Review Article

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The Emerging Concept of Intrinsic Plasticity: Activity-dependent Modulation of Intrinsic Excitability in Cerebellar Purkinje Cells and Motor Learning

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What is memory? How does the brain process the sensory information and modify an organism's behavior? Many neuroscientists have focused on the activity- and experience-dependent modifications of synaptic functions in order to solve these fundamental questions in neuroscience. Recently, the plasticity of intrinsic excitability (called intrinsic plasticity) has emerged as an important element for information processing and storage in the brain. As the cerebellar Purkinje cells are the sole output neurons in the cerebellar cortex and the information is conveyed from a neuron to its relay neurons by forms of action potential firing, the modulation of the intrinsic firing activity may play a critical role in the cerebellar learning. Many voltage-gated and/or Ca^{2+}-activated ion channels are involved in shaping the spiking output as well as integrating synaptic inputs to finely tune the cerebellar output. Recent studies suggested that the modulation of the intrinsic excitability and its plasticity in the cerebellar Purkinje cells might function as an integrator for information processing and memory formation. Moreover, the intrinsic plasticity might also determine the strength of connectivity to the sub-cortical areas such as deep cerebellar nuclei and vestibular nuclei to trigger the consolidation of the cerebellar-dependent memory by transferring the information.

Key words: Cerebellum, Purkinje cells, Excitability, Ion channels, Neuronal plasticity, Learning

INTRODUCTION

Since Hebb's rule was proposed, many neuroscientists have focused on the plastic changes in the synaptic neurotransmission within the given synapses such as long-term potentiation and depression (LTP and LTD, respectively) [1-5]. These persistent alterations of synaptic strength have been suggested to be a cellular basis of memory storage in the brain, which have been supported by the experimental observations in which experience- and use-dependent modulation of the synaptic function are exhibited by certain forms of behavioral training [4, 6, 7]. There is, however, accumulating evidence supporting the idea that information storage may also involve the activity-dependent modulation of neuronal intrinsic excitability (intrinsic plasticity) in addition to

synaptic plasticity [8-12]. The intrinsic plasticity is not confined to the single synapse but accompanies non-synaptic and global changes [13, 14]. Therefore, the incongruity between synaptic and intrinsic plasticity may give rise to a controversy of which the global changes of neuronal excitability would seemingly distort the experience-dependent synaptic plasticity. Notably, synapse-specific and non-specific modifications synergistically contribute to the information processing and memory storage in the defined circuitry [13, 15-17]. When the synaptic plasticity occurs, several voltage-gated ion channels, which are related to the modulation of neuronal excitability, are endowed with activity-dependent up- or down-regulation [18-21]. The activity-dependent modulation of ion channels regulates not only intrinsic excitability but also a dendritic integration of the synaptic inputs [22, 23]. The intrinsic plasticity determines the net output of neurons by integrating the synaptic inputs and consecutively translating them into the action potential (AP) firing. Given that information is conveyed from a neuron (presynaptic) to its following neuron (postsynaptic) by AP firing, synergies between synaptic and intrinsic plasticity would play a role in maximizing information processing such as encoding, transfer and storage.

The inhibitory principal neurons in the cerebellar cortex, the cerebellar Purkinje cells (PCs) integrate excitatory and inhibitory inputs from widely spread dendrite branches. Various sensory information from the pre-cerebellar region and spinal cord project into the cerebellum through the mossy fiber (MF) which forms synapses with the cerebellar granule cells providing excitatory synaptic inputs into the PCs through its axon fibers, parallel fibers (PFs). Cerebellar PCs integrate the sensory information from the PF and then provide inhibitory instructive signals to the neurons in the vestibular nuclei (VN) and/or the deep cerebellar nuclei (DCN) in order to generate motor output. This synaptic gain is modulated in an activity-dependent manner, which has long been considered as the cellular mechanism of cerebellum-dependent motor learning [12, 24-27]. In addition to PF inputs, cerebellar PCs receive the other excitatory synaptic inputs from inferior olivary neuron axon fiber, climbing fiber (CF), encoding the feedback error signal corresponding to performances [28-31]. In order to control goal-directed movement, this sensory feedback of error signals dynamically regulates the cerebellar output [32, 33]. Indeed, the CF inputs onto the cerebellar PCs are regarded as the instructive signals in the cerebellar plasticity as the PF-PC synaptic plasticity is guided by timing rules between PF and CF activation [34, 35]. When the CF inputs are conjunctive and paired with PF inputs, the excitatory synaptic transmission within PF-PC synapses is attenuated, called PF-PC long-term depression (LTD). In contrast, repetitive and strong PF electrical stimulation is found

to induce long-term potentiation (LTP) at this synapses when CF inputs are omitted or non-paired [36]. PF-PC synaptic plasticity, in fact, is the heterosynaptic plasticity guided depending on the timing rules between PF and CF activation. The performance error signals are conveyed by CF to re-compute the motor signal from PCs, enabling finely tuned motor coordination through determining the cerebellar cortical activity. Many implications in cerebellar motor learning have suggested that the bidirectional plasticity of PF-PC synapses may be selectively engaged in specific behavioral paradigms [37, 38]. In spite of abundant studies on PF-PC LTD/LTP associated with cerebellum dependent behaviors, it has been less elucidated how the net output of the cerebellar PCs is regulated in an activity-dependent manner. Since the cerebellar PCs are the sole output of the cerebellar cortex, the plasticity of the intrinsic excitability in the neurons might play a pivotal role in the modulation of cerebellar motor behavior and learning. In this review, we first cover the ion channels regulating the spiking activity of the cerebellar PCs and the cellular mechanisms of the plastic changes in excitability. Furthermore, we discuss the physiological significance of the intrinsic plasticity and how the synergies between synaptic and intrinsic plasticity contribute to behavioral outcomes.

ION CHANNELS AND SPIKING ACTIVITY OF THE CEREBELLAR PCs

The intrinsic excitability is influenced by the conductance of voltage-gated ion channels generating ionic current carried by Na^+ and K^+ ions (I_{NA} and I_K, respectively), which affects the passive and active membrane properties [39-42]. The ion channels are expressed in somatic and/or dendritic regions and modulate the temporal summation of the synaptic inputs and ability to generate AP firing in axon hillock [43, 44]. A balance of ion channel conductance and expressional composition determines the characteristics of spiking activity as well as the neuronal excitability. The cerebellar PCs show a distinct spiking activity which fires spontaneously at high frequency. Many studies illustrated that the changes in ion channel conductance alter the neuronal spiking activity and behavioral outcomes (Fig. 1).

Voltage-gated Na+ channels

Voltage-gated Na^+ channels (Na_V) are involved in determining active properties of neurons including voltage threshold for generating AP at the axon hillock and amplitude of AP spike [40-42]. In the cerebellar PCs, various subtypes of voltage-gated Na^+ channels are expressed, for instance, $Na_V1.1$, $Na_V1.2$ and $Na_V1.6$ have been observed in rodent PCs [45-49]. Observation of $[Na^+]_i$ changes and electrophysiological recordings via outside-out patch clamp
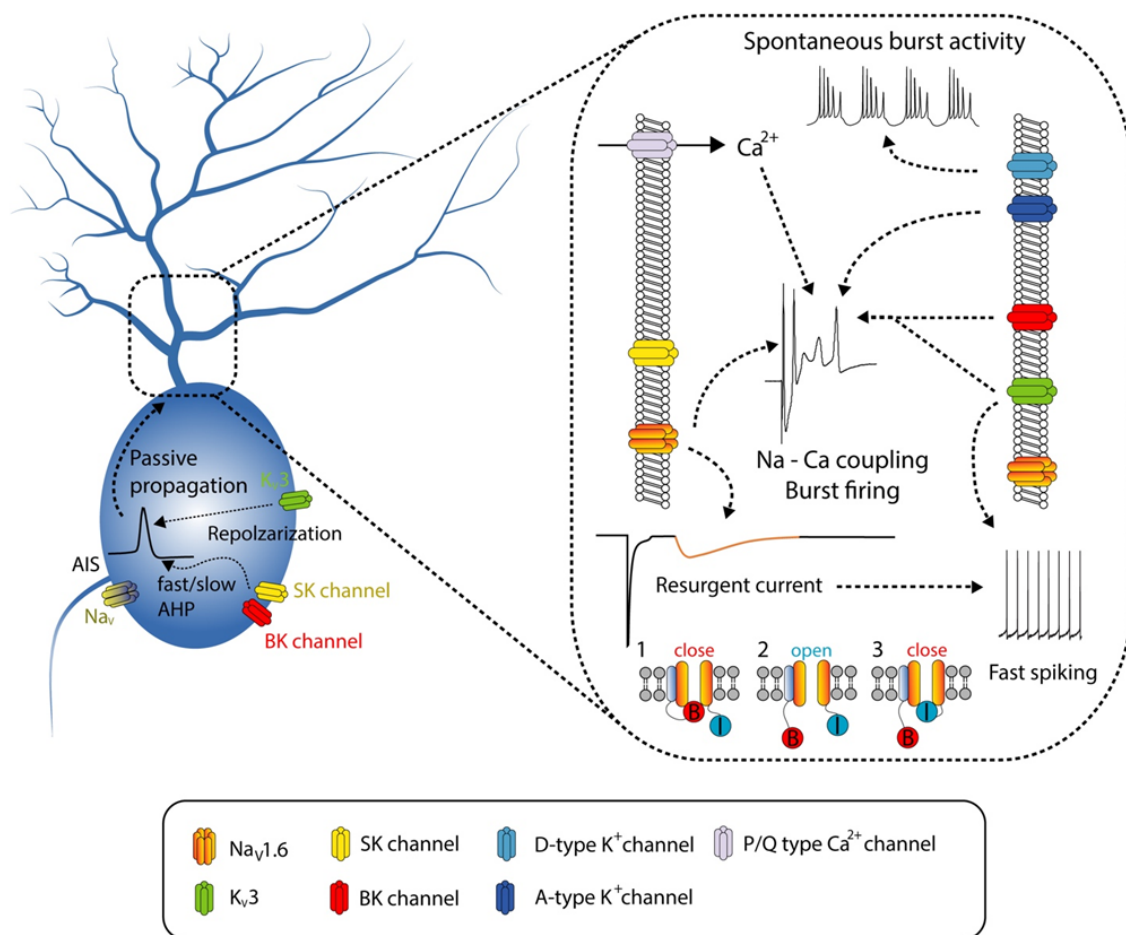
Fig. 1. Schematic illustration for ion channels shaping intrinsic excitability of the cerebellar PCs. Among various ion channels, this review focused on the resurgent Na$^+$ channel (Na$_V$1.6), subthreshold-activated K$^+$ channels (K$_V$1.1, 1.2 and 1.6; K$_V$1.4 and K$_V$4, D-type and A-type K$^+$ channel, respectively), suprathreshold-activated K$^+$ channels (K$_V$3 subfamily) and Ca^{2+}-activated K$^+$ channels (SK and BK channel). Action potential is initiated at the action potential initial segment (AIS) near the axon hillock and then passively propagated into the dendritic area. Somatic SK and BK channels determine the AP spike waveform such as the amplitude of afterhyperpolarization (AHP) and K$_V$3 subfamily regulates repolarization of AP. Because the cerebellar PCs, in particular, are fast-spiking neurons, mechanisms of rapid recovery from Na$_V$ inactivation is required to stably maintain PC spiking behavior. Na$_V$1.6 activity enables to rapidly fire the AP spikes via shortening refractory period. Various ion channels synergistically and dynamically modulate the dendro-somatic activity of the cerebellar PCs.

configuration have revealed that Na$^+$ spikes are generated within the somatic area of PC and then passively spread into the dendrites [49]. Despite some subtypes of Na$_V$ and changes in [Na$^+$]$_i$ in the PC dendrites were observed, backpropagation of AP into the dendrite from soma is absent, suggesting that the [Na$^+$]$_i$ influx and subtypes of Na$^+$ channels expressed in dendrites are not sufficient to generate AP in PC dendrites. In addition, electrophysiological recordings of I_{Na} in the cerebellar PCs have revealed a fast inactivating and/or a persistent conductance of Na$^+$ [47, 50].

Among subtypes of Na$_V$, Na$_V$1.6 shows a distinct feature, which contributes to transient and resurgent components, but not to persistent components (Table 1). Na$_V$1.6 was remarkably observed in the dendritic area in the cerebellar PCs [51]. The resurgent I_{Na}

facilitates reopen Na$_V$ when the membrane potential is repolarized to approximately -40 mV following the long period of depolarization exceeds +30 mV enough to produce maximal inactivation of Na$_V$ [52, 53]. This distinctive current flow contributes to ensuring the fast-spiking activity of the cerebellar PCs via the rapid open-channel block and unblock mechanism because the PCs, in particular, spontaneously fire AP spikes at high frequency. Resurgent current, carried by Na$_V$1.6, has been found to be a mechanism by which the refractory period between AP firing is shortened by rapid recovery from inactivation of I_{Na} [54, 55]. Transgenic mice which resurgent Na$^+$ current is disrupted showed an abnormality in the spiking activity of PCs and manifestation of cerebellar ataxia and failure of motor coordination [54, 56]. Recent studies

Table 1. Active properties of resurgent Na$^+$ channels in the cerebellar PCs and their physiological and pathological roles

Na$_V$1.6 (Resurgent Na$^+$ channel)		
Expression	Dendrite, Node of ranvier	[51]
Gating properties	Sensitivity for tetrodotoxin	[53, 55]
	Evoked by a step repolarization to -30 mV	
	Maximum current at Vm=-30~-40 mV	
	V$_{1/2\,activation}$=-40 mV, rising time=5~6 ms	
	V$_{1/2\,inactivation}$=-62 mV (low Na+), -53 mV (high Na+), τ_{decay}=20~30 ms	
Impact on excitability	Reopening Na$_V$ when the membrane potential is repolarized to approximately -40 mV	[52, 54, 55]
	Shortens the refractory period between action potentials, high-frequency firing appears to be facilitated	
Ablation	Reduced spontaneous firing rates	[54-59]
	Increased spike adaptation	
	Cerebellar ataxia & Dysfunction of motor coordination	
	Impairment of water maze and delayed eyeblink conditioning	

have shown that Na$_V$ channel auxiliary subunit FGF14'b' isoform is involved in controlling the resurgent current and excitability of PCs [57]. In parallel with previous observations, ablation of this auxiliary subunit manifests the abnormality of the firing activity in PCs and motor behavior [57, 58]. Interestingly, inactivation of Na$_V$1.6 affects not only the motor performance but also associative classical conditioning and spatial memory formation. Purkinje cell-specific deletion of Scn8a, encoding Na$_V$1.6, impaired the performance in the delayed eyeblink conditioning and the Morris water maze [59].

Voltage-gated K$^+$ channels and Ca^{2+}-activated K$^+$ channels

K$^+$ channels are another major players in determining the excitability of the neurons. As molecular techniques have been advanced, various subtypes of K$^+$ channels and their gating properties have been characterized [60]. Like Na$^+$ channels, many K$^+$ channels have shown quite distinct and divergent gating properties depending on the types of auxiliary proteins. K$^+$ channels are classified into several types, including voltage-gated channels (K$_V$), Ca^{2+}-activated K$^+$ channels (K$_{Ca}$), inwardly rectifying channels (inward rectifier) and Na$^+$-activated K$^+$ channels. In this review, we focused on the roles of several K$_V$ subfamilies (Table 2) and Ca^{2+}-activated K$^+$ channels (Table 2) in intrinsic firing properties of the cerebellar PCs.

Considering the Hodgkin-Huxley model, ionic flow carried by Na$^+$ and K$^+$ determines the active and passive properties of neurons. Of interest, K$^+$ conductance controls the resting membrane potential, membrane resistance, neuronal excitability, duration of AP and delay time to fire AP spike. Electrophysiological observations and immunohistochemistry data have shown that various types of K$^+$ channels are expressed in cerebellar PC soma and dendrites [61-68]. Gähwiler and Llano [61] observed that outward I_K is found to be elicited by membrane depolarization above -30 mV

from excised somatic membrane. This suprathreshold-activated I_K shows sensitivity for general K$^+$ current inhibitors, tetraethylammonium (TEA) and 4-aminopyrimidine (4-AP), indicating that the I_K is governed by K$_V$3 subfamily of K$^+$ channel [66, 69]. This class of K$^+$ channels (especially K$_V$3.3 and 3.4) repolarizes the Na$^+$ spike discharge to maintain repetitive AP firing in both soma and dendritic area [68, 70-72]. Therefore, dysfunction of this subfamily results in the impairment of repetitive AP spike discharge with a high frequency and an abnormal behavior [73]. In addition, the pharmacological inhibition with 1~10 mM of 4-AP or 2~5 mM TEA affects the dendritic Ca^{2+} spike discharge, indicating that the dendritic K$_V$3 channels contribute to shaping membrane excitability of PC dendrites as well as soma [74]. In spite of lacking backpropagation of Na$^+$ spikes into PC dendrites, membrane depolarization passively propagates into proximal and distal dendrites thereby generating regenerative Na$^+$ spikes and plateau potential which promotes bursting Na$^+$ spikes [48, 75]. In addition, strong depolarization induced by CF activation leads to Ca^{2+} influx into the dendrite, resulting in the Na$^+$ – Ca^{2+} complex spike responses. The mixed Na$^+$ – Ca^{2+} spike discharge activates dendritic K$_V$3.1/3.3 channels, enabling PC spiking activity to be stably maintained with high frequency though preventing Na$^+$ channel inactivation. When [Ca^{2+}]$_i$ is increased by CF activation, large conductance Ca^{2+}-activated K$^+$ (BK) channels are also activated. Interestingly, co-activation of K$_V$3 and BK channels promotes coupling between Na$^+$ and Ca^{2+} spike discharge via reducing Na$^+$ channel inactivation, resulting in burst output of the cerebellar PCs [68].

Differently from K$_V$3 family, some other types of K$^+$ channels show distinct gating properties, for instance, K$_V$1 and 4 subtypes are activated at subthreshold voltage whereas K$_V$3 family shows high voltage-activating and fast deactivating voltage dependency [76]. Storm [77] categorized the subthreshold-activated K$^+$ channels into 2 types depending on their sensitivity for 4-AP

Table 2. Active properties of voltage-gated K$^+$ channels in the cerebellar PCs and their physiological and pathological roles

K_V1.4 & K_V4 (A-type K$^+$ channel)		
Expression	Dendrite	[70]
Gating properties	Sensitivity for high concentration of 4-AP about 1~10 mM (insensitive for DTX)	[60, 77, 85]
	Fast-activating and inactivating channel	
	Activated at subthreshold voltage around -60 mV	
	$V_{1/2\ activation}$=-24.9 mV; $V_{1/2\ inactivation}$=-69.2 mV	
	$\tau_{deactivation}$ at -70 mV : 3~4 ms	
Impact on excitability	Acceleration of AP spike	[82, 84-87]
	Firing frequency firing pattern (rhythmic Na-Ca spike burst)	
	Subthreshold variation of membrane properties	
Impact on plasticity and learning	Eyeblink conditioning derives dendritic excitability underlying downregulation of A-type K+ channel	[116, 117]
K_V1.1, K_V1.2, K_V1.6 (D-type K$^+$ channel)		
Expression	Dendrite	[70]
Gating properties	Sensitivity for low concentration of 4-AP about 0.2~1 mM and DTX (2.8~25 nM)	[60, 77]
	Low-threshold and non-inactivating channel	
	Activated at -40~-50 mV	
	$V_{1/2\ activation}$=-20~-30 mV (K_V1.2: -5~5 mV)	
	$\tau_{deactivation}$=14~23 ms	
Impact on excitability	Spike frequency and adaption, dendritic excitability	[82, 83]
	Amplitude and duration of rebound depolarization	
	Spontaneous bursts	
K_V3.3 & K_V3.4		
Expression	Soma and Dendrite	[69, 70]
Gating properties	Sensitivity for TEA	[68]
	Rapid activating at suprathreshold and rapidly inactivating channel	
	Peak amplitude at 30 mV from -70 mV	
	$V_{1/2\ activation}$=-23.0 mV, τ_{decay}=0.66 ms	
Impact on excitability	Repolarize the membrane potential and maintain repetitive firing	[68, 71]
	Dendritic burst firing through Ca^{2+}- Na$^+$ coupling	
Impact on plasticity and learning	Deletion of K_V3.1/3.3 causes ataxic behavior	[73]

concentration and on the gating properties, A-type and D-type K_V channels, governed by K_V1.4, K_V4.X and K_V1.1, 1.2, 1.6, respectively. D-type K$^+$ channels, sensitive to dendrotoxin (DTX) and low concentration of 4-AP, are well known for determining spike output timing, latency and threshold for AP firing onset and firing frequency [14, 77-81]. In the cerebellar PCs, large depolarization can induce Na$^+$ – Ca^{2+} coupling in dendritic area leading to slowly depolarized potential (SDP), facilitating Na$^+$ burst spike generation. Pharmacological inhibition of the D-type currents shortens the SDP and reduces latency to Ca^{2+} spikes [65], suggesting that the D-type channels are involved in the regulation of dendritic excitability. Application of DTX in cerebellar slices also increases spontaneous rhythmic activity through the enhancement of rebound firing, indicating that D-type I_K plays a modulatory role in defining spiking pattern in PCs [82]. In addition, K_V1.2-containing K$^+$ channels have been shown to inhibit the spontaneous and non-specific Ca^{2+} activity in the PC dendrites to encode motor timing signals. Furthermore, these ion channels contribute to the synaptic

integration of PF inputs [83]. Taken together, low-threshold activated and non-inactivating D-type K$^+$ channels take a part in the integration and generation of finely tuned signals in PCs, thereby signal tuning within physiological appropriate ranges.

Previous studies have shown that A-type and D-type K$^+$ channels play similar physiological roles. For example, the A-type I_K contributes to spike acceleration and determines the firing patterns in the cerebellar PCs [84, 85]. Strikingly, this subfamily of K$^+$ channels is absent in PC somata or dissociated PC, suggesting an exclusive dendritic expression (and axon fiber). In PC dendrites, K_V3 family is also expressed and regulates dendritic active properties. Given the distinct gating properties of A-type K$^+$ channels from K_V3 family, this subfamily of K$^+$ channels is implicated in regulating the subthreshold variations of the membrane potential and processing the synaptic inputs [86, 87].

Instead of dendritic Na$^+$ channels, cerebellar PC dendrites express Ca^{2+} channels with high density. Therefore, strong depolarization causes synchronization of the passively conducted Na$^+$

Table 3. Active properties of Ca^{2+}-activated K$^+$ channels in the cerebellar PCs and their physiological and pathological roles

SK channel (SK2 subfamily)		
Expression	Soma and Dendrite	[90]
Gating properties	Voltage-independent and Ca^{2+} dependent channel	[60, 89]
	Activated by Ca^{2+} influx through P/Q type Ca^{2+} channel	
	Sensitivity for apamin (63 pM)	
Impact on excitability	Regulation of firing frequency	[13, 90, 95, 98]
	Shaping fast afterhyperpolarization (AHP) amplitude	
	Climbing fiber-induced spike pause duration	
	Activity-dependent modulation of climbing fiber-evoked EPSP amplitude and dendritic local Ca^{2+} transient	
Impact on plasticity and learning	Activity-dependent downregulation of SK channel by eyeblink conditioning	[13, 118]
	Inhibition of SK channel prevents LTP-IE induction	
BK channel		
Expression	Soma and Dendrite	[90]
Gating properties	Voltage- and Ca^{2+}-dependent	[60]
	V$_{1/2 \text{ activation}}$=50 mV at 4 μM [Ca^{2+}] to -30 mV at 100 μM [Ca^{2+}]	
Impact on excitability	Generation of burst firing through cooperating with K$_V$3 channels in dendrite	[68, 91-93]
	Climbing fiber-evoked spike pause and burst firing coupled to Ca^{2+} channel	
	Shaping medium or slow component of AHP	
Impact on plasticity and learning	Dysfunction of SK and BK channels is related to cerebellar ataxia	[94-97]

spike and Ca^{2+} spike in dendrites to shape the appropriate spiking activity of PCs. The influx of dendritic Ca^{2+} can activate an ionic flow carried by K$^+$, Ca^{2+}-activated K$^+$ channels. These types of channels are classified into two; small conductance and large conductance Ca^{2+}-activated K$^+$ channel (SK and BK channel, respectively) (Table 3). Both SK and BK channels are also implicated in controlling the spiking activity in PCs [88]. When the dendritic membrane is depolarized by synaptic inputs, local [Ca^{2+}]$_i$ is elevated through P/Q type Ca^{2+} channels, leading to the activation of the SK channels [89]. The SK channels are expressed in PC somata as well as in dendrites. Interestingly, dendrosomatic electrical coupling is governed by SK2 channels in PC soma and dendrite [90]. In addition, somatic and dendritic SK channels show distinct roles in regulating PC activity. SK channels in soma set the maximal level of PC firing frequency, and on the other hand, dendritic SK channels determine the extent of dendritic regions contributing to the firing rates of PCs. The other types of Ca^{2+}-activated K$^+$ channel, BK channels are also involved in the spiking activity in PCs. As we described above, co-activity of K$_V$3 and BK channels modulates dendritic Na$^+$ – Ca^{2+} coupling burst elicited by CF inputs through suppression of Na$^+$ channel inactivation [68]. Both SK and BK channels are activated by Ca^{2+} entering through P/Q type Ca^{2+} channels and regulate PC firing patterns. However, these channels differently contribute to firing behavior in PCs. SK channels have an impact on the setting intrinsic firing frequency [90] whereas BK channels are involved in the regulation of AP waveform and presumably climbing fiber responses [68, 91]. Recent studies have

shown that CF-evoked pause of spontaneous firing and generation of burst firing in PCs require BK channels coupled to Ca^{2+} channels [92, 93]. Because SK and BK channels in PCs play a critical role in the regulation of firing behavior, dysfunction of these channels has been implicated in cerebellar disease such as ataxia. Mutations in P/Q type Ca^{2+} channels resulted in the decrease in the precision of PC pacemaking activity and perfusion of 1-ethyl-2-benzimidazolinone (EBIO), K$_{Ca}$ activator improves the regularity of spontaneous firing and motor performance [94]. In addition, dysregulation of SK and/or BK channels has been reported in various genetic ataxia animal models [94-97], suggesting that modulation of SK and BK channels may be therapeutic targets for cerebella disease and motor dysfunction.

ACTIVITY-DEPENDENT PLASTICITY OF INTRINSIC EXCITABILITY THROUGH ION CHANNEL MODULATION

Activity- and experience-dependent intrinsic plasticity and ion channels

Many theories have implicated that the potentiation or depression of the synaptic transmission at the PF-PC synapses and nonsynaptic intrinsic plasticity are required for cerebellar-dependent learning and memory. Intriguingly, the intrinsic plasticity requires an activity-dependent modulation of ion channels (Fig. 2) [13-15, 98-100]. The ion channels in the cerebellar PCs are regulated by various factors such as an activation of metabotropic receptor or synaptic plasticity-related intracellular signaling [13, 15, 100,
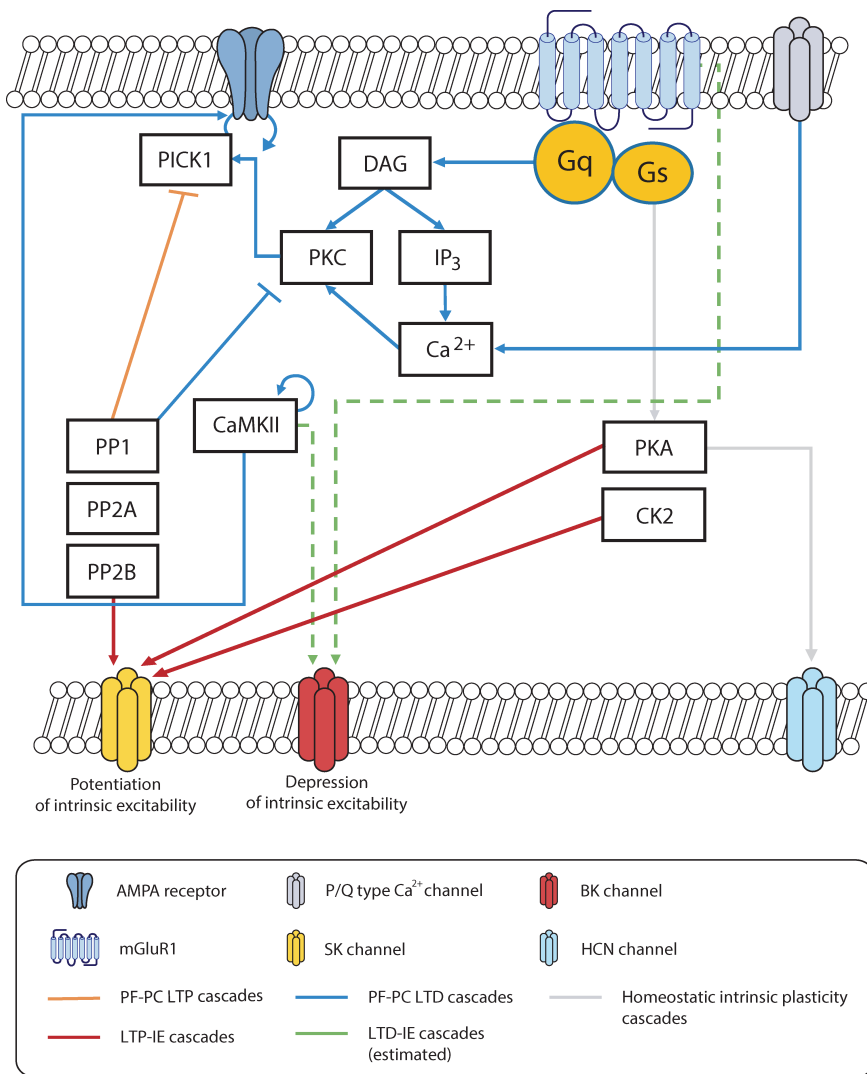
Fig. 2. Schematic illustration for molecular signal cascade for synaptic and intrinsic plasticity in the cerebellar PCs. Intrinsic plasticity indeed shares intracellular signal cascade for synaptic plasticity, in which LTP-IE requires activation of phosphatases such as PP1 and PP2B whereas LTD-IE is dependent on PKC activation. In contrast to abundant studies describing the cellular mechanisms and behavioral outcomes of LTP-IE, detailed mechanisms of LTD-IE and its behavioral impact are still elusive although synaptic LTD has long been considered as cellular basis for cerebellar motor learning. In this review, BK channels are proposed for one plausible ion channels involved in LTD-IE. CaMKII activation is found to be involved in upregulation of BK channel activity (green dot line) in the VN neurons.

101]. When the chronic changes in network activity occur, the neuronal activity is homeostatically regulated in order to maintain the stability of network activity [102-105]. Prolonged application of tetrodotoxin (TTX) to organotypic cultures of cerebellar slices exhibits downregulation of the intrinsic excitability [100]. This homeostatic intrinsic plasticity is derived from an augmentation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel current (I_h) in the cerebellar PCs. Interestingly, the activity-dependent upregulation of I_h in PCs requires an agonist-independent activity of metabotropic glutamate receptor 1 (mGluR1) and its G_S-coupled downstream of PKA signaling. Ablation of HCN channels in PCs exclusively disrupts the integration of inhibitory synaptic inputs and bi-stability of PC firing behavior [22]. Furthermore, HCN channels regulate a balance between excitation and inhibition through the integration of glutamatergic and GABAergic transmission and firing stability, thereby promoting memory

formation at the late stages of cerebellar learning [106].

Recently, plasticity of the intrinsic excitability of the cerebellar PCs has been found to be accompanied by the synaptic plasticity [13, 15, 107]. In fact, PF-PC synaptic LTP derives the long-term potentiation of intrinsic excitability (LTP-IE). LTP-IE requires the downregulation of SK2 channels in dendritic area. The SK channel-dependent intrinsic plasticity may play a role in shaping the cerebellar PC output to adjust the impact of synaptic inputs within optimal ranges. The LTP-IE dampens the impact of PF inputs on the firing behavior of PCs, enabling effects of non-potentiated, weaker synaptic inputs on cellular output signal to minimize [13]. Interestingly, although local dendritic Ca^{2+} signaling is enhanced after formation of LTP-IE, prior induction of intrinsic plasticity prevents subsequent induction of synaptic LTP in PCs. Therefore, the role of intrinsic excitability in signal processing in the PCs is quite distinct from other types of neurons such as hippocampal

pyramidal neurons or cortical neurons because enhanced Ca^{2+} signaling has been regarded as enlargements of synaptic inputs and increases in possibility of subsequent plasticity induction [108, 109]. Considering that one important role of SK channels is setting the firing frequency in physiological limits, SK channel-mediated LTP-IE may ensure that excitatory drive stays within the physiological limits and prevent non-specific subsequent synaptic plasticity induction thereby stabilizing and maximizing the information processing after learning.

In addition to the intrinsic plasticity followed by induction of LTP, the activity-dependent downregulation of PF-PC synaptic function accompanies the intrinsic plasticity. Yang and Santamaria [107] described that the potentiation of excitability in PCs is found following an induction of PF-PC LTD through downregulation of HCN channel conductance. Many observations have shown that the associative eyeblink conditioning training exerts modification of synaptic strength (PF-PC LTD) as well as intrinsic properties of the cerebellar PCs (potentiation of excitability) [110, 111]. Those described that the activity-dependent plasticity of intrinsic excitability may be homeostatically regulated which is similar to hippocampal intrinsic plasticity [18, 19]. Alternatively, Shim et al. [15] demonstrated that the intrinsic excitability is found to be decreased following induction of synaptic LTD. This result is parallel with the previous observation in which population spiking activity is attenuated by synaptic LTD induction [112]. Those contradictory results may be derived from different experimental conditions: Yang and Santamaria [107] delivered PF stimuli with somatic depolarization instead of CF activation whereas Shim et al. [15] synaptically induced synaptic plasticity by delivering simultaneous and conjunctive stimulation of PF and CF within specific time-window. Behavioral training could induce neural plasticity through divergent pathways. Thus, the intrinsic plasticity following synaptic depression may be presumably modulated in various aspects including potentiation or depression of firing rates, responsiveness of synaptic inputs or patterns of spiking activity to achieve maximizing the information storage in the cerebellar circuits.

Several observations have shown that associative eyeblink conditioning accompanies the experience- and use-dependent plasticity in the cerebellar cortex [113-117]. The experience-dependent plasticity of dendritic membrane excitability can be maintained 1 month after conditioning. Recently, it was shown that the delayed eyeblink conditioning increases intrinsic excitability and changes in AP waveforms, presumably derived from SK channel downregulation [118]. There are several evidence supporting that the memory trace in PCs is not just an increase or a decrease in firing rates. In fact, PC activity reflects adaptively timed activity pattern

with intrinsic cellular mechanisms rather than a temporal pattern of excitatory or inhibitory synaptic inputs [113-115]. Taken together, experience-dependent modulation of PC intrinsic excitability is another form of memory engram for the cerebellar-dependent motor learning.

Possible mechanisms for LTD-IE

Since Ito hypothesized that synaptic LTD between PF-PC synapses is the principal elements for the cerebellar-dependent motor learning [24], much of investigation has been extensively focused on the cellular mechanism of the modification of synaptic function to account memory storage in the cerebellum and motor control. Unlikely to synaptic plasticity or LTP-IE, the underlying mechanism for LTD-IE has less been elucidated. Previous reports described that the intrinsic plasticity and synaptic plasticity indeed share intracellular signaling including protein phosphatase and/or protein kinases (Fig. 2). Several signaling molecules are required to induce PF-PC LTD such as protein kinase C (PKC) and CaMKII. In the hippocampus, HCN channel activity is mediated by PKC signaling during mGluR-dependent plasticity induction, which results in intrinsic plasticity. However, mGluR-PKC signaling suppresses I_h, insisting that the LTD-IE in the cerebellar PCs may not be reflected by this signaling cascade. CaMKII is also known for a crucial element of synaptic and intrinsic plasticity. In the VN neurons, the activity-dependent plasticity of excitability requires bidirectional modulation of BK channels mediated by the balance between PKC and CaMKII activity [119-122]. The cerebellar PF-PC synaptic LTD recruits CaMKII signaling which increases open probability of BK channels, thus this type of K_{Ca} may be one possible candidate for the activity-dependent intrinsic plasticity in the cerebellar PCs. Since the cellular and molecular basis for LTD-IE of the cerebellar PCs remain unclear, the detailed mechanisms for the intrinsic plasticity should be further investigated.

Intrinsic plasticity plays a complementary role in integrating synaptic inputs and generating cellular output signal [15, 18, 19, 105, 123, 124]. Synaptic-driven potentiation or depression of excitability show the same polarity with the corresponding direction of synaptic plasticity [13, 15], indicating that the modifications in synaptic strength are synergistically reflected into the final net output of PCs following plasticity. When the synaptic weight is strengthened, the PC output signal is more potentiated, not compensated by intrinsic properties. In addition, cerebellar PC intrinsic plasticity occurs in the branch-dependent manner [98], indicating that synaptic inputs from specific-branches are potentiated by increased membrane excitability limited in conditioned dendritic branches. Otherwise, the plastic changes in synaptic transmission might be contaminated by global changes of excitability and less

reflected into the neuronal firing output signal.

Upside down: to what extent does bidirectional intrinsic plasticity in the cerebellar dependent-motor learning do?

What is the physiological consequence of the bidirectional modulation of intrinsic excitability following plasticity induction or behavioral training? Vestibulo-ocular reflex (VOR) and optokinetic response (OKR) is a representative behavioral paradigm of cerebellar-dependent motor learning. The VOR gain, the ratio of vestibular stimuli to adaptive eye-movement, can be increased or decreased depending on the learning paradigm. Boyden and Raymond [37] reported that the VOR gain-up and -down learning are selectively engaged by the aspects of synaptic plasticity. There was a supporting evidence of this view in which injection of mGluR1 antagonist into the cerebellar flocculus, the core area for VOR learning in the cerebellum, suppresses gain-up learning whereas gain-down learning is not affected by either agonist and antagonist of mGluR1 [38]. In addition, an ultrastructural observation shows a reduction of surface AMPA receptor following the adaptive eye-movement learning, suggesting that the cerebellar LTD would occur during the motor learning [125]. Most recently, it was revealed that the adaptive eye-movement training is associated with modifications of synaptic transmission at the PF-PC synapses [6], indicating the prominent roles of bidirectional synaptic plasticity at PF-PC synapses in the motor learning.

In contrast to Ito's cerebellar LTD theory, many studies have shown that the PF-PC LTD may not be a sufficient cellular mechanism underlying motor learnings in spite of abundant studies describing the critical roles of PF-PC LTD in VOR learning [28, 36, 126-129]. Miles and Lisberger [130, 131] proposed an alternative mechanism for motor learning, which suggests principal roles of VN neurons in the motor memory storage. There is, however, accumulating evidence showing that motor memory storage requires neuronal plasticity at multiple sites including neurons in the cerebellar cortex and VN [12, 27]. Recently, a computational model for motor memory storage provided insights into the mechanism by which motor memory traces are seemingly transferred from cortical neurons (PC) to sub-cortical region (VN) [33, 132]. Cumulative experimental data has shown that encoding the adaptive motor memory in the cerebellar cortex occurs within a few hours and the critical time window for memory transfer is approximately 2.5~4 hours after training [133, 134]. Interestingly, Ito [129] recently suggested that an early adaption is dependent on the cerebellar cortical activity and the late phase of adaptation is accompanied by plasticity in the VN neurons. In parallel, the changes in the population spiking activity in VN neurons are manifested a day after training whereas there is no significant alteration of VN activity within an hour after training [135]. Until recently, this prediction for memory consolidation mechanism has not been investigated in a cellular and circuit level. Recent papers suggested that the intrinsic plasticity of the PCs might be the mechanism of the memory transfer from cerebellar cortex to sub-cortical areas [15, 88]. Collectively, computational implications and experimental observations have both agreed to Ito's recent suggestion in which PF-PC synaptic plasticity may take part in the memory acquisition and plasticity in MF-VN synapses may be involved in long-term memory storage beyond two long-lasting conflicts for VOR memory: Marr-Albus-Ito's cerebellar LTD hypothesis vs. Miles and Lisberger' MF-VN synaptic plasticity theory.

Belmeguenai et al. [13] and Shim et al. [15] insisted that the intrinsic plasticity is modulated by synaptic activity pattern-dependent manner and this bidirectionality may function as an amplifier of the synaptic modification, enabling to transduce the finely tuned signal into the relay neurons such as neurons in DCN or VN. Given that the neural plasticity in the neurons in the cerebellar cortex and VN shows bidirectionality, the intrinsic plasticity would be also selectively engaged by certain forms of learning paradigm and the synergies with synaptic modulation may complete the scenario for the memory storage in the motor circuitry including cerebellum and VN.

THE FURTHER IMPLICATION OF INTRINSIC PLASTICITY IN THE MEMORY CIRCUITS

There are many unanswered questions to be solved in terms of the intrinsic plasticity and behavior. In the classical view of the intrinsic plasticity, changes in excitability have been considered to play pivotal roles in promoting subsequent induction of synaptic plasticity through the up- or downregulation of dendritic ion channels. Thus, the intrinsic plasticity of neurons has been thought as simply a supportive mechanism for activity-dependent modification of synaptic function although the information is conveyed by the AP spikes between neurons. However, some of the suggestions have described that activity-dependent modifications of ion channels can also undergo experience-dependent long-term plasticity beyond changes in synaptic weight [9, 10, 12, 136-139]. Furthermore, cumulative evidence has shown that memory trace should move from one brain region to another brain region to consolidate the long-term memory [125, 134, 140-144]. Intrinsic plasticity may be one plausible mechanism of memory transfer via modulating the strength of connectivity between memory circuits.

Since the majority of researches have been performed to elucidate the mechanisms of cerebellar memory formation by using *in-vitro* cell lines or brain slices, the memory circuits should be

assumed by using mathematical models that are made from bio-chemical observations, physiological recordings and behavioral assessments. Nevertheless, there has been an incongruity between theoretical models and actual experimental data which has to be reconciled. Recently, technical advances have enabled neuro-scientists to overcome the experimental limitations and expand the research scope from modifications in individual neurons to macroscopic alteration involved in shaping the memory *in vivo*. Observations of neuronal ensemble activity from freely moving animals provide insights into how the information is processed within given local circuits. In learning period, neurons that show ensemble activity may encode the similar information and these neurons will be wired to fire together so that the connectivity between them will be strengthened. Indeed, engram cells who store memory show higher excitability than others, indicating that forming stable ensemble may reflect the induction of intrinsic plasticity in the neurons [138]. Collectively, experience-dependent modulation of neuronal excitability determines the net out signals in neurons and generates synchronized population activity to project the information into another brain area.

In the neural circuitry for the cerebellar-dependent motor learning, it is unclear how the information is transferred to the sub-cortical area including DCN and VN neurons from cerebellar PCs is unclear. In the hippocampus, electrophysiological recording and optogenetic manipulation of neural circuits have revealed that the specific frequency of an oscillatory neural activity, called sharp wave ripple (SWR), involves in memory transfer from the hippocampus to cortex [145-148]. During learning, an interplay between synaptic and intrinsic plasticity increases the number of SWR replay events and thereby consolidating the long-term memory. Although the oscillatory activity of the cerebellum has been reported, detailed mechanisms and its physiological roles in formation of memory engram in the motor learning circuits have yet to be investigated. During VOR learning, spike discharge in the cerebellar PCs shows a sinusoidal pattern in response to vestibular head movement [149-151]. Interestingly, the phase of the oscilla-tory pattern of PC firing activity is altered after the training ses-sion, indicating that the response timing to sensory stimuli may be endowed with plastic changes. Therefore, the intrinsic plasticity of PCs may modify the patterns to integrate the synaptic inputs and to generate the spike discharge in response to sensory information.

Since memory is stored throughout the brain region, synergistic modulation of circuit dynamics might play critical roles. Various cutting-edge technologies enable to monitor the activity of large neuronal population and to manipulate the strength of neural circuits and corresponding behavioral outcomes. For example, recent studies using wide-field and high resolution in-vivo two-

photon Ca^{2+} imaging approaches from behaving animals revealed the groundbreaking finding of the how the cerebellar granule cells process the sensory information [152, 153]. These results from *in vivo* experiments lead us to re-evaluate the previously established hypothesis of the intrinsic excitability based on the results largely obtained from *in vitro* experiments. Many studies suggest that the PC modulates the membrane potential of the VN neurons by pro-viding a tonic inhibition or strong hyperpolarization to induce re-bound burst firing of neurons in DCN or VN. Although plasticity in both cerebellar cortex and VN play roles in the VOR memory storage, the neural plasticity occurs at the distinct time window [133-135, 154]. These observations have suggested the serial re-lationship of neural plasticity between the cerebellar cortex and VN, it is, however, still elusive how the memory is transferred from cerebellar cortex to sub-cortical area. Cell type- and engram cell-specific tagging and manipulation with a high temporal and spatial resolution may help elucidating the role of PC output modulation during VOR training and memory transfer period. The modular structure of the cerebellar cortex has been considered as an unit for an information processing, thus ensemble activity of the cer-ebellar PCs may provide strong instructive signals to VN neurons [155]. Investigation from freely moving awake animals may give us the insight into the circuit mechanisms for sensory information processing and memory storage in the cerebellar motor learning circuits.

ACKNOWLEDGEMENTS

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