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Timing Dependence of the Induction of Cerebellar LTD

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Abstract

Long-term depression (LTD) of the granule cell to Purkinje cell synapse is thought to contribute to motor learning. According to the Marr/Albus/Ito model, sensory inputs drive granule cells to fire, thereby exciting Purkinje cells and influencing motor output. Inappropriate motor output causes neurons in the inferior olive to fire and activate Purkinje cells via the powerful climbing fiber (CF) synapse. CF activity is an error signal and the association of CF and granule cell parallel fiber (PF) activity results in LTD at coactivated PF synapses. Here we examine the timing dependence of LTD by using an induction protocol consisting of a single CF activation paired with a PF burst, with the relative timing of CF and PF activation systematically varied. LTD was most prominent when PF activation occurred before CF activation. A plot of LTD magnitude as a function of PF and CF timing was well approximated by a fit in which LTD peaked for PF activity ~ 80 ms before CF activation and the half width was ~ 300 ms. This indicates that the timing dependence of LTD is well suited to allow a CF to depress preceding PF inputs that generated inappropriate motor outputs. We also find that LTD induction and endocannabinoid release have a similar dependence on PF and CF timing. This suggests that the properties of endocannabinoid release may underlie the timing dependence of some forms of motor learning.

Introduction

The Marr/Albus/Ito model maintains that the modification of granule cell parallel fiber (PF) to Purkinje cell (PC) synapses underlies cerebellum-dependent motor learning (Marr, 1969; Albus, 1971; Ito, 2001). Sensory inputs activate granule cells that influence motor output by exciting PCs. If the motor output is unsuitable, neurons in the inferior olive fire convey an error signal to PCs by the powerful climbing fiber (CF) synapses. According to the model, synchronous PF and CF activation results in LTD at PF→PC synapses. Numerous experiments suggest that LTD plays a crucial role in several forms of cerebellar-dependent motor learning (Thompson et al., 1997).

One aspect of motor learning that is not well understood is the manner in which it depends upon the timing of granule cell and CF activity. Inappropriate motor outputs are expected to generate an error signal in the inferior olive such that there would be a delay between PF and CF activity. This suggests that granule cell to PC synapses should be modified when granule cell activity precedes CF activity. Observations by Raymond and Lisberger (1998) of CF activity and PC activity during cerebellar learning provided experimental evidence in support of such a learning rule. In their study, LTD driven by synchronous PF and CF activity could not account for the observed changes in PC firing. Instead, the observed learning required that LTD occurred when CF activity followed PF activity by 100 ms.

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It was not clear whether the timing dependence of LTD at the granule cell→PC synapse conforms to this rule. The timing dependence of LTD has not been extensively studied and reports differ regarding the dependence of LTD on the relative timing of CF and PF activation. Chen and Thompson (1995) found that peak LTD occurred when PF preceded CF activation by 250 ms and little LTD was observed for 0 or 125 ms separation, whereas Wang et al. (2000) found that when PF and CF activation were separated by 150 ms, LTD only occurred when PF activity preceded CF activity.

Another unresolved issue is the mechanism responsible for the timing dependence of LTD. The molecular mechanisms of LTD induction suggest several possibilities (Ito, 2001; Safo et al., 2006). LTD at granule cell to PC synapses relies on nitric oxide (NO) and glutamate release from presynaptic boutons that converge on the postsynaptic cell to activate mGluR1, increase postsynaptic calcium levels and activate PKC and PKG. This results in a reduction in the response of AMPA receptors. Experiments with caged NO and calcium suggest that LTD may rely on two independent coincidence detectors that involve PKG, calcium and NO (Lev-Ram et al., 1997). In addition, endocannabinoids released by PCs and activation of presynaptic type 1 cannabinoid receptors (CB1Rs) regulate the induction of LTD (Safo and Regehr, 2005), and granule cell activity prior to CF activity promotes endocannabinoid release from PCs (Brenowitz and Regehr, 2005). These properties of endocannabinoid signaling suggest that endocannabinoids could contribute to the timing dependence of LTD.

Here we examine the timing dependence of LTD induction by determining the effect of the timing of granule cell activity and CF activity. Peak LTD occurred when PF activity preceded CF activity by 50-150 ms. The dependence of LTD on CF and PF timing was similar to that expected from behavioral studies and described previously for endocannabinoid release (Brenowitz and Regehr, 2005), suggesting that the regulation of endocannabinoid release could control the timing dependence of cerebellar LTD.

Methods

Sagittal cerebellar slices (250 μ m) were cut from the vermis of 13-18 day-old Sprague-Dawley rats as described previously (Safo and Regehr, 2005). The ACSF contained in (mM): 125 NaCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, and 25 glucose, bubbled with 95% O₂ and 5% CO₂. Recordings were performed at 34°C in ACSF with 20 μ M bicuculline or picrotoxin added to block inhibitory currents mediated by GABA_A receptors. All chemicals were purchased from Sigma/RBI (St. Louis, MO) except AM251 which were purchased from Tocris Cookson (Ellisville, MO). Whole-cell current-clamp recordings were performed in PCs using a Multiclamp 700A (Axon Instruments, Foster City, CA) and glass electrodes (1 - 2 M Ω) filled with an internal solution consisting of (mM): 110 KMeSO₃, 10 NaCl, 10 HEPES, 0.5 EGTA, 2 MgCl₂, 0.16 CaCl₂, 4 Na₂-ATP, 0.4 NaGTP and 14 Tris-Creatine phosphate.

Results

We tested the dependence of LTD on the relative timing of CF and PF activation. Numerous induction protocols have been used in previous studies that range from pairing single PF activation with single CF activation repeated at 1 Hz, to using bursts of PFs paired with CF activation. We used an induction protocol consisting of a PF burst (7 stimuli at 100 Hz) paired with a single activation of the CF input, repeated 30 times every 10 seconds (Fig. 1A, D). This induction protocol is similar to that used in some previous studies of LTD, and it has also been shown to be effective in evoking endocannabinoid release (Schreurs et al., 1996; Wang et al., 2000; Brenowitz and Regehr, 2005; Safo and Regehr, 2005).

We found that LTD is critically dependent on the relative timing of PF and CF activation. A representative experiment is shown in which conditioning stimulation consisted of PF activation followed 150 ms later by a single CF stimulation (Fig. 1A). The PF response is initially subthreshold, but high frequency stimulation eventually evokes a series of sodium spikes (Fig. 1B). A single stimulation of the CF results in a complex spike that consists of a series of sodium spikes riding on calcium spikes. As shown by comparing the average EPSPs measured prior to and 20 minutes after the induction protocol, the PF EPSP was reduced to 62% of control (Fig. 1C). In a representative experiment in which CF stimulation preceded PF activation by 150 ms (Fig. 1D, E) the EPSP was 9 % larger after the induction protocol (Fig. 1F).

Summaries of the EPSP amplitude as a function of time are shown for Δt 's of -300 ms to +500 ms (Fig. 1G). LTD was prominent only when PF activation preceded CF action by 50 ms or 150 ms. Little change was observed in the EPSP amplitude when CF activation preceded PF activation (Δt of -300 ms, -150 ms, and -50 ms) and when the interval between PF stimulation and CF activation was 300 ms or 500 ms.

The number of fibers as well as the frequency and duration of the conditioning train can influence the long-term plasticity following conditioning stimulation of PFs alone. High intensity stimulation that activates many fibers can lead to LTD (Hartell, 2002) whereas prolonged high frequency stimulation can produce a presynaptic form of LTP (Salin et al., 1996). We therefore examined the plasticity produced by PF activation alone under our experimental conditions (Fig. 2A). Experiments were conducted as in Figure 1, except that only PFs were activated during conditioning stimulation. In the absence of CF stimulation there was very little long term change in the amplitude of PF EPSPs.

We also determined whether the plasticity following conditioning stimulation as in Figure 1 ($\Delta t = 50$ ms) is dependent upon endocannabinoids, by performing experiments in the presence of the CB1R antagonist AM251. LTD was prevented by the inclusion of AM251 in the bath (Fig. 2B).

The timing dependence of LTD at PF→PC synapses is illustrated by plotting the amplitude of the normalized EPSP at 20-23 minutes following conditioning stimulation for each experiment. There was considerable variability in the magnitude of long-term plasticity in different experiments performed at a given Δt (Fig. 3A), but the average long-term plasticity as a function of Δt shows clear trends (Fig. 3B). The timing dependence of PF plasticity is well approximated by a fit to the equation,

$$X = A + B \exp \left[- \left(\frac{(t - t_0)}{\sigma} \right)^2 \right], \quad (1)$$

with $X = (EPSP_{POST}/EPSP_{PRE})$, $A = 1.12 \pm 0.05$, $B = 0.40 \pm 0.06$, $t_0 = 80 \pm 20$ ms and $\sigma = 180 \pm 40$ ms (Fig. 3B, *solid line*). The observed plasticity suggests that two processes may contribute under these conditions. When there is sufficient separation between CF and PF activation, or when PFs are activated alone, a modest enhancement of ~8 % occurs. The plasticity evoked by PF conditioning stimulation alone, or by PF and CF conditioning stimulation in the presence of AM251 (11%) is of comparable magnitude. The small size of the component makes it difficult to study, but presynaptic LTP that has been described at this synapse could account for this component of plasticity (Salin et al., 1996; Linden and Ahn, 1999). The more apparent component of plasticity is the LTD that peaks when CF activity occurs 80 ms after the PF activity.

To determine the underlying factors that control the timing dependence of LTD it is instructive to compare the timing dependence of LTD with associative synaptically evoked suppression of excitatory synapses (SSE) and local dendritic increase that arise from paired CF and PF activity (Brenowitz and Regehr, 2005). SSE experiments were performed in similar experimental conditions to those used here to study LTD, except that SSE and calcium increases were evoked following single trials with PF bursts of 3 stimuli at 100 Hz and CF bursts of 5 stimuli at 100 Hz. Pairing CF and PF stimuli triggered the release of endocannabinoids that produced a short-term reduction of synaptic strength that lasted for about 10 seconds. As shown in Figure 4A, the extent of SSE was highly sensitive to the relative timing of the CF and PF bursts. The timing dependence of SSE is well approximated by Equation 1 with $X = (EPSP_{POST}/EPSP_{PRE})$, $A=0.1.02 \pm 0.01$, $B=-0.46 \pm 0.02$, $t_0 = 112 \pm 4$ ms and $\sigma = 171 \pm 8$ ms (Fig. 3A, *solid line*). The timing dependence of local calcium increases in Purkinje cell dendrites receiving synaptic inputs is approximated by Equation 2 with $X = Ca_i$, $A=0.76 \pm 0.06$, $B=3.2 \pm 0.4$, $t_0 = 260 \pm 20$ ms and $\sigma = 230 \pm 20$ ms (Fig. 4B, *solid line*). A comparison of the fits to the timing dependence of LTD (Fig. 4C, *black trace*), SSE (Fig. 4C, *red trace*), and dendritic calcium (Fig. 4C, *blue trace*) illustrates that LTD and SSE depend similarly on timing. LTD and SSE induction have standard deviations of 180 ± 40 ms and 171 ± 8 ms respectively and peak when PF activity precedes CF activity by 80 ± 20 ms and 112 ± 4 ms respectively. Although local dendritic calcium increases are also most prominent when CF activation follows PF activation, their timing dependence differs markedly from the timing dependence of LTD.

Discussion

Our studies indicate that LTD at PF→PC synapses is most effectively induced when CF activity follows granule cell activity by about 80 ms. This timing dependence is appropriate for modifying PF→PC synapses that produced inappropriate motor output, generating with a delay an error signal that was conveyed back to the cerebellar cortex by CFs.

Mechanisms That Could Control the Timing Dependence of LTD

To determine the factors that govern the timing dependence of LTD it is useful to compare the properties of LTD with the timing dependence of several properties of transmission at the PF→PC synapses that are regulated by the associative activation of PF and CF synapses. First, CF activation prior to PF activation has been shown to enhance a slow mGluR1-dependent component following a PF burst that is mediated by TRP channels (Batchelor and Garthwaite, 1997; Kim et al., 2003). The timing dependence differs from that of LTD, as the stimulus of the CF enhances the TRP component evoked by subsequent bursts even if they occur tens of seconds later (Batchelor and Garthwaite, 1997). Second, CF activity enhances the local calcium signals in PC dendrites that are evoked by a PF burst (Wang et al., 2000; Brenowitz and Regehr, 2005). The timing dependence of the associative increases in dendritic calcium (Fig. 4B) does not match the timing dependence of LTD (Fig. 4C). Third, when CF activation is paired with a burst of PF activation, the timing dependence of SSE is well approximated by a fit to Equation (1), with $t_0 = 111 \pm 4$ ms and $\sigma = 171 \pm 7$ ms, compared to 80 ± 20 ms and $\sigma = 180 \pm 40$ ms for LTD (Brenowitz and Regehr, 2005). The similarity suggests that the same factors could govern the timing dependence of SSE and LTD. Based on previous studies, the timing dependence of SSE reflects the timing dependence of eCB release, which in turn arises from the supralinear calcium increases that arise when CF synapses and PF synapses are associatively activated and from the timing dependence of mGluR1 driven processes (Brenowitz and Regehr, 2005; Maejima et al., 2005). Thus, these results suggest that as with SSE, the timing dependence of eCB release may make an important contribution to the timing dependence of LTD under some circumstances. Alternative mechanisms could regulate LTD under other circumstances. mGluR1 activation, which is crucial to the induction of LTD (Aiba et al., 1994; Conquet et al., 1994; Kishimoto et al., 2002) can activate multiple pathways that

lead to the production of endocannabinoids, release of calcium from internal stores and PKC activation. It is possible that in some circumstances mGluR1 could control the timing dependence of LTD, through a pathway than that involved in endocannabinoid synthesis, but one that has a similar time dependence. In addition, NO release or the activation of PKC and PKG in PC dendrites could contribute to the timing dependence of LTD (Lev-Ram et al., 1997; Hartell, 2002). Another important factor in relating our LTD experiments with behavioral findings is that we performed our studies in with GABA_A receptors blocked, and that could certainly influence the timing dependence of LTD.

Behavioral studies suggest that there may be diversity in the role of endocannabinoids in different types of motor learning involving the cerebellum. Signaling by endogenous cannabinoids and activation of CB1 receptors is required for delay eyeblink conditioning, a cerebellum-dependent form of learning (Kishimoto and Kano, 2006). In contrast, VOR learning does not require endocannabinoids (Bristol and Raymond, 2006). This is intriguing, because the timing dependence we observe here for LTD agrees with the prediction of optimal LTD for a delay of 100 ms for PF-CF timing for VOR learning (Raymond and Lisberger, 1998), yet we find that LTD induction is endocannabinoid dependent whereas VOR learning is not. This suggests that under some circumstances mechanisms other than those controlling endocannabinoid release may control the timing dependence of LTD.

Comparison to Timing-Dependent Plasticity at Other Synapses

It is interesting to compare the properties of long-term plasticity at PF→PC synapses, which are well suited to mediate motor learning in the cerebellum, to the timing dependence of plasticity observed at other synapses (Abbott and Nelson, 2000; Dan and Poo, 2006). Synapses show a tremendous diversity in the properties of spike timing dependent plasticity (STDP). Endocannabinoid-dependent LTD is known to occur at numerous types of synapses (Chevalleyre et al., 2006), but its contribution to STDP has only been described at cortical synapses (Sjostrom et al., 2003; Bender et al., 2006; Nevian and Sakmann, 2006). In the somatosensory cortex, endocannabinoid-dependent LTD can be studied in isolation by blocking NMDA receptors in the postsynaptic cell (Bender et al., 2006). Under these conditions, peak LTD is observed when the postsynaptic cell is activated 50 ms before synaptic activation occurs ($\Delta t = -50$ ms) and LTD is observed for $\Delta t > -150$ ms and $\Delta t < 50$ ms. This is very different from the LTD we observe. In our experiments CF activation is used instead of a backpropagating action potential, because sodium spikes do not actively propagate within Purkinje cell dendrites (Llinas and Sugimori, 1980; Stuart and Hausser, 1994), and we observe LTD for $\Delta t > -50$ ms and $\Delta t < 300$ ms. While there are numerous differences in the experimental conditions and in the properties of LTD between these cortical and cerebellar synapses, a comparison of these two forms of LTD suggests that endocannabinoids can mediate long-term plasticities with different timing dependencies.

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Figure 1. Timing dependence of the induction of LTD

Granule cell parallel fibers (PFs) and CFs were activated by extracellular electrodes and responses were measured using a whole cell electrode. PFs were stimulated 7 times at 100 Hz and the CF was stimulated once at time Δt , where positive Δt corresponds to PF activation preceding CF activation and Δt is measured from the middle of the stimulus train. This was repeated 30 times every 10 seconds. (A-C) A representative experiment is shown for $\Delta t = 150$ ms with a response to conditioning stimulation (B) and the average EPSPs recorded before (C, black trace, -5 to -2 min) and after (C, grey trace, 20 to 23 min.). (D-F) Another representative experiment is shown for $\Delta t = -150$ ms. (G) Time course of EPSPs evoked by conditioning stimulation with indicated Δt 's. Traces are averages of 6 - 10 experiments and error bars are standard errors.

Figure 2. Climbing Fiber activation and CB1R activation is required for LTD under our experimental conditions

Experiments were conducted as in Figure 1 except that in (A-C) during the conditioning stimulation PFs were stimulated 7 times at 100 Hz without CF activation. Traces from a representative experiment are shown for the response to PF stimulation during the conditioning train (A, left), and the EPSPs before (A, right, black trace) and after the conditioning stimulation (A, right, grey trace). (B) The EPSP is plotted as a function of time for the experiment in (A), and in (C) the EPSP is plotted as a function of time for 6 experiments. (D-F) Experiments were also performed in which PFs were stimulated 7 times at 100 Hz and the CF was stimulated once at time $\Delta t=50$ ms in the presence of AM251. Traces from a representative experiment are shown (D), and the EPSP as a function of time are plotted in (E). (F) A summary of the normalized EPSP amplitude as a function of time is summarized for 5 experiments. Error bars in C and F are standard errors.

Figure 3. Summary of the timing dependence of the induction of LTD

(A) Normalized EPSP amplitude at 20 to 23 minutes post conditioning stimulation for individual experiments for the indicated Δt 's for PF (7 at 100 Hz) and single CF stimulation (*open circles*). Individual experiments are also shown for conditioning trains of PFs (7 times at 100 Hz) without CF activation (*triangles*) and PF and CF activation for $\Delta t=50$ ms in the presence of AM251 (*squares*). (B) Average normalized EPSP amplitude for the experiments in A. The fit to equation 1 is described in the text (*solid line*).

Figure 4. Comparison of the timing dependence of the associative effects of PF and CF stimulation on LTD, SSE and dendritic calcium increases

Previous studies (Brenowitz and Regehr, 2005) have determined the timing dependence of pairing brief PF bursts (3 stimuli at 100 Hz) and CF bursts (5 stimuli at 100Hz) on peak synaptic suppression of excitatory synapses (SSE) (A), and on local calcium signals in Purkinje cell dendrites (B). Fits in A and B are to Equation 1, as described in the text. (C) The time courses of fits LTD (black), SSE, (red) and calcium increases (blue), are compared by normalizing the fits from Fig. 3B, Fig 4A and Fig. 4B respectively.