

Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors

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The molecular mechanisms contributing to the normal age-related decline of cognitive functions or to pathological learning and memory impairment are largely unknown. We demonstrate here that young mice (6–7 weeks) with a genetic deletion of the cannabinoid CB1 receptor performed as well as WT mice, or often better, in a number of learning and memory paradigms, including animal models of skill-learning, partner recognition, and operant conditioning. In contrast, the performance of mature mice (3–5 months) lacking CB1 receptors was much worse than that of age-matched WT animals. In most tests, these mice performed at the same level as old animals (14–17 months), suggesting that the decline in cognitive functions is accelerated in the absence of CB1 receptors. This rapid decline in CB1-deficient animals is accompanied by a loss of neurons in the CA1 and CA3 regions of the hippocampus.

aging | gene knockout | learning | hippocampus | memory

Aging is associated with a decline of cognitive performance in humans (1) and animals (2, 3). However, as in all age-related health issues, there is a wide spectrum of potential outcomes: Although 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, may show signs of cognitive impairment early in their life. In any case, the increasing average lifespan of the human population does result in a growing number of individuals with cognitive deficits, providing an enormous social and economical challenge to society. This challenge can only be met by developing innovative methods for the treatment and prevention of cognitive impairment, based on a better understanding of the normal physiological and accelerated pathological aging process of the brain.

In the present study, we have analyzed the role of the endocannabinoid system in the age-related decline of learning and memory functions. Cannabinoids are aromatic hydrocarbon compounds from the hemp plant *Cannabis sativa* and include the major psychoactive cannabinoid Δ^9 -tetrahydrocannabinol (THC). THC exerts its psychotropic effects by activating the cannabinoid CB1 receptor, which is probably one of the most abundant G protein-coupled receptors in the mammalian brain.

Numerous studies have shown that acute cannabinoid exposure detrimentally affects learning and memory functions (4). In humans, the severity of the cognitive impairment is correlated with the difficulty of the task, with recognition memory being particularly sensitive to disruption by cannabinoids. In animals, cannabinoid administration impairs spatial (5, 6) and working memory (7) and memory consolidation (8). Acute pharmacological blockade of the CB1 receptor has a beneficial effect on the memory. SR141716A, a widely used CB1 receptor antagonist, not only prevented tetrahydrocannabinol-induced memory deficits (9, 10) but, when applied alone, also improved retention of spatial memory (11) and social recognition (12) and reduced memory deficits in aged rodents (12). However, the effects of long-term pharmacological manipulations of the endocannabinoid

system are not clear. Most of the recent studies, for example, did not confirm previous evidence for cognitive impairment in chronic cannabis users.

The pharmacological results are supported by behavioral analyses of mice with a deletion of the CB1 receptor gene *Cnr1* (henceforth referred to as *Cnr1*^{−/−} mice), which were mostly performed with young mice. Collectively, these studies show that some cognitive or memory functions may be altered in *Cnr1*^{−/−} mice when compared with WT mice, because they show enhanced memory retention in the object-recognition task (13, 14), a deficit in reversal learning in the water-maze test (15), and a delayed extinction learning in a fear-conditioning paradigm (16, 17).

The cannabinoid system undergoes characteristic age-related changes. Old rats, when compared with young animals, showed reduced CB1 receptor densities and mRNA expression levels in many brain areas, most prominently in the basal ganglia and in the cerebellum (18, 19). The brainstem of aged rats, in contrast, showed a substantial increase in CB1 mRNA levels. In the cortex, the age-dependent change in CB1 receptor binding or mRNA expression seems to be region-specific; a decrease (19), no change (20), and an increase (21) has been described in different areas. The concentration of endocannabinoids also shows a region specific reduction in aged animals, although it is rather modest (14, 20). The comparison of 26- to 48-week-old mice with 6- to 10-week-old mice did not reveal any differences in CB1 endocannabinoid levels but showed a reduced receptor coupling in the limbic forebrain of older animals. The functional consequences of these age-related changes remain to be shown, but it has been suggested that they contribute to behavioral changes observed in aged animals, such as the age-dependent decline in food and alcohol intake.

Here, we compared the age-related decline in learning and memory functions in WT mice and in *Cnr1*^{−/−} animals. Although young CB1-deficient mice performed as well as, or better than, WT animals in most memory tasks, we found a very surprising accelerated decline of cognitive functions in mature animals, accompanied by neuronal cell loss in the hippocampus.

Methods

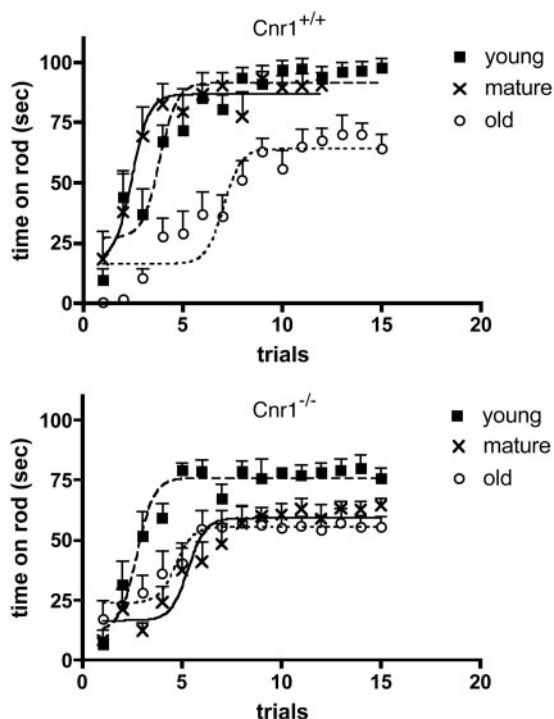
Animals. Experiments were carried out with young (6–8 weeks old), mature (3–5 months old), and old (14–17 months old) male *Cnr1*^{−/−} and *Cnr1*^{+/+} mice on a congenic C57BL6/J background (22). For some experiments, we also used *Cnr1*^{−/−} mice on a CD1 genetic background (43), which were generously provided by Catherine Ledent (Université Libre de Bruxelles, Brussels). All experiments with C57BL6/J-*Cnr1*^{−/−} mice (and WT controls) were performed at University of Bonn; the experiments with CD1-*Cnr1*^{−/−} mice (and WT controls) were performed at Universitat Pompeu Fabra. Animals received water and food *ad*

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Abbreviation: CI, confidence interval.

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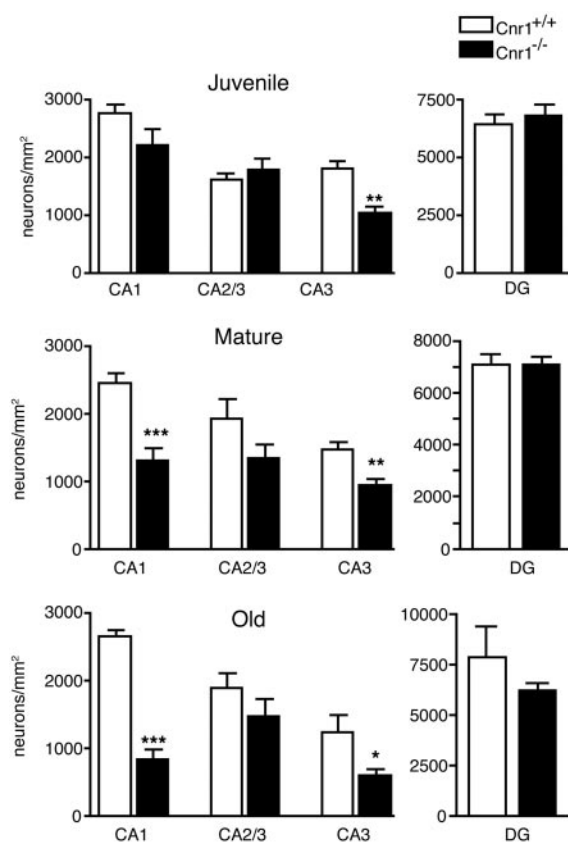


Fig. 5. Neuronal cell densities in the hippocampus. The density of neurons in the CA3 regions was reduced in *Cnr1*^{-/-} mice of all age groups. Significantly lower neuronal densities were also observed in the CA1 region of mature and old *Cnr1*^{-/-} mice. In this region, the neuronal loss seemed to progress with age. Bars represent mean neuronal density expressed as the number of neurons per mm² (\pm SEM), $n = 4-8$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, *Cnr1*^{-/-} vs. *Cnr1*^{+/+} mice (Student's unpaired *t* test).

absence of CB1 receptors. Thus, young *Cnr1*^{-/-} mice performed better than age-matched *Cnr1*^{+/+} mice in the rotarod and partner recognition tests, whereas mature *Cnr1*^{-/-} animals showed significant learning deficits and performed similarly to old mice in most behavioral learning paradigms.

The age-related learning deficit appeared to be generalized rather than related to a specific paradigm. Psychomotor skill-learning, as assessed in a modified version of the rotarod test, involves cortical regions together with the neostriatum and cerebellum (24). Indeed, learning improvement is accompanied by plastic changes in the striatum and motor cortex. "Fast" learning (improvement within the initial training session) involves a recruitment of task-related neurons in both brain structures, whereas the firing pattern is refined during the "slow" learning period (improvement between sessions) (25). Interestingly, it has recently been demonstrated that young C57BL/6J mice performed worse than other mouse strains (NMRI and C57BL/6J \times 129OlaHsd) in skill-learning tasks, although they were the best performers at the adult stage (26). In good agreement with this result, we found that mature WT C57BL/6J animals performed this task better than young mice. It was therefore particularly striking to see that mature *Cnr1*^{-/-} mice had a substantially reduced psychomotor performance and performed at a similar level as old mice. It is also noteworthy that old *Cnr1*^{-/-} mice showed a better rotarod performance when compared with old *Cnr1*^{+/+} animals. Because motor coordination skills are an important contributing factor to the perfor-

mance in this test, it seems possible that sensorimotor performances and cognitive functions are differentially affected by the *Cnr1* mutation.

The operant conditioning paradigm contains elements of working, procedural, and spatial learning. It is well established, and supported by our findings, that old animals have difficulties in the acquisition of operant behaviors (27-29). Indeed, the operant learning ability of *Cnr1*^{+/+} young and mature animals was similar, whereas old mice showed a significant impairment. In *Cnr1*^{-/-} animals, however, we found a continuous age-dependent decrease in performance. *Cnr1*^{-/-} mice are known to eat less than *Cnr1*^{+/+} mice after food deprivation (30, 31) and thus may be less motivated to work for food in the operant behavior paradigm. However, Valverde and colleagues (32) have recently shown that operant behavior for natural rewarding stimuli, such as water and food, was not altered between *Cnr1*^{+/+} and *Cnr1*^{-/-} mice in any of the reinforcement schedules used (FR1 and FR3). In addition, no genotype differences were observed in a progressive ratio schedule of reinforcement between the breaking points obtained in both genotypes for water and food, thus arguing against an altered motivation for these natural stimuli in *Cnr1*^{-/-} animals. Recently, Holter *et al.* (33) have also studied operant behaviors in *Cnr1*^{-/-} mice that were between 11 and 14 weeks of age and thus between the young (6-8 weeks) and mature (14-20 weeks) mice from this study. They demonstrated a slight performance deficit in *Cnr1*^{-/-} mice during the acquisition phase but an equal performance of *Cnr1*^{+/+} and *Cnr1*^{-/-} animals during the end of the training period and the retention phase of the test.

In the social recognition test, old *Cnr1*^{+/+} mice showed a marked deficit in the recognition of the previously seen partner compared with young and mature animals. A similar age-dependent deterioration of social memory was reported in rats (34, 35). We observed again a significant impairment in the social memory task in mature *Cnr1*^{-/-} mice. These animals displayed a normal short-term memory but a complete lack of long-term social memory. This result is contradictory to the previously observed improvement by SR141716A treatment of long-term, but not short-term, social memory performance and the reduction of memory deficits in aged animals with this compound. One possible reason for this discrepancy could be related to the non-CB1-mediated effects of SR141716A, which have been demonstrated repeatedly by using CB1-deficient mouse strains (36, 37). However, we find it more likely that the discrepancy is due to the different physiological effects of the short-term pharmacological blockage vs. long-term genetic ablation of CB1 receptors.

A number of studies have shown that CB1 receptors are expressed in the developing nervous system, and there is some evidence from human and animal studies to suggest that prenatal exposure to cannabinoids affects neurobehavioral development. Thus, it is conceivable that mice develop subtle brain defects in the absence of CB1 receptors and, in consequence, show a poor learning performance. However, young CB1-deficient mice are not impaired in learning and memory tests. In fact, this study, as well as others, strongly suggests that the learning performance is rather improved in young animals in the absence of CB1 receptors. These findings therefore argue against a developmental cause for the learning impairment of mature *Cnr1*^{-/-} mice.

We have therefore considered the possibility that the accelerated decline in learning performance in mature *Cnr1*^{-/-} animals is related to the documented neuroprotective effects of endocannabinoids (38, 39), which are mediated by CB1 receptors (40). Indeed, when we examined the neuronal density in the hippocampus, we found a significant reduction in mature mice in the CA1 or CA3 regions but not in the CA2/CA3 region or in the dentate gyrus, which are known to be differently sensitive to neurotoxic effects (41, 42). These results strongly suggest that the

Corrections

GENETICS. For the article “*DCDC2* is associated with reading disability and modulates neuronal development in the brain,” by Haiying Meng, Shelley D. Smith, Karl Hager, Matthew Held, Jonathan Liu, Richard K. Olson, Bruce F. Pennington, John C. DeFries, Joel Gelernter, Thomas O’Reilly-Pol, Stefan Somlo, Pawel Skudlarski, Sally E. Shaywitz, Bennett A. Shaywitz, Karen Marchione, Yu Wang, Murugan Paramasivam, Joseph J. Lo-Turco, Grier P. Page, and Jeffrey R. Gruen, which appeared in issue 47, November 22, 2005, of *Proc. Natl. Acad. Sci. USA* (**102**, 17053–17058; first published November 8, 2005; 10.1073/pnas.0508591102), the authors note that the data for compound STR have been deposited in the GenBank database (accession no. BV677278).

www.pnas.org/cgi/doi/10.1073/pnas.0509997102

NEUROSCIENCE. For the article “Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors,” by A. Bilkei-Gorzo, I. Racz, O. Valverde, M. Otto, K. Michel, M. Sarstre, and A. Zimmer, which appeared in issue 43, October 25, 2005, of *Proc. Natl. Acad. Sci. USA* (**102**, 15670–15675; first published October 12, 2005; 10.1073/pnas.0504640102), the author name M. Sarstre should have appeared as M. Sastre. The online version has been corrected. The corrected author line appears below.

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