Is Purkinje Neuron Hyperpolarisation Important for Cerebellar Synaptic Plasticity? A Retrospective and Prospective Analysis

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Revised

Is Purkinje neuron hyperpolarisation important for cerebellar synaptic plasticity? A retrospective and prospective analysis

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Abstract

Two recent studies have demonstrated that the dendritic Ca^{2+} signal associated with a climbing fibre (CF) input to the cerebellar Purkinje neuron (PN) depends on the membrane potential (V_m). Specifically, when the cell is hyperpolarised, this signal is mediated by T-type voltage-gated Ca^{2+} channels; in contrast, when the cell is firing, the CF-PN signal is mediated by P/Q-type voltage-gated Ca^{2+} channels. When the CF input is paired with parallel fibre (PF) activity, the signal is locally amplified at the sites of PF-activated synapses according to the V_m at the time of the CF input, suggesting that the standing V_m is a critical parameter for the induction of PF synaptic plasticity. In this review I analyse how the V_m can potentially play a role in cerebellar learning focussing, in particular, on the hyperpolarised state that appears to occur episodically, since PNs are mostly firing under physiological conditions. By revisiting the recent literature reporting *in vivo* recordings and synaptic plasticity studies, I speculate on how a putative role of the PN V_m can provide an interpretation for the results of these studies.

Keywords: Purkinje neuron, membrane potential, calcium channels, synaptic plasticity, Climbing fibre, Parallel fibres.

Introduction

In the vertebrate nervous system, the cerebellum integrates the incoming motor and sensory information to produce a feedback output from the cerebellar nuclei to the motor system that controls movements [1]. This output is precisely shaped by the inhibitory input to cerebellar nuclei neurons from cerebellar Purkinje neurons (PNs) in the cerebellar cortex, which also processes incoming motor information. Specifically, PNs fire action potentials at variable frequencies and these frequencies are modulated by the motor information received from the mossy fibres and processed by cerebellar granule cells (CGCs) that send excitatory parallel fibre (PF) inputs to PNs. The PN firing frequency can either increase or decrease during motor activity according to the balance of excitation versus feed-forward inhibition by molecular layer interneurons (MLIs) also excited by the same PF inputs [2]. But the whole cerebellar system also receives a large sensory excitatory input through the climbing fibres (CFs) from the brainstem inferior olive targeting both PNs and cerebellar nuclei neurons. According to the early theory proposed by Marr [3] and Albus [4], the principal role of the CF input is to provide an "error" signal that weakens the concomitantly active PFs input, in this way permitting a continuous learning of fine motor coordination. This theory was finally validated by the discovery that pairing PF and CF activity can induce long-term depression (LTD) of activated PF synapses [5]. Since then, it became evident that physiological PF synaptic depression is complex and highly heterogeneous in the way it is triggered throughout the cerebellar cortex [6], and that PF plasticity must be bidirectional with synaptic weakening compensated by counterbalancing mechanisms of long-term potentiation (LTP) [7,8].

All CF-mediated forms of homosynaptic plasticity in PNs, although different, require the ability of the CF input to target a triggering signal exclusively to the concomitantly activated PF synapses. This signal that coincidently detects the occurrence of PF and CF inputs and that can initiate a biochemical process leading to a change in the postsynaptic strength is a transient elevation of intracellular Ca^{2+} concentration [9]. More precisely, when the occurrence of a CF excitatory postsynaptic potential (EPSP) is preceded by PF activation, the Ca^{2+} transient associated with the CF-EPSP, at the location of PF synapses only, is larger than the Ca^{2+} transient associated with the CF-EPSP without PF activation (unpaired CF-EPSP), and it is referred as "supralinear" Ca^{2+} signal [10,11]. Whereas CGCs form a complex circuit of ~175,000 contacts in the dendritic spines of PN dendrites [12], the CF input is a unique synapse with over 500 realising sites [13] targeting the soma and the initial dendritic segment and generating a large

depolarisation throughout the dendritic arborisation leading to a widespread Ca^{2+} transient [14-17]. The mechanisms permitting the CF-EPSP to mediate localised Ca^{2+} signals are therefore fundamental for PF synaptic plasticity. A supralinear Ca^{2+} signal, i.e. a larger increase in intracellular Ca^{2+} concentration can be generated by a larger flux of Ca^{2+} into the cytosol, but also by an increase of free Ca^{2+} due to a transient saturation of the endogenous Ca^{2+} buffer (ECB). PN dendrites have an exceptionally high ECB [18] formed mainly by two mobile high-affinity Ca^{2+} binding proteins: Calbindin-D28k and Parvalbumin [19,20]. It has been demonstrated that PN-ECB can be saturated by Ca^{2+} photolysis [21] or by fast Ca^{2+} influx mediated by the PF local depolarisation [16]. More interestingly, it was recently shown that the slow Ca^{2+} influx induced by PF-activated type-1 metabotropic glutamate receptors (mGluR1s) can transiently saturate the ECB [11]. Thus, PF inputs through mGluR1 activation can determine the condition to target a CF-mediated Ca^{2+} signal to the synaptic sites where synaptic plasticity has to occur by locally amplifying the Ca^{2+} transient associated with the CF-EPSP. The source of this Ca^{2+} transient, however, is not the same at any membrane potential (V_m) [17]. When the PN is at a quiescent hyperpolarising state, the CF-mediated depolarisation transient activates T-type voltage-gated Ca^{2+} channels (VGCCs) and A-type voltage-gated K^+ channels that limit the activation of P/Q-type VGCCs preventing dendritic Ca^{2+} spikes. When instead the PN is at a more depolarised firing state, both T and A channels inactivate and the CF-mediated depolarisation activates P/Q-type VGCCs enabling Ca^{2+} spikes. Notably, both Ca^{2+} sources are potentiated by PF-mediated mGluR1 activation, but not in the same way. Ca^{2+} spikes via P/Q channels are boosted by mGluR1-mediated inactivation of A channels [22] whereas T-channels are directly potentiated by mGluR1s at hyperpolarised states [23,24]. Notably, the potentiation of T-type VGCCs is mediated by the same mGluR1 pathway responsible for the slow mGluR1-mediated Ca^{2+} influx [25] suggesting a possible co-localisation of the two processes. Thus, beside the voltage independent amplification through local saturation of the ECB, the supralinear Ca^{2+} signal associated with a CF-EPSP includes an increase in Ca^{2+} influx through the plasma membrane and has a different origin depending on whether the initial V_m is at a hyperpolarised or at a firing state. This fact suggests that the standing V_m of PNs plays a pivotal role in synaptic plasticity and cerebellar learning.

This review is a retrospective and prospective analysis of the possible role of PN V_m in synaptic plasticity. In particular, I focus on the putative role of PN hyperpolarisation since this state is likely episodic while the supralinear Ca^{2+} signal appears to specifically target activated PF synapses. In the following

sections, I first review in detail the biophysical origin of mGluR1-dependent supralinear Ca^{2+} signals. Then, I review some of the most important findings on *in vivo* activity and provide interpretations in light of a possible role of the PN V_m, I analyse the possible activation of K^+ channels that may generate episodes of hyperpolarisation and I speculate on how episodic hyperpolarisation can relate to the rules that govern synaptic plasticity. Finally, I propose a perspective of future research directions to tackle the different hypotheses analysed in this review.

Overview of the origin of mGluR1 supralinear Ca^{2+} signals in Purkinje neurons

I start this analysis with an overview of the recent findings that motivated this review. The first findings concern the biophysical mechanisms governing the dendritic V_m and Ca^{2+} transients associated with the CF-EPSP at different initial V_m [17]. Modifying a previous model [26] to match combined V_m and Ca^{2+} imaging data, the response of a dendritic compartment to a CF depolarising transient was explained in detail with the synergistic activation of six channels. These channels are divided in two different sets that are selectively activated at different initial V_m. By focussing on the two types of VGCCs and on the A-type K^+ channel (Fig. 1a), when the dendrite is hyperpolarised a CF-EPSP activates T-type VGCCs and A-type K^+ channels channels that limit the dendritic depolarising transient below ~10 mV, preventing the activation of P/Q-type VGCCs. When in contrast the dendrite is depolarised, T and A channels are inactivated and the CF-EPSP can eventually activate P/Q channels eliciting Ca^{2+} spikes. A second study [11] has unravelled the mechanisms that allow the Ca^{2+} transient associated with a CF-EPSP, following PF activity, to be amplified locally at the sites of pre-activated PF synapses (Fig. 1b). A first mechanism, independent of the initial V_m, is determined by the Ca^{2+} influx through an mGluR1-activated non-selective cation conductance [27,28]. This channel is believed to be the C3-type transient receptor potential (TRPC3) [29], but it was also shown that mGluR1s can activate GluD2 delta “orphan” glutamate receptors [30]. During the precise time window of mGluR1 action following PF bursting activity, Ca^{2+} entering the cell through this channel binds to ECBs locally lowering the buffer capacity of the cell. Since this is produced by synaptically activated receptors, it can be segregated to activated synapses, i.e. it can co-localise with activated spines [31] depending on their geometry [32]. The mechanism of local ECB saturation amplifies both the Ca^{2+} signal mediated by T-type VGCCs at hyperpolarised states and the Ca^{2+} signal mediated by P/Q-type VGCCs at depolarised states combining, in the two cases, with two distinct further mechanisms

contributing to the supralinear Ca^{2+} signals. The mechanism of boosting P/Q channel Ca^{2+} influx, due to the larger mGluR1-dependent inactivation of A channels [22] is not local. In contrast, the mechanism of boosting T channel Ca^{2+} influx, due to the direct mGluR1-dependent potentiation of these channels [23], is likely occurring exclusively at activated PF synapses. Altogether, these findings lead to the question on whether the V_m is actually a key parameter in the induction of synaptic plasticity and, in particular, whether the apparently rare states of V_m hyperpolarisation are fundamental episodes necessary for cerebellar learning. In the next section I review the state-of-the art literature on the V_m *in vivo* in light of the possible occurrence of hyperpolarised states.

Evidence of physiological hyperpolarisation of Purkinje neurons *in vivo*

Direct measurement of V_m by *in vivo* patch clamp recordings in PNs were obtained both from anesthetised [33-35] and awake animals [2]. In anesthetised rodents, independently of the anaesthesia procedure, PN V_m is characterised by bistability consisting on the ability of alternating between hyperpolarised quiescent states and depolarised firing states associated with simple spikes [33], similarly to what was reported *in vitro* [36]. While PN bistability is due to intrinsic membrane properties (see for example [37]), a CF input can switch the V_m from one state to the other [33]. Notably, the Ca^{2+} transient associated with a CF-EPSP depends on the PN state [34], in the same way as it depends on the initial V_m in brain slices [17]. Yet, the ability of PNs to switch between two states, supposedly associated with the possibility to activate either T-type or P/Q-type VGCCs, does not answer the question on how frequent the hyperpolarised state occurs. Less invasive *in vivo* extracellular recordings have shown that the frequency of simple spike firing is on average higher in awake mice with respect to anesthetised mice, ranging from ~30 Hz to ~100 Hz [38]. The variability in spiking frequency reflects the cerebellar modules where modules expressing zebrin exhibit lower frequencies [39] and this specificity is mediated by TRPC3 channels [40]. In all cases, these relatively high spiking frequencies indicate that hyperpolarising states lasting more than 100 ms must be, under physiological conditions, only episodic. This first conclusion leads to the second important question, i.e. how synaptic activity may lead to these episodic hyperpolarised states. While a CF input alone can theoretically switch from one PN state to another, the occurrence of complex spikes *in vivo*, indicative of a CF input, does not silence simple spiking activity (see for example [38]) since the transient hyperpolarisation mediated by K^+ channels activation, in particular

Ca^{2+}-activated BK channels [41,17] is lasting only a few milliseconds. Thus, episodes of sustained hyperpolarisation can be only induced either by PF activity alone or by concomitant PF activity and CF inputs. A possibility is that hyperpolarisation episodes have a network origin being caused by outward Cl^- currents through $GABA_A$ receptors activated by cerebellar interneurons. PF axons projecting onto PNs also excite MLIs producing feed-forward inhibition to the same PNs and this mechanism regulates behaviours associated with synaptic plasticity and spiking activity in PNs [42,43]. Furthermore, the contribution of feed-forward inhibition in determining the firing rate is enhanced during self-paced locomotion where some PNs increase their spiking frequency whereas others decrease it [2]. This finding leads to a straightforward hypothesis illustrated in Fig. 2. In PNs where the firing frequency increases, and therefore the standing V_m becomes more depolarised, a CF-EPSP would likely activate P/Q-type VGCCs whereas in PNs where the firing frequency decreases, and the standing V_m becomes more hyperpolarised, a CF-EPSP would more likely activate T-type VGCCs. But how MLI inputs to PNs affect the CF response and synaptic plasticity? These questions were investigated by Rowan et al. [44] who showed that concomitant optogenetic stimulation of MLIs reduced the CF-mediated Ca^{2+} transient and when this protocol was paired with PF stimulation it changed the polarity of PF long-term synaptic plasticity from depression to potentiation (i.e. from LTD to LTP). In addition, while CF stimulation combined with head rotation produced an adaptive increase in the vestibulo-ocular reflex, concomitant optogenetic stimulation of MLIs reversed the effect into an adaptive decrease. The straightforward possible interpretation according to the scheme of Fig. 2 would be that MLIs drive the PN to a hyperpolarised state changing the polarity of PF synaptic plasticity. However, activation of molecular layer inhibitory synapses does not only hyperpolarise the V_m, but also increases the dendritic membrane conductance decreasing the propagation of the CF depolarising transient throughout the dendrites. Hence, while molecular layer "disinhinibition" increases the CF-mediated Ca^{2+} transient during behaviourally-induced PF activity, MLI activation suppresses local supralinear Ca^{2+} signals [45], a result in striking contrast with the large supralinear Ca^{2+} signal observed with hyperpolarised states obtained by somatic current injection [11]. Thus, while MLIs may in principle allow, in a portion of PNs, the activation of T-type VGCCs, the results obtained in these studies can be alternatively attributed to a shunting inhibition of the CF depolarising wave. The specific investigation of the occurrence of hyperpolarising episodes *in vivo* is only at a preliminary stage. It must be taken into account that T-type VGCCs recover from inactivation in a few milliseconds [46] and, therefore, an hyperpolarisation episode synchronising with a CF-EPSP can be as

short as 100 ms to allow a local supralinear Ca^{2+} signal mediated by these channels. For example, within feed-forward inhibition, a spike from one single MLI can alone transiently inhibit PN firing [35], but MLIs can be also excited by glutamate spillover from a CF input [47]. The hypothesis that episodes of PN hyperpolarisation playing a role in synaptic plasticity are determined by activation of Cl^- implies that the underlying mechanisms are mainly governed by the cerebellar network. Yet, an alternative hypothesis is that hyperpolarisation episodes are determined by the history of the PN signals and governed by intrinsic mechanisms leading to activation of K^+ currents. This hypothesis is analysed in the next section.

Mechanisms of episodic hyperpolarisation of Purkinje neurons mediated by K^+ channels

A prolonged activation of K^+ conductance can drive PN V_m towards the reversal potential of K^+. This can be produced by a large Ca^{2+} transient activating Ca^{2+}-gated K^+ channels or by K^+ channels activated by other mechanisms. Among the different types of Ca^{2+}-gated K^+ channels, the "small-conductance" K^+ (SK) channel is reported to play a role in different cellular processes in PNs, including in the calcium transients in dendritic spines [48]. Although the expression of this channel decreases with development [49], it was recently reported that SK channels are highly expressed in dendritic spines co-localising with mGluR1s [50] and that they are down-regulated during cerebellar learning increasing PN excitability [51]. In general, SK channels can provide a medium-slow afterhyperpolarisation after PF bursting. The other type of Ca^{2+}-gated K^+ channel expressed in PNs is the "big-conductance" K^+ (BK) channel that co-localise with P/Q-type VGCCs [52] and is activated by dendritic Ca^{2+} spikes [41,17]. But BK channels are also activated by Ca^{2+} influx *via* inositol 1,4,5-triphosphate (InsP3) receptors activated by mGluR1s with a faster kinetics with respect to the slow Ca^{2+} influx [53]. Notably, since InsP3 receptors are highly expressed in PN dendrites [54], several studies have linked PF activation of mGluR1s to Ca^{2+} release from internal stores *via* these receptors [55,56] leading to the hypothesis that this signal is the trigger for PF-LTD [57,58]. While Ca^{2+} release from internal stores does not contribute to the CF dependent supralinear Ca^{2+} signal [11], InsP3 receptor activation requires dendritic depolarisation [53] raising the hypothesis that this event may play a physiological role in generating hyperpolarisation short episodes. Finally, Ca^{2+} release from stores activating Ca^{2+}-gated K^+ channels can also occur *via* ryanodine receptors following an intense stimulation [59], a mechanism that was also associated with the induction of plasticity [60]. Altogether, the ensemble of possible mechanisms leading to activation of Ca^{2+}-gated K^+ channels is

summarised in Fig. 3a. In addition to the pathways analysed above, K^+ channels can be activated by other synaptic mechanisms. PF activity activates type-7 metabotropic glutamate receptors (mGluR7) and this mechanism can transiently silence PN activity after cerebellar learning [61]. Specifically, mGluR7s couple with Kir3/GIRK K^+ channels [62] that are expressed in PNs [63]. The same channels are activated by inhibitory synapses by coupling with metabotropic $GABA_B$ receptors [64]. Altogether, the two mechanisms leading to activation of Kir3/GIRK channels are summarised in Fig. 3b. In light of this general analysis on what can produce hyperpolarisation transients during PF or CF activity, it is crucial to analyse, next, how these mechanisms may relate to the different protocols leading to synaptic plasticity. This issue is addressed in the next section.

How hyperpolarisation can relate to the induction of synaptic plasticity

In the classical theory, the CF input combined with PF activity induces PF-LTD, a prediction that found numerous experimental conformations in brain slices experiments (see for example [9,65-67]). In the opposite direction, repetitive PF activity has been associated with induction of PF-LTP (see for example [16,68,69]), although strong PF stimulation can alone induce PF-LTD [70]. These findings led to the initial hypothesis that the amplitude of a Ca^{2+} transient determines the direction of the PF long-term synaptic plasticity according to an "inverse BMC rule" [7] with respect to the equivalent BMC rule believed to govern cortical synaptic plasticity [71]. Yet, later, it became clear that the induction of PF synaptic plasticity is more complex [8] depending on many parameters. For instance, mGluR1-dependent PF-LTD [72-74] is also critically dependent on the geometry of activated PF fibres [75] since mGluR1s, located at the periphery of synaptic spines [76], are activated only by glutamate spillover by adjacent terminals [77] pointing out a potential importance of the arrangement of activated PFs forming “beams” capable of activating spines in close proximity [78]. The current opinion, however, is now questioning whether general rules of synaptic plasticity induction physiologically apply. First, PF-LTD is not always necessary for vestibular-ocular reflex adaptation [79,80], being replaced by a rebound potentiation of inhibitory synapses to PNs [81]. Conversely, tactile stimulation *in vivo* can evoke a long-lasting increase in PN firing while synergistically reducing the MLI firing thanks to multiple plasticity mechanisms occurring at different cellular levels [82]. Second, PF-LTP can be expressed either at presynaptic terminals [83,84] or at postsynaptic spines [16,69], and the different forms of PF long-term synaptic plasticity, including PF-LTD,

involve presynaptic mechanisms such as NMDA receptor activation [85,86] triggering release of nitric oxide [87] and endocannabinoid receptor activation [88]. Yet, these presynaptic mechanisms are regulated by retrograde postsynaptic mechanisms [89-91], indicating that the synergy between presynaptic activity, controlled only by the principal motor input, and postsynaptic signalling, controlled also by the CF input, is a crucial determinant of synaptic plasticity. Finally, it was found that the rules for induction of PF plasticity are different in the flocculus and in the vermis [92], in the first case being narrowly tuned for a precise interval associated with oculomotor learning, while in the second case being highly variable possibly matching single cell functions. Thus, within the actual complicated scenarios of multiple rules of synaptic plasticity induction [93], the ability of V_m to rapidly switch between two states characterised by different Ca^{2+} sources and pathways may be a critical factor and it may allow the CF input to drive synaptic plasticity in different directions [94].

Further theoretical studies on cerebellar plasticity have challenged the concept of "motor error", since the CF input carries a sensory and not a motor information [95]. Since a "sensory error" must be fully uncorrelated from the motor information in order to instruct the motor system in an independent way, the classical learning rule based on simple concomitant occurrence of PF and CF inputs has been replaced by a decorrelation learning rule based on positive or negative correlation of PF and CF activities [96]. This alternative learning theory brings up an intriguing question: can the PN V_m detect the type of correlation? A recent theoretical and experimental analysis has suggested that correlation detection is possible with two consecutive CF inputs separated by 100 ms [97]. In addition, in a more recent study, PF-LTD induction required the occurrence of two consecutive CF inputs at similar intervals [98]. Under certain circumstances, it is plausible that the first CF input during or just after PF activity can switch the standing V_m from a depolarised state to an hyperpolarised state, priming the dendrite for the second CF input according to a scheme shown in Fig. 4a. The biophysical mechanism underlying this scenario can be the activation of Ca^{2+}-activated K^+ channels by the first paired CF input that activates P/Q channels. An alternative scenario, illustrated in Fig. 4b, can occur if the PF activity alone hyperpolarises the V_m of the PN, for instance through MLI-mediated feed-forward inhibition or by activation of mGluR7s. Thus, the first CF input would activate T channels while the second CF input, occurring when the V_m of the PN is restored to a standing depolarised state, would activate P/Q channels. Notably, the Ca^{2+} transient associated with the second CF input would be amplified by the mGluR1-mediated saturation of the ECB,

regardless of whether the Ca^{2+} influx is mediated by the T-type or the P/Q-type VGCC. Interestingly, these hypotheses are consistent with the idea of "intrinsic" learning and memory in the cerebellum recently proposed in two perspective articles [99,100]. While these hypotheses are both, at this stage, only pure speculations, the careful analysis of the V_m should be included in all future investigations of synaptic plasticity induction protocols.

Perspectives

To answer the general question formulated in the title of this review, three specific questions must be addressed by future experimental investigations: 1. What can cause activity dependent hyperpolarisation? 2. When does hyperpolarisation occur in vivo? 3. How does hyperpolarisation correlate with induction of synaptic plasticity? While activation of K^+ channels can be in principle still investigated in brain slices, these questions must be addressed by measuring dendritic V_m *in vivo*. Interestingly, Roome and Kuhn [101] achieved simultaneous dendritic V_m and Ca^{2+} imaging using an organic voltage indicator, combined with somatic electrical recording from spontaneously active PNs in awake mice. The preliminary results reported in this study suggest that, at a fine time scale, the spatiotemporal processing in PN dendrites is characterised by a high degree of complexity. The most promising approach is the one proposed by Villette et al. based on genetically encoded indicators [102], permitting to record dendritic activity for long periods and to associate these recordings with protocols of cerebellar learning. In summary, the recent findings on the critical role in V_m in selecting Ca^{2+} sources associated with a CF input pave the way for future investigations using the information on V_m as a possible key for cerebellar synaptic plasticity.

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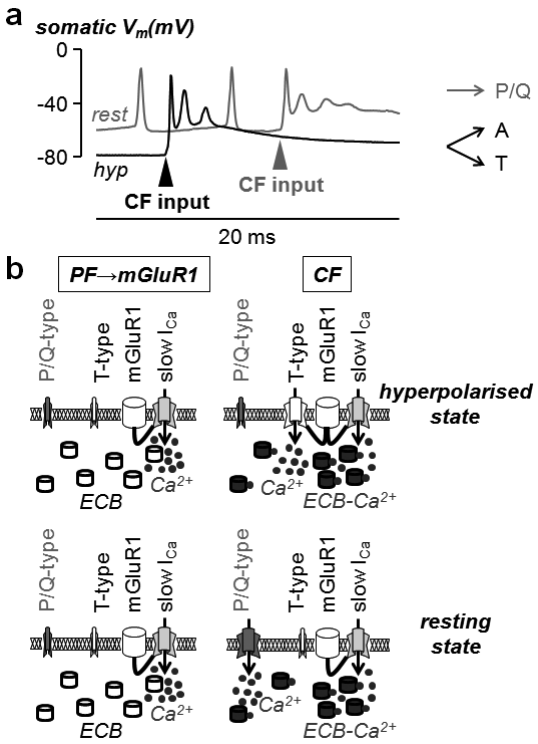

Fig. 1. Ca^{2+} sources responsible for Ca^{2+} transients associated with CF-EPSPs and mechanisms underlying supralinear amplification. **a** Example of CF Somatic V_m associated with CF inputs at initial hyperpolarised V_m (black trace), activating T and A channels, and at resting V_m (gray trace), activating P/Q channels. **b** At both states, the slow Ca^{2+} current (I_{Ca}) gated by mGluR1 activation following PF activity saturates the ECB amplifying the CF Ca^{2+} transient. At the hyperpolarised state (top), this mechanism combines with the co-localised mGluR1-dependent potentiation of T-type VGCCs activated by the CF. At the resting state (bottom), this mechanism combines with the boosted CF activation of P/Q-type VGCCs, but the two mechanisms are not co-localised.

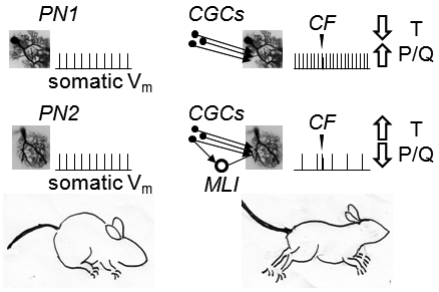

Fig. 2. Hypothesis on the role of feed-forward inhibition in determining the Ca^{2+} source associated with the CF input. On the left, two PNs (PN1 and PN2) firing at the same frequency when the mouse is at rest; on the right, PN1 increases the firing frequency with movement following excitation provided by CGC inputs while PN2 decreases the firing frequency following a dominance of MLI feed-forward inhibition; PN1 would favour activation of P/Q channels following a CF input while PN2 would favour activation of T channels following a CF input.

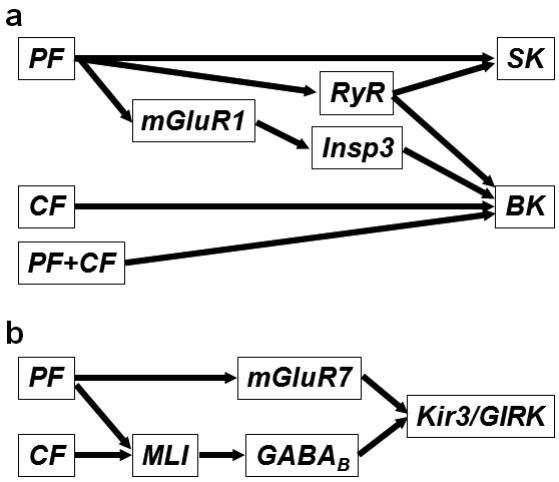

Fig. 3. Possible activity dependent pathways activating K^+ channels and producing hyperpolarising episodes. **a** Possible pathways leading to activation of Ca^{2+}-activated K^+ channels. PF activity can activate SK channels either by directly inducing Ca^{2+} influx or through ryanodine receptors (RyR). PF activity can activate BK channels through RyR activation or through activation of mGluR1s that trigger Ca^{2+} release from stores. BK channels can be also activated by Ca^{2+} influx induced by the CF input either alone or paired with PF activity (PF+CF). **b** Possible pathways leading to activation of Kir3/GIRK K^+ channels. PF activity can activate mGluR7s that activate Kir3/GIRK channels. PF activity or CF inputs (via glutamate spillover) can activate MLIs that activate $GABA_B$ receptors leading to activation of Kir3/GIRK channels.

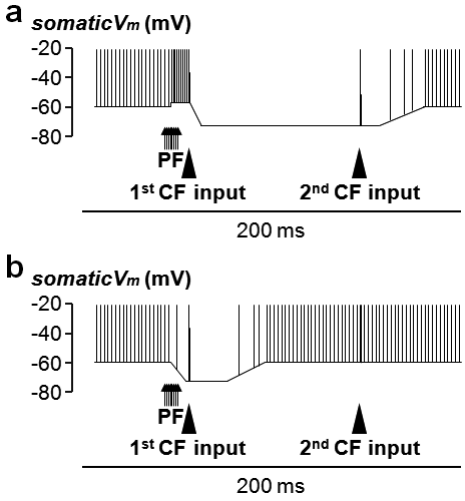

Fig. 4. Possible scenarios of plasticity induction protocols based on two consecutive CF inputs. **a** PF activity further depolarises PN V_m and the first CF input activates P/Q channels leading to an episode of hyperpolarisation (for instance by activating Ca^{2+}-activated K^+ channels); the second CF input occurring when PN V_m is hyperpolarised activates T channels. **b** PF activity hyperpolarises PN V_m (for instance by MLI-mediated feed-forward inhibition or by mGluR7s) and the first CF input activates T channels; the second CF input occurring when PN V_m is restored to a depolarised state P/Q channels. In both cases the Ca^{2+} transient associated with the second CF input is locally amplified by mGluR1-mediated ECB saturation, but the sequence of the two Ca^{2+} signals is different for the first and second CF input.