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REVIEW

Cannabinoid receptor signalling in neurodegenerative diseases: a potential role for membrane fluidity disturbance

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Type-1 cannabinoid receptor (CB_1) is the most abundant G-protein-coupled receptor (GPCR) in the brain. CB_1 and its endogenous agonists, the so-called 'endocannabinoids (eCBs)', belong to an ancient neurosignalling system that plays important functions in neurodegenerative and neuroinflammatory disorders like Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. For this reason, research on the therapeutic potential of drugs modulating the endogenous tone of eCBs is very intense. Several GPCRs reside within subdomains of the plasma membranes that contain high concentrations of cholesterol: the lipid rafts. Here, the hypothesis that changes in membrane fluidity alter function of the endocannabinoid system, as well as progression of particular neurodegenerative diseases, is described. To this end, the impact of membrane cholesterol on membrane properties and hence on neurodegenerative diseases, as well as on CB_1 signalling *in vitro* and on CB_1-dependent neurotransmission within the striatum, is discussed. Overall, present evidence points to the membrane environment as a critical regulator of signal transduction triggered by CB_1, and calls for further studies aimed at better clarifying the contribution of membrane lipids to eCBs signalling. The results of these investigations might be exploited also for the development of novel therapeutics able to combat disorders associated with abnormal activity of CB_1.

LINKED ARTICLES

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

2-AG, 2-arachidonoylglycerol; Aβ, amyloid β peptide; AD, Alzheimer's disease; AEA, *N*-arachidonoylethanolamine or anandamide; ALS, amyotrophic lateral sclerosis; ApoE, apolipoprotein E; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; CB_1, type-1 cannabinoid receptor; CB_2, type-2 cannabinoid receptor; CNS, central nervous system; DAGL, diacylglycerol lipase; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; FMRP, Fragile X mental retardation protein; FXS, Fragile X syndrome; GPCR, G protein-coupled receptor; HD, Huntington's disease; IPSI, inhibitory postsynaptic currents; l_c, liquid-disordered crystalline phase; l_o, liquid-ordered phase; LR, lipid raft; MAGL, monoacylglycerol lipase; MCD, methyl-β-cyclodextrin; mGlu, metabotropic glutamate; MS, multiple sclerosis; NAPE-PLD, *N*-acyl-phosphatidylethanolamine (NAPE)-hydrolysing phospholipase D; NMDA, N-methyl-D-aspartate; PD, Parkinson's disease; PPAR, peroxisome proliferator-activated receptor; PrD, prion-related disorder; PrP, prion protein; TRPV1, type-1 transient receptor potential vanilloid

Introduction

N-arachidonoylethanolamine [anandamide (AEA)] and 2-arachidonoylglycerol (2-AG) activate type-1 (CB_1) (Howlett *et al.*, 2010; Pertwee, 2010) and type-2 (CB_2) cannabinoid receptors (Patel *et al.*, 2010), and are the most active 'endocannabinoids' (eCBs) as yet characterized (Di Marzo, 2009; Maccarrone *et al.*, 2010a). AEA, but not 2-AG, also binds to and activates type-1 transient receptor potential vanilloid (TRPV1) channels, and hence is considered a true 'endovanilloid' (Di Marzo and De Petrocellis, 2010). The nomenclature of the major eCBs-binding receptors (CB_1, CB_2 and TRPV1) conforms to the *British Journal of Pharmacology*'s Guide to Receptors and Channels (Alexander *et al.*, 2009). Here, the hypothesis that changes in membrane fluidity alter function of the eCBs signalling, as well as progression of particular neurodegenerative diseases, is described. To this end, the impact of membrane cholesterol on membrane properties and hence on neurodegenerative diseases, as well as on CB_1 signalling *in vitro* and on CB_1-dependent neurotransmission within the striatum, is discussed.

CB_1 receptor is the most abundant G protein-coupled receptor (GPCR) in the brain (Howlett *et al.*, 2010). Together with its endogenous agonists, CB_1 forms an ancient neurosignalling system that plays important control functions within the central nervous system (CNS) (Katona and Freund, 2008). Alterations in eCBs signalling have been extensively investigated in a wide range of neurodegenerative and neuroinflammatory disorders, spanning from Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), to amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) (Maccarrone *et al.*, 2007; Centonze *et al.*, 2008; Bisogno and Di Marzo, 2010). In addition, prion-related disorders (PrDs) seem to benefit from a nonpsychoactive phytocannabinoid like cannabidiol, that prevents prion accumulation and protects neurons against its toxicity (Dirikoc *et al.*, 2007; Iuvone *et al.*, 2009). Not surprisingly, research on the therapeutic potential of drugs modulating eCBs activity is very intense (Di Marzo, 2009). In recent years it has become increasingly evident the involvement of membrane lipids, especially cholesterol and glycosphingolipids, in regulating the function of GPCRs like β_2-adrenergic and $serotonin_{1A}$ receptors, as well as of several other membrane-associated proteins like caveolins (Pontier *et al.*, 2008; Prinetti *et al.*, 2009; Paila *et al.*, 2010; Shrivastava *et al.*, 2010).

In the following sections the effect of cholesterol on membrane properties will be discussed, as well as its involvement in neurodegenerative diseases where eCBs signalling has been shown to play a role. Then, the effect of membrane cholesterol on CB_1-dependent signal transduction in *in vitro* models will be briefly reviewed, and the molecular determinants that underlie CB_1, but not CB_2, sensitivity to membrane cholesterol perturbation will be presented. Finally, recent evidence that demonstrates a role for membrane cholesterol on CB_1-dependent eCBs signalling in striatal neurotransmission will be summarized, in order to support the concept that membrane environment can indeed control eCBs-dependent neurotransmitter networks also *in vivo*. Incidentally, there evidence that similar changes in plasma membrane fluidity occur in non-neuronal tissues where CB_1 receptors are expressed, for instance within the immune system (Bari *et al.*, 2006). Yet, the effect of alterations of membrane cholesterol on peripheral CB_1-dependent signalling goes beyond the scopes of this review.

Cholesterol and membrane properties: the 'lipid raft' concept

The classical 'fluid-mosaic' model of the biological membrane organization (Singer and Nicolson, 1972) has changed in recent years. Membrane proteins are no longer simply floating in a two-dimensional oriented solution, and the solvent is not only a simple viscous phospholipid bilayer. In fact, lateral compartmentalization and membrane asymmetry impose specific constraints to diffusion and partition between the two monolayers of specific hydrophobic molecules, and affect membrane-bound proteins (Forneris and Mattevi, 2008; Lindahl and Sansom, 2008). Some emerging concepts on membranes are that they are patchy structures, with segregated functional regions of various thickness and composition, and that crowding domains and ectodomains limit lipid exposure to the surrounding aqueous regions (Brown and London, 1998; Galbiati *et al.*, 2001). Therefore, it appears increasingly evident that changes in the physicochemical properties of the biological membrane directly affect the structure, and hence the function, of associated proteins. Rather than serving only as a medium through which membrane proteins diffuse, lipid bilayers have now been demonstrated to form compartmentalized subdomains with different biophysical properties.

Among the most interesting specialized microdomains of the plasma membrane are those enriched in cholesterol, sphingolipids, plasmenylethanolamine and arachidonic acid (Brown and London, 1998; Pike, 2005). These domains, characterized by a more tightly packed state responsible for their resistance to solubilization with non-ionic detergents (Brown and London, 1998), have been referred to as 'lipid rafts' (LRs). Interestingly, the physicochemical properties of LRs seem to be strictly related to their functional roles (Ostermeyer *et al.*, 1999). Phase separation between lipids in different physical states, most often the solid-like gel phase (β-phase) and the liquid-disordered crystalline phase (α-phase, l_c), has been well characterized in model membranes. Sphingolipids differ from most biological phospholipids in that they contain long, largely saturated acyl chains. This allows them to pack tightly together. However, because of the high concentration of cholesterol in the plasma membrane and other membranes in which rafts form, sphingolipids in LRs do not exist in the gel phase. Different kinds of phase separation can occur in binary mixtures of individual phospholipids with cholesterol. In these mixtures, domains in a l_c-like phase coexist with domains in a new state, the so-called 'liquid-ordered' (l_o) phase. Interestingly, acyl chains of lipids in l_o phase are extended and tightly packed, as in the gel phase, but they have a high degree of lateral mobility (Brown and London, 1998). LRs obtained from detergent-resistant membranes isolated from cell lysates seem to exist in this l_o phase or in a state with similar properties (Ge *et al.*, 1999; Ostermeyer *et al.*, 1999). Thus, in the presence of cholesterol, lipid mixtures can undergo a l_o/l_c phase separation, instead of the gel/l_c distribution observed in the absence of cholesterol. Furthermore, cholesterol seems to promote phase separation, apparently because of favourable packing interactions between

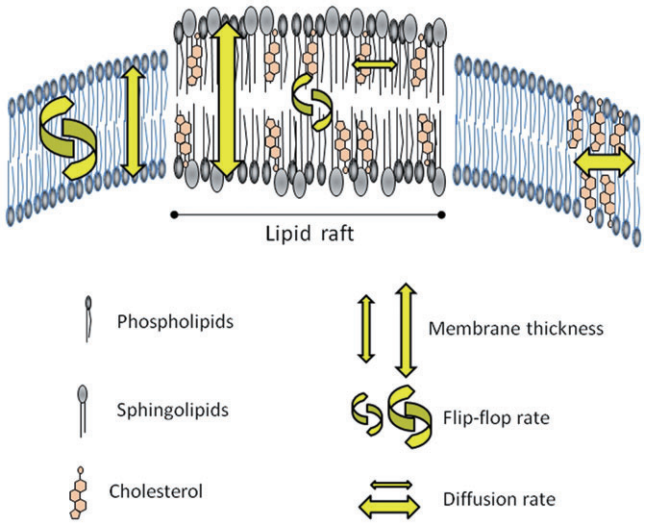

Figure 1

Scheme of a biological membrane containing lipid rafts (LRs). The liquid-ordered regions of LRs are thicker than the liquid-disordered bulk of the membrane, and the cholesterol molecules within this region have a slower rate of lateral and interlayer diffusion (flip-flop). See text for details.

saturated lipids and cholesterol itself (Xu and London, 2000). This 'cholesterol effect' probably explains LRs formation also in cell membranes that contain low levels of sphingolipids, and clarifies why cholesterol depletion can induce LRs disruption, thus affecting raft function.

In the simplest case, LRs can be viewed as signalling platforms that serve to colocalize the required components, such as receptors, coupling factors, effectors and enzymes, facilitating their interaction and supporting signalling. The functions modulated by LRs include cholesterol transport (Fielding and Fielding, 2001; Uittenbogaard *et al.*, 2002; Smart *et al.*, 2004), organization of signalling protein complexes (Galbiati *et al.*, 2001), endocytosis (Pelkmans, 2005), potocytosis (Anderson *et al.*, 1992) and establishment of cell polarity (Ostrom and Insel, 2004). These lipid microdomains can serve also as specific sites of interaction for certain pathogens and toxins (Fivaz *et al.*, 2002), and they play an important role also in the organization of cell signalling machineries such as receptor tyrosine kinases and GPCRs (Barnett-Norris *et al.*, 2005). More generally, the liquid ordered regions of LRs are thicker than the liquid-disordered bulk of the membrane, and the cholesterol molecules within this region have a slower rate of lateral and interlayer diffusion (flip-flop) (Dainese *et al.*, 2010). These properties of LRs are schematically depicted in Figure 1.

Cholesterol and neurodegenerative diseases

Compared with other tissues, mammalian brain contains the highest levels of cholesterol, that in humans represents ~25% of the amount in total body (~20 $mg \cdot g^{-1}$), although human brain accounts for ~2% only of total body weight (Dietschy and Turley, 2004; Vaya and Schipper, 2007). Brain cholesterol is mainly synthesized by oligodendrocytes and astrocytes, without a significant contribution by blood circulation because cholesterol in lipoprotein particles [(most often apolipoprotein E (ApoE)] can not cross the blood–brain barrier (Liu *et al.*, 2010). While oligodendrocytes produce cholesterol to form the myelin sheaths, astrocytes supply cholesterol to neurons, by using ATP-binding cassette A1 and G1 transporters (Karten *et al.*, 2006). In addition, astrocytes are the major ApoE-producing cells within the CNS, and cholesterol/ApoE complexes may enter neurons by receptor-mediated endocytosis (Hayashi *et al.*, 2004), followed by transport of cholesterol to different intracellular organelles (e.g. lysosomes, endoplasmic reticulum, Golgi apparatus) and to the plasma membranes.

The relevance of membrane cholesterol in neurodegenerative processes is demonstrated by the number of research papers devoted to this issue: a PubMed search with 'cholesterol' and 'neurodegenerative diseases' scores more than 1500 entries, 675 of which were released in the last 5 years. Not surprisingly, the topic of cholesterol involvement in the pathogenesis of neurodegenerative diseases has been recently covered by several comprehensive review papers (Adibhatla and Hatcher, 2008; Schweitzer *et al.*, 2009; Liu *et al.*, 2010; Martin *et al.*, 2010; Schengrund, 2010; Zuccato *et al.*, 2010). Besides the effect on membrane properties outlined earlier, it is known that cholesterol in LRs modulates the binding and oligomerization of 'amyloidogenic proteins': these are a series of brain proteins with exceptional conformational plasticity and a high propensity for self-aggregation (Fantini and Yahi, 2010). By controlling the balance between unstructured monomers and α or β conformers (the so-called 'chaperone effect'). LRs can either inhibit or stimulate the oligomerization of amyloidogenic proteins. Cholesterol has a dual role in this process, because it regulates protein-sphingolipid interactions through a fine tuning of sphingolipid conformation (indirect effect), and/or facilitates pore (or channel) formation through direct binding to amyloidogenic proteins (Fantini and Yahi, 2010). Aberrantly folded proteins are hallmarks of amyloidogenic diseases, for instance AD and PrD that, although clinically different, have the same underlying pathogenetic mechanism, that is, an altered protein conformer with high β-sheet structure content: the amyloid β peptide (Aβ) in the case of AD, and the aberrant prion protein, PrP^{sc}, in PrD (Pani *et al.*, 2010). Growing evidence indicates the possible involvement of cholesterol in misfolded protein generation during AD and PrD (reviewed by Pani *et al.*, 2010). Furthermore, Aβ is a product of the amyloid precursor protein (APP) cleavage by secretase, and there is evidence that also the activity of the latter enzyme is modulated by membrane lipid environment (Eckert *et al.*, 2010). Conversely, Aβ disturbs the properties of artificial and isolated biological membranes, as well as those of plasma membranes in living cells. Thus, LRs may be the site where the neurotoxic cascade of Aβ is initiated (Eckert *et al.*, 2010). In addition, also glutamate receptors of the N-methyl-D-asparate (NMDA) subtype show a very complex functional regulation, dependent on neuregulins and receptor tyrosine kinases localized in cholesterol-rich LRs (Schrattenholz and Soskic, 2006). It should be recalled that NMDA receptors are allosteric and ligand-gated calcium channels, with a pivotal role in memory-related signal transduction and other neurotransmission pathways (Chau, 2010). The brain pathologies

Table 1

Impact of membrane cholesterol on neurodegenerative diseases

Disease	Effect	Reference
Alzheimer's disease	LRs disruption by membrane cholesterol depletion (through MCD) leads to a lower rate of β-amyloid accumulation, that impacts tyrosine kinase Fyn and hence tau phosphorylation. Instead, elevated levels of cholesterol enhance the activity of the amyloid precursor protein (APP)-cleaving enzyme BACE1, and that of γ-secretase, also found in LRs.	Simons *et al.* (1998) Bhaskar *et al.* (2005) Kalvodova *et al.* (2005) Grimm *et al.* (2008)
Parkinson's disease	At least four proteins, which when mutated are associated with PD, reside in LRs: Parkin (an E3 ubiquitin-ligase); α-Synuclein; LRRK2 (leucine-rich repeat kinase 2); PINK1 (PTEN-induced kinase). Mutation of α-synuclein (A30P) leads to a reduced association with LRs.	Fallon *et al.* (2002) Fortin *et al.* (2004) Kubo *et al.* (2005) Silvestri *et al.* (2005) Hatano *et al.* (2007)
Huntington's disease	Mutant huntingtin associates with LRs more strongly than the wild-type protein, and inhibits the expression of several genes involved in cholesterol synthesis, vesicle synthesis and trafficking. In addition, mutant huntingtin increases raft-associated glycogen synthase kinase 3-β, a marker of apoptotic stress.	Sipione *et al.* (2002) Valenza and Cattaneo (2006) Valencia *et al.* (2009) Zuccato *et al.* (2010)
Amyotrophic lateral sclerosis	LRs disruption by membrane cholesterol depletion (through MCD) protects motor neurons against brain-derived neurotrophic factor (BDNF)-induced excitotoxicity due to activation of TrkB receptors. Consistently, increased BDNF was found in muscles from ALS patients at an early stage of the disease.	Kust *et al.* (2002) Mojsilovic-Petrovic *et al.* (2006)
Multiple sclerosis	Lipid peroxidation that occurs in MS may be affected by cholesterol, that regulates membrane fluidity.	Carlson and Rose (2006)
Prion-related disorders	LRs are needed for the conversion of normal cellular prion protein (PrP^{C}) into its toxic modified form (PrP^{SC}). Consistently, reduced cholesterol synthesis (by squalestatin) protects cells against prion neurotoxicity.	Johnson and Gibbs (1998) Baron *et al.* (2002) Bate *et al.* (2004)

where membrane cholesterol plays a critical role, and the main targets of cholesterol action, are summarized in Table 1. This table is restricted to neurodegenerative diseases where also eCBs signalling is known to play a major role, whereas other cholesterol-dependent disease conditions, like the Niemann-Pick diseases, were not covered. In fact, Niemann-Pick diseases encompass a heterogenous group of pathologies where lysosomal lipid storage is compromised, leading to cholesterol (and sphingolipids) accumulation in endosomes and lysosomes of neurons due to erroneous cholesterol trafficking (Vance *et al.*, 2006). Yet, for Niemann-Pick disorders a role for eCBs signalling has not yet been recognized, and only a preliminary study showing that sphingomyelin hydrolysis is stimulated by cannabidiol in fibroblasts from a Niemann-Pick patient has been reported (Burstein *et al.*, 1984).

Cholesterol and CB_1 signalling in vitro

A role for membrane cholesterol in the functional regulation of CB_1 has been well documented in neuronal and non-neuronal cells cultured *in vitro* (for an updated review see Dainese *et al.*, 2010). LRs disruption by acute cholesterol depletion with methyl-β-cyclodextrin (MCD) has been shown to double CB_1-dependent signalling via adenylyl cyclase and mitogen-activated protein kinases in neuronal cells (Bari *et al.*, 2005a,b). Instead CB_2 receptor, which is structurally and functionally related to CB_1, and TRPV1 channel, which is also activated by eCBs like AEA (Di Marzo and De Petrocellis, 2010), are completely insensitive to the modulation of membrane cholesterol content (Bari *et al.*, 2006). Consistently, neither CB_2 nor TRPV1 reside in cholesterol-rich LRs (Bari *et al.*, 2006; Maccarrone, 2008; Rimmerman *et al.*, 2008). In this context, it seems noteworthy that 2-AG has been shown to be entirely localized in LRs of dorsal root ganglion cells, where also part of AEA (~30%) can be detected (Rimmerman *et al.*, 2008). However, most of AEA (~70%) was found in non-LR fractions, and it remains to be clarified whether these eCBs are produced directly within LRs, or are transported to (or accumulated within) these microdomains. The different interaction of AEA and 2-AG with membrane microdomains might have significant implications for eCBs-dependent autocrine and/or retrograde-paracrine signalling pathways, and further studies are needed to clarify which structural determinants are responsible for a different localization of two apparently similar eCBs within lipid bilayers (Maccarrone, 2008). The effects of LRs disruption by cholesterol depletion on eCBs-binding receptors are summarized in Table 2. Recently, some details of the molecular basis for the different response of these two receptor subtypes to membrane cholesterol have been clarified, by showing that both CB_1 and CB_2 have a cholesterol-binding domain (CRAC,

Table 2

Effect of LRs disruption by membrane cholesterol depletion on CB_1R, CB_2R and TRPV1 binding and signalling

	Cholesterol depletion				
	Binding		Signalling		
Receptor	K_d	B_{max}	[^{35}S]GTPγS[a]	AC[b]	MAPK[c]
CB_1R	↑	↑	↑	↓	↑
CB_2R	↔	↔	↔	↔	↔
TRPV1	↔	↔	nd	na	na

See text for reference to the original data.
[a]Non-hydrolysable analogue of GTP: it is a measure of the extent of the interaction between receptor and associated G protein.
[b]Adenylyl cyclase.
[c]Mitogen-activated protein kinase.
na, not applicable; nd, not determined.

cholesterol recognition amino acid sequence consensus) in helix 7 (Oddi *et al.*, 2011). In this context, it should be recalled that as yet a unique conserved structural determinant for protein interaction with cholesterol has not been identified, however, CRAC [L/V-X$_{(1–5)}$-Y-X$_{(1–5)}$-R/K] is a well known motif that enables this interaction (Epand, 2006). For instance, CRAC has been demonstrated in caveolin-1, peripheral-type benzodiazepine receptor (Li and Papadopoulos, 1998; Jamin *et al.*, 2005), and in other proteins targeted to LRs (Xie *et al.*, 2010). Interestingly, by sequence alignment of human CB_1 and CB_2 we have demonstrated the presence of a CRAC(-like) motif in the last 11 amino acids of the transmembrane helix 7 of both CB_1 and CB_2, yet with small but very important sequence differences between the two receptor subtypes (Oddi *et al.*, 2011). In particular, we found that in the highly conserved 'CRAC-like' region (82% amino acid identity), CB_1 differs from CB_2 for one residue only: lysine 402 of CB_1 corresponds to glycine 304 in CB_2 (Oddi *et al.*, 2011). Therefore, CB_1 has a true CRAC domain, whereas CB_2 has not. In keeping with this observation, we found that the CB_1(K402G) mutant where the CRAC sequence of CB_1 was converted into that of CB_2 had a reduced propensity to reside in cholesterol-rich membrane regions, and lost its sensitivity to membrane cholesterol enrichment (Oddi *et al.*, 2011). Therefore, these data suggest that one residue in complex proteins like GPCRs can be enough to direct their interaction with membrane lipids, thus affecting their localization within LRs and signal transduction thereof. Additionally, we found that the C-terminal component of CB_1, that is, the intracellular juxtamembrane helix 8, contains a cysteine residue (C415, in green) that could be constitutively palmitoylated (Dainese *et al.*, 2010). The latter reversible post-translational modification can be used by cells to regulate CB_1 targeting to LRs, thus influencing subsequent G protein-dependent signalling. Remarkably, a palmitoylation site corresponding to C415 is absent in CB_2 (Dainese *et al.*, 2010), and running experiments in our laboratory are aimed at ascertaining whether also this residue might contribute to LR localization of CB_1. The positions of CRAC domain and C415 in the three-dimensional structure of CB_1 are depicted in Figure 2.

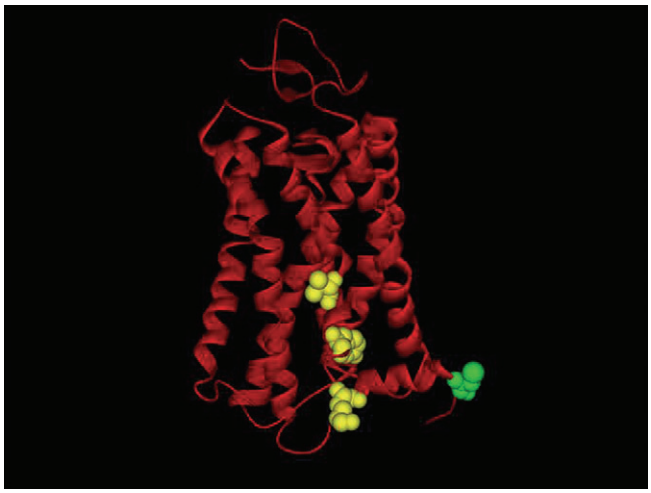

Figure 2

Three-dimensional model of type-1 cannabinoid receptor (CB_1), based on sequence alignment with visual rhodopsin in the inactivated state (PDB code: 1F88). The model was obtained using the protein structure homology-modeling server SWISS-MODEL, integrated in the Deep-View program (Dainese *et al.*, 2010). The three residues (V392, Y397, K402) that form the CRAC sequence are represented as yellow spheres, sized to the Van der Waals radii; these residues belong to the transmembrane helix 7 of CB_1. Additionally, the C-terminal component of CB_1, that is, the intracellular juxtamembrane helix 8, contains a cysteine residue (C415, in green) that could be constitutively palmitoylated. The model was kindly provided by Dr. Enrico Dainese (University of Teramo, Italy). See text for further details.

Overall, we believe that the observation that even a single residue in CB receptors can regulate the activity of eCBs signalling might impact on the therapeutic exploitation of CB_1-dependent versus CB_2-dependent biological activity of these lipid signals. In the next section, evidence showing that within the striatum cholesterol regulation of eCBs signalling through CB_1 can indeed have major consequences on *in vivo* neurotransmission is presented.

Impact of membrane cholesterol on CB_1-dependent neurotransmission within the striatum

The nucleus striatum is a subcortical brain area involved in motor, cognitive and emotional processes (Packard, 2009; Rodriguez-Oroz *et al.*, 2009; Simpson *et al.*, 2010), and is highly enriched in CB_1 receptors controlling both glutamate and GABA transmission, mainly through presynaptic mechanisms (Ferré *et al.*, 2010; Rossi *et al.*, 2010a). Direct pharmacological agonists of CB_1 receptors, in fact, reduce the release of both transmitters, as demonstrated in several studies (Szabo *et al.*, 1998; Gerdeman and Lovinger, 2001; Huang *et al.*, 2001; Köfalvi *et al.*, 2005; Narushima *et al.*, 2006; Maccarrone *et al.*, 2008). Evidence exists that the two sets of CB_1 receptors differ for the preferential eCB activating them, and also have different regulation mechanisms. CB1 receptors controlling striatal glutamate synapses [CB_1R(Glu)], but not those regulating GABA transmission [CB_1R(GABA)], in fact,

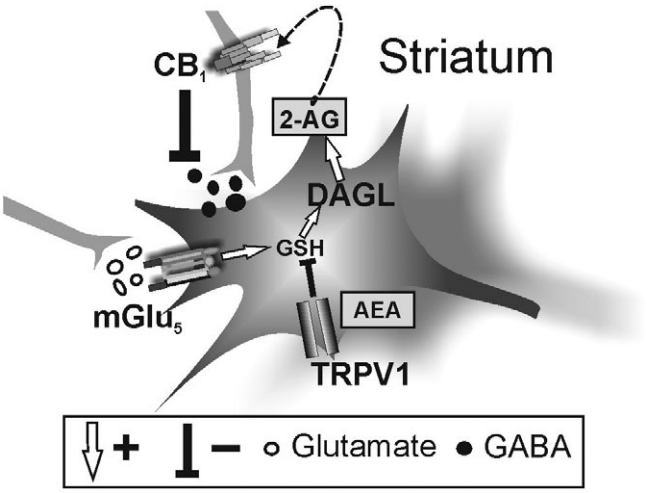

Figure 3

Overall scheme of the interactions between *N*-arachidonoylethanolamine (AEA), type-1 transient receptor potential vanilloid (TRPV1), 2-arachidonoylglycerol (2-AG) and metabotropic glutamate 5 ($mGlu_5$) receptors in the striatum. Stimulation of $mGlu_5$ receptors increases 2-AG synthesis by enhancing glutathione (GSH) production. 2-AG acts as retrograde signal to limit GABA release through the stimulation of presynaptic type-1 cannabinoid receptor (CB_1) receptors. AEA, on the other hand, stimulates TRPV1 channels, presumably located in the somatodendritic region of striatal neurons, and modulates 2-AG metabolism and physiological effects by inhibiting GSH-stimulated diacylglycerol lipase (DAGL) activity (adapted from Maccarrone *et al.*, 2008).

seem to be targeted preferentially by AEoA, because we have found that genetic or pharmacological inhibition of the AEA degrading enzyme fatty acid amide hydrolase (FAAH), that selectively increases AEA levels, inhibits glutamate but not GABA transmission in a CB_1 receptor-dependent manner (Maccarrone *et al.*, 2008; Rossi *et al.*, 2010b). On the other hand, stimulation of endogenous 2-AG synthesis only affects GABA synapses by activating CB_1R(GABA) (Maccarrone *et al.*, 2008).

Several neurotransmitters engage the eCB system in the striatum, and these include dopamine through D_2 receptors (Centonze *et al.*, 2004; Yin and Lovinger, 2006), neurotensin (Yin *et al.*, 2008), acetylcholine through M_1 receptors (Narushima *et al.*, 2007; Uchigashima *et al.*, 2007; Musella *et al.*, 2010) and glutamate through metabotropic glutamate 5 ($mGlu_5$) receptors (Jung *et al.*, 2005; 2007; Maccarrone *et al.*, 2008). Stimulation of $mGlu_5$ receptor enhances the activity of the 2-AG synthesizing enzyme diacylglycerol lipase (DAGL) (Jung *et al.*, 2005; 2007), and the resulting 2-AG synthesis and physiological activity on CB_1 receptors inhibits GABAergic inhibitory postsynaptic currents (IPSCs) in the striatum (Maccarrone *et al.*, 2008). Of note, elevation of AEA content by pharmacological or genetic inhibition of FAAH regulates the mGlu5 receptor/2-AG interaction, through the stimulation of TRPV1 channels and the resulting inhibition of glutathione metabolism (Maccarrone *et al.*, 2008) (Figure 3). Evidence exists that 2-AG is produced postsynaptically in striatal neurons in response to $mGlu_5$ receptor-dependent DAGL stimulation, and that it acts presynaptically on CB_1 receptors to inhibit GABA release (Katona *et al.*, 2006). Accordingly, an ultrastructural study demonstrated that DAGL and $mGlu_5$ receptors are tightly associated on the somatodendritic surface of striatal projection neurons, whereas CB_1 receptors are particularly enriched on GABAergic axon terminals of striatal neurons (Uchigashima *et al.*, 2007).

Membrane cholesterol has been recently found to play a substantial role in $mGlu_5$ receptor/eCB coupling. Depletion of membrane cholesterol with MCD, in fact, failed to alter AEA metabolism in the striatum, because both the AEA synthesizing enzyme *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD) and FAAH were unaffected by the treatment. Instead, the activity of DAGL was significantly enhanced in MCD-treated striatal slices, whereas the activity of the 2-AG degrading enzyme monoacylglycerol lipase (MAGL) was not. As a result, 2-AG striatal contents were increased to levels that are able to stimulate CB_1 receptors in control slices (Maccarrone *et al.*, 2009). Surprisingly, however, the potentiated 2-AG synthesis observed after cholesterol depletion from striatal neuron membranes was not associated with increased activity of this eCB on CB_1 receptors, neither in basal conditions nor after the stimulation of $mGlu_5$ receptors with DHPG. The frequency of spontaneous and miniature GABA-mediated IPSCs was in fact normal in MCD-treated slices, while a frequency reduction of IPSCs normally accompanies the effect of 2-AG on CB_1R(GABA) (Maccarrone *et al.*, 2008; 2009). Furthermore, blockade of CB_1 receptors did not increase IPSC frequency, again supporting the conclusion that MCD-mediated elevation of 2-AG did not result in enhanced activity of this eCB on CB_1 receptors. We did not observe any CB_1 receptor-mediated effect on GABA-mediated IPSCs even after the exacerbation of 2-AG metabolism with DHPG, an effect that was particularly surprising because both CB_1 receptor binding and activity were increased, and not down-regulated, in MCD-treated striatal slices. Furthermore, the sensitivity of CB_1 receptors was also addressed in physiological experiments, demonstrating that the ability of a synthetic agonist of CB_1 receptors to inhibit IPSCs was intact after cholesterol depletion with MCD (Maccarrone *et al.*, 2009).

MCD failed to affect $mGlu_5$ receptor binding, also ruling out that the sensitivity of this receptor was altered following cholesterol depletion (Maccarrone *et al.*, 2009). Instead, by means of double immunofluorescence, we demonstrated a relocalization of both CB_1 receptors and $mGlu_5$ receptors on striatal neurons after MCD treatment, while cellular fluorescence densitometry confirmed a significantly higher CB_1 receptor expression after MCD treatment (Maccarrone *et al.*, 2009). These results are therefore compatible with the idea that lipid rafts not only influence receptor expression and function in membranes but also their spatial orientation. Accordingly, MCD treatment of striatal slices did not alter the general staining of $mGlu_1$ receptors, which has many pharmacological, signal transduction and physiological homologies with the $mGlu_5$ receptors but is expressed outside lipid rafts in neuronal membranes (Becher *et al.*, 2001).

Given the localization of CB_1 receptors (Bari *et al.*, 2005b; 2008) and possibly of $mGlu_5$ receptors (Moffett *et al.*, 2000; Oh and Schnitzer, 2001; Fourgeaud *et al.*, 2003) in lipid rafts, and the substantial role of these cholesterol-enriched membrane subdomains in the limitation of movements of raft-

associated proteins (Lucero and Robbins, 2004; Hanzal-Bayer and Hancock, 2007), it is plausible that MCD disrupts 2-AG-mediated $mGlu_5$ receptor-CB_1 receptor interaction by altering CB_1 receptor and $mGlu_5$ receptor membrane localization (Maccarrone *et al.*, 2009).

Involvement of striatal eCB signalling in physiological and pathological contexts

The data above assign to membrane cholesterol in lipid rafts a previously unexpected role in the regulation of synaptic transmission, with potentially very relevant consequences for the understanding of the cellular correlates of several physiological states and of pathological conditions. The pharmacological response of raft-associated striatal CB_1Rs(GABA) and $mGlu_5R$/2-AG coupling is in fact modulated by a variety of conditions, which include cocaine addiction, stress-induced anxiety, voluntary exercise, caffeine and palatable food assumption, as well as in models of neurological diseases such as HD, MS, ALS and Fragile X syndrome (FXS). It follows therefore that the modulation of cholesterol metabolism in membranes might be considered in the near future as a valuable alternative option for the treatment of frequent and severe neuropsychiatric conditions.

The maximal response of striatal CB_1Rs(GABA) is significantly enhanced by chronic cocaine, when rodents develop overt addictive behaviours, but not by a single administration of the psychostimulant (Centonze *et al.*, 2007a; Rossi *et al.*, 2008; De Chiara *et al.*, 2010a,b). Similar potentiation of CB_1R(GABA) signalling is seen in mice drinking chronically another psychoactive compound, caffeine (Rossi *et al.*, 2009), or exposed to running wheel or given access to a drinking solution containing sucrose (De Chiara *et al.*, 2010a). Of note, voluntary running activity has strong rewarding and reinforcing properties in rodents, and shares many neurochemical and behavioural characteristics with drug-induced reward situations, through the activation of dopamine signalling and the modulation of striatal neuron activity (Werme *et al.*, 2000; 2002; Lett *et al.*, 2001; De Visser *et al.*, 2007). Similarly, sweet foods and drinks also have intense rewarding properties (Lenoir *et al.*, 2007), and many commonalities exist between overconsumption of sugars and drug addiction, including the stimulation of the dopamine signalling in the striatum (Levine *et al.*, 2003; Kelley, 2004; Volkow and Wise, 2005). Thus, the evidence that chemical (cocaine) and natural rewards (running wheel and sucrose) share the common property of enhancing CB_1R(GABA) responses in the striatum suggest that this receptor subtype is involved in the modulation of complex dopamine- and reward-based behaviours. Accordingly, blockade of dopamine D_2 receptors prevents the cocaine-, running- and sucrose-induced sensitization of CB_1R(GABA) receptors (De Chiara *et al.*, 2010a,b).

Because of the ability of rewarding experiences to contrast the behavioural effects of stress, the data presented earlier might indicate that striatal CB_1R(GABA) are involved in the mood disorders, such as anxiety and depression. Accordingly, we have demonstrated that stress-induced anxious-depressive behaviour is associated with the complete loss of the sensitivity of striatal CB_1Rs(GABA), and that cocaine, caffeine, running wheel or sucrose are able to contrast these effects (Rossi *et al.*, 2008; 2009; De Chiara *et al.*, 2010a,b).

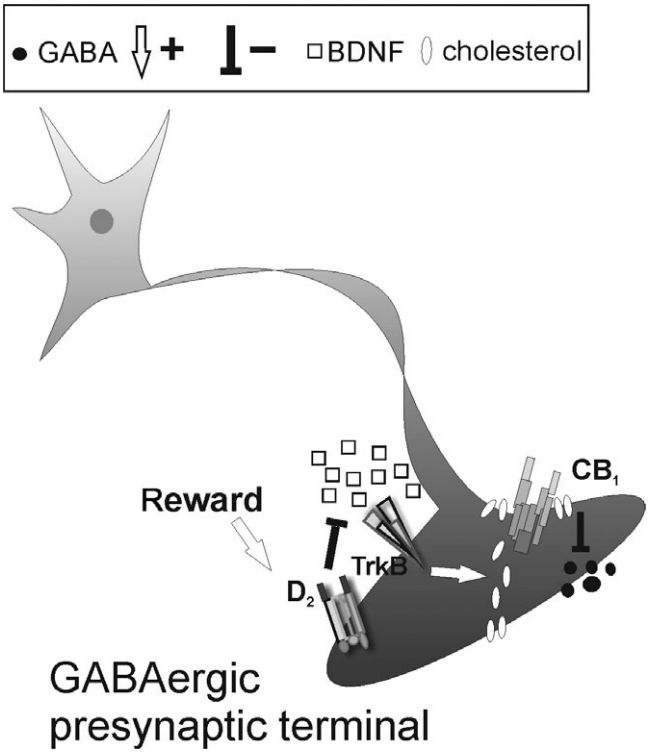

Figure 4

Schematic representation of the interaction between D_2 receptors, brain-derived neurotrophic factor (BDNF) and type-1 cannabinoid receptor (CB_1) in striatal neurons. Stimulation of dopamine D_2 receptors trough chemical (cocaine) and natural rewards (voluntary exercise or sucrose drinking) reduces striatal contents of BDNF, thus reducing cholesterol synthesis and incorporation within CB_1 receptor-containing lipid rafts mediated by the activation of the tyrosine receptor TrkB. This effect results in increased sensitivity of CB_1Rs(GABA), and is limited to striatal GABAergic neuronal elements. The mechanism by which D_2 receptors reduce BDNF concentrations is conversely still unknown (adapted from De Chiara *et al.* 2010a,b).

Membrane cholesterol controls the pharmacological response of striatal CB_1Rs(GABA) (Maccarrone *et al.*, 2009), and it can be hypothesized therefore that compounds able to interfere with lipid raft composition could be effective in the management of mood disorders. In agreement with this speculation, recent evidence showed that brain-derived neurotrophic factor (BDNF) levels in the striatum are regulated by dopamine D_2 receptors, and that BDNF is able to block CB_1R(GABA) activity in the striatum. Importantly, this effect was dependent on increased synthesis and raft concentration of cholesterol mediated by BDNF (De Chiara *et al.*, 2010b) (Figure 4). Importantly, previous evidence showed that striatal infusion of BDNF elicits a depressive behaviour (Eisch *et al.*, 2003), and that the anxious-depressive behaviour induced by social stress is abolished by blockade of BDNF signalling in this brain area (Berton *et al.*, 2006). These observations therefore represent a first indication that raft-associated receptors, such as CB_1Rs(GABA), are involved in mood disorders and that inhibition of cholesterol synthesis might be useful to treat anxious depressive symptoms.

Raft-associated CB_1Rs(GABA) in the striatum are also altered in other pathological conditions, and namely neurodegenerative disorders. One of the earliest neurochemical alterations observed in HD patients, in fact, consists in the loss of CB_1 receptor binding in the striatum, an alteration that significantly precedes the development of identifiable striatal neuropathology, and that might play a critical role in the development of HD symptoms (Richfield and Herkenham, 1994; Glass *et al*., 2000). In addition, down-regulation of CB_1 receptors also occurs in the striatum of transgenic mouse models of HD prior to the development of either neuropsychiatric symptoms or neuronal degeneration (Denovan-Wright and Robertson, 2000; Lastres-Becker *et al*., 2002), and environmental enrichment, which delays the onset of HD symptoms in transgenic mice, is associated with a delayed loss of CB_1 receptors (Glass *et al*., 2004). Together, these findings are compatible with the idea that CB_1 receptors are heavily implicated in HD pathophysiology and, in line with this idea, we have found in R6/2 HD mice that the sensitivity of CB_1Rs(GABA) in the striatum was lost since the early phases of the disease, while the activity of CB_1Rs(Glu) was intact (Centonze *et al*., 2005).

Loss of CB_1R(GABA) responses and preserved CB_1R(Glu) function is also seen in experimental autoimmune encephalomyelitis (EAE), a model of MS in which the eCB system has been proposed to play a major role (Centonze *et al*., 2007b; Rossi *et al*., 2010a). In EAE, in fact, pharmacological stimulation of CB_1 receptors with HU210 fails to inhibit GABAergic IPSCs recorded from striatal neurons, while glutamate synapses are inhibited, as in control conditions, by HU210 (Centonze *et al*., 2007b).

More complex alterations of the eCB system are observed in the striatum of experimental ALS, in which the maximal response of both CB_1Rs(GABA) and CB_1Rs(Glu) is enhanced (Rossi *et al*., 2010c), and in the mouse model of FXS (Maccarrone *et al*., 2009). In FXS mice, altered $mGlu_5$ receptor signalling has been demonstrated in several studies (Huber *et al*., 2002; Bear *et al*., 2004). Recently, we have provided evidence that also $mGlu_5$ receptor/2-AG coupling was altered in these mice. In the striatum of mice lacking fragile X mental retardation protein (FMRP), we found in fact enhanced activity of DAGL, associated with altered sensitivity of GABA synapses to the mobilization of 2-AG by $mGlu_5$ receptor stimulation (Maccarrone *et al*., 2010b). Our data therefore indicate for the first time that $mGlu_5$ receptor-driven eCB signalling in the striatum is under the control of FMRP, and that abnormal $mGlu_5$ receptor/2-AG coupling might play a role in the synaptic defects of FXS.

Conclusions

Modulation of CB_1 receptor activity is receiving increasing attention as a novel, promising strategy in the treatment of neuropsychiatric and neurodegenerative disorders. So far, however, *Cannabis sativa* extracts to activate these receptors (Howlett *et al*., 2010; Pertwee, 2010), or synthetic antagonists to block them have been of limited clinical utility, because severe psychiatric symptoms have been associated with their use (Hill and Gorzalka, 2009). An alternative option intensely explored is the modulation of eCB activity at these receptors, through the regulation of AEA or 2-AG metabolism (Petrosino and Di Marzo, 2010; Rossi *et al*., 2010b). It is still fully unexplored whether the modulation of cholesterol/CB_1 receptor interaction could be a useful approach to modulate eCB signalling in the brain.

Here, we would like to comment that subtle, yet specific, differences might underpin the differential sensitivity of CB_1 and CB_2 to membrane cholesterol, possibly explaining the apparent redundancy of having two largely overlapping receptor subtypes that are activated by similar compounds (eCBs) and trigger similar transduction pathways (Di Marzo, 2009; Maccarrone *et al*., 2010a).

In general, cholesterol may act on the conformation of a membrane receptor by indirectly altering the physicochemical properties of the bilayer, or by directly interacting with the receptor itself, for example, through the CRAC domain and/or palmitoylation sites. More generally, we believe that the comparison between CB_1 and CB_2 might represent an interesting paradigm that goes well beyond eCB signalling. In fact, the modulation of CB_1 by membrane cholesterol might disclose a novel ligand-receptor interaction, where a third player comes into the game: the membrane lipids. As a consequence, the membrane environment might play a role in receptor-dependent signalling, with a potential impact on several neurotransmission pathways, as well as several neurodegenerative/neuroinflammatory diseases where CB_1 is known to play a role. It should be recalled that CB_1-dependent signalling impacts fundamental processes as different as immune response, energy homeostasis, reproduction and skin differentiation (Di Marzo, 2009; Maccarrone *et al*., 2010a), thus it can be anticipated that cholesterol-dependent regulation of CB_1 can have a physiological relevance well beyond the CNS.

In conclusion, membrane environment seems to be critical for the regulation of signal transduction pathways triggered by G protein-coupled receptors like CB_1. Despite the three dimensional complexity of these proteins, we learn from the comparison of CB_1 with CB_2 that just one amino acid residue can direct receptor functioning, calling for attention on the plasma membrane as a key-player in ligand recognition on the cell surface. Further studies aimed at clarifying the impact of these notions for the treatment of disorders associated with abnormal activity of CB_1 are desirable to come in the near future.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Adibhatla RM, Hatcher JF (2008). Altered lipid metabolism in brain injury and disorders. Subcell Biochem 49: 241–268.

Alexander SPH, Mathie A, Peters JA (2009). Guide to receptors and channels (GRAC). 4th edn. Br J Pharmacol 158: S1–S254.

Anderson RG, Kamen BA, Rothberg KG, Lacey SW (1992). Potocytosis: sequestration and transport of small molecules by caveolae. Science 255: 410–411.

Bari M, Paradisi A, Pasquariello N, Maccarrone M (2005a). Cholesterol-dependent modulation of type 1 cannabinoid receptors in nerve cells. J Neurosci Res 81: 275–283.

Bari M, Battista N, Fezza F, Finazzi-Agrò A, Maccarrone M (2005b). Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. J Biol Chem 280: 12212–12220.

Bari M, Spagnuolo P, Fezza F, Oddi S, Pasquariello N, Finazzi-Agrò A *et al.* (2006). Effect of lipid rafts on Cb2 receptor signaling and 2-arachidonoyl-glycerol metabolism in human immune cells. J Immunol 177: 4971–4980.

Bari M, Oddi S, De Simone C, Spagnuolo P, Gasperi V, Battista N *et al.* (2008). Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells. Neuropharmacology 54: 45–50.

Barnett-Norris J, Lynch D, Reggio PH (2005). Lipids, lipid rafts and caveolae: their importance for GPCR signaling and their centrality to the endocannabinoid system. Life Sci 77: 1625–1639.

Baron GS, Wehrly K, Dorward DW, Chesebro B, Caughey B (2002). Conversion of raft-associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP(Sc)) into contiguous membranes. EMBO J 21: 1031–1040.

Bate C, Salmona M, Diomede L, Williams A (2004). Squalestatin cures prioninfected neurons and protects against prion neurotoxicity. J Biol Chem 279: 14983–14990.

Bear MF, Huber KM, Warren ST (2004). The mGluR theory of fragile X mental retardation. Trends Neurosci 27: 370–377.

Becher A, White JH, McIlhinney RA (2001). The gamma-aminobutyric acid receptor B, but not the metabotropic glutamate receptor type-1, associates with lipid rafts in the rat cerebellum. J Neurochem 79: 787–795.

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ *et al.* (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311: 864–868.

Bhaskar K, Yen S-H, Lee G (2005). Disease-related modifications in tau affect the interaction between Fyn and tau. J Biol Chem 280: 35119–35125.

Bisogno T, Di Marzo V (2010). Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. CNS Neurol Disord Drug Targets 9: 564–573.

Brown DA, London E (1998). Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 14: 111–136.

Burstein S, Hunter SA, Renzulli L (1984). Stimulation of sphingomyelin hydrolysis by cannabidiol in fibroblasts from a Niemann-Pick patient. Biochem Biophys Res Commun 121: 168–173.

Carlson NG, Rose JW (2006). Antioxidants in multiple sclerosis: do they have a role in therapy? CNS Drugs 20: 433–441.

Centonze D, Battista N, Rossi S, Mercuri NB, Finazzi-Agrò A, Bernardi G *et al.* (2004). A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal GABAergic transmission. Neuropsychopharmacology 29: 1488–1497.

Centonze D, Rossi S, Prosperetti C, Tscherter A, Bernardi G, Maccarrone M *et al.* (2005). Abnormal sensitivity to cannabinoid receptor stimulation might contribute to altered gamma-aminobutyric acid transmission in the striatum of R6/2 Huntington's disease mice. Biol Psychiatry 57: 1583–1589.

Centonze D, Rossi S, De Chiara V, Prosperetti C, Battista N, Bernardi G *et al.* (2007a). Chronic cocaine sensitizes striatal GABAergic synapses to the stimulation of cannabinoid CB1 receptors. Eur J Neurosci 25: 1631–1640.

Centonze D, Bari M, Rossi S, Prosperetti C, Furlan R, Fezza F *et al.* (2007b). The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. Brain 130: 2543–2553.

Centonze D, Battistini L, Maccarrone M (2008). The endocannabinoid system in peripheral lymphocytes as a mirror of neuroinflammatory diseases. Curr Pharm Design 14: 2370–2382.

Chau PL (2010). New insights into the molecular mechanisms of general anaesthetics. Br J Pharmacol 161: 288–307.

Dainese E, Oddi S, Maccarrone M (2010). Interaction of endocannabinoid receptors with biological membranes. Curr Med Chem 17: 1487–1499.

De Chiara V, Errico F, Musella A, Rossi S, Mataluni G, Sacchetti L *et al.* (2010a). Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. Neuropsychopharmacology 35: 374–387.

De Chiara V, Angelucci F, Rossi S, Musella A, Cavasinni F, Cantarella C *et al.* (2010b). Brain-derived neurotrophic factor controls cannabinoid CB1 receptor function in the striatum. J Neurosci 30: 8127–8137.

De Visser L, Van Den Bos R, Stoker AK, Kas MJ, Spruijt BM (2007). Effects of genetic background and environmental novelty on wheel running as a rewarding behaviour in mice. Behav Brain Res 177: 290–297.

Denovan-Wright EM, Robertson HA (2000). Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. Neuroscience 98: 705–713.

Di Marzo V (2009). The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. Pharmacol Res 60: 77–84.

Di Marzo V, De Petrocellis L (2010). Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. Curr Med Chem 17: 1430–1449.

Dietschy JM, Turley SD (2004). Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 45: 1375–1397.

Dirikoc S, Priola SA, Marella M, Zsürger N, Chabry J (2007). Nonpsychoactive cannabidiol prevents prion accumulation and protects neurons against prion toxicity. J Neurosci 27: 9537–9544.

Eckert GP, Wood WG, Müller WE (2010). Lipid membranes and beta-amyloid: a harmful connection. Curr Protein Pept Sci 11: 319–325.

Eisch AJ, Bolaños CA, de Wit J, Simonak RD, Pudiak CM, Barrot M *et al.* (2003). Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biol Psychiatry 54: 994–1005.

Epand RM (2006). Cholesterol and the interaction of proteins with membrane domains. Prog Lipid Res 45: 279–294.

Fallon L, Moreau F, Croft BG, Labib N, Gu WJ, Fon EA (2002). Parkin and CASK/LIN-2 associate via a PDZ-mediated interaction and are co-localized in lipid rafts and postsynaptic densities in brain. J Biol Chem 277: 486–491.

Fantini J, Yahi N (2010). Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases. Expert Rev Mol Med 12: e27.

Ferré S, Lluís C, Justinova Z, Quiroz C, Orru M, Navarro G *et al.* (2010). Adenosine-cannabinoid receptor interactions. Implications for striatal function. Br J Pharmacol 160: 443–453.

Fielding CJ, Fielding PE (2001). Cellular cholesterol efflux. Biochim Biophys Acta 1533: 175–189.

Fivaz M, Vilbois F, Thurnheer S, Pasquali C, Abrami L, Bickel PE *et al.* (2002). Differential sorting and fate of endocytosed GPI-anchored proteins. EMBO J 21: 3989–4000.

Forneris F, Mattevi A (2008). Enzymes without borders: mobilizing substrates, delivering products. Science 321: 213–216.

Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH (2004). Lipid rafts mediate the synaptic localization of alpha-synuclein. J Neurosci 24: 6715–6723.

Fourgeaud L, Bessis AS, Rossignol F, Pin JP, Olivo-Marin JC, Hémar A (2003). The metabotropic glutamate receptor mGluR5 is endocytosed by a clathrin-independent pathway. J Biol Chem 278: 12222–12230.

Galbiati F, Razani B, Lisanti MP (2001). Emerging themes in lipid rafts and caveolae. Cell 106: 403–411.

Ge M, Field KA, Aneja R, Holowka D, Baird B, Freed JH (1999). Electron spin resonance characterization of liquid ordered phase of detergent-resistant membranes from RBL-2H3 cells. Biophys J 77: 925–933.

Gerdeman G, Lovinger DM (2001). CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85: 468–471.

Glass M, Dragunow M, Faull RL (2000). The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and $GABA_A$ receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97: 505–519.

Glass M, van Dellen A, Blakemore C, Hannan AJ, Faull RLM (2004). Delayed onset of Huntington's disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. Neuroscience 123: 207–212.

Grimm MO, Grimm HS, Tomic I, Beyreuther K, Hartmann T, Bergmann C (2008). Independent inhibition of Alzheimer disease beta- and gammasecretase cleavage by lowered cholesterol levels. J Biol Chem 283: 11302–11311.

Hanzal-Bayer MF, Hancock JF (2007). Lipid rafts and membrane traffic. FEBS Lett 581: 2098–2104.

Hatano T, Kubo SI, Imai S, Maeda M, Ishikawa K, Mizuno Y *et al.* (2007). Leucine-rich repeat kinase 2 associates with lipid rafts. Hum Mol Genet 16: 678–690.

Hayashi H, Campenot RB, Vance DE, Vance JE (2004). Glial lipoproteins stimulate axon growth of central nervous system neurons in compartmented cultures. J Biol Chem 279: 14009–14015.

Hill MN, Gorzalka BB (2009). Impairments in endocannabinoid signaling and depressive illness. JAMA 301: 1165–1166.

Howlett AC, Blume LC, Dalton GD (2010). CB_1 cannabinoid receptors and their associated proteins. Curr Med Chem 17: 1382–1393.

Huang CC, Lo SW, Hsu KS (2001). Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. J Physiol 532: 731–748.

Huber KM, Gallagher SM, Warren ST, Bear MF (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 99: 7746–7750.

Iuvone T, Esposito G, De Filippis D, Scuderi C, Steardo L (2009). Cannabidiol: a promising drug for neurodegenerative disorders? CNS Neurosci Ther 15: 65–75.

Jamin N, Neumann JM, Ostuni MA, Vu TK, Yao ZX, Murail S *et al.* (2005). Characterization of the cholesterol recognition amino acid consensus sequence of the peripheral-type benzodiazepine receptor. Mol Endocrinol 19: 588–594.

Johnson RT, Gibbs CJ, Jr (1998). Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies. N Engl J Med 339: 1994–2004.

Jung KM, Mangieri R, Stapleton C, Kim J, Fegley D, Wallace M *et al.* (2005). Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. Mol Pharmacol 68: 1196–1202.

Jung KM, Astarita G, Zhu C, Wallace M, Mackie K, Piomelli D (2007). A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent endocannabinoid mobilization. Mol Pharmacol 72: 612–621.

Kalvodova L, Kahya N, Schwille P, Ehehalt R, Verkade P, Drechsel D *et al.* (2005). Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids *in vitro*. J Biol Chem 280: 36815–36823.

Karten B, Campenot RB, Vance DE, Vance JE (2006). Expression of ABCG1, but not ABCA1, correlates with cholesterol release by cerebellar astroglia. J Biol Chem 281: 4049–4057.

Katona I, Freund TF (2008). Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med 14: 923–930.

Katona I, Urbán GM, Wallace M, Ledent C, Jung KM, Piomelli D *et al.* (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26: 5628–5637.

Kelley AE (2004). Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44: 161–179.

Köfalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA *et al.* (2005). Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J Neurosci 25: 2874–2884.

Kubo S-I, Neman VM, Chalkley RJ, Anthony MD, Hattori N, Mizuno Y *et al.* (2005). A combinatorial code for the interaction of alphasynuclein with membranes. J Biol Chem 280: 31664–31672.

Kust BM, Copray JC, Brouwer N, Troost D, Boddeke HW (2002). Elevated levels of neurotrophins inhumanbiceps brachii tissue of amyotrophic lateral sclerosis. Exp Neurol 177: 419–427.

Lastres-Becker I, Berrendero F, Lucas JJ, Martin-Aparicio E, Yamamoto A, Ramos JA *et al.* (2002). Loss of mRNA levels, binding and activation of GTP-binding proteins for cannabinoid CB_1 receptors in the basal ganglia of a transgenic model of Huntington's disease. Brain Res 929: 236–242.

Lenoir M, Serre F, Cantin L, Ahmed SH (2007). Intense sweetness surpasses cocaine reward. PloS One 2: e698.

Lett BT, Grant VL, Koh MT (2001). Naloxone attenuates the conditioned place preference induced by wheel running in rats. Physiol Behav 72: 355–358.

Levine AS, Kotz CM, Gosnell BA (2003). Sugars: hedonic aspects, neuroregulation, and energy balance. Am J Clin Nutr 78: 834S–842S.

Li H, Papadopoulos V (1998). Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. Endocrinology 139: 4991–4997.

Lindahl E, Sansom MS (2008). Membrane proteins: molecular dynamics simulations. Curr Opin Struct Biol 18: 425–431.

Liu JP, Tang Y, Zhou S, Toh BH, McLean C, Li H (2010). Cholesterol involvement in the pathogenesis of neurodegenerative diseases. Mol Cell Neurosci 43: 33–42.

Lucero HA, Robbins PW (2004). Lipid rafts-protein association and the regulation of protein activity. Arch Biochem Biophys 426: 208–224.

Maccarrone M (2008). Good news for CB1 receptors: endogenous agonists are in the right place. Br J Pharmacol 153: 179–181.

Maccarrone M, Battista N, Centonze D (2007). The endocannabinoid pathway in Huntington's disease: a comparison with other neurodegenerative diseases. Prog Neurobiol 81: 349–379.

Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A *et al.* (2008). Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. Nat Neurosci 11: 152–159.

Maccarrone M, De Chiara V, Gasperi V, Viscomi MT, Rossi S, Oddi S *et al.* (2009). Lipid rafts regulate 2-arachidonoylglycerol metabolism and physiological activity in the striatum. J Neurochem 109: 371–381.

Maccarrone M, Gasperi V, Catani MV, Diep TA, Dainese E, Hansen HS (2010a). The endocannabinoid system and its relevance for nutrition. Annu Rev Nutr 30: 423–440.

Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A *et al.* (2010b). Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology 35: 1500–1509.

Martin M, Dotti CG, Ledesma MD (2010). Brain cholesterol in normal and pathological aging. Biochim Biophys Acta 1801: 934–944.

Moffett S, Brown DA, Linder ME (2000). Lipid-dependent targeting of G proteins into rafts. J Biol Chem 275: 2191–2198.

Mojsilovic-Petrovic J, Jeong GB, Crocker A, Arneja A, David S, Russell D *et al.* (2006). Protecting motor neurons from toxic insult by antagonism of adenosine A2a and Trk receptors. J Neurosci 26: 9250–9263.

Musella A, De Chiara V, Rossi S, Cavasinni F, Castelli M, Cantarella C *et al.* (2010). Transient receptor potential vanilloid 1 channels control acetylcholine/2-arachidonoylglicerol coupling in the striatum. Neuroscience 167: 864–871.

Narushima M, Uchigashima M, Hashimoto K, Watanabe M, Kano M (2006). Depolarization-induced suppression of inhibition mediated by endocannabinoids at synapses from fast-spiking interneurons to medium spiny neurons in the striatum. Eur J Neurosci 24: 2246–2252.

Narushima M, Uchigashima M, Fukaya M, Matsui M, Manabe T, Hashimoto K *et al.* (2007). Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. J Neurosci 27: 496–506.

Oddi S, Dainese E, Fezza F, Lanuti M, Barcaroli D, De Laurenzi V *et al.* (2011). Functional characterization of putative cholesterol binding sequence (CRAC) in human type-1 cannabinoid receptor. J Neurochem 116: 858–865.

Oh P, Schnitzer JE (2001). Segregation of heterotrimeric G proteins in cell surface microdomains. G(q) binds caveolin to concentrate in caveolae, whereas G(i) and G(s) target lipid rafts by default. Mol Biol Cell 12: 685–698.

Ostermeyer AG, Beckrich BT, Ivarson KA, Grove KE, Brown DA (1999). Glycosphingolipids are not essential for formation of detergent-resistant membrane rafts in melanoma cells. methyl-betacyclodextrin does not affect cell surface transport of a GPI anchored protein. J Biol Chem 274: 34459–34466.

Ostrom RS, Insel PA (2004). The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. Br J Pharmacol 143: 235–245.

Packard MG (2009). Anxiety, cognition, and habit: a multiple memory systems perspective. Brain Res 1293: 121–128.

Paila YD, Ganguly S, Chattopadhyay A (2010). Metabolic depletion of sphingolipids impairs ligand binding and signaling of human serotonin1A receptors. Biochemistry 49: 2389–2397.

Pani A, Mandas A, Dessì S (2010). Cholesterol, Alzheimer's disease, prion disorders: a ménage à trois? Curr Drug Targets 11: 1018–1031.

Patel KD, Davison JS, Quentin J, Pittman OJ, Sharkey KA (2010). Cannabinoid CB2 receptors in health and disease. Curr Med Chem 17: 1394–1410.

Pelkmans L (2005). Secrets of caveolae- and lipid raft-mediated endocytosis revealed by mammalian viruses. Biochim Biophys Acta 1746: 295–304.

Pertwee RG (2010). Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. Curr Med Chem 17: 1360–1381.

Petrosino S, Di Marzo V (2010). FAAH and MAGL inhibitors: therapeutic opportunities from regulating endocannabinoid levels. Curr Opin Investig Drugs 11: 51–62.

Pike LJ (2005). Growth factor receptors, lipid rafts and caveolae: an evolving story. Biochim Biophys Acta 1746: 260–273.

Pontier SM, Percherancier Y, Galandrin S, Breit A, Gales C, Bouvier M (2008). Cholesterol-dependent separation of the beta2-adrenergic receptor from its partners determines signaling efficacy: insight into nanoscale organization of signal transduction. J Biol Chem 283: 24659–24672.

Prinetti A, Loberto N, Chigorno V, Sonnino S (2009). Glycosphingolipid behaviour in complex membranes. Biochim Biophys Acta 1788: 184–193.

Richfield EK, Herkenham M (1994). Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. Ann Neurol 36: 577–584.

Rimmerman N, Hughes HV, Bradshaw HB, Pazos MX, Mackie K, Prieto AL *et al.* (2008). Compartmentalization of endocannabinoids into lipid rafts in a dorsal root ganglion cell line. Br J Pharmacol 153: 380–389.

Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E *et al.* (2009). Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. Lancet Neurol 8: 1128–1139.

Rossi S, De Chiara V, Musella A, Kusayanagi H, Mataluni G, Bernardi G *et al.* (2008). Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. J Neurosci 28: 7284–7292.

Rossi S, De Chiara V, Musella A, Mataluni G, Sacchetti L, Siracusano A *et al.* (2009). Caffeine drinking potentiates cannabinoid transmission in the striatum: interaction with stress effects. Neuropharmacology 56: 590–597.

Rossi S, Bernardi G, Centonze D (2010a). The endocannabinoid system in the inflammatory and neurodegenerative processes of multiple sclerosis and of amyotrophic lateral sclerosis. Exp Neurol 224: 92–102.

Rossi S, De Chiara V, Musella A, Sacchetti L, Cantarella C, Castelli M *et al.* (2010b). Preservation of striatal cannabinoid CB1 receptor function correlates with the antianxiety effects of fatty acid amide hydrolase inhibition. Mol Pharmacol 78: 260–268.

Rossi S, De Chiara V, Musella A, Cozzolino M, Bernardi G, Maccarrone M *et al.* (2010c). Abnormal sensitivity of cannabinoid CB1 receptors in the striatum of mice with experimental amyotrophic lateral sclerosis. Amyotroph Lateral Scler 11: 83–90.

Schengrund CL (2010). Lipid rafts: keys to neurodegeneration. Brain Res Bull 82: 7–17.

Schrattenholz A, Soskic V (2006). NMDA receptors are not alone: dynamic regulation of NMDA receptor structure and function by neuregulins and transient cholesterol-rich membrane domains leads to disease-specific nuances of glutamate-signalling. Curr Top Med Chem 6: 663–686.

Schweitzer JK, Krivda JP, D'Souza-Schorey C (2009). Neurodegeneration in Niemann-Pick Type C disease and Huntington's disease: impact of defects in membrane trafficking. Curr Drug Targets 10: 653–665.

Shrivastava S, Pucadyil TJ, Paila YD, Ganguly S, Chattopadhyay A (2010). Chronic cholesterol depletion using statin impairs the function and dynamics of human serotonin$_{1A}$ receptors. Biochemistry 49: 5426–5435.

Silvestri L, Caputo V, Bellacchio E, Atorino L, Dallapiccola B, Valente EM *et al.* (2005). Mitochondral import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. Hum Mol Genet 14: 3477–3492.

Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998). Cholesterol depletion inhibits the generation of β-amyloid in hippocampal neurons. Proc Natl Acad Sci U S A 95: 6460–6464.

Simpson EH, Kellendonk C, Kandel E (2010). A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron 65: 585–596.

Singer SJ, Nicolson GL (1972). The fluid mosaic model of the structure of cell membranes. Science 175: 720–731.

Sipione S, Rigamonti D, Valenza M, Zuccato C, Conti L, Pritchard J *et al.* (2002). Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Hum Mol Genet 11: 1953–1965.

Smart EJ, De Rose RA, Farber SA (2004). Annexin 2-caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport. Proc Natl Acad Sci U S A 101: 3450–3455.

Szabo B, Dörner L, Pfreundtner C, Nörenberg W, Starke K (1998). Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85: 395–403.

Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007). Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27: 3663–3676.

Uittenbogaard A, Everson WV, Matveev SV, Smart EJ (2002). Cholesteryl ester is transported from caveolae to internal membranes as part of a caveolin-annexin II lipid-protein complex. J Biol Chem 277: 4925–4931.

Valencia A, Reeves PB, Sapp E, Li S, Alexander J, Kegel KB *et al.* (2009). Mutant huntingtin and glycogen synthase kinase 3-β accumulate in neuronal lipid rafts of a presymptomatic knock-in mouse model of Huntington's disease. J Neurosci Res 29: 6106–6116.

Valenza M, Cattaneo E (2006). Cholesterol dysfunction in neurodegenerative diseases: is Huntington's disease in the list? Prog Neurobiol 80: 165–176.

Vance JE, Karten B, Hayashi H (2006). Lipid dynamics in neurons. Biochem Soc Trans 34: 399–403.

Vaya J, Schipper HM (2007). Oxysterols, cholesterol homeostasis, and Alzheimer disease. J Neurochem 102: 1727–1737.

Volkow ND, Wise RA (2005). How can drug addiction help us understand obesity? Nat Neurosci 8: 555–560.

Werme M, Thoren P, Olson L, Brene S (2000). Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. Eur J Neurosci 12: 2967–2974.

Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ *et al.* (2002). Delta FosB regulates wheel running. J Neurosci 22: 8133–8138.

Xie HQ, Liang D, Leung KW, Chen VP, Zhu KY, Chan WK *et al.* (2010). Targeting acetylcholinesterase to membrane rafts: a function mediated by the proline-rich membrane anchor (PRiMA) in neurons. J Biol Chem 285: 11537–11546.

Xu X, London E (2000). The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation. Biochemistry 39: 843–849.

Yin HH, Lovinger DM (2006). Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci U S A 103: 8251–8256.

Yin HH, Adermark L, Lovinger DM (2008). Neurotensin reduces glutamatergic transmission in the dorsolateral striatum via retrograde endocannabinoid signaling. Neuropharmacology 54: 79–86.

Zuccato C, Valenza M, Cattaneo E (2010). Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 90: 905–981.