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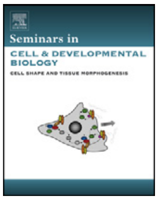

Review

Building an asymmetric brain: Development of the zebrafish epithalamus

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ABSTRACT

The human brain exhibits notable asymmetries. Little is known about these symmetry deviations; however scientists are beginning to understand them by employing the lateralized zebrafish epithalamus as a model. The zebrafish epithalamus consists of the pineal and parapineal organs and paired habenular nuclei located bilateral to the pineal complex. While zebrafish pineal and parapineal organs arise from a common population of cells, parapineal cells undergo a separate program that allows them to migrate left of the pineal anlage. Studying the processes that lead to brain laterality in zebrafish will allow a better understanding of how human brain laterality is established.

Contents

1. Nervous system asymmetry

Nervous system asymmetry is a conserved feature across phyla, from the relatively simple olfactory nerves of the nematode nervous system to the highly specialized human brain [1,2]. The conserved nature of brain asymmetry leads to questions regarding how these lateralities arise and speculation about their evolutionary origins. Early chordates were known to rest on one side of their bodies, causing left–right (L/R) differences in sensory input [3]. Until recently it was thought that gross morphological differences between the left and right hemispheres of the brain were a uniquely human trait [4,5]. Recent investigation into the behavior of humans and chickens, however, suggests that functional lateralization of the brain has roots in an anciently derived species as both chickens and humans use the right side of their brain to understand spatial relations [6].

Interest in brain lateralization dates back to Hippocrates who observed speech and language difficulties in patients suffering from traumatic brain injuries to one side of the head [7]. In the mid-nineteenth century, Broca and Wernicke independently noted that tumors and strokes on the left side of the brain severely impaired patients' ability to speak [2]. While dissection pointed out gross anatomical asymmetries, rigorous scientific investigation began in earnest when Geschwind and Levitsky [8] reopened a long dormant area of study by carefully assessing the size of the left and right planum temporale of the human brain. They conclusively demonstrated significant size differences between these two bilateral structures with the left planum temporale appreciably larger than the right.

Recent interest in the development of brain asymmetry has been sparked because defects in such symmetry have been implicated in various diseases, many of which affect a large number of individuals. For instance, greater symmetry between the left and right planum temporale of young patients has been correlated with an increase in both reading disorders and dyslexia

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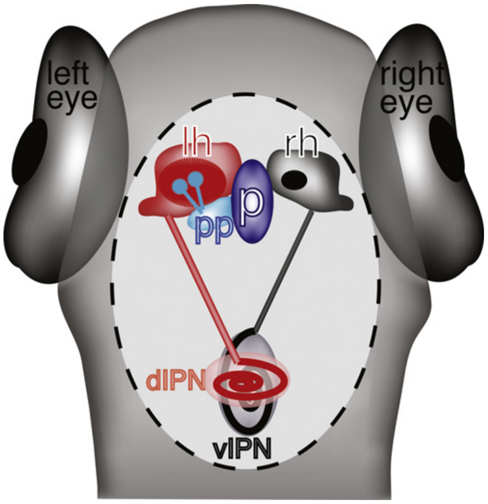

Fig. 1. Schematic of a 4 days post-fertilization (dpf) larval zebrafish epithalamus, viewed from the dorsal aspect. The pineal organ (p) is located in the midline; the parapineal (pp) is located to its left. The left (lh, red) and right (rh, black) habenulae are located on either side of the pineal complex. The region of dense neuropil in the left habenula (red oval) is larger than in the right habenula (black oval). The left habenula primarily sends axonal projections to the dorsal interpeduncular nucleus (dIPN) while the right habenula sends projections to the ventral IPN (vIPN).

[9,10]. Individuals can also be struck later in life by diseases such as Alzheimer's disease which progresses asymmetrically [2]. Asymmetry is also currently a topic of debate in susceptibility to schizophrenia [11]; reduced planar asymmetry in the planum temporale has been correlated with auditory hallucinations [11,12].

In order for scientists to begin to understand the cellular and molecular processes that give rise to brain asymmetries, a more tractable system for genetic and embryological studies is necessary. A fruitful venue for such studies is the dorsal diencephalon (or epithalamus) of the zebrafish, *Danio rerio* (Fig. 1). The diencephalon of all vertebrates arises from a portion of the prosencephalon of the developing neural tube. The rudimentary diencephalon gives rise to the retina, epithalamus, thalamus, and hypothalamus in the adult brain [13]. The epithalamus of both the human and zebrafish consists of the pineal complex and adjacent habenular nuclei. Additionally, the zebrafish pineal complex contains a left sided accessory called the parapineal organ. Though the mammalian brain does exhibit other lateralities, the diencephalon itself does not appear to be asymmetric. The lack of lateralized diencephalic structures in mammals and their presence in fish suggests that mammals may have evolved away from a need for these structures [14].

The use of the zebrafish as a model organism allows for a greater understanding of the genetic and developmental processes that give rise to brain asymmetry. High fecundity, rapid development outside of the mother, available genetic mutants and transgenic tools make the zebrafish particularly well suited to these types of studies [15]. The zebrafish parapineal organ, in particular, can be used to assay various processes common to the development of vertebrate brains. Pineal and parapineal cells undergo separate programs of proliferation, specification, and differentiation from a seemingly uniform population of cells [16,17]. Parapineal cells then migrate to a characteristic position on the left side of the brain. By understanding the processes that give rise to laterality in the zebrafish epithalamus, we may begin to understand the complex developmental processes in the human brain that lead to gross anatomical differences and if altered, to various developmental defects.

1.1. Anatomical asymmetry of the zebrafish epithalamus

The medially placed pineal organ is a bulbous structure that forms at the end of a stalk attached to the dorsal habenular surface. The parapineal organ arises as an outcropping of cells from the anterior region of the pineal complex anlage, which emerges between 28 and 31 h post-fertilization (hpf) and migrates leftward in 95% of individuals within a population [18,19]. As the pineal organ begins to develop the afferent nerves that will eventually form its stalk, the parapineal organ completes its migration and begins to extend afferent processes that coalesce into the parapineal tract. This tract then joins and courses with the habenular commissure (axons that connect the left and right habenulae) before splitting into smaller tracts that extend to the left medial habenular ganglion [20]. The habenular nuclei, in turn, extend long axon tracts, called the fasciculi retroflexus (FR), to a midbrain structure termed the interpeduncular nucleus (IPN). The left FR preferentially innervates the dorsal IPN, while the right FR primarily innervates the ventral IPN, converting L/R laterality to dorsal/ventral differences [21,22].

Besides location of the parapineal to the left side of the pineal organ, other gross asymmetries are also apparent in the zebrafish epithalamus. For example, the stalk upon which the pineal organ is situated is slightly biased to the left in wild type embryos [23]. Additionally, the habenular nuclei also display gross anatomical differences; the left habenulae is 20% greater, volumetrically, than the right habenular nucleus. Antibody labeling with anti-acetylated tubulin, which labels neuropil, also shows a greater region of dense neuropil associated with the left habenula with reduced neuropil is found on the right [18,24].

Consistent with differences in anatomical features, the habenulae also differ in expression of several asymmetrically expressed genes. The gene *leftover* (*lov*) shares a domain similar to the Potassium Channel Tetramerization Domain (KCTD) of voltage gated potassium channels and is expressed throughout the left habenular nucleus, and only in a small section located caudally in the right habenula [24]. Other KCTD-containing genes, *right on* (*ron*) and *dexter* (*dex*) are asymmetrically expressed, but with the opposite pattern; they are more highly expressed in the right habenular nucleus compared to the left [22]. While differential expression of KCTDs may define functional subdomains of the habenular nuclei, the molecular role of KCTD proteins in habenular neurons has not yet been established. Additionally, a glycoprotein required for central axon guidance Contactin2 (Cntn2/Tag1) [25] is expressed in a greater region in the right habenula than the left [26]. Interestingly, the axon guidance molecule Neuropilin 1a (Nrp1a) is more abundant in the left habenular nucleus, and depletion of its axon guidance cue Semaphorin 3d (Sema3d) causes aberrant targeting to the IPN [27] suggesting side specific preference of axon guidance cues.

1.2. Epithalamic asymmetry is a conserved feature of many vertebrates

Asymmetry of the pineal complex (epiphysis) of teleosts is also shared with other vertebrates. The most primitive of vertebrates, the lamprey (*Petromyzon marinus*) and other members of this family have a pineal complex organization similar to the zebrafish [14]. One striking difference however, is that the parapineal organ of lampreys emanates from a separate stalk from the pineal organ and is associated with the parapineal ganglion which shares neurochemical [28] and ultrastructural [29] characteristics with the left habenula. The coelacanth (*Latimeria chalumnae*) has both a pineal and parapineal organ. Coelacanths and teleost fish have a common ancestry that diverged during the Mesozoic period. Interestingly, the coelacanth parapineal organ develops from an anlage similar