Role of CB1 cannabinoid receptors on GABAergic neurons in brain aging

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Brain aging is associated with cognitive decline that is accompanied by progressive neuroinflammatory changes. The endocannabinoid system (ECS) is involved in the regulation of glial activity and influences the progression of age-related learning and memory deficits. Mice lacking the Cnr1 gene ($Cnr1^{-/-}$), which encodes the cannabinoid receptor 1 (CB1), showed an accelerated age-dependent deficit in spatial learning accompanied by a loss of principal neurons in the hippocampus. The age-dependent decrease in neuronal numbers in $Cnr1^{-/-}$ mice was not related to decreased neurogenesis or to epileptic seizures. However, enhanced neuroinflammation characterized by an increased density of astrocytes and activated microglia as well as an enhanced expression of the inflammatory cytokine IL-6 during aging was present in the hippocampus of $Cnr1^{-/-}$ mice. The ongoing process of pyramidal cell degeneration and neuroinflammation can exacerbate each other and both contribute to the cognitive deficits. Deletion of CB1 receptors from the forebrain GABAergic, but not from the glutamatergic neurons, led to a similar neuronal loss and increased neuroinflammation in the hippocampus as observed in animals lacking CB1 receptors in all cells. Our results suggest that CB1 receptor activity on hippocampal GABAergic neurons protects against age-dependent cognitive decline by reducing pyramidal cell degeneration and neuroinflammation.

glial regulation | Morris water maze | stereological quantification | CD40 expression | telemetric EEG recording

The cellular process underlying aging has remained one of the last frontiers in biology. The question of why aging occurs and what determines the speed of aging has amazed mankind since ancient times. Aging of the brain is associated with an increased risk of neurodegenerative diseases as well as with a cognitive decline, even in healthy individuals. Although the onset and progression of learning and memory deficits vary strongly among individuals, age-related deficits in learning are generally observed phenomena in many species, from *Caenorhabditis elegans* (1) to humans (2). Which factors influence the onset and progression of the learning and memory deficits has not been understood yet, but it is hypothesized that the balance between physical, chemical, and biological stressors and antistress responses play a crucial role (3). The immune system is an important element of the antistress system, and it is thought enhanced immune responses play a significant role in the aging process (4–6).

Besides controlling the brain microenvironment, astrocytes participate in immune responses as nonprofessional antigen presenting cells, as well as in cellular repair and scar formation after injury. The expression of glial fibrillary acidic protein (GFAP), a widely used marker of mature astrocytes, is markedly up-regulated after brain injury (7). Microglia, primary immune cells of the brain, continuously survey the central nervous system. They are equipped with receptors that enable them to detect pathogen- or danger-associated molecular patterns (4). In the aging brain, the increasing concentration of abnormal macromolecules promotes the activation of microglia (8). After activation, microglial cells change their morphology from ramified to amoeboid, display a macrophage-like phenotype (9), and increase the expression of ionized calcium binding adapter molecule 1 (Iba1) (10), a microglia-specific protein in the brain (11). Activated microglia can be supportive or toxic to the neighboring neurons (9). The phenotype of the activated microglia is largely determined by the balance between proinflammatory signals and the inhibitory control of neurons (4). Neuron–glia interactions are mediated by surface protein ligand-receptor pairs and also by neurotransmitters (9).

Several lines of evidence suggest that the endocannabinoid system (ECS) might be involved in the regulation of glial activity. Astrocytes and microglial cells can synthesize endocannabinoids because they express enzymes involved in the synthesis (12, 13) and degradation (13, 14) of endocannabinoids. In the central nervous system, cannabinoid type 1 (CB1) receptors are found primarily on neurons and at a marginal level, on glial cells (15). The expression of CB1 receptors varies between brain areas and neuronal cell types. In the hippocampus, GABAergic cells show high, whereas glutamatergic neurons a low CB1 receptor expression (16). The neuronal expression of CB2 receptors in the central nervous system is very low and restricted to some brainstem nuclei and possibly to the cerebellum (17). CB2 receptor expression in astrocytes and microglia generally exceeds the expression of CB1 receptors (18). Thus, the primary receptors for cannabinoid signaling in the brain are CB1 on neurons and CB2 on glia cells.

Activation of CB1 receptor inhibits transmitter release and thus regulates synaptic functions (19). In case of excessive glutamate release, endocannabinoid signaling acts as a circuit breaker (20) and prevents neurons from glutamate excitotoxicity (21). Moreover, the cannabinoid system exerts a control function over the immune system. Activation of CB2 and probably also CB1 receptor, enhances migration and adhesion of immune cells, decreases the release of proinflammatory cytokines (22), and induces apoptosis in dendritic cells (23). These data led to the assumption that endocannabinoid signaling plays a role in the development of neurodegenerative diseases (24) and could be a potential target for novel pharmacotherapies (25). It is known both neurodegenerative changes and chronically enhanced neuroinflammation may contribute to aging of the brain. We therefore hypothesized that the activity of the cannabinoid system, through the regulation of glial responses, may influence the progression of brain aging.

Phenotype analysis of constitutive $Cnr1^{-/-}$ animals revealed that young null mutants have a superior, whereas old ones an

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inferior learning ability. This was interpreted as an early onset of age-related memory impairment (26). In the present study, we first asked whether the early cognitive deficit and neuronal loss is accompanied with enhanced neuroinflammation. Furthermore, we also addressed the question whether CB1 receptor on GABAergic or on glutamatergic neurons protects against age-related changes in the brain.

Results

Increased Age-Related Neuroinflammation in the Hippocampus of Cnr1 Null Mutants. To test the role of the endocannabinoid system in the control of age-related neuroinflammation, we first compared the changes in astrocyte densities between 2, 5, and 12 mo-old $Cnr1^{-/-}$ and $Cnr1^{+/+}$ littermates (27, 28). We found an age-related increase in the density of GFAP-immunopositive cells in the hippocampus, which was significantly enhanced in $Cnr1^{-/-}$ animals in this brain area (Fig. 1*A*). Quantitative stereological analysis also revealed a progressive increase in the number of Iba1-positive microglial cells in the hippocampus during aging, which was more pronounced in the CA1 region of $Cnr1^{-/-}$ mice (Fig. 1*C*). No discernible differences were detected in Iba1 and GFAP staining patterns or intensity in the striatum or in the cortex between the genotypes (Fig. S1). Morphological analysis of the microglial cells revealed that the density of the activated forms of microglia increased age dependently in the hippocampus in $Cnr1^{-/-}$ mice, whereas the amount of microglia with resting, ramified morphology, did not differ between the age groups or genotypes (Fig. S2 *A*–*C*). To validate our histological findings, we next determined the microglial phenotype in more detail. Expression of CD40, a costimulatory protein on antigen presenting cells, is associated with microglial activation (29); therefore, we determined the percentage of CD40-positive cells by flow cytometry from the hippocampi of old $Cnr1^{+/+}$ and $Cnr1^{-/-}$ littermates. In accordance with our histological data, we found a significantly increased percentage of CD40-positive microglia in the $Cnr1^{-/-}$ animals (Fig. 1*B*). We next determined the expression of inflammatory cytokines in 2- and 12-mo-old animals of both genotypes. IL-6, but not TNF or IL-1β, expression was significantly elevated during aging in $Cnr1^{-/-}$ animals (Fig. 1*D*). In contrast, there was no difference in the expression levels between the age groups in $Cnr1^{+/+}$ mice. These data demonstrate that the lack of CB1 receptor leads to an age-dependent enhanced neuroinflammation characterized by hippocampal astrocyte and microglial activation, as well as an elevation of IL-6 levels.

Age-Related Reduction in Hippocampal Neuronal Number and Diminished Neurogenesis in Juvenile $Cnr1^{-/-}$ Mice. To test whether increased neuroinflammation in $Cnr1^{-/-}$ mice is accompanied by a loss of neurons, we compared the number of neurons in the hippocampus, striatum, cingulate, and retrosplenial granular cortex between $Cnr1^{+/+}$ and $Cnr1^{-/-}$ littermates. In good accordance with our previous data (26), we found an age-dependent reduction in neuronal numbers in the hippocampus (Fig. S3 *A* and *B*), but not in other brain areas (Fig. S3*C*). The genotype difference in the number of principal neurons seemed to increase with age. In the CA1 region, the cell density was reduced by 9.5% in

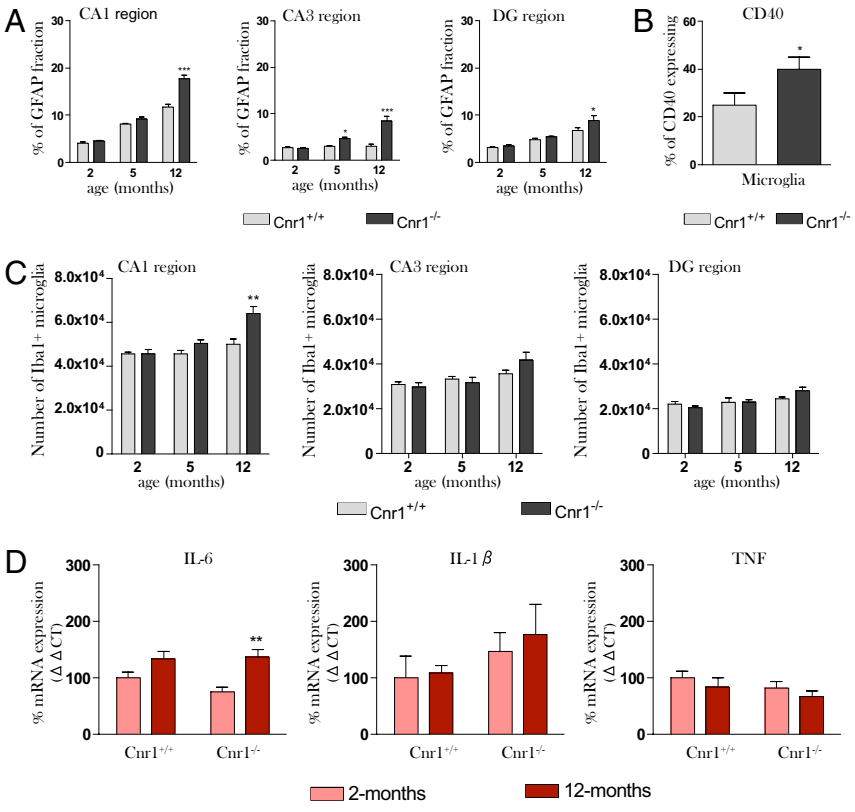

Fig. 1. Progressive reactive gliosis in $Cnr1^{-/-}$ mice. (*A*) The age-dependent increase in the fraction of GFAP-immunopositive (IR) areas in the CA1 and CA3 regions of the hippocampus is exacerbated in $Cnr1^{-/-}$ mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ difference between the genotypes (two-way ANOVA followed by Bonferroni post hoc test, $n = 6$ per group). (*B*) Graph showing the percentage of CD40 expressing microglial cells from the brains of 12-mo-old $Cnr1^{+/+}$ or $Cnr1^{-/-}$ mice. ($*P < 0.05$ Student's t test; $n = 8$–10 mice/group). (*C*) Quantitative stereological analysis of the total number of Iba1-positive cells in the hippocampus ($*P < 0.05$, $**P < 0.01$ two-way ANOVA followed by Bonferroni post hoc test; $n = 4$–5 per group). (*D*) In $Cnr1^{-/-}$ animals, IL-6 but not TNF or IL-1β expression was significantly elevated in aging. In $Cnr1^{+/+}$ mice there was no difference in the expression levels of cytokines between the age groups ($**P <$ 0.01 Student's t test; $n = 6$ mice/group).

2-mo-old and by 17.9% in 12-mo-old Cnr1$^{-/-}$ mice compared with Cnr1$^{+/+}$ mice. The low level of neurogenesis in Cnr1$^{-/-}$ animals (30) could contribute to the reduction of the neuronal numbers in this strain. To test this possibility, we compared neurogenesis between Cnr1$^{+/+}$ and Cnr1$^{-/-}$ mice by quantifying BrdU-labeled neurons in the dentate gyrus. In the 2-mo-old age group we found 48% fewer BrdU-positive cells in the null mutants, indicating a reduced neurogenesis (Fig. S4). However, 5- and 12-mo-old mice showed a similar low level of neurogenesis, suggesting that the initial genotype difference in neurogenesis disappears with aging. Thus, the difference in neurogenesis does not play a significant role in the age-dependent reduction of neuronal number in animals lacking the CB1 receptor.

Age-Related Learning and Memory Deficit in Cnr1$^{-/-}$ Animals in the Water Maze Test. Because glial activation and neuronal loss were most prominent in the hippocampus, we next asked whether they influence hippocampal function. We therefore tested the performance of Cnr1$^{+/+}$ and Cnr1$^{-/-}$ littermates in the Morris water maze (MWM) test, a behavioral paradigm that is highly dependent on hippocampal function (31). In the acquisition phase, we assessed the spatial learning and memory abilities of the mice, whereas on the reversal phase, we investigated the flexibility of memory (Fig. S5*A*). This study revealed that 2-mo-old Cnr1$^{-/-}$ mice have a superior learning ability, because they learned the platform location significantly faster than their WT littermates in both phases of the test (Fig. 2). At the age of 5 mo, Cnr1$^{-/-}$ mice still showed the same performance as their wild-type littermates in the acquisition phase. However, they demonstrated significant deficits in learning a new platform position. Twelve-month-old Cnr1$^{-/-}$ mice showed significantly impaired performances in both phases of the test (Fig. S5*B*). To further investigate the relationship between the learning deficit and enhanced glial activity, we tested 12-mo-old Cnr1$^{+/+}$ and Cnr1$^{-/-}$ mice in the *Y*-maze test. In this model, working memory performance is mostly dependent on the activity of the prefrontal cortex (32), an area largely spared from the neuroinflammatory changes. The ratio of spontaneous alternations, an indicator of working memory strength, did not differ between the genotypes (59.16 ± 1.81 in Cnr1$^{+/+}$ vs. 57.71 ± 1.24 in Cnr1$^{-/-}$ mice; Fig. S5*C*).

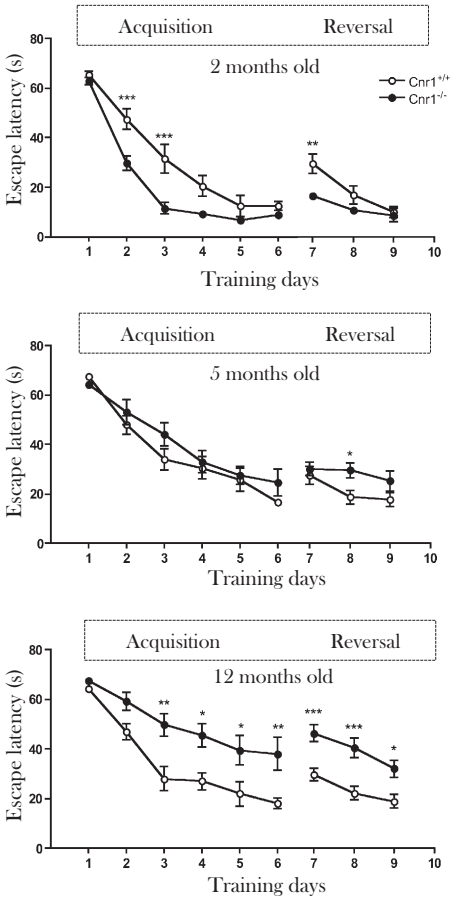

Fig. 2. Age-dependent deficit in spatial learning in *Cnr1*$^{-/-}$ mice. Curves show the escape latencies during the acquisition and reversal trials. *P < 0.05, **P < 0.01, ***P < 0.001 difference between the genotypes (two-way ANOVA followed by Bonferroni's t test; n = 12 per group).

Neuroinflammation and Neuronal Loss Are Not the Consequence of Epileptic Seizures. Increased neuroinflammation could be the result of a disturbed regulation of glial activity, but alternatively, it could be a response to neuronal damage. It was previously published that Cnr1$^{-/-}$ mice are epilepsy prone (28); therefore, we asked whether the increased glial activation is due to recurrent epileptic seizures in the null mutants (33). Major epileptic seizures lead to dendritic reorganization in the hippocampus, which is detectable with Timm staining (34). However, the size of the Timm stained areas did not differ between 12-mo-old Cnr1$^{+/+}$ and Cnr1$^{-/-}$ mice (Fig. S6 *F* and *G*). We next attempted to detect minor epileptic events, which do not lead to dendritic reorganization, but could potentially impair neurons. Thus, Cnr1$^{-/-}$ and Cnr1$^{+/+}$ littermates were implanted with cortical electrodes, and their EEG activity and behavior was continuously monitored for 19 d. We found no sign of epileptic seizures or epileptic EEG activity in Cnr1$^{-/-}$ and Cnr1$^{+/+}$ littermates (Fig. S6 *A*–*E*). We concluded that spontaneous epileptic seizures are not the cause of the enhanced neuroinflammation and early cognitive decline in Cnr1$^{-/-}$ animals.

Lack of CB1 Receptor on GABAergic but Not on Glutamatergic Neurons Leads to Enhanced Glial Responses and Neuronal Loss. Finally, we asked whether CB1 receptor activity in glutamatergic or GABAergic neurons is necessary for the protection against age-related neuronal loss and enhanced glial activity. To answer this question we first compared the histological changes in the brains of 12-mo-old conditional knockout animals lacking CB1 receptors either in forebrain glutamatergic (Glu-Cnr1$^{-/-}$) or GABAergic (GABA-Cnr1$^{-/-}$) neurons (35). The number of neurons, microglial cells, and astrocyte densities was similar in the hippocampus of Glu-Cnr1$^{-/-}$ mice and their wild-type littermates (Fig. 3 *A*–*C*). GABA-Cnr1$^{-/-}$ mice, on the other hand, had a significantly diminished number of principal neurons in all of the three major hippocampal regions examined (Fig. 3*A*). Furthermore, significantly elevated astrocyte densities in the CA1 and CA3 regions (Fig. 3*C*) and increased microglia numbers in the CA1 region (Fig. 3*B*) were detected. The number of resting microglia did not differ between the genotypes, but the subset of microglial cells with activated morphology was significantly increased in the hippocampal areas of the GABA-Cnr1$^{-/-}$ animals (Fig. S2 *D* and *E*). The percentage of CD40 expressing cells did not differ between the genotypes (Fig. S7). However, the expression of the inflammatory cytokines IL-6 and TNF showed a significant age-dependent elevation in GABA-Cnr1$^{-/-}$, but not in Glu-Cnr1$^{-/-}$ or wild-type animals (Fig. 3 *D* and *E*).

Discussion

A potential role of the endocannabinoid system in aging has been suggested in previous studies where an early onset of cognitive deficits in animals lacking CB1 receptors was demonstrated (26, 27). However, the mechanism of the antiaging effect of endocannabinoid system activity has remained largely unknown. In the present work, we show age-related cognitive deficits and neuronal loss in Cnr1$^{-/-}$ animals associated with enhanced neuroin-

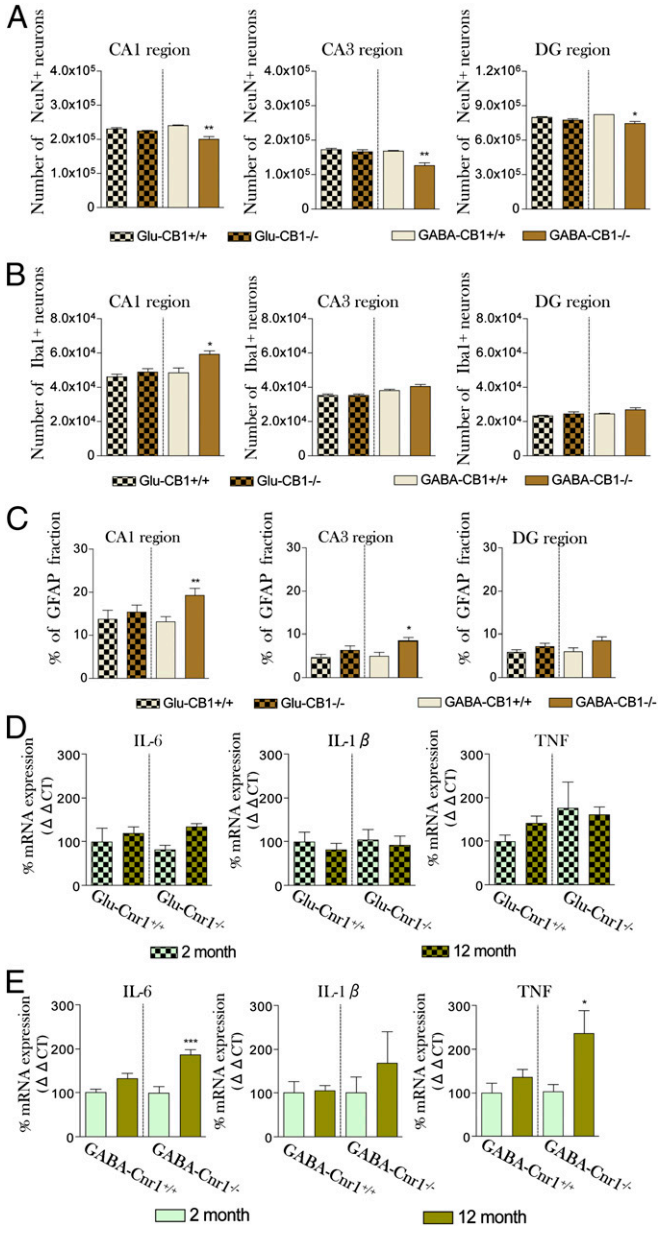

Fig. 3. Neuronal loss and enhanced neuroinflammation in the hippocampus of GABA-Cnr1$^{-/-}$ but not in Glu-Cnr1$^{-/-}$. (*A*) Quantitative stereological analysis revealed a decrease in the number of principal neurons in the hippocampus of 12-mo-old GABA-Cnr1$^{-/-}$ mice. (*B*) Total number of Iba1-positive cells in the CA1 region of the hippocampus is increased in 12-mo-old GABA-Cnr1$^{-/-}$ but not in Glu-Cnr1$^{-/-}$ animals. (*C*) The age-dependent increase in the fraction of GFAP-immunopositive areas in the CA1 and CA3 regions of the hippocampus is more pronounced in 12-mo-old GABA-Cnr1$^{-/-}$ mice than their wild-type littermates. (*$P < 0.05$, **$P < 0.01$ Student's *t* test; $n = 3$ mice/group) (*D*) Comparison of 2- and 12-mo-old animals revealed a similar expression of cytokines in Glu-Cnr1$^{-/-}$ mice and in their control littermates. (*E*) In GABA-Cnr1$^{-/-}$ animals, IL-6 and TNF but not IL-1β expression significantly increased in aging. In their wild-type littermates there was no age-related change in the expression levels of cytokines.

flammation in the hippocampus. It has been shown that enhanced activation of inflammatory cells in the brain impairs neuronal activity (36, 37) and thus this process may significantly contribute to the aging phenotype of Cnr1$^{-/-}$ animals. Although an age-related increase in astrocyte numbers was observed both in Cnr1$^{+/+}$ and in Cnr1$^{-/-}$ null mutant animals, this process was exacerbated in Cnr1$^{-/-}$ mice. Importantly, stereological, morphological, and flow cytometric analyses of microglial cells showed that the number of microglia with activated morphology and the ratio of CD40 expressing microglia was higher in Cnr1$^{-/-}$ than in Cnr1$^{+/+}$ mice. During aging, an increase in the expression levels of proinflammatory cytokines takes place in the brain (6). We detected a significant increase in the expression of IL-6 in 12-mo-old Cnr1$^{-/-}$ mice, whereas the expression of IL-1β, IL-6, or TNF did not differ between 2-mo-old and 12-mo-old wild-type animals, in accordance with previous reports (38). Changes in cell morphology (36) and expression of surface proteins and inflammatory cytokines have different dynamics and onsets (39). Elevation of IL-6 levels has consistently been related to aging (40), and high levels of IL-6 are associated with an increased risk of cognitive decline (41). The fact that IL-6 but not IL-1β or TNF expression is increased suggests that the increase in IL-6 expression is one of the first steps in the gradual activation of microglial cells (42).

Increased neuroinflammation could diminish the number of neurons in the hippocampus of Cnr1$^{-/-}$ mice, but impaired neurogenesis could also contribute to this phenomenon (30, 43). However, a significant difference in BrdU-positive cells between the genotypes was only found when comparing 2-mo-old mice, but not in 5- or 12-mo-old animals, where reduced hippocampal neuronal numbers were detected. Thus, we concluded that differences in the ratio of neurogenesis between Cnr1$^{+/+}$ and Cnr1$^{-/-}$ mice do not play a significant role in the neuronal number reduction in aging in Cnr1$^{-/-}$ animals. Recurrent epileptic seizures together with increased sensitivity to excitotoxicity have been described for Cnr1$^{-/-}$ animals (21, 28). Our present data suggest that spontaneous epileptic attacks are rare in Cnr1$^{-/-}$ animals; therefore, it is unlikely that they influence the aging phenotype.

Importantly, a significant decrease in pyramidal cell numbers precedes neuroinflammation. Reduced neuronal numbers were detected in 2-mo-old Cnr1$^{-/-}$ animals, whereas an increase in inflammatory parameters was first present in 5-mo-old (increase in area covered by astrocytes) and 12-mo-old (elevated microglial activity) Cnr1$^{-/-}$ mice. We hypothesize that degeneration of pyramidal cells triggers a chain of neuroinflammatory changes, leading to further neuronal damages. Neurodegeneration and neuroinflammation thus mutually promote each other and both processes contribute to the development of cognitive deficits.

It was previously reported that young Cnr1$^{-/-}$ animals have a better learning ability in the object (44, 45) and partner (26, 27) recognition tests, as well as in the operant learning paradigm (26). These paradigms sensitively detect disturbances in the function of many brain areas involved in learning and memory. However, we found an age-dependent neuronal loss and neuroinflammatory changes in the hippocampus, but not in the cortex of Cnr1$^{-/-}$ mice. To test whether hippocampal functions are specifically affected, we investigated the learning ability of the animals in the Morris water maze and the *Y*-maze tests, because these models are sensitive for the integrity of the hippocampus and the prefrontal cortex, respectively. Young Cnr1$^{-/-}$ mice showed a longer retention of spatial information, but their performance decreased rapidly during aging (26). We noted that flexibility of learning seems to be an especially sensitive indicator of hippocampal deficits, because our Cnr1$^{-/-}$ mice first showed impairment in the reversal phase of the Morris water maze test. The ratio of spontaneous alternations in the *Y*-maze test, however, did not differ between the genotypes. Our data demonstrate a close association between age-related learning deficits and neuroinflammatory responses in the absence of CB1 receptor activity. First of all, there is a concurrence between onset of the learning deficit and the increase in glial numbers. Second, hippocampus-dependent spatial memory (46) but not the prefrontal cortex-dependent working memory (47) was impaired in 12-mo-old Cnr1$^{-/-}$ mice.

We investigated the involvement of different neuronal populations in the aging phenotype using different conditional

knockout mice lacking CB1 receptors in GABAergic or glutamatergic terminals (35). We found a similar neuronal loss and enhanced glial activity in the hippocampus of GABA-Cnr1$^{-/-}$ mice as observed in constitutive knockouts, although they did not completely recapitulate the neuroinflammatory phenotype of the constitutive knockouts: the density of astrocytes and of the activated form of microglia was increased in both lines, as was the expression of IL-6. However, the percentage of CD40 expressing microglia was enhanced only Cnr1$^{-/-}$ animals and TNF expression was up-regulated only in GABA-Cnr1$^{-/-}$ mice. This difference may suggest that another CB1 receptor expressing-cell type is also involved in glial regulation.

Absence of CB1 receptors on glutamatergic neurons leads to an enhanced excitability of the brain (21, 35), which might promote brain aging. However, our data suggest an enhanced glutamate release does not play a significant role in brain aging. Glu-Cnr1$^{-/-}$ mice showed no signs of altered brain aging, as the severity of the age-related changes in the brain of Glu-Cnr1$^{-/-}$ mice was practically identical to that observed in wild-type littermates.

The expression of CB1 receptors is higher on GABAergic than on glutamatergic neurons (16). Activation of CB1 receptors on the GABAergic neurons leads to a decrease in GABA release (20, 48) and thus to formation of the depolarization-induced suppression of inhibition (DSI) (35) and long-term depression of inhibitory GABAergic synaptic transmission (LTDi) (49, 50). As such, deletion of CB1 receptors from GABAergic neurons might be expected to increase GABA signaling. Although enhanced hippocampal GABA levels are protective against glutamate (51), it has been shown that the excitotoxic effect of kainate is not altered in mice with specific deletion of CB1 receptors in these neurons (21, 35). This suggests the lack of endocannabionoid-mediated inhibitory control on GABAergic hippocampal interneurons in GABA-Cnr1$^{-/-}$ mice does not lead to a tonic elevation in GABA signaling. Progressive neuronal loss and neuroinflammation observed in GABA-Cnr1$^{-/-}$ mice now suggest that CB1 receptor activity on GABAergic neurons in the hippocampus plays a crucial role in age-related changes in the brain, regulating the homeostatic balance between pro- and antiinflammatory processes. Our results now suggest that the endocannabinoid system may serve as a communication channel between glial cells and neurons. This is probably most prominent between microglial cells and GABAergic neurons. Microglial cells produce endocannabinoids at much higher levels than neurons (52) and the expression of CB1 receptor is the highest on GABAergic neurons in the hippocampus (16). Future experiments are necessary to determine how the absence of CB1 receptor signaling on GABAergic neurons leads to an age-dependent increase in glial activity. The lack of CB1 receptor activity may impair microglial feedback signals on GABAergic neurons and reduce the inhibitory control of these neurons on microglial cells. Consequently, the phenotype of microglial cells shifts toward an activated state. Chronically enhanced levels of inflammatory cytokines impairs neuronal function, leading in turn to a further decrease in the efficacy of neuronal control over glial activity and thus to an accelerated age-dependent progression of glial activation. This is consistent with previous studies showing that GABAergic interneurons in the hippocampus are important regulators of microglial activity (53) and with the proposed role of the endocannabinoid system in neuroprotection (54). Our results suggest that enhanced neuroinflammation together with the degeneration of pyramidal cells lead to an early onset of cognitive deficits in the absence of CB1 signaling on GABAergic neurons.

Materials and Methods

Animals. Experiments to test the consequence of a constitutive deletion of the CB1 receptor were carried out with male and female Cnr1$^{-/-}$ and Cnr1$^{+/+}$ littermates on a congenic C57BL6/J background. The conditional GABA and glutamate-specific knockout animals were kept on a predominant C57BL/6N genetic background. Care of the animals and conduction of all experiments followed the guidelines of European Communities Directive 86/609/EEC and the 1998 German Animal Protection Law regulating animal research.

Quantification of Astrocyte and Microglia Densities. GFAP-immunoreactive astrocytes were analyzed using area fraction technique, whereas the optical fractionator method was used to quantify the total number of Iba1-immunoreactive microglia, and NeuN-positive neurons. See detailed description in the *SI Materials and Methods*.

Neurogenesis. Animals were injected with 5-bromo-2′-deoxyuridine (BrdU) and killed 24 h after the last injection. The BrdU-positive cells were counted in the subgranular zone of the dentate gyrus after staining. The method is described in detail in *SI Materials and Methods*.

Timm Staining. Sections from the brain of Cnr1$^{+/+}$ and Cnr1$^{-/-}$ littermates were stained as described in detail in the *SI Materials and Methods*.

Monitoring of Cortical EEG Activity and Behavior. We recorded cortical EEGs from freely moving Cnr1$^{+/+}$ and Cnr1$^{-/-}$ littermates using a telemetric EEG/video monitoring system continuously over 19 d. The method is described in detail in the *SI Materials and Methods*.

Flow Cytometry. Fluorescence staining of isolated CNS mononuclear cell samples from the brains was performed as described in detail in the *SI Materials and Methods*. Percentage of CD40 expressing microglia was calculated.

Hippocampal Expression of IL-1β, IL-6, and TNF. Total RNA was extracted from the isolated hippocampi and mRNA expression was determined as described in detail in the *SI Materials and Methods*.

Behavioral Tests. The spatial learning and memory abilities of mice were assessed in the MWM and working memory in the *Y*-maze task. Detailed description of the device, procedure, and analysis is in the *SI Materials and Methods*.

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