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Cellular and Circuit Mechanisms Underlying Spinocerebellar Ataxias

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Abstract:

Degenerative ataxias are a common form of neurodegenerative disease that affect about 20 individuals per 100,000. The autosomal dominant spinocerebellar ataxias (SCAs) are caused by a variety of protein coding mutations (single nucleotide changes, deletions, and expansions) and in single genes. Affected genes encode plasma membrane and intracellular ion channels, membrane receptors, protein kinases, protein phosphatases, and proteins of unknown function. Although SCA-linked genes are quite diverse they share two key features; first, they are highly, although not exclusively, expressed in cerebellar Purkinje neurons (PNs), and second, when mutated they lead ultimately to the degeneration of PNs. In this review we summarize ataxia-related changes in PN neurophysiology that have been observed in various mouse knockout lines and in transgenic models of human SCA. We also highlight emerging evidence that altered mGluR signaling and disrupted calcium homeostasis in PNs forms a common, early pathophysiological mechanism in SCAs. Together these findings indicate that aberrant calcium signaling and profound changes in PN neurophysiology precede PN cell loss and likely lead to cerebellar circuit dysfunction that explains behavioral signs of ataxia characteristic of the disease.

Introduction

The autosomal dominant class of spinocerebellar ataxias comprises disorders caused by single gene mutations in more than 30 distinct genetic loci. The consequences of these genetic alterations encompass single amino acid changes, deletions, and repeat expansions in protein sequence. The diverse set of genes implicated is enriched in signaling proteins and proteins that interact with them such as ion channels, kinases, phosphatases, and growth factor receptors. However, there are also several genes that encode proteins of undetermined function, as well as proteins involved in histone regulation. Even mutations in the TATA binding protein can cause a form of SCA. Thus, there is no unifying theme for how alterations in these genes lead to a cerebellar disorder characterized at least at the earliest stages by dysfunction of and, at later stages, eventual loss of cerebellar PNs.

A clearly central question is why these genetic insults with such diverse gene products all lead to PN vulnerability. Relatedly, it remains unclear whether there any common pathophysiological mechanisms in SCAs. This short review will not address the considerable progress that has been made in elucidating molecular/cell biological etiologies of the different forms of SCA. We direct readers to several excellent reviews focusing on such topics, particularly those involving details of RNA processing and posttranslational protein modifications in disease pathology (Carlson et al., 2009; Paulson, 2009; Orr, 2013). Rather, we will focus on changes in PN physiology that are common to several mouse models of SCA and that occur early in the disease (i.e., prior to significant PN loss). These pathophysiological changes likely explain ataxic behavior observed at these points in disease

progression and more importantly could serve as a point of leverage in developing treatments for these disorders.

Alterations in spontaneous action potential firing in PNs in SCAs

A hallmark of PN physiology is that they are highly, intrinsically active. This sustained activity does not require excitatory synaptic input but arises as a result of the concerted activity of a unique set of ion channels expressed by PNs (Raman and Bean, 1999; Khaliq et al., 2003). Together these channels prompt PNs to generate their own regular spiking activity such that enzymatically-dissociated PNs fire at approximately 40 Hz with striking, almost metronomic regularity (Raman and Bean, 1999). Under these conditions, coefficients of variation in inter-spike instantaneous frequencies are typically less than 10% over long periods of time (Hausser and Clark, 1997; Smith and Otis, 2003). Thus, individual PNs appear to have a characteristic baseline firing rate or set point, and across a population of PNs, firing rates are much more variable than within any single PN over time. *In vivo*, PN firing varies systematically across different regions with baseline firing rates ranging from 40 to 100 Hz (Zhou et al., 2014). Of course, the interaction between this intrinsically generated spiking and excitatory and inhibitory synaptic inputs generate the more variable and complex patterns of spiking observed *in vivo*. Nonetheless, we use the term "pacemaking" to refer to two features of the robust intrinsic excitability; PNs fire at high spontaneous rates, and, in the absence of synaptic inputs, this firing is remarkably regular.

Such sustained, regular PN firing is degraded in at least six mouse models of SCA and in a number of other non-SCA related transgenic mouse models that exhibit behavioral ataxia. In

a PN-specific, human transgene model of SCA2 (*pcp2- Atxn2*^{127Q}), the progressive dysregulation of transcriptional expression patterns and the severity of behavioral ataxia track the reduction of mean PN firing rates over an 8 month time course of disease progression (Hansen et al., 2013). This model recapitulates basic features of the human disease such as intact motor behavior and normal cerebellar morphology at birth and in early adulthood followed by progression of motor dysfunction and PN loss in later life (Pulst et al., 1996; Hansen et al., 2013). Another PN-specific SCA2 model with a smaller CAG repeat length (*pcp2-ATXN2*^{Q58}) similarly shows impaired firing rates and less regular firing (Kasumu et al., 2012b; Kasumu et al., 2012a). Progressive reductions in pacemaking have also been described in PC-specific models of SCA 1 (*pcp2-ATXN1*^{Q82}) (Hourez et al., 2011), in a global YAC transgenic for SCA3 (*ATXN3*^{Q84}) (Shakkottai et al., 2011), and in a β-III spectrin knockout mouse model of SCA5 (Perkins et al., 2010). Tellingly, the SCA5 model exhibited reductions in a resurgent component of voltage gated Na^+ current known to play a key role in pacemaking (Perkins et al., 2010). Lastly, a PN-specific SCA6 transgenic mouse line expressing a C-terminal fragment, corresponding to exon 47, of the P-type Ca channel containing 27 polyglutamine repeats has been characterized (Mark et al., 2015). PN firing rates in this mouse are reduced and firing becomes markedly irregular. Importantly, in all of these mouse lines utilizing different transgenes, promoters, and SCA subtypes, the loss of pacemaking ability tightly correlates with behavioral ataxia. Moreover, degradation in the physiological output of PNs precedes overt loss of PNs. These findings suggest that reduced PN spiking output could be a common pathophysiological feature of SCAs and that it may contribute to the ataxic symptoms characteristic of the disease class.

Consistent with the hypothesis that reduced PN pacemaking contributes to ataxia, several transgenic mouse lines in which cerebellar genes are deleted show slowed PN firing rates and

an ataxic phenotype. The *moonwalker* mouse line, characterized by a point mutation in the TRPC3 ion channel causing its constitutive activation, shows profound reductions in PN firing frequencies (Sekerkova et al., 2013). A PN-specific TSC1 knockout mouse (*pcp2-TSC1*$^{-/-}$) shows significant reductions in firing with loss of one copy and more severe reductions with loss of both copies of the TSC1 gene (Tsai et al., 2012). Finally, both global (*Rbfox1*$^{+/loxP}$*/Rbfox2*$^{loxP/loxP}$*/Nestin-Cre*$^{+/-}$) and PN-specific (*Rbfox* $^{loxP/loxP}$*/Rbfox2*$^{loxP/loxP}$*/pcp2-Cre*$^{+/-}$) lines in which copies of the RNA splicing genes R*bfox 1* and *2* have been deleted show ataxic behavior and reduced PN firing (Gehman et al., 2012). Here, similar to the SCA5 mouse model described above, there is evidence for disordered expression of splice variants of the resurgent Na^+ channel isoform $Na_V1.6$ encoded by the gene *Scn8a* (Gehman et al., 2012). Although these mouse models do not recapitulate genetic forms of SCAs, the correspondence between reduced PN intrinsic excitability and the behavioral ataxia is supportive of the key role this electrophysiological feature plays in normal motor behavior.

More subtle changes in PN pacemaking have also been linked to ataxia. In mutant mouse lines containing mutations in P-type calcium channel genes, Walter and colleagues describe changes in the regularity of PN firing but not the average firing rate that correlate with behavioral ataxia. They find that treatment of mice with EBIO-1, a positive modulator of SK-type calcium activated potassium channels, reduces the ataxia and causes PN firing to become more regular in the mutant animals (Walter et al., 2006). These findings imply that subtle changes in the pattern of PN output can also lead to ataxic behavior.

Circuit consequences of reduced PN output

PN output is directed exclusively to cerebellar nucleus (DCN) and vestibular nucleus neurons (**Figure 1**). PNs are GABAergic and so their tonic activity provides a potent baseline inhibition of these downstream target neurons, some of which function as premotor neurons, for example in the descending rubrospinal motor pathway. Assuming there is no compensation in downstream pathways, loss of tonic inhibition from PNs would be expected to lead to consequences at a circuit level such as increased cerebellar nuclear neuron excitability and increased motor drive. Confirming this prediction, optogenetic experiments in which PN output is transiently silenced show that cerebellar nucleus neurons burst and that this drives rapid movements (Heiney et al., 2014; Lee et al., 2015). In addition, optogenetic stimuli affecting PN firing are very effective at driving associative learning at a behavioral level (Lee et al., 2015). Such stimuli may drive activity-dependent synaptic plasticity; in PNs, this would result in PF-PN LTD and thus reduced excitatory drive from PFs (the red burst in **Figure 1**), while in DCN, it would result in MF-DCN LTP leading to increased excitatory drive onto DCN neurons (the blue burst in **Figure 1**). All of these circuit changes would be expected to promote the sort of ectopic movements observed after optogenetic training (Lee et al., 2015).

So, given SCA-associated alterations in PN firing, why are ectopic movements, dystonia and/or chorea not observed in SCA? Our working hypothesis is that neurons downstream of PNs, e.g. cerebellar nucleus neurons, red nucleus neurons, and thalamic neurons (see **Figure 1**) compensate for the increased excitability that may result from the reduction in PN inhibition. Future experiments will be required to explore these possibilities and their functional contributions to the ataxic phenotype.

Metabotropic glutamate receptors and intracellular calcium mobilization in SCAs

Other changes that have been observed in PN physiology as a consequence of SCA involve inositol triphosphate receptor (IP_3R) linked calcium signaling networks. PNs express IP_3Rs at extremely high levels and these IP_3-gated, intracellular calcium channels are downstream of the metabotropic glutamate receptor type I (mGluR1), a G- protein coupled glutamate receptor (GPCR). Glutamate released from either of the two excitatory synaptic inputs to PNs (see **Figure 1**), parallel fibers (PFs) or climbing fibers (CFs), activates glutamate gated ion channels (AMPARs) which are responsible for the fast electrical signals but also mGluR1 (Batchelor and Garthwaite, 1997; Brasnjo and Otis, 2001; Dzubay and Otis, 2002). These GPCRs are then coupled to various biochemical pathways mainly via heterotrimeric G protein containing the $G\alpha_q$ subunit (Offermanns, PNAS 1997; Tanaka 2000; Hartmann JNS 2004). A principal set of biochemical pathways activated by $G\alpha_q$ involves phospholipase Cβ (PLCβ) which generates IP_3 and triggers intracellular Ca^{2+} release from ER stores that sit at the base of dendritic spines (Finch and Augustine, 1998; Takechi et al., 1998). Another, more direct limb of the $G\alpha_q$ pathway activates TRPC3 ion channels on the plasma membrane, leading to a slow synaptic current. Activation of TRPC3 requires $G\alpha_q$ (Hartmann et al., 2004) but is independent of PLCβ and of the IP_3R (Dzubay and Otis, 2002; Hartmann et al., 2008; Hartmann et al., 2011) . This suggests a model in which signaling diverges from $G\alpha_q$ to TRPC3 channel and to PLCβ-IP_3R limbs as indicated in **Figure 2**. A third pathway stimulates local protein synthesis regulated by the Fragile X protein (Huber, 2006) (not shown in **Figure 2**).

A central role for mGluR dysregulation in cerebellar ataxia is supported by abundant evidence from mouse and human genetics, implicating each of the signaling proteins in the cascade from mGluR1 to the IP_3R. Importantly, data from dozens of mouse models show that ataxia can result from reductions or increases in several elements of the mGluR signaling cascade, presenting a complex picture of the pathophysiological role of this key feature of PN biology. Below we summarize evidence and speculate on a possible unified view of how this signaling mechanism could be central to this class of disorders.

Evidence suggesting that reduced mGluR signaling plays a role in SCA

Genetic reductions in mGluR signaling have been studied using knockout mice for mGluR1 (Aiba et al., 1994; Conquet et al., 1994), $G\alpha_q$ (Offermanns et al., 1997; Hartmann et al., 2004), PLCβ (Kano et al., 1998; Miyata et al., 2001), IP_3R (Matsumoto et al., 1996; van de Leemput et al., 2007), and TRPC3 (Hartmann et al., 2008); in addition a conditional mGluR1 knockout and PC-specific mGluR1 rescue mouse line have been generated (Ichise et al., 2000; Nakao et al., 2007). All of the genetic deletion mice show ataxia and deficits in cerebellum-dependent forms of motor learning while the rescue mouse restores these cerebellar functions. In humans, loss of function alleles of some of these genes are known to cause specific ataxias. These include SCA15 which is caused by a mutation in the gene for the IP_3R (van de Leemput et al., 2007), as well as a recessive form of congenital ataxia caused by mutations in the mGluR1 gene (Guergueltcheva et al., 2012). Finally, SCA14 is caused by constitutively activating mutations in PKCγ, a protein kinase downstream of mGluR/IP_3R activation (Yabe et al., 2003).

There are also several mouse models of SCA in which it is reported that elements of the mGluR signaling / cytoplasmic calcium homeostasis cascades are downregulated. This has perhaps been studied most thoroughly in SCA1, where mRNAs for the type I IP_3R, PKCγ, homer 3, the EAAT4 glutamate transporter, and the SERCA3 calcium pump are reduced (Lin et al., 2000; Serra et al., 2004; Serra et al., 2006). Messenger RNA for mGluR1 is also reduced in SCA 82Q lines (Serra et al., 2006). At the protein levels things are more complex as mGluR1 levels drop overall as assessed by quantitative Western blot (Zu et al., 2004), but such reductions are accompanied by a loss of dendritic complexity and spine density. At remaining spines mGluR protein levels appear unchanged (Skinner et al., 2001; Zu et al., 2004), raising the possibility that reductions in mGluR protein reflect disease-related alterations in excitatory signaling but are not on their own a driver of pathological symptoms.

Other models also report indirect reductions in mGluR signaling due to changes in protein localization. An SCA3 mouse line expressing a truncated SCA3 transgene with a 69 poly Q repeat exhibits internalization of mGluR protein and a reduction in mGluR-mediated cannabinoid release (Konno et al., 2014). Similarly, in an SCA5 mouse model expressing a mutant form of β–III spectrin there is an apparent mislocalization of dendritic mGluR protein and degraded mGluR-mediated physiology (Armbrust et al., 2014). These data along with those described for SCA1 above suggest that various subtypes of SCA lead to concerted pathophysiology of mGluR1 signaling and calcium homeostasis in PNs. Also consistent with this picture, is the finding that acute pharmacological enhancement of mGluR1 with a positive allosteric modulator improves ataxic behavior in an SCA1 154Q mouse line (Notartomaso et al., 2013).

Evidence suggesting that increased mGluR signaling plays a role in SCA

In the *pcp2-ATXN2*^{Q58} mouse model, work has suggested that mGluR-triggered, IP_3R-mobilized calcium elevations are enhanced in PNs (Liu et al., 2009) due to specific protein-protein interaction between the expanded repeat protein (ataxin 2) and the IP_3R. Subsequent work showed that viral delivery of the IP_3 degradation enzyme inositiol 1,4,5 phosphatase to PNs led to improvement in motor behavior and neuropathology in the SCA2 58Q mouse (Kasumu et al., 2012a).

Although there are no mouse lines that overexpress mGluR1 or downstream signaling elements, there is ample evidence that excessive mGluR signaling and elevated calcium levels can also lead to ataxia. One of the more striking examples comes from the *moonwalker* mouse line mentioned earlier. Here a point mutation in TRPC3 constitutively activates this cation channel which sits downstream from mGluR1 (**Figure 2**). This results in a severe ataxia in mice and concomitant loss of specific types of cerebellar neurons (Becker et al., 2009; Sekerkova et al., 2013). As mentioned above, this defect also alters PN firing. Interestingly, one adult onset case of ataxia has been described with a gain of function point mutation in TRPC3 and it will be interesting to see whether this mutation results in enhanced or constitutive channel activity (Fogel et al., 2015).

Genetic deletion of various other molecules required for normal calcium homeostasis, such as the calcium buffering proteins parvalbumin and calbindin D28K, result in altered PN physiology, calcium signaling, and behavioral ataxia (Airaksinen et al., 1997; Vecellio et al., 2000). There has also been work showing that impairment of calcium homeostasis

exacerbates SCA pathology. Genetic deletion of one copy of the calcium buffering protein calbindin D28K enhances the ataxic phenotype in an SCA1 mouse model (Vig et al., 2012).

Loss of a single copy of the gene encoding the plasma membrane calcium pump PMCA also results in ataxia in mice (Empson et al., 2010), and rare mutations in this gene have been found in human ataxia patients (Zanni et al., 2012; Cali et al., 2015). Taken together, such findings strongly suggest that elevated calcium levels, one of the possible outcomes of increased activity in the mGluR signaling cascade, can compromise the health of PNs and lead to ataxia.

Is it possible to reconcile increases and decreases in mGluR signaling as a cause of ataxia?

Taken together the findings summarized above strongly implicate dysfunction in mGluR1 and calcium signaling pathways as causative for genetic forms of ataxia in mice and humans. However, the data also paradoxically indicate that either reductions or increases in these signaling cascades can lead to ataxia. While it is certainly possible that distinct SCA subtypes are associated with different changes in mGluR1 signaling and calcium homeostasis, we speculate that there could be common pathophysiological mechanisms in this broad class of disease.

A starting point is the assumption that, although the different genetic insults cause disease by different molecular mechanisms (e.g., transcriptional dysregulation, protein aggregation,

impaired calcium homeostasis, altered excitability, etc), as PNs sicken they gradually lose the ability to regulate intracellular calcium. Given the extensive intracellular calcium stores and various pathways for calcium entry across the plasma membrane of PNs, this assumption is almost certainly true at end stages of all types of SCA.

If PNs suffer from elevated basal calcium this could trigger a common disease process characterized by two potent positive feedback mechanisms (the *mGluR/Ca^{2+} excitotoxicity hypothesis of SCA* outlined in **Figure 2**). Batchelor and Garthwaite first demonstrated that there is a robust form of positive feedback regulation exerted by intracellular Ca^{2+} on the mGluR signaling cascade (Batchelor and Garthwaite, 1997). This finding, confirmed by subsequent studies, indicates that slightly elevated (200-300 nM) levels of intracellular Ca^{2+} strongly potentiate mGluR-mediated signals such as TRPC3 currents (Dzubay and Otis, 2002) and IP_3R initiated Ca^{2+} transients (Wang et al., 2000), suggesting that Ca^{2+} potentiates mGluR function at a PLCβ- and IP_3R-independent step early in the signal transduction cascade (see the **1** in **Figure 2**). It is also known that Ca^{2+} directly interacts with the IP_3R (see **2** in in **Figure 2**). At steady state, there is a bell-shaped potentiation/inhibition exerted by Ca^{2+} on the IP_3R such that 200-500 nM Ca^{2+} markedly potentiates while higher concentrations inhibit IP3R function (Bezprozvanny et al., 1991; Finch et al., 1991). In more dynamic circumstances, such as during physiological activity, an order dependence has been described in which IP_3 followed by Ca^{2+} gives the largest enhancements (Sarkisov and Wang, 2008); interestingly this positive feedback has been proposed to contribute to learning rules for PF long term depression (Wang et al., 2000).

These two forms of Ca^{2+} -mediated positive feedback (on TRPC3 currents and on IP_3R function) in **Figure 2** are likely independent of one another implying a strong and multifaceted potentiation exerted by Ca^{2+} on mGluR signaling. In PNs and in cerebellar nucleus neurons, changes in Ca^{2+} are required for forms of synaptic plasticity such as PF-LTD (the red burst in **Figure 1**) and MF LTP (blue burst in burst in **Figure 1)** that are implicated in associative cerebellar learning. The mGluR/Ca^{2+} excitotoxicity hypothesis of SCA suggests that these forms of synaptic plasticity could become saturated as part of the SCA disease process. Moreover, the reductions in expression of mGluR1 signaling elements observed in some SCA models could reflect a compensatory mechanism driving by excessive signaling in this pathway.

Conclusions

It of course remains to be demonstrated whether the highly varied set of molecular alterations seen across SCAs share the two physiological mechanisms discussed here, slowed PN pacemaking, and dysregulated intracellular Ca^{2+} and alterations in the mGluR signaling cascade. Arguing in favor of this possibility is the fact that such mechanisms, pacemaking and IP_3-mediated Ca^{2+} signaling, are defining features of PN physiology, setting PNs apart from most other neuronal types. This could explain why, despite the ubiquity of expression of many of the SCA-related gene products, these diseases, at least at their outset, selectively impact PNs. Although the root cause of the calcium dysregulation could be different for each of the SCAs, this hypothesis could explain why the varied molecular lesions all result in PN pathophysiology and eventually degeneration. A mechanism involving slowly rising calcium levels reinforced by the positive feedback elements described here provides a potential explanation for the slowly progressive nature of the disease. However, experiments in the

numerous SCA mouse models that are available will be required to settle the generality of this mechanism.

How might dysregulated intracellular Ca^{2+} and PN spontaneous firing interact? Ca^{2+}-activated SK potassium channels provide a critical brake on PN excitability; thus, the chronically elevated basal Ca^{2+} hypothesized to occur as part of SCA pathology could lead directly to the slowed pacemaking. It is also reasonable to speculate that chronically elevated Ca^{2+} could dysregulate the expression and or post-translational modulation of the various ion channels required for pacemaking. Indeed, recent results have implicated changes in BK-type, Ca^{2+}-activated and in A-type, voltage-gated potassium channels (Hourez et al., 2011; Dell'Orco et al., 2015). Finally, as mentioned above, the slowed PN firing and elevated Ca^{2+} could together lead to saturated forms of circuit plasticity which would impair motor learning and lead to imbalances in the output of cerebellar circuitry. Finally, both of the putative pathophysiological mechanisms offer potential therapeutic targets by which one might normalize circuit activity and in this way treat the ataxic symptomology.

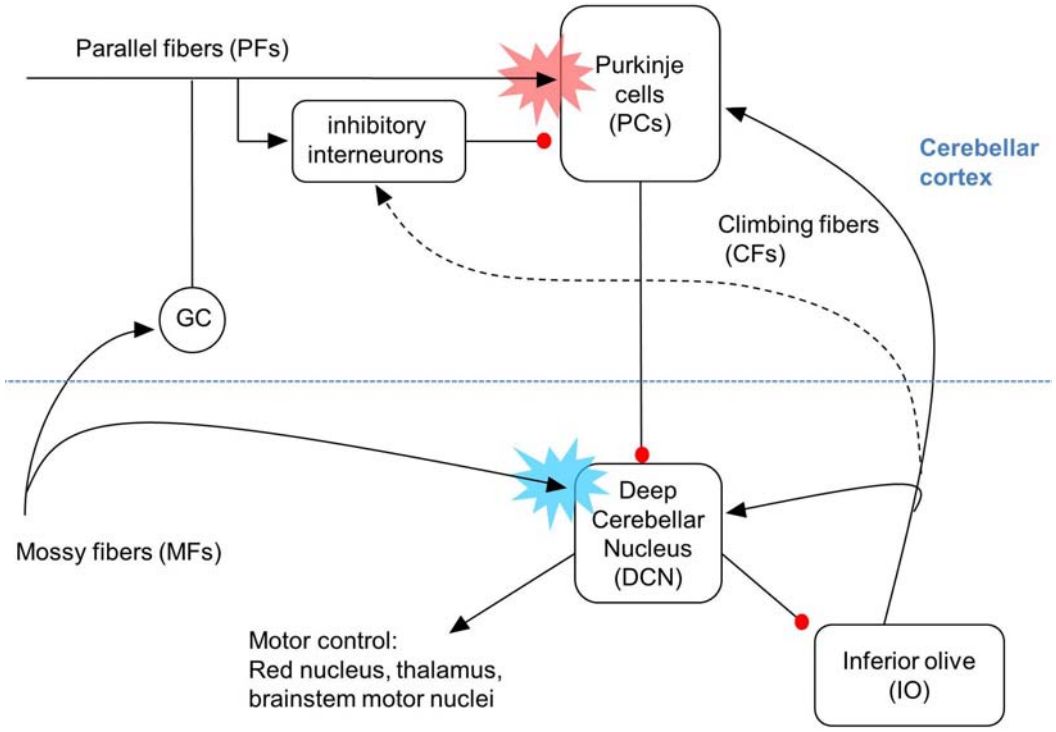

Figure 1. Simplified schematic of the cerebellar circuit. Black arrowheads represent excitatory and red ball ends denote inhibitory connections. The dashed arrow indicates an unconventional connection between CFs and interneurons [22]. The red and blue starbursts indicate hypothesized sites of CF-instructed, associative forms of synaptic plasticity required for associative motor learning (LTD of PF inputs and LTP of MF inputs, respectively). These forms of plasticity may be saturated during the SCA disease process.

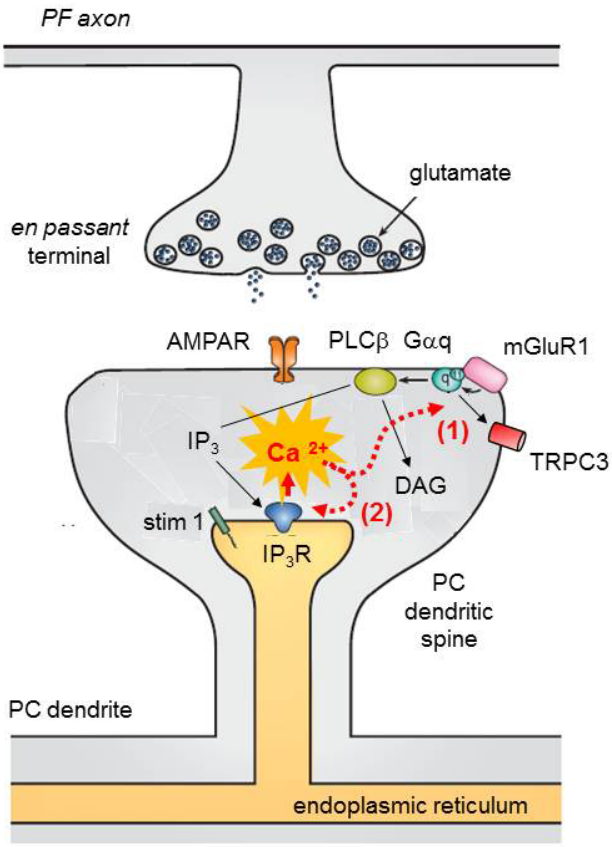

Figure 2. Schematic of the *mGluR / Ca $^{2+}$ hypothesis*. Glutamate release from PFs activates both AMPARs and mGluR1 GPCRs. As indicated by the black arrows, mGluR1 is coupled to PLCβ which leads to release of Ca^{2+} from IP_3Rs on the endoplasmic reticulum. Ca^{2+} exerts positive feedback on mGluR1 transduction at a step early in the cascade (1) as well as at the IP3R (2). Thus, elevations in Ca^{2+} will exacerbate the IP_3R hyperactivity observed in SCA2. Modified from Hartmann et al., 2011.

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