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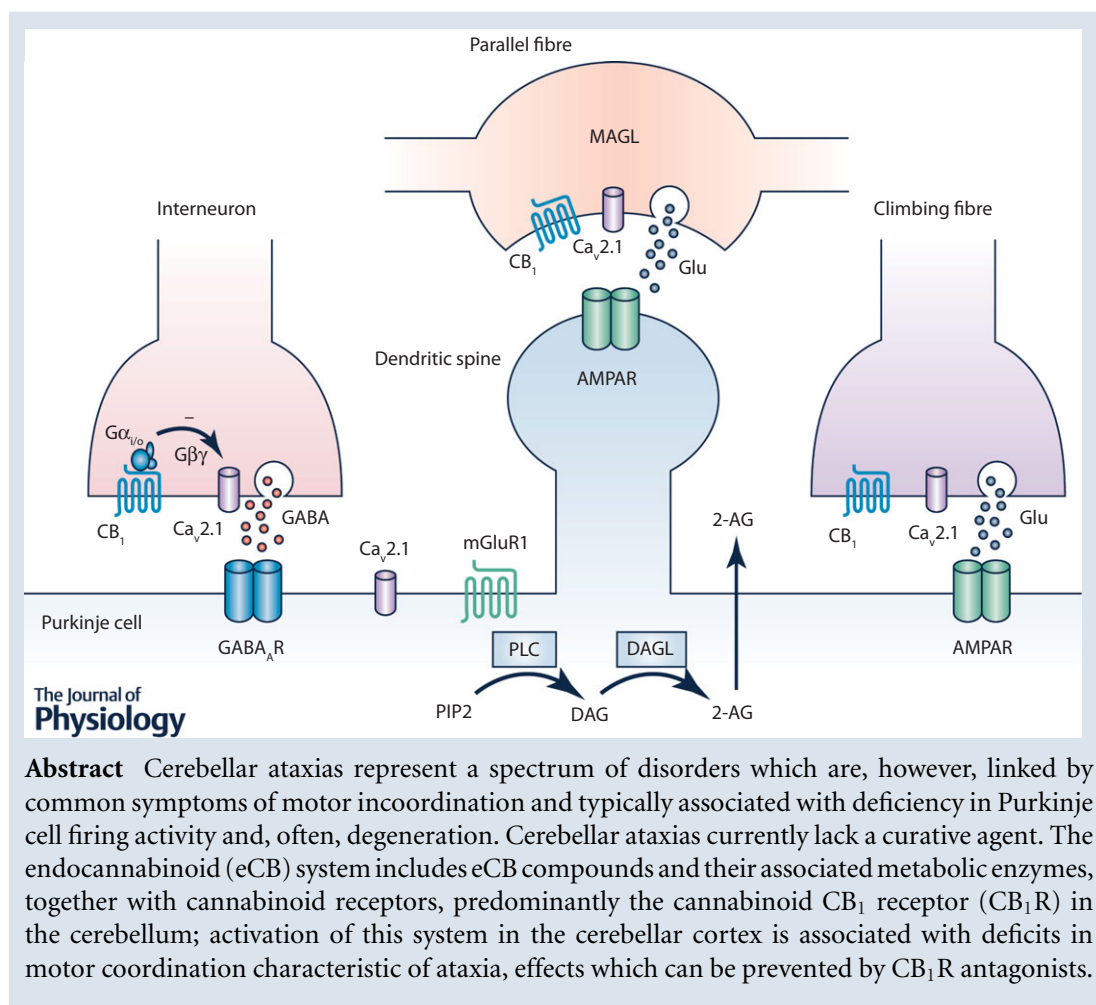
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SYMPOSIUM REVIEW

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

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Of further interest are various findings that CB₁R 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 'ducky²¹' 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 CB₁Rs 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 α (DAGL α); this process is driven by activation of metabotropic receptors including mGluR1 and may also involve Ca⁺-dependent processes including voltage-dependent Ca²⁺ channels such as Ca_v2.1 and also Ca²⁺ permeable ionotropic receptors (see Kano *et al.* 2009). 2-AG is released retrogradely 'on-demand' to active presynaptic CB₁Rs. 2-AG action is terminated by monoacylglycerol lipase (MAGL) localized predominantly to PF terminals. CB₁Rs are highly expression at IN inputs into Purkinje cells at the pinceau. CB₁Rs 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, CB₁Rs predominantly couple to G $\alpha_{i/o}$ to inhibit Ca_v2.1 Ca²⁺ channels via release of G $\beta\gamma$ subunits (shown only at IN–PC synapses for clarity). Activation of presynaptic CB₁Rs inhibits GABA release onto postsynaptic GABA_A receptors and glutamate release predominantly onto postsynaptic AMPA receptors, respectively. As PCs represent the sole output of the cerebellar cortex, CB₁Rs 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; Δ^9 -THC, tetrahydrocannabinol; VDCC, voltage-dependent Ca²⁺ 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 Ca²⁺ channel (VDCC) Ca_v2.1 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 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^{2J} (*dir^{2J}*) 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 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 CB_1R , 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 (Δ^9 -THC) and cannabidiol (CBD) (Hill *et al.* 2012a); and (iii) exogenous synthetic agents, namely CB_1R agonists, for example, WIN 55,212-2, an aminoalkylindole derivative, and CP 55940, which is structurally related to tetrahydrocannabinol, and CB_1R 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 CB_1Rs , which represent the most widespread G protein-coupled receptor (GPCR) in the mammalian cerebellum (Herkenham *et al.* 1991; Tsou *et al.* 1997). CB_1R 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 CB_1R expression at CF inputs onto PC dendritic shafts (Kawamura *et al.* 2006; see Abstract Figure). CB_1Rs 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 CB_1Rs are activated by retrograde 'on demand' release of 2-AG from postsynaptic PCs. The major effect of presynaptic CB_1R activation is a suppression of neurotransmitter release, whereby activation of presynaptic CB_1Rs 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 CB_1R ligand-induced changes to cerebellar cortex network activity are mediated, at least in part, via effects on inhibitory synaptic transmission (Ma *et al.* 2008). CB_1R activation has been

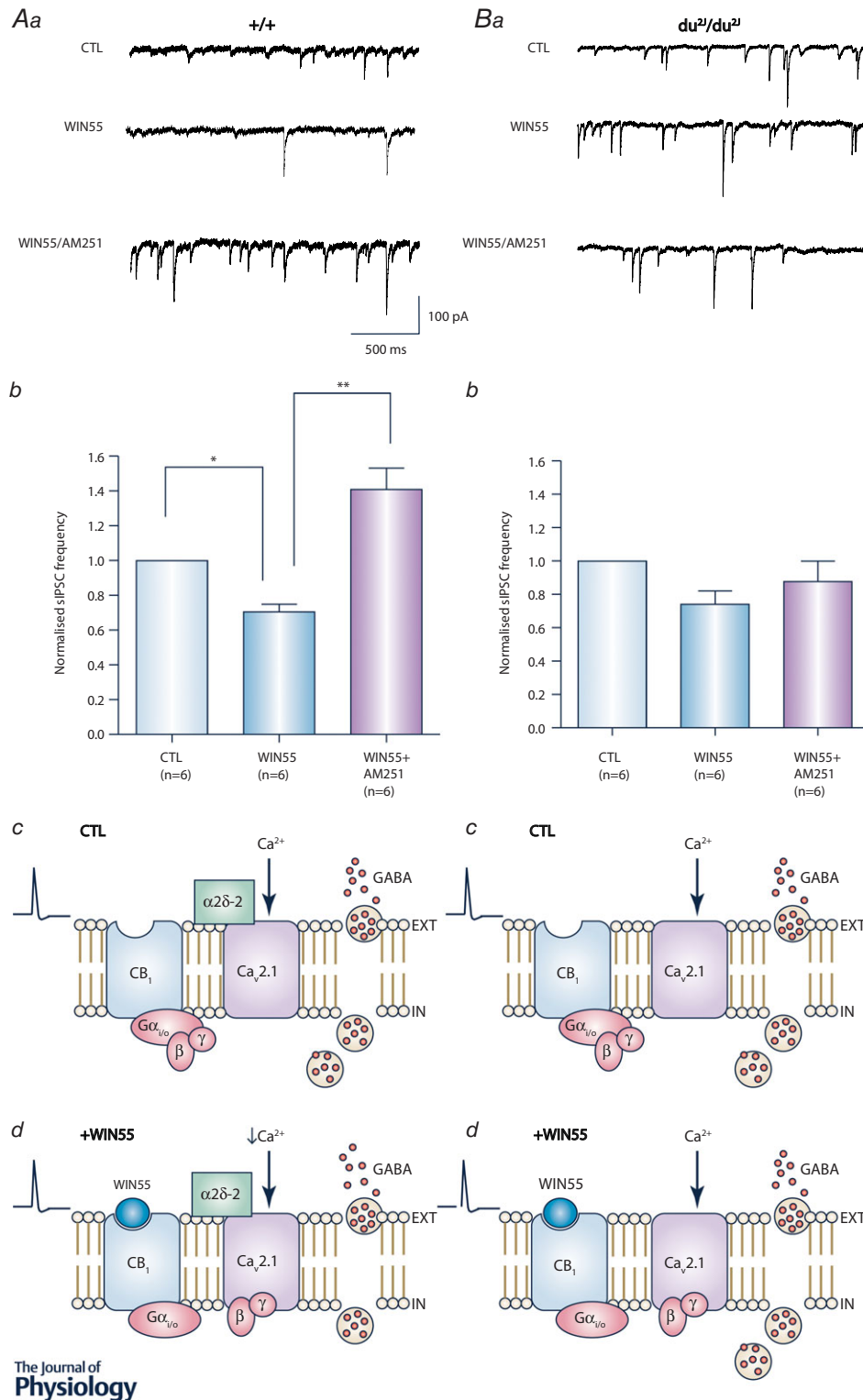


Figure 1. Presynaptic CB₁R modulation of inhibitory transmission at IN–PC synapses is deficient in ataxic ducky^{2J} mice

Aa and Ba, representative spontaneous inhibitory postsynaptic current (sIPSC) traces from +/+ (Aa) and du^{2J}/du^{2J} (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^{2J}/du^{2J} (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 CB₁R at PF–PC synapses (Safo & Regehr, 2005). Here, mGluR1 activation drives 2-AG release (Kano *et al.* 2009; see Abstract Figure). Thus, CB₁R 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, CB₁R activation causes pertussis toxin-sensitive inhibition of Ca_v2 family VDCCs, and can also activate inwardly rectifier K⁺ channels (Mackie & Hille, 1992; Twitchell *et al.* 1997; Guo & Ikeda, 2004). It is likely that CB₁R couple to presynaptic Ca_v2.1 (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_v2.1 (and to a lesser extent Ca_v2.2 and Ca_v2.3) 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).

CB₁R 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, CB₁R agonist effects on miniature EPSCs at PF–PC synapses were only apparent when extracellular Ca²⁺ levels were increased (Yamasaki *et al.* 2006). Moreover, CB₁R antagonists/inverse agonists such as AM251, rimonabant and the pCB Δ⁹-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 CB₁R 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 CB₁R antagonist or CB₁R antisense prevented the induction of an ataxic phenotype. Such data suggest that CB₁R antagonism may be useful in the pathogenic situation. In comparison to administration of CB₁R ligands, data with CB₁R knock-out mice are somewhat more equivocal. Thus, young/mature CB₁R 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 CB₁R. One common feature of CB₁R knock-out mice, chronic marijuana users or animals administered CB₁R 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 CB₁R controlling discrete motor function. Kishimoto & Kano (2006) also report that pharmacological block of CB₁R had no effect on motor function in wild-type mice; however, it is also important to point out that CB₁R deficiency and/or lack of effect of CB₁R 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 *du²¹/du²¹* (*Bc*) conditions. *Ad*, in wild-type conditions, CB₁R activation (i.e. +WIN55) causes the release of Gβγ subunit from CB₁R 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 *du²¹/du²¹* conditions, CB₁R 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_v2.1 in the cerebellum; Barclay *et al.* 2001), normal CB₁R modulation of Ca_v2.1 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 CB₁R-mediated G protein inhibition of Ca_v2.1.

in disease; for example, whilst SR141617A (rimonabant) reversed CB₁R-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 CB₁Rs modulate cerebellar circuitry in ataxic disease, potentially with a progressive onset of effect. Therefore, targeting CB₁Rs may be beneficial in modulating motor incoordination in cerebellar ataxia, as discussed more fully below.

An ataxic mouse model has deficient CB₁R signalling

Whilst the role of ion channels (in particular, Ca_v2.1) 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_v2.1 mutant tottering mice by Zhou and co-workers reported that presynaptic inhibition mediated by GABA_B or α 2-adrenoceptor GPCRs was enhanced at excitatory PF–PC synapses, although this may be as consequence of a switch to a reliance on Ca_v2.2 (N-type) channels for transmitter release (Zhou *et al.* 2003). Tottering mice also had a reduction in GABA_A receptor expression, with specific deficits in granule cells (Kaja *et al.* 2007). We have shown that ataxic *du*^{2J} 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*^{2J}/*du*^{2J} mice were not seen in heterozygous *+/du*^{2J} mice, and the latter also lacked a clear behavioural ataxic phenotype. Importantly, the CB₁R-mediated inhibition at IN–PC synapses seen in litter-matched controls was completely absent in both *+/du*^{2J} and *du*^{2J}/*du*^{2J} mice. These data demonstrate that ataxic α 2 δ -2-deficient mice have aberrant presynaptic CB₁R-mediated signalling. The question arises as to whether deficiency in CB₁R-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*^{2J} mice. We saw no changes in PC firing regularity in response to CB₁R ligands in wild-type or *du*^{2J} mice, consistent with a lack of postsynaptic CB₁R effects in this model. We propose that such deficits occur due to compromised Ca²⁺ channel activity consequent to reduced presynaptic α 2 δ -2 expression in *du*^{2J} mice (Fig. 1). In this regard, α 2 δ -2 subunits have been shown to be essential not only for Ca²⁺ 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 Ca²⁺ chelators (Hoppa *et al.* 2012).

There are few studies measuring CB₁R expression in ataxic animals; we reported no clear changes in expression in the cerebellar cortex of *+/du*^{2J} and *du*^{2J}/*du*^{2J} mice (Wang *et al.* 2013). In a recent study, post-mortem cerebellar tissue from patients with SCAs, CB₁R (and CB₂R) expression was generally up-regulated in PCs, and also in glial cells (Rodríguez-Cueto *et al.* 2014a). Of interest here was that CB₁R 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 CB₁R expression may affect 2-AG release in SCA patients; however, Rodríguez-Cueto and co-workers suggest that this CB₁R 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- α (DAGL α) 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 CB₁R 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 Δ^9 -THC or marijuana improved motor coordination in some multiple sclerosis patients (Clifford, 1983; Meinck *et al.* 1989). At the clinical level, synthetic Δ^9 -THC has been used in management of nausea, emesis and pain, and nabiximols (Sativex) (containing ~1:1 Δ^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 Δ^9 -THC and CBD, as well as the individual administration of Δ^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 CB₁R activation may be useful in cerebellar ataxia contrast to preclinical data where CB₁R 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_v2.1 mutants described above, where both decreases (Maejima *et al.* 2013) and increases in Ca²⁺ current (Gao *et al.* 2012) can produce an ataxia phenotype; moreover, deficits in delay eyeblink conditioning are reported for both CB₁R agonists and CB₁R antagonists/inverse agonists using the same experimental design (Steinmetz & Freeman, 2010). CB₁R agonists have also been shown to possess functional selectivity or 'biased agonism', whereby different ligands (including eCBs) preferentially activate different CB₁R signalling pathways (Laprairie *et al.* 2014; Khajehali *et al.* 2015). Whilst, as argued above, it is likely that CB₁Rs act predominantly on presynaptic Ca_v2.1 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, CB₁R agonists such as Δ^9 -THC have also been proposed to possess anti-oxidant (Hampson *et al.* 1998) and anti-inflammatory (although largely CB₂R-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 CB₁R antagonist prevents the induction of motor incoordination by CB₁R agonists (DeSanty & Dar, 2001; Patel & Hillard, 2001) suggests that CB₁R 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 CB₁R antagonists/inverse agonists has largely been put on hold. Interesting potential alternatives are CB₁R 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 CB₁R antagonists/inverse agonists such as rimonabant. These studies indicate that exogenous allosteric CB₁R 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 CB₁Rs 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 CB₁R partial agonist Δ^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 CB₁R activator (Δ^9 -THC) in combination with CBD as a potential ameliorating agent for unwanted Δ^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 γ (PPAR γ) 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 CB₁Rs 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 CB₁R-independent actions, may also act as a CB₁R 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 CB₁Rs (Ding *et al.* 2014; Gamage *et al.* 2014). Thus, the use of cannabinoids with CB₁R-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 CB₁R-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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