PUBLISHABLE SUMMARY

Spatial navigation is the ability to move around in an environment using an innate sense of direction. It is a skill essential for everyday functioning that we use all the time, whether we are following a familiar route or finding the way to a novel location. Spatial navigation is altered with aging and is impaired in many psychiatric disorders such as Alzheimer disease autism spectrum disorder and, anxiety-related disorders like depression or post-traumatic stress disorder (PTSD). It is an ability that is highly preserved across different species and, therefore, suitable for translational research on cognition. To face the difficulty of navigation in complex environments, both humans and rodents have developed distinct strategies to reach a specific point in space, the most important ones being response learning (RL) and place learning (PL). RL is the acquisition of directed movements along a route to get to a destination (egocentric reference frame). PL is a hippocampus depend navigation strategy and relies on environmental cues which are spatially referenced to the target point and therefore integrated into a cognitive map (allocentric reference frame). Extensive evidence indicates that, due to its wide expression in the CNS, CB1 receptor (CB1R) activity plays a crucial role in the modulation of cognitive processes such as spatial navigation and synaptic plasticity, both in animal models and human. CB1R belongs to the endogenous cannabinoid system (ECS) which comprises at least 2 cannabinoid receptors, CB1R and cannabinoid receptor 2 (CB2R), their endogenous ligands (endocannabinoids), mainly anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), and the enzymes responsible for endocannabinoids biosynthesis and inactivation. Particularly in cortical areas which are essential for memory processes such as hippocampus, the CB1R is present at presynaptic sites in glutamatergic as well as in GABAergic neurons thus controlling both inhibitory and excitatory neurotransmission. Most of the studies on memory were done using exogenous cannabinoids, like of Δ9 tetrahydrocannabinol (Δ9-THC) and only a restricted numbers looked at the involvement of the ECS. Both genetical and pharmacological studies tried to elucidate the role of the ECS in cognition and only limited ones on spatial learning but the results are contradictory and inconclusive. We aimed to investigate the possible role of CB1R in the spatial learning at the behavioral, structural, and electrophysiological levels. For this purpose several genetically modified mice lines, lacking CB1R in different neuronal population underwent complex behavioral protocols to evaluate spatial learning performance in the water-cross maze (WCM). In addition, in vivo structural analyses via manganese-enhanced magnetic resonance imaging (MEMRI) was employed in order to evaluate possible

volumetric changes at the hippocampus level, and electrophysiological studies using voltage-sensitive dye imaging (VSDI) assay were achieved to assess potential modifications in neuronal activity propagations through the hippocampus. CB1 constitutive knockout (CB1-KO) mice were strongly impaired in PL after seven days of training in the WCM. Interestingly, these animals also showed a significantly impaired propagation of activity flow through hippocampus, as revealed by VSDI. However, the severe impairment in hippocampus-dependent learning showed by the constitutive CB1-KO mice did not correlate with any modification in hippocampal volume as assessed in vivo by MEMRI following behavioral testing. A deficiency in the cognitive performance was found in mice lacking CB1R in the cortical glutamatergic neurons (Glu-CB1-KO), as well. However, at the end of the training these mice were able to overcome the deficit in PL. Interestingly, there was an inverse correlation between learning performance and hippocampus volume in these mutant mice. No differences in spatial learning were observed in conditional mice lacking CB1R in GABAergic (GABA-CB1-KO) neurons. However, these animals showed a significantly decreased in total brain volume when compare with their control. Further analysis revealed that after the normalization to total brain volume, there was also a significant decrease in hippocampus volume of GABA-CB1-KO vs WT mice. This difference can be ascribed to the left dorsal part of this brain area since mutant mice revealed smaller left dorsal hippocampus compared to WT and no significant differences in the ventral part. In addition, there were no significant differences in RL in any of the CB1R conditional knockout mice tested. Given that the Nex promoter (Glu-CB1-KO) is active at early prenatal stages and the prejudicial effect of CB1R deletion on spatial learning was present in CB1 constitutive mice and conditional Glu-CB1-KO ones, we conceded the possibility that these detrimental features maybe due to the developmental aspects. Therefore we asked whether the learning deficits observed in CB1-KO mice and Glu-CB1-KO mice could also be induced in vivo in adult animals. We investigated this by bilaterally injecting a Cre expressing virus into the CA3 of dorsal hippocampus of CB1f/f mice and subsequently analyzed spatial learning performance using the hippocampus-depend strategy. We found that mice in which CB1R was absent in the CA3 of the dorsal hippocampus showed an impairment in spatial learning. Therefore, the reduced learning performance observed for CB1-KO and Glu-CB1-KO mice cannot be solely ascribed to developmental effects since the detrimental effect of CB1 deletion on spatial navigation can also be induced in adult animals

In conclusion, CB1R in general is essential for spatial navigation in mice, with CB1 on glutamatergic terminals ensuring efficient place learning. These outcomes have a key value

since CB1R could be exploiting as a potential target when dealing with spatial memory impairments in anxiety related disorders such as PTSD patients.