system, and that the induction conditions of LTD and RP in vivo can be influenced by various factors including activation levels of metabotropic receptors such as mGluR1, GABA_B receptor etc. [15, 23].

Marr-Albus-Ito hypothesis assigned a critical role of LTD in motor learning [1-3]. According to this hypothesis, parallel fiber inputs onto a Purkinje cell followed by undesirable behavioral output are suppressed by LTD depending on an error signal conveyed through a climbing fiber. This scheme has attracted many neuroscientists, and has been supported in general. However, two studies challenged this idea. Firstly, it was shown

that pharmacological blockade of LTD in rats does not affect motor learning [8]. Secondly, mutant mice presumably defective in the last step of LTD expression, which is internalization of AMPA-type ionotropic glutamate receptors and thus seems to affect only LTD, show normal motor learning [9]. In addition, several types of synaptic plasticity other than LTD at parallel fiber – Purkinje neuron synapses such as long-term potentiation (LTP) at the same synapses, RP at inhibitory interneuron – Purkinje neuron synapses, LTP and LTD at parallel fiber – inhibitory interneuron synapses, LTP at mossy fiber – granule cell synapses, LTP in the cerebellar nuclei etc. have been reported [30, 35-39]. With such information, involvement of plasticity mechanisms other than parallel fiber – Purkinje neuron LTD in motor learning has been proposed [2, 10, 11].

Involvement of RP in motor learning was demonstrated using transgenic mice in which γ -peptide, an intracellular region of GABA_A receptor γ 2 subunit, is selectively expressed in Purkinje neurons [16]. The γ -peptide inhibits the interaction of GABARAP protein and GABA_A receptor resulting in suppression of RP [25]. The transgenic mice show suppressed adaptation of vestibulo-ocular reflex but not that of optokinetic response [16]. Thus, the

RP-deficiency suppresses certain motor learning but not all. The results that specific suppression of neither LTD nor RP does not completely inhibit motor learning are in line with the idea that distributed plasticity mechanisms in the cerebellum contribute to motor learning. [2, 10, 11]. Knockout of a signaling protein which can possibly affect both LTD and RP such as mGluR1, Ca²⁺/calmodulin-dependent kinase II, protein kinase G results in motor learning failure [4, 6, 7, 18, 24]. Taken together, among various plasticity mechanisms in the cerebellum, LTD and RP might synergistically work, and compensate the defect of the other in some knockout mice such as those defective in AMPA receptor internalization. In normal animals, LTD might be more effective than RP, because LTD seems to be more easily induced as shown here. Whether and how LTD and RP take place in any learning processes of a behaving animal, are questions to be addressed in future.

References

- [1] M. Ito, Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiol. Rev.* 81 (2001) 1143-1195.
- [2] T. Hirano, Long-term depression and other synaptic plasticity in the cerebellum. *Proc. Japan Acad. B* 89 (2013) 183-195.
- [3] M. Ito, K. Yamaguchi, S. Nagao, T. Yamazaki, Long-term depression as a model of cerebellar plasticity. *Prog. Brain Res.* 210 (2014) 1-30.
- [4] A. Aiba, M. Kano, C. Chen, et al., Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 7 (1994) 377-388.
- [5] N. Kashiwabuchi, K. Ikeda, K. Araki, et al., Disturbed motor coordination, Purkinje cell synapse formation and cerebellar long-term depression of mice defective in the $\delta 2$ subunit of the glutamate receptor channel. *Cell* 81 (1995) 245-252.
- [6] R. Feil, J. Hartmann, C. Luo, et al., Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. *J. Cell Biol.* 163 (2003) 295-302.
- [7] C. Hansel, M. de Jeu, A. Belmeguenai, et al., α CaMKII is essential for

cerebellar LTD and motor learning. *Neuron* 51 (2006) 835-843.

[8] J.P. Welsh, H. Yamaguchi, X.H. Zeng, et al., Normal motor learning during pharmacological prevention of Purkinje cell long-term depression. *Proc. Natl. Acad. Sci. U.S.A.* 102 (2005) 17166-17171.

[9] M. Schonewille, Z. Gao, H.J. Boele, et al., Reevaluating the role of LTD in cerebellar motor learning. *Neuron* 70 (2011) 43-50.

[10] P. Dean, J. Porrill, C.F. Ekerot, H. Jörntell, The cerebellar microcircuit as an adaptive filter: experimental and computational evidence. *Nat. Rev. Neurosci.* 11 (2010) 30-43.

[11] Z. Gao, B.J. van Beugen, C.I. De Zeeuw, Distributed synergistic plasticity and cerebellar learning. *Nat. Rev. Neurosci.* 13 (2012) 619-635.

[12] T. Hirano, S. Kawaguchi, Regulation and functional roles of rebound potentiation at cerebellar stellate cell - Purkinje cell synapse. *Front. Cell. Neurosci.* 8 (2014) 42, 1-8.

[13] T. Hirano, Y. Yamazaki, Y. Nakamura, LTD, RP and motor learning. *Cerebellum* (On line).

[14] M. Kano, U. Rexhausen, J. Dreessen, A. Konnerth, Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals

in cerebellar Purkinje cells. *Nature* 356 (1992) 601–604.

[15] S. Kawaguchi, T. Hirano, Suppression of inhibitory synaptic potentiation by presynaptic activity through postsynaptic GABA_B receptors in a Purkinje neuron. *Neuron* 27 (2000) 339-347.

[16] S. Tanaka, S. Kawaguchi, G. Shioi, T. Hirano, Long-term potentiation of inhibitory synaptic transmission onto cerebellar Purkinje neurons contributes to adaptation of vestibulo-ocular reflex. *J. Neurosci.* 33 (2013) 17209-17220.

[17] K. Tanaka, L. Khiroug, F. Santamaria, et al., Ca²⁺ requirements for cerebellar long-term synaptic depression: role for a postsynaptic leaky integrator. *Neuron* 54 (2007) 787-800.

[18] Y. Kitagawa, T. Hirano, S. Kawaguchi, Prediction and validation of a mechanism to control the threshold for inhibitory synaptic plasticity. *Mol. Systems Biol.* 5 (2009) 280, 1-16.

[19] S. Kawaguchi, N. Nagasaki, T. Hirano, Dynamic impact of temporal context of Ca²⁺ signals on inhibitory synaptic plasticity. *Sci. Reports* 1 (2011) 143, 1-12.

[20] H. Miyakawa, V. Lev-Ram, N. Lasser-Ross, W.N. Ross, Calcium

transients evoked by climbing fiber and parallel fiber synaptic inputs in guinea pig cerebellar Purkinje neurons. *J. Neurophysiol.* 68 (1992) 1178-1189.

[21] R. Shigemoto, T. Abe, S. Nomura, et al., Antibodies inactivating mGluR1 metabotropic glutamate receptor block long-term depression in cultured Purkinje cells. *Neuron* 12 (1994) 1245-1255.

[22] F. Conquet, Z.I. Bashir, C.H. Davies, et al., Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* 372 (1994) 237-243.

[23] Y. Sugiyama, S. Kawaguchi, T. Hirano, mGluR1-mediated facilitation of long-term potentiation at inhibitory synapses on a cerebellar Purkinje neuron. *Eur. J. Neurosci.* 27 (2008) 884-896.

[24] S. Kawaguchi, T. Hirano, Gating of long-term depression by CaMKII through enhanced cGMP signaling in cerebellar Purkinje cells. *J. Physiol.* 591 (2013) 1707-1730.

[25] S. Kawaguchi, T. Hirano, Sustained GABARAP structural change underlies long-term potentiation at inhibitory synapses on a cerebellar Purkinje neuron. *J. Neurosci.* 27 (2007) 6788-6799.

- [26] D.J. Linden, M.H. Dickinson, M. Smeyne, J.A. Connor, A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* (1991) 7, 81-89.
- [27] N. Nagasaki, T. Hirano, S. Kawaguchi, Opposite regulation of sustained CaMKII activity by α and β subunits impacts inhibitory synaptic plasticity. *J. Physiol.* 592 (2014) 4891-4909.
- [28] M. Ito, M. Sakurai, P. Tongroach, Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol.* 324 (1982) 113-134.
- [29] M. Sakurai, Synaptic modification of parallel fibre-Purkinje cell transmission in in vitro guinea-pig cerebellar slices. *J. Physiol.* 394 (1987) 463-480.
- [30] T. Hirano, Depression and potentiation of the synaptic transmission between a granule cell and a Purkinje cell in rat cerebellar culture. *Neurosci. Lett.* 119 (1990) 141-144.
- [31] T. Hirano, Effects of postsynaptic depolarization in the induction of synaptic depression between a granule cell and a Purkinje cell in rat cerebellar culture. *Neurosci. Lett.* 119 (1990) 145-147.

- [32] M. J. Berridge, Inositol trisphosphate and calcium signaling mechanisms. *Biochim. Biophys. Acta.* 1793 (2009) 933-940.
- [33] J. Goto, K. Mikoshiba, Inositol 1,4,5-trisphosphate receptor-mediated calcium release in Purkinje cells: from molecular mechanism to behavior. *Cerebellum* 10 (2011) 820-833.
- [34] T. Doi, S. Kuroda, T. Michikawa, M. Kawato, M. Inositol 1,4,5-trisphosphate-dependent Ca^{2+} threshold dynamics detect spike timing in cerebellar Purkinje cells. *J. Neurosci.* 25 (2005) 950-961.
- [35] V. Lev-Ram, S. Wong, D. Storm, R. Tsien, A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 8389-8393.
- [36] I.C. Duguid, T.G. Smart, Retrograde activation of presynaptic NMDA receptors enhances GABA release at cerebellar interneuron-Purkinje cell synapses. *Nat. Neurosci.* 7 (2204) 525-533.
- [37] H. Jörntell, C.F. Ekerot, Reciprocal bidirectional plasticity of parallel fiber receptive fields in cerebellar Purkinje cells and their afferent interneurons. *Neuron* 34, (2002) 797-806.
- [38] E. D'Angelo, P. Rossi, D. Gall, D, et al., Long-term potentiation of

synaptic transmission at the mossy fiber-granule cell relay of cerebellum.

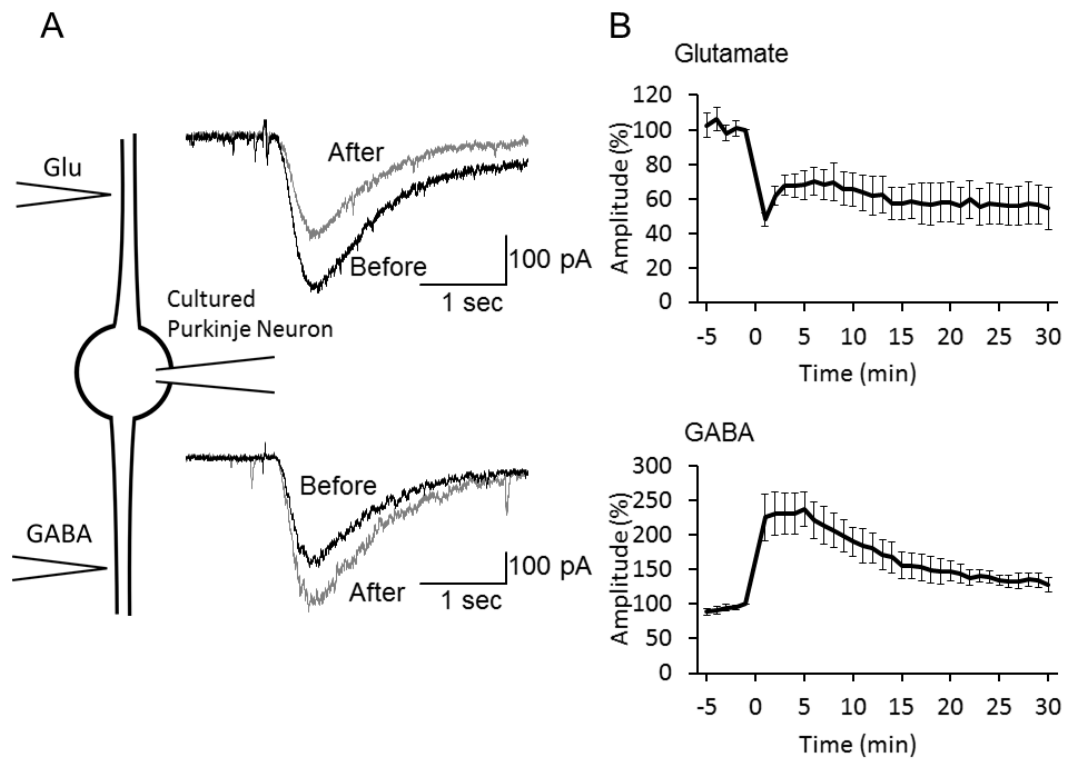
Prog. Brain Res. 148 (2005) 69-80.

[39] J. Pugh, I. Raman, Potentiation of mossy fiber EPSCs in the cerebellar nuclei by NMDA receptor activation followed by postinhibitory rebound current. Neuron 51 (2006) 113-123.

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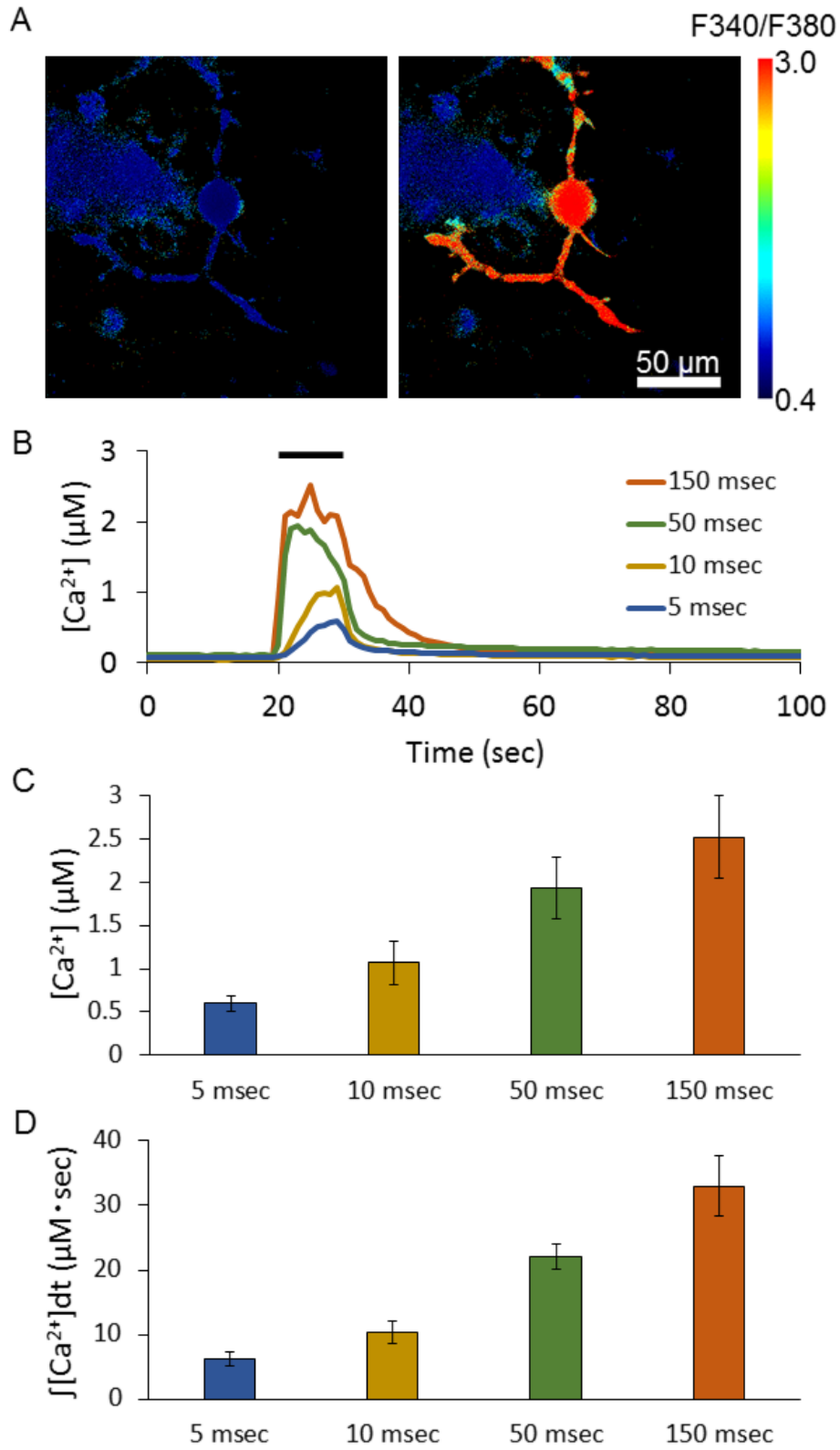
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Figure 1



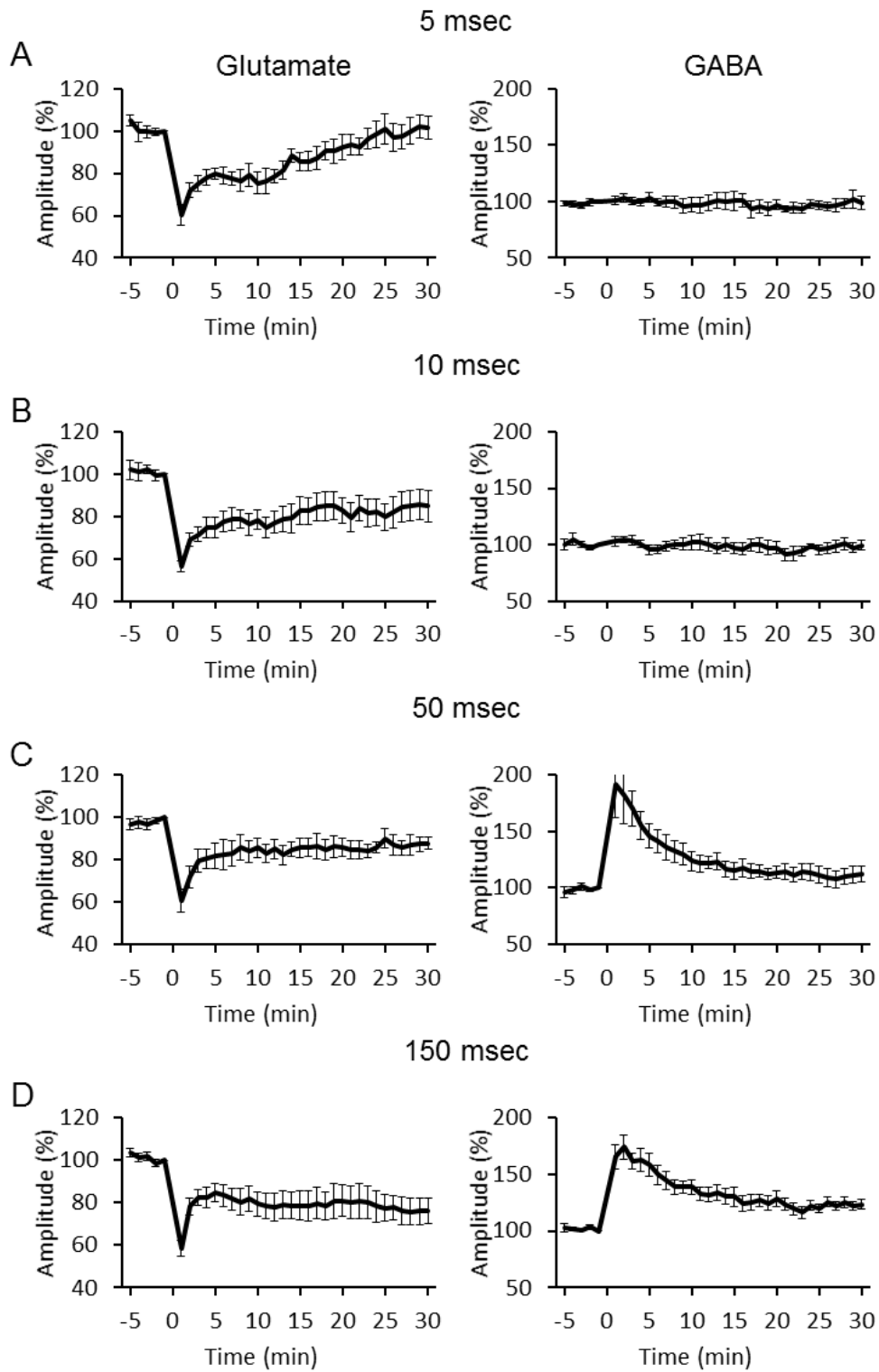
Simultaneous induction of LTD and RP. A, Glutamate and GABA were applied to dendrites of a whole-cell voltage-clamped cultured Purkinje neuron, and the induced currents were recorded. Insets show current traces recorded before (black lines) and after (grey lines) conditioning depolarization (5, 500 msec pulses to 0 mV at 0.5 Hz). B, Time courses of amplitude of glutamate- or GABA-induced current. Depolarization was applied at 0 min.

Figure 2



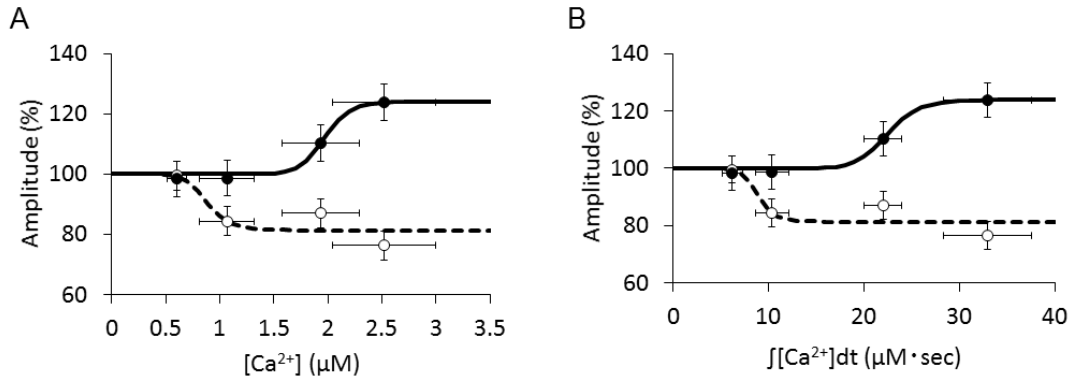
The increase in intra-dendritic Ca^{2+} concentration induced by 50 depolarization pulses to 0 mV of various durations at 5 Hz. A, Pseudo-color images of a Purkinje cell loaded with fura-4F before (left) and during (right) depolarization (50 msec pulses). B, The averaged time courses of Ca^{2+} concentration in proximal dendrites of Purkinje neurons before and after application of depolarization (upper bar). C, The peak Ca^{2+} concentration during depolarization pulses. D, The time integration of Ca^{2+} concentration increase induced by depolarization pulses.

Figure 3



Time courses of the peak amplitudes of glutamate- and GABA-induced currents after 50 depolarization pulses to 0 mV of various durations (A, 5 msec; B, 10 msec; C, 50 msec; D, 150 msec) applied at 5 Hz. The 10, 50, 150 msec pulses induced LTD of glutamate response, whereas RP of GABA response was induced only by 150 msec pulses, although the transient increase of GABA response was induced by 50 msec pulses.

Figure 4



The relations between the Ca^{2+} concentration and LTD or RP. A, B, The peak amplitudes of glutamate-induced current (broken lines) and GABA-induced currents (solid lines) are plotted against the peak intracellular Ca^{2+} concentration (A) or the time integration of Ca^{2+} concentration (B).