

Rapid communication

Enhancement of memory in cannabinoid CB₁ receptor knock-out mice

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Abstract

We have used cannabinoid CB₁ knock-out mice in a two-trial object recognition test to assess the role of cannabinoid CB₁ receptors in memory. Cannabinoid CB₁ knock-out mice are able to retain memory for at least 48 h after the first trial whereas the wild-type controls lose their capacity to retain memory after 24 h. These results suggest that endogenous cannabinoid CB₁ receptors play a crucial role in the process of memory storage and retrieval. © 1999 Elsevier Science B.V. All rights reserved.

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Cannabinoids exert their actions in the brain of humans and animals through cannabinoid CB₁ receptors (Howlett et al., 1990) which have recently been cloned (Matsuda et al., 1990). Although several studies have been performed to investigate the physiological role of endogenous cannabinoids and cannabinoid CB₁ receptors, this still remains to be fully elucidated.

One of the central behavioral effects of Δ^9 -tetrahydrocannabinol in several animal species, including humans (Schwartz et al., 1989; Heyser et al., 1993), and of synthetic agonists in rodents (Lichtman et al., 1995), is the impairment of memory. Recently, cannabinoid CB₁ receptor knock-out (KO) mice, which do not respond Δ^9 -tetrahydrocannabinol (Ledent et al., 1999), have been generated.

Cannabinoid CB₁-KO mice (CB₁^{-/-}) and wild-type (WT) mice (CB₁^{+/+}), 11–12 weeks old, were generated as described (Ledent et al., 1999) and provided by Transgenic Alliance (France). Animals were housed four per cage ($n = 11$ –12 animals per group) with free access to food and water. Room temperature was $22 \pm 2^\circ\text{C}$ and the light–dark cycle was 12:12 h (lights on at 0600 h). Experiments were performed in the morning.

Memory was assessed by using the two-trial object recognition test for the assessment of memory, as previously described (Dodart et al., 1997). In brief, on day one mice were trained to explore an empty open field for 50 min. During this session the locomotor activity was analysed for 15 min using a video analyser system (Videotrack[®], View Point, France). On day two mice are allowed to explore the same open field in the presence of an object A (a plastic figure, 2.5 cm high), for 10 min. The time spent exploring this object was recorded using a system for the collection and analysis of observational data (Observer, Noldus, the Netherlands). At 3, 24 and 48 h after the exploration trial of object A, the retention performance was evaluated by allowing the same animal to re-explore the open field for 10 min in the presence of two objects: the familiar object A and a novel object B (a plastic flag, 3.5 cm high). The time spent exploring each object (time A and time B) were recorded. Recognition memory was assessed by comparing the time spent exploring each object during the second trial. A recognition index was calculated for each animal and expressed as a ratio ((time B $\times$ 100)/time A + time B). The recognition index is around 50% when animals do not remember and time A and time B are equivalent but recognition index is more than 50% when time B is superior to time A, indicating that they have remembered the familiar object. Non-parametric Kruskal and Mann–Whitney *U*-tests were

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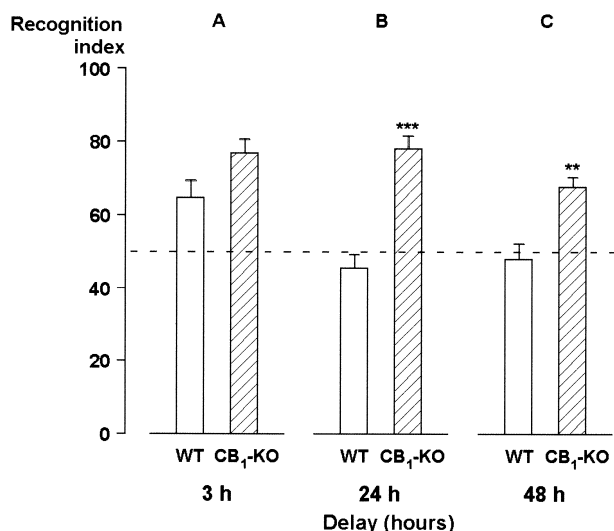


Fig. 1. Enhancement of memory in cannabinoid CB₁-KO mice compared to WT mice. Recognition index was measured at 3 h (A), 24 h (B) and 48 h (C) after the training session. Values are means $\pm$ S.E.M. Significantly different from wild-type mice **: $P < 0.01$ and ***: $P < 0.001$.

used to compare the recognition indexes between groups. Time A and time B were compared by using a within-group analysis (Wilcoxon matched pairs signed-ranks test).

Fig. 1 shows that cannabinoid CB₁-KO mice and the WT controls initially have a similar high recognition index, as measured 3 h after the exploration training of object A. This suggests that both types of animals have a comparable ability for recognition. When the memory test is performed 24 or 48 h after the training session the recognition index in the WT animals declines as demonstrated by their inability to recognise the familiar object. By contrast cannabinoid CB₁-KO mice at 24 and 48 h after training, still have an recognition index above 50%, suggesting that they are able to recognise the familiar object, and that they spend more time investigating the new one.

The reduction in the time spent in exploration is not due to a decrease in locomotor activity, as measured through a video analyzer system (data not shown). This strengthens

the hypothesis that the difference observed between two groups is due to differences in memory retention.

Our results confirm and extend previous pharmacological evidence, obtained Δ^9 -tetrahydrocannabinol, synthetic analogues and cannabinoid CB₁ antagonists (Schwartz et al., 1989; Heyser et al., 1993; Terranova et al., 1996), demonstrating that modulation of cannabinoid CB₁ receptors affects memory. The present results in cannabinoid CB₁-KO mice further suggest that an endogenous cannabinoid tone is present in physiological conditions and is involved in the modulation of memory.

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