

# Asymmetry in the epithalamus of vertebrates

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## ABSTRACT

The epithalamus is a major subdivision of the diencephalon constituted by the habenular nuclei and pineal complex. Structural asymmetries in this region are widespread amongst vertebrates and involve differences in size, neuronal organisation, neurochemistry and connectivity. In species that possess a photoreceptive parapineal organ, this structure projects asymmetrically to the left habenula, and in teleosts it is also situated on the left side of the brain. Asymmetries in size between the left and right sides of the habenula are often associated with asymmetries in neuronal organisation, although these two types of asymmetry follow different evolutionary courses. While the former is more conspicuous in fishes (with the exception of teleosts), asymmetries in neuronal organisation are more robust in amphibia and reptiles. Connectivity of the parapineal organ with the left habenula is not always coupled with asymmetries in habenular size and/or neuronal organisation suggesting that, at least in some species, assignment of parapineal and habenular asymmetries may be independent events.

The evolutionary origins of epithalamic structures are uncertain but asymmetry in this region is likely to have existed at the origin of the vertebrate, perhaps even the chordate, lineage. In at least some extant vertebrate species, epithalamic asymmetries are established early in development, suggesting a genetic regulation of asymmetry. In some cases, epigenetic factors such as hormones also influence the development of sexually dimorphic habenular asymmetries. Although the genetic and developmental mechanisms by which neuroanatomical asymmetries are established remain obscure, some clues regarding the mechanisms underlying laterality decisions have recently come from studies in zebrafish. The Nodal signalling pathway regulates laterality by biasing an otherwise stochastic laterality decision to the left side of the epithalamus. This genetic mechanism ensures a consistency of epithalamic laterality within the population. Between species, the laterality of asymmetry is variable and a clear evolutionary picture is missing. We propose that epithalamic structural asymmetries per se and not the laterality of these asymmetries are important for the behaviour of individuals within a species. A consistency of the laterality within a population may play a role in social behaviours between individuals of the species.

*Key words:* Epithalamus; habenula; pineal complex; parapineal organ; asymmetry; evolution; genetics.

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## INTRODUCTION

The epithalamus has been historically conceived as a distinct neuroanatomical moiety within the diencephalon of all vertebrates. Named because of its topographical situation 'above' (*'epi'*) the thalamus, the epithalamus was originally considered as one of the fundamental longitudinal subdivisions of the diencephalon, together with the dorsal thalamus,

ventral thalamus and hypothalamus. Recent ontogenetic studies, however, have revealed that at least the dorsal thalamic and ventral thalamic subdivisions are not longitudinal, but instead are oriented perpendicular to the longitudinal axis of the brain. In the context of this emerging neuromeric model of brain organisation (Rubenstein et al. 1998), the epithalamus stems from the same neuromere as the dorsal thalamus, denominated parencephalon posterius or

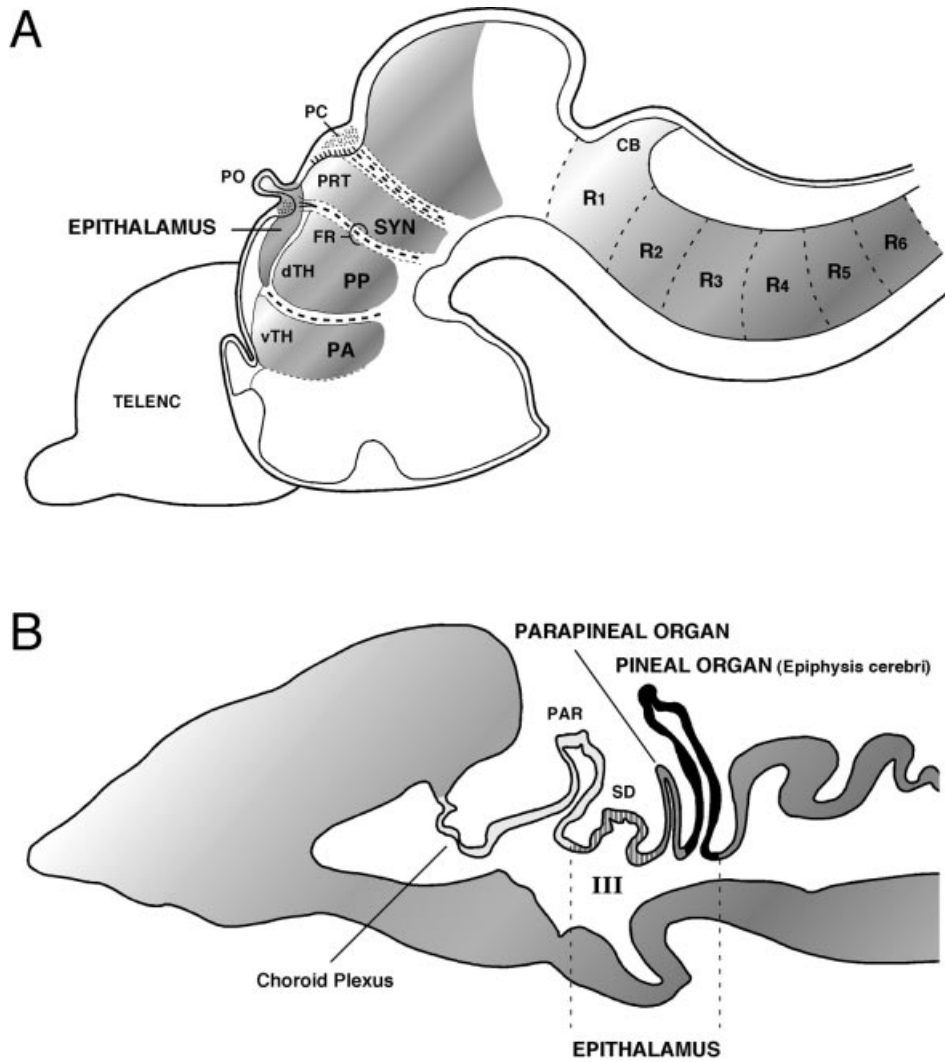


Fig. 1. The epithalamus of vertebrates. Diagrams of sagittal sections of the mouse brain (A, modified from Nieuwenhuys, 1998e) and of a basal vertebrate (B, modified from Kardong, 1995) with anterior to the left and dorsal up. (A) According to the neuromeric model, the epithalamus stems from the same neuromere as the dorsal thalamus (dTH), named parencephalon posterius (PP). (B) Several medial evaginations are present along the epithalamic roof of the diencephalon, the 2 most significant being the photoreceptive pineal and parapineal organs. CB, cerebellum; FR, fasciculus retroflexus; PA, parencephalon anterius; PAR, paraphysis; PC, posterior commissure; PO, pineal organ; PRT, preectum; R1–R6, rhombomeres; SD, saccus dorsalis; SYN, synencephalon; TELENC, telencephalon; vTH ventral thalamus; III third ventricle.

P2 (Fig. 1A) (for a historical overview of the diencephalic subdivisions see Nieuwenhuys, 1998e, pp. 196–225).

The epithalamus is constituted by 2 sets of neuronal conglomerates with strikingly dissimilar cytoarchitectonic organisation: the habenula and the pineal complex. Whereas the habenula is formed by a bilateral set of nuclei surrounding the lateral walls of the third ventricle, the pineal complex comprises a pair of median evaginations situated along the diencephalic roof plate. The habenular commissure divides the diencephalic roof plate into a larger rostral and a smaller caudal part. The rostral part gives rise to the saccus dorsalis, a membranous evagination of

unknown function that reaches the posterior end of the velum transversum (Fig. 1B). The caudal part of the diencephalic roof plate gives rise to a pair of saccular or tubular evaginations known as pineal organ or epiphysis cerebri, and parapineal organ or parietal eye. Together, the pineal and parapineal organs constitute the so-called pineal complex (Fig. 1B).

From the 19th century, a large number of cytoarchitectonic studies have shown that in many species the left and right sides of the habenula display a remarkable asymmetry in size and sometimes also in neuronal organisation. Other, more sporadic studies have revealed that asymmetry is also seen in the

parapineal organ, and that asymmetry in both the habenula and parapineal organ is not restricted to cytoarchitecture but is also reflected in neuronal connectivity, neurochemistry and gene expression during embryogenesis. Despite its widespread occurrence, the functional consequences of epithalamic asymmetry upon animal behaviour remain largely unknown. In this manuscript, we review the neuro-anatomical basis of asymmetry in both the habenula and the pineal complex, with special attention to studies of cytoarchitecture, neurochemistry and connectivity. We explore evolutionary hypotheses on the origin of epithalamic asymmetries, and discuss these findings in the context of recent experimental data suggesting a genetic regulation of the laterality of asymmetry in the epithalamus.

#### ASYMMETRY OF THE HABENULA

The habenula and its associated fibre tracts form part of a conserved conduction system linking the fore-brain and the ventral midbrain (Butler & Hodos, 1996). The habenula of lampreys (Yañez & Anadón, 1994) and teleosts (Yañez & Anadón, 1996), for example, contributes to a system of projections between nuclei of the caudal telencephalon and the interpeduncular nuclei of the ventral midbrain. Similar projections form a subset of the much more complex set of connectivities found in amniotes (Herkenham & Nauta, 1977, 1979; Diaz & Puelles, 1992*a, b*). Indeed, it has been proposed that the habenula of lampreys and teleosts is homologous to the medial component of the habenula of lizards and mammals (Yañez & Anadón, 1994, 1996). The lateral habenula may be a late acquisition in the evolution of vertebrates, perhaps reflecting the increasing importance of cortical circuits in amniotes (Yañez et al. 1996). The habenula in mammals facilitates functional interactions amongst neural structures in the limbic forebrain and the midbrain (Wang & Aghajanian, 1977; Sutherland, 1982), and roles have been proposed in olfactory responses, mating and feeding behaviours, in the generation of sleep patterns, secretion of hormones (noradrenaline, adrenaline, corticosterone), in the response to stress, and in avoidance learning (reviewed in Sandyk, 1991). In support of a role in reproductive behaviour, asymmetries of the habenula show sex- and seasonal-dependent variations in some species (see below).

#### *Mixinoidea*

In hagfishes, the habenula shows a unique feature that may be attributed to the long and independent

evolutionary history of this class (Wicht & Nieuwenhuys, 1998). Although of bilateral origin (Conel, 1931), the habenula of the adult hagfish forms a single body located at the midline of the brain. Left and right habenular components can be distinguished only at a microscopic level, each containing a corpus habenularis, a lateral nucleus and a ventral nucleus (Jansen, 1930). The habenula is considerably larger on the right side mostly due to hypertrophy of the right corpus habenularis (Fig. 2*A*), a subdivision that also contains distinct cell-free patches of neuropil (Wicht & Northcutt, 1992).

#### *Petromizontoidea*

The habenula in the other group of jawless vertebrates, the lampreys, show a remarkable asymmetry in both size and neuronal organisation (Table). This phenomenon is already detected at larval stages but becomes more substantial after metamorphosis (Cole & Youson, 1982). While neurons on the left are restricted to the periventricular area, in the right hypertrophied habenula they are also arranged in distinct superficial cell layers (Nieuwenhuys, 1977; Yañez & Anadón, 1994) (Fig. 2*B*). Asymmetry also extends to efferents from the habenula that course ipsilaterally in the habenulo-interpeduncular tract or fasciculus retroflexus, the right tract appearing much larger than the left tract (Johnston, 1902).

#### *Chondrichthyes*

In almost all species of cartilaginous fishes examined, the habenula is enlarged on the left side (Fig. 2*C*) (Kemali & Miralto, 1979; Smeets et al. 1983). One exception to this rule is the dogfish *Scyliorhinus canicula* in which contradictory reports have placed the enlarged nucleus on either the left (Farner 1978; Smeets et al. 1983; Rodriguez-Moldes et al. 1990) or the right (Anadón et al. 2000). Asymmetry of the habenula extends to neuronal organisation and fibre myelination (Kemali et al. 1980; Miralto & Kemali 1980; Smeets et al. 1983), and to the distribution of the calcium-binding protein calbindin- $D_{28k}$  (Rodriguez-Moldes et al. 1990). Whereas the habenula on the right contains small densely packed cells and lacks calbindin- $D_{28k}$ -immunoreactivity, on the left it is organised into a nucleus medialis, of densely packed cells similar to the right nucleus but showing calbindin- $D_{28k}$ -immunoreactivity, and a nucleus lateralis consisting of larger neurons associated with myelinated fibres.

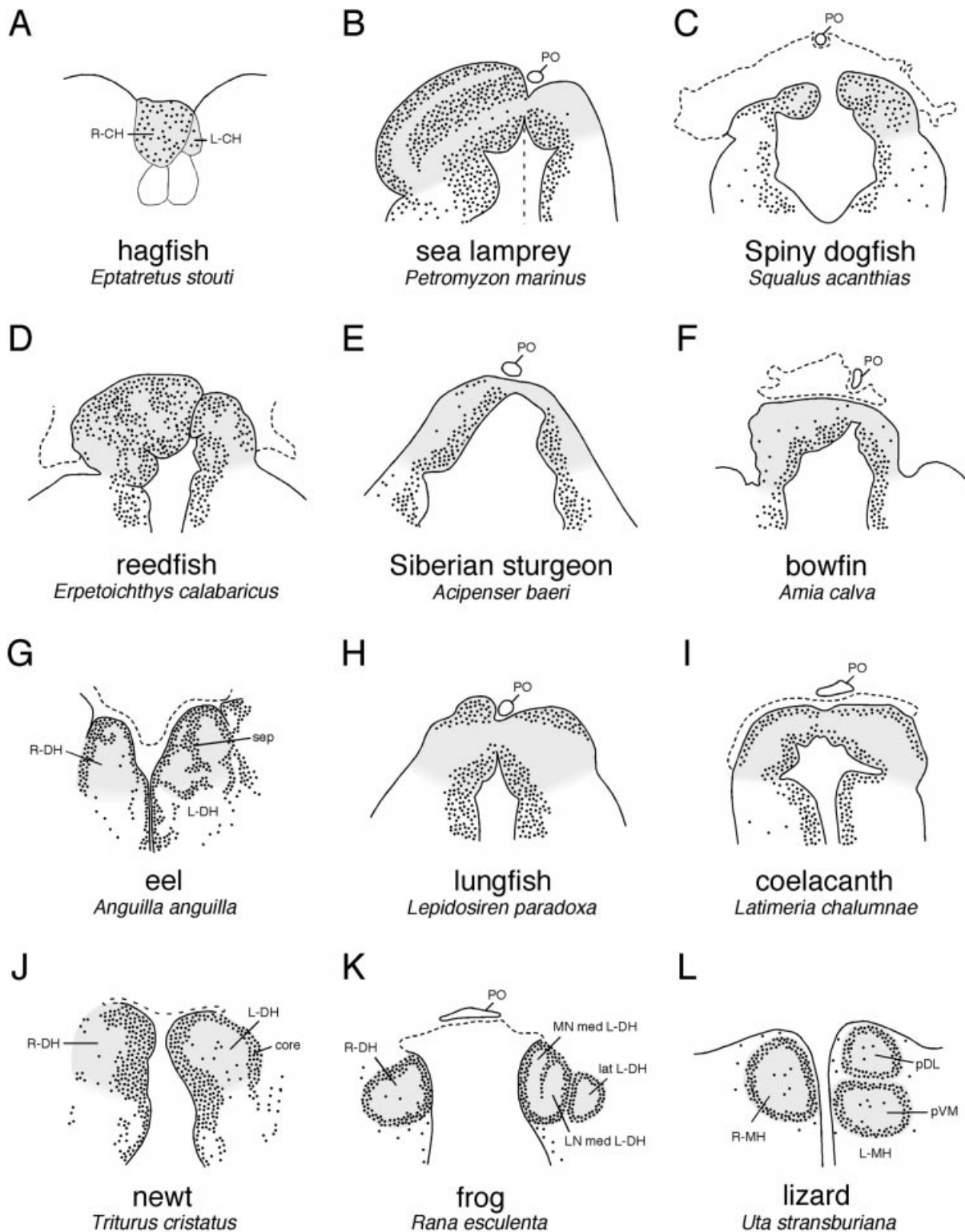


Fig. 2. The habenula is asymmetric in extant representatives of different vertebrate groups. Each panel corresponds to a transverse section at the level of the epithalamus, the asymmetric components of the habenula being highlighted in grey, with the right side illustrated on the left of the schematic. The dashed lines correspond to the diencephalic tela choroidea. Small dots indicate neuronal nuclei. Further classification details are given in the Table. lat L-DH, lateral component of left dorsal habenula; L-CH, left corpus habenularis; L-DH, left dorsal habenula; L-MH, left medial habenula; LN med L-DH, lateral neuropil of the medial component of the left dorsal habenula; MN med L-DH, medial neuropil of the medial component of the left dorsal habenula; pDL, pars dorsolateralis; PO, pineal organ; pVM, pars ventromedialis; R-CH, right corpus habenularis; R-DH, right dorsal habenula; R-MH, right medial habenula; sep, septum. Modified from Braitenberg & Kemali, 1970 (G, J and K); Engbretson et al. 1981 (L); Meek & Nieuwenhuys, 1998 (F); Nieuwenhuys 1998a, b, d, c (D, E, H and I, respectively); Smeets, 1998 (C); Wicht & Northcutt, 1992 (A); Yañez & Anadón, 1994 (B).

Table. Systematic analysis of studies describing asymmetry in the habenula of vertebrates

Species examined	Asymmetry of the habenula					References
	Size↑	Cyto.	Ult.	Histo-immuno.	Con.	
AGNATHA						
<b>Mixinoidea</b>						
<i>Myxine glutinosa</i>	R	✓				Jansen (1930)
<i>Epiplatys stouti</i>	R	✓				Wicht & Northcutt (1992)
<i>Bdellostoma stouti</i>	R					Conel (1931)
<b>Petromyzontoidea</b>						
<i>Lampreta fluviatilis</i>	R	✓			✓pp	Nieuwenhuys (1977), Yañez et al. (1999)
<i>Petromyzon marinus</i> L	R	✓			✓pp	Johnston (1902), Yañez & Anadón (1994), Yañez et al. (1999)
CHONDRICHTHYES						
<b>Elasmobranchii</b>						
<i>Scyliorhinus canicula</i> L	L	✓		Calbindin-D28		Farner (1978), Smeets et al. (1983), Rodriguez-Moldes et al. (1990), Anadón et al. (2000)
<i>Scyllium stellare</i>	L	✓	✓			Kemali & Miralto (1979), Miralto & Kemali (1980), Kemali et al. (1980)
<i>Raja clavata</i>	L	✓				Smeets et al. (1983)
<i>Squalus acanthias</i>	L	✓				Smeets et al. (1983)
<b>Holocephali</b>						
<i>Hydrolagus collei</i>	L	✓				Smeets et al. (1983)
OSTEICHTHYES						
<b>Actinopterygii</b>						
Cladistia						
<i>Polypterus bichir</i>	R	✓				Nieuwenhuys & Bodenheimer (1966)
<i>Polypterus delhezi</i>	R	✓				Nieuwenhuys & Bodenheimer (1966)
<i>Polypterus ornatipinnis</i>	R	✓				Nieuwenhuys & Bodenheimer (1966)
<i>Polypterus palmas</i>	R					Braford & Northcutt (1983)
<i>Erpetoichthys calabaricus</i>	R	✓				Nieuwenhuys (1998 a)
Chondrostei						
<i>Acipenser rubicundus</i>	R					Johnston (1901)
<i>Acipenser baeri</i>	R	✓		ChAT		Adrio et al. (2000)
<i>Scaphirhynchus platyrhynchus</i>	R					Nieuwenhuys (1998 b)
<i>Polyodon spathula</i>	L					Hocke Hoogenboom (1929)
Ginglimodi						
<i>Lepisosteus osseus</i>	R					Braford & Northcutt (1983)
Halecomorphi						
<i>Amia calva</i>	R					Meek & Nieuwenhuys (1998)
Teleostei						
● Euteleostei						
<i>Anguilla anguilla</i>	L	✓				Braitenberg & Kemali (1970)
<i>Danio rerio</i>	L	✓			✓pp	Concha et al. (2000)
<i>Oncorhynchus kisutch</i>	L	✓		Serotonin		Ekström & Ebbesson (1988)
<i>Oncorhynchus mykiss</i>	R				✓pp	Yañez & Anadón (1996), Yañez et al. (1996)
<i>Osmerus eperlanus</i>	R	✓				Holmgren (1920)
<i>Bathypterois articular phenox</i>	R					Shanklin (1935)
<i>Cyclothone acclinidens</i>	R					Gierse (1904), quoted in Shanklin (1935)
<b>Sarcopterygii</b>						
Dipnoa						
<i>Lepidosirem paradoxa</i>	R					Nieuwenhuys (1998 d)
<i>Protopterus</i>	R					Schnitzlein & Crosby (1968)
Crossopterygii						
<i>Latimeria chalumnae</i>	L					Nieuwenhuys (1998 c)

Table. (cont.)

Species examined	Asymmetry of the habenula					References
	Size <sup>↑</sup>	Cyto.	Ult.	Histo-immuno.	Con.	
AMPHIBIA						
Urodela						
<i>Triturus cristatus</i>	s	✓				Braitenberg & Kemali (1970)
Anura						
<i>Rana esculenta</i>	L	✓	✓	NADPH-D Substance-P		Braitenberg & Kemali (1970), Kemali & Sada (1973), Kemali & Guglielmotti (1977, 1984), Kemali et al. (1990), Guglielmotti & Fiorino (1998, 1999)
<i>Rana catesbiana</i>	L	✓				Frontera (1952)
<i>Rana pipiens</i>	L	✓		Melatonin R.		Frontera (1952), Wiechmann & Wirsig-Wiechmann (1993)
<i>Rana temporaria</i>	L	✓				Morgan et al. (1973)
<i>Hyla sp.</i>	L	✓				Frontera (1952)
REPTILIA						
<i>Uta stansburiana</i>	L	✓		Substance-P	✓/pe	Engbretson et al. (1981, 1982)
<i>Lacerta sicula</i>	s	✓			✓/pe	Kemali & Agrelli (1972), Korf & Wagner (1981)
AVES						
<i>Gallus gallus</i>	L					Gurusinghe & Erhlich (1985), Gurusinghe et al. (1986)
MAMMALIA						
<i>Albino rat</i>	L					Wree et al. (1981)
<i>Talpa europaea</i>	s	✓				Kemali (1984)
<i>Albino mouse</i>	R					Zilles et al. (1976)

Asymmetry of the habenula may be detected in size (enlarged left [L] or right [R] side; symmetric habenula [s]), cytoarchitecture (Cyto), ultrastructure (Ult), histochemistry (Histo), Immunohistochemistry (Immuno) and connectivity (Con). The left habenula in some species receives afferents from the parapineal organ (pp) or parietal eye (pe). NADPH-D, nicotinamide-adenine-dinucleotide-phosphate-diaphorase; Melatonin R, melatonin receptors; ChAT, choline-acetyltransferase.

### *Osteichthyes*

The bony fishes constitute by far the largest class of extant vertebrates encompassing 2 subclasses: the Actinopterygii or ray-finned fishes, and the Sarcopterygii or fleshy-finned fishes (Meek & Nieuwenhuys, 1998). Actinopterygian fishes can be further subdivided into 5 major radiations: Cladistia, Chondrostei, Ginglymodi, Halecomorphi and Teleostei (Lauder & Liem, 1983). In the Cladistia, or Brachipterygii (arm-finned fishes), the habenula displays a marked asymmetry as the right side is larger and contains a wider layer of tightly packed neurons than the left (Fig. 2D) (Nieuwenhuys & Bodenheimer, 1966; Braford & Northcutt, 1983; Nieuwenhuys, 1998a). Asymmetry of the habenula is also reflected by an increased cross-sectional area of the right vs the left fasciculus retroflexus (Braford & Northcutt, 1983). In most species of chondrosteian fishes the habenula is markedly enlarged on the right (Fig. 2E; Table) although the opposite is observed in *Polyodon* (Hocke Hoogenboom, 1929). In the Siberian sturgeon *Acipenser baeri*, more choline-acetyltransferase

(ChAT) immunoreactive (ir) fibres but less ChAT-ir cells are observed on the right than on the left side of the habenula. Furthermore, efferents coursing in the right fasciculus retroflexus are more abundant in number and larger in caliber than in the left fasciculus, and are also immunoreactive to ChAT (Adrio et al. 2000). In other ganoids (non-teleost actinopterygian fishes) like the longnose gar *Lepisosteus osseus*, (Ginglymodi) (Braford & Northcutt, 1983) and the bowfin *Amia calva* (Halecomorphi) (Fig. 2F) (Meek & Nieuwenhuys, 1998) the habenula is somewhat enlarged on the right, and this asymmetry is again reflected by an enlarged right fasciculus retroflexus (Braford & Northcutt, 1983).

Within the teleosts, studies of habenular cytoarchitecture have been done in species belonging to three of the four major subdivisions (Lauder & Liem, 1983). The habenula is described as symmetric in Osteoglossomorpha (*Pantodon buchholzi*: Butler & Saidel, 1991), Clupeomorpha (*Clupea harengus*: Butler & Northcutt, 1993) and in most Euteleostei (*Fundulus heteroclitus*: Peter et al. 1975; *Carassius auratus*: Peter & Gill, 1975; Braford & Northcutt,

1983; *Haplochromis burtoni*: Fernald & Shelton, 1985; *Ictalurus punctatus*: Striedter, 1990; *Coris julis*, *Syngnathus acus*, *Gasterosteus aculeatus*, *Pleuronectes platessa*, *Gaidropsaurus mediterraneus*: Gómez-Segade & Anadón, 1988; *Apteronotus leptorhynchus*: Maler et al. 1991). A few exceptions to bilateral symmetry, however, have been reported within euteleosts (Table). The habenula in this group can be subdivided into a ventral nucleus of densely packed small cells, and a dorsal nucleus of larger, more loosely packed neurons arranged in strands (Meek & Nieuwenhuys, 1998). In the eel *Anguilla anguilla* (Braitenberg & Kemali, 1970), the coho salmon *Oncorhynchus kisutch* (Ekström & Ebbesson, 1988) and the larval zebrafish *Danio rerio* (Concha et al. 2000), the habenula is enlarged on the left. Besides this difference in size, the left dorsal habenula is more lobate than the right dorsal nucleus in the eel (Fig. 2G) (Braitenberg & Kemali, 1970), contains a distinct serotonergic subnucleus in the coho salmon (Ekström & Ebbesson, 1988) and shows an enlarged neuropil in the larval zebrafish (Fig. 6A) (Concha et al. 2000). Although the enlarged neuropil regions of zebrafish are likely to arise from afferent projections reaching the habenula through the stria medullaris, a possible involvement of local habenular circuits has not been discounted. A reversed direction of asymmetry has been described in some species of salmonids in which the habenula is described as 'somewhat' larger on the right and having a looser arrangement of neurons than on the left (Holmgren, 1920; Yañez & Anadón, 1996).

It is commonly believed that all terrestrial vertebrates have evolved from the sarcopterygian group, which is constituted by the Dipnoi, or lungfishes (Nieuwenhuys, 1998d) and the Crossopterygii, or tassel-finned fishes. In lungfishes, a 'slightly' enlarged habenula on the right is described in some species (Fig. 2H) (Schnitzlein & Crosby, 1968; Nieuwenhuys, 1998d) although in some others the habenula is reported as being symmetric (*Neoceratodus forsteri*: Holmgren & van der Horst, 1925). In crossopterygian fishes, on the other hand, asymmetry of the habenula is clearly observed in the coelacanth *Latimeria chalumnae* where the left side is enlarged (Fig. 2I) (Nieuwenhuys, 1998c).

### Amphibia

Asymmetry in the habenula of modern amphibians has been described in species belonging to the orders Urodela (newts and salamanders) and Anura (frogs and toads). The habenula in both orders can be subdivided into dorsal and ventral nuclei (ten

Donkelaar, 1998a, c), but asymmetry is only observed in the dorsal nucleus. In the newt *Triturus cristatus*, neurons of the left dorsal habenula organise into a layer that extends far more laterally than in the right dorsal nucleus thus defining an enclosed region poor in nuclei (Fig. 2J) (Braitenberg & Kemali, 1970). In contrast to this single description of asymmetry in urodeles, the habenula of anurans and in particular that of the frog *Rana esculenta* is probably the most extensively studied example of epithalamic asymmetry in vertebrates. The left dorsal habenula of anurans is considerable larger than the right dorsal nucleus (Frontera, 1952; Braitenberg & Kemali, 1970; Morgan et al. 1973), a feature that shows both seasonal and sex-dependent variations (see below). Furthermore, while neurons in the right dorsal habenula are distributed around a single region of neuropil, a more complex assemblage of subdivisions is observed in the left dorsal habenula (Gaupp et al. 1899; Röthig, 1923; Braitenberg & Kemali, 1970; Morgan et al. 1973; Guglielmotti & Fiorino, 1999). The left dorsal habenula is divided into a lateral subnucleus similar in structure to the right dorsal habenula, and a medial subnucleus showing unique features (Braitenberg & Kemali, 1970). This medial subnucleus can be further compartmentalised into a medial and a lateral neuropil (Fig. 2K) based on cytoarchitecture (Guglielmotti & Fiorino, 1999), ultrastructure (Kemali & Guglielmotti, 1977), histochemistry (NADPH-diaphorase: Guglielmotti & Fiorino, 1999), immunohistochemistry (substance-P: Kemali & Guglielmotti, 1984; melatonin receptor expression: Wiechmann & Wirsig-Wiechmann, 1993) and connectivity (Guglielmotti & Fiorino, 1998). Importantly, habenular asymmetry appears to be established early in development and probably originates from afferent projections, as suggested by the correspondence between the increased nitric oxide synthase (NOS) activity found in neuropil of the left medial habenula at the same stage that NOS(+) cells are detected in areas of the forebrain known to project to the habenula (Guglielmotti & Fiorino, 1999).

### Reptilia

The habenula has been reported as asymmetric in some species of lizards (e.g. *Lacerta sicula*: Kemali & Agrelli, 1972; *Uta stansburiana*: Engbretson et al. 1981) but appears symmetric in others (e.g. *Tupinambis nigropunctatus*: Cruce, 1974), and in species of turtles (ten Donkelaar, 1998b), ophidians (Nagasaki, 1954) and crocodiles (Huber & Crosby, 1926; Tamura et al. 1955). The habenula of the lizard

*Uta stansburiana* (Engbretson et al. 1981), as in other species of reptiles (Butler & Northcutt, 1973), can be subdivided into a lateral nucleus containing scattered cells, and a medial nucleus with linear arrays of cells arranged around cell-free regions. As in amphibians, asymmetry is restricted to one of these subdivisions, the medial nucleus, and involves a further compartmentalisation of the left medial nucleus into 2 components, denominated pars dorsolateralis and pars ventromedialis (Fig. 2L). While the pars ventromedialis displays a cytoarchitecture similar to the right medial habenula, the pars dorsolateralis has unique cytoarchitectonic (Kemali & Agrelli, 1972; Engbretson et al. 1981), connectional (Engbretson et al. 1981; Korf & Wagner, 1981) and immunohistochemical (Substance-P: Engbretson et al. 1982) features. Volumetric studies have shown that the presence of the pars dorsolateralis in the left medial habenula of the lizard *Uta stansburiana* accounts for the marked (60%) difference in size between the left and right nucleus (Engbretson et al. 1981).

#### *Aves/Mammalia*

Reports of habenular asymmetry in birds and mammals are scarce. Overall, the habenula in these classes appears symmetric. However, subtle differences between the right and left habenula can be detected when using quantitative volumetric studies. In the chick, a sex-dependent asymmetry of the medial component of the habenula is observed (see below). In mammals, analyses of the developing and mature habenula in two different species reveal contrasting results. While in albino rats the medial habenula is slightly but significantly enlarged on the left (Wree et al. 1981), an enlarged right lateral habenula is observed in albino mice (Zilles et al. 1976). Asymmetry in neuronal organisation has also been detected in the macrosomatic mole in which a row of dark cells is seen lying along the lateral border of the habenula only on the left (Kemali, 1984).

#### ASYMMETRY OF THE PINEAL COMPLEX

The pineal complex is formed by either one or two evaginations from the roof of the diencephalon, known as pineal and parapineal organs. The pineal organ has been described in almost all species of vertebrates examined and appears to show little sign of major asymmetry (although see Liang et al. 2000). The parapineal organ, on the other hand, is much less conserved in evolution but shows asymmetric connectivity and is sometimes also asymmetrically positioned within the epithalamus. Below we focus on

describing the neuroanatomy of the parapineal organ in different vertebrate species and for a comparative survey of the pineal organ the reader should refer to other reviews (e.g. Gladstone & Wakeley, 1940; Ariëns Kappers, 1965; Oksche, 1965; Ekström & Meissl, 1997; Falcón, 1999).

A parapineal organ is described in lampreys, the bowfin, teleosts, the coelacanth, and in some reptiles, and is absent (at least in adult stages) in other extant vertebrate groups such as hagfishes, cartilaginous fishes, amphibians, birds and mammals.

#### *Petromizontoidea*

In lampreys, pineal and parapineal organs are both well developed and occupy a position at the dorsal midline of the head beneath a patch of translucent skin (e.g. *Geotria australis*: Dendy, 1907; Eddy & Strahan, 1970; *Lampreta fluviatilis*: Meiniel & Collin, 1971; *Lampreta planeri*: Cole & Youson, 1982; *Petromyzon marinus* L.: Studnicka, 1905; Cole & Youson, 1982). The only exceptional species described as lacking a parapineal organ and having a poorly developed pineal organ is *Mordacia mordax* (Eddy & Strahan, 1970). In the lamprey, the pineal vesicle is directly connected to the roof of the diencephalon by a tube-like stalk, whereas the parapineal vesicle is associated with a nucleus termed the parapineal ganglion which itself connects to the diencephalon by a stalk (Fig. 3A) (Studnicka, 1905; Dendy, 1907). Cells in the parapineal vesicle predominantly project towards the parapineal ganglion although some bipolar cells have long axons projecting to the left habenula. Cells in the parapineal ganglion receive afferent fibres from the telencephalic subhippocampal nuclei and project to the left habenula and the interpeduncular nuclei (IPN) in the ventral midbrain (Fig. 3A') (Yañez et al. 1999). The similarity between projections associated with the parapineal ganglion and those associated with the left habenula suggests that the parapineal ganglion corresponds to a component of the left habenula that has undergone migration to a novel location in lampreys (Studnicka, 1905; Dendy, 1907). Indeed, the parapineal ganglion exhibits neurochemical (calretinin: Yañez et al. 1999) and ultrastructural (Meiniel & Collin, 1971) properties similar to those of the left habenula.

Pineal and parapineal vesicles exhibit common neurochemical properties such as immunoreactivity to S-antigen, rod-opsin, serotonin and choline-acetyltransferase (Meiniel, 1978; Vigh-Teichmann et al. 1983; Tamotsu et al. 1990; Yañez et al. 1999). However, important differences in the cellular or-



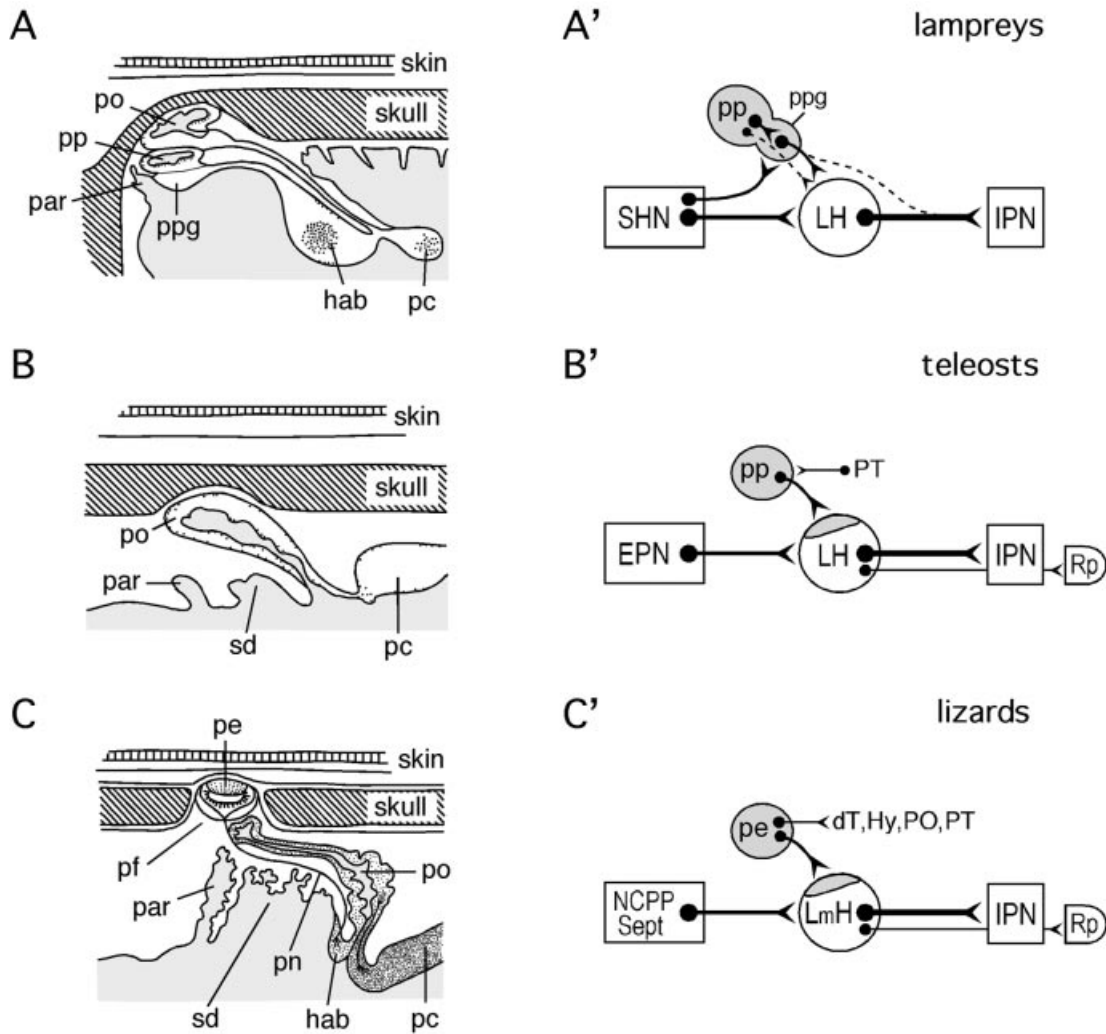


Fig. 3. The pineal complex of lampreys, teleosts and lizards, and their asymmetric connectivity with the left habenula. (A–C) Schematics of sagittal sections showing the pineal complex in lampreys (A), teleosts (B) and lizards (C). Anterior is to the left and dorsal up. In lampreys, the parapineal vesicle (pp) is associated with a parapineal ganglion (ppg). In teleosts, the parapineal organ is displaced to the left side of the midline and is not shown on the diagram. The parapineal organ or parietal eye (pe) of lizards develops into a vesicle located in a foramen of the parietal bone (pf), and is connected to the epithalamus by a parietal nerve (pn). (Modified from Kardong, 1995.) (A'–C') Schematics showing asymmetric connectivity of the pineal organs of lampreys (A'), teleosts (B') and lizards (C') to the left habenula. In lampreys, projections from the parapineal vesicle (pp) are mainly directed to the parapineal ganglion (ppg), although some bipolar cells send axons to the left habenula. The parapineal ganglion receives afferents from the subhippocampal nuclei (SHN) and sends projections to the left habenula and interpeduncular nuclei (IPN) of the ventral midbrain. Note that this pattern of connectivity resembles that of the telencephalo-habenulo-interpeduncular system of lampreys and other vertebrate species. In teleosts, the parapineal projects to a restricted region of the left habenula (LH) and receives a small afferent input from the preteectum (PT). The habenula of teleosts receives afferents primarily from the entopeduncular nucleus (EPN) of the telencephalon and projects to the IPN and the raphe nucleus (Rp). The parietal eye of lizards primarily projects asymmetrically to the left medial habenula (LmH), although symmetric projections to the dorsal thalamus (dT), hypothalamus (Hy), preoptic area (PO) and preteectum are also observed. The habenula of lizards receives projections primarily from the nucleus septalis impar (Sept) and the nucleus of the posterior pallial commissure (NCPP) and sends axonal projections to the IPN and raphe. Connectivity data adapted from Engbreton et al. (1981), Korf & Wagner (1981), Diaz & Puelles (1992a, b); Yañez & Anadón (1994, 1996), Yañez et al. (1996, 1999). Par, paraphysis; hab, habenula; pc, posterior commissure; sd, saccus dorsalis.

ganisation of pineal and parapineal vesicles can be detected by light and electron microscopy (Cole & Youson, 1982; Meyer-Rochow & Stewart, 1992). The dorsally located pineal vesicle shows a distinct pigmented retinal layer containing cone-like photoreceptor cells (type-I cells), rudimentary photoreceptor or photoneuroendocrine cells (type-II cells),

ganglion cells, and supporting cells. The parapineal vesicle, on the other hand, contains mainly type-II cells with poorly developed photoreceptive components. The scarcity of type-I photoreceptors and ganglion cells suggests a rather rudimentary photoreceptive function for the parapineal organ of lampreys.

*Osteichthyes*

The pineal complex in the bowfin *Amia calva* (Halecomorphi) and in teleosts is generally situated beneath the roof of the skull, although in a few species of extant teleosts it emerges from a foramen in the skull to reach a position underneath the skin (Steyn & Webb, 1960). In addition to the prominent pineal organ, which contains photoreceptors, supporting and other neuronal cells and serves a photoneuroendocrine role (reviewed in Ekström & Meissl, 1997), an asymmetrically positioned parapineal organ has been described in the bowfin (Hill, 1894; Kingsbury, 1897) and in many teleost species (Fig. 3B) (for a list of species see Borg et al. 1983; plus Vigh-Teichmann et al. 1991, Concha et al. 2000). Both pineal and parapineal organs originate during embryogenesis as evaginations of the diencephalic roof plate, the pineal developing earlier and in a more posterior position than the parapineal (Hill, 1891; Eycleshymer & Davis, 1897). While the pineal organ preserves its median connection with the diencephalon, the parapineal organ appears to move laterally (Holmgren, 1965) to become positioned caudal to the left habenula in the horizontal plane of the habenular commissure (Fig. 6A, C) (Borg et al. 1983; Concha et al. 2000). A further movement of the parapineal organ often takes place to situate it posterior to the pineal stalk in the adult (Holmgren, 1965). Parapineal cells display ultrastructural features of rudimentary photoreceptors (Rüdeberg, 1969; van Veen, 1982; Ekström et al. 1983), and in some cases show immunoreactivity to the visual proteins opsin (Vigh-Teichmann et al. 1980, 1983, 1991; Ekström et al. 1987; Concha et al. 2000), S-antigen (Ekström et al. 1987) and transducin (van Veen et al. 1986; Ekström et al. 1987). Unmyelinated nerve fibres emanate from the parapineal organ and constitute the parapineal tract, which courses towards the left medial habenula (Rüdeberg, 1969; van Veen et al. 1980; van Veen, 1982; Concha et al. 2000) terminating in a well defined rostro-dorsal field (Fig. 3B') (*Oncorhynchus mykiss*: Yañez et al. 1996). This terminal field appears to correspond to the serotonergic subnucleus described in the left medial habenula (*Oncorhynchus kisutch*: Ekström & Ebbesson, 1988). The arrangement of rudimentary photosensory cells and nerve tracts suggests a role, although perhaps rather rudimentary, in photoreception.

In the coelacanth *Latimeria chalumnae*, pineal and parapineal organs occupy a deep position in the head covered by adipose tissue and by the roof of the skull. They form a pair of saccular vesicles in open

communication with each other and with the diencephalic ventricle. A photoreceptive function of both pineal and parapineal organs is suggested by the presence of photoreceptors, supporting and other neuronal cells (Hafeez & Merhige, 1977). In contrast to what is seen in teleosts, the parapineal in the coelacanth appears as the more substantial organ within the pineal complex (Hafeez & Merhige, 1977).

*Amphibia*

The pineal complex of anurans is composed of a frontal organ and a pineal organ. The frontal organ is located under the skin between the lateral eyes and contains photoreceptors and other neurons that send axons into the frontal tract, which penetrates the skull to reach the intracranial pineal organ (reviewed in Van de Kamer, 1965). The extracranial situation of the frontal organ and its ability to generate responses to light has been interpreted by some authors as an indication of homology to the parietal eye of reptiles, and by consequence to the parapineal organ of other vertebrates (e.g. Ariëns Kappers, 1965; Jarvik, 1980; Tsuneki, 1987). However, embryological and connectivity data does not support this view and suggests that the frontal organ is instead a pineal derivative. Indeed, the frontal organ probably develops from a parietal vesicle located caudal to the pineal (Becarri, 1943, although see Ariëns Kappers, 1965; Tsuneki, 1987) and projects symmetrically to mesencephalic and diencephalic regions in a pattern similar to the pineal organ (Eldred et al. 1980).

*Reptilia*

In reptiles, a pineal organ together with a parietal eye in some species constitutes the pineal complex. Parietal eyes have been described in sphenodon (Rhynchocephala) (Dendy, 1911) and in species of lizards inhabiting higher temperate latitudes (Gundy et al. 1975; Quay, 1979). In contrast to the saccular or tubular intracranial pineal organ, the parietal eye emerges from a foramen in the skull and is connected to the diencephalon by a parietal nerve (Fig. 3C) (Gladstone & Wakeley, 1940). During embryogenesis, the parietal eye develops either as an independent evagination situated rostral to the pineal organ, or as a rostral component of the pineal evagination (reviewed in Ariëns Kappers, 1965). This ontogenetic origin together with its pattern of efferent connections suggests the parietal eye of lizards and the parapineal organ of lampreys and teleosts are homologous structures (e.g. Holmgren 1965; Oksche, 1965;

Engbretson et al. 1981; Yañez et al. 1996, 1999). The pineal organs of reptiles contain rudimentary photoreceptor cells with secretory features that appear to play a photoneuroendocrine role (Oksche & Hartwig, 1979; Tosini, 1997). The structure of the parietal eye, on the other hand, resembles that of the lateral eyes of other vertebrates. It contains a so-called cornea, a lens, a highly structured retina-like sensory epithelium with pigmented cells, highly developed photoreceptor cells capable of transducing photic information, and ganglion cells able to transmit this information to the brain (reviewed in Eakin, 1973; Quay, 1979; Engbretson, 1992). Axonal projections of the ganglion cells contribute to the parietal nerve, which innervates the pars dorsomedialis of the left medial habenula (Engbretson et al. 1981; Korf & Wagner, 1981) and other bilateral diencephalic targets (Fig. 3C') (Korf & Wagner, 1981). Electrophysiological studies have shown that the parietal eye is a fully functional photoreceptive structure showing chromatic responses to light stimuli (e.g. Solessio & Engbretson, 1993, 1999). It is thus possible that in lizards, and maybe also in lampreys and teleosts, information on the daily light oscillations reaches and asymmetrically modulates the activity of the habenulo-interpeduncular system.

#### EVOLUTIONARY CONSIDERATIONS ON EPITHALAMIC ASYMMETRY

##### *Distribution of neuroanatomical asymmetries*

*Parapineal organ.* The parapineal is a single organ, probably of midline origin, and asymmetry is detected in the pattern of its connectivity and in teleosts also in the position of the organ in relation to the dorsal midline. The peculiar distribution of the parapineal organ amongst the vertebrate taxa indicates either that the parapineal organ evolved or was lost several times independently during evolution. Comparative embryological and connective data suggests that, despite marked morphological differences, the parapineal organs of the different extant groups share several important features that suggest a common evolutionary origin. First, the parapineal organ is always accompanied by the presence of a more caudally situated pineal organ. Indeed, during embryogenesis, pineal and parapineal organs develop either as independent evaginations or as part of a common evagination of the roof of the diencephalon, the pineal always being located caudal to the parapineal (Oksche, 1965). Second, the presence of photosensory cells and other neurons, which in some cases have been shown to exhibit electric responses to photo-

stimulation, suggest a photoreceptive function for the parapineal organ. Finally, an asymmetric fibre projection from the parapineal organ towards the left habenula has been observed in all species examined. These observations suggest that the lack of parapineal organs in certain extant vertebrate groups is most likely due to regressive changes that occurred multiple times independently during evolution (Tsuneki, 1987; Yañez et al. 1999). However, developmental analyses have been limited to rather few studies, and it remains uncertain whether any vestige of a parapineal might be present in groups of animals within which no equivalent structure has been detected in the adult.

*Habenula—neuroanatomy of asymmetries.* Asymmetry of the habenula, in terms of size, neuronal organisation and probably connectivity and neurochemistry, is present in species representative of virtually all classes of vertebrates and thus appears to be plesiomorphic—primitive—to this group (Table). It is unclear, however, whether asymmetries are widespread among species within individual vertebrate groups as only a few representative species have been analysed, and indeed asymmetry may have been overlooked in many cases. Further research sampling more species and examining species without obvious asymmetries in greater detail will be required to clarify the extent and diversity of habenular asymmetries within the vertebrates.

Many aspects of the neuroanatomical asymmetries of the epithalamus have not been explored sufficiently to gain a clear evolutionary picture of this phenomenon. However, a few tendencies can still be depicted concerning the evolution of morphology, neuronal organisation, and connectivity of the habenula. A first observation is that, within a species, size asymmetries between the left and right sides of the habenula are often accompanied by asymmetries in neuronal organisation. However, the latter may also take place without a marked asymmetric expansion of habenular size, e.g. *Rana esculenta* (Braitenberg & Kemali, 1970) and *Lacerta sicula* (Kemali & Agrelli, 1972).

A second observation is that, between species, asymmetries in size and neuronal organisation follow different evolutionary courses. Whereas size asymmetries are conspicuous in fish (with the exception of teleosts), less evident in amphibians and reptiles, and rare in birds and mammals, asymmetries in neuronal organisation are poorly developed in most fishes, become sophisticated in amphibians and reptiles, and are again rare in birds and mammals. One may speculate that the presence of a marked size asymmetry of the habenula is a primitive character that has been lost in teleosts, amphibians and amniotes. If

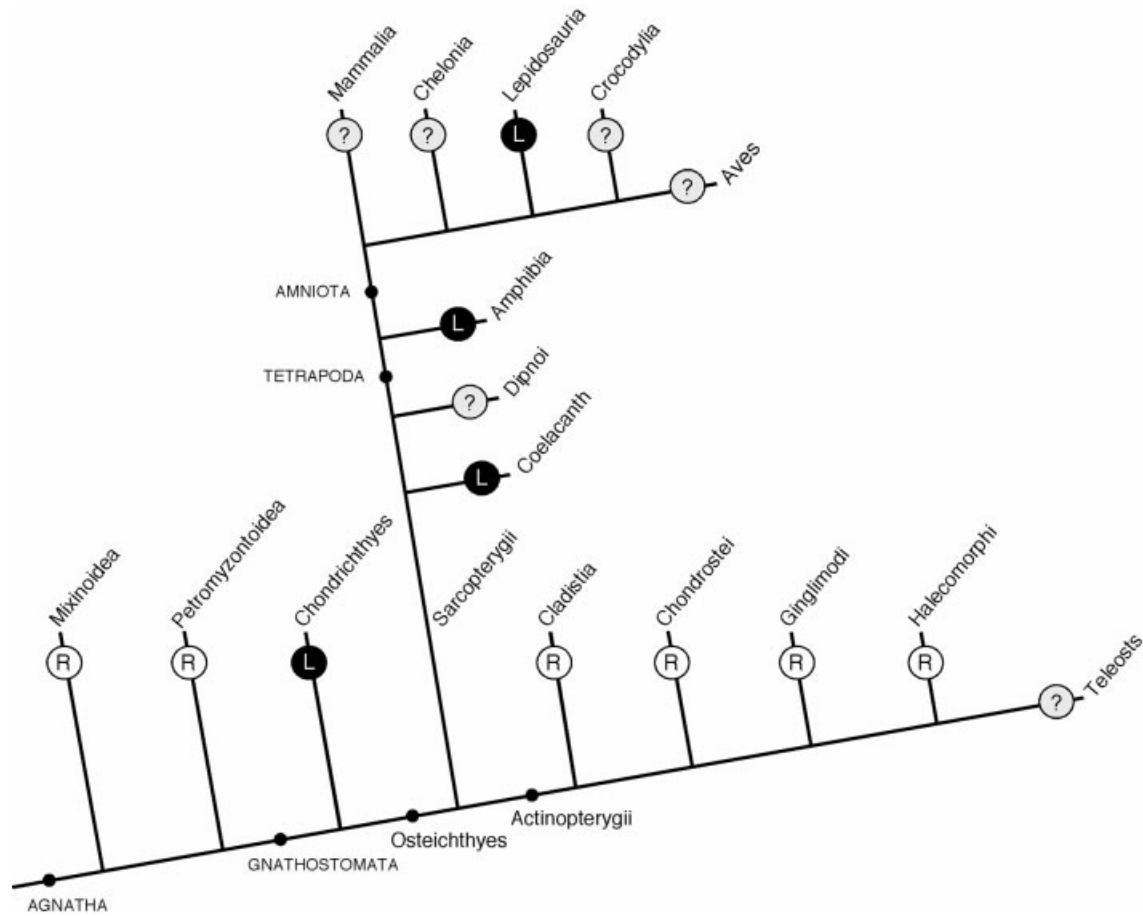


Fig. 4. Phylogenetic distribution of right versus left lateralities of asymmetry in the habenula of vertebrates. Laterality of habenular asymmetry was determined within each group by taking into consideration both size and neuronal complexity, and represented in a classic cladogram of vertebrate lineages (modified from Lauder & Liem, 1983; Walker & Liem, 1994; Zhu et al. 2001). L and R, left or right side of the habenula being larger and/or with a more complex arrangement of neurons than the contralateral side;?, lack of consistency in the laterality of habenular asymmetry within a group, or lack of sufficient studies addressing asymmetry beyond minor differences in size between the left and right components of the habenula.

asymmetry in size has a functional significance, then asymmetry in neuronal organisation may have taken over this function in frogs, lizards, and maybe also in teleosts. One correlation is that size asymmetries are more often conspicuous in species where the habenula is perhaps able to extend freely towards the tela choroidea (e.g. lampreys, hagfishes, elasmobranchs and brachiopterygian fishes) but is more subtle when habenular expansion may be compromised by neighbouring tissues, such as the optic tectum (e.g. teleosts) or cerebral hemispheres (birds and mammals).

*Habenula—laterality of asymmetries.* The laterality of habenular asymmetry is consistent within some vertebrate groups (e.g. right laterality: lampreys, hagfishes, and most non-teleost actinopterygian fishes; left laterality: chondrichthyes, amphibians and reptiles), but is variable within other groups e.g. teleosts (Table). By examining the phylogenetic distribution of right versus left laterality (Fig. 4), we

speculate that a right-directed asymmetry is plesiomorphic to agnathans and ganoids, and that there is a tendency in evolution to transform from right-directed to left-directed asymmetry in lineages leading to amphibians and reptiles. Marked asymmetries of the habenula appear then to be lost in birds and mammals. In the context of this hypothesis, the left directed asymmetry exhibited by chondrichthyes would be a derived (apomorphic) feature. The consistency of laterality within teleosts is uncertain as only a few species have been analysed, with most studies focussing upon size differences. As differences in size between the left and right habenula are only subtle in teleosts further studies examining neuronal complexity are required.

One further limitation of most current studies is that asymmetries have only been examined within a few individuals of most species. Thus it is usually not known how consistent the laterality is between

members of the same species. Given the availability of reagents that detect asymmetries in the epithalamus of very young animals (Concha et al. 2000), it should now be possible to perform population studies in animals for which fry/larvae are available in large numbers.

Behavioural asymmetries consistently biased to one side within a population have ecological advantages (Güntürkün et al. 2000; Rogers, 2000) but may impair the survival of the species, as they would help predators to predict prey behaviour (Corballis, 1998). This disadvantage could, however, be overcome in social species where individuals explore the environment in pairs or groups (Bisazza et al. 1999; Vallortigara et al. 1999). Indeed, as the laterality of habenular asymmetry is consistent within large populations of individuals within some species (e.g. Concha et al. 2000), asymmetry may not only play a role in individual behaviour but also in the behaviour of animal communities. In fact, in a few cases, it has been found that the direction of laterality of behaviour is consistent within populations of fish displaying social behaviours but randomised between members of non-social species (Vallortigara et al. 1999). Whether this behavioural asymmetry is also reflected at a neuro-anatomical level is yet to be determined.

*Coordination of habenular and parapineal asymmetries.* The parapineal organ, or parietal eye, projects asymmetrically to the left habenula in lampreys, teleosts and reptiles. A causal relationship between the presence of parapineal innervation and the development of habenular asymmetry has been proposed for reptiles (Engbretson et al. 1981). In species of lizards having a well developed parietal eye, a unique neuronal subdivision develops in the left medial habenula (e.g. *Uta stansburiana*: Engbretson et al. 1981). Comparable neuroanatomical asymmetries, however, are absent in species of lizards, such as the tegu lizard *Tupinambis nigropunctatus* (Cruce, 1974), lacking a parietal eye. Additionally, no evidence of habenular asymmetry has been observed in other groups of reptiles lacking either a parietal eye (turtles: ten Donkelaar, 1998b) or both a parietal eye and a pineal organ (alligator: Huber & Crosby, 1926). However, despite this correlation, a causal link between parietal nerve innervation and habenular asymmetry has not been determined.

In teleosts, the parapineal organ projects to a circumscribed region of the left habenula (Yañez et al. 1996) that displays immunoreactivity to serotonin (Ekström & Ebbesson, 1988), and is probably coincident with a region of enlarged neuropil (Concha et al. 2000). Studies of habenular asymmetry in species

lacking a parapineal organ or in situations in which this organ has been removed during embryogenesis are again lacking. However, in zebrafish fry that exhibit randomised CNS laterality, the parapineal and enlarged habenula are always located on the same side (Concha et al. 2000). This indicates that either the asymmetry of one structure is dependent upon that of the other or that a single laterality determining event coordinates habenular and parapineal asymmetry.

In lampreys the parapineal organ projects to the left habenula but counterintuitively it is the right and not the left habenula that is enlarged and displays a more complex arrangement of neurons. Indeed, as parapineal structures have not been described in a majority of vertebrates with epithalamic asymmetry, it is clear that for most species, habenular asymmetry can not be absolutely dependent upon parapineal innervation.

We propose that a projection from the parapineal organ in lizards and teleosts is likely to be involved in the elaboration of some aspects of asymmetry in neuronal organisation of the habenula but further experimental studies are required to directly address this issue. The situation in lampreys suggests that if the parapineal organ does play a role in the establishment of the laterality of habenular asymmetry, this may be overridden by other mechanisms. A mechanism to generate habenular asymmetry independent of parapineal innervation must also operate in vertebrate groups such as hagfishes, cartilaginous fishes, amphibian, birds and mammals apparently lacking a parapineal organ. An important implication is that several independent mechanisms may be operating in concert or competition to determine the final state of laterality and asymmetry in the habenula.

#### *The evolutionary origin of epithalamic asymmetry*

It remains to be determined when and how epithalamic asymmetries arose during evolution and the nature of the ontogenetic mechanisms that led to the development of asymmetry. One approach to elucidating these issues will be to examine extant protochordates to determine if epithalamic asymmetries are likely to have been present at the origin of the vertebrate lineage. Of course, one problem is to determine if protochordates have a region of the central nervous system (CNS) homologous to the epithalamus. In the cephalochordate, amphioxus, this does indeed seem to be the case as comparative neuroanatomical studies suggest that the major part of the anterior brain in amphioxus is homologous to the vertebrate diencephalon (Lacalli et al. 1994). In

particular, it has been proposed that the photoreceptive lamellar body may be homologous to the pineal complex of vertebrates (Lacalli et al. 1994; Ruiz & Anadón, 1991). This idea has gained support from a recent study suggesting that precursor cells of the lamellar body may express gene homologous to the epithalamically expressed vertebrate *floating head* (*flh*) gene (I. Masai & S. Wilson, unpublished observations). Although homology seems likely, to our knowledge there has not been any detailed analysis of asymmetry in this region of the amphioxus brain.

In contrast to amphioxus, there is documentation of asymmetric development in the sensory vesicle of tunicates (Lacalli & Holland, 1998; Lacalli, 2001). For instance, within the dorsal aspect of the sensory vesicle of the ascidian *Halocynthia*, cells derived from the left and right sides of the anterior neural plate give rise either to the photoreceptive ocellus melanocyte or alternatively to the otolith melanocyte. The identity as either ocellus or otolith depends upon cellular interactions that occur upon neural tube closure when cells from the left meet cells from the right at the dorsal midline (Nishida, 1992). Thus, there appears to be no correlation between the left/right origin of the melanocyte precursor and its final identity. Instead, at the dorsal midline, cells from the left and right intercalate and it is the anterior cell that forms the otolith and the posterior cell that forms the ocellus (Nishida, 1987; Nishida & Satoh, 1985, 1989). If one melanocyte precursor is ablated or if the precursors fail to interact at the midline, the remaining cell (or cells) develop as an ocellus (or ocelli) (Crowther & Whittaker, 1984; Nishida & Satoh, 1989). Thus both left and right precursors have equivalent potential to develop as ocelli, but interactions at the midline suppress ocellar fate and promote otolith identity in the anterior cell. As melanocyte fate decisions appear to be regulated by mechanisms that interpret position along the anterior to posterior axis, it is uncertain whether they are related to those more directly involving the left/right axis of vertebrates. A second issue that remains to be resolved is the degree to which the sensory vesicles/dorsal ganglia of tunicates are homologous to the diencephalon (or epithalamus) of vertebrates (Lacalli & Holland, 1998). Fortunately, some tunicate species are excellent model systems for gene function analysis (Sordino et al. 2001) and so ongoing studies addressing the conservation/divergence of gene function in the tunicate brain should help resolve these issues of homology.

Together, these studies are consistent with the possibility that an epithalamic region of the brain was

present in chordates prior to the origin of vertebrates. However, as yet, it is uncertain whether asymmetry is likely to have been present in this region of the CNS.

It has been proposed that epithalamic asymmetries between the left and right habenula may have been imposed during evolution by modifications of an initially symmetrical sensory apparatus (e.g. Braitenberg & Kemali, 1970). For instance, 2 paired and bilaterally symmetric photoreceptive organs (parietal eyes), each connected to the corresponding half of the epithalamus, may have rotated around each other to end up with either one caudal and one rostral or one dorsal and one ventral (Fig. 5*Ai*). If the rotation of the initially bilaterally symmetric parietal organs resulted in one being rostral and one caudal, then the rostral photoreceptive organ (parapineal) may have retained the connection with the left habenula. However, as the caudal photoreceptive organ (pineal) moved further away from the right habenula, it is conceivable that a break in the connection may have taken place (Fig. 5*Aii*), and that the pineal secondarily developed novel bilateral projections (Fig. 5*Aiii*).

Evidence in support of a bilateral origin of the pineal complex comes from analysis of fossil fish. Large pineal impressions are observed in the skull of fossil Ostracoderm fishes indicating that the pineal complex of ancestral/ancient vertebrates was more substantial than that of most living vertebrates (Gladstone & Wakeley, 1940; Edinger, 1955, 1956). Furthermore, the presence of paired pineal foramina (or single median but longitudinally divided foramina) and of single but nonmedial foramina in these fossil skulls supports the idea that the pineal complex was originally a paired organ (Gladstone & Wakeley, 1940; Edinger, 1956). Interestingly, the presence of asymmetry between the left and right foramina of some species (Fig. 5*C*) raises the possibility that asymmetry in the epithalamus was already present in the pineal complex of ancient vertebrates.

If indeed the parapineal and pineal were originally embryological unilateral components of the pineal complex (Fig. 5*A*) then one might expect that in extant vertebrates, the 2 components of the pineal complex would each have predominantly left sided or right sided origins during embryogenesis. Definitive fate-mapping studies have yet to be performed but gene expression analyses (Masai et al. 1997), and preliminary fate-mapping analyses (M. Concha & S. Wilson, unpublished observations) indicate a bilateral origin for the epiphyseal component of the pineal complex. Indeed, in embryos with defective neural tube closure, pineal organs develop on each side of the

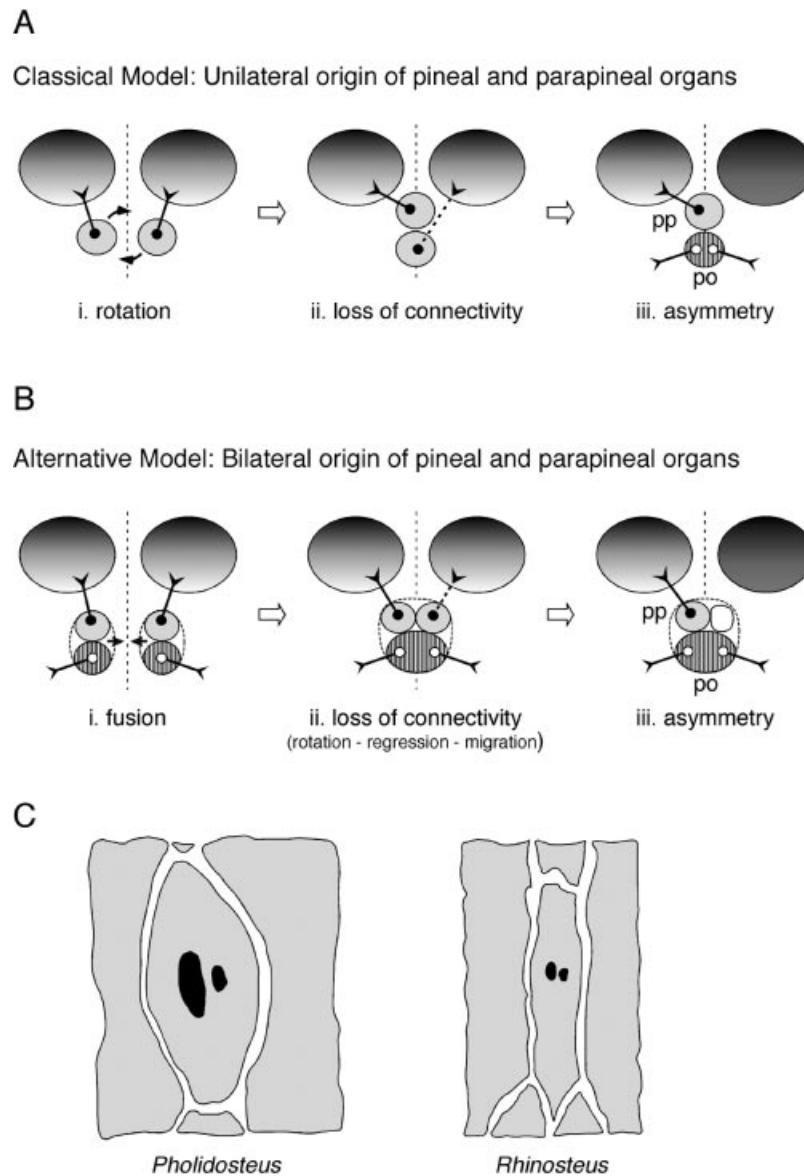


Fig. 5. Evolutionary origins of pineal complex components. (A) Classic model of parapineal asymmetry (after Braitenberg & Kemali, 1970). In this model, it is proposed that the pineal complex originally consisted of paired photoreceptive organs each projecting to the ipsilateral habenula (i). Through a rearrangement of the organs at the midline (ii), the right organ loses connectivity with the right habenula and gives rise to the pineal organ (iii). (B) Modified version of the model described in (A) that more easily accommodates a bilateral embryonic origin of cells contributing to the pineal organ. For further details see text. (C) Paired pineal complex foramina in the skulls of extinct Ostracoderm fishes. Schematic representations of fossils of Upper Devonian members of the placoderm order Arthrodira: *Pholidosteus* (A) and *Rhinosteus* (B). Adapted from Gladstone & Wakeley, 1940 (after Stensiö, 1934). Po, pineal organ; pp, parapineal organ.

epithalamus (M. Concha & S. Wilson, unpublished observations).

The model described above can be adapted in several ways to accommodate a bilateral origin of the pineal organ. For instance, the paired parietal organs may each have consisted of both pineal and parapineal components (Fig. 5B). During evolution, a fusion of these paired organs at the midline and subsequent separation along the rostro-caudal axis may have

given rise to separate pineal and parapineal organs (Fig. 5Bi). A second step would have involved a loss of connectivity of the right parapineal organ with the right habenula (Fig. 5Bii), perhaps involving a rotational mechanism analogous to that described for the classic model (Fig. 5Aii). The fate of the right parapineal cells may then have been to regress and die, to migrate caudally and incorporate into the pineal organ or migrate across the midline and contribute to

the left parapineal. Importantly, all of these hypotheses should be testable by performing fate map analyses to determine the origin and fate of parapineal precursor cells in extant species.

#### GENETICS AND THE DEVELOPMENT OF ASYMMETRY

Although considerable progress has been made in elucidating the genetic pathways that establish heart and visceral asymmetries, little is known concerning the establishment of neuroanatomical asymmetries in vertebrates (reviewed in Burdine & Schier, 2000; Capdevila et al. 2000). However some insights into the genetics of brain asymmetry have recently come from studies using the zebrafish (Bisgrove et al. 2000; Concha et al. 2000; Liang et al. 2000). The zebrafish offers several advantages over other systems for the study of neuroanatomical asymmetries. These include the availability of many mutant lines carrying mutations in genes known to play a role in the development of asymmetry (e.g. Bisgrove et al. 2000; Chen et al. 1997), the ability to score asymmetry defects in large populations of mutant animals, and the possibility of correlating neuroanatomical asymmetries (e.g. Concha et al. 2000) with behavioural asymmetries (Miklosi et al. 1998; Miklosi & Andrew, 1999). Recent studies have begun to elucidate the genetic mechanisms by which the laterality of CNS asymmetries are established (Concha et al. 2000). However, the issues of how asymmetry is first established in the embryo, whether this process is conserved between species and the nature of the mechanisms that are responsible for the development of the neuroanatomical asymmetry itself are still obscure.

To address how neuroanatomical asymmetries in the epithalamus develop we need to have a much better understanding of the development and genetics of the habenula and pineal complex. At present, we know little of the genetics of habenular development except for a list of genes expressed in this region, and a few studies of mutations that cause defects in habenular development (e.g. Xiang et al. 1996; Chen et al. 2000; Shanmugalingam et al. 2000). The genetics of development of the pineal complex is slightly better understood but studies to date have focussed on the pineal organ rather than the asymmetric parapineal organ. However, if pineal and parapineal organs share a common evolutionary origin, then an understanding of the development of the pineal organ may also shed light on the development of the parapineal organ.

Indeed, both pineal and parapineal organs express a common set of genes during early developmental stages (M. Concha & S. Wilson, unpublished results). In zebrafish, the pineal organ develops under the control of the homeobox-containing gene *flh* as evidenced by the absence of pineal neurons in mutants lacking Flh activity. Additionally, the extent of Flh activity can influence the size of the pineal (Masai et al. 1997) suggesting that evolutionary changes in Flh activity could contribute to the size and location of pineal complex components. Although the function of Flh orthologues have not been assessed in other species, related genes are expressed in the pineal organs of frogs (von Dassow et al. 1993) and chicks (Stein & Kessel, 1995).

#### *Genetics of laterality decisions*

Recent experimental data has indicated a role for the Nodal pathway in regulating laterality decisions in the habenula and parapineal organ (Concha et al. 2000) and in the positioning of the pineal stalk (Liang et al. 2000). In the larval zebrafish, asymmetries are characterised by the presence of an enlarged left habenula with increased neuropil (Fig. 6A) and a parapineal organ situated to the left side of the brain that projects to the left habenula (Fig. 6C) (Concha et al. 2000). Importantly, these 2 types of asymmetry are linked in such a way that the parapineal organ is always located on the side of the enlarged habenula. It is possible that both asymmetries depend on the same laterality determining event or alternatively, as discussed above, that the projection of the parapineal organ may regulate subsequent asymmetric habenular development.

Genes functioning in the Nodal pathway are expressed in the epithalamus, either bilaterally or asymmetrically on the left side, prior to the development of neuroanatomical asymmetries in the same region (e.g. Bisgrove et al. 2000; Concha et al. 2000; Liang et al. 2000). Those genes normally expressed on the left side of the epithalamus (e.g. *cyc* and *pitx2*) are either absent or expressed bilaterally in embryos carrying mutations that affect Nodal signalling (Fig. 6D) (Bisgrove et al. 2000; Concha et al. 2000; Liang et al. 2000). In both situations, neuroanatomical asymmetries are still established but their laterality is randomised (Fig. 6E, F), and situations in which a parapineal organ is either absent (right isomerism) or located on both sides of the brain (left isomerism) are never observed (Concha et al. 2000). This implies that Nodal signalling is not required for asymmetric development per se but is essential to



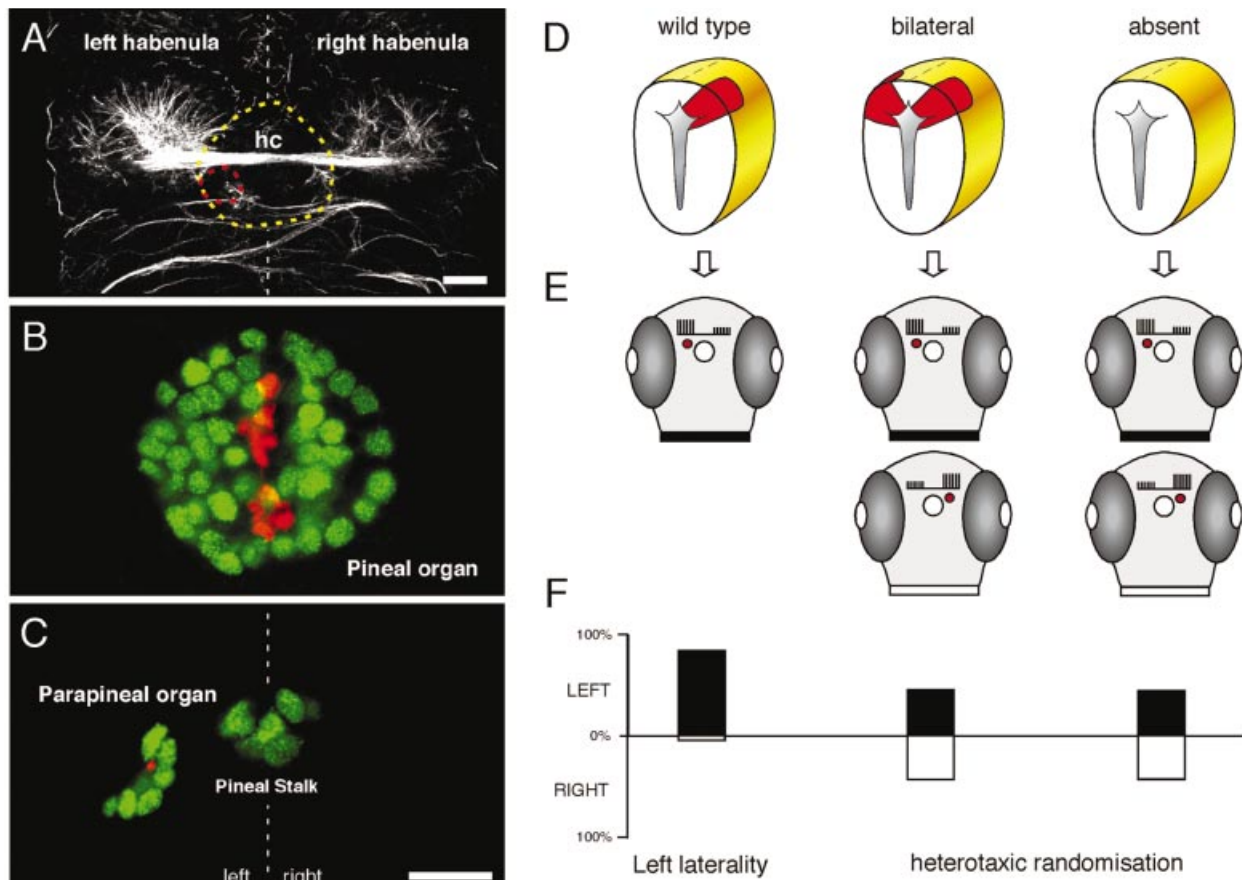


Fig. 6. Genetic analysis of epithalamic laterality in zebrafish (*A–C*) Confocal images of the habenular nuclei (*A*), neurons in the pineal organ (*B*) and neurons in the parapineal organ (*C*) of larval zebrafish. The dashed lines outline the positions (dorsal to this section) of the pineal and parapineal in (*A*). The left side of the habenula has more labelling of neuropil (*A*) and the parapineal is situated to the left of the midline (*C*). The pineal organ contains neurons symmetrically distributed on both left and right sides of the midline (*B*) (quantification of neurons on left and right sides of the pineal has not been analysed). The photoreceptive nature of both pineal and parapineal organs is indicated by opsin immunoreactivity (red) of some neurons. (*D*) Schematics of frontal views of the diencephalon in wild-type zebrafish embryos and in embryos with disrupted Nodal signalling. Expression of several Nodal pathway genes, such as the Nodal ligand *cyclops* and the downstream effector of Nodal signalling *pitx2* is restricted to the left side of the epithalamus in wildtype embryos. In embryos with disrupted Nodal signalling, expression of left-sided genes is either bilateral or is absent in the brain. (*E*) Schematics of dorsal views of the brains of wild-type embryos or embryos with altered expression of Nodal pathway genes in the brain. The pineal is represented by a large white circle, the parapineal by a small red circle and the enlarged habenula by vertical black lines. In the wild-type situation, both the parapineal and enlarged side of the habenula are on the left. In embryos in which left-sided nodal pathway genes are either expressed bilaterally or are absent, asymmetry still develops, but laterality is disrupted. In all cases, the parapineal is located on the same side of the brain as the enlarged habenula. (*F*) Distribution of epithalamic lateralities within populations of zebrafish fry. Within wildtype populations, over 95% show left sided epithalamic asymmetry (for both parapineal and habenula). Within fry that had either bilateral or absent expression of left-sided Nodal pathway genes, 50% show left sided epithalamic asymmetry and 50% show right sided epithalamic asymmetry. For further details on all panels, see Concha et al. (2000).

determine the laterality of the asymmetry. In other words, the consequence of the asymmetric activation of Nodal signalling is to bias to the left, an otherwise stochastic decision regarding the laterality of the CNS asymmetry. This genetic mechanism ensures that asymmetries are always localised on the same (left) side within the population. In fact, asymmetries of the habenula and parapineal organ are left biased in more than 95% of zebrafish (Fig. 6*F*) (Concha et al. 2000). It is possible that zebrafish, as a social species, benefit from having the asymmetry of the epithalamus

consistently biased to the left although functional studies are needed to confirm this hypothesis.

#### EPIGENETICS AND THE DEVELOPMENT OF ASYMMETRY—THE ROLE OF HORMONES

Epigenetic factors such as hormones are able to induce or modulate the development of structural asymmetries in the vertebrate brain. One of the most widely studied examples of this is the role of gonadal hormones in the establishment of sexually dimorphic

brain morphologies. Sex-dependent structural asymmetries have been found, for example, in cortical and hippocampal regions (Diamond et al. 1983; Van Eden et al. 1984; Lipp et al. 1984; Murphy, 1985; Wisniewski, 1998) and in specific nuclei such as the rat amygdaloid nucleus (Melone et al. 1984) and the chick and frog habenula (Gurusinghe & Ehrlich, 1985; Gurusinghe et al. 1986; Kemali et al. 1990). The medial habenula of chicks exhibits a sex-dependent asymmetry, as in males but not in females the habenula is enlarged on the right side (Gurusinghe & Ehrlich, 1985). An involvement of testosterone in this asymmetry has been demonstrated by the administration of this hormone to young animals. While testosterone has no effect on the asymmetry of males it induces asymmetry in females to favour the right side, as in males (Gurusinghe et al. 1986). Asymmetries of the dorsal habenula in frogs are present in both males and females, but are more pronounced in spring than in winter, especially in females (Kemali et al. 1990). Since frogs are sexually active in springs, it is likely that this sex- and seasonal-dependent asymmetry is a result of the influence of reproductive hormones (Kemali et al. 1990).

The mechanisms by which sex-dependent asymmetries are established in the habenula of frogs and chicks are unknown. One possibility is that asymmetries result from a difference in the rate of ontogenetic development between the left and right sides of the brain (Corballis & Morgan, 1978). Proliferation rates may be differentially susceptible to hormonal influences with a consequent differential growth between the left and right sides of the brain (Nordeen & Yahr, 1982).

#### CONCLUDING REMARKS

Although asymmetries are documented in the epithalamic region of the diencephalon in most groups of vertebrates that have been studied, surprisingly little is known concerning how these asymmetries arise or their role in modulating asymmetric behaviours. However, ongoing genetic and embryological studies in model species are likely to significantly enhance our understanding of the mechanisms that establish epithalamic asymmetry within the next few years. It remains an even greater challenge to dissect the function of both the symmetric and asymmetric components of the epithalamus. Once again, however, genetic and behavioural studies in animals in which epithalamic development is specifically compromised should help resolve the functional roles of this ancient and highly conserved region of the vertebrate brain.

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