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Review

The bright side of psychoactive substances: cannabinoid-based drugs in motor diseases

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Abstract

Introduction: psychoactive substances are associated with the idea of drugs with high addictive liability, affecting mental states, cognition, emotion and motor behavior. However these substances can modify synaptic transmission and help to disclose some mechanisms underlying alterations in brain processing and pathophysiology of motor disease. Hence, the "bright side" of e cannabinoid-based drugs must be thoroughly examined to be identified within the latter framework.

Areas covered: we will analyze the preclinical and clinical evidence of cannabinoid-based drugs, discussing their therapeutic value in basal ganglia motor disorders such as Parkinson's disease and Huntington disease.

Expert commentary: despite the knowledge acquired in the last years, the therapeutic potential of cannabinoid-based drugs should be further tested by novel routes of investigation. This should be focused on the role of cannabinoid signaling system in mitochondrial function as well as on the physical and functional interaction with other key receptorial targets belonging to this network.

Key words: Parkinson's disease; Huntington disease; endocannabinoid system; motor diseases; cannabinoid-based drugs; neuroprotection.

1. Cannabinoids and the "tune up" of brain locomotor circuits

The major and measurable impact of psychoactive substances (PS) is on motor function, and the extent to which PS affect, alter or modulate psychomotricity and locomotor patterns can be assumed as index of their whole action on central nervous system functioning. Indeed, a non-exhaustive list of PS should include at least ethanol, nicotine, hypnotics and sedatives, opioids, dopamine-active compounds and cannabinoids. All these PS have a dramatic impact on motor function producing sedation and motor incoordination (ethanol, hypnotics and sedatives, opioids), arousal, sensitization and locomotor-enhancing effects (nicotine), hyperlocomotion and motor sensitization (cocaine, amphetamines) and dose- and context-dependent opposite effects on motor activity (cannabinoids).

Thus, quite a few of these PS are considered of key importance for their action in the control of motor function and consequently for the therapeutic potential in specific pathophysiologic conditions. Here, we discuss some motor dysfunctions and disorders in which endocannabinoids (eCBs), synthetic and plant derivative cannabinoids modulate motor symptoms and can be

explored as therapeutic option for the treatment of movement disorders. However, given the broad definition of movement disorders we will focus on one selected hypokinetic disorder such as Parkinson's disease (PD) and one hyperkinetic and akinetic-rigid (in advanced stage) disorder such as Huntington's disease (HD). It is worth mention that focusing on PD and HD does not imply that cannabinoids are not involved in other extrapyramidal disorders such as Gilles de la Tourette's syndrome (TS), tardive dyskinesia and dystonia and would not be of interest to assess the effects of cannabinoid-based drugs in these disorders.Nevertheless, one may ask why cannabinoid-based drugs should be favored candidates for therapeutic intervention over other PS targeting for instance the cholinergic receptors. The legitimate question calls into play their special role in synaptic plasticity and balance of the basal ganglia (BG) output and their interaction with the major BG neurotransmitter systems (glutamatergic, dopamine-(DA)ergic, GABAergic and cholinergic) to select appropriate motor responses. The identification and cloning, in the brain and in immune organs of cannabinoid receptor type-1 (CB_1) and type-2 (CB_2), respectively, have opened new opportunities to understand how Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of *Cannabis sativa*, and its synthetic analogs, act to produce their pharmacological responses [1]. Both CB_1 and CB_2 receptors are seven-transmembrane domain proteins coupled to G-proteins type Gi/o and, less frequently, to the Gs type [2]. The existence of these two receptors entailed the presence of endogenous ligands or eCBs, i.e. *N*-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoyl-glycerol (2-AG) that activate these receptors, were identified in mammals [1]. These molecules are biosynthesized (Figure 1) *via* the processing of membrane lipid precursors, i.e. *N*-arachidonoyl-phosphatidylethanolamine (N-ArPE) and *sn*-2-arachidonate containing diacylglycerols (DAG), by the action of *N*-acyl-phosphatidylethanolamine-selective phospholipase D (NAPE-PLD) and diacylglycerol lipases (DAGL) α and β, in AEA and 2-AG, respectively. eCBs are then inactivated by intracellular hydrolyzing enzymes, i.e. fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL), respectively [3,4]. Receptors, eCBs and the proteins responsible for their metabolism are the key components of the complex endogenous signaling network known as the eCB system. Recent comprehensive reviews highlighted as complexity and redundancy of eCB molecular targets and metabolic pathways required a full revision of this definition [5-7]. Briefly, eCBs as well as some plant derived cannabinoids, phytocannabinoids, bind to molecular targets different from CB_1 and CB_2 such as orphan G-protein-coupled receptors (GPR55), peroxisome proliferator-activated nuclear receptors (PPARs) and transient

receptor potential (TRP) channels. Moreover, several metabolic enzymes contributed to biosynthesis or inactivation of the main eCBs. In particular, N-ArPE might release AEA in one step by NAPE-PLD action or multiple steps that involve: 1) α,β -hydrolase-4 (ABHD4) followed by glycerophosphodiester phosphodiesterase 1 GDE1; 2) soluble phospholipase A_2 ($sPLA_2$) followed by *lyso*phospholipase D (*lyso*-PLD); 3) phospholipase C (PLC) enzymes followed by PTPN22 phosphatase. Again, eCB degradative pathways are not limited to the action of FAAH or MAGL but other enzymes such as α,β-hydrolase-6 (ABHD6) and -12 (ABHD12) are able to hydrolyze 2-AG as well as lipoxygenases and cycloxygenase-2 might oxidize eCBs to produce several potential novel lipid mediators [3,7]. On a final note, except for THC and Δ^9-tetrahydrocannabivarin (THCV), other pharmacologically active phytocannabinoids do not bind to CB receptors but modulate eCB metabolism and/or activate eCB off-target receptors [3,5,7]. The eCB system signaling as well as synthetic cannabinoids and phytocannabinoids represent an important field of research in order to design and develop novel therapeutic agents for symptom relief or control of disease progression in several human diseases. In the next sections, we will collect and discuss data concerning the therapeutic value of compounds that acting on the eCB system might contribute to counteract or slow down motor disease progression. The major mechanisms involving protection of nigrostriatal neurons and recruitment of anti-inflammatory responses (microglial toxicity) in PD and HD will be scrutinized and reported.

2. Parkinson's disease

Caudate-putamen, globus pallidus (GP), subthalamic nucleus (STN) and substantia nigra (SN) form the BG. The BG are a highly interconnected set of nuclei responsible for motor skill and the correct and balanced selection of appropriate movements. The PD pathogenesis is characterized by the progressive loss of DA neurons in the SN *pars compacta* (SNpc) and neurodegeneration of the DA innervation of the dorsal striatum (i.e., DA nigrostriatal system). In PD is also recognizable the deposition of α-synuclein aggregates in surviving nigral neurons as well as in other brain regions. Moreover, there is also evidence of neuroinflammatory processes such as microglial cell activation and production of proinflammatory mediators and T cell infiltrations [8]. However, despite the severe impact of neuroinflammation to PD pathogenesis there is no consensus about the underlying mechanisms responsible of inflammatory responses. Accordingly, there are no disease-modifying therapies available and currently approved drugs can only improve PD symptomatology but also elicit motor complications after long-term

treatment. The existing therapies are based on the idea of DA replacement and restoring of DA signaling such as 3,4-dihydroxyphenyl-l-alanine (L-DOPA), DA reuptake inhibitors (e.g., amantadine) and DA agonists (e.g., ropinirole, pramipexole) [9].The serious consequences of DA loss for the selection-execution of movements and accurate motor skills uncover the key importance played by DA in BG function. The BG network itself can be conceptualized as a DA-dependent system consisting of two dominant pathways: the direct one associated to the D1-like DA receptors, dynorphin-, and substantia P-expressing neurons, and the indirect pathway predominantly expressing encephalin and D2-like DA receptors [10]. The large part (90-95%) of striatal neurons are the GABAergic medium spiny neurons (MSNs) that link the cortical input and the striatum to the different output nuclei (Figure 2). The tight functional interaction between DA signaling and eCBs at BG level is epitomized by the effects induced by the alteration of eCB-mediated action on motor activity and by the fact that these effects largely depend on the DAergic system. The MSNs project to SN *pars reticulata* (SNpr) and the internal segment of GP (GPi) and originate the striatonigral direct pathway whereas the striatopallidal indirect pathway projects to the SNpr via the GP *pars externa* (GPe) and the STN [11]. In this regard, the MSN can be viewed as a central gateway that integrates information incoming from different cortical regions and mediates changes in synaptic strength in striatal circuits to shape adaptive behavioral responses. The execution of movements is the result of the fine-tuned balance between the D1 receptor-dependent facilitatory signaling and activation of motor programs through the direct striatonigral pathway and the D2 receptor-dependent inhibition of the indirect striatopallidal pathways (Figure 2).

2.1 PD, CB_1 distribution and eCB-dependent plasticity

mRNA expression of CB_1 receptors are maximally present at BG level [12]. The localization of CB_1 receptors on presynaptic axon terminals of glutamatergic corticostriatal projecting neurons represents a key factor in the fundamental contribution of the eCB system to different forms of striatal plasticity. CB_1 receptors are also densely located on presynaptic terminals of GABAergic MSNs projecting to SNpr [13]. CB_1 receptors have been detected both in direct striatonigral and D1-expressing neurons and in striatopallidal and D2-expressing neurons [12]. Also other efferent striatal outputs such as GP in humans, the entopeduncular nucleus in rodents, and the GABAergic projections from the GP to the STN contain high level of CB_1 receptors [13].

The DA transmission controls the plasticity of glutamatergic synapses at dorsal striatal level, and the long-term depression (LTD) at corticostriatal synapses is a well-known form of non NMDA and activity-dependent plasticity. Notably, this form of plasticity is eCB-mediated and requires the stimulation of D2 receptors as demonstrated by the potentiation of eCB signaling and LTD enhancement upon D2 receptor activation [14,15].

The progressive loss of DAergic neurons and DA innervation of striatal circuits severely undermine corticostriatal plasticity, and in particular D2 receptor-dependent eCB-induced LTD. The impairment of corticostriatal eCB-induced LTD is responsible for the development of cardinal symptoms in PD such as tremor and bradykinesia and also for the appearance of detrimental side effects such as L-DOPA-induced dyskinesia [14,16]. From here, it emerges that striatal eCB-LTD represents a key target for novel therapeutics options in PD (Figure 2).

2.2 The eCB signaling system: control of motor behavior and neuroinflammation in PD

CB_1 receptor binding is increased in PD patients and 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP)-treated marmosets at caudate-putamen level [17] while a decrease in CB_1 receptor availability has been observed in the SN of PD patients [18]. The eCB system undergoes a drastic remodeling during the course of PD pathogenesis. The disease modifies not only receptors but also levels of eCBs. AEA levels were found considerably amplified in cerebrospinal fluid of PD patients [19] and L-DOPA or DA receptor agonist treatment was shown to restore back AEA levels to control subjects [20]. Nevertheless, AEA levels do not change according to disease development, stages and severity, leading to suggest that these alterations might be considered as adaptive changes secondary to DA depletion [21]. Experimental reserpine- or 6-hydroxy(OH)DA-induced DA depletion abolish LTD in indirect pathway MSNs and the treatment with the FAAH inhibitor, URB597, rescues the eCB-induced LTD and ameliorate Parkinsonian motor deficits (catalepsy, motor hypoactivity) but only when co-administered with the D2 receptor agonist quinpirole [14]. This study further corroborates the notion that eCBs exert an inhibitory control on movement and motor execution and these effects depend on DAergic transmission. Systemic AEA and THC administration or synthetic CB agonists (e.g., WIN 55,212-2 or CP 55,940) reduce locomotor activity both in intact and in DA-depleted animals [22]. Since there are no CB_1 receptors on DAergic neurons, the effects on DAergic transmission are indirectly mediated by CB_1-containing GABAergic and glutamatergic neurons. This may help to understand why the eCB system results overactivated in PD as

consequence of the loss of DAergic innervation and facilitation of DA-evoked motor behavior [18,23].

In the context of the interaction between microglial cells, neurons and astrocytes there is the possibility to investigate the role of CB_2-mediated signaling in PD-associated neuroinflammatory responses. Remarkably, CB_2 receptors are densely expressed in the brain in the activated microglia while their expression is low in microglia quiescent state [24]. Although controversial for a long time, several recent evidence support the concept of CB_2 receptors neural expression in prefrontal cortex, hippocampus, SN and in GP [24]. CB_2 receptors appeared involved in the degeneration of nigrostriatal DAergic neurons as for the increase of CB_2 receptors at the level of the microglial activation recruited by MPTP-induced neural lesion [25] (Table 1). This study further shows that administration of CB_1/CB_2 agonist, WIN 55,212-2, or selective CB_2 receptor agonist, JWH015, reduced microglial activation and infiltration [25].

In different models of PD, the unilateral intra-striatal infusion of either 6-OHDA or the bacterial endotoxin lipopolysaccharide (LPS) produced an increase of CB_2 receptor gene expression and, for LPS alone, also the increase of AEA and 2-AG levels [26]. This study reported a correlation between CB_2 receptor overexpression and increased microglial activation, thus suggesting microglia as possible source for the increase in CB_2 receptor expression and CB_2 receptors as potential targets against PD-associated neuroinflammation. An increase of CB_2 receptor expression was also found earlier in the LPS-based inflammation model of PD and a neuroprotective effects after administration of the selective CB_2 receptor agonist HU-308 [27]. Recently, the same group [28] identified the presence of CB_2 receptors in nigrostriatal neurons of the human SN of PD patients that resulted expressed at lower level than control subjects. Notably, a later study [29] has found an upregulation of CB_2 receptor in glial cells in the SN of PD patients together with a parallel increase of activated microglia and infiltrated macrophages. This study also reveals that the pharmacological activation of CB_2 receptors *via* the administration of HU-308 counteracted LPS-induced proinflammatory responses in mice (i.e., elevation of striatal CD68 immunofluorescence and inducible nitric oxide synthase (iNOS) gene overexpression). The administration of HU-308 was also shown to be partially effective against 6-OHDA-induced DA depletion whereas the use of selective and non-selective CB_1 receptor agonists failed to confer protection [30] (Table 1).

2.3 The therapeutic potential of eCB-based agents in PD: to boost or to shrink?

In animal models of PD the activity of the eCB system appear enhanced as a result of the amplification of CB_1 and CB_2 mRNA levels and decreased FAAH activity [30-32]. The key objective of PD-like animal models is to improve the motor impairment evoked by the experimental depletion of DAergic source to BG (Table 1).

The administration of CB_1 receptor agonists produces inhibition of both motor behavior and DA release in the BG, thus revealing its inadequacy in counteracting the motor deficits in PD and also revealing the potential exacerbation of motor symptoms as for the induction of bradykinesia [33]. Nevertheless, the stimulation of CB_1 receptors can disclose its potential as option to reduce the impact of the disabling involuntary movements induced by protracted treatment with L-DOPA. In a recent study [34], L-DOPA-induced dyskinetic movements (LIDs) were reduced by subchronic administration of WIN 55,212-2 in rats unilaterally lesioned *via* 6-OHDA in the medial forebrain bundle. The stimulation of CB_1 receptors can provide an anti-excitotoxic response and a neuroprotective action via the reduction of glutamate release [36]. The overactivity of glutamatergic input through the corticostriatal pathway is believed to underlie LIDs, and reduction of glutamate release by CB_1 receptor stimulation might therefore be used to reduce the incidence of LIDs. Moreover, the use of CB_1 receptor agonists against LIDs might also be supported by the decrease of GPe GABAergic output induced by the stimulation of CB_1 receptors located on the presynaptic terminals of the indirect striatopallidal pathway (see figure 2). However, at the highest dose tested, the preferential CB_1 receptor agonist HU-210 has shown only partial efficacy towards L-DOPA-induced abnormal involuntary movements (AIMs) and also elicited unwanted motor suppressant effects [37]. The anti-excitotoxic response is an important consequence of CB_1 receptors stimulation that has been described also for the decrease of glutamate release from STN-nigral neurons thus corroborating the idea that CB_1 receptor agonists might have a therapeutic value in alleviating PD-associated tremors [38].

Interestingly, the stimulation of CB_1 receptors and the increase of AEA availability *via* FAAH inhibition do not show the same anti-dyskinetic potential. While WIN 55,212-2 administration attenuated L-DOPA-induced abnormal AIMs, FAAH inhibition was ineffective against AIMs except when co-administered with the TRP vanilloid (TRPV1) antagonist capsazepine [39]. The latter results are in agreement with the hypothesis that the hypokinetic effects evoked by AEA might be attributable to the involvement of TRPV1-mediated transmission and, in particular, that the increase of AEA levels induce a decrease of DA release from nigrostriatal terminals [40].

On the other hand, blockade of CB_1 receptors has been reported to improve spontaneous motor activity after severe nigral DAergic degeneration in rats and therefore after the effects of CB_1 receptor antagonism, *via* SR 141716A, on striatonigral D1-mediated activity are reduced or suppressed [41].

With regard to the beneficial effects of CB_1 receptor blockade in late-stages of PD, the lack of CB_1 receptor expression in SNpc might account for the overactivity of eCB system and the upregulation of CB_1–mediated activity observed in PD. From this view the clinical use of CB_1 receptor antagonists appears to provide a higher therapeutical potential that is been also associated to the increase of glutamate release induced by SR 141716A [42].

Moreover, there is evidence of antagonistic relationship between eCB-mediated signaling and D1- and D2-dependent motor behavior. The potentiation of eCB signaling by the inhibition of AEA transport abolishes D1- and D2-dependent grooming and oral stereotypies, respectively [12]. The contralateral turning induced by intra-striatal unilateral D1 receptor stimulation is potentiated by CB_1 receptor blockade, thus demonstrating the negative modulation exerted by CB_1 receptors on D1-mediated neurotransmission [12].

In another study, SR 141716A administration improved hypokinesia in intracerebroventricular 6-OHDA lesioned rats [43]. Thus, despite the moderate degree of DA loss obtained in a model of bilateral DAergic lesion, the blockade of CB_1 receptors induced positive effects on bradykinetic-like symptoms [43]. According to the enhancement of D2-dependent motor activity induced by SR 141716A administration, these results corroborate the idea that inhibition of CB_1 receptor-mediated signaling might occlude the eCB-induced inhibition of DAergic receptors. The efficacy of SR 141716A administration in improving forepaw stepping in rats with unilateral 6-OHDA-lesioned was demonstrated both alone and in concomitant administration with low dosage L-DOPA [44]. Nevertheless, it should be mentioned that SR 141716A administration failed to improve Parkinsonian-like symptoms in MPTP-treated in primates [34]. Mixed results were obtained in MPTP-treated rhesus monkeys were the selective CB_1 antagonist CE-178,253 was ineffective against motor disabilities except when co-administered with subthreshold doses of L-DOPA, thus enhancing the anti-Parkinsonian effects of L-DOPA treatment [45].

The block of CB_1-mediated signaling may reveal its potential as strategy to reduce LIDs [46]. As shown in MPTP-treated marmosets, SR 141716A administration can improve motor function and reduce L-DOPA-induced dyskinesia [46]. Similar findings were described later in the 6-OHDA

rat model of PD in which the co-administration of SR 141716A and L-DOPA reduced the AIMs elicited by repeated L-DOPA treatment also exerting some anti-degenerative effects in terms of preservation of DAergic nigral cells [47]. This study provides an interesting therapeutic perspective in which CB_1 receptor blockade parallels, and not follows, L-DOPA treatment and therefore might prevent the development of the AIMs.

With regard to the neuroprotective potential provided by CB_1 receptor modulation there is also evidence that CB_1 receptor stimulation can protect against MPTP-induced DAergic neuronal loss by the way of microglial activation [48] (Table 1). Nevertheless, the increase of CB_1-mediated signaling might aggravate some Parkinsonian symptoms such as bradykinesia. Moreover, an approach based on CB_1-receptor appears irreconcilable with the progressive loss of CB_1 receptors that is observed in neurodegenerative diseases. For this reason, a neuroprotective eCB-based approach would rely on other non-mutually exclusive targets as for the particular role played by CB_2 receptors in neuroinflammation.

2.4 Targeting eCB signaling in PD patients

Clinical studies/trials have been extensively reviewed by Kluger and co-authors [49]. Briefly, these studies described that PD patients who made use of cannabis or received nabilone (i.e., a THC mimetic) reported an improvement of motor impairment and in particular of bradykinesia, tremor and L-DOPA-induced dyskinesia. Moreover, there is evidence for possible beneficial effects of eCB system stimulation and decrease of PD-associated comorbidities such as psychosis and REM sleep behavior disorder. However, another trial that implemented a four-week oral cannabis administration in PD patients failed to show any significant anti-Parkinsonian activity. Although with a limited sample size, also a case study in which patients smoked marijuana at different THC concentrations did not report improvement of tremor symptoms. In a randomized placebo-controlled study, the administration of SR141716A was ineffective against motor symptoms and also LIDs [49].

3. Huntington disease

Huntington disease (HD) is a neurodegenerative genetic disorder caused by a polyglutamine expansion mutation (a CAG trinucleotide expansion) in exon 1 of the IT15 gene coding for huntingtin protein (Htt) on chromosome 4 [50]. The most characteristic symptoms of disease are

abnormal involuntary movements called chorea, which are produced by neuronal dysfunction in the striatum, and dementia caused by neuronal decline in the cortical structures. Moreover, multiple molecular and cellular events including aggregation of mutated Htt, transcriptional dysregulation, altered energy metabolism, excitotoxicity, impaired axonal transport and altered synaptic transmission contributed to neuronal dysfunction and death [50]. Most HD patients carry CAG repeats in the range of 38–55 and develop neurological symptoms in mid-life, larger repeats (>60Q) can cause juvenile onset HD [50]. Unfortunately, HD is a disease with no cure and Tetrabenazine is the only pharmacological treatment approved by the Food and Drug Administration against chorea associated with HD.

3.1 The link between HD and CB_1 receptor-mediated protective action

One of the earliest changes observed in HD patients and animal models of HD is transcriptional dysregulation of a subset of genes including CB_1 receptors. The CB_1 is high expressed in the brain areas that control motor and social behaviors as well as learning and memory function impaired in HD [13]. CB_1 receptors are significantly reduced in MSN projections of the caudate-putamen [51-53] and is thought to participate in HD pathogenesis. The downregulation of CB_1, observed in post-mortem tissue of HD patients and in most mouse models of HD, including R6/2, R6/1, YAC128 and HdhQ150 mice [52,54-56], seems to occur at early stages of the disease and prior the onset of choreic symptoms and neuronal death. Moreover, CB_1 receptor genetic ablation in mice deteriorated HD symptoms and pathology, while treatment with THC, or WIN 55,212-2 delayed the onset of motor and neurochemical alterations [36,57-59] (Table 1). A variant of the CB_1 gene (CNR1 rs4707436) that is related with lower levels of CB_1 has been associated with age at onset in HD patients [60]. Further, mutant Htt affects CB_1 promoter activity [59] through repressor element 1 silencing transcription factor, REST, which is implicated in the pathogenesis of HD [61,62].

Recently, the development of conditional mutant mice lacking CB_1 in glutamatergic excitatory neurons or GABAergic inhibitory neurons has helped to clarify the CB_1 receptor-dependent neuroprotective activity in HD. This neuroprotective potential appears due to a unique and well-defined population of CB_1 receptors located on cortical glutamatergic neurons that project to the striatum. The identification of this population of CB_1, preserved during HD progression, supported the development of therapeutic approaches aimed at targeting glutamatergic CB_1 receptors in spite of their loss [63]. In addition, eCB, synthetic cannabinoids and

phytocannabinoids exhibited biased signaling at CB_1, and activation of CB_1 by Gαi/o- and Gβγ-selective ligands might be therapeutically beneficial in HD [64-66]. Indeed, *in vitro* treatment of striatal cell model of HD with AEA normalized CB_1 protein levels and this effect was associated with improved cell viability, ATP production, BDNF-2 expression and inhibition of GABA release [64-66].

3.3 eCBs, CB_2 receptor and the therapeutic promise in HD

However, recent evidence suggested that CB_2 receptor is much more widely distributed in the CNS than originally thought, where it plays multiple and unexpected neuroprotectant roles. Compounds that selectively activate the CB_2 receptor also appear to be effective in different animal models of HD. In particular, the expression of CB_2 receptors is upregulated in the striatum of R6/2 mice at both pre-symptomatic and symptomatic stages and genetic ablation of CB_2 receptors exacerbated disease symptomatology and neurochemical alterations in same model [67]. Moreover, CB_2 receptor stimulation by the selective CB_2 agonist HU-308 attenuated glial activation and protected striatal neurons from damage induced by intrastriatal injection of quinolinic acid or the mitochondrial complex II inhibitor malonate [67,68].

Besides the pivotal role of cannabinoid receptors, the participation of other elements of the eCB system in HD pathology might also be considered. In particular, FAAH activity was reported to be downregulated and eCB upregulated in peripheral lymphocytes from HD patients [69]. Striatal FAAH messenger RNA levels were upregulated in symptomatic R6/2 mice and in the caudate-putamen of patients with HD compared to control subjects [59] while Bari and co-workers [70] reported that striatal FAAH enzymatic activity was reduced in 12-week-old R6/2 mice. Again, the gene expression of MAGL remained unchanged in the striatum of R6/2 along disease progression [59] while the enzymatic activities of the main eCB biosynthetic enzymes, DAGL and NAPE-PLD, decreased in 12-week-old R6/2 mice compared to control wild-type mice [70]. Finally, a region-specific decline of eCB levels were reported in the striatum of 3-nitropropionic acid (3-NP)-lesioned rats and in symptomatic R6/2mice [71,72].

The preclinical data reported above support the development of novel therapeutic strategies that by targeting the eCB system might contribute to new routes for drug design against HD. This might include the "direct" activation of eCB system by using specific agonists, the use of "indirect" cannabinoid receptor agonists as inhibitors of eCB inactivation or as alternative strategy the use of positive allosteric modulators.

On the other hand, the inhibitors of eCB catabolism have been tested in different animal models of HD showing controversial results and additional studies are needed to understand how elevating eCB levels affects the signs and symptoms of disease [57,72-76]. An another approach of enhancing eCB signaling is the use of positive allosteric modulators (PAMs) able to potentiate agonist binding to the CB receptors and at the same time inhibiting agonist activity in numerous functional assays [77,78]. However, further investigation is necessary to better understand the correlation between *in vitro* and *in vivo* pharmacology of PAMs [66,78]. On a final note, also the beneficial effect of the phytocannabinoids Δ^9-THC and cannabidiol (CBD), alone or in combination in the form of mouth spray, Sativex®, has been investigated in several animal models of HD. Multiple mechanisms of action including CB_1 or CB_2 receptors, additional eCB-binding receptors like PPARs, non-eCB targets like $5HT_{1A}$ receptors or even anti-oxidant properties have been supposed to be responsible of their beneficial action [79,80]. Controversial results were obtained by using phytocannabinoids [68,73,81], and improvements of hyperkinesia and behavioral alterations have been reported in clinical trials with nabilone [82]. Unfortunately, a recent phase II clinical trial with Sativex® was unsuccessful [83] probably due to short treatment period and low dose administered. More recently, cannabigerol (CBG), another phytocannabinoid with non-psychotropic profile, was investigated in both R6/2 and 3NP-lesioned mice models of HD [81]. CBG preserved striatal neurons death as well as neurological deterioration and improved motor deficits, although these effects were much more evident in 3NP-lesioned mice than in R6/2 mice. Since CBG exhibited poor affinity for CB_1 and/or CB_2 receptors, the mechanisms responsible for the beneficial effects of CBG in HD await to be better investigated [81].

4. **Expert commentary**

The eCB system is part of a wider lipid-signaling pathway capable to provide unique anti-oxidative, anti-excitotoxic, anti-inflammatory and neuroprotective properties. The expression and distribution of the major eCB system components all over the BG network and their tight interaction with DAergic, GABAergic and glutamatergic systems contribute to support the view of the therapeutic potential of eCB system in motor diseases. The protective action conferred by eCB-based drugs against age-dependent pathologies such as neuroinflammatory insult and neurodegeneration further supports the exploitation of eCB signaling in motor diseases. Ageing is associated with mitochondrial dysfunction that also occurs in occurs in PD and in HD. Since

CB_1 receptors have been found localized on brain mitochondria and contribute to reduce mitochondrial respiration and alter eCB-mediated synaptic plasticity [84,85] the eCB system can play a relevant role in mitochondrial function. Mitochondrial ROS production and in particular the oxidative stress induced by the exposure of mitochondria to paraquat were attenuated *via* CP55,940 and JWH-015 [86]. However, while increasing CB_1 receptor activity might impair mitochondrial function, enhancing CB_2 receptor activity exerts a neuroprotective action against inhibition of mitochondrial function as demonstrated in a rat model of HD [68]. Collectively, the possibility to limit microglial overactivation, infiltrating macrophages and confer defense against mitochondrial dysfunction given by CB_2 receptor stimulation appears the most promising strategy to fight inflammation, regulate autophagy and reduce neural death in neurotoxic models of PD and HD and potentially in multiple BG-associated motor diseases.

The examination of the key outcomes emerging from the use of cannabinoid-based drugs in BG-associated motor diseases reveals the existence of a gap between preclinical and clinical investigations. In the current scenario, preclinical studies in animal models of PD and HD are only partially convincing providing limited evidence as for the impact of cannabinoid-based drugs to attenuate bradykinesia, tremor, hypokinesia and choreic motor signs. On the other hand, clinical trials appear not to be sufficient to draw conclusive assumptions. As above mentioned, the clinical use of CB_1 receptor antagonists might reveal unexpected therapeutical chances in case of advanced stages of PD [41] or marked refractariety to DA replacement therapy. This therapeutic opportunity appears confirmed by the predictions made possible by recent models of BG functioning as for the case of the dynamic "centre-surround model"[87]. This model emphasizes the role of BG in facilitating motor programs but also in the inhibition of competing and interfering movements that might elicit movement disorders such as PD and HD. The "centre-surround model" is based on the idea of the selective facilitation of motor programs and concurrent "surround" inhibition of competing motor patterns. As elsewhere underlined [88], the "centre-surround model" can help to account for the failure to accomplish desired movements or inhibit undesired movements as for PD and HD, respectively. Within this context, we believe that the "centre-surround model" could be used to evaluate the predictive potential at the light of the idea of the overactivity of the CB system in movement disorders (see Figure 3 for the model adaptation to PD). Hence, CB_1 receptor blockade is expected to reduce the inhibition in striatopallidal projecting neurons and alleviate hypokinesia.

Another stimulating therapeutic field for the investigation of cannabinoid-based drugs is offered by the anti-dyskinetic potential for the clinical management of PD-associated motor symptoms [46,47]. We believe that cannabinoid-based drugs possess a great therapeutic potential that should be further explored taking into account the knowledge developed on the interaction between CB_1 receptors and other targets of the BG network that have provided robust evidence as non-DAergic substitutes to alleviate motor deficits in PD. Among non-DAergic mechanisms, the role of the adenosine A_{2A} receptor subtype acquires a particular relevance. Besides to D2-A_{2A} receptor interaction and its role in striatal plasticity, there is evidence that A_{2A} and CB_1 receptors co-localize in corticostriatal glutamatergic terminals and in dendrites of MSNs of the indirect pathway. D2-A_{2A} and also A_{2A}-CB1 receptors can form heteromeric complexes in the striatum [89] providing the morphological basis for functional A_{2A}-CB1 receptor interaction subserving the control of motor output [90]. Moreover there is evidence of physical A_{2A}- CB_1 receptor interaction on the same corticostriatal glutamatergic terminal and that A_{2A} receptor activation reduce the CB1 receptor-mediated inhibition of synaptic transmission with major effects on motor coordination and PD pathophysiology [91]. Moreover, as recently reported in different PD models, the altered expression of A_{2A}/CB_1/D2 heteromers induced by DA depletion can be restored by L-DOPA treatment and therefore contribute to normalize the functioning of BG network [92,93]. Although different hypotheses might be formulated to understand the influence of presynaptic A_{2A} receptor populations on CB_1 receptor signaling at corticostriatal synapses, it is also clear that the A_{2A} receptor is a key target for the modulation of CB_1 receptor functioning.

5. Five-year view

One of the major challenge for the use of cannabinoid-based drugs as therapeutic option for the treatment of BG-associated movement disorders is linked to the assessment of their efficacy on disease progression. Although the studies summarized in this review provided either marginal or substantial evidence to support their use in clinical practice, they have been performed in cellular or animal models whereas the few clinical trials have been focused on the alleviation of specific symptoms rather than on the control of disease progression. This could, at least in part, help to explain the controversial results obtained in clinical studies.

In our view, the approval for clinical use of Sativex and Epidiolex might facilitate in the near future the clinical utilization and development of cannabinoid-based drugs. These formulations,

or additional combination of phytocannabinoids, seem to be suitable to treat pathological situations in which the involvement of different toxic mechanisms contributes to the damage.

There are nonetheless specific clues that should be followed within a short-term perspective.

1) First, it should be examined the opportunity to implement combined therapies and develop dual-acting drugs targeting the A_{2A}-CB_1 receptors taking advantage of their physical and functional interaction to rebalance the DAergic signaling and striatal plasticity.

2) Next in order, it should be further explored the impact of cannabinoid-based drugs on mitochondrial function. eCBs interfere with mitochondrial respiration by receptor- and nonreceptor-mediated mechanisms [94] and consequently affect energy homeostasis and metabolic-associated functions. Remarkably, overactivation and/or alteration of eCB system does not occur only in motor diseases but also in other neurodegenerative conditions. From this point of view, the use of CB_1 receptor antagonist/inverse agonists might offer new elements of analysis and investigation along with the neuroprotective effects of CB_2 receptor stimulation on mitochondrial function.

3) Other targets of eCBs and/or related compounds such as TRP channels and PPARs should be also carefully considered. For instance, other *N*-acylethanolamines (e.g., oleoylethanolamide and palmitoylethanolamide) are endogenous activators of PPARs and hold a neuroprotective and/or anti-excitoxic potential. Moreover, it should be further investigated the role played by the different members of TRP channels (e.g. vanilloid-, melastatin- and ankirin-type), their activation by exogenous or endogenous cannabinoid-related compounds as well as their potential synergic or antagonistic effects in BG.

4) Finally, the role of eCB system in the regulation of autophagy machinery represents a novel and promising field of investigation. eCBs have been shown to induce autophagy in several cancer cell lines, thus contributing to cytoprotection. Considering the key role of oxidative stress and autophagy dysregulation in PD and HD there is a solid rationale to solicit future investigations on the activity of cannabinoid-based drugs in autophagy function and rebalance of cellular homeostasis.

6. Key issues

- The existence of a “bright” side of psychoactive substances is corroborated by the therapeutic use of cannabinoid-based drugs.

- eCBs and cannabinoid-based drugs can "tune up" brain locomotor circuits and play a key role in BG neural plasticity.
- eCB system shapes information processing within the BG network and participate to the function and physiology of BG.
- Time- and space-selective alterations of eCB system are linked to the onset and progression of several BG-associated diseases.
- eCBs and cannabinoid-based drugs are one of the first lines of investigations to treat BG-associated motor diseases such as PD and HD.
- The widespread diffusion of eCB targets within the different excitatory and inhibitory nuclei of BG might account for the disappointing and often inconsistent results obtained by the use of selective cannabinoid-based "magic" drug bullets.
- The great potential provided by CB_2 receptors in terms of defense against neuroinflammation and mitochondrial dysfunction is another frontier for development of cannabinoid-based drugs.
- The exploration of the potential therapeutic provided by co-localization of CB_1 receptors with other family of receptors (e.g., A_{2A}, TRPs and PPARs) involved in BG-associated motor diseases offer novel opportunities in clinical trials.

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Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Legend to figures and tables

Figure 1

Schematic representation of the eCB system and the interactions with some phytocannabinoids. *N*-acyl-phosphatidylethanolamine-selective phospholipase D, NAPE-PLD; 2-arachidonoyl-glycerol, 2-AG; *N*-arachidonoyl-ethanolamine, AEA; cannabidiol, CBD; cannabinoid receptor type-1, CB_1; cannabinoid receptor type-2, CB_2; cycloxygenase-2, COX-2; diacylglycerol lipases, DAGL; fatty acid amide hydrolase, FAAH; glycerophosphodiester phosphodiesterase 1, GDE1;

G-protein-coupled receptors 55, GPR55; α,β –hydrolase domain containing 4, ABHD4; α,β –hydrolase domain containing 6, ABHD6; α,β –hydrolase domain containing 12, ABHD12; lipoxygenase, LOX; lysophospholipase D, lyso-PLD; monoacylglycerol lipase, MAGL; peroxisome proliferator-activated nuclear receptors, PPARs; phospholipase C, PLC; protein tyrosine phosphatase non-receptor type 22, PTPN22; soluble phospholipase A_2, $sPLA_2$; Δ^9-tetrahydrocannabinol, THC; Δ^9-tetrahydrocannabivarin, THCV; transient receptor potential (TRP) channels.

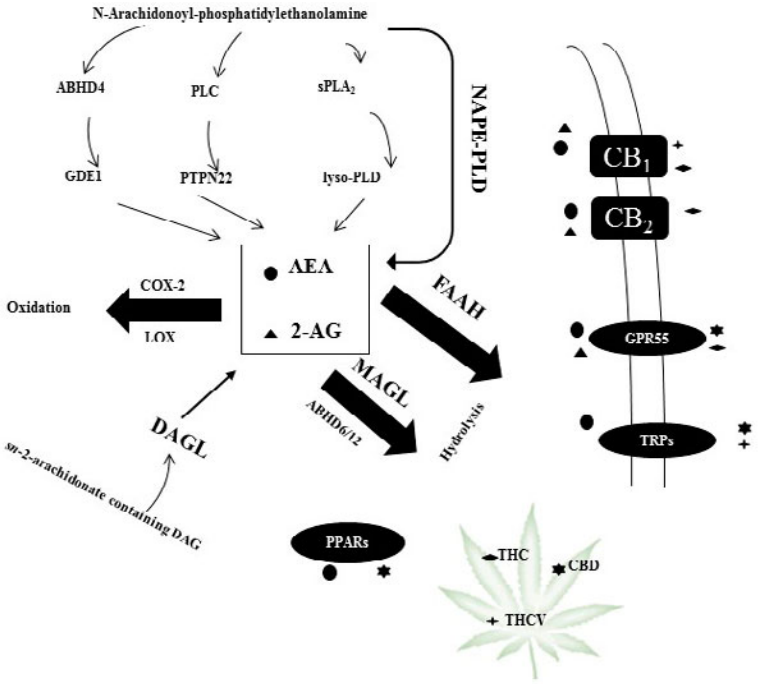


Figure 2

Schematic model of direct and indirect pathways of BG and distribution of CB_1 receptors including their relationship with DA D2 and adenosine A_{2A} receptors. The striatum is the main input of BG and the output nuclei of BG are the GPe and GPi/SNr as striatonigral D1 receptor-expressing direct pathway and striatopallidal D2 receptor-expressing indirect pathway, respectively. The GABAergic projections of D1-mediated direct pathway inhibit GPi/SNr cells and stimulate motor behavior, whereas the D2-mediated indirect pathway inhibit the GPe, disinhibit the subthalamic nucleus and stimulate/excite the GPi/SNr. Arrow-ending solid lines are glutamatergic excitatory output, dotted lines are GABAergic inhibitory output and solid circle lines depict DAergic pathway. Adenosine A_{2A} receptor, $A_{2A;}$ cannabinoid receptor type-1, CB_1; dopamine D1 receptor, D1; dopamine D2 receptor, D2; globus pallidus *pars externa*, GPe; globus pallidus *pars interna*, GPi; glutamate, Glu; medium spiny neurons, MSN; substantia nigra *pars compacta*, SNpc; substantia nigra *pars reticulata*; subthalamic nucleus, STN.

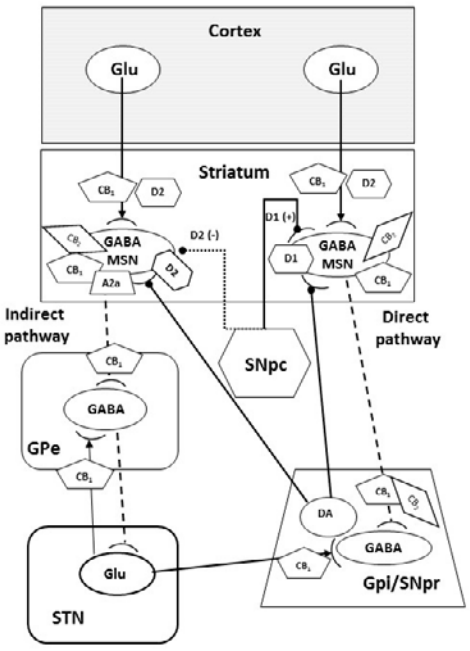
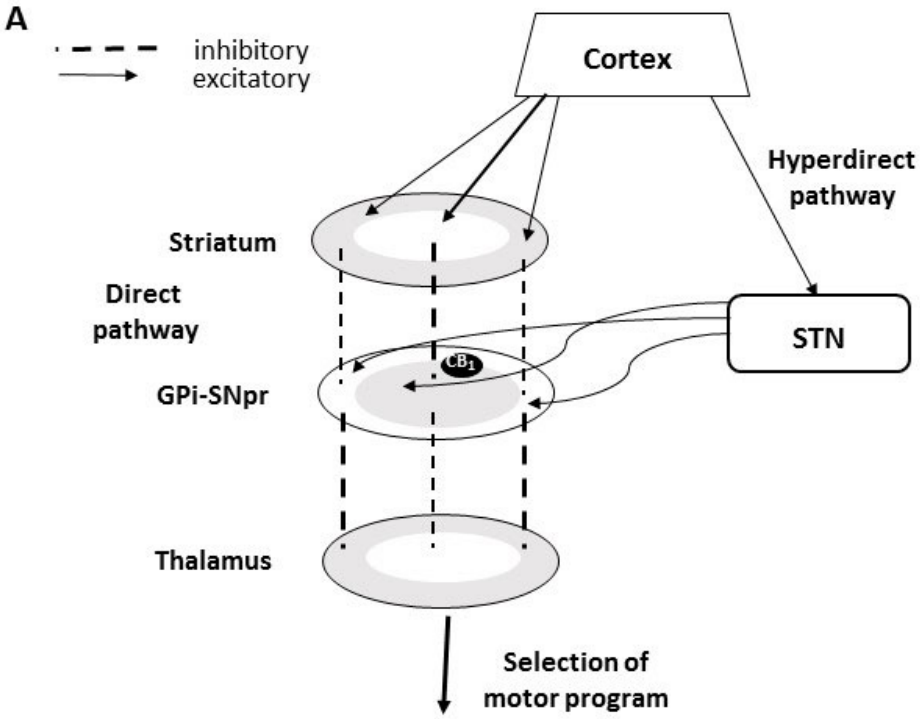


Figure 3

Schematic diagram of the dynamic "centre-surround model" (adapted from [88]). According to its early formulation [87], the model attempts to explain the role of BG in the execution of voluntary movements that include the projection between cortex and STN (i.e., hyperdirect pathway) and the widespread fibers connecting the STN to the GPi/SNpr of the direct pathway. Here, to reduce the complexity, the indirect pathway is omitted. A) In normal conditions, inputs from the cortex to the striatum (i.e., direct pathway) or to the GPi/SNr (i.e., hyperdirect pathway) can induce inhibition (grey area) or excitation (white area) of the efferent stations. The dynamism of the model depends on the "corollary" signals triggered when a voluntary movement is initiated. Corollary signals are conveyed from cortex to GPi/SNr via the hyperdirect pathway contributing to inhibit the thalamic area, competing motor programs and facilitate motor execution. Other corollary signals are transmitted in parallel through the direct pathway contributing to inhibit certain neuronal populations of GPi/SNr (see the centre area) and consequently disinhibit excitatory thalamic areas (see the centre white area of the thalamus, at the bottom). B) In PD, the activity of the hyperdirect pathway is increased whereas the activity of the direct pathway is decreased. This reduction of activity is hypothesized to increase the inhibitory output from the GPi/SNr to the excitatory thalamic areas and decrease the inhibitory output to the inhibitory thalamic areas (see the peripheral grey area of the thalamus, at the bottom) leading to hypokinesia.

Arrow-ending solid lines are glutamatergic excitatory and dotted lines are GABAergic inhibitory inputs and outputs. The different thickness of excitatory solid lines or inhibitory dotted lines designate the relative degree of excitatory or inhibitory stimulation through the cortex-striatum-GPi/SNr-thalamus axis. The different number of CB_1 receptors indicate the relative degree of functional activity. Cannabinoid receptor type-1, CB_1; globus pallidus *pars interna*, GPi; substantia nigra *pars reticulata*, SNpr; subthalamic nucleus, STN.



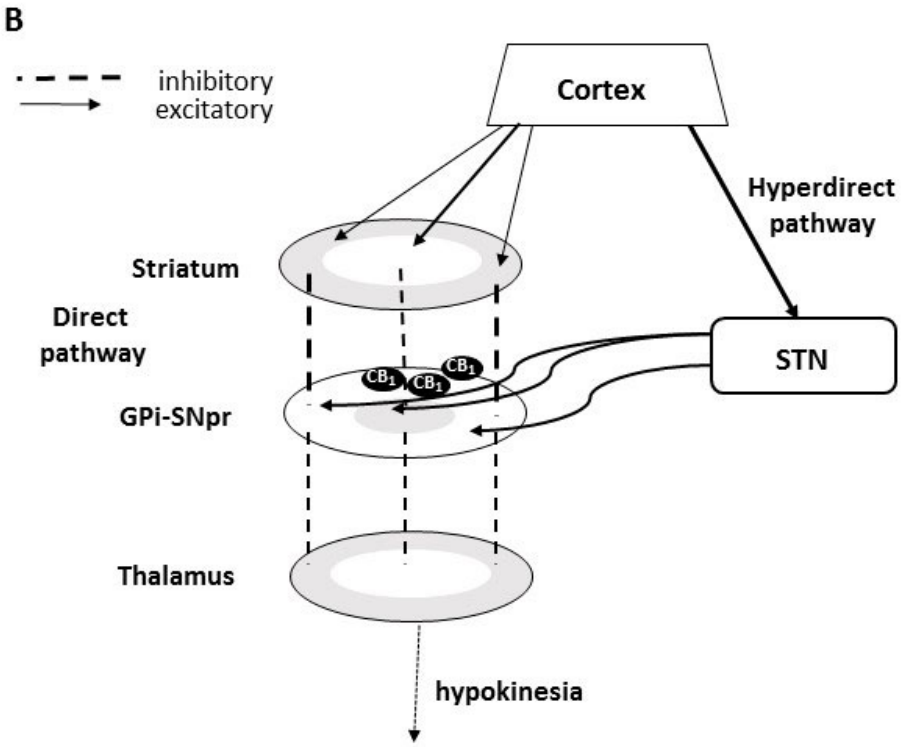


Table 1

Experimental use of cannabinoid-based drugs in BG disorders: selected studies.

Disease	Target	Drug	Effect	Model	Refs
Parkinson's disease	CB_1/CB_2 Agonist	THC	Increased bradykinesia	MPTP-treated mice	[34]
	CB_1/CB_2 Agonist	WIN 55,212-2	Reduced microglial activation	MPTP-treated mice	[25]
			Neuroprotection	MPTP-treated mice	[48]
			Reduced dyskinetic movements	6-OHDA-treated rats	[35]
	CB_1 Antagonist/inverse agonist	SR 141716A	Improved hypokinesia	6-OHDA-treated rats	[43]
			Neuroprotection	MPTP-lesioned marmosets	[46]
	CB_2 Agonist	JWH015	Reduced microglial activation	MPTP-treated mice	[25]
	CB_2 Agonist	HU-308	Neuroprotection	LPS-induced inflammation	[27]
			Neuroprotection	6-OHDA- treated rats	[30]
Huntington's disease	CB_1/CB_2 Agonist	THC	Attenuated motor deficit	R6/2 mice	[59]
			Neuroprotection	striatal neuroblasts	[59]
			Neuroprotection	3-Nitropropionic acid induced striatal lesions	[58]
			Neurotoxic	Malonate-induced toxicity in rats	[57]
	CB_1/CB_2 Agonist	WIN 55,212-2	Neuroprotection	Quinolinic acid-induced toxicity in rats	[36]
	CB_2 Agonist	HU-308	Neuroprotection	Quinolinic acid-induced toxicity in rats	[67]
			Neuroprotection	Malonate- induced toxicity in rats	[68]

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*-Papers of interest

**-Papers of considerable interest

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