Exposure to cannabinoid receptor 1 ligands induces miswiring of GnRH3 and AgRP1 axons in the brain of zebrafish embryos

Giulia Zuccarini [1], Ilaria D'Atri [1,2], Erika Cottone [1], Valentina Pomatto [1], Yoav Gothilf [3], Patrizia Bovolin [1], Giorgio R. Merlo [4]

[1] Department Life Science and Systems Biology, University of Torino
[2] University of Exeter Medical School, Exeter, UK
[3] Department Neurobiology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Israel
[4] Department Molecular Biotechnology and Health Sciences, University of Torino

Cannabinoid receptors 1 (CB1) are G-protein coupled receptors, widely expressed in the central nervous system, and are part of the endocannabinoid system. This system is composed of neuro-modulatory lipids, the endocannabinoids, of which anandamide (AEA) and 2-arachydonoylglycerol (2-AG) are the best characterized, their synthetic and degradative enzymes and their receptors. CB1 receptors have been recognized as regulators of brain development, including wiring of neuronal connections: they activate intracellular pathways for the control of neuronal actomyosin cytoskeleton and participate in the regulation of axonal guidance. CB1 receptors are also targets of Phyto-cannabinoids (e.g. Cannabis-derived Δ9-THC), and it has been reported that children exposed in utero to cannabis present neurobehavioral and cognitive impairments, suggesting that the exposure to exogenous ligands during embryonic development could affect the endocannabinoid system and the cellular mechanisms regulated by it.

In the zebrafish embryo, previous data showed that CB1-knockdown causes abnormal axonal growth in forebrain areas, characterized by failure in fasciculation of axonal tracts in particular at the level of the anterior commissure. Since this area is rich in Gonadotropin Releasing Hormone (GnRH) and Agouti-related peptide (AgRP) expressing fibers, the aim of this study is to assess whether pharmacological modulation of CB1 could modify GnRH and AgRP axonal pathfinding during zebrafish early neurodevelopment.

During early developmental stages, CB1 is expressed in forebrain areas where both GnRH3 and AgRP1 fibers are present and co-localize with GnRH3-expressing axons. Transgenic GnRH3:EGFP and agrp1:mCherry embryos treated with increasing concentrations of different CB1 ligands (agonist, antagonist and reverse agonist) from 0 to 72-96 hpf, showed axon misrouting and abnormal pathfinding of both GnRH3 and AgRP1 fibers in the anterior commissure. Moreover, morpholino-mediated knockdown of the receptor confirmed similar phenotypes. Following exposure with either CB1 agonist, antagonist or reverse agonist, CB1 gene expression was significantly up-regulated, as well as GnRH3 and AgRP1 expression. Moreover, CB1 pharmacological modulation influenced the expression of some genes involved in brain development, axonal growth and cell migration, such as Stmn2a/b, Negr1 and Sez6a/b, highlighting the possible involvement of new molecular mechanisms not yet studied.

These results indicate that CB1 acts as regulator of axonal pathfinding on GnRH and AgRP neurons during development. The implications of these results are important in the contest of pre-natal exposure to cannabis.