Norepinephrine Facilitates Induction of Long-term Depression through β-Adrenergic Receptor at Parallel Fiber-to-Purkinje Cell Synapses in the Flocculus

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Abstract—The cerebellum is involved in motor learning, and long-term depression (LTD) at parallel fiber-to-Purkinje cell (PF-PC) synapses has been considered to be a primary cellular mechanism for motor learning. In addition, the contribution of norepinephrine (NE) to cerebellum-dependent learning paradigms has been reported. Thus, the roles of LTD and of NE in motor learning have been studied separately, and the relationship between the effects of NE and LTD remains unclear. Here, we examined effects of β-adrenergic receptor (β-AR) activity on the synaptic transmission and LTD at PF-PC synapses in the cerebellar flocculus. The flocculus regulates adaptation of oculomotor reflexes, and we previously reported the involvement of both LTD and β-AR in adaptation of an oculomotor reflex. Here we found that specific agonists for β-AR or NE did not directly change synaptic transmission, but lowered the threshold for LTD induction at PF-PC synapses in the flocculus. In addition, protein kinase A (PKA), which is activated downstream of β-AR, facilitated the LTD induction. Altogether, these results suggest that NE facilitates LTD induction at PF-PC synapses in the flocculus by activating PKA through β-AR.

Key words: cerebellum, synaptic plasticity, motor learning, optokinetic response.

INTRODUCTION

The cerebellum plays an important role in motor learning. A type of synaptic plasticity, long-term depression (LTD) at parallel fiber (PF)-to-Purkinje cell (PC) synapses, has been considered to be a cellular mechanism of motor learning in the cerebellum. Late professor Masao Ito was the first researcher to demonstrate LTD, and he proposed that LTD in the flocculus contributes to adaptation of the vestibulo-ocular reflex, a type of reflex eye movement (Ito, 1982, 1989). Since then, various transgenic mice with impaired cerebellar LTD have exhibited motor-learning deficits (Alba et al., 1994; Kashiwabuchi et al., 1995; De Zeeuw et al., 1998). However, some rodents in which LTD was pharmacologically or genetically suppressed showed no significant disruption of motor learning ability (Welsh et al., 2005; Schonewille et al., 2011), and it is suggested that LTD is not essential for motor learning. In 2016, Ito’s group (Yamaguchi et al., 2016) demonstrated that the stronger conditioning stimulation induces LTD in the mutant mice which was previously reported to be defective in LTD, rebutting the conclusion of Schonewille et al. (2011). Recently, LTD was shown to occur during motor learning in rodents by examining PF-PC synapses after induction of adaptation of the optokinetic response (OKR) (Wang et al., 2014; Inoshita and Hirano, 2018). OKR is a reflex eye movement which works to stabilize the visual image during head motion, and its adaptation is regulated by a small region of cerebellar cortex the flocculus (Robinson 1981; Boyd et al., 2004). One-hour OKR training decreases the density of postsynaptic AMPA type glutamate receptors (Wang et al., 2014), depresses the postsynaptic responsiveness, and suppresses the LTD induction at PF-PC synapses in the flocculus (Inoshita and Hirano, 2018). These results altogether indicate that LTD occurs in the flocculus during OKR training.

In addition, the contribution of norepinephrine (NE) to cerebellum-dependent learning paradigms has been reported (Keller and Smith, 1983; Bickford, 1993; Gould, 1998; Pompeiano, 1998; Cartford et al., 2002; Schweighofer et al., 2004; Wakita et al., 2017). In OKR adaptation, the β-adrenergic receptor (β-AR), which is activated by NE, plays an important role. Injection of β-AR agonist into the mouse flocculus increases the efficacy of OKR, and injection of β-AR antagonist...
suppresses the training-dependent gradual increase of OKR gain (Wakita et al., 2017).

Although involvement of either β-AR or LTD in OKR adaptation has been demonstrated, there has been no report about the relationship between the β-AR activity and LTD. Here, we examined PF-PC synapses by whole-cell patch-clamp recording in cerebellar slices, and found that activation of β-AR lowers the threshold for the LTD induction through activation of PKA in the flocculus.

**EXPERIMENTAL PROCEDURES**

**Animals**

Eight- to 10-weeks old C57BL/6 male mice were used for electrophysiological recordings. Experimental procedures were carried out in accordance with the guidelines laid down by the National Institutes of Health of the USA and by Kyoto University, and approved by the local committee for handling experimental animals in the Graduate School of Science, Kyoto University.

**Slice preparation**

250 μm coronal slices of the flocculus or sagittal slices of the vermis were prepared from the cerebellum of an 8- to 10-weeks old male mouse in a solution containing (in mM): 93 N-methyl-D-glucamine, 2.5 KCl, 1.3 NaH₂PO₄, 30 NaHCO₃, 2 thiourea, 5.1 Na-ascorbate, 3.1 Na-pyruvate, 0.5 CaCl₂, 10 MgCl₂, 25 glucose and 20 HEPES, titrated to pH 7.4 with HCl. Then, slices were transferred to Krebs’ solution containing the following (in mM): 124 NaCl, 1.8 KCl, 1.2 KH₂PO₄, 1.3 MgCl₂, 2.5 CaCl₂, 26 NaHCO₃, and 10 glucose, saturated with 95% O₂ and 5% CO₂, and were maintained at room temperature (22–24 °C) for 1–9 h.

**Electrophysiology**

In most of experiments, a PC was whole-cell voltage-clamped at −80 mV with a glass pipette filled with an internal solution containing the following (in mM): 150 CsCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP and 15 sucrose, titrated to pH 7.3 with CsOH. The temperature of extracellular Krebs’ solution was kept at room temperature. PF-PC excitatory postsynaptic current (EPSC) was evoked by applying a 200 μs voltage pulse at 0.05 Hz through a glass electrode that contained Krebs’ solution and had been placed in the molecular layer. To induce LTD, a 100 ms voltage pulse to −20 mV coupled with PF stimulation at 15 and 65 ms after the onset of depolarization was applied twice or 10 times at 1 Hz as a weak or strong conditioning stimulation, respectively. During the PF and CF conjunctive stimulation experiment or the paired pulse recording, a PC was whole-cell voltage-clamped at −70 mV with a glass pipette filled with an internal solution containing the following (in mM): 130 K-gluconic acid, 10 KCl, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.2 Na-GTP and 15 sucrose, titrated to pH 7.3 with KOH, and the temperature of Krebs’ solution was kept at 30–32 °C. The current recorded during 1.5 msec from the onset of stimulation was not shown in the record to exclude the relatively large stimulation artifact.

**Data analysis**

Data are presented as mean ± SEM. The normality of data was examined with the Shapiro-Wilk test. Dunnett’s test was used to compare EPSC amplitudes and paired pulse ratio in more than two conditions. The two-tailed paired t-test was used to compare EPSC amplitudes or time courses before and after application of the conditioning stimulation. The two-tailed Students’ t-test was used to compare EPSC amplitudes or time courses in other cases. α value was set at 0.05.

**RESULTS**

**Activation of β-AR facilitated LTD induction at PF-PC synapses in the flocculus**

Considering that injection of β-AR agonist enhances OKR (Wakita et al., 2017), we first investigated whether activation of β-AR affects synaptic transmission at PF-PC synapses in the flocculus. Bath application of β-AR agonist, isoproterenol (ISO) to floccular slices did not significantly change EPSC amplitude during 30 min (Fig. 1A, B) (at 5 min, 103.9 ± 8.6%, p = 1; 10 min, 100.1 ± 5.0% p = 0.98; 20 min, 101.6 ± 5.3% p = 1; 30 min, 102.1 ± 3.5% p = 1, n = 8, compared with −2 min, Dunnett’s test). EPSC time courses were not changed by ISO application (Table 1). These results indicate that β-AR activity does not directly affect synaptic transmission at PF-PC synapses in the flocculus. We next examined the effects of β-AR activity on LTD at PF-PC synapses. In order to test whether β-AR activation affects LTD or not, we applied relatively weak conditioning stimulation with or without ISO in the
extracellular solution. The weak conditioning stimulation (100 ms voltage pulse to \(-20\) mV coupled with PF stimulation at 15 and 65 ms after the onset of voltage pulse twice with 1 sec interval) failed to induce LTD at PF-PC synapses in the ISO-free solution (Fig. 1C, E) (at 30 min, 96.1 \(\pm\) 7.8\% \(n = 6\), \(p = 0.95\), paired t-test vs at \(-2\) min). On the other hand, in the ISO-containing solution the same conditioning stimulation induced LTD at PF-PC synapses in both ISO-free (at 30 min, 45.3 \(\pm\) 4.8\% \(n = 5\)) and ISO-containing solution (at 30 min, 49.2 \(\pm\) 7.6\% \(n = 5\), and the induced LTD amplitudes were comparable (Fig. 1F–H) (\(p = 0.68\), Student’s t-test). These results suggest that activation of \(\beta\)-AR affects neither synaptic transmission directly nor the extent of LTD at PF-PC synapses in the flocculus, but facilitates induction of LTD.

**NE facilitated LTD induction through \(\beta\)-AR activation**

The endogenous agonist of \(\beta\)-AR is NE. We examined effects of NE on the synaptic transmission and LTD at PF-PC synapses in the flocculus. Bath application of NE did not significantly change the EPSC amplitudes (Fig. 2A, C) (at 5 min, 104.3 \(\pm\) 6.2\% \(p = 0.95\); 10 min, 103.9 \(\pm\) 7.4\% \(p = 0.94\); 20 min, 93.1 \(\pm\) 6.9\% \(p = 0.33\); 30 min, 93.9 \(\pm\) 7.3\% \(p = 0.34\), \(n = 7\), compared with 2 min, Dunnett’s test). EPSC time courses were also not altered by NE (Table 1). Weak conditioning stimulation that did not induce LTD in the control solution, induced LTD in the NE-containing solution (Fig. 2D, F) (at 30 min, 52.8 \(\pm\) 4.9\% \(n = 6\), \(p < 0.001\), paired t-test vs at \(-2\) min). EPSC time courses did not change after the LTD induction (Table 1). These results are similar to those obtained in the ISO-containing solution.

We next checked whether effects of NE were mediated by \(\beta\)-AR activation or not. Bath application of NE together with a \(\beta\)-AR antagonist, propranolol (PRO), did not significantly change EPSC amplitudes (Fig. 2B,
PKA activation mediated facilitation of LTD induction

β-AR is coupled to stimulatory G protein (Gs), which activates adenylyl cyclase and results in activation of protein kinase A (PKA). PKA is known to participate in several signaling pathways regulating ion channels, receptors, and gene transcription (Skalhegg and Tasken, 2000; Taylor et al., 2012). We tested whether PKA mediated the ISO-induced facilitation of LTD induction. We focused on postsynaptic PKA activity, because LTD at PF-PC synapses is expressed postsynaptically, via a decrease in the number of postsynaptic AMPA receptors. Table 1. 10–90% rise time and half decay time of EPSC in various conditions

C) (at 5 min, 103.1 ± 4.9% p = 1; 10 min, 107.1 ± 7.4% p = 1; 20 min, 91.8 ± 5.9% p = 0.25; 30 min, 88.8 ± 6.7% p = 0.16, n = 5, compared with −2 min, Dunnett’s test) nor EPSC time courses (Table 1). In the solution containing both NE and PRO, weak conditioning stimulation did not induce LTD (Fig. 2E, F) (at 30 min, 99.3 ± 3.5% n = 6, p = 0.29, paired t-test vs at −2 min, p < 0.001, Student’s t-test, vs NE). These results suggest that NE does not directly affect the synaptic transmission, but facilitates LTD induction by activating β-AR.
0 min. EPSC amplitudes are normalized by setting the amplitude between PRO-containing (white) solution. The time when the conditioning stimulation was applied is set at amplitudes before and after the conditioning stimulation in the NE-containing (black) or both NE and PRO (white). The time when bath application of drugs started is set at 0 min. Data are presented as mean ± SEM. ***

Fig. 2. Effects of NE and/or PRO on the synaptic transmission or LTD at PF-PC synapses in the flocculus. (A, B) Representative EPSC traces recorded before (left) or 30 min after (right) the bath application of NE (A) or both NE and PRO (B) to the extracellular solution. (C) Time courses of normalized EPSC amplitudes before and after the bath application of NE (black) or both NE and PRO (white). The time when bath application of drugs started is set at 0 min. (D, E) Representative EPSC traces recorded before (left) or 30 min after (right) the weak conditioning stimulation in the NE-containing (D) or both NE and PRO-containing (E) solution. (F) Time courses of normalized EPSC amplitudes before and after the conditioning stimulation in the NE-containing (black) or both NE and PRO-containing (white) solution. The time when the conditioning stimulation was applied is set at 0 min. EPSC amplitudes are normalized by setting the amplitude between -1 min and 0 min at 100%. Data are presented as mean ± SEM. ***p < 0.001, Student’s t-test at 30 min.

receptors (Kuroda et al., 2001; Linden, 2001; Kawaguchi and Hirano, 2013). It has also been reported that PKA activity in the presynaptic parallel fiber terminal facilitates glutamate release (Salin et al., 1996). In order to restrict the inhibition of PKA to postsynaptic PC, we infused a membrane-impermeable PKA inhibitor, PKI(6-22), to a PC via a patch pipette 15 min prior to bath application of ISO, and applied the weak conditioning stimulation at least 10 min after the start of ISO application. In the presence of PKI(6-22) in a PC, the weak conditioning stimulation failed to induce LTD at PF-PC synapses in ISO-containing extracellular solution (Fig. 3A, C) (at 30 min, 89.7 ± 7.7% n = 6, p = 0.31, paired t-test vs at -2 min). In order to exclude the possibility that the long-term whole-cell clamp condition causing wash-out affected the LTD induction, we applied the weak conditioning stimulation 25 min after the onset of whole-cell recording in the ISO-containing solution without PKI(6-22), which successfully induced LTD in the ISO containing solution (Fig. 3B, C) (at 30 min, 50.4 ± 1.3% n = 5, p < 0.001, paired t-test at -2 min, p = 0.001, Student’s t-test vs PKI(6-22)). We confirmed that intracellular PKI(6-22) application affected neither EPSC amplitude nor EPSC time courses (Table 1). These results suggest that a PKA-dependent pathway is necessary for the ISO-induced facilitation of LTD induction at PF-PC synapses in the flocculus.

Activation of PKA facilitated LTD induction

The results described above imply that the facilitation of LTD induction is mediated by PKA activation. Thus, we next investigated whether PKA activation in a postsynaptic PC facilitated the LTD induction or not. A PKA activator, 8-OH-cAMP, was applied to a postsynaptic PC through a patch-pipette at least 25 min before the conditioning stimulation so as not to activate presynaptic PKA at PF-PC synapses (Salin et al., 1996; Linden and Ahn, 1999). In the presence of 8-OH-cAMP, the weak conditioning stimulation induced LTD even in the ISO-free solution (Fig. 4) (at 30 min, 45.2 ± 5.9% n = 5, p = 0.005, paired t-test vs at -2 min). We confirmed that intracellular 8-OH-cAMP application affected neither EPSC amplitude nor EPSC time courses (Table 1). This result suggests that activating PKA in a PC is sufficient for facilitating the LTD induction in the flocculus.

NE facilitated LTD induction by climbing fiber and parallel fiber conjunctive stimulation

Next, we examined effects of NE on the LTD induction in a more physiological condition, in which not depolarization of a PC but CF stimulation was coupled with PF stimulation in a current clamp condition at more physiological temperature (30–32 °C). It was reported that LTD occurrence depends on the interval between PF and CF activation, and 120 ms interval was optimal in the flocculus (Suvrathan et al., 2016). Thus, we applied CF stimulation delayed by 120 ms from PF activation 400 times at 1 Hz as a conditioning stimulation (CF conditioning). This CF conditioning did not significantly change the EPSC amplitude in the NE-free solution (at 30 min, 101.7 ± 5.2% n = 7, p = 0.90, paired t-test vs at -2 min), whereas it induced LTD at PF-PC synapse in the NE-containing solution (at 30 min, 77.2 ± 5.1%
n = 6, p = 0.01, paired t-test vs at 2 min) Fig. 5. These results indicate that NE facilitates the LTD induction in a more physiological condition.

**NE or β-AR activation does not change presynaptic release from PF**

Effects of NE on presynaptic release property of PF was examined. Paired pulse ratio (PPR) of EPSC amplitude reflects the presynaptic release probability. NE or ISO did not change PPR (control, 1.21 ± 0.03, n = 6; NE, 1.28 ± 0.07, n = 6, p = 0.54; ISO, 1.26 ± 0.03, n = 6, p = 0.70, Dunnett’s test) with an interval of 100 ms at PF-PC synapses in the flocculus Fig. 6. These results suggest that presynaptic release probability of PF is not affected by NE or ISO.

**Effects of NE on PF-PC synapses in the vermis is different from those in the flocculus**

Finally, we addressed whether the facilitatory effects of NE on the LTD induction is common throughout the cerebellar cortex or not, because there are differences in the LTD induction conditions among cerebellar regions (Suvrathan et al., 2016). Thus, we examined effects of ISO or NE on PF-PC synapses in the cerebellar vermis (see Fig. 7). ISO did not change the synaptic transmission (at 5 min, 107.2 ± 8.0% p = 1; 10 min, 102.7 ± 11.6% p = 1; 20 min, 100.4 ± 12.4% p = 0.99; 30 min, 104.5 ± 9.0% p = 1, n = 6, compared with /C0 2 min, Dunnett’s test) and time course (Table 1) of EPSC, and the weak conditioning stimulation induced LTD in the ISO-containing solution (at 30 min, 61.4 ± 6.7% n = 5, p = 0.007, paired t-test vs at −2 min) at PF-PC synapses in the vermis, similarly to the flocculus. However, we found that NE application by itself decreased EPSC amplitude (at 5 min, 94.5 ± 5.3% p = 0.34; 10 min, 84.9 ± 4.4% p = 0.03; 20 min, 70.7 ± 6.0% p < 0.001; 30 min, 59.6 ± 3.6% p < 0.001, n = 5, compared with −2 min, Dunnett’s test), and that the weak conditioning stimulation did not induce LTD in the NE-containing solution (at 30 min, 97.8 ± 10.2% n = 6, p = 0.84, paired t-test vs at −2 min) in the vermis. These results were different from those in the flocculus, and suggest that in the vermis NE by itself suppresses the PF-PC synaptic transmission and occludes the LTD induction through AR other than β-AR. Thus, regulations of synaptic transmission and LTD at PF-PC synapses by NE are different between the flocculus and the vermis.

**DISCUSSION**

The results suggest that NE facilitates induction of LTD through β-AR and PKA activation in the flocculus. Our
findings not only clarified the relationship between LTD and NE, but also demonstrated that PKA activity in the postsynaptic PC contributes to a metaplastic mechanism regulating the threshold of LTD induction. The facilitatory effect of NE on the LTD induction might be physiologically important for the cerebellum-dependent learning, because it has been reported that LTD is not so easily induced in physiological conditions (Suvrathan et al., 2016; Titley et al., 2019).

Mechanisms of facilitation of LTD induction through β-AR

Ca²⁺, protein kinase C, protein kinase G and Ca²⁺/calmodulin-dependent kinase II (CaMKII) are critical molecules for induction of cerebellar LTD, whereas whether PKA is involved in LTD induction has been unclear. Although the contribution of PKA to LTD regulation was reported to be much weaker than that of protein kinase G, a model for intracellular signaling suggests that the activity of PKA could contribute to LTD by suppressing protein phosphatase 2A activity counteracting protein kinase C, through a CaMKII, phosphodiesterase, cGMP and protein kinase G cascade (Kawaguchi and Hirano, 2013).

Another possible molecular mechanism working downstream of PKA is suppression of the interaction between GluD2 receptor (GluRd2) and Delphilin. GluRd2 is a membrane protein related to ionotropic glutamate receptor, which is selectively expressed in a PC (Araki et al., 1993; Lomeli et al., 1993), and its expression is further restricted to postsynaptic membrane of PF-PC synapses (Takayama et al., 1996; Landsend et al., 1997). In GluRd2 knockout mice, LTD, motor learning and motor coordination are impaired (Funabiki et al., 1995; Hirano et al., 1995; Kashiwabuchi et al., 1995; Kishimoto et al., 2001). On the other hand, delphilin is a GluRd2-interacting protein (Miyagi et al., 2002), and the knockout mice show enhancement of both the LTD induction and OKR adaptation (Takeuchi et al., 2008). PKA activation prevents interaction of delphilin and GluRd2 by phosphorylating C terminal amino acids of GluRd2 (Sonoda et al., 2006). Thus, separation of GluRd2 from delphilin by PKA activation might facilitate the LTD induction.

Relation between NE, LTD and OKR adaptation

We previously showed that injection of ISO to the flocculus by itself enhances OKR (Wakitaka et al., 2017), whereas here we found that ISO did not affect basal PF-PC synaptic transmission, which suggests that OKR amplitude is not a simple reflection of PF-PC transmission. On the other hand, we also reported that OKR adaptation is accompanied by a decrease in EPSC amplitude at PF-PC synapses (Inoshita and Hirano, 2018). How can these apparently contradictory results be reconciled? One possibility is that spontaneous neural activities in vivo might cause LTD in ISO-injected mice. Since activation of
β-AR lowers the threshold for LTD induction, spontaneous neural activities might be sufficient to induce LTD at PF-PC synapses in a basal condition. Alternatively, β-AR activation might enhance inhibitory synaptic transmission between molecular layer interneurons and a PC (Lin et al., 1991; Liano and Gerschenfeld, 1993). It was reported that PKA activation, which is potentially caused by β-AR activation, facilitates induction of rebound potentiation, a long-term enhancement of inhibitory synaptic transmission between a molecular layer interneuron and a PC (Kawaguchi and Hirano, 2002; Sugiyama et al., 2008; Kitagawa et al., 2009). Enhancement of inhibitory synaptic transmission results in suppression of PC activity similarly to LTD, and might contribute to OKR adaptation.

Effects of β-AR activity on PF-PC synaptic transmission

A previous report showed that β-AR activation facilitates transmission at PF-PC synapses (Lippiello et al., 2015). However, here bath application of ISO did not significantly change the amplitude of EPSC at PF-PC synapses in the flocculus and vermis. In addition, neither synaptic release nor time course of EPSC significantly changed by ISO application. These different effects of the β-AR activity on PF-PC synapses might be ascribed to differences in the age of experimental animals. Here we used 8- to 10-weeks old mice, whereas Lippiello et al. (2015) used younger mice, 10- to 24-days old.

Differences between the flocculus and the vermis

Here, we showed that effects of NE on PF-PC synapses were different between in the vermis and in the flocculus, although effects of ISO were similar. NE decreased the PF-PC synaptic transmission in the vermis but not in the flocculus, and NE facilitated the LTD induction in the flocculus but not in the vermis. Failure of LTD induction by the weak conditioning stimulation in the NE-containing solution in the vermis, might be caused by saturation of LTD induction.

NE activates α1-, α2- and β-AR. All these AR subtypes are expressed in the molecular layer and in PCs, although the expression pattern of each subtype is different (Sutin and Minneman, 1895; Rainbow et al., 1984; Schambra et al., 2005). Differential effects of NE in the flocculus and vermis might be ascribed to different distribution of AR subtypes. Unfortunately, distribution patterns of ARs in the flocculus has not been reported.

Considering that effects of ISO on the PF-PC synaptic transmission and the LTD induction in the vermis were similar to those in the flocculus, ARs not activated by ISO such as α1- or α2-AR might be responsible for the differential NE effects on the PF-PC synaptic transmission and LTD. It was reported that activation of α1- or α2-AR depresses the PF-PC synaptic transmission and that α2-AR activation tends to increase PPR in juvenile mice, suggesting that the α2-AR activation may affect presynaptic release probability (Lippiello et al., 2015).

While α2-AR is coupled to Gi protein, α1-AR is mainly coupled to Gq protein which activates phospholipase C. Phospholipase C produces IP3 and diacylglycerol, which then activate protein kinase C. Protein kinase C in a PC is a critical molecule in the LTD induction (Kuroda et al., 2015).
Thus, α1-AR activation by NE in the vermis might induce LTD through protein kinase C activity, decrease PF-PC EPSC amplitudes, and occlude the additional LTD induction. Future investigation in the vermis is necessary to confirm the above speculation.

Physiological roles of NE

NE affects cerebellar cortical synapses other than PF-PC synapses. NE regulates presynaptic release properties in both GABAergic and glutamatergic synapses. Activation of β-AR enhances GABA release from molecular layer interneurons (Lin et al., 1991; Llano and Gerschenfeld, 1993), and activation of α1-AR also enhances inhibitory GABA release to a PC independently of β-AR activity (Herold et al., 2005). On the other hand, α2-AR activation suppresses the glutamate release from a CF (Carey and Regehr, 2009).

In addition, NE is involved in regulation of multiple types of synaptic plasticity other than the PF-PC LTD. Activation of β-AR facilitates induction of long-term potentiation at PF-PC synapses in juvenile mice (Lippiello et al., 2015). Presumably, it also enhances induction of rebound potentiation, long-lasting potentiation of inhibitory transmission occurring at molecular layer interneuron to PC synapses through activation of PKA (Kawaguchi and Hirano, 2002; Sugiyama et al., 2008; Kitagawa et al., 2009).

How these multiple actions of NE in the cerebellar cortex are coordinated is an important question to be addressed in the future. Clarification of expression pattern of each AR subtype with respective affinity to NE and that of NE release characteristics in different cerebellar cortical regions seem critical for comprehensive understanding of NE functions in the cerebellum.

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DECLARATION OF INTEREST

None.

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