Selective inhibition of spontaneous but not Ca\(^{2+}\)-dependent release machinery by presynaptic group II mGluRs in rat cerebellar slices

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mGluR inhibition of spontaneous release
Abstract

Two main forms of neurotransmitter release are known: action potential-evoked and spontaneous release. Action potential-evoked release depends on Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels whereas spontaneous release is thought to be Ca\(^{2+}\)-independent.

Generally, spontaneous and action potential-evoked release are believed to use the same release machinery to release neurotransmitter. This study shows, using the whole cell patch clamp technique in rat cerebellar slices, that at the interneuron – Purkinje cell synapse activation of presynaptic group II metabotropic receptors suppresses spontaneous GABA release through a mechanism independent of voltage gated Ca\(^{2+}\) channels, store-operated Ca\(^{2+}\) channels and Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores, suggesting that the metabotropic receptors target the release machinery directly. Voltage gated Ca\(^{2+}\) channel-independent release following increased presynaptic cAMP production is similarly inhibited by these metabotropic receptors.

In contrast, both voltage gated Ca\(^{2+}\) channel-dependent and presynaptic NMDA receptor-dependent GABA release were unaffected by activation of group II metabotropic glutamate receptors. Hence, the mechanisms underlying spontaneous and Ca\(^{2+}\)-dependent GABA release are distinct in that only the former is blocked by group II metabotropic glutamate receptors.

Thus, the same neurotransmitter, glutamate, can activate or inhibit neurotransmitter release by selecting different receptors that target different release machineries.
Neurotransmitter release is generally taken as an example of regulated exocytosis: Upon an appropriate stimulus (usually the action potential, AP), a neuron releases neurotransmitter onto other neurons. The function of the AP is to depolarize the terminal, thereby opening voltage-gated Ca\(^{2+}\) channels (VGCCs). Ca\(^{2+}\) influx through these channels then triggers the fusion of the neurotransmitter-filled vesicle with the presynaptic membrane, thereby releasing its content into the synaptic cleft. However, neurotransmitter release can also occur in the absence of APs. This form of exocytosis is called spontaneous release because of the apparent lack of any obvious stimulus that might trigger this release. Generally, spontaneous release occurs at a much slower rate than AP-dependent release.

It is unclear why these two neurotransmitter release pathways exist in parallel but it seems that the AP-dependent pathway is used primarily for information transfer whereas the spontaneous pathway may also serve such diverse functions as synapse structure maintenance (McKinney et al. 1999), regulation of dendritic protein synthesis (Sutton et al. 2004), shaping of nerve cell firing (Cohen and Miles 2000; Carter and Regehr 2002) and contribution to synaptic plasticity (Jensen et al. 1999; Kombian et al. 2000).

It has been shown for some synapses that spontaneous and AP-induced neurotransmitter release use the same vesicle pool and release machinery (Scanziani et al. 1995; Rosenmund and Stevens 1996; Wu and Saggau 1997; Prange and Murphy 1999). At other synapses, however, spontaneous and evoked release employ different release
machineries and/or vesicle pools (Deitcher et al. 1998; Koenig and Ikeda 1999; Humeau et al. 2000; Maruyama et al. 2001; Calakos et al. 2004; Sara et al. 2005).

AP-dependent and spontaneous neurotransmitter release are both subject to modulation by G-protein coupled receptors and in many systems, activation of these receptors leads to a decrease in presynaptic neurotransmitter release (reviewed in Wu and Saggau 1997; Miller 1998; Offermanns 2003).

In principle, G-protein coupled receptors can reduce neurotransmitter release by inhibiting VGCCs, or by opening inwardly rectifying K\(^+\) channels which prevents or reduces terminal depolarization. Alternatively, activation of G-protein coupled receptors could modulate Ca\(^{2+}\) release from intracellular stores or even directly inhibit the neurotransmitter release machinery itself (Miller 1998; Blackmer et al. 2001; Offermanns 2003; Stevens 2004; Blackmer et al. 2005; Gerachshenko et al. 2005; Sullivan 2005).

At the interneuron – Purkinje cell synapse in rat cerebellar slices it has been shown that activation of presynaptic metabotropic glutamate receptors (mGluRs) decreases both spontaneous and AP-dependent GABA release (Llano and Marty 1995; Glitsch et al. 1996). It is not clear whether activation of mGluRs blocks both forms of exocytosis through similar or distinct intracellular signaling pathways.

This study demonstrates that spontaneous and depolarization-induced GABA release are differentially regulated by presynaptic group II mGluRs in that only spontaneous GABA release is inhibited. Hence, group II mGluRs selectively target the vesicles/release machinery responsible for spontaneous release.
Materials and Methods

Tissue preparation

Sagittal cerebellar slices were obtained from Sprague Dawley rats aged 13-15 days, as previously described (Glitsch et al. 1996; Llano et al. 1991). Briefly, rats were decapitated following cervical dislocation and the cerebellar vermis was dissected out. The vermis was cooled in ice-cold bicarbonate-buffered saline (BBS, composition see below) for 2-4 minutes and then glued to the stage of a vibratome. Slices of 200 μm thickness were cut and incubated in oxygenated bicarbonate-buffered saline (BBS) at room temperature for at least 40 minutes before their use in experiments.

Electrophysiology

Tight-seal whole cell recordings were obtained from Purkinje cells, as described in Glitsch et al. (1996) and Llano et al. (1991). A 63x water immersion objective was used for visualization of the slices during experiments. Experiments were performed using an EPC9/2 patch-clamp amplifier (Heka Electronics, Lambrecht, Germany). Patch pipettes had resistances of 2-3 MΩ with the standard internal and extracellular solutions used (composition see below). If series resistance exceeded 10 MΩ or the holding current at -60mV holding potential exceeded -500pA, experiments were abandoned. Capacitative currents were cancelled and series resistance was compensated by between 50-80%, as described in Llano et al. (1991).

Experiments were performed at room temperature and slices were continuously perfused with oxygenated BBS at a rate of 2ml/min. Drugs were applied to the slice by either
dissolving them directly in BBS or by dissolving prepared stock solutions into BBS to the desired concentration. BBS was applied via the bath perfusion system at a high perfusion speed (>5ml/min) for up to one minute. To study the effect of a drug on synaptic transmission, synaptic currents were recorded for 3 min under control conditions, starting 2 min after establishing the whole-cell configuration. Then the drug was washed in and recordings were resumed for 3 min under test conditions. In some cases, a third recording was made. For some experiments, slices were pre-incubated in the presence of the drugs to ensure maximal drug exposure.

Only one cell per slice was used for each experiment to avoid contamination of the results by potential long-term side effects of the drugs used.

Solutions and Drugs

The standard external solution BBS contained (in mM): 125 NaCl, 2.5 KCl, 26 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2 and 10 glucose (pH 7.4 when oxygenated with carbogen). For experiments with elevated extracellular K⁺ concentration, 12.5mM NaCl was replaced by 12.5mM KCl.

The intracellular solution contained (in mM): 150 CsCl, 10 HEPES, 1 EGTA, 0.1 CaCl2, 4 Na-ATP, 0.4 Na-GTP, 4.6 MgCl2 (pH adjusted to 7.3 with CsOH).

(2S,2’R,3’R)-2-(2’,3’-Dicarboxycyclopropyl)glycine (DCG-IV), Tetrodotoxin (TTX), 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; disodium salt), N-Methyl-D-aspartic acid (NMDA) and forskolin were obtained from Tocris Cookson (Bristol, UK). CdCl₂, nifedipine, ryanodine and thapsigargin were obtained from Sigma Aldrich (Poole, UK).
Apart from nifedipine, ryanodine, forskolin, 2-Aminoethoxydiphenylborane (2-APB) and thapsigargin, which were dissolved in DMSO, all drugs were either dissolved in water or BBS. Control experiments for drugs dissolved in DMSO were carried out in the same final concentration of DMSO to ensure that DMSO per se did not have any effects on synaptic transmission. NBQX was dissolved in equimolar NaOH. Drug stocks were aliquotted and frozen at -20°C. All experiments were carried out in the presence of 0.25\textmu M TTX and 10\textmu M NBQX or CNQX; the DCG-IV concentration used was always 5\textmu M. The TTX concentration used was high enough to completely suppress any APs both pre- and postsynaptically, even in the presence of elevated external potassium (see also Glitsch and Marty, 1999).

**Analysis**

Synaptic currents were analysed using an event-detection routing kindly provided by Dr. P. Vincent. Each event above the selected detection threshold was visually confirmed to be a synaptic current (Vincent and Marty, 1993).

Data are given as mean±SE for n numbers of cells. Student T test was carried out using InStat 2.03 for Macintosh and SPSS 12.0 for Windows and paired tests were used where appropriate. Results were considered significant with $p \leq 0.05$.

Data were normalized by calculating the average control value (average control amplitude or average control frequency of mIPSCs). Control and test values were then divided by this average control value and converted to % normalized amplitude or % normalized number of events.
Results

It was previously shown that activation of presynaptic group II mGluRs reduced GABA release from presynaptic interneurons in rat cerebellar slices in the presence of TTX (Llano and Marty 1995; Glitsch et al. 1996). Fig. 1A shows that activation of type II mGluRs with the specific group II mGluR agonist DCG-IV (5μM, a maximally effective dose, Glitsch et al. 1996) leads to a reduction in mIPSC frequency. Aggregate data from 16 Purkinje cells is summarized in Figs. 1B and 1C. Application of 5μM DCG-IV led to a decrease in mIPSC frequency by 63.4±3.6%, which was highly significant (P<0.0001; Fig. 1B) whilst mIPSC amplitude was unaffected (P=0.4876; Fig. 1C).

Although this pattern is similar to the findings reported previously (Glitsch et al. 1996), there are some minor differences. In the previous study, there was a small but significant reduction in mIPSC amplitude (15±4% reduction) following DCG-IV application which was not observed in the present study. Also, the effect of DCG-IV on mIPSC frequency was less pronounced in the previous study (45±4.5% reduction) compared with that here (63.4±3.6% reduction). Moreover, in the previous study 17% of Purkinje cells showed no decrease in mIPSC frequency following DCG-IV applications, whereas here all Purkinje cells displayed a decrease in mIPSC frequency (n=45). The reason for these discrepancies are unclear but are likely to reflect a difference in rat ages and/or strains used (rat ages here P13-15, previous study P11-12; rat strain here Sprague Dawley, previous study Wistar).
Activation of presynaptic group II mGluRs leads to a decrease in mIPSC number without affecting mIPSC amplitude. A Raw data recorded from one typical Purkinje cell showing mIPSCs under control (ctl) conditions (left) and in the presence of 5\textmu M DCG-IV (right). There is a clear reduction in mIPSC frequency in the presence of the group II mGluR agonist. Scale bars: 1sec/100pA. B Summary of the effect of 5\textmu M DCG-IV on mIPSC frequency in 16 Purkinje cells. The reduction in mIPSC frequency is highly significant (P<0.0001). C Aggregate data showing that 5\textmu M DCG-IV has no inhibiting effect on mIPSC amplitude for the same 16 Purkinje cells.

Frequency (freq.) and amplitudes (amp.) of mIPSCs were normalized (n.) to average control frequency and amplitude, respectively.

Spontaneous neurotransmitter release could in principle be the result of spontaneous opening of voltage-gated Ca^{2+} channels. Experiments were designed to test whether
activation of presynaptic group II mGluRs reduced spontaneous GABA release by inhibiting VGCCs (recently reviewed in Elmslie 2003).

Fig. 2A shows that in the presence of 200μM Cd\(^{2+}\), mIPSCs can still be recorded. There was a significant reduction in mIPSC frequency (30.5±14.8%; P=0.004; Fig. 2B) which would suggest that at least some mIPSCs result from the spontaneous opening of VGCCs. However, these results have to be seen in conjunction with the corresponding, significant reduction in mIPSC amplitude following Cd\(^{2+}\) application (16.4±4.3%; P=0.013; Fig. 2C; n=9). It is well documented in the literature that currents through GABA\(_A\) receptors are reduced in the presence of Cd\(^{2+}\) (Smart and Constanti 1990; Celentano et al. 1991; Casagrande et al. 2003). The small reduction in mIPSC frequency observed after addition of Cd\(^{2+}\) (Fig. 2B) can therefore most easily be explained by the small reduction in mIPSC amplitude that occurs in the presence of Cd\(^{2+}\) (Fig. 2C). This will result in a reduction in mIPSC frequency because more synaptic events will fall under the detection threshold, thereby effectively reducing the number of events that are detected.

Supplementary figure 1 shows a correlation between average mIPSC amplitude under control conditions and percent reduction in mIPSC frequency following Cd\(^{2+}\) application. The smaller the initial average mIPSC amplitude (and hence the more small mIPSCs contribute to the small average amplitude), the bigger the relative reduction in mIPSC frequency, whereas the bigger the initial average mIPSC amplitude (and the less small amplitude mIPSCs contribute to it), the smaller the mIPSC frequency reduction following Cd\(^{2+}\) application. These results suggest that it is indeed the reduction in mIPSC amplitude that gives rise to an apparent decrease in mIPSC frequency after Cd\(^{2+}\) application because
cells with a small initial average mIPSC amplitude are more affected than those with a large average mIPSC amplitude.

Importantly, even in the presence of Cd\(^{2+}\), addition of 5\(\mu\)M DCG-IV produced a highly significant reduction in mIPSC frequency (Fig. 2A, aggregate data summarized in Fig. 2B; \(P=0.001\)). The extent of reduction observed in the presence of Cd\(^{2+}\) was similar to that under control conditions following application of DCG-IV (when comparing frequency of mIPSCs in presence of Cd\(^{2+}\) and frequency of mIPSC in presence of Cd\(^{2+}\) and DCG-IV; \(P=0.3338\)).

These findings are in good agreement with other studies that have shown that neurotransmitter release in the presence of TTX is independent of presynaptic VGCCs (Scanziani et al. 1992; Thompson et al. 1993; Scanziani et al. 1995; Miller 1998; Bouron 2000; Harvey and Stephens 2004).

Generally, only P/Q-, N- and R-type voltage-gated Ca\(^{2+}\) channels are believed to play a role in neurotransmitter release (reviewed in Catterall 2000; Stevens 2004) and Cd\(^{2+}\) is known to fully block all these channels at concentrations similar or lower than that used here. However, to ensure that there was no unexpected role for T-type Ca\(^{2+}\) channels in spontaneous neurotransmitter release, experiments were repeated in 200\(\mu\)M Ni\(^{2+}\) which blocks T-type Ca\(^{2+}\) channels (as well as R- and to some extent P-type Ca\(^{2+}\) channels).

In the presence of 200\(\mu\)M Ni\(^{2+}\), DCG-IV reduced presynaptic GABA release by 55.6\(\pm\)11.4\% (\(P=0.028\), n=4; Fig. 2D). Even in the combined presence of 200\(\mu\)M Ni\(^{2+}\) and
200μM Cd\textsuperscript{2+}, DCG-IV still suppressed mIPSC frequency by 60.3±13% (P=0.009, n=4; Fig. 2E). As was the case with Cd\textsuperscript{2+}, Ni\textsuperscript{2+} produced a small reduction in mIPSC frequency and amplitude (data not shown for effect of Ni\textsuperscript{2+} on amplitude).

The reduction in mIPSC frequency following DCG-IV application in the presence of both Ni\textsuperscript{2+} and Cd\textsuperscript{2+} was not significantly different to the reduction induced by DCG-IV under control conditions (P=0.745; Fig. 1B/2E), suggesting that VGCCs are not involved in the group II mGluR-mediated reduction in presynaptic GABA release. Moreover, these results show that spontaneous GABA release from presynaptic interneurons is largely independent of presynaptic VGCC activity.
Inhibition of presynaptic Ca\textsuperscript{2+} channels with Cd\textsuperscript{2+} does not interfere with the group II mGluR-mediated inhibition of presynaptic GABA release.

A Raw data recorded from one typical Purkinje cell showing mIPSCs under control conditions (left), in the presence of 200\textmu M Cd\textsuperscript{2+} (middle) and in the presence of 200\textmu M Cd\textsuperscript{2+} + 5\textmu M DCG-IV (right). Scale bars:
2sec/100pA. B Histogram summarises the normalized frequency (n. freq.) of mIPSCs under control conditions (ctl), in the presence of 100 - 200μM Cd^{2+} (Cd) and in the presence of Cd^{2+} + DCG-IV (D); data from 9 Purkinje cells. Results obtained with 100 and 200μM Cd^{2+} were similar and have been pooled. The reduction in mIPSC frequency in Cd^{2+} is significant (P=0.044), as is that in the presence of Cd^{2+} + DCG-IV is (P=0.001). C Summary of data from the same 9 Purkinje cells as in B. Histogram summarises the effects of the various treatment on normalized amplitudes (n. amp.) of mIPSCs; symbols as in B. The reduction in mIPSC amplitude in Cd^{2+} was significant (P=0.013) but there was no further effect of DCG-IV on mIPSC amplitude. D Summary of data obtained in 4 Purkinje cells. Histogram compares normalized frequency of mIPSCs (n. freq. mIPSCs) under control conditions (ctl), in the presence of 200μM Ni^{2+} (Ni) and in the additional presence of DCG-IV (+D). There is a significant reduction in mIPSC frequency (P=0.028). E Summary of data obtained in 4 Purkinje cells. Histogram compares normalized frequency of mIPSCs under control conditions, in the presence of 200μM Ni^{2+} and 200μM Cd^{2+} (NiCd) and in the additional presence of DCG-IV (+D). There is a significant reduction in mIPSC frequency (P=0.009). However, there is no difference in the extent of reduction observed after applying DCG-IV between control conditions (Fig.1), in the presence of Cd^{2+} only or Ni^{2+} only or the combined presence of both Cd^{2+} and Ni^{2+}.

In non-excitatory cells, Ca^{2+} entry through store-operated channels can drive secretory events (recently reviewed by Parekh and Putney 2005). However, the store-operated channel blocker 2-APB (20μM) had no significant inhibitory effect on spontaneous release (13.2±11% reduction in mIPSC frequency with no effect on mIPSC amplitude; the reduction in mIPSC frequency was not significant with P=0.15; n=4), suggesting that this Ca^{2+} influx pathway does not underlie spontaneous GABA release at the interneuron – Purkinje cell synapse.

There is evidence that Ca^{2+} release from presynaptic Ca^{2+} stores contributes to GABA release both at the interneuron – Purkinje cell synapse (Llano et al. 2000; Bardo et al.
2002, Galante and Marty 2003) and at other synapses (for review see Bouron 2001; Bouchard et al. 2003). Although group II mGluR activation can reduce spontaneous GABA release in the absence of functional VGCC, it is possible that they modulate Ca\(^{2+}\) release from stores.

Slices were preincubated in 20\(\mu\)M ryanodine (a concentration previously shown to release Ca\(^{2+}\) from intracellular stores, Llano et al. 1994; Llano et al. 2000), 2\(\mu\)M thapsigargin (a SERCA pump blocker that prevents refilling of intracellular stores) and 200\(\mu\)M Cd\(^{2+}\) (on average 47±4.2 min incubation; n=9). Under these conditions, Ca\(^{2+}\) influx through VGCCs should be blocked by Cd\(^{2+}\) and intracellular Ca\(^{2+}\) stores should be depleted by the combination of ryanodine and thapsigargin.

As can be seen in Fig.3A, even in the presence of these drugs, there was still a clear and significant reduction in mIPSC frequency after application of DCG-IV. Aggregate data is summarized in Fig. 3C (left hand panel). DCG-IV still significantly reduced mIPSC frequency following exposure to ryanodine/thapsigargin/Cd\(^{2+}\), and the extent of this reduction was similar to that seen in the presence of Cd\(^{2+}\) but where intracellular Ca\(^{2+}\) stores were intact (Figs. 3B and 3C right hand panel; n=9).

It cannot be ruled out that a presynaptic ryanodine- and thapsigargin-insensitive Ca\(^{2+}\) store is involved in regulating spontaneous GABA release and that this store is targeted by group II mGluRs. Although such a store has been proposed for certain non-excitable cells (Genazzani and Galione 1996), its existence remains controversial (Gerasimenko et al. 2003; Gerasimenko and Gerasimenko 2004).
These data demonstrate that spontaneous GABA release can still be blocked by activation of group II mGluRs even in the absence of functional VGCC and functional ryanodine- and thapsigargin-sensitive Ca\(^{2+}\) stores.

**Fig. 3**

DCG-IV reduces presynaptic GABA release by a mechanism that is independent of presynaptic Ca\(^{2+}\) stores.
A Raw data recorded from one representative Purkinje cell showing mIPSCs after preincubation (35min) in 20μM Ryanodine, 2μM Thapsigargin + 200μM Cd^{2+} (left) and after applying 5μM DCG-IV (right). There is a clear reduction in mIPSC frequency with DCG-IV. Scale bars: 1sec/50pA. B Typical raw data obtained from a Purkinje cell showing mIPSCs in the presence of 0.1% DMSO + 200μM Cd^{2+} (left) and after applying 5μM DCG-IV (right). Scale bars: 2sec/150pA. C Summary of the effect DCG-IV following preincubation with ryanodine, thapsigargin + Cd^{2+} (labeled RTCd; 47±4.2min; n=9; frequency (freq.) of mIPSCs normalized (n.) to average mIPSC frequency in ryanodine, thapsigargin + Cd^{2+}) compared with the effect of DCG-IV on mIPSC frequency in the presence of DMSO + Cd^{2+} alone (n=6; mIPSC frequency normalized to average mIPSC frequency in DMSO + Cd^{2+}). In both cases, the reduction in mIPSC frequency is significant (P=0.0317 and P=0.0221, respectively) and there is no difference between the extent of mIPSC reduction by DCG-IV after preincubation with ryanodine, thapsigargin + Cd^{2+} and in the presence of DMSO + Cd^{2+} only (P=0.8978). As a control, it was shown that the same stock and concentration of thapsigargin induced pronounced Ca^{2+} release from intracellular stores in RBL cells (Prof. A. Parekh, personal communication).

The preceding results clearly demonstrate that activation of presynaptic group II mGluRs by DCG-IV reduces spontaneous GABA release through a mechanism that is independent of VGCC, store-operated channels and intracellular Ca^{2+} stores, suggesting that group II mGluRs target directly the release machinery (Cartmell and Schoepp 2000; Blackmer et al. 2001; Offermanns 2003; Blackmer et al. 2005; Gerachshenko et al. 2005; Sullivan 2005). In some mammalian synapses, spontaneous and depolarization-induced neurotransmitter release utilize the same release machinery and the same pool of synaptic vesicles (Scanziani et al. 1995; Rosenmund and Stevens 1996; Wu and Saggau 1997; Prange and Murphy 1999).

If this is also true for the interneuron – Purkinje cell synapse, then one would expect activation of presynaptic group II mGluRs, which reduces spontaneous release, to also
impair depolarization-induced transmitter release. To test this, terminals were depolarized directly by raising extracellular $K^+$ concentration sixfold (external $K^+$ raised by 12.5mM to 15mM; subsequently referred to as elevated $K^+$).

Raising external $K^+$ to 15mM depolarizes the presynaptic terminals, thereby activating VGCCs. This results in a prominent increase in presynaptic GABA release (Fig. 4A). The IPSCs recorded in the presence of TTX and elevated $K^+$ are a mixture of both VGCC-mediated IPSCs and mIPSCs and will henceforth be called tIPSCs (t standing for total). If both depolarization-dependent and spontaneous GABA release link to the same pool of vesicles/release machinery, then addition of DCG-IV should lead to a pronounced reduction in depolarization-mediated GABA release.

Fig. 4A shows that DCG-IV, at a concentration and exposure time that substantially reduced spontaneous release, failed to inhibit increased GABA release following presynaptic depolarization with 15mM $K^+$. Whilst elevated $K^+$ significantly increased tIPSC frequency (Fig.4C; $P=0.0075; n=9$), subsequent addition of DCG-IV had no significant effect (Fig. 4C).

In the experiments of Fig. 4A, cells were first exposed to elevated $K^+$, and after synaptic activity had been recorded, DCG-IV was added (still in elevated $K^+$). It is possible that prolonged exposure to elevated $K^+$ leads to a time-dependent increase in tIPSC frequency and this could conceivably mask potential inhibitory effects of DCG-IV. To test this, tIPSCs were monitored in the continuous presence of elevated $K^+$ (without adding DCG-IV).

As shown in Fig. 4B, there is indeed a further increase in tIPSC frequency with prolonged exposure to elevated $K^+$. Aggregate data is summarized in Fig. 4D, which
compares the tIPSC frequency in control conditions, after 1 min exposure to elevated K+ (called K) and then after 6 min exposure (called pK). After 6 min exposure, tIPSC frequency increased significantly (P=0.022) compared with tIPSC frequency after 1 min. Because of this time-dependent increase in tIPSC frequency in elevated K+, it was important to compare tIPSC frequency in elevated K+ with that in elevated K+ + DCG-IV but after equal exposure time to elevated K+.

Fig. 4E compares the normalized tIPSC frequency obtained after prolonged (6min) exposure to elevated K+ with the normalized tIPSC frequency obtained in the additional presence of DCG-IV (and exposed to elevated K+ for the same time; n=9 for each condition).

Although the frequency of tIPSC in the presence of DCG-IV was slightly reduced when compared with elevated K+ alone, this reduction was not significant (Fig. 4E; P=0.1605). Moreover, a small reduction in tIPSC frequency is not unexpected, since even in the presence of elevated K+ (which increases presynaptic GABA release about threefold) there will be a certain number of mIPSCs within this tIPSC population that will be blocked following activation of presynaptic group II mGluRs, giving rise to the small reduction of tIPSC frequency in the presence of DCG-IV.

The important finding is that the difference between extent of reduction in tIPSC frequency after addition of DCG-IV in the presence of elevated K+ and under control conditions (i.e. no elevated K+; Fig. 4F) was highly significant (P=0.0083). If both spontaneous and depolarization-induced GABA release were subject to inhibition following activation of presynaptic group II mGluRs, then there should be no difference in the extent of reduction of mIPSC frequency after DCG-IV application in the presence
and absence of elevated K⁺. The fact that the effect of DCG-IV is significantly less in the presence of elevated K⁺ means that the increased GABA release following activation of VGCCs in elevated K⁺ is not subject to group II mGluR-mediated modulation.

A

control +15mM K +5µM DCG-IV

B

control +15mM K prolonged K

C

D

n. freq. tIPSCs [%] n. freq. tIPSCs [%] n. freq. tIPSCs [%] n. freq. tIPSCs [%]

K+D

K

ctl

D

F

n. freq. mIPSCs [%] n. freq. mIPSCs [%]

K+D

pK

ctl +D
Fig. 4

Activation of presynaptic group II mGluRs does not interfere with depolarization-induced GABA release.

A Raw data recorded from one typical Purkinje cell showing mIPSCs under control conditions (left), in the presence of 15mM K⁺ (middle; 1min exposure) and in the additional presence of 5μM DCG-IV (right). There is a clear increase in total (t) IPSC frequency with elevated K⁺. The addition of DCG-IV does not have much of an effect on tIPSC frequency. Scale bars: 1sec/200pA. B Raw data recorded from one typical Purkinje cell showing mIPSCs under control conditions (left), in the presence of 15mM K⁺ (middle; 1min exposure) and in the prolonged presence of elevated K⁺ (right; 11min min exposure). There is a clear further increase in tIPSC frequency with prolonged exposure to 15mM K⁺. Scale bars: 2sec/200pA. C Summary of data obtained from 9 Purkinje cells that were exposed to 5μM DCG-IV in the presence of 15mM K⁺. The increase in tIPSC frequency was very significant (P=0.0075; middle grey bar labeled K) but there was no apparent effect on tIPSC frequency after applying DCG-IV in the continued presence of elevated K⁺ (labeled K+D; right black bar). tIPSC frequency (freq.) were normalized (n.) to average control (ctl) tIPSC number (left white bar). D Summary of 9 Purkinje cells in which the effect of prolonged exposure to 15mM K⁺ (pK) was assessed. The increases in tIPSC frequency after 1min exposure is significant (P=0.0011) as is the increase in tIPSC frequency with prolonged exposure (11min; P=0.0222). tIPSC frequency was normalized to control tIPSC frequency (left white bar). E Summary of effects of DCG-IV on 15mM K⁺. Left white bar: average tIPSC frequency after prolonged exposure normalized to tIPSC frequency after prolonged exposure. Right black bar: average tIPSC frequency in presence of both elevated K⁺ and DCG-IV, normalized to tIPSC frequency after prolonged exposure to elevated K⁺ only. There is no significant difference between tIPSCs frequency recorded in the prolonged presence of 15mM K⁺ only or in the presence of both DCG-IV and 15mM K⁺ (P=0.1605). F Aggregate data of 10 cells in which 5μM DCG-IV (D) was applied under control conditions. The reduction in tIPSC frequency in the presence of DCG-IV is highly significant (P<0.0001). The difference between reduction of tIPSC frequency with DCG-IV in the presence of 15mM K⁺ and under control conditions is highly significant (P=0.0083).
In order to confirm that elevated $K^+$ did indeed increase tIPSC frequency via activation of presynaptic VGCCs, control experiments were carried out in which the effect of 100 and 200$\mu$M Cd$^{2+}$ on elevated K$^+$-induced GABA release was investigated. Results for 100 and 200$\mu$M Cd$^{2+}$ were identical and therefore pooled. On average, 15mM K$^+$ increased tIPSC frequency by 160.6±27.5% (1min exposure) and also increased mean tIPSC amplitude by 11.8±5.3% (n=24). Both increases were significant (P<0.0001 and P=0.002, respectively).

In the presence of 100 - 200$\mu$M Cd$^{2+}$, the elevated K$^+$-induced increase in tIPSC frequency was largely suppressed (reduced by 80.5±21.2% without affecting mean amplitude). This finding is consistent with the idea that only high-threshold VGCCs are involved in elevated K$^+$-dependent presynaptic GABA release (recently reviewed in Catterall 2000; Stevens 2004). Moreover, these results show that 100$\mu$M Cd$^{2+}$ is able to suppress VGCC activity, yet at the same and higher concentrations it barely affects spontaneous release (Fig. 2).

Because of the somewhat unexpected time-dependence of presynaptic GABA release following exposure to elevated K$^+$, it was considered whether there might be a role for L-type VGCCs in presynaptic GABA release. However, when experiments with elevated K$^+$ levels were repeated in the presence of 10$\mu$M nifedipine, a potent L-type Ca$^{2+}$ channel blocker, there was no difference in the extent of increase in presynaptic GABA release between control and test (i. e. in the presence of nifedipine) conditions (control increase with 15mM K$^+$: 257.2±67.7% (n=6); increase with 15mM K$^+$ in the presence of nifedipine: 249.6±53.1%; n=5), suggesting that L-type Ca$^{2+}$ channels are not involved in the elevated K$^+$-mediated increase in presynaptic GABA release.
Taken together, these results show that activation of presynaptic group II mGluRs with DCGIV does not lead to a reduction in VGCC-mediated presynaptic GABA release.

Forskolin, a potent stimulator of cAMP production through direct activation of adenylate cyclases, leads to increases in presynaptic neurotransmitter release in a number of preparations. In cerebellum, both spontaneous and action-potential induced neurotransmitter release can be enhanced following forskolin application (Glitsch et al. 1996; Salin et al. 1996; Chen and Regehr 1997; Kondo and Marty 1997).

It was therefore of interest to see whether the forskolin-induced increase in mIPSC frequency could be blocked by activation of group II mGluRs. Fig. 5A shows that application of 10μM forskolin (recordings resumed after 2 min perfusion with forskolin) led to a clear increase in mIPSC frequency and this increase was statistically significant compared with DMSO (solvent for forskolin) controls (P=0.0181; Fig. 5D). Subsequent addition of DCG-IV (in forskolin) led to a clear and significant reduction in mIPSC frequency (see Fig. 5A for one example; P=0.0116). To ensure maximal activation of adenylate cyclases by forskolin, slices were preincubated in 10μM Forskolin for 12.6±1min (n=5) prior to exposure to DCG-IV. As can be seen in Fig. 5E, there is a pronounced and significant increase in absolute number of mIPSCs with this procedure compared with a 2 min perfusion of forskolin (P=0.0233).

Importantly, however, despite the significant increase in mIPSC frequency, DCG-IV still potently and significantly suppressed presynaptic GABA release following preincubation in forskolin (one example is shown in Fig. 5B; on average 59.5±11% reduction in mIPSC frequency following exposure to DCG-IV; P=0.0089; n=5). Figs. 5C and F (right two
bars) show that 0.2% DMSO, which is equivalent to the final concentration of DMSO used in the forskolin experiments, had no effect on DCG-IV’s ability to block presynaptic GABA release.

There was no significant difference in the extent of reduction of mIPSC frequency with DCG-IV obtained after preincubation or 2 min exposure to forskolin (P=0.4107). Fig. 5F therefore compares aggregate data in which the effect of DCG-IV on forskolin-mediated increase in presynaptic GABA release was tested. On average, DCG-IV reduced mIPSC frequency by 54±9.9% in the presence of forskolin (left pair of columns; very significant reduction with P=0.0046). This is similar to the extent of reduction induced by DCG-IV under control conditions in the absence of forskolin (Fig. 5F right pair of columns; no significant difference between extent of reduction with DCG-IV in forskolin and DMSO only; P=0.3918).

This suggests that forskolin-induced increases in presynaptic GABA release are subject to inhibition by activation of presynaptic group II mGluRs.
Fig. 5

Forskolin-stimulated increases in presynaptic GABA release are inhibited by activation of presynaptic group II mGluRs.

A Raw data recorded in one representative Purkinje cell in which the effect of 10µM forskolin on DCG-IV was tested. Left trace shows control mIPSCs in the presence of 0.2% DMSO (solvent for forskolin), middle
trace mIPSCs in the additional presence of forskolin (2min incubation) and right trace mIPSCs in the presence of forskolin and 5μM DCG-IV. Scale bars: 2sec/300pA. B Raw data recorded in one representative Purkinje cell. Left trace shows mIPSCs after preincubation with 10μM forskolin (12min), right trace shows mIPSCs in the additional presence of 5μM DCG-IV. Scale bars: 2sec/200pA. C Raw data recorded in one representative Purkinje cell showing control mIPSCs in the presence of 0.2%DMSO (left trace) and in the additional presence of 5μM DCG-IV (right trace). Scale bars: 2sec/300pA. D Aggregate data of 9 cells in which the effect of 10μM forskolin on mIPSC frequency is summarised. There is a significant increase in mIPSC frequency after forskolin (F) application (P=0.0181). mIPSC frequency (freq.) was normalised (n.) to average mIPSC frequency in solvent. E Summary showing that preincubation in forskolin (pre F) for 12.6±1 min (n=5) significantly increases absolute (abs.) mIPSC number compared to acute exposure to forskolin (n=5; 2min exposure to forskolin prior to recording; P= 0.00233). F Summary showing that DCG-IV (D) still significantly reduces mIPSC frequency in the presence of forskolin (all forskolin data pooled= F pool; left two columns; P=0.0046; n=10; mIPSC frequency normalized to average mIPSC frequency in forskolin) and that DMSO does not interfere with the ability of DCG-IV to reduce mIPSC frequency (right two columns; significant reduction with P=0.0241; n=5; mIPSC frequency normalized to average mIPSC numbers in DMSO). There is no significant difference between the extent of reduction in mIPSC frequency with DCG-IV in forskolin or DMSO only (P=0.3918).

Given that activation of group II mGluRs reduces GABA release following exposure to forskolin but not to elevated K⁺, it was of interest to see whether the forskolin-induced increase in mIPSC frequency was independent of activation of presynaptic VGCCs (Kondo and Marty 1997; Yoshihara et al. 1999). Figs. 6A and C (left two columns) show that this is indeed the case. Even in the presence of 200μM Cd²⁺ and 200μM Ni²⁺, forskolin still induced a significant increase in mIPSC frequency (88.1±60.8% increase; P=0.017) that was virtually identical to that observed in the absence of 200μM Cd²⁺ and
200μM Ni^{2+} (99.7±33.1%; statistically significant increase compared to control with \( P=0.044 \); Figs. 6B and C right two columns).

Taken together, these results show that the forskolin-induced increase in mIPSC frequency is independent of the activity of VGCCs.
Voltage-gated Ca\textsuperscript{2+} channel blockers Cd\textsuperscript{2+} and Ni\textsuperscript{2+} do not suppress the Forskolin-induced increase in presynaptic GABA release.

A Raw data obtained from one representative Purkinje cell. Left, control mIPSCs in 0.05% DMSO; middle, mIPSCs in presence of 200\mu M Ni\textsuperscript{2+} and 200\mu M Cd\textsuperscript{2+}; right, mIPSCs in additional presence of 10\mu M forskolin. Scale bars: 2sec/100pA. B Raw data recorded in one representative Purkinje cell. Left, control mIPSCs in 0.05% DMSO; right, mIPSCs in additional presence of 10\mu M forskolin. Scale bars: 2sec/100pA. C Aggregate data summarising the effect of forskolin (F) in the presence of 200\mu M Ni\textsuperscript{2+} and 200\mu M Cd\textsuperscript{2+} (left two columns). The increase in mIPSC frequency with forskolin was significant (P=0.017; n=4); mIPSC frequency (freq.) was normalized (n.) to average mIPSC frequency in the presence of Ni\textsuperscript{2+} and Cd\textsuperscript{2+}. Right two columns, control experiments in which the effect of 10\mu M forskolin alone on mIPSC frequency was tested (n=4; mIPSC frequency normalized to average mIPSC frequency in DMSO). The increase in mIPSC frequency was significant (P=0.044). There was no difference in the extent of increase in mIPSC frequency following forskolin application in the presence or absence of Ni\textsuperscript{2+} and Cd\textsuperscript{2+} (P=0.307).

Application of either elevated K\textsuperscript{+} or forskolin not only led to an increase in IPSC frequency but also an increase in average IPSC amplitude. On average, forskolin increased mIPSC amplitude by 16.7±9.8\% (P=0.031; n=13; Fig. 7A). 15mM K\textsuperscript{+} increased tIPSC amplitude by 12.4±5.4\% (P=0.02; n=27; Fig. 7B).

Fig. 7C and D compare amplitude distributions of IPSCs recorded from Purkinje cells exposed to either forskolin (Fig. 7C) or elevated K\textsuperscript{+} (Fig. 7D). Amplitude histograms were normalized to total number of events recorded over a 3-min-period to investigate if certain amplitudes are affected by application of forskolin/elevated K\textsuperscript{+} to a lesser or greater extent. The upper panel shows the control distribution of amplitudes (bin width is 10pA) while the middle panel shows the amplitude distribution obtained in either forskolin (Fig. 7C) or elevated K\textsuperscript{+} (Fig. 7D). The bottom panels show the amplitude
histogram that results from subtracting the control histogram from the test histogram (i.e., top histogram subtracted from middle histogram) to uncover the forskolin/elevated K⁺-induced changes.

For both treatments, there was a decrease in number of small amplitude IPSCs and an increase in number of larger amplitude IPSCs, showing that both forskolin and elevated K⁺ achieve an increase in average IPSC amplitude by both reducing small amplitude events and increasing large amplitude events.

The loss of small amplitude currents and increase in bigger amplitude currents probably reflects the fact that due to increased GABA release there is a bigger chance of two release events occurring at the same time, thereby giving rise to one big rather than two small events. Alternatively, these results could be explained by a general increase in IPSC size and a reduction in small size mIPSCs with a parallel increase in large size mIPSCs.
Forskolin and elevated K\(^+\) have similar effects on the amplitude distribution of IPSCs.

A. Forskolin increases average mIPSC amplitude significantly by 16.7\% \pm 9.8\% (P=0.031; n=13). B. 15mM K\(^+\) increases average mIPSC amplitude significantly by 12.4\% \pm 5.4\% (P=0.002; n=27). C. Average amplitude distribution of IPSCs under control conditions (upper graph) and in the presence of 10\mu M forskolin (middle graph; n=13). Bottom graph is obtained by subtraction of middle graph (forskolin) from upper graph (control) and shows forskolin-induced changes in IPSC amplitude distribution. D. As in C, but with 15mM K\(^+\) instead of forskolin (n=27). Amplitude distributions were normalized to total number of IPSCs. Bin width is 10pA. Both forskolin and elevated K\(^+\) slightly reduce the frequency of small IPSCs and increase the frequency of larger ones.

It was previously published that activation of presynaptic NMDA receptors on cerebellar interneurons leads to an increase in GABA release from interneurons onto Purkinje cells (Glitsch and Marty 1999). Given that both receptors use the same neurotransmitter,
glutamate, it was of interest to see whether NMDA could still enhance presynaptic GABA release in the presence of DCG-IV.

Originally, experiments were designed in which first 30μM NMDA was applied and then 5μM DCG-IV added (analogous to elevated K⁺ experiments). However, control experiments showed that prolonged exposure to 30μM NMDA alone lead to a pronounced desensitization of the NMDA response, resulting in a decrease in presynaptic GABA release by 30±19% (n=7). This reduction in presynaptic GABA release due to prolonged NMDA exposure alone was statistically not significantly different from the decrease in presynaptic GABA release observed in the prolonged presence of NMDA and additional presence of DCGIV (47±21% reduction, n=5; P=0.547).

However, there was a concern that the desensitization of the presynaptic NMDA receptors might mask potential DCG-IV effects. Therefore, experiments were repeated to verify that NMDA could still increase presynaptic GABA release following activation of presynaptic group II mGluRs. Fig. 8 shows that this was the case. Following a reduction in mIPSC frequency with 5μM DCG-IV (Fig. 8A middle panel), there is a clear increase in mIPSC frequency after addition of 30μM NMDA (Fig. 8A right panel). Fig. 8B compares the increase in mIPSC frequency following stimulation with NMDA in the absence (right two bars) and presence (left two bars) of 5μM DCG-IV. Application of NMDA following activation of type II mGluRs with DCG-IV resulted in a robust increase in mIPSC frequency (left black bar) that was not significantly different to that observed in the absence of DCG-IV (right black bar).
These results suggest that the NMDA-induced increase in GABA release, like the 15mM K⁺-induced GABA increase, is not subject to modulation by presynaptic group II mGluRs.

**Fig. 8**

*Activation of group II mGluRs does not interfere with NMDA receptor activation-induced increase in presynaptic GABA release.*

A Raw traces from one representative Purkinje cell showing mIPSC frequency under control (left trace) conditions, after applying 5μM DCG-IV (middle trace) and then in the additional presence of 30μM NMDA (right trace).
B Summary of the effect of 30µM NMDA on mIPSC frequency in the presence of 5µM DCG-IV (left two bars) and under control (ctl) conditions; n=5, respectively. mIPSC frequency (freq.) was normalized (n.) to average mIPSC frequency in presence of DCG-IV (left two bars) or under control conditions (right two bars). There was no significant difference between NMDA-induced increases in mIPSC frequency obtained under control conditions and NMDA-induced increases in mIPSC frequency obtained in the presence of DCG-IV.
Discussion

The main finding of this study is that there are two different GABA release pathways at the interneuron - Purkinje cell synapse that are differentially regulated by presynaptic group II mGluRs. The GABA release machinery of the Ca\(^{2+}\) influx-dependent pathway (activated either by terminal depolarization following application of 15mM K\(^+\) or NMDA receptor activation) is not subject to inhibition by group II mGluRs whereas the VGCC-independent pathway (spontaneous and cAMP-dependent GABA release following application of forskolin) is.

Neither spontaneous nor cAMP-dependent GABA release depend on Ca\(^{2+}\) influx through VGCCs. Indeed, in cerebellum it has been shown that both mIPSCs and the cAMP-dependent GABA release persist in the absence of external Ca\(^{2+}\) (Llano and Gerschenfeld 1993; Kondo and Marty 1997; Llano et al. 2000). The fact that activation of group II mGluRs results in a decrease in both spontaneous and cAMP-dependent GABA release under conditions when Ca\(^{2+}\) influx and Ca\(^{2+}\) release are suppressed suggests that group II mGluRs are inhibiting a late step in the release process, probably the release machinery itself (Cartmell and Schoepp 2000; Blackmer et al. 2001; Offermanns 2003; Blackmer et al. 2005; Gerachshenko et al. 2005; Sullivan 2005).

In contrast, the increase in GABA release following stimulation with 15mM external K\(^+\) or NMDA receptor activation (which can increase intracellular Ca\(^{2+}\) concentration by Ca\(^{2+}\) influx through the NMDA receptor itself or by depolarising the presynaptic membrane, thereby activating VGCCs) is unaffected by stimulation of presynaptic group II mGluRs.
It is well known that AP-dependent neurotransmitter release, which recruits VGCCs, can be modulated by G-protein coupled receptors. In theory, this inhibition of AP-dependent neurotransmitter release can be the result of modulation of ion channel function (inhibition of VGCCs or opening of K⁺ channels, which will hyperpolarize the terminal, thus making it harder for an incoming AP to trigger neurotransmitter release) and/or inhibition of the actual presynaptic release machinery (for recent reviews see Miller 1998; Cartmell and Schoepp 2000; Bouron 2001; Offermanns 2003; Sullivan 2005). Inhibitory effects of presynaptic G-protein coupled receptors on presynaptic voltage-gated ion channels would decrease AP-evoked neurotransmitter release only without affecting the Ca²⁺ influx-independent spontaneous release (Prince and Stevens 1992; Doze et al. 1995; Gereau and Conn 1995; McQuision and Colmers 1996).

If spontaneous and AP-induced neurotransmitter release use the same pool of synaptic vesicles and the same release sites (i.e. release machineries), as has been shown for some synapses (Rosenmund and Stevens 1996; Van der Kloot 1996; Ryan et al. 1997; Murthy and Stevens 1999; Prange and Murphy 1999 and references therein), then activation of presynaptic G-protein-coupled receptors should interfere directly and equally with the release machinery of both spontaneous and AP-evoked neurotransmitter release (as is shown in Scanziani et al. 1995; Dittmann and Regehr 1996; Nicola and Malenka 1997). However, this was clearly not the case here. Activation of group II mGluRs affected only spontaneous GABA release. There is increasing evidence that at some synapses, spontaneous and AP-evoked neurotransmitter release may require different release machineries (e.g. Geppert et al. 1997; Deitcher et al. 1998; Humeau et al. 2000;
The results of the present study suggest that the interneuron – Purkinje cell synapse is one such example of a synapse that uses different release machineries/vesicles for spontaneous and AP-evoked neurotransmitter release. To the author’s knowledge this is the first study showing that the release machineries of depolarisation/AP-induced and spontaneous neurotransmitter release can be differentially regulated by presynaptic G-protein coupled receptors in the CNS.

At first glance, the results presented here may seem confusing. On the one hand, it has been shown for the interneuron – Purkinje cell synapse that both AP-induced and spontaneous GABA release are blocked following activation of group II mGluRs (Glitsch et al. 1996). However, the results of this study suggest that this block is not achieved by one common pathway, the modulation of the release machinery, but by two independent pathways: AP-induced GABA release appears to be reduced due to activation of presynaptic K⁺ channels (since VGCCs could still be activated in this study in the presence of DCG-IV) while spontaneous release is reduced due to effects on the release machinery. A clue to answer the question why one common pathway is not used to reduce presynaptic GABA release may lie in the NMDA experiments carried out in this study.

Activation of NMDA receptors leads to clear increases in presynaptic GABA release in cerebellar slices which has three interesting properties: 1. it triggers presynaptic GABA release even in the presence of TTX, suggesting that the NMDA receptors responsible for
this increase are on or near the terminal; 2. it only triggers release of GABA from inhibitory synapses, while glutamate release from excitatory synapses is unaffected; 3. it only increases the frequency of small mIPSCs, suggesting that mainly monoquantal release is affected (Glitsch and Marty 1999). On the other hand, activation of group II mGluRs, which have a very similar K_D for glutamate, suppresses presynaptic GABA release. These results are puzzling in that the same neurotransmitter can both increase and depress GABA release over a similar concentration range.

However, the present findings could resolve this paradox: glutamate will activate presynaptic group II mGluRs, thereby efficiently blocking both AP-induced and spontaneous GABA release. Spontaneous release at the interneuron – Purkinje cell synapse can be multiquantal and hence give rise to relatively large inhibitory potentials postsynaptically (Llano et al. 2000). At the same time, activation of presynaptic NMDA receptors will still be able to trigger increases in GABA release because the Ca^{2+} influx-dependent GABA release machinery is not affected by group II mGluR activation. However, NMDA receptor activation will only give rise to increases in smaller, most likely monoquantal release of GABA.

Physiologically, a likely source for glutamate activating these presynaptic receptors, are neighbouring excitatory synapses. There are two excitatory inputs into Purkinje cells that use glutamate as their neurotransmitter, the parallel fibre and the climbing fibre input. These two inputs differ substantially in their ability to excite the postsynaptic Purkinje cell: While a given parallel fibre will generally make only one or a few excitatory synapse(s) with a given Purkinje cell (mainly on the more distal dendrites), a given climbing fibre will make hundreds of synapses with one Purkinje cell (proximal dendrites
of the Purkinje cell). Activation of a parallel fibre can trigger a simple spike in Purkinje cells but may not always depolarize the postsynaptic Purkinje cell sufficiently to induce Purkinje cell firing. Activation of the climbing fibre, on the other hand, triggers a complex spike in Purkinje cells and usually initiates a burst of APs in the Purkinje cell. Because the climbing fibre input is the dominant glutamatergic input for Purkinje cells, it is likely that the climbing fibre – Purkinje cell synapse is the source of glutamate that activates presynaptic NMDA receptors and group II mGluRs on interneurons.

Purkinje cells display long term depression (LTD) of the parallel fibre input when parallel fibre and climbing fibre inputs coincide. This has been shown to be the result of AMPA receptor internalization in the postsynaptic membrane of the parallel fibre – Purkinje cell synapse. As a consequence, a subsequent AP in the parallel fibre will not trigger an excitatory postsynaptic potential in the Purkinje cell because the synapse(s) in question has(have) been silenced. This process is believed to be a powerful mechanism underlying cerebellar learning and memory formation (reviewed in Ito 2001).

With a view to the findings of this study, it is tempting to propose the following model: Climbing fibre firing will result in glutamate release which can spill over to neighbouring interneuron – Purkinje cell synapses. At these synapses, binding of glutamate to presynaptic group II mGluRs will suppress both AP-dependent and spontaneous GABA release, which can be multiquantal and hence give rise to large and somewhat variable hyperpolarizing potentials postsynaptically. At the same time, activation of presynaptic NMDA receptors will trigger increased, presumably monoquantal, GABA release which gives rise to more uniform and smaller postsynaptic hyperpolarisations. As a net result, the postsynaptic Purkinje cell will now be left more easily excitable (but not over-
excitable due to the NMDA-induced GABA release). As a consequence of this, the firing of a presynaptic parallel fibre might now be able to trigger Purkinje cell firing. In this model, parallel fibre inputs could give rise to much bigger postsynaptic depolarizations than they normally would if they occur within a given time window following climbing fibre activity, simply because the postsynaptic Purkinje cell would be in a more excitable state.

Hence, coinciding activity of parallel fibre and climbing fibre will result in an inhibition of parallel fibre inputs via LTD, whereas a parallel fibre input that follows a climbing fibre input would be potentiated.

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There is a correlation between average mIPSC amplitude and extent of reduction in mIPSC frequency following Cd\(^{2+}\) application.

This graph plots the average mIPSC amplitude under control conditions (i.e. before Cd\(^{2+}\) application) against the percent reduction in mIPSC frequency following Cd\(^{2+}\) application. The higher the initial average mIPSC amplitude, the smaller the percent reduction in mIPSC frequency with Cd\(^{2+}\), and the smaller the initial average mIPSC amplitude, the bigger the percent reduction in mIPSC frequency with Cd\(^{2+}\). Cells with a small initial average mIPSC amplitude have more small mIPSCs contributing to the
average mIPSC amplitude. Following application of Cd$^{2+}$, which partially blocks the postsynaptic GABA$_A$ receptors, more mIPSCs will fall below the detection threshold, thereby resulting in a bigger apparent reduction of mIPSC frequency.