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Review

The endocannabinoid system in normal and pathological brain ageing

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The role of endocannabinoids as inhibitory retrograde transmitters is now widely known and intensively studied. However, endocannabinoids also influence neuronal activity by exerting neuroprotective effects and regulating glial responses. This review centres around this less-studied area, focusing on the cellular and molecular mechanisms underlying the protective effect of the cannabinoid system in brain ageing. The progression of ageing is largely determined by the balance between detrimental, pro-ageing, largely stochastic processes, and the activity of the homeostatic defence system. Experimental evidence suggests that the cannabinoid system is part of the latter system. Cannabinoids as regulators of mitochondrial activity, as anti-oxidants and as modulators of clearance processes protect neurons on the molecular level. On the cellular level, the cannabinoid system regulates the expression of brain-derived neurotrophic factor and neurogenesis. Neuroinflammatory processes contributing to the progression of normal brain ageing and to the pathogenesis of neurodegenerative diseases are suppressed by cannabinoids, suggesting that they may also influence the ageing process on the system level. In good agreement with the hypothesized beneficial role of cannabinoid system activity against brain ageing, it was shown that animals lacking CB1 receptors show early onset of learning deficits associated with age-related histological and molecular changes. In preclinical models of neurodegenerative disorders, cannabinoids show beneficial effects, but the clinical evidence regarding their efficacy as therapeutic tools is either inconclusive or still missing.

Keywords: oxidative stress; macromolecule clearance; neuroinflammation; glial regulation; neurogenesis; neurodegenerative diseases

1. FUNCTIONAL, HISTOLOGICAL AND MOLECULAR CHANGES IN THE AGEING BRAIN

Ageing is associated with a decline of motor coordination [1], sensory abilities [2], attention and cognitive performance [3], which together are responsible for the increasing deficits in learning and memory tasks. However, as with all age-related health issues, there is a wide spectrum of potential outcomes: While many senior citizens still enjoy their cognitive abilities at an advanced age, others, especially those who suffer from neurodegenerative disorders such as Alzheimer's disease (AD), may show signs of cognitive impairment early in their life. Thus, a better understanding of the molecular and cellular processes that contribute to, or protect against, cognitive decline, may offer novel routes for therapy and prevention.

The mitotic activity within the central nervous system is low and the newly generated cells are mostly glia cells. Neurogenesis is restricted to the subventricular zone and to the subgranular zone of the hippocampus in mammals. Only a small percentage of the new neurons integrate to the existing neuronal networks, the majority undergo apoptosis and die. Although the brain is mostly a post-mitotic organ, the onset and progression of age-related changes is independent from the chronological age of the neurons but correlates with the lifespan of the species: neurons from 3-year-old mice show signs of accelerated senescence, whereas neurons from the brain of a 3-year-old dog are free from these changes. At present, the reason for this huge interspecies difference, despite identical mechanisms influencing the process of brain ageing, is not fully understood.

The onset and progression of age-related decline in brain functions also differs strongly between the cognitive domains in humans [3] and in animals from primates [4] and rodents [5] to zebrafish [6]. The reason for this large variance is partly the differences in the ability to recruit additional brain areas for task solving, which can partially counterbalance the effect of functional decline [7]. Another factor, which may contribute to the differences in the effect of ageing on cognitive functions, is the large variance in the sensitivity to age-related changes between brain areas and neuronal types [8,9]. Nevertheless, it is generally assumed that age-related progression in synaptic dysfunction and neuronal plasticity impairment are the direct causes of the alterations in neuronal connectivity [10] and thus functional deficits in ageing [11].

Probably the most consistent change during healthy ageing in the brain structure in humans is the shrinkage in brain volume and the expansion of the ventricular system. The frontal and parietal cortex

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together with the putamen, thalamus and accumbens are the most affected areas, whereas the volume of the brain stem remains largely unchanged in ageing [12]. Atrophic changes in white matter [13] play a major role in the reduction of brain volume. The white matter, of which the integrity is crucial for the communication between brain areas, contains mostly large myelinated axons. Probably both degeneration and loss of nerve fibres, and deterioration of myelin sheets with age, contribute to the reduction of white matter volume [14,15]. Although it is tempting to speculate that the degeneration of white matter contributes to the development of age-related cognitive deficits, the correlation between these structural and functional changes is weak [16]. In normal healthy ageing, the global number of microglia [17] or neurons [18] does not decrease significantly. However, a significant decline in neuronal number was detected in several brain regions such as the subiculum and hilus regions of the hippocampus [19] and in the entorhinal cortex [20]. These changes correlate well with the severity of declarative memory decline [21]. Although the intensity of adult neurogenesis strongly decreases in ageing, it is improbable that this effect contributes to the reduction of neuronal numbers in the hippocampus [22]. However, because the newly generated neurons participate in pattern separation involving the generation of new neuronal networks [23], this effect can contribute to age-related cognitive deficits. The extent and branching of dendrites is largely preserved during ageing [24] (but see also [25]) with a region-specific pattern [26,27], which suggests that during healthy ageing it is probably functional rather than structural alterations that are responsible for the progression of cognitive ageing.

Although detailed stereological analyses of the ageing brain revealed that the histological structure of the brain is largely preserved, the expression and composition of neurotransmitter receptors, the amount of trophic factor and their receptors characteristically changes in normal ageing and in neurodegenerative disorders [28]. Not surprising therefore that the intracellular signalling systems where the receptor signals converge also undergo characteristic changes during ageing in both neurons and glial cells. Substantial evidence from a variety of species indicates that cAMP response element-binding protein (CREB) is a molecular switch that converts alterations in receptor activity into transcriptional changes leading to long-lasting adaptation [29]. A crucial role of CREB signalling in memory formation was found in both invertebrates and vertebrates [30]. Generally, decreased CREB activity was associated with learning impairments in healthy aged animals [31,32] and with cognitive deficits in animal models of neurodegenerative disorders [33–35]. Importantly, the level of phosphorylated CREB and the activity-induced increase in CREB phosphorylation is diminished in ageing [36,37], and this itself may influence the ageing process [38,39]. Altered calcium signalling in ageing neurons significantly contributes to diminished CREB activity. It is suggested that the calcium homeostasis is disturbed in ageing, because the amplitude of the calcium-dependent after-hyperpolarising potential is increased in aged neurons [40], which makes them less excitable. The reason for this phenomenon is that the expression of L-type calcium channels increases with age [41], whereas the expression of proteins involved in the termination of calcium signal such as calcium extrusion ATPases [42,43] and calcium-binding proteins [44] is diminished in old neurons. These changes may lead to an enhanced calcium signal after activation and elevated activity of calcium-dependent signalling pathways. Age-dependent alterations in the intracellular signalling system have a huge impact on the transcriptional activity of the cells [30,38,45].

Change in the expression profile during ageing in the brain is a characteristic phenomenon [46] observed from humans [47] and mice [48] to *Drosophila* [49]. The differences in expression patterns are more prominent between age groups than between sexes, ethnicities or individuals in humans [50]. Generally, among the most affected genes are members of the calcium signalling and the CREB pathway [24], genes playing central roles in synaptic plasticity, stress responses and inflammation [47,51]. The measure of age-dependent changes in gene expression correlated with memory performance [52,53] and could be partially reversed by techniques known to positively influence the process of brain ageing [51,54,55]. It should nevertheless be noted that the detected changes in gene expression could be responsible for the neuronal deficits but they could also be adaptive, helping to maintain the structural integrity of neuronal networks in the ageing environment [56]. In neurodegenerative disorders, an acceleration of expression changes is observed in genes involved in inflammatory and apoptotic processes; thus, there is partially a continuum between age-related and neuropathological expression changes. On the other hand, there is a group of genes where the expression change is specific for the disease and the expression pattern significantly differs from the pattern observed in age-matched healthy controls [57–59]. The reason for the altered gene expression is, besides the changes in the intracellular signalling system, also an alteration of the epigenetic structure. During ageing, there is a characteristic change in chromatin structure owing to histone modifications [60,61], which can profoundly influence the accessibility of genes to transcription factors and thus the cognitive functions [62].

2. PROCESSES CONTRIBUTING TO BRAIN AGEING

The balance between the generation and clearance of toxic metabolic by-products and damaged macromolecules crucially influences the progression of ageing. Evolutionarily highly conserved homeostatic mechanisms such as anti-oxidant, DNA maintenance, and proteosome systems and autophagic processes, keep control over the continuously generated toxic or non-functional by-products and damaged macromolecules. Thus, the expression of genes encoding elements of these systems significantly influences the onset and course of the ageing process. Dividing cells can further reduce the intracellular concentration of non-degradable particles by each division. However, neurons are practically fully post-mitotic cells, therefore they are

especially sensitive to the accumulation of damaged, oxidized macromolecules. Moreover, production of reactive oxygen species (ROS) is tightly connected to energy production and thus to mitochondrial activity [63]. Because the brain has one of the highest energy demands in higher organisms, the balance between mitochondrial ROS production and activity of anti-oxidative systems also has a high impact on the progression of its ageing [64,65]. Similar molecular mechanisms contribute to the pathogenesis of neurodegenerative diseases as involved in brain ageing. The selective and significant loss of neurons in neurodegenerative disorders reflects the differences in the vulnerability of neuronal populations to different forms of cellular stress such as protein misfolding, high biosynthetic or secretory demands, oxidative stress, alterations in the dynamics of calcium signalling, etc. [66].

The mitochondrial activity is tightly regulated, because reduced activity leads to a diminished energy support, whereas increased activity results in an overproduction of ROS [67]. In ageing, a reduction in the expression of mitochondrial genes is observed [47,52], which could be a compensatory change to reduce ROS load. Furthermore, as mentioned earlier, one of the largest class of genes upregulated in ageing are involved in oxidative defence and DNA repair [47,68]. Nevertheless, an increase in the amount of oxidized macromolecules in ageing is a generally observed phenomenon in a wide range of species [69,70]. Enhanced oxidative stress in the brain generally correlates with cognitive decline [46,71] and with an enhanced risk for the development of neurodegenerative diseases [72,73]. Evidence of increased oxidative damage was found in the brain of AD patients [74,75]. This damage was present in the early stage of AD in humans [76] suggesting that it plays a causal role in the pathogenesis of the disease. In mouse models of AD, signs of oxidative stress also preceded the appearance of learning and memory deficits [77–79] further supporting the hypothesis about the potential causal role of oxidative damage in AD. Enhanced oxidative stress was found in the dopaminergic neurons in the ageing substantia nigra of humans suffering from Parkinson's disease [80,81] and in the noradrenergic neurons in the locus coeruleus of AD patients [82]. The selective loss of neurons in these cases is probably the result of the increased ROS production owing to the metabolism of catecholamines [83].

As a result, catecholaminergic neurons have depleted anti-oxidant levels making them vulnerable to oxidative stress. Not surprising therefore that anti-oxidants were intensively tested as potential therapeutic agents against the negative consequences of brain ageing and neurodegenerative disorders [84–86]. Generally, it was found that anti-oxidant treatment or activation of anti-oxidative pathways improve brain functions and partially restores age-dependent changes in gene expression both in normal ageing [87,88] and in models of accelerated ageing [89,90]. Clinical data suggest that dietary anti-oxidants have some protective effects against AD, Parkinson's disease and amyotrophic lateral sclerosis [91] and also in pharmacological and genetic models of neurodegenerative disorders [92–94]. We have to note that ROS have an important intracellular signalling function: oxidative stress responses may regulate autophagy and induce synaptic growth [95,96] suggesting that long-lasting diminished ROS production by pharmacological treatment could have a negative impact on brain functions.

The increasing concentration of toxic metabolic end products, misfolded macromolecules and non-functional organelles, is a typical characteristic of the ageing brain and it is thought to significantly contribute to cognitive decline [46]. Neurodegenerative diseases such as AD [97], Parkinson's [98] and Huntington's [99] disease, are each associated with the accumulation of specific protein aggregates, which play a key role in the pathogenesis of the disease. Thus, the intensity and efficacy of clearance of the damaged molecules largely determines the progression of neurodegenerative disorders [100–102] but also the pace of brain ageing in healthy individuals [46,103,104]. The significance of autophagic processes in normal ageing is shown by a genome-wide association study which found a significant association between genetic variance in autophagy associated genes and survival in healthy aged humans [105]. It is generally observed that improving clearance with the induction of autophagy [10,106] or proteasome activity [107,108] slows down the ageing process and improves cognitive functions. Moreover, it was shown that reduced autophagy causes ageing-like decrements [109,110] and facilitates the development of neurodegenerative diseases [111]. Not surprising therefore that induction of autophagy was intensively tested in various models of neurodegenerative diseases. Although it is known that autophagy *per se* contributes to neuronal death [112–114], it was concluded that facilitation of clearance processes is a potential strategy in the treatment of neurodegenerative disorders [115].

The increasing amount of altered macromolecules and release of signal molecules from ageing damaged neurons activate the immune system of the brain [116,117]. The major cell types of the immune system in the brain are astrocytes and microglial cells. Astrocytes, which are the most numerous glial cell population of the brain, secrete cytokines and chemokines after induction and therefore can contribute to an inflammatory environment in the brain. Microglial cells originate from a macrophage lineage and they are the main form of active immune defence within the central nervous system. Glial cells and neurons are in a mutual interaction: astrocytes but also microglia control neuronal activity providing trophic factors [118,119], energy [120,121] and regulation of synaptic transmission [122] as well as neurogenesis [123]. Neurons exert an inhibitory control on the immune activity of glial cells [124]. Normal, healthy brain ageing is associated with an increased number of activated microglia [125–127] and with an enhanced level of pro-inflammatory cytokines. The increased pro-inflammatory environment in the brain accelerates brain ageing [128,129], aggravates cognitive deficits impairing neuronal functions [130–132] and reduces neurogenesis [133,134]. Activated microglia can be both anti-inflammatory (which supports the neurons) and pro-inflammatory (which damages neurons).

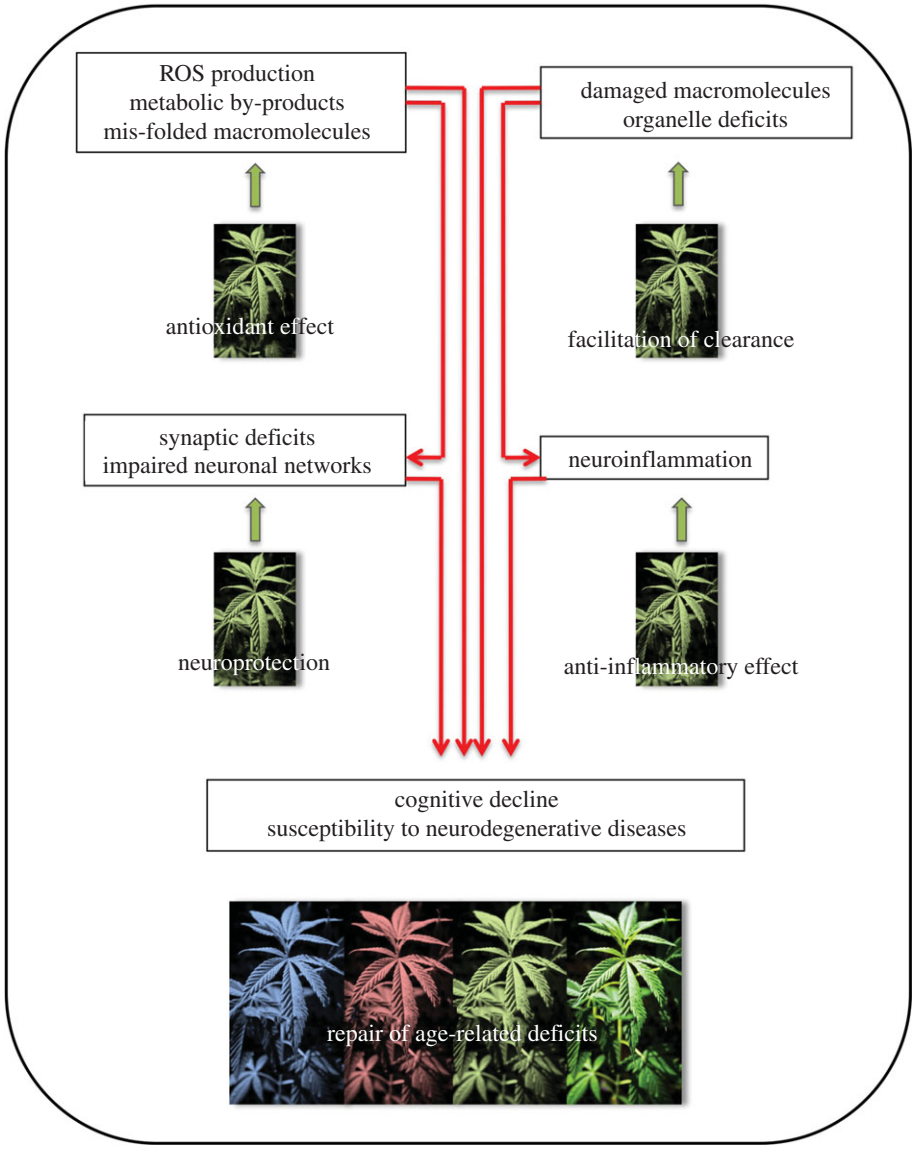

Figure 1. Accumulation of damaged macromolecules, organelle deficits lead to impairment in neuronal functions and neuroinflammation. These mutually interacting processes are the major driving forces of brain ageing. Activity of cannabinoid system antagonizes these changes and thus decreases the progression of brain aging.

It is generally observed that in the ageing brain microglial activation and the resulting pro-inflammatory environment contributes to cognitive impairments [135,136] and promotes neurodegeneration [137]. In the brain of patients suffering from neurodegenerative diseases [138–140] and in animal models of these disorders [141–144], signs of massive neuroinflammation are detected. Importantly, the phenotype of both astrocytes and microglia changes in ageing. During ageing, a characteristic secretory phenotype was observed in different cell types [145], as well as in astrocytes in the brain [146]. Senescent astrocytes are dysfunctional and provide a reduced support for neurons [147]. Microglia in the brain of old individuals show activated, amoeboid-like morphology, their cytoplasm contains lipofuscin granules, and they are primed for exaggerated immune response. Moreover, senescent microglia are less functional because they have serious autophagic dysfunction [148]. It is thought the reduced autophagy combined with an enhanced pro-inflammatory character leads to enhanced neurotoxicity and contributes to neurodegenerative changes [149,150]. On the basis of significant contribution of immune system activity to the development of cognitive deficits, it was suggested that rejuvenation of immunity could reverse age-related cognitive deficits [151].

3. THE CANNABINOID SYSTEM MODULATES AGE-RELATED MOLECULAR AND CELLULAR PROCESSES

There are several lines of evidence that cannabinoid system activity modulates critical molecular and cellular processes influencing the pace of the ageing process (figure 1).

(a) *Oxidative stress*

Phytocannabinoids and structurally related synthetic compounds are known to possess anti-oxidant properties [152,153]. Although the structurally unrelated endogenous cannabinoid 2-AG is able to inhibit ROS formation *in vitro* [154], when considering its *in vivo*

anti-oxidant properties one should keep in mind that it is metabolized by the prostaglandin/leukotriene pathway [155] resulting indirectly in pro-oxidant actions. It was suggested that besides the chemical structure of the cannabinoids, their metal-ion chelating property could also contribute to their anti-oxidant capacity [156]. The protective effect of cannabinoids against oxidative stress *in vitro* in neurons is dependent on their anti-oxidant properties as cannabinoid receptors are not involved in this process [157,158]. However, in astrocytes, the CB1 receptor mediates the protective effect of cannabinoids, because cannabinoids prevented H_2O_2-induced loss of viability of astrocytes in cell culture in a CB1-receptor-dependent manner [159]. For the neuroprotective effect of cannabinoids both their anti-oxidant and receptor binding properties contribute, as was shown in a rodent model of Parkinson's disease [160,161]. The neuroprotective effect of the CB1 receptor agonists Δ^9-THC and WIN55,212-2 was blocked by the CB1 antagonist rimonabant on hippocampal neurons in culture [162] suggesting further the importance of cannabinoid receptor activity in neuroprotection. It is important to note that the cannabinoids modulate oxidative load in a cell-type-specific manner: in glioma [163] and leukaemia cell lines [164] but also in hepatic cells [165,166], cannabinoids induce cell death by increasing oxidative stress.

(b) *Clearance of damaged macromolecules*

The cannabinoid system can reduce oxidative load besides reducing the amount of ROS by influencing the removal of damaged macromolecules. It has been recently shown that the majority of CB1 receptors do not reach the cell surface but instead show an intracellular localization. A significant part of the intracellular CB1 receptors is present at lysosomes and late endosomes [167,168]. Cannabinoids at physiological concentrations increase lysosomal stability and integrity [169]. On the other hand, Δ^9-THC in high concentration can increase lysosomal permeability through CB1 receptor binding, which may contribute to the neurotoxic effect of the drug [170]. Whether decreased CB1 receptor activity owing to genetic variation or epigenetic changes in humans influences lysosomal function and thus contributes to the development of neurodegenerative diseases is not known.

Cannabinoids have been previously implicated in inducing autophagy in various cancer cell types: glioma [171] hepatocellular carcinoma [172], pancreatic adenocarcinoma [173], and these effects are at least, in part, dependent on the CB1 receptor. We have found an altered autophagosomal and lysosomal turnover in Cnr1$^{-/-}$ mice (A. Piyanova 2012, unpublished results), suggesting a regulatory role for endocannabinoid signalling in autophagy also in non-cancerous tissues. This hypothesis was recently supported by Redlich *et al*. [174], who showed that the endocannabinoid palmitoylethanolamide increased the phagocytosis of murine microglial cells. The autophagy dysregulation owing to reduced CB1 receptor signalling is probably more important in ageing, as there is a general increase in oxidative load as well as formation of lipofuscin-like aggregates in the neurons and microglial cells.

(c) *Mitochondrial activity*

Cannabinoid system activity influences the amount of intracellular ROS not only via their anti-oxidant buffering capacity but also by regulating mitochondrial activity [175]. It has been recently shown that CB1 receptors are also present on mitochondrial membranes and regulate the activity of mitochondria [176]. Whether cannabinoids decrease or increase mitochondrial activity is not fully known: an early study showed that cannabinoids can decrease oxidative metabolism of isolated mitochondria [177], which was later supported by showing that CB1 agonists decrease oxygen consumption, ROS production, membrane potential [175] and oxidative phosphorylation [178]. On the other hand, an increase in brain mitochondrial oxidative phosphorylation was shown *ex vivo* in anandamide or Δ^9-THC treated rats, which was antagonized by the CB1 receptor blocker SR141716A [179]. Under cellular stress, cannabinoids seems to be protective for the mitochondria: the cannabinoid receptor agonist CP55,940 and JWH-015 both attenuated mitochondrial damage against paraquat-induced oxidative stress [180] and the endogenous cannabinoid 2-AG decreased calcium-induced cytochrome c release from mitochondria [181].

(d) *Regulation of glial activity*

The cannabinoid system also influences ROS levels indirectly through the regulation of glial activity [182]. Both astrocytes and microglia express CB1 and CB2 cannabinoid receptors in an activity-dependent manner [183–185]. In the microglia, the expression of CB2 receptor exceeds the expression of CB1 receptors and correlates with microglial phenotype and activity [186]. Activation of glial CB2 receptors attenuates glial activation [187] and prevents neurodegeneration and reduces symptoms in mouse model of Huntington's disease [188]. These data suggest that cannabinoids regulate glial activity primarily through CB2 receptors. Glial cells not only receive cannabinoid signals but can themselves produce cannabinoids such as anandamide [189], 2-AG [190] and palmitoylethanolamide [191], and also express the enzymes involved in the synthesis and degradation of endocannabinoids [192–194]. Because both neurons and glia express elements of the cannabinoid system, it was hypothesized that the cannabinoid system plays an important role in neuron–glia communication. In support of this hypothesis, it was shown that cannabinoids modulate glial activity by directly binding to the glial cannabinoid receptors [185,195,196] and indirectly by modulating neuronal activities [197]. During central nervous system inflammation, as in multiple sclerosis [195], AD [198] or HIV encephalitis [199], a general upregulation of cannabinoid system activity is observed. Increased activity of the cannabinoid system is generally anti-inflammatory: elevation of anandamide levels [200,201] or activation of the cannabinoid receptor by synthetic receptor agonists [202,203] inhibits the production of pro-inflammatory mediators and reduces microglial migration *in vitro*. This effect may contribute to the beneficial effect of the cannabinoid system against neurodegeneration [204]. On the other hand, increased 2-AG levels increase inhibitory signalling and impair

the control of retrograde neurotransmission thus contributing to the synaptic impairments in AD [198].

(e) ***Synaptic plasticity and neurogenesis***

The age-dependent decline in brain-derived neurotrophic factor (BDNF) expression plays a crucial role in the chain of events leading to functional deficits. BDNF signalling regulates morphological and physiological synaptic plasticity [205], and restoration of BDNF levels was crucial for the rescue of synaptic plasticity in aged animals [206]. Application of CB1 receptor agonists increased BDNF expression both *in vitro* and *in vivo* [207,208], which may significantly contribute to the neuroprotective effect of the cannabinoids. This hypothesis was supported by Marsicano showing that induction of BDNF expression contributes to the protective effect of CB1 receptor activity against excitotoxicity [209]. CB1 receptor activity can enhance TrkB signalling partly by activating MAP kinase/ERK kinase pathways [210] but also by directly transactivating the TrkB receptors [211]. The fact that genetic deletion of CB1 receptors leads to a decreased BDNF expression suggests that endogenous cannabinoids exert a tonic control over BDNF expression [212]. Clinical data testing the BDNF levels in marijuana users and control individuals [213] also support the role of CB1 receptor activity on BDNF expression.

Besides influencing synaptic plasticity, cannabinoid system activity also facilitates embryonic and adult neurogenesis. Neural progenitor cells express the elements of the cannabinoid system, and thus actively use endocannabinoids as signalling molecules [214]. Activation of cannabinoid receptors by agonists [215,216] or by elevation of endocannabinoid levels [215,217,218] promotes cell proliferation, neurogenesis and neuronal diversification [219]. It was shown that CB1 receptor activity is necessary for the up-regulation of neurogenesis and proliferation after excitotoxic stress [220]. In good agreement with these findings, genetic deletion of CB1 receptors leads to defective neurogenesis [221]. The consequence of this effect on the learning phenotype is unclear but it is unrelated to the early loss of cognitive abilities in this strain [197].

4. CANNABINOID SYSTEM MAY CHANGE DURING AGEING IN THE BRAIN

Because cannabinoid system activity regulates mechanisms underlying normal and pathological ageing, age-dependent change in the activity of the cannabinoid system might contribute to the process of ageing. Although the results of different groups are sometimes conflicting, a decline in cannabinoid system activity in ageing is probable. Berrendero, in one of his earlier works, found a lower level of CB1 receptor level in rats in a brain-region-specific manner: the reduction was most prominent in the cerebellum and cerebral cortex, whereas it was less pronounced but still significant in the limbic and hypothalamic structures as well as in the hippocampus [222]. A similar decrease in both CB1 receptor binding and mRNA levels was found in most of the basal ganglia in rats during ageing [223]. Also, in isolated rat hippocampal synaptosomes, a reduction in CB1 receptor densities in ageing was described [224]. On the contrary, Liu *et al.* [225] found no differences in CB1 receptor protein levels between four- and 24-month-old rats in the hippocampus. They found, however, a significant difference in the CB1 receptor levels in the adjacent structures: they were reduced in the postrhinal, whereas elevated in the entorhinal and temporal cortices in the old animals. Wang *et al.* [226], testing C57BL/6J mice, did not find differences in CB1 densities in the hippocampus, limbic forebrain, amygdala and cerebellum. On the other hand, he reported a significantly reduced coupling between the receptors and Gi proteins in the limbic forebrain during ageing, which could be responsible for a reduced CB1 receptor signalling even when receptor levels are unchanged. In humans, a sex-dependent increase in CB1 receptor binding during ageing in the basal ganglia, lateral temporal cortex and in limbic areas was reported [227]. Whether the level of endogenous cannabinoids changes in ageing is unclear: some studies have reported diminished anandamide levels during ageing in CB1-receptor-deficient mice [228,229], whereas others found no significant differences in the endocannabinoid levels during ageing in different brain regions in WT or CB1-receptor-deficient mice [226]. There are also considerable differences in the endogenous cannabinoid concentrations reported in those earlier studies compared with more recent work (for review, see Buczynski & Parsons [230]). It is now known that multiple factors can influence the endogenous cannabinoid levels, such as post-mortem tissue handling and sample extraction methods, thus contributing to discrepancies between the studies from different groups.

5. AGE-DEPENDENT EFFECT OF THE CANNABINOID SYSTEM ON LEARNING AND MEMORY

Considering that the cannabinoid system regulates processes involved in ageing one would expect that (i) reduced activity of the cannabinoid system in genetically modified animals is accompanied by accelerated progression of ageing, and (ii) cannabinoid receptor agonists have different effects in young and old individuals. And in reality, genetic deletion of CB1 receptors leads to an age-related change in the learning and memory abilities of mice. Young CB1 receptor knockout ($CB1^{-/-}$) mice showed a superior performance in a broad range of models including object [231] and social recognition tests, in skill and operant learning models [232] as well as enhanced long-term potentiation [233]. The learning ability of 12-month-old $CB1^{-/-}$ mice is, however, impaired and this impairment was accompanied by a loss of principal neurons in the hippocampus. Importantly, the early onset of age-related changes was specific for the cognitive functions in the brain, because neither the motor nor sensory abilities were affected [5]. The peripheral organs also did not show signs of early ageing except in the skin, where an atrophy of the subdermal fat layer was present in 12-month-old $CB1^{-/-}$ but not in wild-type mice [5]. A following study showed that the early onset of cognitive decline and neuronal loss was accompanied by neuroinflammatory changes in the

hippocampus, but not in the cortex or striatum [197]. It was suggested that CB1 receptors on the GABAergic neurons play a principal role in the regulation of glial activity and thus in protection against age-related changes: deletion of CB1 receptors from the forebrain GABAergic but not glutamatergic neurons resulted in a similar ageing phenotype as found in the constitutive knockouts [197]. Interestingly, similar neuroinflammatory changes were not present in the cortex and striatum, brain areas having high expression of CB1 receptors and containing abundant GABAergic neurons. Thus, we can conclude that CB1 receptor activity plays an important role in anti-ageing defence. On the other hand, genetic deletion of CB2 receptors does not lead to similar enhanced lipofuscin accumulation or glial activation in the brain (A. Bilkei-Gorzo 2012, unpublished observation), therefore this receptor type is probably not involved in age-related processes. The question of whether 2-AG or anandamide is responsible for the anti-ageing activity of CB1 receptor is not known: although animals with genetic deletion of key enzymes of endocannabinoid metabolism are available [234], their age-related phenotype has not yet been published. On the basis of previous results, one would expect a delayed or slowed ageing in animals having increased CB receptor signalling. In good accordance with this assumption, animals lacking the anandamide degrading enzyme fatty acid amino hydrolase (FAAH) show an attenuated ageing associated decline in cardiac function and decreased expression of inflammation-associated genes [235]. Whether genetic variation in genes encoding the elements of the cannabinoid system in humans contributes to the variation in the progression of age-related cognitive decline is an open question.

The age dependency of cannabinoids on cognitive functions was demonstrated by a number of studies showing that adolescents are more sensitive to the adverse long-term effects of cannabinoid receptor agonists on cognitive abilities as adults [236–238]. On the basis of biological effects of cannabinoids, it was suggested that in old individuals cannabinoid receptor ligands may have even beneficial effects against age-related cognitive deficits [239]. Only a low number of publications exists focusing on the influence of cannabinoids on brain functions in healthy aged animals, but their results support this hypothesis: the CB1 receptor agonist WIN-55212-2 attenuated spatial memory impairment, reduced the number of activated microglia [240] and triggered neurogenesis [216] in aged rats. Similar to CB1 agonists, CB2 agonists or blockers of FAAH also enhanced proliferation of neuronal progenitor cells in old individuals [215]. These reports and the accelerated ageing phenotype of CB1 knockout animals together suggest that elevation of cannabinoid system activity ameliorates symptoms of brain ageing.

6. POTENTIAL ROLE OF CANNABINOIDS IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

It was suggested that modulators of cannabinoid system activity could be a therapeutic tool for the treatment of neurodegenerative diseases [241,242]. At first sight, it is striking that cannabinoid agonists, substances known to impair cognitive functions, could be beneficial in neurodegenerative cognitive disorders. However, cannabinoid receptor activation could reduce oxidative stress and excitotoxicity, suppress neuroinflammatory processes and thus alleviate the symptoms of neurodegenerative motor [243] and cognitive diseases [244]. *In vitro* studies together with pharmacological experiments on animal models of AD generally supported the speculations about the therapeutic value of CB1 receptor agonists in the treatment of AD [245]. Cannabinoids protected against microglia-mediated neurotoxicity elicited by amyloid beta protein in rat cortical coculture [246]. *In vivo*, chronic treatment with CB1 agonists in the pre-symptomatic and early symptomatic phases ameliorated cognitive deficits and reduced microglial activity and cortical amyloid β-protein levels in APP2576 and APP/PS1 transgenic mice [247,248]. In rats, CB1 receptor agonists prevented cognitive impairment and microglial activation induced by intracerebroventricular injection of amyloid β-protein [246]. Despite the promising preclinical results, the detailed clinical evaluation of cannabinoids in AD patients is still missing. One pilot study, however, reported a significant reduction in nocturnal motor activity and agitation after dronabinol treatment in patients in late stages of dementia [249]. Cannabinoid treatment effectively attenuated the loss of dopaminergic neurons in rat models of Parkinson's disease [160,161] and proved to be neuroprotective in human neuronal cell culture exposed to Parkinson's disease relevant toxins [250]. The authors suggested that the anti-oxidative effect and suppression of microglial activity by cannabinoids together play a major role in these models [161,251]. The three published clinical trials in Parkinson's disease patients did not provide a clear answer whether cannabinoids modify the progression or the outcome of the disease. A double-blind, placebo-controlled crossover study that tested the symptom relieving effect of cannabis testing nine patients reported a significant reduction in levodopa-induced dyskinesia [252]. The following two clinical trials using a larger number of patients could not find any improvement in levodopa treatment induced dyskinesia or parkinsonian motor disability using cannabis [253] or the CB1 receptor antagonist rimonabant [254]. The situation is similar in the case of Huntington's disease and amyotrophic lateral sclerosis: a limited number of promising preclinical results using animal models of the diseases are reported but the supporting clinical data are missing [255–258]. Both cannabinoid receptor agonists and elevation of cannabinoid levels led to a significant improvement in disease progression and alleviation of symptoms in rodent models of multiple sclerosis [259,260]. Moreover, a case–control human genetic study reported an association between the genetic variance of CNR1 and primary progressive multiple sclerosis [261]. Unlike with other neurodegenerative disorders, numerous clinical studies were carried out testing different cannabis plant preparations and synthetic cannabinoids on patients with multiple sclerosis. It is clear from these studies that cannabinoid treatment alleviates the symptoms of the disease, reducing pain and sleep

disturbances and improving general well being [262]. There are some conflicting results published regarding the effect of cannabis plant extracts on spasticity [263,264], but in a recent meta-analysis a trend for reduced spasticity was reported [265]. Generally, the treatment was well tolerated and maintained its efficacy after long-lasting administration [266], which is a prerequisite in the treatment of a chronic progressive disease. The recently completed CUPID study (http://sites.pcmd.ac.uk/cnrg/cupid.php) focused on the effect of THC on the progression of multiple sclerosis. In this large study, which involved 493 patients and ran for 8 years, the researchers found beneficial effects in patients in the initial phase of the diseases but no evidence for slowing down the progression of the disease generally.

7. CONCLUSION

Experimental evidences show that cannabinoid system activity is neuroprotective regulating critical homeostatic processes and that cannabinoid signalling is possibly decreasing in ageing. Thus, elevation of cannabinoid receptor activity either by pharmacological blockade of the degradation of cannabinoids or by receptor agonists could be a promising strategy for slowing down the progression of brain ageing and for alleviating the symptoms of neurodegenerative disorders.

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