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Long-term synaptic plasticity in cerebellar stellate cells

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Abstract

Inhibitory transmission controls the action potential firing rate and pattern of Purkinje cell activity in the cerebellum. A long-term change in inhibitory transmission is likely to have a profound effect on the activity of cerebellar neuronal circuits. However little is known about how neuronal activity regulates synaptic transmission in GABAergic inhibitory interneurons (stellate/basket cells) in the cerebellar cortex. We have examined how glutamate released from parallel fibres (the axons of granule cells) influences postsynaptic AMPA receptors in stellate cells and modulates GABA release from these neurons. First, we found that burst stimulation of presynaptic parallel fibres changes the subunit composition of post-synaptic AMPA receptors from GluR2-lacking to GluR2-containing receptors. This switch reduces the Ca^{2+} permeability of AMPA receptors and the EPSP amplitude, and prolongs the duration of the synaptic current, producing a qualitative change in synaptic transmission. This switch in AMPA receptor phenotype can be induced by activation of extrasynaptic NMDA receptors and involves PICK1 and the activation of PKC. Second, activation of presynaptic NMDA receptors triggers a lasting increase in GABA release from stellate cells. These changes may provide a cellular mechanism underlying associative learning involving the cerebellum.

Neuronal activity can induce a lasting change in the efficacy of synaptic transmission in the cerebellum. Long-term synaptic plasticity within the cerebellar circuit is thought to underlie the associative learning that involves the cerebellum (1–5). For example associative eyelid conditioning leads to a sustained decrease in action potential firing in Purkinje cells (3). This is often attributed to a reduction in synaptic transmission at the parallel fibre to Purkinje cell synapse (“cerebellar LTD”; see review by Hansel et al, (6)). However Purkinje cells are also innervated by inhibitory stellate and basket cells in the molecular layer of the cerebellar cortex and an enhanced inhibitory transmission is also expected to modulate the firing rate and pattern of Purkinje cells (7–9). We have therefore examined whether physiologically relevant neuronal stimuli can alter synaptic transmission in cerebellar stellate cells.

Stellate cells receive excitatory inputs from parallel fibres (PF), the axons of granule cells. Both LTP and LTD have been observed at this synapse and LTP requires NO production (10). The excitatory post-synaptic currents (EPSCs) at the parallel fibre to stellate cell synapse are predominantly mediated by AMPA receptors that lack GluR2 subunits (11,12). These receptors are permeable to Ca^{2+} and have a large single channel conductance (13,14). In contrast receptors that contain GluR2 subunits are Ca^{2+} impermeable and have slow decay kinetics (15). Our previous studies showed that repetitive activation of synaptic Ca^{2+} permeable AMPA receptors can induce a switch in the subunit composition of synaptic AMPA receptors from GluR2-lacking to GluR2-containing receptors (11). A recent *in vivo* study demonstrated that sensory stimulation can evoke a burst of action potential firing in

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granule cells (16). We therefore tested whether a burst stimulation of parallel fibres (FPs) that activated extrasynaptic NMDA receptors (12,17) could also alter the synaptic AMPA receptor phenotype.

In these experiments the subunit composition of synaptic AMPA receptors in stellate cells was determined from the current – voltage relationship of EPSCs when spermine was included in the patch electrode. An inwardly rectifying I-V relationship would suggest the presence of Ca²⁺ permeable, GluR2-lacking AMPA receptors, whereas a linear one would indicate GluR2-containing receptors (18–21). We found that bursts of PF stimulation in the presence of an AMPA receptor blocker altered the I-V relationship from being inwardly rectifying to linear, giving rise to an increase in rectification index. These changes occurred at a near physiological temperature (Figure 1A). This result indicates that the number of GluR2-containing AMPA receptors increased at the synapse (22). The increase in rectification index was completely blocked by an NMDA receptor blocker, CPP, and could be induced by a brief application of NMDA and glycine (22). Therefore activation of NMDA receptors triggers the switch from GluR2-lacking to GluR2 containing receptors. This leads to a reduction in current amplitude at -60 mV and a prolonged decay time of the EPSCs.

This NMDA receptor-mediated change requires the activation of PKC, since inclusion of PKC inhibitors, PKCI 19–36 and chelerythrine, in the postsynaptic electrode abolished the alteration in AMPA receptor phenotype. Conversely activation of PKC by OAG and (–) indolactam-V increased the expression of GluR2-containing receptors at the synapse (22). It is known that activated PKC interacts with PICK1, which binds to GluR2 subunits and facilitates the targeting of GluR2 to spines in hippocampal neurons (23). To test for a role of PICK1 in the insertion of GluR2 receptors we used a peptide inhibitor (pep2-AVKI) that selectively binds to PICK1 and disrupts its interactions with GluR2 subunits. As predicted, inclusion of pep2-AVKI in the patch electrode prevented the increase in synaptic GluR2 receptors, but did not interfere with the loss of GluR2-lacking receptors (22). Thus PICK1 is involved in the change in AMPA receptor phenotype that is induced by the activation of synaptic AMPA receptors (14,24) as well as by activation of extrasynaptic NMDA receptors (22).

To test whether the receptor switch requires transcriptional activity, we monitored the rectification index of EPSCs for several hours following the PF stimulation. We observed that the switch occurred within 30 minutes and lasted for at least 4 hours. Actinomycin D, a transcriptional inhibitor, did not block the PF stimulation-induced switch in AMPA receptor phenotype (Liu et al, manuscript submitted).

Taken together these results suggest that activation of extrasynaptic NMDA receptors can increase the expression of synaptic GluR2-containing AMPA receptors. This requires the activation of PKC and the interaction between PICK1 and GluR2 subunits, but does not depend on transcription. Thus synaptic activity can modulate AMPA receptor trafficking leading to an increase in the expression of synaptic GluR2-containing receptors (Figure 1B).

A possible mechanism suggested by our work and that of other studies (11,13,14,22,24) is that PICK1 is involved in the activity-dependent insertion of GluR2-containing AMPA receptors to extrasynaptic (or synaptic) sites in stellate cells (Figure 1B). These extrasynaptic GluR2-containing AMPA receptors may then move laterally into the synapse. Both PKC activation and PICK1-GluR2 interactions are also required for LTD at the parallel fibre to Purkinje cell synapse, suggesting that PICK1 facilitates the removal of synaptic GluR2-containing receptors in Purkinje cells (25–28). A study using a mutant mouse that lacks PICK1 revealed that GluR2 proteins accumulate at extrasynaptic regions and

intracellularly within Purkinje cell spines (28). In contrast, deletion of PICK1 reduces somatic GluR2-containing AMPA receptors in stellate cells (24). While synaptic plasticity at both the PF to stellate cell and PF to Purkinje cell synapse requires PICK-GluR2 interactions, the mechanism(s) by which PICK1 produces opposing actions at these two synapses, remains to be determined.

Stellate cells innervate Purkinje cells. At this synapse inhibitory synaptic transmission undergoes a lasting potentiation following postsynaptic depolarization due to the activation of excitatory climbing fiber inputs (29,30). This potentiation arises from an increase in the postsynaptic GABA_A receptor sensitivity and can be suppressed by activation of postsynaptic GABA_B receptors (31,32). Stellate cells also innervate other stellate cells forming inhibitory networks. We therefore examined whether glutamatergic synaptic activity could also regulate GABA release from stellate cells. Evoked GABA release was monitored at an autaptic synapse, which allowed us to detect GABA release from a single axon. We found that the amplitude of inhibitory synaptic currents (IPSCs) increased following burst PF stimulation and lasted for at least one hour (33). This was associated with a decrease in the paired pulse ratio, a reduction in the coefficient of variation of IPSC amplitude and an increase in the failure rate. These changes indicate that the increase in evoked IPSC amplitude arose from a potentiation in GABA release from stellate cells. PF stimulation also induced an increase in the miniature IPSC (mIPSC) frequency in stellate cells, but not in the amplitude of mIPSCs (33), again indicative of enhanced GABA release from stellate cells.

This increase in the secretion of GABA could be induced by application of NMDA and glycine and was completely blocked by CPP (an NMDA receptor blocker). It was not affected by NO synthase inhibitor, CBR1, mGluR or β -adrenergic receptor blockers. Therefore activation of NMDA receptors is required for the induction of the lasting increase in GABA release (I-LTP). Removal of Ca²⁺ from extracellular solution during NMDA/glycine application prevented I-LTP, but inclusion of BAPTA in the patch electrode did not block I-LTP. This suggests that Ca²⁺ entry *via* presynaptic NMDA receptors is important for the induction of I-LTP (33).

Since the probability of GABA release from stellate cells has been shown to decrease during development (34), we tested whether I-LTP was more likely to be induced in mature stellate cells that have an initially low release probability. Indeed we found that I-LTP could be induced in stellate cells from P18 and older mice (33), but not from younger animals (35).

In conclusion, our results show that burst stimulation of PFs can alter synaptic transmission in GABAergic stellate cells. We find several sites of modulation. First there is a change in the postsynaptic AMPA receptor phenotype. This is predicted to modify action potential firing in stellate cells and thus inhibitory transmission in the cerebellum. Second, PF stimulation directly modulates GABA release from stellate cells. A lasting increase in GABA release from stellate cells is also likely to alter the action potential firing pattern and reduce the firing rate of both Purkinje cells and synaptically connected stellate cells. Both of these changes therefore appear likely to alter the output of this small cerebellar inhibitory circuit and contribute to associative cerebellar learning.

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References

1. Raymond JL, Lisberger SG, Mauk MD. The cerebellum: a neuronal learning machine? *Science*. 1996; 272(5265):1126–31. [PubMed: 8638157]
2. Ito M. Long-term depression. *Annu Rev Neurosci*. 1989; 12:85–102. [PubMed: 2648961]
3. Thompson RF. Neural mechanisms of classical conditioning in mammals. *Philos Trans R Soc Lond B Biol Sci*. 1990; 329(1253):161–70. [PubMed: 1978361]
4. Medina JF, Mauk MD. Computer simulation of cerebellar information processing. *Nat Neurosci*. 2000; 3 (Suppl):1205–11. [PubMed: 11127839]
5. Hansel C, Linden DJ. Long-term depression of the cerebellar climbing fiber--Purkinje neuron synapse. *Neuron*. 2000; 26(2):473–82. [PubMed: 10839365]
6. Hansel C, Linden DJ, D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nat Neurosci*. 2001; 4(5):467–75. [PubMed: 11319554]
7. Hausser M, Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron*. 1997; 19(3):665–78. [PubMed: 9331356]
8. Jaeger D, Bower JM. Synaptic control of spiking in cerebellar Purkinje cells: dynamic current clamp based on model conductances. *J Neurosci*. 1999; 19(14):6090–101. [PubMed: 10407045]
9. Midtgaard J. Stellate cell inhibition of Purkinje cells in the turtle cerebellum in vitro. *J Physiol*. 1992; 457:355–67. [PubMed: 1297838]
10. Rancillac A, Crepel F. Synapses between parallel fibres and stellate cells express long-term changes in synaptic efficacy in rat cerebellum. *J Physiol*. 2004; 554(Pt 3):707–20. [PubMed: 14617674]
11. Liu SQ, Cull-Candy SG. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature*. 2000; 405(6785):454–8. [PubMed: 10839540]
12. Clark BA, Cull-Candy SG. Activity-dependent recruitment of extrasynaptic NMDA receptor activation at an AMPA receptor-only synapse. *J Neurosci*. 2002; 22(11):4428–36. [PubMed: 12040050]
13. Liu SJ, Cull-Candy SG. Activity-dependent change in AMPA receptor properties in cerebellar stellate cells. *J Neurosci*. 2002; 22(10):3881–9. [PubMed: 12019307]
14. Liu SJ, Cull-Candy SG. Subunit interaction with PICK and GRIP controls Ca²⁺ permeability of AMPARs at cerebellar synapses. *Nat Neurosci*. 2005; 8(6):768–75. [PubMed: 15895086]
15. Liu SJ, Zukin RS. Ca²⁺-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci*. 2007; 30(3):126–34. [PubMed: 17275103]
16. Chadderton P, Margrie TW, Hausser M. Integration of quanta in cerebellar granule cells during sensory processing. *Nature*. 2004; 428(6985):856–60. [PubMed: 15103377]
17. Carter AG, Regehr WG. Prolonged synaptic currents and glutamate spillover at the parallel fiber to stellate cell synapse. *J Neurosci*. 2000; 20(12):4423–34. [PubMed: 10844011]
18. Bowie D, Mayer ML. Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. *Neuron*. 1995; 15(2):453–62. [PubMed: 7646897]
19. Kamboj SK, Swanson GT, Cull-Candy SG. Intracellular spermine confers rectification on rat calcium-permeable AMPA and kainate receptors. *J Physiol*. 1995; 486 (Pt 2):297–303. [PubMed: 7473197]
20. Koh DS, Burnashev N, Jonas P. Block of native Ca(2+)-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. *J Physiol*. 1995; 486 (Pt 2):305–12. [PubMed: 7473198]
21. Washburn MS, Numberger M, Zhang S, Dingledine R. Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. *J Neurosci*. 1997; 17(24):9393–406. [PubMed: 9390995]
22. Sun L, Liu SJ. Activation of extrasynaptic NMDA receptors induces a PKC-dependent switch in AMPA receptor subtypes in mouse cerebellar stellate cells. *J Physiol*. 2007
23. Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB. PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface

- levels of the AMPA-type glutamate receptor subunit 2. *J Neurosci.* 2001; 21(15):5417–28. [PubMed: 11466413]
24. Gardner SM, Takamiya K, Xia J, Suh JG, Johnson R, Yu S, et al. Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron.* 2005; 45(6):903–15. [PubMed: 15797551]
 25. Leitges M, Kovac J, Plomann M, Linden DJ. A unique PDZ ligand in PKC α confers induction of cerebellar long-term synaptic depression. *Neuron.* 2004; 44(4):585–94. [PubMed: 15541307]
 26. Dev KK, Nishimune A, Henley JM, Nakanishi S. The protein kinase C α binding protein PICK1 interacts with short but not long form alternative splice variants of AMPA receptor subunits. *Neuropharmacology.* 1999; 38(5):635–44. [PubMed: 10340301]
 27. Xia J, Chung HJ, Wihler C, Huganir RL, Linden DJ. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron.* 2000; 28(2):499–510. [PubMed: 11144359]
 28. Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S, et al. Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. *Neuron.* 2006; 49(6):845–60. [PubMed: 16543133]
 29. Kano M, Rexhausen U, Dreessen J, Konnerth A. Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. *Nature.* 1992; 356(6370):601–4. [PubMed: 1313949]
 30. Kano M, Fukunaga K, Konnerth A. Ca²⁺-induced rebound potentiation of gamma-aminobutyric acid-mediated currents requires activation of Ca²⁺/calmodulin-dependent kinase II. *Proc Natl Acad Sci U S A.* 1996; 93(23):13351–6. [PubMed: 8917594]
 31. Kawaguchi S, Hirano T. Suppression of inhibitory synaptic potentiation by presynaptic activity through postsynaptic GABA(B) receptors in a Purkinje neuron. *Neuron.* 2000; 27(2):339–47. [PubMed: 10985353]
 32. Kawaguchi SY, Hirano T. Signaling cascade regulating long-term potentiation of GABA(A) receptor responsiveness in cerebellar Purkinje neurons. *J Neurosci.* 2002; 22(10):3969–76. [PubMed: 12019316]
 33. Liu SJ, Lachamp P. The activation of excitatory glutamate receptors evokes a long-lasting increase in the release of GABA from cerebellar stellate cells. *J Neurosci.* 2006; 26(36):9332–9. [PubMed: 16957089]
 34. Pouzat C, Hestrin S. Developmental regulation of basket/stellate cell-->Purkinje cell synapses in the cerebellum. *J Neurosci.* 1997; 17(23):9104–12. [PubMed: 9364057]
 35. Liu SJ. Biphasic Modulation of GABA Release From Stellate Cells by Glutamatergic Receptor Subtypes. *J Neurophysiol.* 2007; 98(1):550–6. [PubMed: 17537903]

Figure 1. Activity-dependent switch in AMPA receptor subtypes at the parallel fibre to stellate cell synapse

(A) Synaptic currents in a stellate cell exhibit an inwardly rectifying current (I)-voltage (V) relationship, a characteristic feature of GluR2-lacking AMPA receptors. Stimulation of PFs with a burst of 4 stimuli at 100 Hz, repeated 10 times at 1 Hz, changed the I-V relationship of spontaneous EPSCs from inwardly rectifying to linear. This suggests that synaptic AMPA receptors switched from GluR2-lacking to GluR2-containing receptors. This experiment was performed at 36°C. (B) Schematic of the mechanism underlying modulation at the parallel fibre-stellate cell synapse. During PF burst stimulation, glutamate released from parallel fibres activates synaptic AMPA receptors and extrasynaptic NMDA receptors. Ca^{2+} influx via AMPA and NMDA receptors promotes PKC activation. PKC activates PICK and PICK facilitates the delivery of GluR2-containing AMPA receptors to synaptic or extrasynaptic sites.