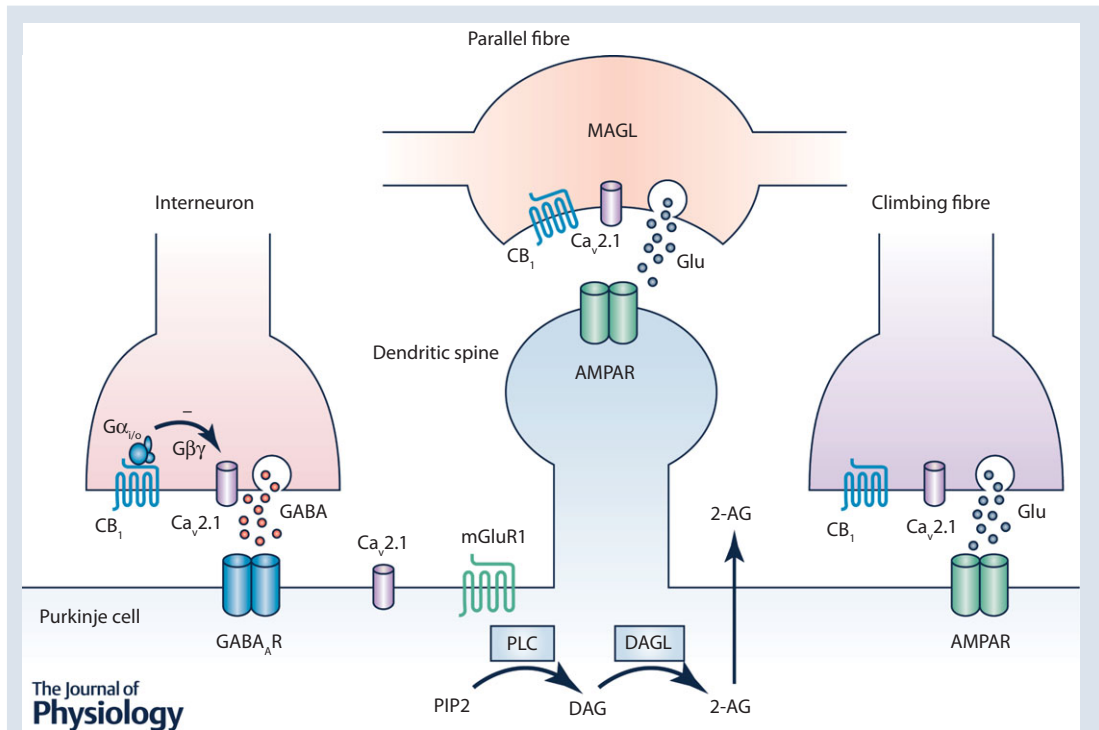


# Does modulation of the endocannabinoid system have potential therapeutic utility in cerebellar ataxia?

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**Abstract** Cerebellar ataxias represent a spectrum of disorders which are, however, linked by common symptoms of motor incoordination and typically associated with deficiency in Purkinje cell firing activity and, often, degeneration. Cerebellar ataxias currently lack a curative agent. The endocannabinoid (eCB) system includes eCB compounds and their associated metabolic enzymes, together with cannabinoid receptors, predominantly the cannabinoid CB<sub>1</sub> receptor (CB<sub>1</sub>R) in the cerebellum; activation of this system in the cerebellar cortex is associated with deficits in motor coordination characteristic of ataxia, effects which can be prevented by CB<sub>1</sub>R antagonists.

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Of further interest are various findings that CB<sub>1</sub>R deficits may also induce a progressive ataxic phenotype. Together these studies suggest that motor coordination is reliant on maintaining the correct balance in eCB system signalling. Recent work also demonstrates deficient cannabinoid signalling in the mouse 'ducky<sup>21</sup>' model of ataxia. In light of these points, the potential mechanisms whereby cannabinoids may modulate the eCB system to ameliorate dysfunction associated with cerebellar ataxias are considered.

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**Abstract figure legend** Presynaptic CB<sub>1</sub>Rs modulate Purkinje cell output in the cerebellar cortex. Interneurons (INs) make inhibitory contacts predominantly at the so-called pinceau region surrounding the Purkinje cell (PC) soma and also potentially at dendritic shafts. Parallel fibres (PFs) arise from granule cells, which in turn receive input from mossy fibres, and make weak but numerous excitatory contacts predominantly with postsynaptic dendritic spines. Climbing fibres (CFs) arise from the inferior olive and make strong monosynaptic contacts with individual PCs within the dendritic tree, predominantly at dendritic shafts. 2-Arachidonylglycerol (2-AG), the predominant endocannabinoid (eCB) in the cerebellar cortex, is synthesized from diacylglycerol (DAG) by diacylglycerol lipase  $\alpha$  (DAGL $\alpha$ ); this process is driven by activation of metabotropic receptors including mGluR1 and may also involve Ca<sup>+</sup>-dependent processes including voltage-dependent Ca<sup>2+</sup> channels such as Ca<sub>v</sub>2.1 and also Ca<sup>2+</sup> permeable ionotropic receptors (see Kano *et al.* 2009). 2-AG is released retrogradely 'on-demand' to active presynaptic CB<sub>1</sub>Rs. 2-AG action is terminated by monoacylglycerol lipase (MAGL) localized predominantly to PF terminals. CB<sub>1</sub>Rs are highly expression at IN inputs into Purkinje cells at the pinceau. CB<sub>1</sub>Rs are highly expressed at perisynaptic regions and also at extrasynaptic and synaptic PF sites, but at lower density on CF terminals. At all presynaptic terminals, CB<sub>1</sub>Rs predominantly couple to G $\alpha_{i/o}$  to inhibit Ca<sub>v</sub>2.1 Ca<sup>2+</sup> channels via release of G $\beta\gamma$  subunits (shown only at IN–PC synapses for clarity). Activation of presynaptic CB<sub>1</sub>Rs inhibits GABA release onto postsynaptic GABA<sub>A</sub> receptors and glutamate release predominantly onto postsynaptic AMPA receptors, respectively. As PCs represent the sole output of the cerebellar cortex, CB<sub>1</sub>Rs are ideally localized to control cerebellar function.

**Abbreviations** 2-AG, 2-arachidonylglycerol; CBD, cannabidiol; CBDV, cannabidivarin; CF, climbing fibre; eCB, endocannabinoid; GPCR, G protein-coupled receptor; IN, interneuron; LTD, long term depression; MAGL, monoacylglycerol lipase; PC, Purkinje cell; pCB, phytocannabinoid; PF, parallel fibre; SCA, spinocerebellar ataxia;  $\Delta^9$ -THC, tetrahydrocannabinol; VDCC, voltage-dependent Ca<sup>2+</sup> channel.

### Cerebellar ataxias are a diverse group of disorders lacking a therapeutic agent

Cerebellar ataxias comprise a group of progressive neurological diseases associated with deficits in motor coordination and are typically associated with dysfunction and/or degeneration of Purkinje cells (PCs), the sole efferent output of the cerebellar cortex. There are a range of acquired ataxias and different hereditary forms of the disease (Klockgether, 2011). Thus, ataxia can be acquired from, amongst others, traumatic head injury, bacterial infection (meningitis or encephalitis), viral infection (chickenpox or measles), disruption of blood flow (stroke or transient ischaemic attack, haemorrhage), CNS disease (cerebral palsy or multiple sclerosis), sustained long-term alcohol misuse, under-active thyroid gland and cancer autoimmune conditions (lupus), and can also be iatrogenic. Hereditary ataxias may be autosomal-dominant diseases, including forms of spinocerebellar ataxia (SCA), several of which are associated with polyglutamine repeats in the dysfunctional protein; for example: ataxin 1 in SCA1; ataxin 2 in SCA2;

Cacna1a encoding the voltage-dependent Ca<sup>2+</sup> channel (VDCC) Ca<sub>v</sub>2.1 subunit in SCA6 (also in episodic ataxia 2). There are also autosomal-recessive diseases such as Friedreich's ataxia and ataxia telangiectasia associated with deficits in, respectively, the mitochondrial protein frataxin and a serine/threonine protein kinase termed ataxia telangiectasia mutated protein (Klockgether, 2011). Despite this range of causes and implicated proteins, deleterious effects are largely limited to the cerebellar cortex and are typically associated with cerebellar dysfunction and/or degeneration and are manifest as motor incoordination. This commonality of symptoms offers hope for providing treatment options; however, at present there is no known cure for cerebellar ataxia. There are treatments to ameliorate associated symptoms. For example, vitamin E and anti-oxidants, such as co-enzyme Q10 and its synthetic analogue idebenone, have been suggested to have some benefit, largely in Friedreich ataxia. However, as yet, such agents lack proven efficacy in controlled clinical trials (Cooper *et al.* 2008; Lynch *et al.* 2010), although some improvement in comparison to controls was seen in cross-over trials,

suggesting that patients with vitamin E-deficient and co-enzyme Q10-deficient ataxia may receive some benefit (Cooper *et al.* 2008). In addition, administration of thyrotropin-releasing hormone (TRH) was reported to ameliorate cerebellar ataxia in rolling Nagoya mice (Shibusawa *et al.* 2008) and the TRH analogue taltirelin is approved to improve motor performance in ataxic patients in Japan.

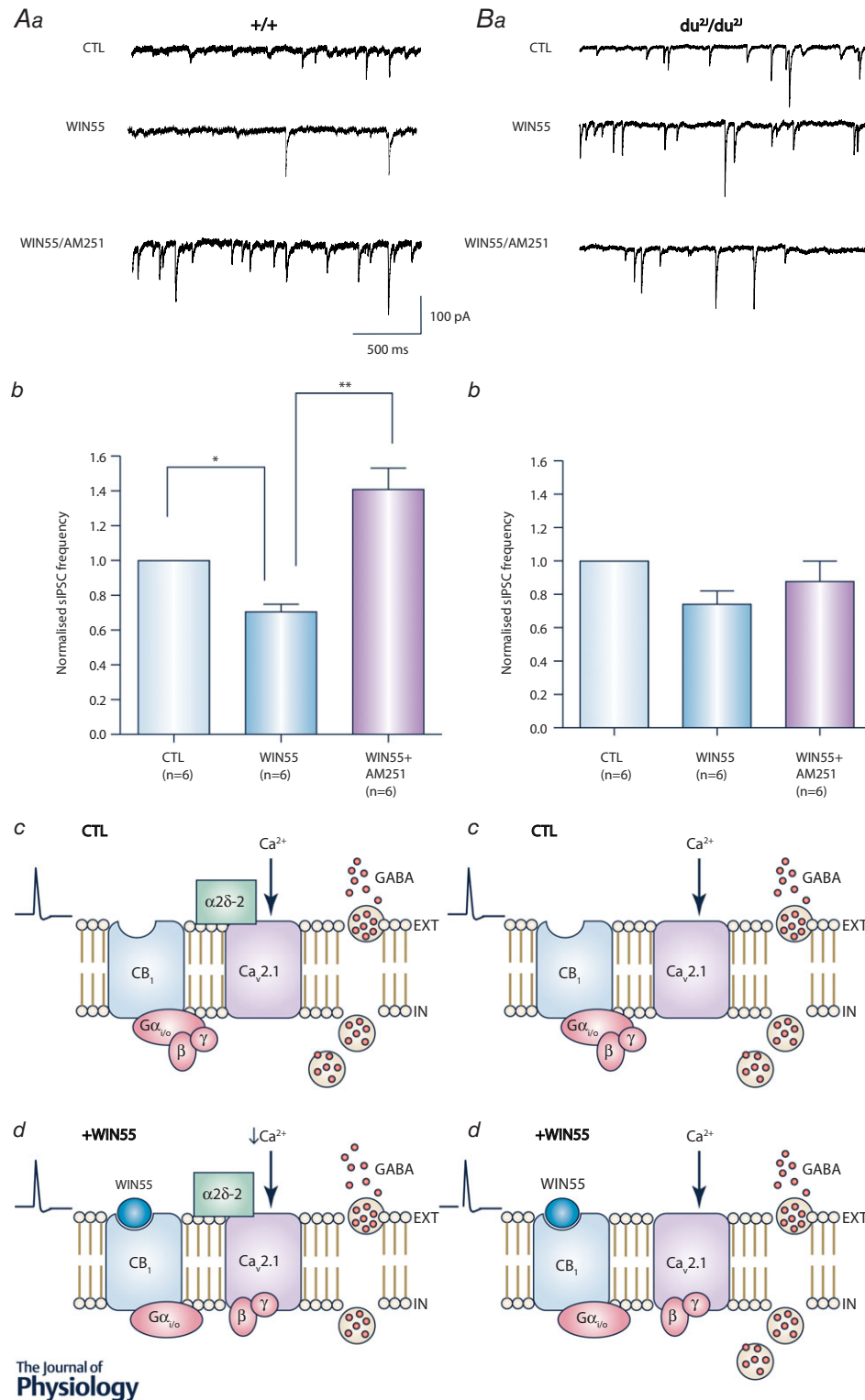
The elucidation of function of proteins associated with inherited ataxias within the cerebellar cortex may also lead to future therapeutic advances relevant across different forms of ataxia. Amongst target proteins, the  $\text{Ca}_V2.1$  ( $\alpha 1A$ ) VDCC represents a widely studied protein.  $\text{Ca}_V2.1$  subunits are highly expressed in the cerebellum (Westenbroek *et al.* 1995; Kulik *et al.* 2004). In particular,  $\text{Ca}_V2.1$  is expressed postsynaptically in PCs (which led to the designation of these subunits as carriers of P-type  $\text{Ca}^{2+}$  current (Mintz *et al.* 1992)), at presynaptic terminals of inhibitory interneurons (INs) arising from basket and stellate cells, and of excitatory, parallel fibres (PFs) and climbing fibres (CFs); such inputs regulate PC, and thus cerebellar cortex, output activity (Regehr & Mintz, 1994; Mintz *et al.* 1995; Stephens *et al.* 2001; Lonchamp *et al.* 2009). Several mouse  $\text{Ca}_V2.1$  mutants display ataxia (Pietrobon, 2010; Rajakulendran *et al.* 2012). Correspondingly, genetic deletion of  $\text{Ca}_V2.1$  is associated with a clear ataxic behavioural phenotype (Jun *et al.* 1999). Moreover, conditional PC-specific  $\text{Ca}_V2.1$  knock-down was shown to be sufficient to induce impaired synaptic transmission and ataxia (Mark *et al.* 2011; Todorov *et al.* 2012), the former study termed their mice 'purky'. Cell-specific work was extended to excitatory inputs into PCs, where it was shown that selective  $\text{Ca}_V2.1$  knockdown in PFs (arising from mossy fibre inputs) in so-called 'quirky' mice, also gave rise to an ataxic phenotype (Maejima *et al.* 2013). Of further interest here, is that mutations which increase  $\text{Ca}_V2.1$  current also give impaired synaptic transmission and irregular PC firing (a cerebellar epitome predicted to lead to motor incoordination) (Gao *et al.* 2012); these reports suggest that correct VDCC activity must be maintained for PC firing fidelity. To add to purky and quirky, we also have 'ducky' mice (Barclay *et al.* 2001). Ducky, and the related ducky<sup>2J</sup> (*dir<sup>2J</sup>*) strain, have mutations predicted to lead to deficits in  $\alpha 2\delta$ -2 auxiliary VDCC subunit protein, which is expressed at high levels in normal cerebella (Cole *et al.* 2005) and is associated predominantly with  $\text{Ca}_V2.1$  in the cerebellum (Barclay *et al.* 2001). In different ducky strains, the ataxic phenotype is associated with a reduction in postsynaptic PC whole-cell  $\text{Ca}^{2+}$  current (Brodbeck *et al.* 2002; Donato *et al.* 2006), together with irregular PC firing (Donato *et al.* 2006; Walter *et al.* 2006; Wang *et al.* 2013). Thus, several potential therapeutic targets have been suggested, largely confined to protein associated with inherited ataxias; however, as

discussed above, as yet we have no cure for ataxia. The remainder of this review will focus on the potential to target the endocannabinoid (eCB) system to ameliorate cerebellar ataxia and, in particular, eCB compounds and their associated metabolic enzymes and G protein-coupled  $\text{CB}_1\text{R}$ , one of the most ubiquitously expressed proteins in the mammalian cerebellum, and a protein which also modulates  $\text{Ca}_V2.1$  activity at the presynapse.

### Cannabinoid signalling and its potential links to cerebellar ataxia

Cannabinoids represent a diverse number of compounds, including (i) eCBs, for example, the lipid mediator 2-arachidonylglycerol (2-AG), which is the major eCB in the cerebellum (Szabo *et al.* 2006); (ii) plant-derived phytocannabinoids (pCBs), for example, the major herbal *Cannabis* constituents tetrahydrocannabinol ( $\Delta^9$ -THC) and cannabidiol (CBD) (Hill *et al.* 2012a); and (iii) exogenous synthetic agents, namely  $\text{CB}_1\text{R}$  agonists, for example, WIN 55,212-2, an aminoalkylindole derivative, and CP 55940, which is structurally related to tetrahydrocannabinol, and  $\text{CB}_1\text{R}$  antagonists/inverse agonists, for example, rimonabant (Pertwee *et al.* 2010), and newer allosteric modulators, for example, Org27569 (Price *et al.* 2005) and PSNCBAM-1 (Horswill *et al.* 2007).

Within the CNS, cannabinoids predominantly activate  $\text{CB}_1\text{Rs}$ , which represent the most widespread G protein-coupled receptor (GPCR) in the mammalian cerebellum (Herkenham *et al.* 1991; Tsou *et al.* 1997).  $\text{CB}_1\text{R}$  expression is reported to be very low at PC cell bodies; rather, expression is high at excitatory PF inputs into PCs, reportedly with a perisynaptic over extrasynaptic and synaptic localization, with lower  $\text{CB}_1\text{R}$  expression at CF inputs onto PC dendritic shafts (Kawamura *et al.* 2006; see Abstract Figure).  $\text{CB}_1\text{Rs}$  are expressed at higher levels on presynaptic terminals of inhibitory INs, predominantly basket cells, but also stellate cells, which form a specialized region surrounding the PC axon initial segment known as the pinceau (the French word for paintbrush) (Tsou *et al.* 1997; Kawamura *et al.* 2006; Rodríguez-Cueto *et al.* 2014). Presynaptic  $\text{CB}_1\text{Rs}$  are activated by retrograde 'on demand' release of 2-AG from postsynaptic PCs. The major effect of presynaptic  $\text{CB}_1\text{R}$  activation is a suppression of neurotransmitter release, whereby activation of presynaptic  $\text{CB}_1\text{Rs}$  inhibits action potential-evoked and spontaneous inhibitory postsynaptic currents (IPSCs) at IN-PC synapses (see Fig. 1) or excitatory postsynaptic currents (EPSCs) at PF-PC and CF-PC synapses (Takahashi & Linden, 2000; Szabo *et al.* 2004; Kano *et al.* 2009). We have also used multi-electrode array recording to demonstrate that  $\text{CB}_1\text{R}$  ligand-induced changes to cerebellar cortex network activity are mediated, at least in part, via effects on inhibitory synaptic transmission (Ma *et al.* 2008).  $\text{CB}_1\text{R}$  activation has been



**Figure 1. Presynaptic CB<sub>1</sub>R modulation of inhibitory transmission at IN–PC synapses is deficient in ataxic ducky<sup>2J</sup> mice**

Aa and Ba, representative spontaneous inhibitory postsynaptic current (sIPSC) traces from +/+ (Aa) and du<sup>2J</sup>/du<sup>2J</sup> (Ba) PCs showing effect of WIN55 (5 μM), and also subsequent application of AM251 (2 μM). Ab and Bb, summary bar graphs showing that WIN55 significantly reduced, and AM251 significantly increased, normalized sIPSC frequency in +/+ (Ab), but was without effect in du<sup>2J</sup>/du<sup>2J</sup> (Bb), conditions. \**P* < 0.05; \*\**P* < 0.01;

widely associated with a number of different forms of short- and long-term synaptic plasticities which modulate cerebellar learning (Kano *et al.* 2009; Ohno-Shosaku & Kano, 2014). Thus, 2-AG release mediates the short-term suppression of inhibitory GABA release from IN terminals (depolarization-induced suppression of inhibition) or excitatory glutamate release (depolarization-induced suppression of excitation) (Szabo *et al.* 2006; Tanimura *et al.* 2009). Seminal work by Ito (1989) linked long term depression (LTD) by associative stimulation of PF and CF inputs to PCs, to motor learning in the cerebellum. It is also known that the metabotropic glutamate receptor 1 (mGluR1) pathway is critically involved in cerebellar development and LTD (Aiba *et al.* 1994), and it was further proposed that CF inputs into individual PCs were required for normal motor coordination (Chen *et al.* 1995). More recent work has established that cerebellar LTD is a post-synaptic phenomenon requiring 2-AG release from PCs and activation of presynaptic CB<sub>1</sub>R at PF–PC synapses (Safo & Regehr, 2005). Here, mGluR1 activation drives 2-AG release (Kano *et al.* 2009; see Abstract Figure). Thus, CB<sub>1</sub>Rs have a privileged position in the function and control of overall output of the cerebellar cortex and, as such, represent good potential targets to modulate dysfunctional signalling associated with cerebellar ataxias.

In cell lines and native neurons, CB<sub>1</sub>R activation causes pertussis toxin-sensitive inhibition of Ca<sub>v</sub>2 family VDCCs, and can also activate inwardly rectifier K<sup>+</sup> channels (Mackie & Hille, 1992; Twitchell *et al.* 1997; Guo & Ikeda, 2004). It is likely that CB<sub>1</sub>Rs couple to presynaptic Ca<sub>v</sub>2.1 (P/Q-type) VDCCs at IN–PC synapses to reduce action potential evoked GABA release (Forti *et al.* 2000; Stephens *et al.* 2001; Lonchamp *et al.* 2009) and to Ca<sub>v</sub>2.1 (and to a lesser extent Ca<sub>v</sub>2.2 and Ca<sub>v</sub>2.3) at PF–PC synapses to reduce action potential-evoked glutamate release (Brown *et al.* 2004); these effects are most likely mediated by direct binding of G protein Gβγ subunits to VDCCs (Abstract Figure and Fig. 1).

CB<sub>1</sub>R agonists also cause clear reductions in frequency of ‘miniature’ IPSCs at IN–PC synapses (Takahashi & Linden, 2000; Yamasaki *et al.* 2006; Ma *et al.* 2008), consistent with an inhibition of action potential-independent GABA release. These effects are proposed to occur downstream of actions on

voltage-dependent ion channels and are also consistent with direct effects on the synaptic release machinery, and also may be mediated by Gβγ subunits (Stephens, 2009). By contrast, CB<sub>1</sub>R agonist effects on miniature EPSCs at PF–PC synapses were only apparent when extracellular Ca<sup>2+</sup> levels were increased (Yamasaki *et al.* 2006). Moreover, CB<sub>1</sub>R antagonists/inverse agonists such as AM251, rimonabant and the pCB Δ<sup>9</sup>-tetrahydrocannabinol all increase inhibitory GABA release at IN–PC synapses (Ma *et al.* 2008). Such effects are consistent with the presence of a strong, modulatable eCB tone in the cerebellum (Kreitzer *et al.* 2002; Galante & Diana, 2004), which provides further opportunity for therapeutic intervention in cerebellar ataxia.

Importantly, activation of presynaptic CB<sub>1</sub>Rs by synthetic cannabinoids and eCBs has been shown to promote cerebellar dysfunction, causing severe motor incoordination and modelling cerebellar ataxia (Lichtman *et al.* 1998; DeSanty & Dar, 2001; Patel & Hillard, 2001); in these studies pre-treatment with a CB<sub>1</sub>R antagonist or CB<sub>1</sub>R antisense prevented the induction of an ataxic phenotype. Such data suggest that CB<sub>1</sub>R antagonism may be useful in the pathogenic situation. In comparison to administration of CB<sub>1</sub>R ligands, data with CB<sub>1</sub>R knock-out mice are somewhat more equivocal. Thus, young/mature CB<sub>1</sub>R deficient mice are reported not to exhibit clear motor discoordination or changes to gait (Steiner *et al.* 1999; Kishimoto & Kano, 2006); however, deficits in motor function were reported in mature and older mice, in comparison to unaffected younger mice, in rotarod tests (Bilkei-Gorzo *et al.* 2005). One interpretation of these studies is that a progressive ataxic pathogenesis may be associated with long-term loss of CB<sub>1</sub>R. One common feature of CB<sub>1</sub>R knock-out mice, chronic marijuana users or animals administered CB<sub>1</sub>R agonists is a reported deficit in delay eyeblink conditioning, a cerebellar-dependent, motor learning process (Kishimoto & Kano, 2006; Skosnik *et al.* 2008; Steinmetz & Freeman, 2010). These data are consistent with CB<sub>1</sub>R controlling discrete motor function. Kishimoto & Kano (2006) also report that pharmacological block of CB<sub>1</sub>R had no effect on motor function in wild-type mice; however, it is also important to point out that CB<sub>1</sub>R deficiency and/or lack of effect of CB<sub>1</sub>R antagonism in a non-pathogenic situation does not preclude a role

repeated measurement one-way ANOVA followed by Tukey's honest significant difference test. *Ac* and *Bc*, summary diagrams for +/+ (*Ac*) and *du<sup>21</sup>/du<sup>21</sup>* (*Bc*) conditions. *Ad*, in wild-type conditions, CB<sub>1</sub>R activation (i.e. +WIN55) causes the release of Gβγ subunit from CB<sub>1</sub>R and subsequent inhibitory coupling of Gβγ to Cav2.1 at the presynapse to inhibit the action potential-evoked GABA release seen in control (CTL). *Bd*, by contrast, in *du<sup>21</sup>/du<sup>21</sup>* conditions, CB<sub>1</sub>R activation (i.e. +WIN55) has no effect on the GABA release seen in control (CTL). AM251 effects were also absent (see Wang *et al.* 2013). Thus, we propose that at synapses lacking α2δ-2 subunits (which associate predominantly with Ca<sub>v</sub>2.1 in the cerebellum; Barclay *et al.* 2001), normal CB<sub>1</sub>R modulation of Ca<sub>v</sub>2.1 is lacking. This deficit may relate to incorrect control of synaptic release by α2δ subunits (Hoppa *et al.* 2012); alternatively, it is possibly that lack of α2δ subunits may cause changes to CB<sub>1</sub>R-mediated G protein inhibition of Ca<sub>v</sub>2.1.

in disease; for example, whilst SR141617A (rimonabant) reversed CB<sub>1</sub>R-induced dysfunction, it had no effects itself on motor incoordination in non-ataxic animals (Lichtman *et al.* 1998; DeSanty & Dar, 2001). Together, these data suggest that CB<sub>1</sub>Rs modulate cerebellar circuitry in ataxic disease, potentially with a progressive onset of effect. Therefore, targeting CB<sub>1</sub>Rs may be beneficial in modulating motor incoordination in cerebellar ataxia, as discussed more fully below.

### An ataxic mouse model has deficient CB<sub>1</sub>R signalling

Whilst the role of ion channels (in particular, Ca<sub>v</sub>2.1) has been broadly studied in animal models of ataxia, there has been much less work on the presynaptic receptors that modulate neurotransmitter release and the postsynaptic receptors responsible for onward signalling in such models. A study in Ca<sub>v</sub>2.1 mutant tottering mice by Zhou and co-workers reported that presynaptic inhibition mediated by GABA<sub>B</sub> or  $\alpha$ 2-adrenoceptor GPCRs was enhanced at excitatory PF–PC synapses, although this may be as consequence of a switch to a reliance on Ca<sub>v</sub>2.2 (N-type) channels for transmitter release (Zhou *et al.* 2003). Tottering mice also had a reduction in GABA<sub>A</sub> receptor expression, with specific deficits in granule cells (Kaja *et al.* 2007). We have shown that ataxic *du*<sup>2J</sup> mutant mice exhibit increased irregularity of PC and, to a lesser extent, granule cell firing in multi-electrode array recordings from cerebellar brain slices (Wang *et al.* 2013). Of note, clear effects on PC firing regularity in *du*<sup>2J</sup>/*du*<sup>2J</sup> mice were not seen in heterozygous *+/du*<sup>2J</sup> mice, and the latter also lacked a clear behavioural ataxic phenotype. Importantly, the CB<sub>1</sub>R-mediated inhibition at IN–PC synapses seen in litter-matched controls was completely absent in both *+/du*<sup>2J</sup> and *du*<sup>2J</sup>/*du*<sup>2J</sup> mice. These data demonstrate that ataxic  $\alpha$ 2 $\delta$ -2-deficient mice have aberrant presynaptic CB<sub>1</sub>R-mediated signalling. The question arises as to whether deficiency in CB<sub>1</sub>R-mediated signalling is involved in ataxia pathogenesis or whether it occurs as a result of the disease. It appears that, in this model, both alleles need to be affected in order for an ataxic phenotype to be seen (Wang *et al.* 2013), and thus progressive deficits may be associated with *du*<sup>2J</sup> mice. We saw no changes in PC firing regularity in response to CB<sub>1</sub>R ligands in wild-type or *du*<sup>2J</sup> mice, consistent with a lack of postsynaptic CB<sub>1</sub>R effects in this model. We propose that such deficits occur due to compromised Ca<sup>2+</sup> channel activity consequent to reduced presynaptic  $\alpha$ 2 $\delta$ -2 expression in *du*<sup>2J</sup> mice (Fig. 1). In this regard,  $\alpha$ 2 $\delta$ -2 subunits have been shown to be essential not only for Ca<sup>2+</sup> channel trafficking (Dolphin, 2012), but also for synaptic function, the latter by increasing transmitter release probability and also protecting release from inhibitory effects of intracellular Ca<sup>2+</sup> chelators (Hoppa *et al.* 2012).

There are few studies measuring CB<sub>1</sub>R expression in ataxic animals; we reported no clear changes in expression in the cerebellar cortex of *+/du*<sup>2J</sup> and *du*<sup>2J</sup>/*du*<sup>2J</sup> mice (Wang *et al.* 2013). In a recent study, post-mortem cerebellar tissue from patients with SCAs, CB<sub>1</sub>R (and CB<sub>2</sub>R) expression was generally up-regulated in PCs, and also in glial cells (Rodríguez-Cueto *et al.* 2014a). Of interest here was that CB<sub>1</sub>R expression was reported in PC soma and pinceau in SCA patients, but was confined largely to the pinceau in control patients. It is possible that upregulated postsynaptic CB<sub>1</sub>R expression may affect 2-AG release in SCA patients; however, Rodríguez-Cueto and co-workers suggest that this CB<sub>1</sub>R expression is associated with degenerating PCs and may represent a marker for degeneration and/or a protective response against such degeneration. A further study in SCA patients reported an up-regulation of eCB degradative fatty acid amide hydrolase and monoacylglycerol lipase (MAGL) enzymes (Rodríguez-Cueto *et al.* 2014b), proposed to lead to reduced eCB levels in disease. In these studies, a compensatory up-regulation of cannabinoid receptor expression may occur as a consequence of reduced eCB levels; alternatively, it may be argued that eCBs are suppressed in order not to overactivate the system. Thus, it may also be possible to target eCB metabolizing enzymes for future therapeutic development. In this regard, potential avenues to increase 2-AG include inhibition of MAGL, localized predominantly to the PF terminal (Tanimura *et al.* 2012), or activation of the biosynthetic enzyme diacylglycerol lipase- $\alpha$  (DAGL $\alpha$ ) localized predominantly to the base of postsynaptic dendritic spines (Yoshida *et al.* 2006) (see Abstract Figure). It is also clear that we will need to investigate potential changes to eCB signalling in different animal models of ataxia to inform development of the most useful therapeutic strategies and also to determine if any such changes represent useful markers for different forms of ataxia.

### Do cannabinoids have therapeutic utility in cerebellar ataxia?

There are anecdotal reports that cannabis smokers can achieve symptom relief for several CNS disorders. Such evidence has fuelled the investigation of use of CB<sub>1</sub>R agonists as potential neuroprotective agents for a range of conditions including epilepsy, neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease and appetitive disorders (Fernández-Ruiz *et al.* 2011; Hill *et al.* 2012a). Earlier evidence for ataxia is largely confined to two case studies which suggest that oral  $\Delta^9$ -THC or marijuana improved motor coordination in some multiple sclerosis patients (Clifford, 1983; Meinck *et al.* 1989). At the clinical level, synthetic  $\Delta^9$ -THC has been used in management of nausea, emesis and pain, and nabiximols (Sativex) (containing ~1:1  $\Delta^9$ -THC:CBD)

represents the first phytocannabinoid medicine, used as an oromucosal spray for pain and spasticity associated with multiple sclerosis (Hill *et al.* 2012a); Sativex was also stated to delay onset of ataxia symptoms in the Medicines and Healthcare products Regulatory Agency (MHRA) Public Information Report UK/H/961/01/DC. Such reports contributed to fuelling a major review on the clinical effects of cannabinoids in ataxia associated with multiple sclerosis (Mills *et al.* 2007); although cannabinoids showed promise, it was concluded that better standardized measures of ataxia were needed to fully establish the utility of cannabis-based medicines in ataxia. The role of cannabinoids in disease-associated movement disorders and tremor has been further discussed more recently by Arjmand *et al.* (2015) and Kluger *et al.* (2015), who similarly concluded that further work on cannabinoids in different models of ataxia is warranted. In this regard, an interesting recent report suggests that a 'Sativex-like' combination of  $\Delta^9$ -THC and CBD, as well as the individual administration of  $\Delta^9$ -THC or CBD, was able to improve motor deficits in a viral model of multiple sclerosis (Feliú *et al.* 2015). Clearly, studies which suggest CB<sub>1</sub>R activation may be useful in cerebellar ataxia contrast to preclinical data where CB<sub>1</sub>R agonists induce an ataxic phenotype (Lichtman *et al.* 1998; DeSanty & Dar, 2001; Patel & Hillard, 2001); however, these data do support the hypothesis that maintaining the correct balance in eCB system signalling is a major factor for proper control of motor coordination. This hypothesis is further supported by data from Ca<sub>v</sub>2.1 mutants described above, where both decreases (Maejima *et al.* 2013) and increases in Ca<sup>2+</sup> current (Gao *et al.* 2012) can produce an ataxia phenotype; moreover, deficits in delay eyeblink conditioning are reported for both CB<sub>1</sub>R agonists and CB<sub>1</sub>R antagonists/inverse agonists using the same experimental design (Steinmetz & Freeman, 2010). CB<sub>1</sub>R agonists have also been shown to possess functional selectivity or 'biased agonism', whereby different ligands (including eCBs) preferentially activate different CB<sub>1</sub>R signalling pathways (Laprairie *et al.* 2014; Khajehali *et al.* 2015). Whilst, as argued above, it is likely that CB<sub>1</sub>Rs act predominantly on presynaptic Ca<sub>v</sub>2.1 to reduce transmitter release in the cerebellar cortex, alternative signalling pathways include inhibition of cAMP or stimulation of phosphorylation of signal regulated kinases (Howlett *et al.* 2010). Thus, it could be argued that by using knowledge of biased agonism that we can target specific pathways associated with diseases, including cerebellar ataxia. Finally here, CB<sub>1</sub>R agonists such as  $\Delta^9$ -THC have also been proposed to possess anti-oxidant (Hampson *et al.* 1998) and anti-inflammatory (although largely CB<sub>2</sub>R-mediated) (Fernández-Ruiz *et al.* 2011) properties; in common with other degenerative diseases, such properties may benefit the amelioration of cerebellar ataxia symptoms.

The demonstration that pre-treatment with a CB<sub>1</sub>R antagonist prevents the induction of motor incoordination by CB<sub>1</sub>R agonists (DeSanty & Dar, 2001; Patel & Hillard, 2001) suggests that CB<sub>1</sub>R antagonists/inverse agonists may be protective in ataxia. The archetypal agent rimonabant was introduced as an anti-obesity agent, but was withdrawn due to fears of increased suicidality and depression in patients (Nathan *et al.* 2011). Since then, therapeutic development of CB<sub>1</sub>R antagonists/inverse agonists has largely been put on hold. Interesting potential alternatives are CB<sub>1</sub>R negative allosteric antagonists, such as Org-27569 and PSNCBAM-1. These compounds have a somewhat unique pharmacological profile as they increase orthosteric agonist binding, but decrease agonist activity; more intriguingly, allosteric antagonism action is ligand-dependent and also shows biased antagonism for different signalling pathways (Baillie *et al.* 2013). We have shown that such functional selectivity for PSNCBAM-1 extends to effects on orthosteric ligands at IN-PC synapses in the cerebellar cortex (Wang *et al.* 2011); thus, PSNCBAM-1 attenuated CP55940 agonist and AM251 antagonist effects, but had no clear effects against WIN 55,212-2. Moreover, when applied alone, PSNCBAM-1 was not associated with potentially deleterious effects on eCB tone, a concern associated with use of CB<sub>1</sub>R antagonists/inverse agonists such as rimonabant. These studies indicate that exogenous allosteric CB<sub>1</sub>R ligands have potential to fine tune eCB orthosteric agonist effects in a ligand- and/or cell signalling-selective manner within the cerebellar cortex; moreover, biased antagonism effects may allow for further useful therapeutic development.

This review has focused on cannabinoids as agents acting on the eCB system in the cerebellum; moreover, the modulation of CB<sub>1</sub>Rs has been highlighted. It may transpire that, for exogenous compounds, targeting alternative modes of action offers improved therapeutic potential in diseases such as ataxia. The last few years have seen increasing calls for the use of medical marijuana to treat a range of disorders; of course, use of marijuana is intimately associated with psychoactive effects of the CB<sub>1</sub>R partial agonist  $\Delta^9$ -THC. Therapeutically, a more attractive option may be use of non-psychoactive pCBs. Thus, CBD and cannabidivarin (CBDV) have reported utility in epilepsy and, potentially, other CNS disorders (Hill *et al.* 2012b, 2013; Devinsky *et al.* 2014). The demonstration that Sativex can improve motor activity in multiple sclerosis (Feliú *et al.* 2015) is consistent with beneficial effects of a CB<sub>1</sub>R activator ( $\Delta^9$ -THC) in combination with CBD as a potential ameliorating agent for unwanted  $\Delta^9$ -THC effects (McPartland *et al.* 2015); for example, CBD alone was also effective in improving motor deficits, potentially via an action on peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) receptors (Feliú

*et al.* 2015). In this regard, CBD has recently been awarded orphan drug status for the severe childhood epilepsy Dravet syndrome and is currently progressing well through clinical trials. CBD and CBDV have only low affinity at CB<sub>1</sub>Rs and CBD has been proposed, amongst other possibilities, to act at alternative GPCRs or at transient receptor potential ion channels or, possibly, to augment eCB tone via effects on metabolic enzymes (Hill *et al.* 2012a; McPartland *et al.* 2015). A recent study has proposed that CBD, as well as having CB<sub>1</sub>R-independent actions, may also act as a CB<sub>1</sub>R negative allosteric antagonist (Laprairie *et al.* 2015); therefore, CBD may share useful properties of this class of agents discussed above. It is also of interest that the hypophagic effects of the allosteric antagonist Org27569 have been suggested to occur independently of CB<sub>1</sub>Rs (Ding *et al.* 2014; Gamage *et al.* 2014). Thus, the use of cannabinoids with CB<sub>1</sub>R-independent and/or allosteric actions should also be considered.

In the future, it will be of interest in particular to test agents such as Sativex, and perhaps CBD as an individual compound, in cerebellar ataxia. There are a number of general points to consider, including whether deficits in CB<sub>1</sub>R-mediated signalling are hallmark characteristics of different forms of ataxia, how best to target such deficits and whether aberrant cannabinergic signalling represents a useful biomarker for early or asymptomatic cerebellar ataxia. The answer to such questions will go some way to determining if modulation of the eCB system has therapeutic utility in cerebellar ataxia.

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## Additional information

### Competing interests

The author has no conflict of interest.

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