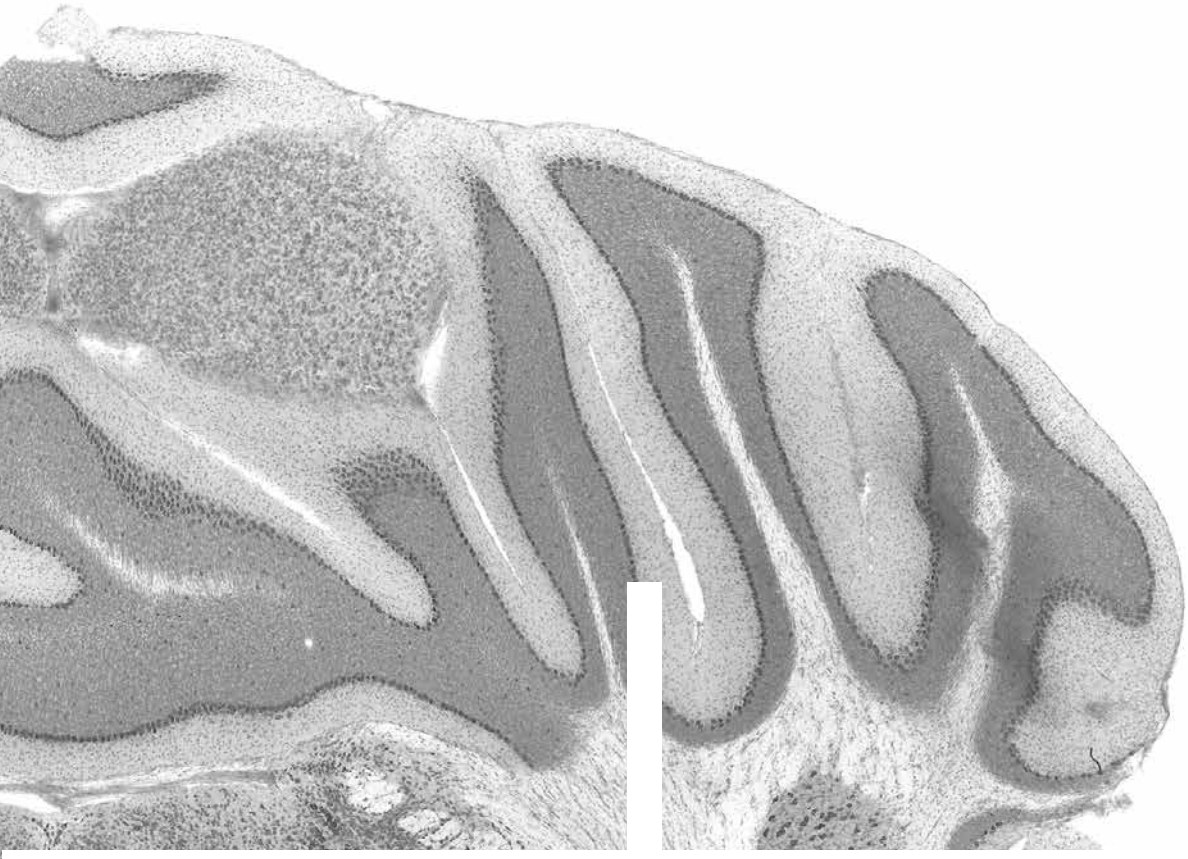

Climbing fibres in spinocerebellar ataxia: A mechanism for the loss of motor control

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Abstract

The spinocerebellar ataxias (SCAs) form an ever-growing group of neurodegenerative disorders causing dysfunction of the cerebellum and loss of motor control in patients. Currently, 41 different genetic causes have been identified, with each mutation affecting a different gene. Interestingly, these diverse genetic causes all disrupt cerebellar function and produce similar symptoms in patients. In order to understand the disease better, and define possible therapeutic targets for multiple SCAs, the field has been searching for common ground among the SCAs. In this review, we discuss the physiology of climbing fibres and the possibility that climbing fibre dysfunction is a point of convergence for at least a subset of SCAs.

Keywords: Spinocerebellar ataxia; climbing fibres; synaptic plasticity; glutamate; common pathology; mouse models

Introduction

The spinocerebellar ataxias (SCAs) form a group of genetically heterogeneous neurodegenerative disorders causing dysfunction of the cerebellum and loss of motor control in patients. To date, there are 44 different SCA types with as many genetic causes (Table 1). Interestingly, these different genetic variations all result in very similar symptoms of ataxic gait and poor coordination of the hands, speech and eye movements, all caused by dysfunction of the cerebellum. This dysfunction is caused by an altered cerebellar output, indicating impairment of the sole output of the cerebellar cortex: Purkinje cells (PCs). This change occurs either because the PCs receive changed input, or because the PCs themselves are impaired, or a combination of both. Because most ataxias display similar symptoms and PC loss, researchers have been looking for common mechanisms on a molecular level. Gene-gene interaction networks have previously shown that even when SCAs are divided into groups based on mutation type, they still show great overlap in gene co-expression mechanisms[1,2]. Gene-gene interaction networks emerging in all cerebellar ataxias include neurogenesis, cell cycle and proliferation, cell communication, and synaptic transmission, all of which include calcium signaling. This suggests that a common molecular mechanism could be found in one of these networks.

Several studies exploring SCA1, SCA7, SCA14 and SCA23 have found problems in synaptic transmission, specifically with one of the excitatory inputs of the cerebellum: the climbing fibres[3–7]. Climbing fibres are the axons of inferior olive neurons, and innervate PCs in the cerebellum[8–10]. They exert enormous amount of control over their individual PCs, as will be discussed in this review, and therefore greatly influence the output of the cerebellum. Even though the climbing fibre deficits previously described are not identical, this crucial cerebellar input could be the common ground among SCA pathologies. Finding common underlying pathology could provide insights into key aspects of the disease, and possibly provide therapeutic opportunities for many, if not all, SCA types. Therefore, it would be of great scientific and clinical value to find common pathology before the disease has progressed to PC loss.

Climbing fibre defects have now been detected in mouse models representing four different SCA types, of which two are polyQ (non-conventional) SCA types and two are conventional SCA types. The related disease genes in each type have entirely different functions (Table 1). This presents an opportunity to investigate climbing fibres more closely in relation to cerebellar neurodegeneration and SCA. In this review, we will take a closer look at the development and function of climbing fibres, and their known deficits in SCA1, SCA7, SCA14, and SCA23. We will also discuss the excitatory neurotransmitter glutamate which is utilized by climbing fibres to convey information, and which offers another line of investigation for a shared disease mechanism among SCA types.

Table 1. Overview of all known mutations causing spinocerebellar ataxia

SCA subtype	Gene	Protein	Function	Mutation Type	Reference
SCA1	*ATXN1*	Ataxin-1	Transcription regulation	$(CAG)_n$	(Orr et al., 1993)
SCA2	*ATXN2*	Ataxin-2	RNA metabolism	$(CAG)_n$	(Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996)
SCA3	*ATXN3*	Ataxin-3	Deubiquitination, transcription regulation	$(CAG)_n$	(Kawaguchi et al., 1994)
SCA4	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Flanigan et al., 1996)
SCA5	*SPTBN2*	Beta-III Spectrin	Neuronal membrane skeleton	Deletion, MM	(Ikeda et al., 2006)
SCA6	*CACNA1A*	Calcium Voltage-gated Channel, P/Q Type, Alpha 1A Subunit	Calcium signaling	$(CAG)_n$	(Zhuchenko et al., 1997)
SCA7	*ATXN7*	Ataxin-7	Transcription regulation	$(CAG)_n$	(David et al., 1997)
SCA8	*KLHL1AS/ ATXN8*	Kelch-like 1/Ataxin-8	*Unknown*	Intronic $(CTG)_n$	(Koob et al., 1999)
SCA9	*Reserved*	*Unknown*	*Unknown*	*Unknown*	(Higgins et al., 1997)
SCA10	*ATXN10*	Ataxin-10	Neuritogenesis	$(ATTCT)_n$	(Matsuura et al., 2000)
SCA11	*TTBK2*	Tau Tubulin Kinase 2	Implicated in tau phosphorylation	Deletion	(Houlden et al., 2007)
SCA12	*PPP2R2B*	Protein Phosphatase 2 (formerly 2A), Regulatory Subunit B	Regulation of PP2 activity, transcription regulation	5′-UTR $(CAG)_n$	(Holmes et al., 1999)
SCA13	*KCNC3*	Potassium Voltage-Gated Channel, Shaw-related Subfamily, Member 3	Potassium signaling	MM	(Waters et al., 2006)
SCA14	*PRKCG*	Protein Kinase C, gamma	Protein phosphorylation	MM	(Chen et al., 2003)
SCA15	*ITPR1*	Inositol 1,4,5-Triphosphate Receptor, Type 1	Calcium signaling	Deletion	(van de Leemput et al., 2007)
SCA16	*ITPR1*	Inositol 1,4,5-Triphosphate Receptor, Type 1	Calcium signaling	Deletion	(Iwaki et al., 2008)
SCA17	*TBP*	TATA-Box-Binding Protein	Transcription regulation	$(CAG)_n$	(Nakamura et al., 2001)
SCA18	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Devos et al., 2001)
SCA19	*KCND3*	Potassium Voltage-Gated Channel, Shal-related Subfamily, Member 3	Potassium signaling	MM	(Duarri et al., 2012)
SCA20	*Unknown*	*Unknown*	*Unknown*	Chromosomal Duplication	(Knight et al., 2004)
SCA21	*TMEM240*	Synaptic transmembrane protein	*Unknown*	MM	(Delplanque et al., 2014)

SCA22	*KCND3*	Potassium Voltage-Gated Channel, Shal-related Subfamily, Member 3	Potassium signaling	MM	(Lee et al., 2012)
SCA23	*PDYN*	Prodynorphin	Synaptic transmission	MM, frameshift	(Bakalkin et al., 2010)
SCA24	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Swartz et al., 2002)
SCA25	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Stevanin et al., 2005)
SCA26	*eEF2*	Eukaryotic translation elongation factor 2	Protein synthesis	MM	(Hekman et al., 2012)
SCA27	*FGF14*	Fibroblast Growth Factor 14	Signal transduction, Regulation of voltage-gated sodium channels	MM	(van Swieten et al., 2003)
SCA28	*AFG3L2*	AFG3 ATPase Family Gene 3-Like 2	ATP-dependent protease essential for axonal development	MM	(Mariotti et al., 2008)
SCA29	*ITPR1*	Inositol 1,4,5-Triphosphate Receptor, Type 1	Calcium signaling	MM	(Huang et al., 2012)
SCA30	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Storey et al., 2009)
SCA31	*TK2 or BEAN*	*Unknown*	*Unknown*	Intronic $(TGGAA)_n$	(Sato et al., 2009)
SCA32	*Reserved*				
SCA33	*Reserved*				
SCA34	*ELOVL4*	Elongation of Very Long Chain Fatty Acids Protein 4	Elongation of fatty acids	MM	(Cadieux-Dion et al., 2014)
SCA35	*TGM6*	Transglutaminase 6	Crosslinking of proteins, conjugation of polyamines to proteins	MM	(Wang et al., 2010)
SCA36	*NOP56*	NOP56 Ribonucleoprotein Homolog	60S ribosomal subunit biogenesis (early & middle stages)	Intronic $(GGCCTC)_n$	(Kobayashi et al., 2011)
SCA37	*Unknown*	*Unknown*	*Unknown*	*Unknown*	(Serrano-Munuera et al., 2013)
SCA38	*ELOVL5*	Elongation of Very Long Chain Fatty Acids Protein 5	Elongation of fatty acids	MM	(Di Gregorio et al., 2014)
SCA39	*Unknown*	*Unknown*	*Unknown*	Chromosomal Duplication	(Johnson et al., 2015)
SCA40	*CCDC88C*	Coiled-Coil Domain Containing 88C	Regulation of protein phosphorylation, regulation of Wnt signaling	MM	(Tsoi et al., 2014)
SCA41	*TRPC3*	Transient Receptor Potential Cation Channel, Subfamily C, Member 3	Receptor-activated non-selective calcium permeant cation channel	MM	(Fogel et al., 2015)
DRPLA	*ATN1*	Atrophin I	Transcriptional corepressor	$(CAG)_n$	(Koide et al., 1994)
Undefined	*RNF170*	Ring Finger Protein 170	E3 ubiquitin ligase activity	MM	(Valdmanis et al., 2011)
Undefined	*GRID2*	Glutamate Receptor, Ionotropic, Delta 2	Ionotropic glutamate receptor activity	MM	(Coutelier et al., 2015)

Climbing fibre development

PCs receive input from various cell types. Inhibitory inputs are provided by several types of interneurons in the molecular layer of the cerebellum (Figure 1). Excitatory inputs are provided by parallel fibres and climbing fibres. Parallel fibres are the axons of granule cells located in the cerebellar granule layer (Figure 1) and innervate the distal dendritic tree of PCs, with around 200,000 parallel fibres synapsing approximately once or twice upon a PC[9,10]. As mentioned in the Introduction, climbing fibres are the axons of inferior olive neurons, and innervate the proximal PC dendritic tree in a one-to-one ratio, with the climbing fibre synapsing onto the PC between 250 and 1500 times[8–15].

During development, climbing fibres arrive at the cerebellum between embryonic day 15.5 (E15.5) and E16.5[16], when PCs are still migrating[17], and make synaptic contact with PC dendrites rather than soma[17] as early as E16.5[16], however, whether these are functional contacts is not yet known. The postnatal development of climbing fibres has been reviewed extensively by Watanabe and Kano[14]. In short, during the first three postnatal weeks, climbing fibres go through six stages of development. During the creeper stage, starting at postnatal day 0 (P0), the fibres creep among PC somata and form transient synapses on the PC's immature dendrites. Around P5, they surround PC somata with a high density and innervate the somata in the pericellular stage. Until approximately P7, PCs are innervated by, on average, five climbing fibres. Between P3 and P7, one of these climbing fibres undergoes functional differentiation and strengthening. Recently, C1ql1-Bai3 signaling has been implicated in regulating the selection of "winner" climbing fibres via an anterograde signal supplied by the inferior olive neuron itself, and is required for maintaining the "winner" climbing fibre's synapses from P7[18]. The "winner" climbing fibre then starts to displace its synapses to the apical region of the PC soma around P9, during what is called the capuchon stage. By P12, the "winner" climbing fibre will start the dendritic stage of development, meaning it will translocate to the dendrites of the PC and make synapses there.

As soon as one climbing fibre has become the "winner" (around P7), the early phase of climbing fibre synapse elimination starts, lasting until about P11. This process consists of pruning of somatic climbing fibre synapses, and is dependent upon P/Q-type voltage-dependent Ca^{2+} channels, as evidenced by the improper wiring of climbing fibres in mice lacking the P/Q-type voltage-dependent Ca^{2+} channel $Ca_v2.1$, which is also the channel affected in SCA6[19,20] (Table 1). At the same time, semaphorin3A in PCs acts on plexinA4 on climbing fibres as a retrograde signal to maintain or strengthen the active synapse and prevent elimination[21,22], further strengthening the position of the "winner" climbing fibre. Massive elimination of somatic synapses enables the "winner" climbing fibre to monopolize innervation of its PC in postnatal week 3. Late phase climbing fibre synapse elimination occurs from P12-P17 and two mechanisms are currently known to influence this process. The first is the GluRδ2-Cbln1 pathway, wherein GluRδ2 strengthens parallel fibre-Purkinje cell (PF-PC) synapses structurally while simultaneously weakening these synapses functionally

Figure 1. A schematic overview of the excitatory wiring of the cerebellum

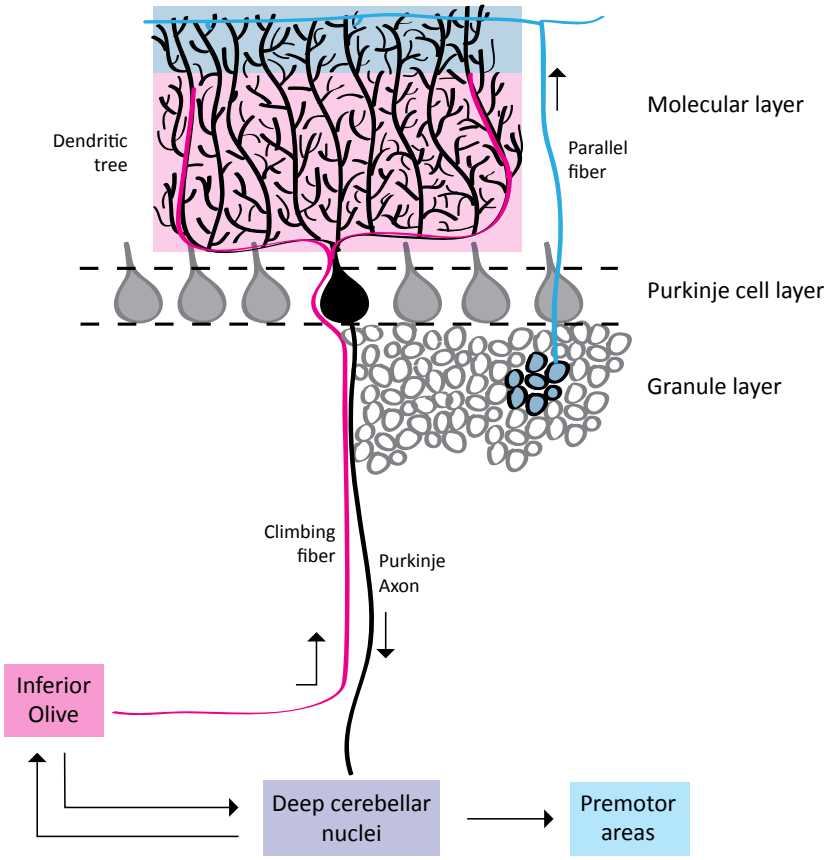

Figure 1. A schematic overview of the excitatory wiring of the cerebellum

The Purkinje cells (PCs), located in the Purkinje cell layer, are the sole output of the cerebellar cortex. The PCs receive inhibitory (not shown) and excitatory inputs that synapse onto the dendritic tree, which is located in the molecular layer. Climbing fibers (in pink) originate from neurons in the inferior olive, travel to the cerebellum, and synapse between 250 and 1500 times upon a single PC, in a unique 1:1 ratio. The proximal dendritic tree is climbing fiber territory, indicated in light pink. Parallel fibers (in green) originate from granule cells in the granule layer of the cerebellum, and synapse upon the distal dendritic tree (indicated in light green) of a PC. In contrast to climbing fibers, parallel fibers only synapse once or twice upon a single PC. The parallel fiber territory of the PC dendritic tree is occupied by as many as 200,000 parallel fibers, thus creating the many PF-PC synapses found on a PC.

and precerebellin 1 (Cbln1) facilitates synaptic connectivity. Both climbing fibre and parallel fibre innervation are severely altered in GluRδ2-KO mice, as distal PC dendrites are innervated by climbing fibres instead of parallel fibres[23,24], and Cbln-null mice show a similar phenotype[25]. This mechanism shows the dependence of proper climbing fibre wiring upon parallel fibre synaptogenesis.

In the second mechanism influencing late phase climbing fibre elimination, mGluR1-PKCγ signaling plays a central role. The normal development of mGluR1-null mice up to 2 weeks postnatal followed by abnormal synapse elimination towards the end of postnatal week 2,

suggests that mGluR1-PKCγ signaling is essential for late phase, but not early phase, climbing fibre synapse elimination[14]. Incidentally, PF-PC synapse formation is completely normal in these mice, indicating that impaired late phase synapse elimination is not secondary to deficits in parallel fibre synaptogenesis in these mice[14], but is dependent upon mGluR1-PKCγ signaling in PCs. Notably, mutations in PKCγ cause SCA14 (Table 1). Downstream of mGluR1, semaphorin7A mediates synaptic elimination by acting on plexinC1 and integlinB1 in a retrograde manner[21,22], further indicating the importance of mGluR1 signaling in this process, with the suggestion that mGluR1-semaphorin7A signaling promotes elimination rather than shielding "winner" synapses from elimination. This hypothesis was strengthened by the recent observation that C1ql1-Bai3 signaling maintains "winner" climbing fibre synapses independently from mGluR1 signaling, and typically on the more distal climbing fibre-Purkinje cell (CF-PC) synapses[18].

Climbing fibre activation

The activation of a climbing fibre has a dual role: 1) triggering synaptic plasticity at dendritic PC synapses (see *Synaptic plasticity and climbing fibre control,* below) and 2) generating a distinct output in the PC axon, the complex spike[8,26–28]. The complex spike represents a critical signal for cerebellar functioning, conveying timing information for motor function[29]. It consists of a fast initial spike followed by several slower spikelets with smaller amplitudes, separated by 2-3 milliseconds[8]. The initial fast spike is caused by opening of the α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA-Rs) at the CF-PC synapse[14], while the smaller spikelets are the result of interaction between local sodium currents in the PC soma[30–33] and the typical activation of climbing fibres, which tend to fire in high-frequency bursts of 1 to 6 spikes[34]. As the complex spike is crucial for cerebellar functioning and motor control, inadequate generation of complex spikes would cause abnormal cerebellar functioning and ataxic phenotypes. Whether the character of climbing fibre activation is "all-or-nothing" or more graded, so as to encode more information, is still being debated (for a review, see[13]). However, more evidence of pre- and postsynaptic modulation is emerging, making the case for a graded, more instructive complex spike[13].

Synaptic plasticity and climbing fibre control

Pre- and postsynaptic modulation consist of long-term potentiation (LTP) and long-term depression (LTD) of the excitatory synaptic inputs of the PC, namely the climbing and parallel fibres. Synaptic plasticity is of great importance to the functioning of the cerebellum, as it has long been implicated in motor functioning and learning[35–40]. Changes in the efficiency of specific synapses can change the content of the message entirely. Of course, if the changed synaptic efficiency is physiological and intentional, it can be very useful. However, when the changes are

Table 2. Overview of the different forms of synaptic plasticity

Synapse	Plasticity	Critical molecules	Effect/Expression
PF-PC	post-synaptic LTD	mGluR1 AMPA receptors Ca2+↑↑ cPLA2α CB1R NMDA receptors	Reduced post-synaptic expression of AMPARs leading to reduced excitability probability
PF-PC	post-synaptic LTP	Ca2+↑ cPLA2α CB1R NO NSF	Increased post-synaptic expression of AMPARs leading to increased excitability probability
PF-PC	pre-synaptic LTD	CB1R NMDA receptors NO synthase	*Unknown*
PF-PC	pre-synaptic LTP	adenylyl cyclase I PKA	Decreased rate of synaptic failures
CF-PC	post-synaptic LTD	mGluR1 Ca2+↑↑ PKC	Reduced post-synaptic expression of AMPARs leading to reduced excitability probability, and consequently reduced probability of induction of postsynaptic PF-LTD

pathological, they can unravel the finely tuned cerebellar neuronal machinery quite quickly. The parallel fibre-Purkinje cell (PF-PC) synapse can express LTD pre-[38,41,42] and postsynaptically[11,35,39,43–45], as well as LTP pre-[13,35,46–50] and postsynaptically[51–56]. CF-PC synapses can express LTD postsynaptically[11,35,57]. These processes are reviewed in detail below. An overview can be found in Table 2.

Postsynaptic parallel fibre long-term depression

Postsynaptic parallel fibre LTD (PF-LTD) has long been described as a mechanism for cerebellar motor learning[38–40], and is dependent upon the simultaneous activation of climbing and parallel fibres[35,39,43,44]. At the PF-PC synapse, glutamate acts upon mGluR1 and AMPA-Rs, increasing intracellular calcium levels. Combined with the complex spike generated by climbing fibre generation, the intracellular calcium levels in the PC are pushed to the high threshold needed for PF-LTD induction[35,56]. Subsequently, PKCγ and αCaMKII are activated[35,58–61], as well as several other signaling cascades, including $cPLA_2$α/COX2, cannabinoid receptor 1 (CB1R), *N*-methyl-D-aspartate receptors (NMDA-Rs), and nitric oxide (NO)[35,62–67]. These signaling cascades ultimately lead to a reduction in the number of functional AMPA-Rs at the postsynaptic membrane of the PF-PC

synapse[35,68,69], thus making excitation of the postsynaptic membrane less likely during activation of the parallel fibre. However, a recent publication proposed that PF-LTD is facilitated by AMPA-Rs[70], suggesting that the expression of functional AMPA-Rs at the post-synaptic membrane is not completely lost during PF-LTD. Furthermore, the degree of activation of mGluR1 is important during the critical stages of postsynaptic LTD induction, as increased activation of mGluR1 has been shown to facilitate this process at both excitatory inputs of the PC[71,72].

Postsynaptic parallel fibre long-term potentiation

Postsynaptic parallel fibre LTP (PF-LTP) can reverse its counterpart, postsynaptic PF-LTD, as it has been shown that PF-LTP causes extinction of learned associations in trained animals[53,73,74]. Postsynaptic PF-LTP requires a lower calcium threshold than postsynaptic PF-LTD[56], and is induced via low frequency stimulation of parallel fibres[51–54]. Low frequency parallel fibre stimulation subsequently leads to activation of $cPLA_2\alpha$, resulting in liberation of arachidonic acid and the production of 2-arachidonolylglycerol, which binds presynaptic type 1 cannabinoid receptors (CB1Rs) in a retrograde manner[51]. Activation of the CB1R then triggers NO activation, leading to low levels of NO anterogradely crossing the synapse[51,54,55]. At the postsynaptic membrane, it promotes nitrosylation of *N*-ethyl-maleimide-sensitive factor (NSF)[38,75], which in turn binds the AMPA-R subunit GluR2 and mediates insertion of AMPA-Rs into the membrane[38,76,77]. Postsynaptic PF-LTP is thus expressed as an increase in functional AMPA-Rs at the postsynaptic membrane, and is clearly the cellular inverse of postsynaptic PF-LTD. Interestingly, postsynaptic PF-LTP and PF-LTD share a number of critical molecules, including calcium, $cPLA_2\alpha$, CB1R and NO. A notable divergence is glutamate binding to mGluR1 and NMDA-Rs, which PF-LTD is dependent upon, but PF-LTP is not[71,78–81]. However, in a SCA5 mouse model expressing mutant βIII-spectrin, mGluR1 shows altered localization, and mGluR1-mediated postsynaptic PF-LTP is deficient[82]. Furthermore, $cPLA_2\alpha$ and CB1R may play dual roles in synaptic plasticity[51,62,63,83–86], as does calcium –and therefore climbing fibre activity– which is considered to be a deciding factor in the induction of LTP or LTD[38,56].

Presynaptic parallel fibre long-term depression

The presynaptic form of PF-LTD has been less well studied. It involves endocannabinoid signaling and presynaptic CB1R activation, is dependent upon NMDA-R activation, and upregulated by NO synthase, while a consensus has not yet been reached on the involvement of mGluR1[38,41,42]. It is also currently unclear which physiological conditions require this type of synaptic plasticity[38].

Presynaptic parallel fibre long-term potentiation

Presynaptic PF-LTP is evoked by 4-8Hz parallel fibre stimulation, and depends on the activation of calcium/calmodulin-sensitive adenylyl cyclase I and the subsequent activation of cAMP-dependent kinase protein A (PKA)[35,46–50]. It is associated with a decrease in the rate of synaptic failures and the extent of paired-pulse facilitation[35].

Postsynaptic climbing fibre long-term depression

Climbing fibres can express postsynaptic LTD, and similarly to postsynaptic PF-LTD, CF-LTD requires post-synaptic Ca^{2+} influx and activation of mGluR1 and PKC[11,87,88], with both expressed as a reduced number of AMPA-Rs at the postsynaptic membrane[87]. The consequences of CF-LTD are a reduction in the slow component of the complex spike[11,57], a reduction in the complex spike afterhyperpolarization[89], and decreased complex spike-evoked dendritic Ca^{2+} transients[57], all of which reduce the probability of subsequent induction of parallel fibre plasticity requiring a high calcium threshold[57,88]. In addition, CF-LTD is not associated with changes in the synaptic glutamate transient[87]. The observed decrease in complex spike-evoked Ca^{2+} transients[57] has a significant effect on the probability for PF-LTD induction[56], could have a neuroprotective function[56] and/or could provide a critical component of cerebellar learning[88].

The climbing fibre as control switch

The PF-PC synapse is clearly a very plastic synapse. However, neither the parallel fibre nor the PC have much control over this plasticity. The regulation of these processes seems to be mostly outsourced to the climbing fibre, which acts as a control switch. Climbing fibre activation evokes complex spikes and, consequently, large dendritic calcium transients, triggering postsynaptic PF-LTD induction, whereas the absence of complex spikes during climbing fibre inactivity leads to the induction of postsynaptic PF-LTP[56]. Changes in climbing fibres can therefore lead to altered synaptic plasticity and cause an ataxic phenotype, which will be discussed in the section on "*Climbing fibre deficits and glutamate dysregulation in mouse models of spinocerebellar ataxia*". Furthermore, to create a sort of "safety lock" to prevent simultaneous induction of pre- and postsynaptic plasticity, presynaptic PF-LTP is also under the influence of climbing fibre activity[86]. Climbing fibre activity can trigger endocannabinoid release at the PF-PC synapse[90–92] and in a retrograde manner bind and activate CB1R, which then blocks adenylyl cyclase I to suppress presynaptic PF-LTP while PF-LTD is expressed postsynaptically[86]. Without this "safety lock", the decrease in response elements during postsynaptic PF-LTD could be accompanied by an increase in neurotransmitter release, nullifying the effect of reducing postsynaptic AMPA-Rs.

The importance of glutamate and calcium in synaptic plasticity

Climbing fibres exert a lot of control over the plasticity of the excitatory synapses onto the PC and, because climbing fibres are themselves excitatory inputs, glutamate plays a major role in controlling plasticity. During climbing fibre activation, multiple glutamate-containing vesicles are released into the synaptic cleft[93], where there are at least three types of glutamate receptors present: AMPA-Rs, NMDA-Rs and mGluRs. The AMPA-Rs located at the CF-PC synapse largely mediate EPSCs[12,94,95], and

do not contribute to the PC calcium surge because they contain the GluD2 subunit, which makes them impermeable to Ca^{2+}[96]. NMDA-Rs are expressed in mature PCs and, next to the NR1 subunit, mostly contain subunits NR2A and NR2B[97,98]. NMDA-Rs are activated by climbing fibre stimulation, and influence the number and timing of spikelets, thereby contributing to the complex spike waveform[97]. NMDA-Rs are permeable to Ca^{2+}[99], and therefore likely contribute to the postsynaptic Ca^{2+} surge associated with climbing fibre activation and postsynaptic LTD induction. As discussed earlier (in the sections on "*Postsynaptic parallel fibre long-term depression*" and "*Postsynaptic climbing fibre long-term depression*"), mGluR1 is critical for induction of postsynaptic LTD at both PF- and CF-PC synapses, as mGluR1 potentials significantly enhance complex spike-associated Ca^{2+} transients throughout the PC dendrite[100]. mGluR1 signaling has two paths: IP3-mediated Ca^{2+} release from internal stores and activation of transient receptor potential canonical (TRPC) channels. Because the IP3 receptor (mutated in SCA15 and SCA16[101,102]) expressed in PCs has a remarkably low sensitivity to IP3[103], it is much more likely that mGluR1 synaptic transmission is mediated by TRPC3[104,105], a non-selective cation channel with a high permeability for calcium[106] and high expression in the cerebellum[107]. Interestingly, Moonwalker mice (mice with a mutation in the SCA41 gene TRPC3[108]) and *Trpc3* knockout mice demonstrate ataxic phenotypes, with the Moonwalker having a more severe phenotype[109,110]. Additionally, glutamate transporters play an important role in synaptic plasticity by controlling the amount of glutamate available in the synaptic cleft and, indirectly, the degree of activation of mGluR1[71,72]. Thus, a major consequence of glutamate-mediated transmission is clearly an increase in intracellular calcium. To reduce intracellular Ca^{2+} and prevent excitotoxicity, PCs express an abundance of Ca^{2+}-binding EF-hand protein buffers, Ca^{2+} pumps and exchangers (for review see Arundine and Tymiansky[111] and Wojda *et al.*[112]). However, in such a highly regulated environment, the slightest change in Ca^{2+} buffering capacity due to extra- or intracellular changes may cause or further PC dysfunction[113]. Furthermore, it has been proposed that changes in cellular functions directly or indirectly leading to Ca^{2+} dysregulation are eventually responsible for dark cell degeneration[114], a type of cell death observed in mouse models of SCA1, SCA2, SCA3, SCA5, SCA7, SCA28, and AMPA-induced delayed excitotoxicity and of hypoxia[6,113], to which PCs seem to be particularly sensitive.

Climbing fibre deficits and glutamate dysregulation in mouse models of spinocerebellar ataxia

In mouse models of SCA1, SCA7, SCA14, and SCA23 climbing fibre deficits have been found. These deficits range from developmental changes to retraction of climbing fibres in adult mice. In addition, mouse models for SCA5 and SCA28 display alterations in glutamate signaling, a finding which is in line with climbing fibre deficits, as both result in PC dysfunction.

Mouse models for SCA1 expressing expanded *ATXN1[82Q]* exhibit abnormal motor behaviour

by 6 weeks of age, including slightly reduced cage activity, gentle swaying of the head while walking, and early signs of general incoordination, accompanied with mild PC dysfunction[115], and PC loss around 24 weeks of age[116]. In addition to PC dysfunction, climbing fibre deficits are a critical component of the SCA1 pathology, and can be observed around 6 weeks of age, long before PC loss begins[3,4,116]. These transgenic mice demonstrate diminished arborization of climbing fibres along PC dendrites[3], a reduction in PC responsiveness to climbing fibre activation[4,116] and, perhaps most importantly, compromised development of climbing fibres as indicated by reduced ascension and disrupted pruning of climbing fibre termini on PC somata and apical dendrites[4]. CF-PC synaptic transmission deficits required *ATXN1[82Q]* to be located in the nucleus, suggesting *ATXN1[82Q]* alters the expression of one or more genes in the PC that are crucial for its innervation by climbing fibres[4]. The simultaneous appearance of abnormal phenotypical behaviour and climbing fibre deficits suggests that these deficits are a likely cause of the ataxic phenotype of SCA1 transgenic mice.

Conditional transgenic mice expressing mutant *ATXN7[92Q]* are a model for SCA7, and demonstrate progressive ataxia and impaired motor function starting at 20 weeks of age, and loss of Calbindin – a PC marker – at 40 weeks of age[117]. Examination of the CF-PC synapses showed proximal aggregation of climbing fibre synapses at 40 weeks of age in transgenic mice, while 20-week-old transgenic mice display normal climbing fibre morphology[5], suggesting that expression of mutant ataxin-7 causes redistribution of climbing fibre termini between 20 and 40 weeks of age in mice. Interestingly, SCA7 mutant mice display an ataxic phenotype before changes in climbing fibre distribution can be observed. It is possible that in SCA7, pathology is caused by dysfunction of PCs mediated by dysfunction of Bergmann glia, which clear excess glutamate from the CF-PC synaptic cleft. If excess glutamate is not effectively cleared, this can affect Ca^{2+} signaling in the PC and lead to PC dysfunction.

A mouse model for SCA14 has been generated by injection of a lentiviral vector expressing mutant PKCγ into the cerebellar cortex[118]. One-week-old mice lentivirally treated with mutant PKCγ spent less time on an accelerating rotarod than age-matched non-treated mice, whereas mice treated at P21-P25 did not display an ataxic phenotype[118]. Injection of lentivirus during the development of climbing fibres (P6-7) caused PCs expressing mutant PKCγ to be innervated by multiple climbing fibres, while injection after maturation of climbing fibres (P21-25) did not[118], indicating early expression of mutant PKCγ impairs determination of a "winner" climbing fibre. Postsynaptic PF-LTD could not be induced in either model, while presynaptic PF-LTD was not disrupted[118], which could be expected, as PKCγ is involved in postsynaptic, but not presynaptic, PF-LTD. These models suggest that even when climbing fibres develop normally, the CF-PC synapse cannot function properly when mutant PKCγ is expressed. Therefore, in SCA14, as in SCA1, SCA7, and SCA23, one change in the highly regulated process of synaptic plasticity is the likely cause of the typical ataxic phenotype.

The transgenic mice modelling SCA23 express mutant Prodynorphin, and display a slowly

progressive spinocerebellar ataxia starting at 3 months of age, with mild PC loss at 12 months of age and progressive retraction of climbing fibres starting at 3 months of age[7]. In these mice, climbing fibres retract from the PC dendrite due to expression of mutant Prodynorphin and, consequently, secretion of mutant peptide Dynorphin A, leading to deficits in synaptic transmission. Interestingly, climbing fibre pathology is mainly restricted to the anterior vermis of the SCA23 cerebellum, and coincides with the onset of the ataxic phenotype[7] suggesting that, as in SCA1, it is not PC loss but climbing fibre deficits that are a likely cause of the pathological phenotype.

Spnb3$^{-/-}$ mice expressing low levels of truncated βIII-spectrin are a model for SCA5, and are prone to a mild, non-progressive ataxia and stimulus-induced seizures starting between 6 and 8 months of age[119], while mice completely lacking βIII-spectrin model SCA5 by displaying gait abnormalities, tremor, deteriorating motor coordination, Purkinje cell loss and cerebellar atrophy[6]. Spectrins are important structural proteins of the plasma membrane skeleton, and control the disposition of selected membrane channels, receptors, and transporters. βIII spectrin is found on PC somata and dendrites, and it directly binds to glutamate transporter EAAT4, GluRδ2, and other proteins[6,119]. Spnb3$^{-/-}$ mice do not show specific climbing fibre deficits up to one year of age, however, their βIII spectrin deficiency diminished, among other factors, the EAAT4 and GluRδ2 expression at the postsynaptic membrane[119]. Interestingly, in mice lacking βIII-spectrin, PF-LTD has been shown not to be impaired (Gao et al., 2011). Nonetheless, this is another mutation causing spinocerebellar ataxia that leads to changes in glutamate (and therefore calcium) signaling disrupting the delicate balance of synaptic plasticity in the cerebellum.

A mouse model for SCA28, haploinsufficient for mitochondrial protease *Afg3l2*, also demonstrated problems with glutamate homeostasis. These mice exhibit a progressive decline in motor function and dark cell degeneration of mitochondrial origin from 8 months of age onward[120]. AFG3L2 is part of a quality control protein complex located on the inner membrane of the mitochondrium, which selectively degrades damaged proteins, exerts a chaperone-like activity on respiratory chain complexes, and is essential for axonal development[121–124]. In cultured *Afg3l2*-deficient PCs, mitochondria ineffectively buffer Ca^{2+} peaks, resulting in increased intracellular Ca^{2+} levels, triggering PC dark cell degeneration. Partial genetic silencing of mGluR1 or treatment with ceftriaxone, an antibiotic that promotes synaptic clearance of glutamate, reduced Ca^{2+} influx into PCs, and improved ataxic phenotypes in SCA28 mice[120]. This indicates that glutamate dysregulation is a hallmark of SCA28 as well.

In addition, recently, three new mutations in *GRID2* encoding GluRδ2 were identified in a large Algerian family with adult-onset slowly progressive ataxia in seven adults and congenital ataxia in one child and in a large cohort of congenital ataxia patients[125]. In the Algerian family, the Leu656Val mutation was identified, and found to be heterozygous in the adult patients but homozygous in the child with congenital ataxia. This mutation is located in the third transmembrane domain of GluRδ2, which is involved in transmission of information between the ligand-binding domain and the pore, and AMPA-R trafficking. In the congenital ataxia cohort, two missense de novo mutations

were identified: Ala654Thr and Ala654Asp[125]. Interestingly, these mutations affect the same amino acid as the well-known Lurcher mutation, and Ala654Thr is actually the same mutation[126]. The affected amino acid is located in the highly conserved SYTANLAAF motif, crucial for gating of the channel, and mutations in this motif in other ionotropic glutamate receptor subunits are known to change channel function significantly[127–129]. Lurcher mouse PCs have been shown to have increased conductance, giving rise to a constitutively active inward current, which is not affected by the presence of glutamate, but reduced when extracellular Na^+ is replaced with *N*-methyl-D-glucamine, a relatively large organic cation[126], indicating the channel is constitutively open. These changes in PC conductivity suggest changes in membrane depolarization and Ca^{2+} transients, and therefore changes in PC functioning.

Conclusion

The cerebellar circuitry is a finely tuned neuronal machine, crucial for motor learning and functioning. We discussed how climbing fibres are an important part of the machinery and any changes in their development or physiology can be catastrophic for cerebellar functioning. Not only are the climbing fibres a major excitatory input for the PC, they also exert an enormous amount of control over the other excitatory PC input, the parallel fibres and their synaptic plasticity. This synaptic plasticity is another crucial factor for normal cerebellar function and motor behaviour. When any component of the highly regulated processes in the cerebellum is altered the consequences for neural functioning are catastrophic.

The disorders discussed here are all caused by mutations in different genes (Table 1), but they all lead to a disruption of the same cerebellar synapse. This is a very interesting phenomenon, and suggests this may be a common pathology among the SCAs. As has been demonstrated, it is PC dysfunction rather than PC loss that gives rise to the symptoms of ataxia, and climbing fibres play a major role in the proper functioning of PCs. Having much of the control over induction of plasticity in both parallel fibres and climbing fibres, the climbing fibres have control over the excitability of the PC and, consequently, over cerebellar output and motor control. Of course, improper execution of motor function is a central symptom in ataxia, which could be explained in part by dysfunctional climbing fibres. However, further research is needed to determine whether climbing fibres and glutamate signaling really form some common ground underlying the spinocerebellar ataxias, and whether this phenomenon could be useful as a therapeutic target. Additionally, genes with functions in the CF-PC synapse and glutamate signaling may be candidate disease genes for ataxia cases without a genetic diagnosis.

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