A late phase of LTD in cultured cerebellar Purkinje cells requires persistent dynamin-mediated endocytosis

David J. Linden

The Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted 7 September 2011; accepted in final form 26 October 2011

Linden DJ. A late phase of LTD in cultured cerebellar Purkinje cells requires persistent dynamin-mediated endocytosis. J Neurophysiol 107: 448–454, 2012. First published November 2, 2011; doi:10.1152/jn.00824.2011.—Long-term synaptic depression (LTD) of cerebellar parallel fiber-Purkinje cell synapses is a form of use-dependent synaptic plasticity that may be studied in cell culture. One form of LTD is induced postsynaptically through an mGlu1/Ca influx/protein kinase Ca (PKCa) cascade, and its initial expression requires phosphorylation of ser-880 in the COOH-terminal PDZ-ligand region of GluA2 and consequent binding of PICK1. This triggers postsynaptic clathrin/dynamin-mediated endocytosis of GluA2-containing surface AMPA receptors. Cerebellar LTD also has a late phase beginning 45–60 min after induction that is blocked by transcription or translation inhibitors. Here, I have sought to determine the expression mechanism of this late phase of LTD by applying various drugs and peptides after the late phase has been established. Neither bath application of mGluR1 antagonists (JNJ-16259685, LY-456236) nor the PKC inhibitor GF-109203X starting 60–70 min after LTD induction attenuated the late phase. Similarly, achieving the whole cell configuration with a second pipette loaded with the peptide PKC inhibitor PKC(19–36) starting 60 min postinduction also failed to alter the late phase. Late internal perfusion with peptides designed to disrupt PICK1/entactin interaction or PICK1 dimerization failed to impact late phase LTD expression. However, late internal perfusion with two different blockers of dynamin, the drug dynasore and a dynamin inhibitory peptide (QVPSRPNRAP), produced rapid and complete reversal of cerebellar LTD expression. These findings suggest that the protein synthesis-dependent late phase of LTD requires persistent dynamin-mediated endocytosis, but not persistent PICK1-GluA2 binding nor persistent activation of the upstream mGluR1/PKCa signaling cascade.

parallel fiber; glutamate; mGlu1; AMPA receptor; clathrin

CONSOLIDATION, CONTEXTUALIZATION and long-term storage of memory appear to require de novo gene expression and protein synthesis. These events are also essential for the late phase of certain forms of synaptic plasticity thought to underlie memory storage, such as long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD) (Costa-Mattioli et al. 2009; Redondo and Morris 2011). However, while the expression mechanisms of the protein synthesis-independent early phase of various forms of LTP and LTD have been extensively studied, little is known about the expression mechanisms underlying the protein synthesis-dependent late phase.

Cerebellar LTD of parallel fiber-Purkinje synapses has been suggested to form a portion of the engram for certain forms of motor learning (Albus 1971; Ito 1982; Marr 1969). While this view was supported by analysis of mutant mice which lacked both cerebellar LTD and showed impairments in associative eyelid conditioning or vestibulo-ocular reflex adaptation (Aiba et al. 1994; Koekkoek et al. 2003), it has also been challenged by a number of recent findings (Ke et al. 2009; Schonewille et al. 2011; Welsh et al. 2005). This form of LTD is induced postsynaptically through an mGlu1/protein kinase Ca (PKCa) cascade and is expressed by PICK1-dependent clathrin/dynamin-mediated endocytosis of GluA2-containing surface AMPA receptors (Chung et al. 2003; Leitges et al. 2004; Steinberg et al. 2006; Wang and Linden 2000). A late phase of cerebellar LTD in cultured Purkinje cells, beginning 45–60 min after induction, is blocked by transcription or translation inhibitors or by divorcing the synapses from the nucleus through formation of a stable dendritic outside-out macropatch (Linden 1996; Murashima and Hirano 1999). More specifically, the late phase of cerebellar LTD requires binding of the transcription factor SRF and its cofactor MAL to an identified site (SRE 6.9) within the promoter region of the immediate-early gene Arc and subsequent Arc transcription and translation (Smith-Hicks et al. 2010). Arc, which binds the key endocytotic proteins dynamin and endophilin (Chowdhury et al. 2006) and is normally found at low basal levels in Purkinje cells, is induced in response to LTD stimuli (Smith-Hicks et al. 2010).

The present experiments are designed to interfere with various portions of the signaling cascade that have been characterized for the induction and expression of the early phase of cerebellar LTD to determine if persistent activation of any of these signaling events is required to maintain a protein synthesis-dependent late phase. In particular, given that a late phase of LTD requires delayed expression of Arc and that depression of synaptic transmission by Arc requires its interaction with dynamin and endophilin (Smith-Hicks et al. 2010), I hypothesize that a late phase requires persistent clathrin/dynamin-mediated endocytosis of GluA2-containing AMPA receptors, similar to that previously reported in the early phase of chemical LTD evoked in hippocampal cultures by bath application of the mGluR1/5 agonist DHPG (Waung et al. 2008).

METHODS

Cell culture and whole cell patch-clamp recording were performed as previously described (Smith-Hicks et al. 2010). Briefly, patch electrodes were filled with a solution containing (in mM) 135 CsCl, 10 HEPES, 0.5 EGTA, 4 Na2-ATP, and 0.4 Na-GTP, adjusted to pH 7.35 with CsOH. Cells were bathed in 140 NaCl, 5 KCl, 2 CaCl2, 0.8 MgCl2, 10 HEPES, 10 glucose, 0.0005 TTX, and 0.3 picrotoxin, adjusted to pH 7.35 with NaOH, which flowed at a rate of 0.5 ml/min. Electrodes were pulled from N51A glass and polished to yield a resistance of 2–4 MΩ. Iontophoresis electrodes (1-μm tip diameter)
were filled with 10 mM glutamate (in 10 mM HEPES, pH 7.1) and were positioned ~20 μm away from large-caliber dendrites. Test pulses were delivered using negative current pulses (300–900 nA, 30- to 70-ms duration). LTD-inducing pairing stimuli consisted of six 3-s-long depolarizations to 0 mV, each delivered together with a test pulse of glutamate.

Membrane currents were recorded with an Axopatch 200A amplifier and digitized at 10 kHz. Signals were low-pass filtered at 5 kHz and acquired using Axograph X software (Axograph Scientific, Sydney, Australia). Recordings in which R_input or R_series varied by more than 15% were excluded from the analysis.

Bis-fura-2 ratio imaging of intracellular free Ca^{2+} in the dendritic shafts of cultured Purkinje cells was accomplished by measuring the background corrected fluorescence ratio at 340- and 380-nm excitation using a cooled CCD camera system. In these experiments, EGTA was removed from the internal saline and replaced with 100 μM bis-fura-2 (Invitrogen).

The drugs JNJ-16259685, LY-456236, GF-109203X, DHPG, and dynasore, and the peptide PKC(19–36) were purchased from Ascent Scientific. GluA2 phospho-SVKI peptide, GluA2 scrambled dynamin inhibitory peptide (QPPASNPRVR) were purchased from Ascent Scientific, GluA2 phosphorylated-SVKI peptide, GluA2 SGK1 peptide, PICK1 WT GST fusion protein, and PICK1A159–166 fusion protein were gifts from the laboratory of Richard Huganir (Johns Hopkins Univ. School of Medicine).

RESULTS

To investigate the mechanism of expression of the late phase of cerebellar LTD, a well-established cell culture model system was used. Whole cell voltage-clamp recordings were made from Purkinje cells in cultures derived from embryonic wild-type mouse cerebellum. Two iontophoresis electrodes were used to stimulate separate dendritic branches in an alternating fashion. Following acquisition of baseline responses to test pulses of glutamate, test pulses were halted at the “control” electrode, and LTD was induced by applying glutamate/d depolarization conjunctive stimulation at the “paired” electrode. This resulted in input-specific induction of LTD (Fig. 1) as has been previously described. It has previously been reported that induction of cerebellar LTD requires activation of mGlu1 in Purkinje cell dendrites (Linden et al. 1991), so it is not surprising that bath application of two different potent and selective mGlu1 antagonist drugs prior to and during conjunctive stimulation (from Δt = 10 to 5 min) produced a complete blockade of LTD (JNJ-16259685, 50 nM, paired pathway, 97 ± 5.6% of baseline at Δt = 30 min, n = 7; LY-456236, 2 μM, paired pathway, 90 ± 4.6% of baseline at Δt = 30 min, n = 7). As a test of the hypothesis that continued mGlu1 activation is necessary for maintenance of the late phase, LTD was induced and then either JNJ-16259685 or LY-456236 was bath applied starting at Δt = 70 min (Fig. 1). This time point was chosen because previous work has shown that this is a point when LTD expression is sensitive to prior treatment with protein synthesis inhibitors (Linden 1996) or interference with the transcription factor SRF (Smith-Hicks et al. 2010). Neither of these drugs modified the late phase of LTD (JNJ-16259685, 50 nM, paired pathway, 53 ± 6.9% of baseline at Δt = 120 min, n = 9; LY-456236, 2 μM, paired pathway, 53 ± 8.6% of baseline at Δt = 120 min, n = 8) indicating that persistent activation of mGlu1 is not required. It is worthwhile to note that JNJ-16259685 is a non-competitive inhibitor (Maibere et al. 2005), so its failure to affect the late phase of LTD cannot be attributed to an inability to compete for the glutamate binding site.

LTD induction also requires activation of PKC within Purkinje cell dendrites (Chung et al. 2003; De Zeeuw et al. 1998; Linden and Connor 1991), and the relevant PKC isofrom is PKCa, due to its unique QSAV sequence that confers PICK1 binding (Leitges et al. 2004). As an initial test of the hypothesis that continued PKC activation is required for the late phase, we used the cell-permeant PKC inhibitor GF-109203X (Fig. 2A). Bath application of this drug prior to and during conjunctive stimulation produced a complete blockade of LTD (GF-109203X, 100 nM, paired pathway, 102 ± 6.4% of baseline at Δt = 30 min, n = 6). This confirms previous reports with other PKC inhibitors (Chung et al. 2003; De Zeeuw et al. 1998; Linden and Connor 1991), and, more importantly, shows that this preparation of the drug is active and effectively penetrates cultured Purkinje cells. However, when GF-109203X was bath applied starting at Δt = 60 min, no alteration of the late phase of LTD was observed (paired pathway, 53 ± 9.0% of baseline at Δt = 120 min, n = 8). Inhibitors of the classical isoforms of PKC can act either at the regulatory site, to interfere with binding of Ca or diacylglycerol, or an the catalytic site, to
interfere with the binding of ATP. GF-109203X competes with ATP at the catalytic site (Toullec et al. 1991), so it would be expected to inhibit both intact PKC\(\alpha\) as well as forms of PKC\(\alpha\) that might have been processed by proteolysis or some other modification to inactivate the regulatory site.

As a further test of the hypothesis that continued PKC activation is required for the late phase, I made use of the membrane-impermeant pseudosubstrate peptide PKC(19–36) (sequence: RFARKGALRQKNVHEVKN). When this peptide was included in the internal saline of the recording electrode at a concentration of 10 \(\mu\)M and whole cell mode was achieved >25 min prior to conjunctive stimulation, LTD was completely abolished (Fig. 2B; paired pathway, 111 ± 9.1% of baseline at \(t = 30\) min, \(n = 7\)) consistent with prior reports (Linden and Connor 1991). To deliver PKC(19–36) after LTD was established, a second recording pipette filled with the same internal saline supplemented with the peptide was used. This second pipette achieved whole cell mode on the soma at \(t = 61\) min. In ~20% of cases, this manipulation persistently destabilized the recording quality as indexed by \(R_{\text{input}}\) or \(I_{\text{hold}}\); recordings were discarded if these parameters persistently changed by more than 15%. However, in the remaining cases, the recording restabilized within 10 min, so monitoring resumed to evaluate the time course of the late phase. Dual whole cell application of PKC(19–36) at \(t = 61\) min had no effect on either the late phase of LTD (paired pathway, 55 ± 8.7% of baseline at \(t = 120\) min, \(n = 10\)) or responses in the unpaired control pathway (100 ± 8.0% of baseline at \(t = 120\) min). Thus, persistent PKC activity does not appear to be required for the late phase of LTD.

PKC phosphorylates ser-880 in the COOH-terminal PDZ-ligand domain of the GluA2 AMPA receptor. This phosphorylation decreases the affinity of the PDZ ligand for the postsynaptic scaffold protein GRIP1 and allows binding of another PDZ-domain containing protein, PICK1. Induction of cerebellar LTD can be abolished by preventing GluA2 ser-880 phosphorylation (Chung et al. 2003; Steinberg et al. 2006) or preventing PICK1–GluA2 PDZ-domain interactions (Xia et al. 2000). It is not known whether persistent PICK1–GluA2 interactions are necessary to maintain LTD during the late phase. To address this question, I disrupted PICK1–GluA2 interactions by applying GluA2 phospho-SVKI peptide (KEGYNVYGIESGKA) to bind and sequester native PICK1. When applied in the recording pipette from the onset of whole cell recording, phospho-SVKI, but not an inactive SGKA control peptide (KEGYNVYGI-ESGKA), produced a near-complete blockade of LTD (Fig. 3; phospho-SVKI, paired pathway, 91 ± 8.6% of baseline at \(t = 30\) min, \(n = 7\); SGKA, paired pathway, 51 ± 7.9% of baseline at \(t = 30\) min, \(n = 7\)). However, when either phospho-SVKI or SGKA peptides were applied starting at \(t = 60\) min using the aforementioned second patch pipette technique, no alteration of the late phase was observed (phospho-SVKI, paired pathway, 56 ± 7.9% of baseline at \(t = 61\) min (indicated by the horizontal bar). In the late condition, a second recording pipette filled with the same internal saline supplemented with the peptide was used. This second pipette achieved whole cell mode on the soma at \(t = 61\) min as indicated by the arrowhead; PKC(19–36) early, \(n = 7\); PKC(19–36) late, \(n = 10\). Scale bars = 2 s, 50 pA.

Fig. 2. Late internal or external application of PKC inhibitors fails to reverse established LTD. A: LTD was induced by glutamate depolarization pairing at \(t = 0\) min. Early application of the membrane-permeant PKC inhibitor drug GF-109203X (100 nM) was given from \(t = -10\) to \(+5\) min as indicated by the black horizontal bar. Late bath application of GF-109203X (100 nM) was given starting at \(t = 61\) min as indicated by the gray horizontal bar; GF-109203X (100 nM) early, \(n = 6\); GF-109203X (100 nM) late, \(n = 8\). Scale bars = 2 s, 50 pA. B: the membrane-impermeant peptide pseudosubstrate inhibitor of PKC, PKC(19–36), was added to the internal saline at a concentration of 10 \(\mu\)M. In the early condition, the peptide was added to the recording electrode at the whole cell configuration and was held for at least 25 min before LTD induction at \(t = 0\) min (indicated by the horizontal bar). In the late condition, a second recording pipette filled with the same internal saline supplemented with the peptide was used. This second pipette achieved whole cell mode on the soma at \(t = 61\) min as indicated by the arrowhead; PKC(19–36) early, \(n = 7\); PKC(19–36) late, \(n = 10\). Scale bars = 2 s, 50 pA.
for the late phase of LTD, two different dynamin inhibitors were used, the drug dynasore, a noncompetitive inhibitor of dynamin GTPase activity (Macia et al. 2006), and the dynamin inhibitory peptide QVPSRNPR, which blocks dynamin binding to the adaptor protein amphiphysin and thereby prevents endocytosis by interfering with the formation of a clathrin/dynamin complex (Grabs et al. 1997). When dynasore or dynamin inhibitory peptide were added to the internal saline of the recording electrode, LTD induction was completely blocked (Fig. 5, dynasore, early, paired pathway, 102 ± 8.0% of baseline at t = 30 min, n = 8; dynamin inhibitory peptide, early, paired pathway, 99 ± 7.5% of baseline at t = 30 min, n = 8), consistent with previous reports using similar dynamin inhibitors (Wang and Linden 2000). When either dynasore or dynamin inhibitory peptide was applied internally starting at t = 61 min using the second pipette method, this resulted in a rapid increase in glutamate responses such that LTD had reverted to baseline values by t = 110 min (dynasore, late, paired pathway, 101 ± 8.5% of baseline at t = 110 min, n = 10; dynamin inhibitory peptide, late, paired pathway, 96 ± 9.0% of baseline at t = 110 min, n = 10). Responses in the control pathway were unaltered by late dynamin inhibition (dynasore, late, control pathway, 115 ± 7.4% of baseline at t = 110 min, n = 10; dynamin inhibitory peptide, late, control pathway, 112 ± 7.0% of baseline at t = 110 min, n = 10). Furthermore, second pipette application of an inactive scrambled dynamin inhibitory peptide (QPPASNPRVR) has no ef-

Fig. 3. Late internal application of a GluA2 peptide designed to block GluA2-PICK1 interactions fails to reverse established LTD. These experiments used the dual somatic pipette technique shown in Fig. 2B. In the early condition, the peptide was added to the recording electrode and the whole cell configuration was held for at least 25 min before LTD induction at t = 0 min (indicated by the horizontal bar). In the late condition, a second recording pipette filled with the same internal saline supplemented with the peptide was used. This second pipette achieved whole cell mode on the soma at t = 61 min as indicated by the arrowhead; phospho-SVKI (100 μM), early, n = 7; phospho-SVKI (100 μM), late, n = 9; SGKA (100 μM), early, n = 7; SGKA (100 μM), late, n = 8. Scale bars = 2 s, 50 pA.

Fig. 4. Late internal application of a PICK1 fusion protein designed to block GluA2-PICK1 interaction nor persistent PICK1 dimerization are required for the late phase of LTD. These findings suggest that neither persistent PICK1-GluA2 nor persistent PICK1-GluA2 interaction nor persistent PICK1 dimerization are required for the late phase of LTD.

The expression of the early phase of LTD requires clathrin/dynamin-mediated endocytosis of GluA2-containing AMPA receptors (Wang and Linden 2000). To test the hypothesis that persistent clathrin/dynamin-mediated endocytosis is required...
fect on either paired or control pathways (scrambled dynamin inhibitory peptide, late, control pathway, 104 ± 6.8% of baseline at t = 110 min, n = 10), arguing against a nonspecific effect of the second patch protocol.

To test these reagents for side effects on Ca signals, Purkinje cells were loaded with Ca indicator and either dynasore or dynamin inhibitory peptide. Neither of these drugs produced a significant attenuation of Ca transients evoked by either depolarizing steps (1-s-long depolarizations from −70 to 0 mV) or brief pressure pulses of the mGlu1/mGlu5 agonist DHPG (Fig. 6). Together, these results indicate that persistent clathrin/dynamin-mediated endocytosis is required for a late phase of cerebellar LTD.

**DISCUSSION**

The main finding of this study is that drugs or peptides designed to interrupt key processes in the initial signaling cascade for cerebellar LTD induction did not affect the time course of a late phase of LTD when introduced 60–70 min after induction. The late phase does not require persistent activation of mGlu1 or PKC nor does it require persistent interaction between GluA2 and PICK1, nor PICK1 dimerization through its BAR domain (Figs. 1–4). However, two different treatments that block dynamin function, the drug dynasore and a dynamin inhibitory peptide (but not a scrambled control peptide), both produced a rapid and complete abolition of the late phase of LTD (Fig. 5). Importantly, these two treatments block clathrin-mediated endocytosis by targeting different sites on the dynamin molecule. Dynamin inhibitory peptide prevents dynamin’s proline rich domain from binding the SH3 domain of the crucial adaptor molecule.

**Fig. 5.** Late internal application of dynamin inhibitors reverses established LTD. These experiments used the dual somatic pipette technique for late drug application. A: dynasore (50 μM) early, n = 8; dynasore (50 μM) late, n = 10. Scale bars = 2 s, 50 pA. B: dynamin inhibitory peptide (20 μM) early, n = 8; dynamin inhibitory peptide (20 μM) late, n = 10; scrambled dynamin inhibitory peptide (20 μM) late, n = 10. Scale bars = 2 s, 50 pA.

**Fig. 6.** The drugs and peptides used in this study do not have unintended side effects on depolarization-evoked or mGlu1 agonist-evoked dendritic Ca transients. Purkinje cells were pipette loaded with a Cs-based internal saline supplemented with bis-fura-2 (100 μM) to allow for dendritic Ca imaging after 25 min of equilibration in whole cell mode. Voltage-clamped Purkinje cells were challenged with step depolarization to 0 mV (1-s duration) or a micro-pressure pulse of the mGlu1/5 agonist DHPG (10 psi, 2 s) as an index of voltage-sensitive Ca channel function and mGluR1 function, respectively. N = 10 cells/group.
amphiphysin, thereby preventing the dynamin motor from associating with the clathrin lattice (Grabs et al. 1997). Dynasore has no effect on amphiphysin binding but rather inhibits dynamin’s GTPase activity, which is required for generating scission force (Macia et al. 2006). Neither of these treatments had side effects on either mGluR1 function or depolarization-evoked Ca transients (Fig. 6). Thus, it appears as if a late phase of cerebellar LTD requires persistent clathrin/dynamin-mediated endocytosis. In my view, the most parsimonious interpretation of the present findings is that expression of the late phase of LTD requires clathrin/dynamin-mediated endocytosis of AMPA receptors and does so in a fashion that is independent of PICK1-GluA2 binding or PICK1 BAR domain dimerization. However, it should be noted that both dynasore and dynamin inhibitory peptide will inhibit clathrin/dynamin-mediated endocytosis generally, so it is possible that the requirement for this process in the late phase of LTD is related to endocytosis of some target other than GluA2-containing AMPA receptors that downregulate postsynaptic AMPA receptor function indirectly.

Or, to put it another way, at a macroscopic level, the expression mechanisms of early and late phase LTD are similar. Both involve a reduction of GluA2-containing surface AMPA receptors and both require clathrin/dynamin-mediated endocytosis and are expressed postsynaptically as a reduction in synaptic GluA2-containing AMPA receptors. Yet, at a molecular level, important differences emerge: the early phase requires GluA2-PICK1 binding (Chung et al. 2003) and PICK1 dimerization (Steinberg et al. 2006) but not Arc expression (Smith-Hicks et al. 2010), whereas the late phase has exactly the opposite requirements.

There are several caveats that should be sounded in interpreting these findings. First, while a late phase of LTD requires clathrin/dynamin-mediated endocytosis, this does not mean that this process is sufficient for the expression of the late phase. For example, it may be that late phase LTD expression may only be sustained if delivery of new or recycled AMPA receptors to synapses is also downregulated to achieve a new equilibrium with reduced postsynaptic strength. In future experiments, it will be useful to interfere with these processes as a test of this idea. Second, these experiments are conducted in a cell culture system, so the possibility remains that these processes may proceed differently in the intact, unanesthetized brain with ongoing neural activity. Third, due to the limits of patch-clamp recording, these cells were not recorded for periods longer than 120 min after LTD induction, so the possibility remains that an even later phase of LTD exists with yet different mechanisms of expression.

It is notable that application of dynamin inhibitors, either before or 60 min after induction of LTD, produced only small and slow increases in control pathway responses (Fig. 5). This is consistent with a previous observation in cultured Purkinje cells that inclusion in the internal saline of peptides that interfere with dynamin-amphiphysin binding failed to alter mEPSC amplitude (Wang and Linden 2000). While we have no independent measure of delivery of AMPA receptors to synapses following application of dynamin inhibitors, one possibility is that there is a compensatory reduction in AMPA receptor delivery that occurs when endocytosis is blocked.

There are some precedents for examining the signaling requirements for late phases of LTP. In developing Xenopus retino-tectal synapses, perfusion with the NMDA receptor antagonist D-AP5 blocked a subsequent late phase of LTP when applied 0–30 min (but not 60 min) after induction (Gong et al. 2011). A similar effect was seen with the voltage-sensitive Na channel blocker TTX: late LTP was blocked when TTX was applied immediately after induction but not when applied 1 h later. These experiments suggest a requirement for postinduction neural activity in triggering late LTP but do not address the mechanism of late LTP expression.

Work on a late phase of LTP by Saktor and coworkers has implicated persistent expression and constitutive activity of the PKC-like enzyme PKMζ (Yao et al. 2008). In this model, LTP induction inhibits the prolyl isomerase Pin1. Pin1 functions as an inhibitor of dendritic translation of a number of mRNAs including that encoding PKMζ, so PKMζ translation is thereby derepressed (Sacktor 2011). PKMζ is a splice form of PKC that lacks regulatory domains, so is constitutively active. Application of a peptide inhibitor designed to specifically inhibit PKMζ and derivatized to permeate cell membranes has been shown to reverse established late LTP when applied even hours to days after induction. Importantly, PKMζ inhibits Pin1 activity, setting the stage for a feedback loop to sustain LTP indefinitely (Westmark et al. 2010).

The persistent PKMζ model for late LTP described above is in contrast to the present model for late phase cerebellar LTD, which does not require sustained PKC (or presumably PKMζ) activity. In the present model, PKC activation is required for the early phase but not the late phase of LTD, whereas hippocampal LTD is reported to have the opposite requirements. It will be useful to examine the detailed expression mechanisms of late LTP and LTD (and other forms of information storage such as intrinsic plasticity) to see if common themes or modules emerge.

ACKNOWLEDGMENTS

Thanks to Devorah VanNess for excellent technical assistance and to members of the Linden and Worley labs for helpful suggestions. Richard Huganir’s lab provided the PICK1 GST fusion proteins.

GRANTS

This work has been supported by National Institute of Mental Health Grants R37 MH-51106 and P50 MH-084020.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.J.L. conception and design of research; D.J.L. performed experiments; D.J.L. analyzed data; D.J.L. interpreted results of experiments; D.J.L. prepared figures; D.J.L. drafted manuscript; D.J.L. edited and revised manuscript; D.J.L. approved final version of manuscript.

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J Neurophysiol • doi:10.1152/jn.00824.2011 • www.jn.org


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