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Development and connectivity of the habenular nuclei

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ABSTRACT

Accumulating evidence has reinforced that the habenular region of the vertebrate dorsal forebrain is an essential integrating center, and a region strongly implicated in neurological disorders and addiction. Despite the important and diverse neuromodulatory roles the habenular nuclei play, their development has been understudied. The emphasis of this review is on the dorsal habenular nuclei of zebrafish, homologous to the medial nuclei of mammals, as recent work has revealed new information about the signaling pathways that regulate their formation. Additionally, the zebrafish dorsal habenulae have become a valuable model for probing how left-right differences are established in a vertebrate brain. Sonic hedgehog, fibroblast growth factors and Wingless-INT proteins are all involved in the generation of progenitor cells and ultimately, along with Notch signaling, influence habenular neurogenesis and left-right asymmetry. Intriguingly, a genetic network has emerged that leads to the differentiation of dorsal habenular neurons and, through localized chemokine signaling, directs the posterior outgrowth of their newly emerging axons towards their postsynaptic target, the midbrain interpeduncular nucleus.

Contents

1. Introduction

The habenulae (Hb), are conserved, bilateral structures in the dorsal diencephalon and consist of medial (mHb) and lateral (lHb) nuclei in mammals (equivalent to dorsal and ventral nuclei, respectively, in fish and amphibians [1,2]). Habenular efferents project through the prominent fasciculus retroflexus (FR) fiber bundles, with neurons in the medial/dorsal nuclei primarily innervating the unpaired interpeduncular nucleus (IPN) in the ventral midbrain and those of the lateral/ventral nuclei predominantly targeting the raphe nuclei [3,4].

The Hb are known to modulate diverse states such as fear and anxiety, aversion and reward, pain, sleep, and reproduc-

Abbreviations: ANB, anterior neural boundary; A-P, anterior-posterior; BMP, bone morphogenetic Protein; CSPG, chondroitin sulfate proteoglycans; d, dorsal; dpf, days post fertilization; D-V, dorsal-ventral; Fgf, fibroblast growth factor; FR, fasciculus retroflexus; GABA, gamma aminobutyric acid; Hb, habenula; hpf, hours post fertilization; HSPG, heparin sulfate proteoglycans; IPN, interpeduncular nucleus; l, lateral; LPM, lateral plate mesoderm; L-R, left-right; m, medial; MDO, middiencephalic organizer; p(1-3), prosomeres 1-3; PT, pretectum; pTh, prethalamus; RN, raphe nucleus; Shh, sonic hedgehog; ThEPC, thalamic-epithalamic early projecting cluster; v, ventral; Wnt, wingless-type MMTV integration site family member; ZLI, zona limitans intrathalamica.

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tive and aggressive behaviors [5–14]. Habenular activity has also been implicated in clinically relevant conditions, with dysregulation strongly associated with depressive disorders [15–17], and drug addiction [18–20]. Historically, our understanding of Hb function has come from gross lesioning experiments in the rodent brain [5,6,21,22], which can be imprecise and damage other brain regions. Such studies also fail to address the neuronal subpopulations within the Hb that mediate specific functions. State-of-the-art transgenic approaches in the mouse, however, have enabled selective manipulation of distinct cholinergic and peptidergic Hb neurons and monitoring of their activation in a variety of behavioral tests. Although increasing information is known about the identity and functions of some Hb neurons, notably the cholinergic and *tachykinin*-expressing populations, [23–25], and tracing experiments have uncovered pre- and post-synaptic partners [4,7,26,27], the neuronal complexity of the Hb and the precise circuitry underlying their diverse neuromodulatory roles are only now beginning to be appreciated. Additionally, the dorsal habenulae (dHb) of many non-mammalian vertebrates show striking L-R differences in their size, gene expression, and neuronal connections, whose formation and functional significance have become a topic of intense interest [28–31].

This review presents an overview of our current knowledge of habenular development, with a primary emphasis on the establishment of the dHb-IPN pathway in the developing zebrafish brain. The genetic network regulating development of the dHb is complex, often with a single signaling pathway involved in multiple, temporally distinct events. While some headway has been made, significant work is needed to obtain a complete picture of the regulation of Hb differentiation. Outstanding questions include how the appropriate number of neurons is generated, how neuronal diversity is achieved, and how precise connectivity with the IPN is established. A deeper molecular understanding of habenular development will not only provide fundamental insights into the diversification of brain nuclei, but will also allow the construction of more precise tools to manipulate select neuronal populations.

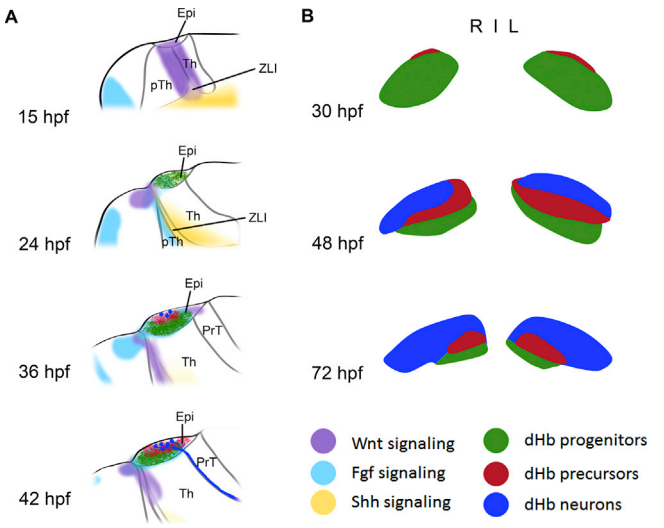

Fig. 1. Schematic of diencephalic and habenular development.
(A) Development of the zebrafish dorsal diencephalon from 15 to 42 h post fertilization (hpf, lateral view, anterior left). At 15 hpf, Wnt (purple), Fgf (light blue), and Shh (yellow) signals pattern the forebrain. Shh expression and signaling has not yet expanded dorsally at the presumptive ZLI. By 24 hpf, Wnt, Fgf and Shh all influence the presumptive epithalamus, and expression of *dbx1b* (green) can be detected. By 36 hpf, expression of *cxcr4b* (red) has initiated and the first dHb neurons appear (dark blue). At 42 hpf, the number of dHb neurons has increased, and their efferent axons project towards the midbrain target, extending between the border of p2 and p1 (Th and PrT, respectively). In zebrafish, the vHb have not yet differentiated. Abbreviations: Epithalamus (Epi), Prethalamus (pTh), Thalamus (Th), Pre-tectum (PrT), Zona limitans intrathalamica (ZLI). Drawings are adapted from [40]. (B) Frontal view of the developing zebrafish dHb drawn from live images at the indicated stages [54]. The *dbx1b*-expressing dHb progenitors (green) lie ventromedial to *cxcr4b*-expressing neural precursors (red) and dHb neurons (blue). As dHb development proceeds, the transition from progenitors to mature neurons results in progressive restriction of *dbx1b* expression to a smaller, ventromedial domain, with *cxcr4b* transcripts concentrated in cells positioned between the progenitor and neuronal populations.

2. Mechanisms underlying habenular development

2.1. Specification and regionalization of the diencephalon

The posterior forebrain or diencephalon is comprised of three distinct regions referred to as prosomeres (p3-p1 along the A-P axis), which themselves can be subdivided into presumptive brain regions: p3 consists of the pre-thalamus (pTh), p2 of the epithalamus (containing the pineal complex and flanking Hb nuclei), zona limitans intrathalamica (ZLI) and thalamus, and p1 of the pretectum (PT) [32–34]. Boundaries between prosomeres are determined by gene expression and there is little mixing of cells between them or the brain regions that develop within them [35,36]. A number of regions along the A-P axis of the developing brain serve as signaling centers controlling diencephalic regionalization (Fig. 1A). Those most relevant for p2, and therefore epithalamic specification/habenular development, are the anterior neural boundary (ANB) in the very early telencephalon and the later-arising, mid-diencephalic organizer (MDO), located at the ZLI. The MDO is a source of Wnt, Fgf and Shh morphogens and is involved in setting up the p2-p3 border and in patterning p2 [36–40]. The position, size and molecular composition of the MDO is thus critical for generation of the thalamic and epithalamic regions.

The Hb, along with the pineal gland, arise from the anterodorsal region of prosomere 2 (p2) in the roof of the developing diencephalon [33]. Consequently, formation of the Hb depends on the specification and regionalization of the diencephalon and its prosomeres. This is accomplished by input from signaling pathways along the dorsal-ventral (D-V) and anterior-posterior (A-P) axes; the former by opposing gradients of bone morphogenetic proteins (BMPs) and Sonic Hedgehog (Shh) signals and the latter by dynamic signaling centers that refine A-P subregions over time [37–40]. Wingless-INT proteins (Wnts) and Fibroblast growth factors (Fgfs) direct A-P patterning during the early subregionalization of the neural tube and establishment of the diencephalon. Canonical Wnt signals have been shown to promote posterior brain fates, such as the diencephalon, while simultaneously acting to antagonize forebrain fates [41,42]. Non-canonical Wnts and inhibitors of canonical Wnt signaling are necessary for formation of anterior brain structures and to oppose diencephalic fates [39,41,43].

Additionally, Fgf signals play important roles in the differentiation and patterning of the forebrain, influencing gene expression, neuronal identity and commissural connections [39,44,45]. The opposing action of Fgf and Wnt signaling also determines the boundary between the telencephalon and diencephalon [37,46].

Recent studies demonstrate that the adoption of thalamic or epithalamic neuronal cell fates is intimately connected. For example, in mice lacking Transcription Factor 7 Like 2 (Tcf7l2), a transcription factor in the canonical Wnt signaling pathway, post-mitotic thalamic neurons inappropriately acquire molecular properties of habenular neurons, which themselves now transfate and display features of thalamic neurons [47]. Similarly, loss of the *gastrulation brain homeobox 2* (*Gbx2*) gene results in the adoption of habenular neuronal fate at the expense of thalamic neuronal identity, apparently by indirectly altering the developmental program of proliferating thalamic progenitors [48].

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