“FEDERICO II” UNIVERSITY OF NAPLES

VETERINARY SCIENCES PhD THESIS
XXX CYCLE

A functional study of the endocannabinoid system in zebrafish neurodevelopment: implications in vision and locomotion

Candidate
Rosa Maria Sepe

Tutors
Dr. Paolo Sordino
Prof. Paolo De Girolamo

Coordination - *Prof. Giuseppe Cringoli*

1. ABSTRACT:

The endocannabinoid system (ECS) is constituted by a group of endogenous arachidonate-based lipids [endocannabinoids (eCBs)], and their receptors, capable of regulating neuronal excitability as well as a variety of physiological processes.

The 2-Arachidonoylglycerol (2-AG) is a retrograde neurotransmitter present at relatively high levels in the central nervous system, with cannabinoid neuromodulatory effects on synaptic transmission in the adult brain. Recently, several lines of evidence have demonstrated the presence of eCBs, their receptors and metabolizing enzymes, also in early stages of brain development, suggesting an important role of ECS in the regulation of neural progenitor proliferation and specification as well as migration and differentiation.

In this study we focus on the importance of 2-AG signaling in central nervous system development, with the aim to investigate the role of 2-AG in the development and differentiation of neurons, and in the formation of neuronal circuits that control spontaneous locomotion and visual system, using zebrafish as model organism.

The vertebrate *Danio rerio* (zebrafish) represents a valid animal model system to study eCB biology, since phylogenetic analyses of the zebrafish ECS have demonstrated that it is highly conserved with the mammalian counterpart.

Here we report the presence of a complete endocannabinoid system during zebrafish development and show that the genes coding for enzymes that catalyze the anabolism and catabolism (*dagla* and *mgll* respectively) of the endocannabinoid, 2-AG, as well as its main receptor in the brain,

cannabinoid receptor type 1 (*cnr1*), are co-expressed in defined regions of neurogenesis and axogenesis.

Through the use of morpholino-induced transient knockdown of the zebrafish *dagla* and its pharmacological rescue, we suggest that the synthesis of 2-AG is implicated in the control of axon formation in defined areas of the developing brain, such as optic tectum, cerebellum and optic nerve. Animals lacking Daglα display defective axonal growth and fasciculation, and abnormal physiological behaviors in tests measuring stereotyped eye movement and motion perception. Moreover the use of *dagla* morpholino in the zebrafish transgenic line 'ath5:gap-gfp', in which it is possible to follow the retinal ganglion cells (RGC) pathfinding, reveals abnormalities in RGC fiber tracts and in the correct arborization in the optic tectum.

Furthermore, pharmacological treatments using antagonists of the two main zebrafish eCBs receptors (CB1 and CB2) suggest their putative role in the correct formation of the neuropile in transgenic line 'ath5:gap-gfp' and in the correct lamination of neuroretina in SoFa line, in which all the major retina neuronal subtypes are labeled simultaneously.

In details, pharmacological treatments using antagonists of CB1 receptor suggest its putative role in the correct formation of the neuropile in zebrafish transgenic line 'ath5:gap-gfp'. On the other hands, by using zebrafish SoFa line, in which all the major retina neuronal subtypes are simultaneously labeled, similar treatments highlight the possible role of CB2 in the correct lamination of neuroretina by regulating the number and positioning of interneurons (amacrine cells) in the inner plexiform layer of developing retina.

Animals treated with these antagonists display also defective swimming behavior, suggesting, in addition, the implication of CB1 and CB2 receptors in the correct formation of neuronal circuits that control spontaneous locomotion.

In conclusion, our results point to the important role of eCBs as mediators in axonal outgrowth with implications in the control of vision and movement, highlighting that the well-established role of 2-AG in axon guidance is required in brain areas that control locomotor and optokinetic functions. On the other hand, the eCBs receptors CB1 and CB2 can specifically regulate the formation of retinotectal system, the differentiation and lamination of zebrafish neuroretina, as well as direct the control of swimming behaviour.