DELETION OF CANNABINOID CB2 RECEPTORS INDUCES MEMORY IMPAIRMENT ASSOCIATED WITH SYNAPTIC PLASTICITY ALTERATIONS

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The identification of CB_2r in areas involved in learning and memory, such as the HIP, the close interaction between CB_2r and the GABAergic system, and the cognitive alterations observed in CB2KO mice, suggest that CB_2r might be an important neurobiological substrate for cognitive processes. In this study, the role of CB_2r on aversive memory consolidation was further evaluated. Mice lacking CB_2r (CB2KO) and their corresponding littermates (WT) were exposed to the step-down inhibitory avoidance test (SDIA). MAP2, NF200, and synaptophysin (SYN)-immunoreactive fibers were studied in the hippocampus (HIP) of both genotypes. The number of synapses, postsynaptic density thickness, and the relation between the synaptic length across the synaptic cleft and the distance between the synaptic ends were evaluated in the HIP (dentate gyrus (DG) and CA1 fields) by electron microscopy. Brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (NR3C1) gene expressions and mTOR/p70S6K signaling cascade were evaluated in the HIP and prefrontal cortex (PFC). Finally, the effects of acute administration of CB_2r-agonist JWH133 or CB_2r-antagonist AM630 on memory consolidation were evaluated in WT mice by using the SDIA.

The lack of CB_2r impaired aversive memory consolidation and reduced MAP2, NF200 and SYN-inmunoreactive fibers and the number of synapses in DG of CB2KO mice. BDNF and NR3C1 gene expression were reduced in the HIP of CB2KO mice. An increase of p-p70S6K (T389 and S424) and p-AKT protein expression was observed in HIP and PFC of CB2KO mice. Interestingly, administration of AM630 impaired whereas JWH133 enhanced aversive memory consolidation. Further functional and molecular assessments would have been helpful to further support our conclusions.

These results revealed that CB_2r are involved in memory consolidation, suggesting that this receptor could be a promising target to develop novel treatments for different cognitive impairments-related disorders.

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1. STEP DOWN INHIBITORY ADVOIDANCE

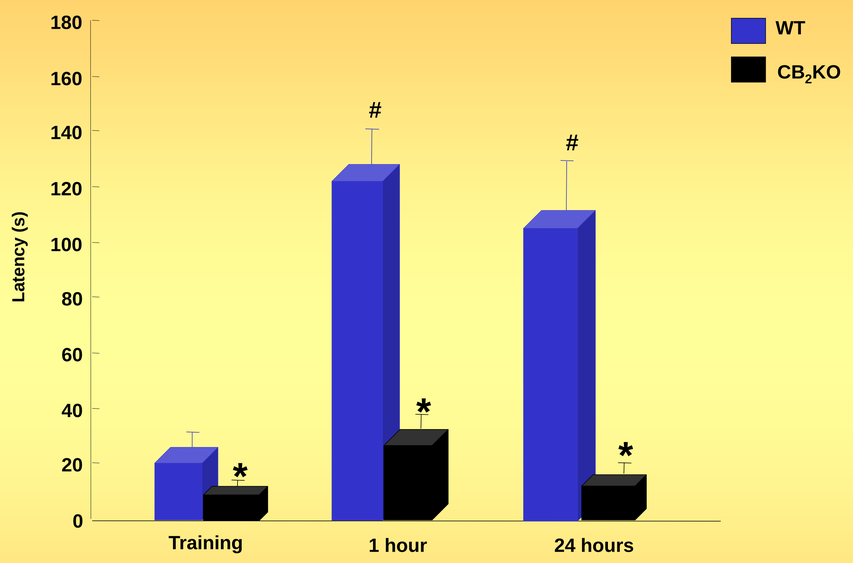

Figure 1. Step-down inhibitory avoidance test (SDIA). CB2KO and their corresponding littermates WT mice were exposed to a scrambled foot shock (0.5 mA for 2 seconds) and the step-down latency (s) was measured 1 hour (short-term memory) and 24 hours later (long-term memory). Columns represent the mean and vertical lines ± S.E.M of step-down latency (s). *, values that are significantly ($p < 0.05$) different from their corresponding control group.

2. INMUNOHISTOCHEMISTRY: MAP2, NF200 AND SYNAPTOPHYSIN

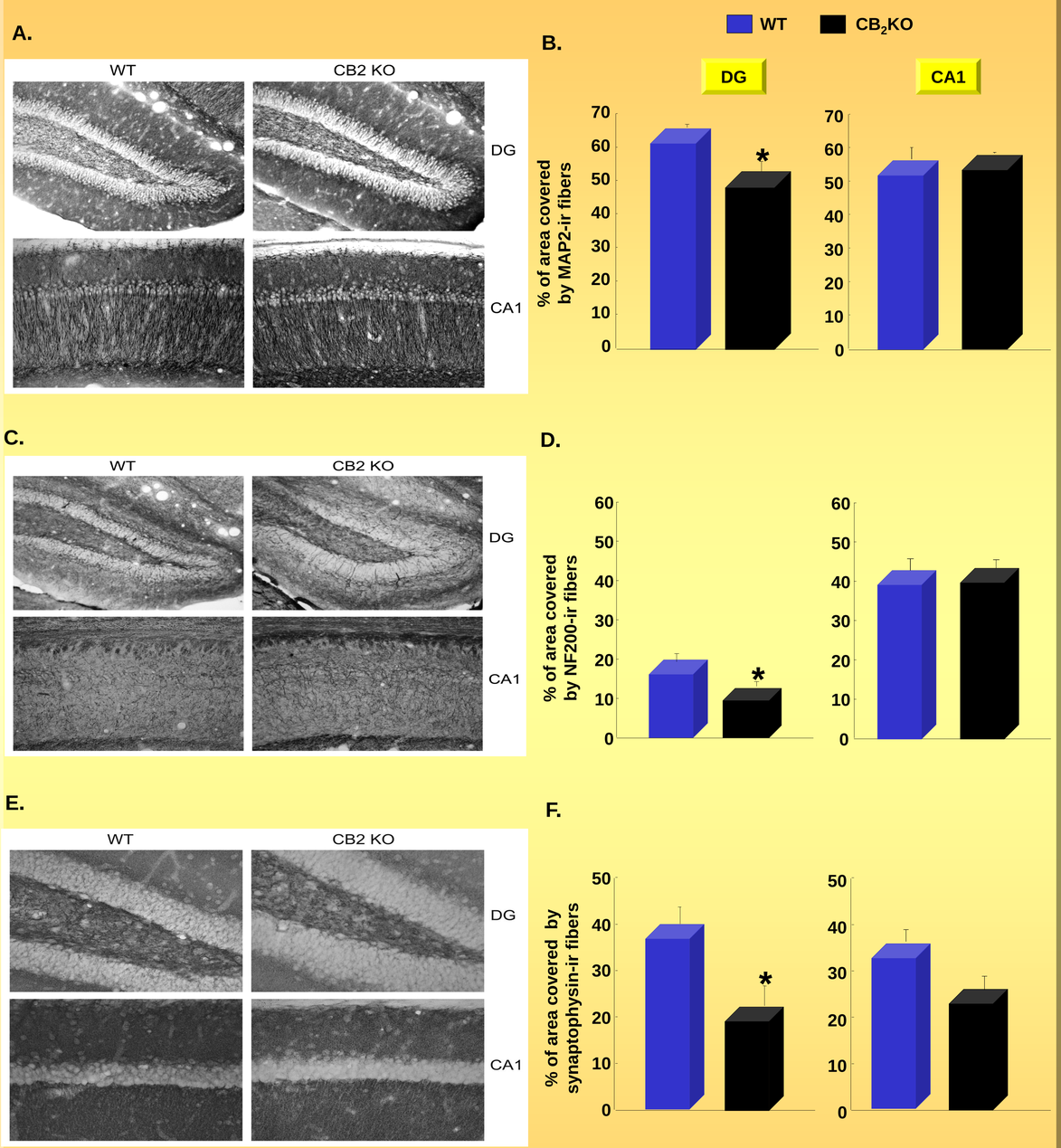

Figure 2. MAP2, NF200 and SYN immunostaining in the DG and CA1 fields of the HIP of CB2KO and WT mice. Immunohistochemistry and densitometric analysis of MAP2 (A, B), NF200 (C,D) and SYN (E, F) in the DG and CA1 from CB2KO and WT mice. Columns represent the mean and vertical lines ± S.E.M of % of area covered by inmunoreactive (ir) fibers. *, values that are significantly ($p < 0.05$) different from their control group. Images are representative of several slices.

3. ELECTRON MICROSCOPY ANALYSES IN THE DG AND CA1 OF HIPPOCAMPUS

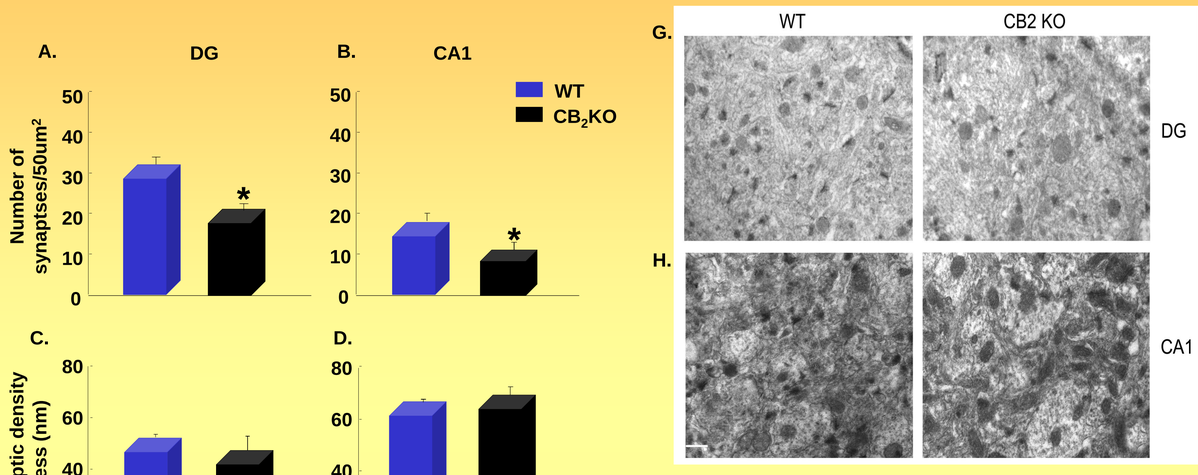

Figure 3. Electron microscopy analyses in the DG and CA1 fields of HIP from CB2KO and WT mice. Panel A and B: Number of synapses in the DG and CA1 regions. Panel C and D: postsynaptic thickness in the DG and CA1 regions. Panel E and F: Synaptic cleft morphology measured as the relation between the synaptic length across the synaptic cleft and the distances between the synaptic ends in the DG and CA1 regions. Panel G and H: electron microscopy images in CA1 and DG. Data are expressed as mean± S.E.M. *, values that are significantly ($p < 0.05$) different from their control group.

4. BDNF, GR AND mTOR PATHWAY

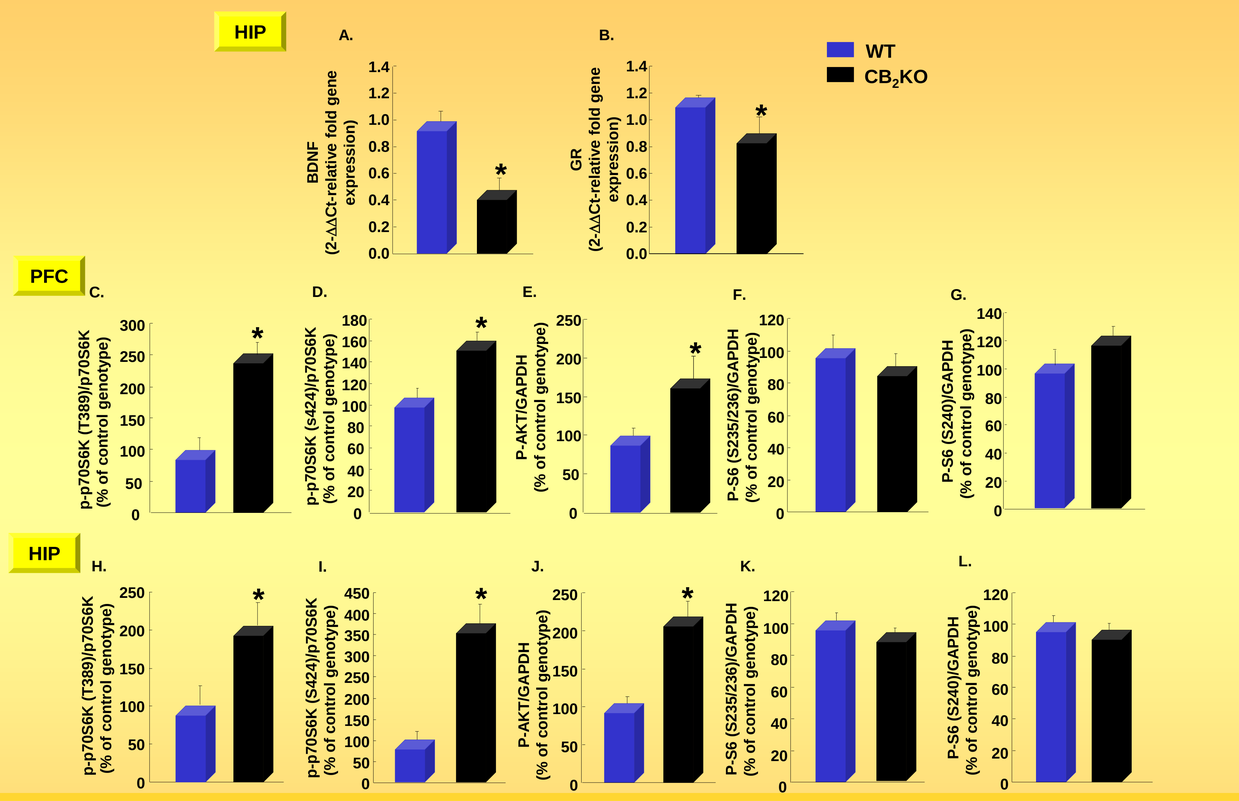

Figure 4. Evaluation of relative BDNF (A) and NR3C1 gene expression (B) and mTOR pathway (C-J) in PFC and HIP from CB2KO and WT mice. Panel A: Relative BDNF gene expression in the HIP of CB2KO and WT mice. Panel B: Relative NR3C1 gene expression in the HIP of CB2KO and WT mice. Panel C-E: Optical density quantification of the immunoreactive bands for phospho-p70S6K (T389) (C), phospho-p70S6K (S424) (D), AKT (E), S6 (S235/236) (F) and S6 (S240) (G) in the PFC of CB2KO and WT mice. Panel H-L: Optical density quantification of the immunoreactive bands for phospho-p70S6K (T389) (H), phospho-p70S6K (S424) (I), AKT (J), S6 (S235/236) (K) and S6 (S240) (L) in the HIP of CB2KO and WT mice. p70S6K (T389) phosphorylation was measured by immunoblot as a read-out of mTOR activity. Data are expressed as mean± S.E.M. *, values that are significantly ($p < 0.05$) different from their control group.

5. DOSE-RESPONSE EFFECTS OF AM630 OR JWH133 ON SDIA

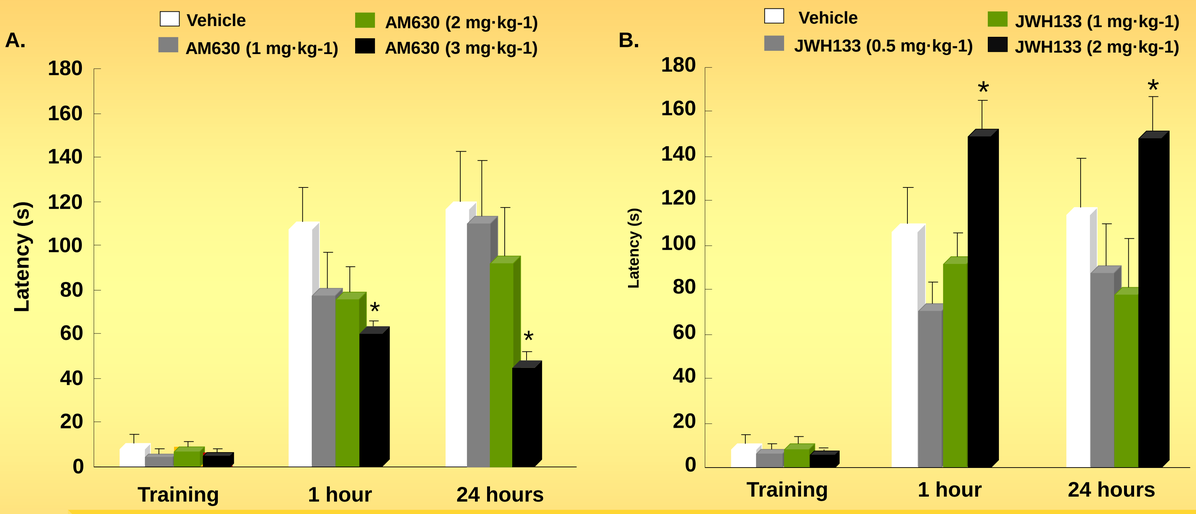

Figure 5. Dose-response effects of acute AM630 (1, 2 or 3 mg·kg-1, ip) (A) or JWH133 (0.5, 1 or 2 mg·kg-1, i.p.) (B) administration on step-down inhibitory avoidance test. AM630 or JWH133 was administered immediately after a scrambled foot shock (0.5 mA for 2 seconds) and the latency was evaluated 1 hour (short term memory) and 24 hours (long-term memory) later. Columns represent the mean and vertical lines ± SEM of latency (s). *, values that are significantly ($p < 0.05$) different from their control group.

CONCLUSIONS

- The results presented here show that the CB_2r play a pivotal role in the neurobiology of cognitive process. The lack of CB_2r resulted in an impairment of short and long-term memory processes associated with the alterations of different targets involved in the neuroplasticity of the HIP, such as MAP2, NF200, SYN, BDNF and GR. Furthermore, the increased phosphorylation of proteins involved in the mTOR signaling pathway revealed potential alterations in the translational process that controls the protein synthesis underlying synaptic neuronal plasticity and memory. In addition, acute administration of JWH133 resulted in an enhancement of cognitive function.
- Taken together, these findings strongly support the role of CB_2r in the regulation of memory and point to this receptor as a potential new target for the treatment of cognitive impairment-related disorders.

NO POTENTIAL CONFLICT OF INTEREST