40 YEARS OF RESEARCH ON THE "REPRODUCTIVE BRAIN" OF TELEOST FISHES

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This year marks the 40th anniversary of the first article ever published on the effects of a brain hypothalamic factor on pituitary gonadotrophin in a teleost fish, the carp [1]. Since this pioneer work, one generation of researchers has written more than 1250 papers dedicated to various aspects of the brain control over the reproductive axis of fish. What we have learnt is that, overall, the neuroendocrine circuits controlling the pituitary gonadotropic activity and the main molecular actors involved in this control are highly conserved in vertebrates, indicating that they are at least 450 million years old and, probably, that many of these actors, if not all, were inherited from invertebrates.

A first issue is to understand how the reproductive brain develops and is sexualized in accordance with the gonadal sex. While it is admitted in birds and mammals that the brain of males and females are different, this issue is poorly addressed in fish. It is probably due partly to the fact that there are important species differences. In general, it is admitted that the brain of fish exhibits a high sexual plasticity as exemplified by the fact that many species can change sex naturally or after hormonal treatment. This indicates that the brain of fish is not permanently sexualized as documented in other vertebrate models. How this can be achieved is likely linked to the fact that the brain of fish conserves throughout life properties of the embryonic brain of mammals, such as persistence of radial glia progenitors, high neurogenic activity and elevated aromatase activity. This probably allows sexual steroids to shape the brain in a region and sex-specific manner through a modulation of neurogenesis [2].

In terms of neuroendocrine control, the key player is GnRH that is the most potent neurohormone on LH release. Three GnRH genes are found in fish, the history of which is still matter of active debates. GnRH1 neurons in the preoptic area express a decapeptide, which varies from species to species and stimulates the synthesis and release of FSH and LH. In some families, GnRH1 seems to be replaced by GnRH3. However, there is some evidence to suggest that some GnRH genes could remain undiscovered in certain species, such as salmon or zebrafish. Recently, GnRH2, a universal ancestral GnRH variant was shown to act as a melatonin-stimulating factor in the European sea bass through direct projection in the pineal [3]. In sea bass, this new role involves one of 5 GnRH-Rs, and this pineal receptor is different from the one present in the pituitary. The other GnRH-Rs are still in search of functions and precise sites of expression. The GnRH system develops during early stages of embryogenesis. In the sea bass and other species, GnRH neurons and fibres reach their respective final destination in adult much earlier than the time of the first sexual maturation. This suggests that, as in mammals, this system develops early and needs to be activated at the time of puberty, which takes us to kisspeptins.

With the discovery of kisspeptin, year 2003 has seen a real revolution in the field of reproductive neuroendocrinology and puberty control. In mammals, kiss neurons are now acknowledged as essential activators of the GnRH systems and considered as integrators of metabolic, seasonal and hormonal signals. Most fish species have conserved 2 copies of an ancestral kiss gene, kiss1 and kiss2. However, the expression of these two genes and their relative importance in reproduction certainly varies from species to species. The zebrafish is so far the only species in which kiss expression has been deciphered at both the messenger and protein levels [4]. However, data in medaka [5] and sea bass (Escobar *et al.*, this meeting) suggest that the use of kiss1 and kiss2 genes might vary from species to species. The pituitary could also be a source of kisspeptins as indicated in zebrafish and sea bass. In any case, the role of kisspeptin on fish puberty has not been firmly established so far and we need more data regarding the roles of kisspeptins on GnRH neuron activity.

Another key question concerns the brain targets of hormones such as sex steroids, but also metabolic hormones or melatonin, and their effects on the circuits controlling reproduction. Although outstanding progress has been made, notably in the localization of receptors, we are far from understanding the respective roles of these hormones. It seems now clear that estrogens do not influence GnRH neurons directly. The possibility exists that, similar to rodents, kiss neurons are targets for estradiol as documented in zebrafish, medaka and sea bass. Thus as in mammals, kiss neurones could forward estrogen information to GnRH neurons. The functional significance of the high aromatase expression found in fish brain is still unclear and this makes it difficult to understand the roles of aromatizable vs. non aromatizable androgens. In addition, recent data point to

the fact that neurosteroids are produced *de novo* in the brain of developing and adult fishes [6]. The roles of these neurosteroids vs. peripheral steroids are still open to speculation. Importantly, it was shown that testosterone can be actively transformed, through 5α-reductase and 3ß-hydroxysteroid-deshydrogenase, into the potent estrogenic androgen, 5 alpha-androstan-3 alpha,17 beta-diol [7]. This makes the situation very complicated to fully understand the roles of androgens vs. estrogens. Androgen receptors are widely distributed in the brain of fish as well as nuclear and membrane progesterone receptors (nPR). These nPR are up-regulated by estradiol in both larvae and adults. To add further complexity, we have evidence that radial glial cells express membrane estrogen receptors. Whether these correspond to the GPR30-expressing cells that we have identified in zebrafish is under investigation.

Leptin and melatonin receptors are widely expressed in the brain of fish. How exactly those signals are processed and transformed into reproductively relevant information is not known. Preliminary data indicate that kiss neurons are tightly related to leptin receptors and that their expression varies according to energy status. Kiss neurons are thus likely to be direct targets for leptin. On another hand, there is increasing indication that melatonin influences kiss neuron activities.

This lecture intends to provide a comprehensive and integrated view of the current situation and to raise a number of unsolved questions and bottlenecks in our understanding and mastering of fish reproductive physiology.

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