Asymmetry of the Brain: Development and Implications

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Abstract

Although the left and right hemispheres of our brains develop with a high degree of symmetry at both the anatomical and functional levels, it has become clear that subtle structural differences exist between the two sides and that each is dominant in processing specific cognitive tasks. As the result of evolutionary conservation or convergence, lateralization of the brain is found in both vertebrates and invertebrates, suggesting that it provides significant fitness for animal life. This widespread feature of hemispheric specialization has allowed the emergence of model systems to study its development and, in some cases, to link anatomical asymmetries to brain function and behavior. Here, we present some of what is known about brain asymmetry in humans and model organisms as well as what is known about the impact of environmental and genetic factors on brain asymmetry development. We specifically highlight the progress made in understanding the development of epithalamic asymmetries in zebrafish and how this model provides an exciting opportunity to address brain asymmetry at different levels of complexity.

INTRODUCTION

Epithalamus: the most dorsal segment of the diencephalon in the posterior forebrain

Corpus callosum: main bundle of neural fibers that connects the left and right hemispheres

A fundamental property of physical and biological systems is their symmetrical organization. Symmetry, and especially bilateral symmetry, has generally been associated with harmony and perfection, but also with a form of boring rigidity. Instead, asymmetry is often associated with chaos, but at a subjective dose, its unpredictable nature makes it attractive (110). As such, throughout history, the search for balance between symmetry and asymmetry has inspired man widely in art and architecture, as illustrated by, for example, M.C. Escher's fascinating drawings (**http://www.mcescher.com/gallery/**). Although symmetry is ubiquitous in the wild, it is in reality frequently broken, leaving us surrounded by many illustrations of obvious morphological asymmetries: e.g., fiddler crabs claws, both eyes of flat fish on the same side of the head, and the asymmetric distribution of internal organs (125). However, asymmetry can be subtle, as is the case in the brain. Although high variability exists between individuals, the left and right hemispheres of our brain display anatomical and molecular left-right (LR) asymmetries that correlate with their functional specialization in particular cognitive processes. Studies over the past few decades have shown that brain asymmetry is not unique to humans but is rather a general trait of animal brains. Despite the widespread representation of brain lateralization across evolution, our knowledge of how anatomical asymmetries are established is still poor. In this review, we present, in a nonexhaustive way, some of the different approaches that have been developed in humans and in model organisms to better understand the development of brain asymmetries and their impact on brain function. This review focuses on what has been learned from studies of the establishment of LR asymmetries in the zebrafish epithalamus. In addition, we discuss some issues for which model organisms such as zebrafish will be helpful in the future.

FUNCTIONAL ASYMMETRIES OF THE HUMAN BRAIN AND NEUROANATOMICAL CORRELATES

Pioneering work from the middle of the nineteenth century by M. Dax and P. Broca, and, later, K. Wernicke, showed that damage in specific areas of the left brain is associated with problems in either producing or understanding language (22, 39, 104). A few decades after initial studies of aphasic patients, lesions in the right brain were shown to be associated with a decrease in emotional expression and inappropriate indifference (43). Injuries in the right brain have also been associated with a syndrome called left hemi-neglect, in which patients fail to pay attention to their left visual field (80, 161). Depending on the severity, patients appear oblivious to the left side of what they are looking at and are unable to draw the left side of objects properly. For example, when asked to describe from memory a particular place from one side (e.g., the Milan central square looking out from the cathedral), left hemi-neglect patients are unable to describe the left half of the scene. However, if they are asked to imagine the same place from the opposite side (facing the cathedral this time), they describe only the right side of the area that they neglected in their previous recollection (18). As left-sided damage almost never results in a reciprocal behavior (lack of attention for the right visual field), these observations suggested that the right brain plays a specific role in spatial attention that cannot be compensated for by the left.

Hemispheric specialization for other cognitive functions has been revealed by various studies, among which is the famous analysis of so-called split-brain patients, initiated by Gazanigga & Sperry (62, 63). The hemispheres of these patients have been disconnected by ablation of the corpus callosum, and as a result, the sensory information coming from lateralized stimuli presented to a given hemisphere is not passed to the contralateral hemisphere. Thus, these patients represented a unique opportunity to address the performance of each hemisphere in different tasks. Most

split-brain patients cannot name objects presented in their left visual field or touched by their left hand, but they have no problem doing so when objects are presented on the right; however, they are still able to acknowledge the presence of stimuli on the left by pointing to a matching object or by drawing it with their left hand, confirming that the right brain perceived the information (63). Subsequent studies using a similar strategy showed that the left hemisphere is dominant not only for language but also for problem solving, logical interpretations, and viewing details, whereas the right hemisphere is specialized for global viewing and visuospatial tasks such as solving puzzles and drawing geometrical figures (62).

Hemispheric specialization for a given cognitive function most likely reflects differences in the neural circuits of each hemisphere and correlates with asymmetry in gray and white matter of the left and right side of the brain. Two well-documented neuroanatomical asymmetries are found in the human cortex and overlap with regions involved in language: the planum temporale (PT), an area of the brain that lies just posterior to the auditory cortex in the temporal lobe, displays, in 65% of adult brains, a greater gray matter volume on the left side (65); the perisylvian fissure (PF), one of the most prominent fissures that divides the frontal and parietal lobes on each side of the brain, is significantly longer in the left hemisphere than in the right hemisphere (65). Another structural asymmetry has been described in frontal and occipital cortical protrusions: This asymmetry, called cerebral petalia, consists of a greater protrusion of the surface of one hemisphere beyond that of the opposite hemisphere, usually the extension of the right frontal lobe beyond the left on the anterior side of the brain and the left occipital lobe beyond the right on the posterior side; however, the functional relevance of petalia asymmetry remains unclear (97, 158).

The past decade has seen an explosion of noninvasive imaging approaches, such as PET (positron emission tomography), MRI (magnetic resonance imaging), and DTI (diffusion tensor imaging), that have allowed the visualization of the structure of gray matter, axonal tracts, and neuronal activities in the brains of living subjects (44, 77) [see **Figure 1*a–c*** for illustrations (7)]. With continuous innovations and progress regarding resolution, these imaging approaches now permit precise associative correlations to be established between structural and functional brain asymmetries. For instance, a large number of studies on healthy and aphasic patients led to the precise mapping of cortical areas and tracts that form the lateralized network involved in language (77, 132, 165). On the contralateral side, the dominance of the right hemisphere in visuospatial function has been associated with a volumetric asymmetry in the ventral superior longitudinal fasciculus (45). Finally, anatomical differences in the central sulcus (CS) have been linked to either experience-shaped or innate aspects of handedness. Indeed, whereas right-handers display a leftward asymmetry in CS surface area, this asymmetry is inverted in left-handers, but not in left-handers forced to use their right hand during childhood, illustrating the impact of motor experience on brain asymmetry (89). However, Sun et al. (155) reported structural differences in the shape of the CS between right-handers and both left-handers or "left to right switched" handers.

BRAIN ASYMMETRIES ACROSS EVOLUTION

Brain Lateralization Is Widespread in Animals

LR asymmetry is a general feature of animal brains, and a variety of functional and anatomical asymmetries have been described in both vertebrates and invertebrates. As in humans, for instance, all primates investigated so far show an asymmetry in their facial expression while communicating an emotion—i.e., a stronger emotion on the left side of their face (100)—suggesting that right hemisphere dominance for processing of emotions probably predates the human-monkey split. Meanwhile, the left hemisphere has been implicated in the generation or perception of

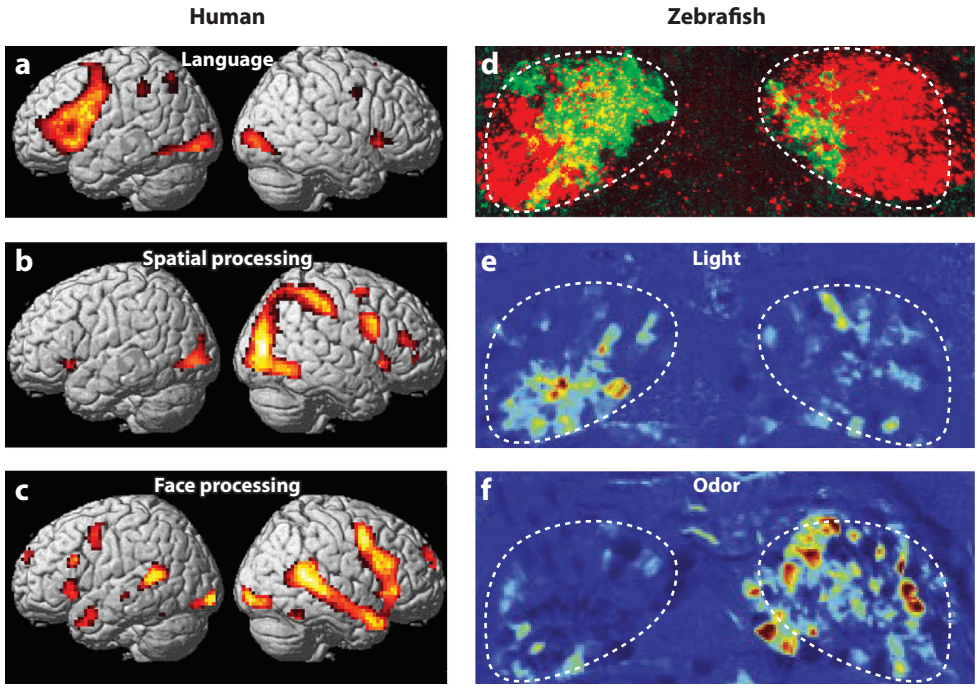

Figure 1

Illustrations of brain asymmetries in human and zebrafish. (*a*) Asymmetrical activation elicited for speech production, (*b*) spatial processing, (*c*) and face processing (recorded using fMRI). Activations are displayed laterally on cortical surface–rendered brains (reproduced from Reference 7). (*d*) Molecular asymmetry in zebrafish habenulae: asymmetric expression of *kctd8* (*red*, broader in the right habenula) and Tg[Et(gata2a:EGFP)^{pku588}] (*green*, broader in the left habenula) (81). Color-coded calcium signals showing the left-right lateralized response of dorsal habenular neurons to a nonlateralized presentation of (*e*) light and (*f*) odor stimuli (reproduced from Reference 49).

vocalizations in primates and mice, suggesting a conserved role of this hemisphere in communication (122). For instance, the ultrasonic calls that are emitted by young mice to evoke maternal caring behavior are preferentially recognized by the left hemisphere of their mother's brain (52).

Interestingly, in both mammals and insects, brain lateralization has been associated with learning and the retrieval of short- versus long-term memory. For instance, the vast majority of *Drosophila* that possess an asymmetric body (AB), a small nucleus inside the brain that is usually only found in the right hemisphere, display more efficient long-term memory than those with a bilateral AB (126). In honeybees, recall of short-term memory is favored if odors are presented to the right antenna, whereas the reverse is true for long-term memory (141). Finally, mice with impaired hippocampal asymmetry display decreased performances in spatial learning and working memory (67). Whether different lateralized pathways are also used in humans to recall short- or long-term memory has yet to be investigated. However, fMRI studies of individuals with unilateral hippocampal damage suggest that specific types of memory are lateralized, with verbal and nonverbal memory involving predominantly the left and right hippocampus, respectively (8, 115).

Hemispheric specialization for stimuli perception and motor responses has also been reported in many animals through the observation of widespread lateralized behaviors. Although the

directionality of these asymmetries is not always consistent at the population level, handedness/paw preference has been observed in primates, rodents, parrots, and toads (137). In vertebrates, lateralized behaviors are, however, mostly inherent to differential eye use. For instance, toads are more likely to attack conspecifics on their left side and prey on their right side (163). Similarly, Poeciliid fish display a consistent rightward turning bias in front of an opaque barrier, but a leftward bias when bypassing a simulated predator (16).

As shown in humans (55, 66), functional lateralization of the animal brain is further suggested by reports of asymmetrical distribution of neurotransmitters in rodents (9, 123) and zebrafish (42, 79). Asymmetries at the structural level have also been widely reported. An asymmetry in the PT has been observed in chimpanzees, macaques, and vervet monkeys, although in these last two species, sidedness is not consistent at the population level (103). Rodent brains are also asymmetrical at various structural levels, although here again orientation of these asymmetries is subject to variation depending on age, gender, species, and environmental factors (24, 46, 84, 150). One of the best documented conserved neuroanatomical asymmetries is found in the epithalamus of many phyla, including reptiles, frogs, and fish (35), and the zebrafish has emerged as a model to address the development of this asymmetry (see Genetics of Zebrafish Epithalamus Development below).

Advantage of Functional Lateralization of the Brain

The fact that brain lateralization has been either conserved or independently acquired during evolution suggests it somehow provides an advantage and, thus, its loss is detrimental. Furthermore, variation in the development of brain asymmetry is suspected to contribute to various neuropathologies in humans. Indeed, disorders such as autism and dyslexia are associated with atypical patterns of functional and structural asymmetries (21, 76). The prevalence of left- or mixed-handedness is strikingly increased from approximately 10% in the general population to between 20% (48) and 40% (166) in schizophrenic patients.

Above all, brain lateralization is assumed to provide cognitive advantages. This is supported by quantitative functional MRI studies that correlate the strength of functional lateralization with the level of cognitive ability in humans (68). Brain lateralization might provide benefits by allowing the hemispheres to perform parallel tasks (142). Strong evidence for this idea comes from studies in model organisms in which the development of laterality can be manipulated. For instance, light-induced visual lateralization is known to improve the performance of chicks in dual tasks, i.e., discriminating between seeds and pebbles while monitoring a model predator (136a). Similarly, lateralized fish perform better than nonlateralized fish when they are tested for their ability to capture shrimp in the presence of a predator (37a). Functional lateralization could also be a result of space constraint in the brain. In mouse, the bias in front-paw use is inversely correlated with the side of the face displaying supernumerary whiskers, and this has been suggested to result from competition for cortical space between the size of motor and somato-sensory areas (10).

Hemispheric specialization underlies LR asymmetry in perceiving and responding to stimuli, but these biases in lateralized behavior can present disadvantages when stimuli are present on both sides of the animal environment (38). Thus, functional lateralization must provide enough advantages for animal life to compensate the associated ecological cost (reviewed in 31, 138, 162).

Despite the broad representation of brain asymmetries across the animal kingdom, little is known about how anatomical asymmetries are established during development. In the next section, we present some environmental factors and genetic determinants described to influence the development of brain asymmetry in human and other vertebrate models and, specifically, what we have learned from studying the development of epithalamic asymmetries in the zebrafish model.

DEVELOPMENT OF BRAIN ASYMMETRY

Environment/Physiological Factors

Habenulae: a pair of conserved nuclei in the epithalamus of vertebrate forebrain

It is largely assumed that the development of language lateralization and handedness in humans, for instance, has genetic components (see Genetic Determinants of Brain Asymmetries in Humans) (37, 121). Both, however, are very plastic, as they can be easily reversed in early life: early lesions in the left hemisphere can trigger right dominance for language or at least an increase in the implication of the right hemisphere, and cultural pressure has clearly been applied to push left-handers to right-handedness. Thus, although genetic factors are involved in the establishment of brain laterality, these probably trigger only a weak/modest hemispheric dominance that is then reinforced by environmental and/or physiological factors in pre- and postnatal life (reviewed in 29, 54).

Light. The best characterized and most dramatic illustration of the impact of environment on brain lateralization is that of light stimulation on the asymmetric development of the visual pathway in chickens (135, 140) and pigeons (105). In the final days of development, avian embryos are turned in the egg so that only the right eye can be stimulated by light passing through the shell. Chicks hatching from eggs with their left eye occluded preferentially use their right eye/left hemisphere to distinguish grains from pebbles, whereas those manipulated so their right eye is occluded showed reverse asymmetries in this same test; chicks incubated in the dark for the last three days before hatching show no asymmetry in left or right hemisphere use (136).

An effect of light on brain lateralization has also been proposed in zebrafish. Indeed, Budaev & Andrew (23) showed that eye preference for approaching or avoiding a predator depends on early exposure of embryos to light; the authors also suggested that light could influence asymmetric development of the habenulae, a pair of conserved nuclei in the forebrain of vertebrates (see Genetics of Zebrafish Epithalamus Development), rather than the visual pathway. However, absence of light was shown to delay the differentiation of habenular neurons rather than eliminate asymmetric expression of molecular markers (41). Likewise, Dreosti et al. (49) failed to detect an effect of dark incubation on functional lateralization of the habenulae for visual and olfactory stimuli (see Discussion and Future Directions). Thus, how light exposure affects lateralized behavior in zebrafish remains unclear but its influence on habenular asymmetry is probably subtle at best.

Posture. If light is necessary to set up asymmetry in the chick visual pathway, the directionality of this asymmetry is given by the almost invariant asymmetric posture of the embryo in the egg. The factors that determine chick embryo posture are unknown, but it is interesting that asymmetrical posture of the developing fetus has also been proposed as a determinant that could influence or reinforce early brain lateralization in humans (reviewed in 54, 131). During the final trimester, most fetuses are in a head-down orientation on the mother's left side, a preferred position due possibly to physical constraints imposed by the mother's visceral asymmetry. This posture may favor the movement of the right arm by providing more space on the right side and/or the turning of their head toward the right to face the direction of any light that might be able to penetrate the mother's abdomen, thus contributing to motor lateralization. It may also influence language lateralization by triggering a greater stimulation of the right ear, which is closer to the mother's body wall. More studies of both language lateralization and handedness in cephalic and breech infants are needed to better understand whether posture is indeed influencing or reinforcing brain asymmetry in humans.

Hormones. One suggested environmental effect, although controversial, is the season of conception (64). Although not confirmed by other studies (85, 114, 159), independent reports in humans found significantly more left-handers among the males born in winter (152, 160). One

hypothesis is that seasonal variation in the mother's hormones, such as high levels of androgens during spring, could influence fetal development and contribute, together with genetic factors, to induce a left shift. This hypothesis is interesting given the genetic association found between handedness and the androgen receptor presented below (112). An influence of sex hormones on the development of brain lateralization also explains gender differences observed in human cerebral structural asymmetries (44) and in handedness (128, 133), and gender variation in lateralized behaviors reported in animals such as fish (16). It is noteworthy that seasonal changes in habenular structural asymmetry have also been reported in frog (86), suggesting a conserved role for hormones in modulating brain structure and asymmetries.

As described below, the establishment of zebrafish epithalamic asymmetries depends on genetic factors, but environment could also play subtle roles in decreasing/reinforcing these asymmetries. The zebrafish certainly provides an appropriate model to study the impact of environment and experience on interindividual variability of brain asymmetries.

Genetic Determinants of Brain Asymmetries in Humans

In humans, handedness and asymmetry in language processing seem to be established early in development. Fetuses move their right arms and suck their right thumbs more often than their left as early as 15 weeks, a preference that correlates with handedness later (75). Similarly, various studies suggest that language perception and production are lateralized in young infants before the development of true language (reviewed in 54). This early onset of hemispheric dominance for handedness and language skills and the significant degree of heritability for handedness suggest that their development involves genetic factors.

Genetics of handedness. Until recently, the available data on the genetics of handedness fit with models involving a single gene with two alleles (a dominant allele favoring right-handedness and a recessive one favoring random choice) (6, 109). However, intensive research efforts over the past decades have failed to identify a unique handedness gene and have rather suggested that handedness is a multigenic trait (111a). Linkage analysis and more recent genome-wide association studies (GWASs) have highlighted various gene loci with modest association to handedness. One of these genes is *LRRTM1* (leucine-rich repeat transmembrane neuronal 1), for which minor alleles were found associated with handedness in families of schizophrenic patients but not in healthy controls (57). Another interesting candidate coming from GWASs on dyslexic patients is PCSK6 (preprotein convertase subtilisin/kexin 6) (148). PCSK6 displays a specific SNP (single nucleotide polymorphism) associated with handedness in dyslexic patients and a second SNP associated with the degree rather than the orientation of handedness in healthy individuals. The association of genetic variation in PCSK6 with handedness is intriguing, as this gene encodes an enzyme involved in the maturation of Nodal/Tgfβ factors, a family of cytokines with a well-described role in setting up body asymmetries (see A Link Between Body and Brain Laterality); furthermore, PCSK6 knockout mice display visceral heterotaxia (36). Finally, Medland et al. (112) showed an association between handedness and the number of polymorphic polyglutamine CAG repeats in the androgen receptor (located on the X chromosome). This candidate gene is interesting given the association between handedness and gender, with males tending to be more represented among left-handers (reviewed in 133). Moreover, structural asymmetries in the PT area and the occipital cortex are more pronounced in XY males than in XX females or XXY males, further suggesting that the development of cerebral structural asymmetries is influenced by testosterone levels (147). These genetic associations need to be further confirmed in independent studies with larger samples of individuals. The rather modest incidence of each of these allelic variations on handedness and the difficulty to confirm results in others cohorts therefore support a multigenic model of handedness and argue for an additive contribution of environmental factors.

Genetics of language. A few candidate genes have been associated with language development. Among these, the most exciting is the *FOXP2* gene that encodes the forkhead-box P2 transcription factor (FOXP2). *FOXP2* carries a specific series of SNPs in members of a family that display severe speech impairments (94). Interestingly, healthy people carrying some specific SNPs in the *FOXP2* gene tend to exhibit reduced lateralization in a dichotic listening task, a test that reflects language lateralization (120). Different SNPs in *FOXP2* have also been associated with interindividual variability in the activation of brain areas while reading (130). Nonetheless, the function of FOXP2 factor in language development remains unclear. Studies in the zebra finch suggest a postnatal function for FoxP2 in promoting neuronal plasticity. In this songbird, *FoxP2* expression increases during song learning in area X, a structure necessary for this task (72). Furthermore, lentiviral-mediated RNAi loss of *FoxP2* function results in inaccurate vocalization (71). This result is interesting given that vocalization in songbirds is known to be lateralized (122). However, studies in mice rather suggest a prenatal role for FOXP2 in brain development and synaptic plasticity (70). *FoxP2* mutant pups have also been reported to display impaired vocalization (149), but recent studies suggest that this phenotype might be an indirect consequence of developmental delay associated with the loss of FOXP2 activity (61). Thus, future studies are needed to understand whether FOXP2 is required for symmetric development of brain regions and the circuitry involved in language or whether it is specifically involved in the establishment/plasticity of the asymmetric circuitry required for language processing.

So far, no genetic factors have been described to be associated with both language lateralization and handedness, although their inheritance is partially linked with 95% of right-handers having left dominance for language and only 75% of left-handers doing so; the remaining right-handed and most left-handed subjects usually show symmetric language processing, and a few left-handers (approximately 6%) display a right dominance for language (90). This link has been moderated in a recent study (108), supporting further that language lateralization and handedness probably involve mostly independent mechanisms and gene loci. However, they might be expected to share a few asymmetric determinants, and GWAS analysis on rare atypical subjects that display left-handedness and right hemispheric dominance for language might help to identify genetic variants involved in the covariance of handedness and language lateralization.

Transcriptomic approach. The mechanisms that break symmetry in the brain must at some level involve the asymmetric expression of genes involved in neural development (154). To gain insights into the molecular basis of brain lateralization, Sun et al. (153) compared gene expression levels in the perisylvian region of left and right human fetal cortex. They found 27 genes expressed differentially between the two hemispheres at 12 weeks of gestation that tended to become more symmetric later. One gene consistently expressed at higher levels in the right perisylvian fissure encodes LMO4, a transcription factor known to be essential for cortical development in mice (153). Interestingly, *Lmo4* is expressed asymmetrically in the adult mouse brain (although without fixed directionality). If confirmed, these results suggest that early transcriptional asymmetry can prefigure human cortical asymmetry. It will be interesting to assess whether SNP variation in these genes correlates with the degree of asymmetries in the perisylvian fissure in humans and to address further the functions of these genes in model systems.

The need for model systems. The coupling of genome-wide analysis and neuroimaging in humans has emerged as a powerful method for highlighting correlations between allelic variations in genes and structural and functional asymmetry in the brain. However, it is difficult to assess the extent of a gene's contribution to a particular brain asymmetry in humans. To gain insights into the development and functional relevance of brain asymmetries, the field has turned to model systems

in which these issues can be addressed at different levels of complexity: from gene function to cell identity, neuronal circuitry, and cognitive ability. In this regard, the zebrafish has proven well-suited, as it displays both neuroanatomical and behavioral asymmetries and is amenable to forward genetic screens and in vivo imaging of neuronal networks. The zebrafish is not the only model system being used in asymmetry studies; the nematode *Caenorhabditis elegans* has also provided important insights into this issue (78, 156). Here, however, we focus only on recent advances made in understanding the development of the zebrafish epithalamus.

Genetics of Zebrafish Epithalamus Development

The epithalamus, the most dorsal part of the diencephalon, is composed of the pineal complex and a bilateral pair of nuclei, known as the habenulae, both of which display prominent asymmetries in many vertebrates, including zebrafish (see **Figure 1*d*** and **Figure 2**; for review, see 12, 31, 73, 144). The habenulae are part of a conserved conduction system connecting the forebrain to midbrain nuclei (14). They have been implicated in various behaviors such as experience-dependent fear responses in zebrafish (1, 96) or behavior guided by error predictions and reward in primates and humans (95, 107), and have been associated with nicotine addiction (56, 146). The pineal complex consists of two photoreceptive structures: the epiphysis or pineal gland (Pi) located on the midline and the parapineal (pp), a nucleus on the left side of the midline (**Figure 2**). Although the epiphysis is known to be involved in the conserved regulation of circadian rhythms in many animals, the pp nucleus is found only in some species of vertebrates and its physiological function remains unclear. Over the past 15 years, embryological and genetic manipulations in zebrafish have allowed considerable progress to be made in understanding the developmental pathways that control the asymmetric morphogenesis of the epithalamus and, in an independent way, the direction of this asymmetry (laterality); these results are described in further detail below and summarized in **Table 1** (a modified and updated version of the table presented in Reference 34).

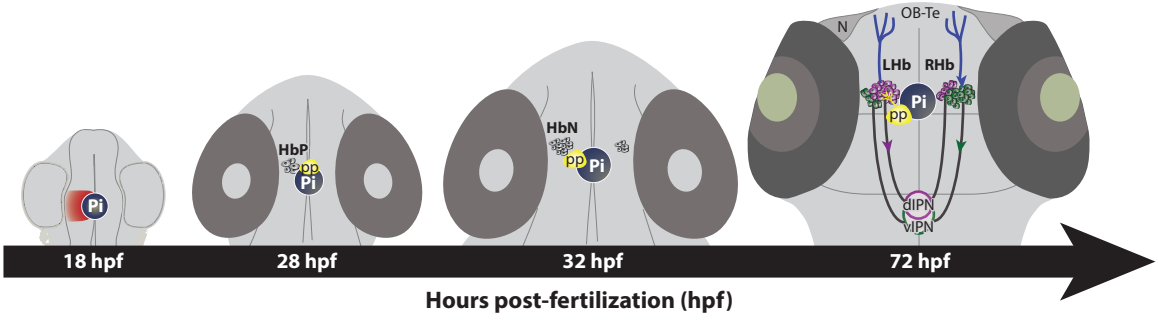

Figure 2

Schematics describing the development of left-right asymmetry in the zebrafish epithalamus. The *nodal*-related gene *ndr2/Cyclops* is expressed on the left side of the epithalamus at 18 hpf (hours post-fertilization) (*red*). At 28 hpf, the parapineal (pp) is specified from the anterior part of the pineal gland (Pi) and starts migrating leftward. Dorsal habenular progenitors (HbP) are born earlier in the left habenula (Hb) than in the right and are detected from 28 hpf; this early asymmetry in the timing of habenular neurogenesis results in an asymmetric pool of habenular neurons (HbN), detected from 32 hpf. By three days post-fertilization, asymmetries of the epithalamus are fully developed: Dorsal habenular neurons have acquired a specific identity for the lateral subnucleus (dHbl; *purple*) or the medial subnucleus (dHbm; *green*) that are thought to project predominantly to the dorsal or ventral part of the interpeduncular nucleus (dIPN or vIPN, respectively). The left (LHb) and right (RHb) dorsal habenula receive axonal afferents from the pp and the olfactory bulb (OB), respectively. See **Table 1** for more information. Abbreviations: N, nose; Te, telencephalon.

Table 1 Summary of genetic contexts or embryonic manipulation and the associated phenotypes in the zebrafish epithalamus

Genetic or experimental context	Mutant allele/morpholino (MO)/chemical	*cyc/pitx2c* expression (18 hpf)	Early Hb neurogenesis (28–32 hpf)	pp position (32–72 hpf)	Habenular identity (72 hpf)			References
					Marker	Neuropil	Projections	
Wild type		L	Asym	L	L-R	L-R	L>dIPN; R>vIPN	2, 32, 58, 59, 99, 143
Laterality defects	**Nodal absent**							
oep/Cripto/Tdgf1	Rescued[c] *oep*^{tm134,tz57}	Absent	nd	Rand	Rand	Rand	Rand	2, 32, 58, 59, 99
southpaw/Ndr3	MO-*ndr3*	Absent	Sym	Rand	Rand	Rand	Rand	58, 102, 143
cyclops/Ndr2	*cyc*^{m294}	Bilateral	nd	nd	nd	nd	nd	32, 99
Chemical inhibition	SB431542 (16 hpf)	Absent	Sym	Rand	nd	nd	nd	143
	Nodal bilateral							
Midline ablation	–	Bilateral	nd	nd	nd	nd	nd	32
no tail/T/Brachyury	*ntl*^{b160}, MO-*ntl*	Bilateral	Sym	Rand	Rand	Rand	Rand	2, 32, 58, 98
casanova/Sox32	*cas*^{ta56}	Bilateral	nd	Rand	Rand	nd	–	32, 58, 98
floating head/Not	*flh*^{n1}	Bilateral	nd	Rand	Rand	nd	nd	59
Asymmetry defects	**pp defect**							
pp laser ablation		L	Asym	No pp	R-R	R-R	Both Hb->vIPN	2, 33, 58, 59, 143
fgf8 (hypomorph)	*ace*^{ti282}	L	nd	Midline	Red[a]	Red[a]	nd	134
fgf8 (null)	*fgf8a*^{x15}	nd	nd	No pp	nd	nd	nd	28
from beyond/Tbx2b	*fby*^{c144}	L	nd	No pp	R-R	R-R	Both Hb->vIPN	151
	Notch pathway							
midbomb/E3 Ubi. Lig.1	*mib*^{ta52b} (Notch LOF)	nd	nd	nd	L-L	nd	nd	3
UAS:NICDxHS:Gal4	Notch GOF	nd	nd	nd	R-R	nd	nd	3
	WNT pathway							
Axin1 (Wnt GOF)	*mbl*^{tm213}	Bilateral, L	nd	L (delayed)	R-R	R-R	Both Hb->vIPN	26

tcf7l2 (Wnt LOF)	*tcf7l2*$^{ex1/u763/u754}$	L	Asym	L	L-L	L-L	Both Hb->dIPN	82
Chemical inhibition (Wnt LOF)	IWRendo (34–36 h)	nd	nd	nd	L-L/R	nd	nd	82
	Others							
pitx2c	MO-*pitx2c*	L	nd	L (enlarged)	L-L/R	L-L	nd	47, 60
sec61al1	*sec61al1*^{c163}	L	nd	L	L-L	nd	Both Hb->vIPN	47
daam1a	MO-*daam1*	Rand[b]	nd	Rand[b]	Rand/Red[a]	Rand/Red[a]	Rand/Red[a]	30
Unc51-like kinase 2	MO-*ulk2*	nd	nd	nd	nd	Red[a]	nd	157
leftover/Kctd12.1	*ktcd12.1*^{vu442}	nd	nd	nd	nd	Expanded[a]	nd	157
right on/Kctd12.2	*ktcd12.2*^{fh312}	nd	nd	nd	nd	Expanded[a]	nd	157
neuropilin 1a	MO-*nrp1*	nd	nd	L	L-R	L-R	Both Hb->vIPN	92
semaphorin 3D	MO-*sema3d*	nd	nd	L	L-R	L-R	Both Hb->vIPN	92

[a]Habenular markers and/or neuropils are reduced or expanded in both Hb.

[b]Data not shown in the referenced study.

[c]Rescued *oep*: Zebrafish embryos mutant for the *oep/Cripto/Tdgf1* gene in which the early developmental defects have been rescued by injection, at one cell stage, of the *oep* mRNA encoding the corresponding Oep protein; the phenotypes described are due to LOF of the *oep/Cripto/Tdgf1* gene at late stage, when the gene is expressed in the epithalamus.

Abbreviations: Asym, asymmetric; Both Hb->vIPN/dIPN, both habenulae innervate the ventral or dorsal IPN; GOF, gain of function; Hb, habenulae; IPN, interpeduncular nucleus; L, left; L>dINP, left habanula innervates predominantly the dorsal IPN; L-L, both habenulae display a left identity; L-L/R, the rHb displays a mixed identity as it expresses markers for both lateral and medial Hb nuclei; LOF, loss of function; L-R, wild-type pattern for the left and right habenula; MO, morpholino (antisense oligonucleotide that blocks translation or splicing of a target mRNA and results in a partial or full loss of function of the corresponding gene); nd, not determined; pp, parapineal; R, right; Rand, randomized laterality; Red, reduced; R-R, both habenulae display right identity; R>vIPN, rHb innervates the ventral IPN; Sym, symmetric.

Development of asymmetry in the pineal complex. In zebrafish, the pp delaminates from the anterior region of the pineal territory, migrates leftward, and projects axons to the left habenula (**Figure 2**). Both the transcription factor Tbx2 and the secreted factor Fgf8 are required for pp specification (28, 151); Fgf8 is additionally needed for pp migration, as in embryos carrying a hypomorphic mutant allele of *fgf8* gene, pp cells are specified but often fail to leave the dorsal midline (134).

Although Fgf8 appears essential for pp migration, the leftward orientation of migration depends on the TGFβ signaling molecule Nodal-related 2 (Ndr2/Cyclops). Ndr2/Cyclops is expressed in the left epithalamus approximately eight hours before the pp starts migrating (17, 32, 99); at this stage, Ndr2/Cyclops is required for the expression of a feedback inhibitor of the Nodal pathway, Lefty1, and the paired-type homeobox transcription factor Pitx2c. However, Nodal activity only biases the orientation of pp migration as, in situations of absent or bilateral Nodal signaling in the epithalamus, the pp still migrates but randomly either to the left or the right side (32, 33, 59). Interestingly, recent work indicates that a balance of FGF signaling at mid-somitogenesis is also required for normal formation of the midline in the brain and the expression of Ndr2/Cyclops on the left (119). Although the ligand involved in this process might not be Fgf8, it suggests that Fgf signaling has reiterative functions during the establishment of brain asymmetry.

Development of habenular asymmetry. The fact that the orientation of pp migration is randomized in contexts of absent or bilateral Cyclops expression suggests that the Nodal pathway is a determinant of left orientation rather than a symmetry-breaking factor. However, several years ago, we showed that Nodal signaling promotes asymmetry in the appearance of early habenular neurons (**Figure 2**) (143). The functional relevance of this subtle asymmetry in the pool of early born habenula neurons remains unclear. How the Nodal pathway imposes asymmetry in the timing of habenular neurogenesis is also not yet known. It is possible that both the roles for the Nodal pathway in biasing the orientation of pp migration and driving the asymmetry in early habenular neurogenesis involve a modulation of Fgf8 function. Indeed, Fgf8 is required for habenular neurogenesis, as mutations in the gene result in reduced habenulae in both zebrafish and mouse (106, 134). This role of Fgf8 might involve its ability to control the expression of the Dbx1b transcription factor, specifically in progenitors of the dorsal habenular (40). Another putative target of the Nodal pathway could be the Hedgehog pathway, which has also been implicated in development of the epithalamus in mouse and zebrafish (27, 72a).

At later stages, the zebrafish habenulae are composed of a dorsal, LR-asymmetric domain and a ventral, symmetric nucleus, which are globally homologous to the medial and lateral habenular subnuclei in mammals, respectively (4). Within the dorsal habenula (dHb), two main subnuclei have been defined that display LR differences in molecular markers and innervation profiles: a medial subdivision [dHbm (green nuclei in **Figure 2**), originally defined by the expression of the *Tg*(*brn3a:GFP*) transgene] is bigger in the right habenula (RHb), projects predominantly to the ventral region of the interpeduncular nucleus (IPN), and contains cholinergic and glutamatergic neurons; a lateral subdivision [dHbl (purple nuclei in **Figure 2**)] expresses the *kctd12.1* [(potassium channel tetramerization domain 12.1)/*left-over* gene] is larger on the left side, predominantly innervates the dorsal part of the IPN, and contains mostly glutamatergic neurons (2, 13, 58, 79). However, this classification is oversimplified, as further analyses of new habenular markers indicate that the dHb is actually made of more than these two subdomains (42).

Dorsal habenular precursors in the zebrafish adopt lateral or medial character at frequencies that vary between the left and right sides. How do these precursors choose between these two specification programs? Pioneering experiments have shown that pp cell ablation prior to migration leads habenular neurons to adopt a predominantly right character (33, 59). It has also been shown

that neurons with a left character are born before those with right character, a heterochronic process that could be regulated by Notch signaling (3). However, the molecular mechanisms underlying the timing of neuronal birth, its impact on habenular specification, and its link with pp function are not clear.

Studies from the Wilson and, more recently, Carl labs have revealed that the acquisition of dHbl or dHbm fate requires the precise control of Wnt signaling (26, 81, 82) (**Table 1**). Indeed, genetic contexts that result in increased Wnt signaling (*axin1* mutant) lead to a double right habenular phenotype (26). Conversely, mutations in the *Tcf7l2* gene, which encodes a transcription factor that mediates Wnt signaling, result in the majority of right habenular neurons acquiring left dHbl identity in a pp-independent manner (82) (**Table 1**). *Tcf7l2* is expressed bilaterally in nascent habenular neurons and is cell-autonomously required for the acquisition of the dHbm character. While this happens on the right side, on the left the pp is likely to suppress Wnt activity in the environment into which the neuron is born; consequently, the precursor becomes a dHbl neuron on the left.

How and when the pp modulates Wnt signaling in the left habenula is still unknown. However, this function seems to be dependent on a tight control of pp size. Indeed, in a manner somewhat similar to the loss of *Tcf7l2* function, the abrogation of Pitx2c activity leads to the right habenula adopting aspects of left character (60). In the latter context, however, there is a concomitant increase in pp cell numbers. Reducing the number of pp cells to wild-type levels in Pitx2c loss-of-function embryos, through partial laser ablation of pp precursors, restores habenular asymmetry. Thus, restricting pp cell number is crucial for the correct elaboration of epithalamic asymmetry, perhaps by limiting the extent to which the pp can modulate the Wnt pathway. An important future step will be to understand the molecular basis of this pp-habenular interaction.

Asymmetry in axonal and dendritic connectivity. The right and left dorsal habenula also present asymmetries in connectivity, with the axons exiting the medial and lateral subdivisions predominantly innervating the ventral or dorsal part of the IPN, respectively. Guidance of left habenular neurons to the dorsal IPN has been shown to require Neuropilin1a, a class 3 semaphorin receptor exclusively expressed in the left habenula (92); in contrast, the mechanisms underlying the projection of right habenular neurons to the ventral IPN are not known.

Another asymmetry is found in the size and organization of habenular neuropils, which are larger on the left than the right side (32). Various asymmetrically expressed factors, such as Kctd12.1, Kctd12.2, and Daam1a (dishevel associated activator of morphogenesis), have been associated with the growth of habenular neuropils while not apparently being required for the specification of habenular neuronal subtypes (30, 157) (**Table 1**). In addition to dendritic extensions from habenular neurons, these neuropil domains contain afferent connections from pp cells to the left habenula (33) and from a subset of mitral cells of the olfactory bulb to the right habenula (74, 116). However, nothing is known about the signals involved in asymmetrically targeting these axons.

Pitx2-dependent and -independent targets of Nodal in the brain. Although progress has been made toward characterizing the development of brain asymmetries in the zebrafish epithalamus, many questions remain to be answered. First, Nodal activity is required to bias the orientation of pp migration such that at later stages the pp can influence the development of habenular neural subtypes. How Nodal achieves this is unclear. In the absence of a Nodal signal, pp migration, which is normally randomized in this context, can be oriented toward the left or the right by an exogenous source of Fgf8 (134). This suggests that the role of Nodal involves the modulation of Fgf8 signaling activity, and future studies are needed to understand the cross talk between these

two pathways. How the early information provided by transient asymmetric Nodal expression is maintained and propagated until the pp migrates is also unknown. It was assumed that the function of Nodal involves its downstream target Pitx2. Indeed, Pitx2 is required, downstream of Nodal in the lateral plate mesoderm (LPM) for the LR-asymmetric morphogenesis of different internal body organs (25, 129, 145, 169). Although Pitx2 controls pp size, it is intriguing that *pitx2c* loss-of-function does not randomize pp migration orientation (60). This suggests that Nodal targets other than Pitx2c must be involved in biasing pp laterality. One future challenge will be to identify these Nodal targets in the epithalamus and to investigate whether similar Nodal-dependent/Pitx2c-independent targets also exist in the LPM. Transcriptional analysis performed on left versus right brain tissues, complemented by high-throughput proteomic profiling approaches (88), should provide new insights into the mechanisms underlying symmetry-breaking events.

DISCUSSION AND FUTURE DIRECTIONS

A Link Between Body and Brain Laterality

How and when is asymmetry set up in the brain and to what extent does it depend on the establishment of the LR body axis? The last decade of intensive research has brought many insights into how LR asymmetry is first established in the embryo, although this issue is still debated. Data in *Xenopus* and chick suggest that LR axis is established very early through gap junction–dependent cell communication (164). In other species, such as mouse and zebrafish, asymmetry in the LR axis is propagated, reset, or initiated at the gastrulation stage by a ciliated structure called the node/Kupffer's vesicle (127). In the node, the beating of motile cilia drives a leftward flow of extra-embryonic fluid. This asymmetric information at the node is then transmitted to the left LPM, where it sets up expression of Nodal and its downstream targets, Lefty and Pitx2.

In zebrafish, it has been shown that the activation of Nodal signaling in the LPM is required for the later transient expression of Nodal in the left epithalamus (102). As such, in the vast majority of wild-type zebrafish embryos, there is a perfect coupling between body and brain asymmetries. In contrast, there is, to date, no evidence for Nodal expression in either human or rodent brains and no obvious coupling between body and brain asymmetries in humans; for instance, cases of situs inversus (SI) that show inverted visceral asymmetry display a normal ratio of right-left handedness (111). However, some observations suggest that a few links between body and brain asymmetries may exist and need to be investigated further. For instance, two independent studies, on six SI individuals in total, found a systematic reversion in the structural asymmetry of the frontal and occipital petalia (83, 87); in contrast, the asymmetry in the PT was not reversed in these SI subjects. In a recent GWAS, Brandler et al. (20) found an enrichment of SNPs in four genes involved in ciliogenesis (*GLI3*, *MNS1*, *RFX3*, and *PDK2*) that are strongly associated with relative hand skills. Finally, in mice carrying mutations in the LR dynein gene (*LRD*), a gene required for the motility of node cilia, the hippocampus displays right isomerism in the distribution of GluRe2 receptor irrespective of the laterality of the viscera (67). This suggests that hippocampal asymmetry is not directly imposed by early visceral asymmetry but might be dependent on the same laterality event or factors that break body asymmetry (67). Taken together, these data suggest that the development of different brain asymmetries involves mechanisms that are generally independent from visceral asymmetries in mammals. Nonetheless, the early mechanisms that establish visceral LR asymmetry might to some moderate extent influence the development of brain asymmetries. Alternatively, some of the genes and/or molecular mechanisms involved in visceral asymmetry could be reused later during cerebral development to establish brain asymmetries.

Nodal Signaling Module and Nervous System Asymmetries

Increasing evidence suggests that the TGFβ/Nodal signaling module has been reiteratively used to convey directional asymmetry of the body in various protostome and deuterostome lineages (118, 124). For the past 15 years, it has also been known that the same module is employed in the zebrafish epithalamus to establish neuroanatomical asymmetries. Examination of other members of the vertebrate phyla suggests that this genetic program has also been conserved in the brain (**Figure 3**) but that its importance varies (93). For instance, in lamprey and catshark the Nodal-Lefty1-Pitx2 module is expressed asymmetrically on the left side of the epithalamus (expression of *nodal*-related genes highlighted in red in **Figure 3**). Unlike in the zebrafish, however, where the absence of Nodal signaling leads to randomization of the epithalamus asymmetry, in both the catshark and the lamprey abrogation of Nodal signaling leads to right isomerism of the habenular nuclei (93).

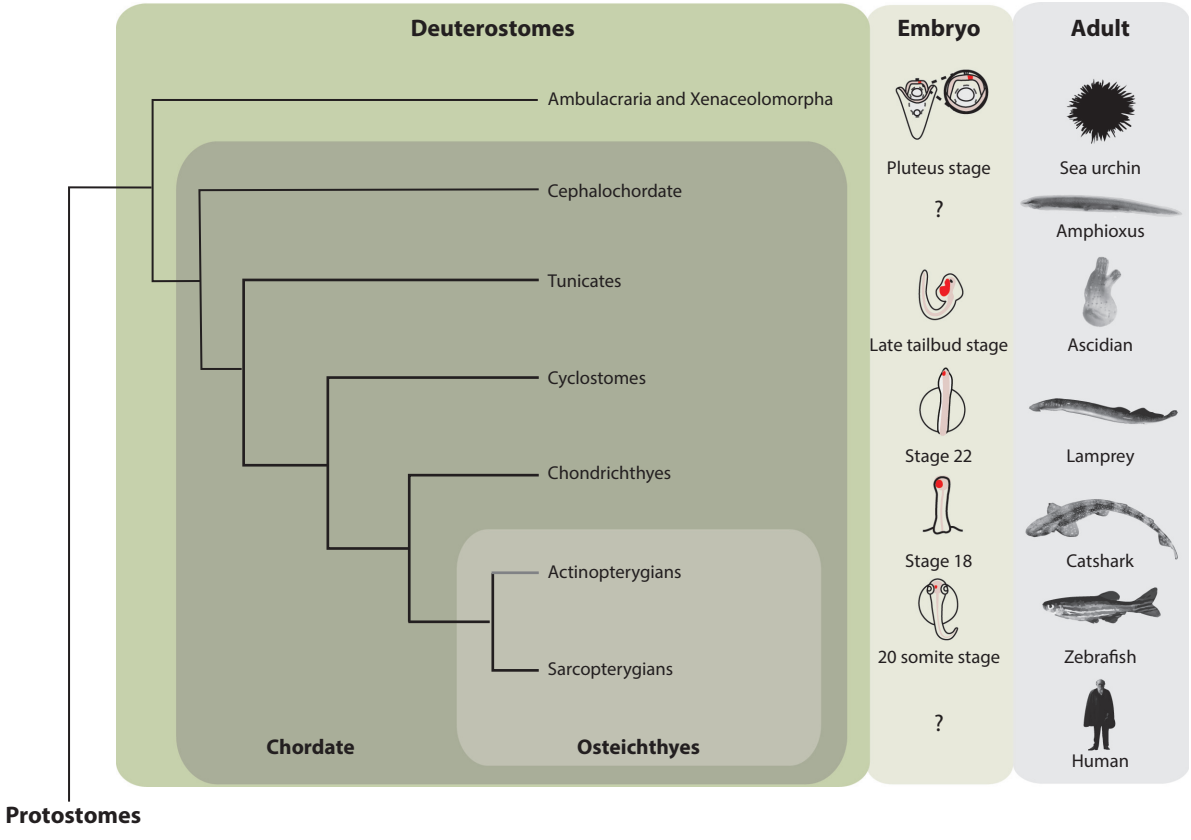

Figure 3

Is the Nodal signaling cassette a conserved module to create asymmetries in the nervous system? Comparison of the expression patterns of *nodal*-related genes in the nervous system (NS) of deuterostome model organisms with respect to their phylogeny. Expressions of *nodal*-related genes contributing to asymmetries other than the one observed in the NS have been omitted from the schematic. The embryonic expression domains of *nodal*-related genes are highlighted in red, and the NS is shown in pink on the different embryos. *nodal*-related genes are expressed in a few cells on the right side of the ciliary band in the sea urchin and on the left side of the sensory vesicles in ascidians. *nodal*-related genes are expressed in the left side of the diencephalon (forebrain) in cyclostomes, chondrichthyans, and in all teleosts, highlighting the ancestral role of Nodal signaling in setting asymmetry of the vertebrate forebrain. No expression has been reported so far in the NSs of protostomes, cephalochordates, or sarcopterygians. Expression profiles of *nodal*-related genes in nonvertebrate organisms do not infer any homology with the vertebrate diencephalon.

These findings suggest that in an ancestral vertebrate, habenular asymmetries may have been driven by asymmetric Nodal activity in the brain. Interestingly, these species display marked differences regarding the pp organ: In lampreys, the pp organ does not migrate from the midline or project asymmetrically to the left habenula, and catsharks apparently lack a pp organ (35, 93, 167). Thus, in the zebrafish in which the pp assumes a predominant role in the elaboration of epithalamic asymmetry, the Nodal-dependent/pp-independent asymmetry in habenular neurogenesis could reflect the ancient role for Nodal in the establishment of habenular asymmetry (143).

Study of additional phyla, such as reptiles and amphibians, for which habenular asymmetries have been documented, should provide insights into the extent of the different strategies for setting up epithalamic asymmetries. It should be noted, however, that asymmetric expression of *nodal*-related genes has also been reported in the nervous system of various deuterostomes, where they act as a symmetry-breaking factor. In sea urchin, for instance, the *nodal/pitx2* module is found expressed in a cluster of cells located on the right side of the apical tuft (part of the diffuse nervous system in larvae) that likely correspond to neurons (50, 51). In tunicates, phyla sharing close ancestry with vertebrates, ascidian larvae typically show LR asymmetry in the positioning and morphogenesis of the sensory vesicle situated in anterior brain and tilted to the right (19, 117); *nodal* is expressed in the left epidermis and involved in the asymmetric morphogenesis of this sensory vesicle (168). If expression of *nodal*-related genes has not been reported to date in amniote diencephalic regions, this might be the consequence of the loss of such expression during evolution or the relatively discrete and labile expression that current methods of analysis have failed to reveal. Assessing the possible homology of directional asymmetries of the nervous system more broadly requires a clearer picture of how nervous systems have evolved. Our understanding from the number of species already studied lends support to the idea that the deuterostome ancestor used a Nodal module to establish asymmetry in the central nervous system and that this module was possibly lost or that its function was subsequently modified in various deuterostome lineages.

Relevance of Population Level–Biased Lateralization

As discussed in a previous section (Advantage of Functional Lateralization of the Brain), brain lateralization might provide significant advantages to the individuals. However, lateralized behaviors in animals, as handedness in humans, are not only detected in individuals but also often manifest at the population level, where most individuals share the directionality of the given asymmetrical behavior. This population-biased lateralization is, here again, suspected to provide advantages for population survival and fitness. In that context, why does a reverted minority exist and persist through evolution?

Social advantage of a population level–biased lateralization. Population-biased lateralization, i.e., the fact that, within a population, individuals often display the same sideness of a given asymmetrical behavior, is thought to be important for social behavior as it seems more frequently observed in social than solitary species. By analyzing turning bias in 16 species of fishes, for instance, Bisazza et al. (15) reported that social schooling species display a population bias in their behavior more often than solitary species. Similar observations have been made in social invertebrates: In a learning assay associating odor with sugar, the social honeybee *Apis mellifera* performs better when odors are presented to their right antenna than to their left antenna (98). Although nonsocial bees present individual-level lateralization in this learning assay, they showed no population-level lateralization (5). Moreover, within the same hive, right antenna honeybees were more likely to interact with other right antenna bees than left antenna bees (139), further

confirming that this lateralized behavior is important for social interaction. Nonetheless, the fact that this bias is lost in solitary species suggests that the alignment of asymmetry at a population level is a balance between gain and cost.

The advantage of a reverted minority. If lateralized behavior provides advantages in social contexts, why is the laterality established in a given population not fully penetrant? In wild-type zebrafish, the asymmetry-breaking events leading to asymmetrical activation of the Nodal module in the brain are prone to errors because the outcome is more variable than that observed for the module in the LPM. Indeed, although almost 100% of the wild-type embryos have the Nodal pathway activated on the left side of the LPM, between 5% and 10% of the embryos either fail to activate Nodal signaling or do so bilaterally in the epithalamus, and subsequently adopt a random directionality of brain asymmetry (17, 32, 99). This variability is intriguingly reminiscent of the one found in hemispheric dominance for language or motor skills in humans (only 90% of right-handers). Why would a mechanism with such a variable outcome be kept through evolution? Biased directionality could simply persist because of its nonlethal and neutral character. However, it could be maintained, according to a frequency-dependent selection, because some individuals can benefit from belonging to a minority; for instance, an inverted individual might have a novelty effect advantage, as competitors or predators will not easily predict its behavior (162). In addition, if there was an evolutionary advantage for a given population to harbor a consistent but not fully penetrant laterality, the laterality ratios observed in a population could therefore represent an optimum ratio to insure sustainability of the species. Various theories have been proposed to explain the maintenance of a relatively fixed proportion of left-handedness in humans but experimental evidence is lacking (69, 101). Here again, animal models such as the zebrafish provide an opportunity to explore the minority advantage and optimum ratio hypotheses by comparing the behavior of cohorts artificially made up of different ratios of left- and right-brained individuals.

The Zebrafish Epithalamus: A Model to Link Neuronal Asymmetry and Behavior

The asymmetries observed in molecular markers and connectivity pattern suggest that the left and right habenulae in zebrafish have different functions. Although the dorsal habenulae have been implicated in fear- and anxiety-associated behavior in zebrafish (1, 96), it is not clear whether habenular asymmetries have an impact on this behavior. Recently, Dreosti et al. (49) have shown that the left and right habenulae display different responses to visual and olfactory stimuli. Using live calcium imaging, the authors showed that visual stimuli predominantly activate neurons in the left habenula, whereas olfactory stimulus most frequently activates neurons in the right habenula (**Figure 1*e*,*f***). Krishnan et al. (91) further showed that the pattern of neuronal activity in the right habenula depends on the identity and concentration of olfactory cues (aversive versus attractive). The functional specialization of habenular neurons clearly correlates with the previously characterized neuroanatomical asymmetry of the habenulae. Indeed, manipulations that result in embryos with right or left habenular isomerism lead to a loss of habenular responsiveness to light or odor, respectively (49, 82). Thus, correct LR asymmetry in the dorsal habenula is crucial to segregate the processing of visual and olfactory stimuli. Whether this segregation provides any advantages to the processing of sensory information remains an open question. Nonetheless, these data provide important insights into how a defect in establishing brain asymmetries might result in a loss of brain functions and potentially lead to pathologies.

Although the right habenula is known to receive direct afferences from cells in the olfactory bulb, it is not clear how the left habenula is connected to the visual pathway. As the left habenula receives afferent connections from the photoreceptive pp, one might expect that it is involved in asymmetric activation of habenular neurons by light. However, the response of habenular neurons to light is not lost upon ablation of the pp, although it is lost in the absence of the eyes (49). It would nonetheless be interesting to test whether the response of left habenular neurons to other visual stimuli, such as varying light wavelengths or intensity, could be modulated by the pp. Also, one cannot exclude the possibility that input from the pp modulates the response of left habenular neurons to other sensory stimuli. In this regard, it will be interesting to test whether mechanosensory or noise stimuli are processed in the habenulae and in an asymmetric way.

As described in other vertebrates, zebrafish display eye preference depending on the visual task being performed, the right eye being usually preferred to approach novel objects or scenes (113). Some lateralized behaviors have been shown to be reversed in zebrafish larvae that display an inverted habenula/pp asymmetry (11) and, reciprocally, selection for fishes with atypical behavioral lateralization increases the frequency of individuals with reversed epithalamic asymmetry (53); these data suggest a role for epithalamic asymmetries in lateralized visual behavior. Therefore, it would be interesting to analyze in further detail whether different visual stimuli, such as novel versus familiar stimuli, are differentially processed by the habenulae. The zebrafish also presents an opportunity in the future to understand how a particular circuit, such as the habenulae-IPN conduction system, interacts with other circuitries to trigger lateralized behavior.

CONCLUSION

The past few decades of studies on animal models have shown that brain asymmetry is an important feature of highly complex vertebrate brains but also of simpler invertebrate brains. Moreover, these investigations have provided important insights into the molecular and genetic mechanisms that underlie the development of brain asymmetry with a consistent sidedness. Such data might not always be easy to extrapolate to humans. It is nonetheless possible that some of the genetic factors implicated in the establishment of asymmetries in the nervous system of animal models such as zebrafish or *C. elegans* may have been co-opted to generate asymmetry in the mammalian cortex. Further studies are clearly needed to address whether this is the case. In the future, work on model systems should also contribute, for instance, to understanding of how genetically encoded asymmetric circuits mature with age, and whether/how they are shaped/reinforced by experiences. This issue is particularly relevant, as in humans functional lateralization for language and visuospatial tasks has been described to increase progressively during childhood and adolescence (reviewed in 43). The zebrafish epithalamus provides a powerful system to address these possibilities. Moreover, the increasing number of tools and resources available to manipulate brain asymmetries, together with the possibility of performing behavior tests and imaging neural activity in the brain, makes the zebrafish a very appropriate model to address general concepts concerning the functional relevance of brain lateralization at individual and population levels. We believe that only a comparative approach that integrates data from development, genetics, and molecular research fields to neuroimaging and behavior fields, in humans and in model systems, will provide significant insight into the ontogeny and phylogeny of brain lateralization and its importance for normal brain function.

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LITERATURE CITED

1. Agetsuma M, Aizawa H, Aoki T, Nakayama R, Takahoko M, et al. 2010. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat. Neurosci.* 13:1354–56
2. Aizawa H, Bianco IH, Hamaoka T, Miyashita T, Uemura O, et al. 2005. Laterotopic representation of left-right information onto the dorso-ventral axis of a zebrafish midbrain target nucleus. *Curr. Biol.* 15:238–43
3. Aizawa H, Goto M, Sato T, Okamoto H. 2007. Temporally regulated asymmetric neurogenesis causes left-right difference in the zebrafish habenular structures. *Dev. Cell* 12:87–98
4. Amo R, Aizawa H, Takahoko M, Kobayashi M, Takahashi R, et al. 2010. Identification of the zebrafish ventral habenula as a homolog of the mammalian lateral habenula. *J. Neurosci.* 30:1566–74
5. Anfora G, Frasnelli E, Maccagnani B, Rogers LJ, Vallortigara G. 2010. Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behav. Brain Res.* 206(2):236–39
6. Annett M. 1979. Family handedness in three generations predicted by the right shift theory. *Ann. Hum. Genet.* 42(4):479–91
7. Badzakova-Trajkov G, Häberling IS, Roberts RP, Corballis MC. 2010. Cerebral asymmetries: complementary and independent processes. *PLOS ONE* 5(3):e9682
8. Banks SJ, Sziklas V, Sodums DJ, Jones-Gotman M. 2012. fMRI of verbal and nonverbal memory processes in healthy and epileptogenic medial temporal lobes. *Epilepsy Behav.* 25(1):42–49
9. Barnéoud P, le Moal M, Neveu PJ. 1990. Asymmetric distribution of brain monoamines in left- and right-handed mice. *Brain Res.* 520(1–2):317–21
10. Barnéoud P, der Loos HV. 1993. Direction of handedness linked to hereditary asymmetry of a sensory system. *PNAS* 90(8):3246–50
11. Barth KA, Miklosi A, Watkins J, Bianco IH, Wilson SW, Andrew RJ. 2005. fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Curr. Biol.* 15:844–50
12. Beretta CA, Dross N, Gutierrez-Triana JA, Ryu S, Carl M. 2012. Habenula circuit development: past, present, and future. *Neurogenesis* 6:51
13. Bianco IH, Carl M, Russell C, Clarke JD, Wilson SW. 2008. Brain asymmetry is encoded at the level of axon terminal morphology. *Neural Dev.* 3:9
14. Bianco IH, Wilson SW. 2009. The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain. *Philos. Trans. R. Soc. Lond. B* 364:1005–20
15. Bisazza A, Cantalupo C, Capocchiano M, Vallortigara G. 2000. Population lateralisation and social behaviour: a study with 16 species of fish. *Later. Asymmetries Body Brain Cogn.* 5(3):269–84
16. Bisazza A, Facchin L, Pignatti R, Vallortigara G. 1998. Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behav. Brain Res.* 91(1–2):157–64
17. Bisgrove BW, Essner JJ, Yost HJ. 2000. Multiple pathways in the midline regulate concordant brain, heart and gut left-right asymmetry. *Development* 127:3567–79
18. Bisiach E, Luzzatti C. 1978. Unilateral neglect of representational space. *Cortex J. Devoted Study Nerv. Syst. Behav.* 14(1):129–33
19. Boorman CJ, Shimeld SM. 2002. The evolution of left-right asymmetry in chordates. *BioEssays* 24:1004–11

20. Brandler WM, Morris AP, Evans DM, Scerri TS, Kemp JP, et al. 2013. Common variants in left/right asymmetry genes and pathways are associated with relative hand skill. *PLOS Genet.* 9(9):e1003751
21. Brandler WM, Paracchini S. 2014. The genetic relationship between handedness and neurodevelopmental disorders. *Trends Mol. Med.* 20(2):83–90
22. Broca P. 1865. Sur le siège de la faculté du langage articulé ("nous parlons avec l'hémisphère gauche", p384). *Bull. Soc. Anthropol.* 6:377–93
23. Budaev S, Andrew RJ. 2009. Patterns of early embryonic light exposure determine behavioural asymmetries in zebrafish: a habenular hypothesis. *Behav. Brain Res.* 200(1):91–94
24. Bulman-Fleming B, Wainwright PE, Collins RL. 1992. The effects of early experience on callosal development and functional lateralization in pigmented BALB/c mice. *Behav. Brain Res.* 50(1–2):31–42
25. Campione M, Steinbeisser H, Schweickert A, Deissler K, van Bebber F, et al. 1999. The homeobox gene *pitx2*: mediator of asymmetric left-right signaling in vertebrate heart and gut looping. *Development* 126(6):1225–34
26. Carl M, Bianco IH, Bajoghli B, Aghaallaei N, Czerny T, Wilson SW. 2007. Wnt/axin1/beta-catenin signaling regulates asymmetric nodal activation, elaboration, and concordance of CNS asymmetries. *Neuron* 55:393–405
27. Chatterjee M, Guo Q, Weber S, Scholpp S, Li JY. 2014. Pax6 regulates the formation of the habenular nuclei by controlling the temporospatial expression of *Shh* in the diencephalon in vertebrates. *BMC Biol.* 12(1):13
28. Clanton JA, Hope KD, Gamse JT. 2013. Fgf signaling governs cell fate in the zebrafish pineal complex. *Dev. Camb. Engl.* 140(2):323–32
29. Cochet H, Byrne RW. 2013. Evolutionary origins of human handedness: evaluating contrasting hypotheses. *Anim. Cogn.* 16(4):531–42
30. Colombo A, Palma K, Armijo L, Mione M, Signore IA, et al. 2013. Daam1a mediates asymmetric habenular morphogenesis by regulating dendritic and axonal outgrowth. *Development* 140(19):3997–4007
31. Concha ML, Bianco IH, Wilson SW. 2012. Encoding asymmetry within neural circuits. *Nat. Rev. Neurosci.* 13(12):832–43
32. Concha ML, Burdine RD, Russell C, Schier AF, Wilson SW. 2000. A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. *Neuron* 28:399–409
33. Concha ML, Russell C, Regan JC, Tawk M, Sidi S, et al. 2003. Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality. *Neuron* 39:423–38
34. Concha ML, Signore IA, Colombo A. 2009. Mechanisms of directional asymmetry in the zebrafish epithalamus. *Semin. Cell Dev. Biol.* 20:498–509
35. Concha ML, Wilson SW. 2001. Asymmetry in the epithalamus of vertebrates. *J. Anat.* 199(1–2):63–84
36. Constam DB, Robertson EJ. 2000. SPC4/PACE4 regulates a TGFβ signaling network during axis formation. *Genes Dev.* 14(9):1146–55
37. Corballis MC. 2014. Left brain, right brain: facts and fantasies. *PLOS Biol.* 12(1):e1001767
37a. Dadda M, Bisazza A. 2006. Does brain asymmetry allow efficient performance of simultaneous tasks? *Anim. Behav.* 72(3):523–29
38. Dadda M, Zandona E, Agrillo C, Bisazza A. 2009. The costs of hemispheric specialization in a fish. *Proc. Biol. Sci.* 276:4399–4407
39. Dax M. 1865. Lésions de la moitié gauche de l'encéphale coïncident avec l'oubli des signes de la pensée (lu à montpellier en 1936). *Gaz. Hebd. Médecine Chir. Tome 2* 2:259–62
40. Dean BJ, Erdogan B, Gamse JT, Wu S-Y. 2014. Dbx1b defines the dorsal habenular progenitor domain in the zebrafish epithalamus. *Neural Dev.* 9(1):20
41. De Borsetti NH, Dean BJ, Bain EJ, Clanton JA, Taylor RW, Gamse JT. 2011. Light and melatonin schedule neuronal differentiation in the habenular nuclei. *Dev. Biol.* 358(1):251–61
42. deCarvalho TN, Subedi A, Rock J, Harfe BD, Thisse C, et al. 2014. Neurotransmitter map of the asymmetric dorsal habenular nuclei of zebrafish. *Genesis* 52(6):636–55

43. Demaree HA, Everhart DE, Youngstrom EA, Harrison DW. 2005. Brain lateralization of emotional processing: historical roots and a future incorporating "dominance." *Behav. Cogn. Neurosci. Rev.* 4(1):3–20
44. Dennis EL, Thompson PM. 2013. Mapping connectivity in the developing brain. *Int. J. Dev. Neurosci.* 31(7):525–42
45. De Schotten MT, Dell'Acqua F, Forkel SJ, Simmons A, Vergani F, et al. 2011. A lateralized brain network for visuospatial attention. *Nat. Neurosci.* 14(10):1245–46
46. Diamond MC, Johnson RE, Young D, Singh SS. 1983. Age-related morphologic differences in the rat cerebral cortex and hippocampus: male-female; right-left. *Exp. Neurol.* 81(1):1–13
47. Doll CA, Burkart JT, Hope KD, Halpern ME, Gamse JT. 2011. Subnuclear development of the zebrafish habenular nuclei requires ER translocon function. *Dev. Biol.* 360(1):44–57
48. Dragovic M, Hammond G. 2005. Handedness in schizophrenia: a quantitative review of evidence. *Acta Psychiatr. Scand.* 111(6):410–19
49. Dreosti E, Vendrell Llopis N, Carl M, Yaksi E, Wilson SW. 2014. Left-right asymmetry is required for the habenulae to respond to both visual and olfactory stimuli. *Curr. Biol.* 24(4):440–45
50. Duboc V, Lapraz F, Besnardeau L, Lepage T. 2008. Lefty acts as an essential modulator of nodal activity during sea urchin oral-aboral axis formation. *Dev. Biol.* 320:49–59
51. Duboc V, Rottinger E, Besnardeau L, Lapraz F, Lepage T. 2005. Left-right asymmetry in the sea urchin embryo is regulated by nodal signaling on the right side. *Dev. Cell* 8:1–12
52. Ehret G. 1987. Left hemisphere advantage in the mouse brain for recognizing ultrasonic communication calls. *Nature* 325(6101):249–51
53. Facchin L, Argenton F, Bisazza A. 2009. Lines of *Danio rerio* selected for opposite behavioural lateralization show differences in anatomical left-right asymmetries. *Behav. Brain Res.* 197(1):157–65
54. Fagard J. 2013. Early development of hand preference and language lateralization: Are they linked, and if so, how? *Dev. Psychobiol.* 55(6):596–607
55. Fink M, Wadsak W, Savli M, Stein P, Moser U, et al. 2009. Lateralization of the serotonin-1a receptor distribution in language areas revealed by pet. *NeuroImage* 45(2):598–605
56. Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. 2011. Habenular α5 nicotinic receptor subunit signaling controls nicotine intake. *Nature* 471(7340):597–601
57. Francks C, Maegawa S, Laurén J, Abrahams BS, Velayos-Baeza A, et al. 2007. *Lrrtm1* on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia. *Mol. Psychiatry* 12(12):1129–39
58. Gamse JT, Kuan YS, Macurak M, Brosamle C, Thisse B, et al. 2005. Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. *Development* 132:4869–81
59. Gamse JT, Thisse C, Thisse B, Halpern ME. 2003. The parapineal mediates left-right asymmetry in the zebrafish diencephalon. *Development* 130:1059–68
60. Garric L, Ronsin B, Roussigné M, Booton S, Gamse JT, et al. 2014. Pitx2c ensures habenular asymmetry by restricting parapineal cell number. *Development* 141(7):1572–79
61. Gaub S, Groszer M, Fisher SE, Ehret G. 2010. The structure of innate vocalizations in *Foxp2*-deficient mouse pups. *Genes Brain Behav.* 9(4):390–401
62. Gazzaniga MS. 2005. Forty-five years of split-brain research and still going strong. *Nat. Rev. Neurosci.* 6(8):653–59
63. Gazzaniga MS, Bogen JE, Sperry RW. 1962. Some functional effects of sectioning the cerebral commissures in man. *PNAS* 48(10):1765–69
64. Geschwind N, Galaburda AM. 1985. Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Arch. Neurol.* 42(5):428–59
65. Geschwind N, Levitsky W. 1968. Human brain: left-right asymmetries in temporal speech region. *Science* 161(3837):186–87
66. Glick SD, Ross DA, Hough LB. 1982. Lateral asymmetry of neurotransmitters in human brain. *Brain Res.* 234(1):53–63

67. Goto K, Kurashima R, Gokan H, Inoue N, Ito I, Watanabe S. 2010. Left-right asymmetry defect in the hippocampal circuitry impairs spatial learning and working memory in IV mice. *PLOS ONE* 5(11):e15468
68. Gotts SJ, Jo HJ, Wallace GL, Saad ZS, Cox RW, Martin A. 2013. Two distinct forms of functional lateralization in the human brain. *PNAS* 110(36):E3435–44
69. Groothuis TGG, McManus IC, Schaafsma SM, Geuze RH. 2013. The fighting hypothesis in combat: How well does the fighting hypothesis explain human left-handed minorities? *Ann. N. Y. Acad. Sci.* 1288(1):100–9
70. Groszer M, Keays DA, Deacon RMJ, de Bono JP, Prasad-Mulcare S, et al. 2008. Impaired synaptic plasticity and motor learning in mice with a point mutation implicated in human speech deficits. *Curr. Biol.* 18(5):354–62
71. Haesler S, Rochefort C, Georgi B, Licznerski P, Osten P, Scharff C. 2007. Incomplete and inaccurate vocal imitation after knockdown of *FoxP2* in songbird basal ganglia nucleus area X. *PLOS Biol.* 5(12):e321
72. Haesler S, Wada K, Nshdejan A, Morrisey EE, Lints T, et al. 2004. *FoxP2* expression in avian vocal learners and non-learners. *J. Neurosci.* 24(13):3164–75
72a. Halluin C, Madelaine R, Naye F, Leoture S, Peers B, Roussigné M, Blader P. 2015. Habenular neurogenesis in zebrafish is regulated by a Hedgehog, Pax6, proneural gene cascade. *PLOS ONE* Submitted
73. Halpern ME, Liang JO, Gamse JT. 2003. Leaning to the left: laterality in the zebrafish forebrain. *Trends Neurosci.* 26:308–13
74. Hendricks M, Jesuthasan S. 2007. Asymmetric innervation of the habenula in zebrafish. *J. Comp. Neurol.* 502:611–19
75. Hepper PG, Shahidullah S, White R. 1991. Handedness in the human fetus. *Neuropsychologia* 29(11):1107–11
76. Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Kennedy DN, et al. 2005. Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain J. Neurol.* 128(Pt. 1):213–26
77. Hervé P-Y, Zago L, Petit L, Mazoyer B, Tzourio-Mazoyer N. 2013. Revisiting human hemispheric specialization with neuroimaging. *Trends Cogn. Sci.* 17(2):69–80
78. Hobert O. 2014. Development of left/right asymmetry in the *Caenorhabditis elegans* nervous system: from zygote to postmitotic neuron. *Genesis* 52(6):528–43
79. Hong E, Santhakumar K, Akitake CA, Ahn SJ, Thisse C, et al. 2013. Cholinergic left-right asymmetry in the habenulo-interpeduncular pathway. *PNAS* 110(52):21171–76
80. Husain M, Rorden C. 2003. Non-spatially lateralized mechanisms in hemispatial neglect. *Nat. Rev. Neurosci.* 4(1):26–36
81. Hüsken U, Carl M. 2013. The Wnt/beta-catenin signaling pathway establishes neuroanatomical asymmetries and their laterality. *Mech. Dev.* 130(6–8):330–35
82. Hüsken U, Stickney HL, Gestri G, Bianco IH, Faro A, et al. 2014. Tcf7l2 is required for left-right asymmetric differentiation of habenular neurons. *Curr. Biol.* 24(19):2217–27
83. Ihara A, Hirata M, Fujimaki N, Goto T, Umekawa Y, et al. 2010. Neuroimaging study on brain asymmetries in situs inversus totalis. *J. Neurol. Sci.* 288(1–2):72–78
84. Kaplan S, Rağbetli MÇ, Canan S, Sahin B, Marangoz C. 2003. Numerical density of pyramidal neurons in the hippocampus of 4 and 20 week old male and female rats. *Neurosci. Res. Commun.* 32(1):37–48
85. Karev GB. 2008. Season of birth and parental age in right, mixed and left handers. *Cortex* 44(1):79–81
86. Kemali M, Guglielmotti V, Fiorino L. 1990. The asymmetry of the habenular nuclei of female and male frogs in spring and in winter. *Brain Res.* 517(1–2):251–55
87. Kennedy DN, O'Craven KM, Ticho BS, Goldstein AM, Makris N, Henson JW. 1999. Structural and functional brain asymmetries in human situs inversus totalis. *Neurology* 53(6):1260–65
88. Kim M-S, Pinto SM, Getnet D, Nirujogi RS, Manda SS, et al. 2014. A draft map of the human proteome. *Nature* 509(7502):575–81
89. Klöppel S, Mangin J-F, Vongerichten A, Frackowiak RSJ, Siebner HR. 2010. Nurture versus nature: long-term impact of forced right-handedness on structure of pericentral cortex and basal ganglia. *J. Neurosci.* 30(9):3271–75

90. Knecht S, Dräger B, Deppe M, Bobe L, Lohmann H, et al. 2000. Handedness and hemispheric language dominance in healthy humans. *Brain* 123(12):2512–18
91. Krishnan S, Mathuru AS, Kibat C, Rahman M, Lupton CE, et al. 2014. The right dorsal habenula limits attraction to an odor in zebrafish. *Curr. Biol.* 24(11):1167–75
92. Kuan YS, Yu HH, Moens CB, Halpern ME. 2007. Neuropilin asymmetry mediates a left-right difference in habenular connectivity. *Development* 134:857–86
93. Lagadec R, Laguerre L, Menuet A, Amara A, Rocancourt C, et al. 2015. Ancestral role of nodal signalling in breaking L/R symmetry in the vertebrate forebrain. *Nat. Commun.* 6:6686
94. Lai CSL, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 413(6855):519–23
95. Lawson RP, Seymour B, Loh E, Lutti A, Dolan RJ, et al. 2014. The habenula encodes negative motivational value associated with primary punishment in humans. *PNAS* 111(32):11858–63
96. Lee A, Mathuru AS, Teh C, Kibat C, Korzh V, et al. 2010. The habenula prevents helpless behavior in larval zebrafish. *Curr. Biol.* 20:2211–16
97. LeMay M. 1976. Morphological cerebral asymmetries of modern man, fossil man, and nonhuman primate. *Ann. N. Y. Acad. Sci.* 280(1):349–66
98. Letzkus P, Ribi WA, Wood JT, Zhu H, Zhang S-W, Srinivasan MV. 2006. Lateralization of olfaction in the honeybee *Apis mellifera*. *Curr. Biol.* 16(14):1471–76
99. Liang JO, Etheridge A, Hantsoo L, Rubinstein AL, Nowak SJ, et al. 2000. Asymmetric nodal signaling in the zebrafish diencephalon positions the pineal organ. *Development* 127:5101–12
100. Lindell AK. 2013. Continuities in emotion lateralization in human and non-human primates. *Front. Hum. Neurosci.* 7:464
101. Llaurens V, Raymond M, Faurie C. 2009. Why are some people left-handed? An evolutionary perspective. *Philos. Trans. R. Soc. Lond. B* 364(1519):881–94
102. Long S, Ahmad N, Rebagliati M. 2003. The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry. *Development* 130:2303–16
103. Lyn H, Pierre P, Bennett AJ, Fears S, Woods R, Hopkins WD. 2011. Planum temporale grey matter asymmetries in chimpanzees (*Pan troglodytes*), vervet (*Chlorocebus aethiops sabaeus*), rhesus (*Macaca mulatta*) and bonnet (*Macaca radiata*) monkeys. *Neuropsychologia* 49(7):2004–12
104. Manning L, Thomas-Antérion C. 2011. Marc Dax and the discovery of the lateralisation of language in the left cerebral hemisphere. *Rev. Neurol.* 167(12):868–72
105. Manns M, Güntürkün O. 1999. Monocular deprivation alters the direction of functional and morphological asymmetries in the pigeon's (*Columba livia*) visual system. *Behav. Neurosci.* 113(6):1257–66
106. Martinez-Ferre A, Martinez S. 2009. The development of the thalamic motor learning area is regulated by Fgf8 expression. *J. Neurosci.* 29(42):13389–400
107. Matsumoto M, Hikosaka O. 2007. Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447:1111–15
108. Mazoyer B, Zago L, Jobard G, Crivello F, Joliot M, et al. 2014. Gaussian mixture modeling of hemispheric lateralization for language in a large sample of healthy individuals balanced for handedness. *PLOS ONE* 9(6):e101165
109. McManus IC. 1985. Handedness, language dominance and aphasia: a genetic model. *Psychol. Med. Monogr. Suppl.* 8:1–40
110. McManus IC. 2005. Symmetry and asymmetry in aesthetics and the arts. *Eur. Rev.* 13(Suppl. S2):157–80
111. McManus IC, Martin N, Stubbings GF, Chung EMK, Mitchison HM. 2004. Handedness and situs inversus in primary ciliary dyskinesia. *Proc. R. Soc. Lond. B* 271(1557):2579–82
111a. McManus IC, Davison A, Armour JAL. 2013. Multilocus genetic models of handedness resemble single-locus models in explaining family data and are compatible with genome-wide association studies. *Ann. N. Y. Acad. Sci.* 1288:48–58
112. Medland SE, Duffy DL, Spurdle AB, Wright MJ, Geffen GM, et al. 2005. Opposite effects of androgen receptor CAG repeat length on increased risk of left-handedness in males and females. *Behav. Genet.* 35(6):735–44

113. Miklósi A, Andrew RJ, Savage H. 1997. Behavioural lateralisation of the tetrapod type in the zebrafish (*Brachydanio rerio*). *Physiol. Behav.* 63(1):127–35
114. Milenković S, Rock D, Dragović M, Janca A. 2008. Season of birth and handedness in Serbian high school students. *Ann. Gen. Psychiatry* 7(1):2
115. Milner B. 1968. Visual recognition and recall after right temporal-lobe excision in man. *Neuropsychologia* 6(3):191–209
116. Miyasaka N, Morimoto K, Tsubokawa T, Higashijima S, Okamoto H, Yoshihara Y. 2009. From the olfactory bulb to higher brain centers: genetic visualization of secondary olfactory pathways in zebrafish. *J. Neurosci.* 29:4756–67
117. Morokuma J, Ueno M, Kawanishi H, Saiga H, Nishida H. 2002. *HrNodal*, the ascidian *nodal*-related gene, is expressed in the left side of the epidermis, and lies upstream of *HrPitx*. *Dev. Genes Evol.* 212(9):439–46
118. Namigai EKO, Kenny NJ, Shimeld SM. 2014. Right across the tree of life: the evolution of left-right asymmetry in the bilateria. *Genesis* 52(6):458–70
119. Neugebauer JM, Yost HJ. 2014. Fgf signaling is required for brain left-right asymmetry and brain midline formation. *Dev. Biol.* 386(1):123–34
120. Ocklenburg S, Arning L, Gerding WM, Epplen JT, Güntürkün O, Beste C. 2013. Foxp2 variation modulates functional hemispheric asymmetries for speech perception. *Brain Lang.* 126(3):279–84
121. Ocklenburg S, Beste C, Arning L, Peterburs J, Güntürkün O. 2014. The ontogenesis of language lateralization and its relation to handedness. *Neurosci. Biobehav. Rev.* 43:191–98
122. Ocklenburg S, Ströckens F, Güntürkün O. 2013. Lateralisation of conspecific vocalisation in non-human vertebrates. *Later. Asymmetries Body Brain Cogn.* 18(1):1–31
123. Oke A, Lewis R, Adams RN. 1980. Hemispheric asymmetry of norepinephrine distribution in rat thalamus. *Brain Res.* 188(1):269–72
124. Oliverio M, Digilio MC, Versacci P, Dallapiccola B, Marino B. 2010. Shells and heart: Are human laterality and chirality of snails controlled by the same maternal genes? *Am. J. Med. Genet. A* 152A(10):2419–25
125. Palmer AR. 2009. Animal asymmetry. *Curr. Biol.* 19(12):R473–77
126. Pascual A, Huang K-L, Neveu J, Préat T. 2004. Neuroanatomy: brain asymmetry and long-term memory. *Nature* 427(6975):605–6
127. Pennekamp P, Menchen T, Dworniczak B, Hamada H. 2015. Situs inversus and ciliary abnormalities: 20 years later, what is the connection? *Cilia* 4(1):1
128. Peters M, Reimers S, Manning JT. 2006. Hand preference for writing and associations with selected demographic and behavioral variables in 255,100 subjects: the BBC internet study. *Brain Cogn.* 62(2):177–89
129. Piedra ME, Icardo JM, Albajar M, Rodriguez-Rey JC, Ros MA. 1998. Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. *Cell* 94(3):319–24
130. Pinel P, Fauchereau F, Moreno A, Barbot A, Lathrop M, et al. 2012. Genetic variants of FOXP2 and KIAA0319/TTRAP/THEM2 locus are associated with altered brain activation in distinct language-related regions. *J. Neurosci.* 32(3):817–25
131. Previc FH. 1991. A general theory concerning the prenatal origins of cerebral lateralization in humans. *Psychol. Rev.* 98(3):299–334
132. Price CJ. 2012. A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. *Neuroimage* 62(2):816–47
133. Raymond M, Pontier D, Dufour A-B, Moller AP. 1996. Frequency-dependent maintenance of left handedness in humans. *Proc. R. Soc. Lond. B* 263(1377):1627–33
134. Regan JC, Concha ML, Roussigné M, Russell C, Wilson SW. 2009. An Fgf8-dependent bistable cell migratory event establishes CNS asymmetry. *Neuron* 61(1):27–34
135. Rogers LJ. 1982. Light experience and asymmetry of brain function in chickens. *Nature* 297(5863):223–25
136. Rogers LJ. 1990. Light input and the reversal of functional lateralization in the chicken brain. *Behav. Brain Res.* 38(3):211–21

136a. Rogers LJ, Zucca P, Vallortigara G. 2004. Advantages of having a lateralized brain. *Proc. R. Soc. Lond. B* 271(Suppl. 6):S420–22
137. Rogers LJ. 2009. Hand and paw preferences in relation to the lateralized brain. *Philos. Trans. R. Soc. Lond. B*. 364(1519):943–54
138. Rogers LJ. 2014. Asymmetry of brain and behavior in animals: its development, function, and human relevance. *Genesis* 52(6):555–71
139. Rogers LJ, Rigosi E, Frasnelli E, Vallortigara G. 2013. A right antenna for social behaviour in honeybees. *Sci. Rep*. 3:2045
140. Rogers LJ, Sink HS. 1988. Transient asymmetry in the projections of the rostral thalamus to the visual hyperstriatum of the chicken, and reversal of its direction by light exposure. *Exp. Brain Res*. 70(2):378–84
141. Rogers LJ, Vallortigara G. 2008. From antenna to antenna: lateral shift of olfactory memory recall by honeybees. *PLOS ONE* 3(6):e2340
142. Rogers LJ, Zucca P, Vallortigara G. 2004. Advantages of having a lateralized brain. *Proc. R. Soc. Lond. B* 271(Suppl. 6):S420–22
143. Roussigné M, Bianco IH, Wilson SW, Blader P. 2009. Nodal signalling imposes left-right asymmetry upon neurogenesis in the habenular nuclei. *Development* 136:1549–57
144. Roussigné M, Blader P, Wilson SW. 2012. Breaking symmetry: the zebrafish as a model for understanding left-right asymmetry in the developing brain. *Dev. Neurobiol*. 72(3):269–81
145. Ryan AK, Blumberg B, Rodriguez-Esteban C, Yonei-Tamura S, Tamura K, et al. 1998. Pitx2 determines left-right asymmetry of internal organs in vertebrates. *Nature* 394(6693):545–51
146. Salas R, Sturm R, Boulter J, Biasi MD. 2009. Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *J. Neurosci*. 29(10):3014–18
147. Savic I. 2014. Asymmetry of cerebral gray and white matter and structural volumes in relation to sex hormones and chromosomes. *Neuroendocr. Sci*. 8:329
148. Scerri TS, Brandler WM, Paracchini S, Morris AP, Ring SM, et al. 2011. PCSK6 is associated with handedness in individuals with dyslexia. *Hum. Mol. Genet*. 20(3):608–14
149. Shu W, Cho JY, Jiang Y, Zhang M, Weisz D, et al. 2005. Altered ultrasonic vocalization in mice with a disruption in the *Foxp2* gene. *PNAS* 102(27):9643–48
150. Slomianka L, West MJ. 1987. Asymmetry in the hippocampal region specific for one of two closely related species of wild mice. *Brain Res*. 436(1):69–75
151. Snelson CD, Santhakumar K, Halpern ME, Gamse JT. 2008. Tbx2b is required for the development of the parapineal organ. *Development* 135:1693–1702
152. Stoyanov Z, Nikolova P, Pashalieva I. 2011. Season of birth, Geschwind and Galaburda hypothesis, and handedness. *Later. Asymmetries Body Brain Cogn*. 16(5):607–19
153. Sun T, Patoine C, Abu-Khalil A, Visvader J, Sum E, et al. 2005. Early asymmetry of gene transcription in embryonic human left and right cerebral cortex. *Science* 308(5729):1794–98
154. Sun T, Walsh CA. 2006. Molecular approaches to brain asymmetry and handedness. *Nat. Rev. Neurosci*. 7:655–62
155. Sun ZY, Klöppel S, Rivière D, Perrot M, Frackowiak R, et al. 2012. The effect of handedness on the shape of the central sulcus. *NeuroImage* 60(1):332–39
156. Taylor RW, Hsieh YW, Gamse JT, Chuang CF. 2010. Making a difference together: reciprocal interactions in *C. elegans* and zebrafish asymmetric neural development. *Development* 137:681–91
157. Taylor RW, Qi JY, Talaga AK, Ma TP, Pan L, et al. 2011. Asymmetric inhibition of Ulk2 causes left-right differences in habenular neuropil formation. *J. Neurosci*. 31(27):9869–78
158. Toga AW, Thompson PM. 2003. Mapping brain asymmetry. *Nat. Rev. Neurosci*. 4(1):37–48
159. Tonetti L, Adan A, Caci H, Fabbri M, Natale V. 2012. Season of birth and handedness in young adults. *Later. Asymmetries Body Brain Cogn*. 17(5):597–601
160. Tran US, Stieger S, Voracek M. 2014. Latent variable analysis indicates that seasonal anisotropy accounts for the higher prevalence of left-handedness in men. *Cortex* 57:188–97
161. Vallar G. 1998. Spatial hemineglect in humans. *Trends Cogn. Sci*. 2(3):87–97
162. Vallortigara G, Rogers LJ. 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci*. 28:575–89; discuss. 589–633

163. Vallortigara G, Rogers LJ, Bisazza A, Lippolis G, Robins A. 1998. Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *NeuroReport* 9(14):3341–44
164. Vandenberg LN, Levin M. 2013. A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality. *Dev. Biol.* 379(1):1–15
165. Vigneau M, Beaucousin V, Hervé PY, Duffau H, Crivello F, et al. 2006. Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. *NeuroImage* 30(4):1414–32
166. Webb JR, Schroeder MI, Chee C, Dial D, Hana R, et al. 2013. Left-handedness among a community sample of psychiatric outpatients suffering from mood and psychotic disorders. *SAGE Open* doi: 10.1177/2158244013503166
167. Yáñez J, Pombal MA, Anadón R. 1999. Afferent and efferent connections of the parapineal organ in lampreys: a tract tracing and immunocytochemical study. *J. Comp. Neurol.* 403(2):171–89
168. Yoshida K, Saiga H. 2011. Repression of *Rx* gene on the left side of the sensory vesicle by nodal signaling is crucial for right-sided formation of the ocellus photoreceptor in the development of *Ciona intestinalis*. *Dev. Biol.* 354(1):144–50
169. Yoshioka H, Meno C, Koshiba K, Sugihara M, Itoh H, et al. 1998. *Pitx2*, a bicoid-type homeobox gene, is involved in a lefty-signaling pathway in determination of left-right asymmetry. *Cell* 94(3):299–305