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Exposure to cannabinoid receptor 1 ligands induces miswiring of hypothalamic axons in the brain of zebrafish embryos

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Introduction: CB1 cannabinoid receptors (CB1) are the targets of endocannabinoids (AEA and 2-AG) and marijuana (Cannabis spp)-derived phytocannabinoids (e.g. 9-THC). Interestingly, children exposed in utero to cannabis present neurobehavioral and cognitive impairments. CB1 receptors are widely expressed in the CNS and they have been recently recognized as regulators of brain development, including wiring of neuronal connections. eCBs have been proposed to activate intracellular pathways that involve Rho-GTPases and the control of neuronal actomyosin cytoskeleton, participating in the regulation of axonogenesis. In the zebrafish (zf) embryo, previous data showed that CB1 knockdown causes abnormal axonal growth with failure in fasciculation in particular at the level of the anterior commissure. Since this area is particularly rich in Gonadotropin Releasing Hormone (GnRH) and Agouti-related peptide (AgRP) expressing fibers, we assessed whether pharmacologic modulation and downregulation of the CB1 receptor expression could modify GnRH and AgRP axonal pathfinding and fasciculation during zebrafish early neurodevelopment.

Methods: We initially assessed the relationship between the CB1 receptor and the GnRH3 fibers by performing fluorescence immunohistochemistry. Then we treated transgenic GnRH3::EGFP and agrp1::mCherry zf embryos with increasing concentrations of CB1 agonist and antagonists from 0 to 72/96hpf; and we also performed morpholino-mediated CB1 knockdown. We analyzed parameters such as survival, hatching time and, above all, morphology of neuron pathfinding. Expression levels of key genes potentially involved in CB1-mediated effects were monitored by Real-Time RT-PCR.

Results: We found CB1 immunoreactivity along anterior commissure, post-optic commissure and longitudinal fibers tracts, suggesting that CB1 co-localize with GnRH axons. Following CB1-ligands treatment, we found axon misrouting and abnormal pathfinding in the anterior commissure of both GnRH and AgRP fibers. Moreover, morpholino-mediated downregulation of CB1 expression resulted in similar phenotypes and in a reduction of the number of GnRH3::GFP positive cells in the olfactory epithelium. Finally, we observed that CB1 pharmacological inhibition influenced the expression of some genes involved in axonal growth and cell migration, such as Stmn2a/b and Negr1.

Conclusions: These results indicate that during early zebrafish development, CB1 acts as a regulator of axonal pathfinding on GnRH and AgRP cells. Future experiments will elucidate if the CB1 miss-regulation also affects GnRH neuron migration from the olfactory placode to the hypothalamus, with consequent effects on sexual maturation and reproduction.