An Ancestral Axial Twist Explains the Contralateral Forebrain and the Optic Chiasm in Vertebrates

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Abstract

Among the best-known facts of the brain are the contralateral visual, auditory, sensational, and motor mappings in the forebrain. How and why did these evolve? The few theories to this question provide functional answers, such as better networks for visuomotor control. However, these theories contradict the data, as discussed here.

Instead we propose that a 90-deg turn on the left side evolved in a common ancestor of all vertebrates. Compensatory migrations of the tissues during development restore body symmetry. Eyes, nostrils and forebrain compensate in the direction of the turn, whereas more caudal structures migrate in the opposite direction. As a result of these opposite migrations the forebrain becomes crossed and inverted with respect to the rest of the nervous system.

We show that such compensatory migratory movements can indeed be observed in the zebrafish (\textit{Danio rerio}) and the chick (\textit{Gallus gallus}). With a model we show how the axial twist hypothesis predicts that an optic chiasm should develop on the ventral side of the brain, whereas the olfactory tract should be uncrossed.

In addition, the hypothesis explains the decussation of the trochlear nerve, why olfaction is non-crossed, why the cerebellar hemispheres represent the ipsilateral bodyside, why in sharks the forebrain halves each represent the ipsilateral eye, why the heart and other inner organs are asymmetric in the body. Due to the poor fossil record, the possible evolutionary scenarios remain speculative. Molecular evidence does support the hypothesis. The findings may shed new insight on the problematic structure of the forebrain.

Keywords: body axis, embryogenesis, trochlearis cranial nerve IV, homeobox transcription factor, holoprosencephaly, situs inversus

1. Introduction

Contralateral means “on the other side”. It refers to the fact that each side of the brain receives its input from the eye (or visual field) on the opposite side, and also controls and senses the opposite bodyside. The contralateral organisation of the forebrain is a general and prominent vertebrate feature, but explanations are rare and usually only address either the motor or the visual aspect (Vulliemoz et al., 2005; Shinbrot and Young, 2008). Although much information has become available about molecular and genetic mechanisms, the general pattern remains puzzling (Stern, 2002; Lee et al., 2004; Jeffery and Erskine, 2005).

The only theory to date that tries to explain the general pattern of contralateral representations in the brain, is by Ramón y Cajal (1898). Ramón y Cajal was the first to develop a conceptual framework to explain the pattern of contralateral representations and neuronal decussations\textsuperscript{1} in a functional manner (Ramón y Cajal, 1898; Llinás, 2003; Vulliemoz et al., 2005; Loosemore, 2009). He carefully described the fibre crossings in several species of bony fish, amphibians, reptiles, birds and mammals.

Cajal’s theory can best be explained at hand of one of his own figures (reproduced in fig. 1). Central to his theory is the hypothesis that the brain creates a panoramic representation of the outer world. In a lateral-eyed animal, the two retinas have non-overlapping visual fields. In such animals, including humans, not all optic tract fibers cross the midline. Instead, each

\textsuperscript{1}Decussation comes from the latin numeral X (ten), is used to refer to midline-crossings of axons or nerves. Chiasm is derived from the greek letter \chi, and is used for the midline crossing of the optic tract.
hemisphere of the brain represents the contralateral visual hemifield of both eyes (Jeffery and Erskine, 2005). It is generally accepted that this organisation is highly advantageous for depth perception. The local mismatch between the images on corresponding locations of the two retinas (known as disparity) provides accurate information about the distance of objects with respect to the eyes (Regan, 2000). Already according to Cajal, the incomplete crossing of the retinal projections in the optic chiasm of frontal-eyed land vertebrates is a later adaptation to achieve depth vision from binocular disparity.

To complete his theory, Cajal proposed that the contralateral motor and somatosensory representations in the forebrain are an adaptation to the contralateral visual representation (fig. 1, lower part). He argued that the motor and somatosensory representations must be crossed in order to match the right visual hemifield with the right body-side and the left visual hemifield with the left body-side.

Figure 1: Ramón y Cajal’s theory for the contralateral organisation of the forebrain. He proposed that the contralateral somatosensory and motor representations are adaptations to the visual system (see text for explanation). O, optic tract; C, primary and secondary visual centres; M, decussating motor pathways; S, decussating sensory pathways; R, motor efferents of the spine; G, spinal ganglions and sensory afferents. Note that Cajal drew the spinal section upside down and the cortical hemispheres as fused. The secondary visual centres presumably refer to primary motor and somatosensory cortex. Reproduced from Ramón y Cajal (1898).

Cajal’s theory is still popular and, at first sight, elegant. However, a closer look reveals several problems. It is questionable, in the first place, whether a continuous, aligned projection in the brain is an advantage at all. All vertebrates can move their eyes, and moving the eyes makes the retinal images shift with respect to each other, so a true alignment of the visual fields is impossible.

A more serious problem of Cajal’s theory is that the hemispheres of the forebrain are comparatively badly connected. If an aligned projection would have a special evolutionary advantage, one should have expected that the primary visual cortex on each side of the brain should be a most densely interconnected part of the brain, especially in lateral eyed species. There is no evidence for this. The corpus callosum, which Ramón y Cajal thought to fulfil this function, only evolved in mammals. In other vertebrates the cortical hemispheres are connected only by the relatively small anterior commissure.

At the level of the sensorimotor representations in the brain, Cajal’s explanation is also seriously problematic. The theory assumes that it is optimal to connect each side of the visual world with the ipsilateral sensorimotor system. However, theoretical models show that even for the simplest imaginable animal it is optimal to have bilateral connections between visual and motor systems (Braitenberg, 1984). Flechsig (1899) already questioned this part of Cajal’s theory in his foreword to the german translation of (Ramón y Cajal, 1898), arguing that there is no direct projection of the primary visual cortex to the motor cortex. Some studies have used similar reasoning as Ramón y Cajal, but found that the crossed connections of visual hemifield and body and leg musculature is optimal (Loeb, 1918; Bertin, 1994).

Functional explanations focus on vertebrates. This is problematic, because molluscs (cephalopods) and arthropods also evolved highly sophisticated visual and motor systems. In spite of the well-developed interhemispheric connections, none of these clades seems to have evolved systematic contralateral representations (Budelmann, 1995; Hausen, 1984).

According to an entirely different approach, Ebbesson’s parcellation theory, lateralisation is an epiphenomenon of an increase in brain size (Ebbesson, 1980). The primitive condition, according to this view, is a bilateral representation, whereas the evolution of contralateral representations is coincidental and as likely as the evolution of ipsilateral representations (Ebbesson and Íto, 1980). However, there are examples of extensive bilateral connections in the optic chiasm, in several lineages of vertebrates. Thus, the theory implies that a complete decussation in the optic chiasm of vertebrates should have evolved independently many times. It seems highly unlikely that this could have happened without altogether losing the optic chiasm even in a single case.

Thus, although a contralateral organisation may be advantageous in some special cases, there is currently no theory to explain the extent of decussations and the extraordinary evolutionary conservation. Neither does consistent evidence exist for an evolutionary advantage of having vi-
sion, audition, somato-sensation, and motor control all contralaterally organised, in the forebrain. Furthermore, to my knowledge no existing theory explains why olfaction should be the only ipsilateral sense in the forebrain.

The current work proposes a very different explanation, which neither leads to better visual processing nor to better motor control or sensorimotor integration. It is proposed that opposite compensatory deformations to an axial rotation in the early embryo explain the pattern of midline-crossing and non-crossing connections in the nervous system. A model is developed to explain how these patterns can be brought about and how an optic chiasm develops as its consequence.

2. The axial twist hypothesis

As we will see below (section 4) all extant and fossil vertebrates possess an optic chiasm. The model is therefore based on the hypothesis that an ancestor of all vertebrates has turned on its left side, by a 90° turn about the body axis (i.e. anti-clockwise from the perspective of the embryo). As the fossil record of vertebrates extends back to the cambrian, this event should have occurred at least 450 Myr ago. Here we will focus on the early embryological compensations to this side-turn and the morphological implications in extant vertebrates. Possible evolutionary scenarios will be presented in the Discussion (section 7.2).

The left-turn makes that the embryo is located on its left side (fig. 2A-B). The body has thus lost bilateral symmetry with respect to the vertical sagittal plane. Being fishes, early vertebrates were active swimmers and so probably had a strong evolutionary advantage of a bilaterally symmetric locomotor system. By contrast, for inner body structures, such as heart and digestive organs, there is no evolutionary advantage to compensate the turn, so we expect that these will make a clear left-turn during embryology.

There are two ways to regain bilateral symmetry. Compensations opposite to the turn (i.e. clockwise) will bring the body parts back into their original symmetric position. On the other hand, compensations in the same direction of the turn (i.e. anti-clockwise) will bring the body parts into a symmetric position that is inverted, both on the left-right and the dorso-ventral body axes, with respect to the original position.

We propose that developmental compensations have evolved in both directions simultaneously, leading to a twist in the nervous system (fig. 2B-C). In figure 2B the embryo is turned on its left side. The rostral head region compensates in the direction of the turn, 90° anti-clockwise, so the anterior head region becomes inverted (cf. 2A and C). The more caudal regions, including the midbrain and the mouth, compensate against the direction of the body-turn, 90° clockwise (fig. 2B-C). As a result, the rostral head region in the adult vertebrate is twisted and inverted with respect to the caudal body parts.

The embryological model thus has three components. (1) A 90° anti-clockwise turn, (2) 90° anti-clockwise compensations in the rostral head region and (3) 90° clockwise compensations elsewhere. Note that the anti-clockwise turn may be difficult to discern from the compensatory movements, since vertebrate embryos are not free-swimming but attached to a large yolk mass.
the target, the optic tectum, is not. As a result, the primary molecular markers will have inverted along with the forebrain and therefore lead the tracts in a medial ventral direction. Since the tectum is not located ventral but dorsal and on the opposite side, the axons are subsequently guided across the midline toward the dorsal, contralateral tectum (fig. 2D). The second target of the optic nerves is the thalamus, contralateral to the retina. The thalamic branch splits off behind the optic chiasm.

In the following sections we will show that: 1. The morphology of the forebrain is consistent with a dorsoventral inversion. 2. The hypothesis better explains the pattern of midline crossings and ipsilateral connections in the central nervous system than does any of the existing theories. 3. The axial twist model is consistent with recent observations of the early development of vertebrates: zebrafish and chick. 4. The model also explains morphological peculiarities such as the asymmetric inner organs and the decussation of the trochlear nerve. 5. The twist is predicted by a plausible evolutionary scenario which assumes that the common ancestor of vertebrates turned on its left side and compensated for this in its further development (analogous to some flatfishes).

3. Dorsoventral inversion of the forebrain

The forebrain of vertebrates is in many ways a puzzling structure and is often thought to have evolved independently of the rest of the brain (for example when comparing vertebrates and cephalochordates (the lancelet)). The axial twist hypothesis provides a simple explanation if the forebrain is inverted dorsoventrally with respect to the mid- and hindbrain.

Figure 3 shows a generalised schema of the five-vesicle embryological stage of the vertebrate brain (Von Kupffer, 1906; Nieuwenhuys, 1998). There are four commissures, the cerebellar, posterior and habenular commissures are located dorsally, whereas the anterior commissure is located ventrally. Each of the four commissures is associated with a sensory centre in one of the main subdivisions of the brain. The anterior commissure is associated with the olfactory tract, which is the only sensory system in the central nervous system that targets a ventral brain region. The afferent spinal nerve roots target the dorsal lobes of the spinal chord (cf. fig. 1), the sensory ganglia in the medulla and pons are located dorso-laterally, the cerebellum and tecti mesencephali are located dorsally. The visual tract is a transitional structure since its rostral target, the thalamic LGN, is ventral, whereas its more caudal target, the optic tectum, is located in the dorsal midbrain.

Consequently, the gross anatomy of the forebrain is clearly consistent with an anterior dorsoventral inversion. The transitional twist is rostral from the habenular commissure and caudal from the optic chiasm (arrows in fig. 3).

4. Midline crossings and ipsilateral connections in the nervous system

The pattern of contralateral and ipsilateral representations in the brain is complicated and needs a more detailed treatment. First we should mention, however, that there are many bilateral connections in the central nervous system, between homologous regions on both sides (for example, the four major commissures in the brain, cf. figure 3), as well as for long-range connections (for example the projections of the thalamo-spinal projections in the lamprey; see Discussion). Such bilateral connections have important functions, as mentioned in the Introduction (section 1).

In all vertebrates, extant and fossil, the tractus of the olfactory bulb is the first cranial nerve, n.I (and probably n.0, the terminalis as well), which connects to the telencephalon (Nieuwenhuys et al., 1998; Janvier, 1996). The olfactory tract never crosses, so smell is an ipsilateral sense. This is consistent with the hypothesis telling that both, olfaction and the forebrain, are inverted.

For the visual sense, the situation is more complex. In all vertebrates, the retina is the origin of the second cranial nerve, the optic tract (n.II). In all vertebrates the optic nerves cross or merge in the optic chiasm. The latter is located ventral to the brain, or, in cyclostomes (lamprey and hagfishes) in the ventral part of the midbrain (Nieuwenhuys et al., 1998; Ronan and Northcutt, 1998). As mentioned above, frontal-eyed vertebrates often are specialised for binocular vision. In those cases, each side of the brain receives projections from the contralateral visual hemifield of both eyes, rather than only from the contralateral eye. Otherwise, the vast majority of optic tract fibers projects to the contralateral side of the brain.

The optic tracts project to the optic tectum (=superior colliculus in mammals) of the dorsal midbrain from the contralateral eye (or the contralateral visual hemifield). This is consistent with the hypothesis, because the eyes are inverted but the midbrain is not. In many vertebrates
the telencephalon receives visual inputs. These inputs can come from the optic tectum, or from the lateral geniculate nucleus (LGN) of the thalamus. In mammals and birds, the forebrain is predominant in visual processing. Visual fibers enter directly from the optic chiasm into the contralateral LGN (Nieuwenhuys, 2002). Since the thalamus is part of the forebrain, the projections from the thalamus to the visual cortex do not cross the midline so that the visual cortex receives inputs from the contralateral eye (or contralateral visual hemifield).

In sharks a large part of the telencephalon, the central nucleus, has a visual function (Luiten, 1981b). Interestingly, the central nucleus of nurse sharks represents the ipsilateral eye, as was found in electrophysiological experiments (Cohen et al., 1973). This remarkable difference from other vertebrates is nevertheless consistent with the axial twist hypothesis. The central nucleus gets its input predominantly from the optic tectum, passed via thalamic nuclei (Smets, 1981a,b). Luiten (1981a,b) showed that nerve fibers decussate between the tectum and the thalamus. Thus, in accordance with our hypothesis, the visual information crosses the midline twice: first in the optic chiasm and again in the ventral mesencephalic tegmentum (Luiten, 1981b; Ebbesson and Schroeder, 1971).

Thus, whether the hemispheres of the telencephalon represent the ipsilateral or the contralateral eye depends on whether the visual input comes mainly from the optic tectum (chondrichthyans) or from the LGN (mammals and birds). Note, that none of the existing theories, not even Cajal’s, can explain the ipsilateral representation (with two decussations) in shark brains.

For the auditory system, projections from the rhombencephalic auditory centres (cochlear nuclei, superior olivary complex, nuclei of the lateral lemniscus) to the temporal auditory cortex predominantly cross the midline, to the contralateral hemisphere (van der Loo et al., 2009; Heffner and Heffner, 1989). This is as predicted by our hypothesis.

5. The early development of vertebrates

As explained above (section 2) the model distinguishes three components: the anti-clockwise turn, the anterior anti-clockwise compensations and the more caudal clockwise compensations (fig. 2). Note that the model does not prescribe a temporal order for these occurrences: if the developmental processes would occur in the free-swimming stage, the larva should be adapted to swimming during each phase. The early vertebrate embryos do not swim nor move though. Being fed by a large yolk mass, the selective pressures for the temporal order in which the three processes occur presumably are defined by the level of symmetry obtained for the sensory and locomotor systems in the first free-swimming or free-moving stage. Thus, it can be expected that the opposite clockwise and anti-clockwise compensations in the head region might be the first to develop because these involve heavy twisting after which the anterior head region is effectively inverted.

Compensatory movements must not involve the heart and other inner organs. This is because these are not part of sensory systems or the locomotor system and therefore there is no selective pressure that these organs should be symmetric in the body. Consequently, the clockwise compensations may manifest themselves as an anti-clockwise turn of the bowels with respect the body.

Such anti-clockwise turning movements of inner organs do indeed occur in the early embryological development of vertebrates and involve the heart, stomach, gut, liver and pancreas. This developmental turning of viscera presumably occurs in all vertebrates. Detailed descriptions exist for teleost fish (e.g. Barth et al., 2005) and tetrapods (e.g. Hamburger and Hamilton, 1951).

Brain development in teleosts such as the zebrafish, *Danio rerio*, depends critically on the **organiser** (in birds and mammals known as Hensens’ node). A group of cells in the prospective anterior head region determines the development of the forebrain (Houart et al., 1998). Recently, technical advances have enabled the continuous three-dimensional tracing of large numbers of cells in the live, developing teleost embryo (Rembold et al., 2006; England et al., 2006; Keller et al., 2008). Ultimately, Keller et al. (2008) traced the first 1600 min (26:40 h) of all cells of a zebrafish. Some of the movies show the movements of a group of cells that will form the left eye (cf. their supplementary movie 11). Keller et al. did not describe the movements of the cells of the prospective right eye, and did consequently not report a turning movement of the anterior head region.

A detailed inspection of the published movies (www.embl.de) reveals that almost all cells that will form the right eye are invisible until 880 min (14:40 h) after the first cell division, because they are hidden behind (towards the yolk) the prospective forebrain cells. Between 880–1000 min (14:40–16:40 h), these cells appear (fig. 4 and supplementary material, movies Appendix A.1 and Appendix A.2). During this period, the cell populations that will form the two eyes move by 90° about the body axis. Simultaneous with the rostral movements, cells of the later mid- and hindbrain move rightward, in the opposite direction to the forebrain region (fig. 4). This pattern is fully consistent with the predictions of the model for compensations of an axial turn (fig. 2B-C). At present, the authors have no information as to when and how the left-turn occurs in the zebrafish embryo.

A traditionally well-studied tetrapod embryo is the chick (*Gallus gallus domesticus*). The early development is usually distinguished into standardised stages (Hamburger and Hamilton, 1951; Bellairs and Osmond, 2005). A left-turn occurs between stage 13-17, starting at the head and ending at the tail. From then on, the chick lies with its left side towards the yolk sac. Since this movement involves the entire body, including the heart, it really is a turn and not a series of compensatory movements (fig. 5D). We hypothesise that this left turn is indeed the 90° anti-clockwise turn of the model (fig. 2A-B). Is it possible to identify the
compensatory deformations as well?

It has been known for almost a century (Cooke, 2004) that a transient asymmetric development already occurs at a very early stage (Cooke, 1995). The chick embryo develops perfectly symmetrically up to stage 3. At that stage, Hensen’s node has formed, and a small group of cells from the rostral end of the node moves rostrally to the head process (Duband and Thiery, 1982). Lopez-Sanchez et al. (2001) found that cells from Hensen’s node migrate anteriorly toward the rostral endoderm between stage 4 and 8, where they form a “fountain-like” pattern (fig. 5A). This pattern is highly asymmetrical to the left of the midline. More recently, Kirby et al. (2003) studied a slightly earlier stage where they found that this asymmetric distribution is not yet restricted to the rostral “fountain” but involves the entire forehead region. As a result, Hensen’s node transiently opens to the left in stage 8 (Cooke, 1995, 2004). These rostral asymmetric movements, which occur between stage 4 and 8, match well with the hypothetical anti-clockwise compensations (arrows in fig. 5A1) as envisioned by the model (fig. 2B-C). These movements only involve the rostral head region and therefore are not a part of the anti-clockwise whole-body turn which starts much later, in stage 13.

Asymmetric movements have been observed in the chick embryo between stage 10 and 11 (fig. 5C). In stage 9, the heart begins to develop in the body midline. During stage 10-11 the heart turns anti-clockwise as predicted. As explained above, this turn of the heart reflects the clockwise, compensatory movements indicated by the arrows in figure 5C.

The interpretation that the turn of the heart reflects compensatory movements of the body is not in contradiction with the possibility that an asymmetric heart (and bowels) might be advantageous (Cooke, 2004). On the contrary: had the asymmetric position in the body been disadvantageous, then the heart would have evolved to compensate along with the surrounding tissue at least in some lineages.

We have now seen that the embryology of one teleost and one tetrapod are consistent with the predictions of the axial twist hypothesis. The compensatory movements of the rostral head region in the chick will have to be confirmed experimentally, for example by three-dimensional tracing techniques (Keller et al., 2008). Also, for the zebrafish future analyses of the individual cell movements that will give rise of the two eyes as well as the compensatory movements of the individual cells in the more caudal region should be quantified. Since these predictions are very specific, these observations present strong evidence for the hypothesis. Although some the observations are well-known and well-documented in the literature, there is to our knowledge no alternative theory that predicts them.
Figure 5: Stages of the early embryological development of the chick. (A) Anti-clockwise (embryo’s perspective) compensatory movements in the rostral region between stage 4 and 8 (presented is stage 6). A1: schematic dorsal view of the embryo. The white disk shows the location of a quail graft, implanted 24 h before (stage 3b) at the rostral end of the primitive streak. The dark spots are marked quail cells. The horizontal bars show the locations of transverse sections A2 and A3. Dashed lines mark the primitive streak (midline) and the borders of the region with quail cells in the rostral section (A2), used for alignment of the sections with the dorsal view. A2, A3: the two layers depict ectoderm and endoderm, with quail cells in black. Arrows: hypothetical anti-clockwise movements of rostral head region. (B) Overview of the sections in the stages 9, 10, 11, 13 and 14 of panels C and D. (C) Clockwise compensatory movements during stage 9-11. The ventricle (v) and truncus arteriosus (ta) are drawn in the same orientation in each stage, whereas the embryo migrates around this orientation (arrow). The atrium (a) is located in a different section in after stage 10. Shown are the neural tube (n) flanked by the otic vesicles (from stage 10), the notochord and pharynx (ph), flanked by the two dorsal aortas. e = endoderm. (D) During stage 13-17 a left-turn of the body occurs (presented are stage 13 and 14). As shown by the arrows, the heart also turns. Panel A: adapted from Lopez-Sanchez et al. (2001). Panel B-D: adapted from Bellairs and Osmond (2005). Scale bars are 300 µm.

6. The decussation of the trochlear nerve

In addition to the optic tract there is another cranial nerve that decussates, the trochlearis nerve. Most vertebrates have six muscles to move the eye. These muscles are innervated by cranial motor nerves III (oculomotorius), IV (trochlearis) and VI (abducens). Four of the muscles are innervated by the ipsilateral oculomotorius, one by the ipsilateral abducens nerve (which originates from the hindbrain). The superior oblique muscle is the only one innervated by the contralateral trochlearis motor nerve.

This control of the eye movements is remarkable, because it is highly conserved and because it displays a complex mixture of ipsilateral and contralateral innervation of eye muscles, so that the eyes are controlled by both sides of the mid- and hindbrain (Fritzsch et al., 1990). Nevertheless, eye movements are among the fastest and most accurate that vertebrates can make.

The trochlearis is the only motor nerve with a complete chiasma², and it is the only one to leave the brainstem dorsally, even in the lamprey (Larsell, 1947) and hagfish. This conservation is remarkable because the antag-

²CORRECTION MADE ON 10 MAY 2014: The original version erroneously stated “that decussates”, which is wrong because the branch of the oculomotor nerve (III) that innervates the superior rectus eye muscle does decussate. We thank J. Voogd for kindly pointing this out (see Fig. 17.8 of Nieuwenhuys et al., 2008).
onistic inferior oblique muscle is innervated by the ipsilateral oculomotorius nerve. When Fritzsch and Sonntag (1990) cut the trochlearis in Xenopus embryos, the nerve regenerated, but with a tendency to innervate the ipsilateral eye without any observable behavioural consequences. Also, in mice with a defective netrin receptor Unc5c, the trochlear will partially or fully innervate the ipsilateral superior oblique muscle (Burgess et al., 2006). Thus, there does not seem to exist a functional advantage for the decussation of the trochlear nerve.

Nevertheless, the decussation of the trochlearis nerve never disappeared in evolution. Instead, the trochlearis nerve leaves the brain contralaterally and dorsally at the isthmus rhombomere, just as if its insertion were turned upside-down. In this property, the trochlear shows an intriguing parallel to the optic tract. Whereas the latter originates from the dorsal tectum and decussates ventrally, the trochlear originates from the ventral midbrain, to decussate dorsally.

The explanation provided by the axial twist hypothesis is similar to that for the optic chiasm (see section 2). In the non-twisted vertebrate ancestor the trochlearis nerve will have innervated a muscle in the rostral head region, probably associated with the eye. After the adaptation to the left-turn the target of this trochlear motor nerve has twisted along with the eyes, and is no longer dorsal and ipsilateral, but contralateral.

An interesting question is why the other two nerves, III and VI, do not decussate. The nucleus of the oculomotorius (n. III) is located slightly more rostral than that of the trochlearis (n. IV), but still in the midbrain and more caudal than the twist. A possible explanation is that the superior oblique muscle, that is innervated by the trochlearis nerve, is the only muscle that was already associated with the eye before the axial twist evolved. In this view the target muscles of the abducens and oculomotor nerves were originally not located in the rostral head region, but evolved as eye muscles later.

7. Discussion

The present paper introduced a new hypothesis to explain why vertebrates possess an optic chiasm and why the forebrain is organised contralaterally, except for olfaction. On the basis of the hypothesis, an embryological model was developed. It was shown that the dorso-ventral structure of the brain as well as the pattern of decussating and non-decussating connections between the forebrain and other regions and with the eyes is consistent with the hypothesis. On the basis of the model, predictions were made for the early development of vertebrate embryos, which were tested for the zebrafish and the chick. Finally, it was shown that even the decussation of the trochlearis motor nerve can be explained with the model.

The discussion will treat decussations and chiasms in other brain regions and in non-vertebrate animals. The possible evolutionary scenario of the axial twist will be discussed. In the outlook we will briefly treat developmental malformations and molecular evidence.

7.1. Other decussations and chiasms

Vertebrates are not the only animals with decussating connections and the axial twist hypothesis does not explain all decussating connections in vertebrates.

The optic nerve of insects has two so-called chiasms. However, these chiasms invert the order of the fibres within each optic tract rather than crossing the midline of the body. Thus each side of the insect brain processes predominantly the ipsilateral eye.

Insects like most invertebrates possess a ventral nervous system structured like a rope ladder. The central nervous system of insects does have many bilateral connections, commissures, between the body sides. The growth mechanisms of bilateral connections have been studied in detail in Drosophila and shows many parallels to vertebrates (Evans and Bashaw, 2010). In an orderly temporal sequence of attraction and repulsion axons grow towards the midline and beyond. The decussations of such neurons differ from the decussations that are explained by the axial twist hypothesis. These axons do not grow in rostral or caudal axial directions but medially. Once they have crossed the midline they may commence to grow rostrally or caudally. This is different from the decussations that are due to the axial twist: the axons (for example of the pyramidal tracts) at first grow in an axial direction before they cross the midline.

Neurons in vertebrates that decussate in a manner similar to the decussating neurons of Drosophila, are the spinal and reticular Müller and Mauthner cells of lamprey (Rovainen, 1967). The axons of these cells decussate within the segment of their origin and continue on the opposite side in rostral (Müller) or caudal (Mauthner) directions. It has been proposed that these neurons may be homologous to the Mauthner cells of teleosts (Kimmel et al., 1982). Mauthner cells in teleost fish are reticulospinal cells, developing in the reticular formation (Kimmel et al., 1982). Teleost fish usually possess just a single pair of these huge cells which drive the C-start escape responses.

Unilateral high-frequency electrical stimulation to the ventral thalamus of lampreys (Lampetra fluviatilis) evokes alternating rhythmic activity in the left and right ventral spinal roots (El Manira et al., 1997). One might expect that the stimulation arrives slightly earlier on the ipsilateral side than contralateral to the stimulation, but this is not the case. The contralateral reaction of the spinal root leads the ipsilateral side by about 400 ms. Application of strychnine to the rhombencephalic reticular nucleus speeds up the oscillation (El Manira et al., 1997), but the contralateral side still leads by about 35 ms. Similarly, stimulation of contralateral optic tract evokes a response in the middle rhombencephalic reticular nucleus after 11 ms contralaterally and 38 ms ipsilaterally (Zompa et al., 1999).
and Dubuc, 1996, intracellular recording). These results are as predicted by the axial twist hypothesis. Due to the twisted forebrain, the contralateral thalamo-spinal and retino-rhombencephalic connections behave as if they connect the ipsilateral bodyside, whereas the ipsilateral connections are delayed to compensate the twisted morphology.

7.2. Evolution

An important question is the evolutionary origin and survival of the axial turn that eventually evoked the twisting compensations mentioned earlier. Did the twist evolve in an early vertebrate? In a chordate, or even an early deuterostome?

Our two most distant living vertebrate relatives are the jawless lampreys and hagfish. Adult hagfish (Ronan and Northcutt, 1998) and lampreys (Nieuwenhuys and Nicholson, 1998) both possess an optic chiasm. The chiasm in lampreys and hagfish differs from that of gnathostomes in that it is located inside the ventral brain. In Lampetra fluviatilis, the majority of rostrally projecting spinal chord neurons have decussating axons (Tang and Selzer, 1979). Similarly, in hagfish 90% of the optic tract neurons decussate to the contralateral optic tectum (Ronan and Northcutt, 1998; Kishida et al., 1987; Wicht and Northcutt, 1990). The diencephalon receives bilateral visual inputs (Wicht and Northcutt, 1990). Nevertheless, the general structure and connections of the forebrain of both, lampreys and hagfish, is clearly contralateral as in gnathostomes. Besides, Janvier (Janvier, 1996; Janvier et al., 1991) presented evidence for a left-sided heart in osteostreans, a group of early, jawless vertebrates, similar to the left-sided heart of extant vertebrates (cf. fig. 5). Thus, the conclusion that the axial twist evolved in a common ancestor of extant vertebrates, including lampreys and hagfish, seems justified.

The apparent ancientness of the axial twist places enormous difficulty on the question after the original mechanism. Why did a common ancestor of the extant vertebrates turn on its side? There are at least two conceivable scenarios. A deep-bodied animal may have turned on the side, with some resemblance to the evolution of extant flatfishes. Alternatively, a flattened animal may have turned towards an upright position (fig. 6).

The first scenario (fig. 6A) requires that an early, deep-bodied (laterally flattened) clade of vertebrates has existed which was active, free-swimming. It should have possessed paired eyes and nostrils, but no paired appendages. This animal may have become flat-bodied to hide on the sea floor, in order to overcome predation. Crucially, the deep-bodied ancestor must have had its eyes and nostrils below the body axis, because only then would these compensate in the direction of the turn and land on a crossed position, as in figure 6A3.3 Thus, the first scenario predicts that the ancestor was an active animal that foraged by sight and smell from the sea floor and therefore had these senses placed ventrally or ventro-laterally, close to the mouth. It might have rested and hidden on its left side, lying on the sea floor.

The second scenario (fig. 6B) is complementary to the first. A flat, bottom dwelling animal might have turned on its left side. This might have been advantageous for anguilliform swimming close to the seafloor, or for capturing swimming prey or even for filter feeding. In becoming more active, it might have abandoned the bottom-dwelling orientation altogether. Again, to evolve a crossed position of the eyes and nostrils requires that the resulting, deep-bodied form had eyes and nostrils below the body axis. This scenario would be consistent with a theory by Dzik, who found that an early cambrian animal, Yunnanozoon lividum, was possibly a vertebrate with bilateral ventro-rostral eyes (Chen et al., 1995). According to Dzik (1995), the precambrian ancestor of Yunnanozoon might have been a precambrian Dickinsonia. The ventro-rostral position of the presumed eyes and the deep-bodied form of Yunnanozoon, and the flattened bottom-dwelling dickinsonians would match well with the axial twist hypothesis.

The axial twist hypothesis is not consistent with the calcichordate hypothesis (Jefferies, 1986), which proposes that a sessile ancestor (Dexiothelecia) of solutes (an extinct clade of blastozaan echinoderms David et al., 2000) lied down on its right side and gradually became more symmetric via cephalochordates to vertebrates. Jefferies’ phylogeny (Jefferies, 1997) is not supported by phylogenetic and fossil evidence (Lefebvre, 2005; David et al., 2000). Although echinoderm embryos do turn to the left, their complex twisting is very different to that in vertebrates (de Lussanet, 2011).

The asymmetrically developing Cephalochordates (such as Amphioxus) are more closely related to vertebrates than are echinoderms (Bourlat et al., 2006; Delsuc et al., 2006). The mouth of cephalochordates develops at first on the left side of the body before it migrates (anti-clockwise) to the ventral position of the adult. Note that this is the inverse of vertebrates. Cephalochordates only possess median, non-paired, visual and olfactory organs (small cell clusters) and show no signs of a twist (Lacalli, 2004). However, since cephalochordates are laterally flattened, the mouth appearing on the left side and the first gill slits on the other, they match well with Dzik’s Dickinsonia hypothesis, and so with the scenario of figure 6B.

Thus, although at present the evolutionary mechanism behind the axial twist hypothesis is open, there are at least two plausible mechanisms in accordance with the observations from different sources including morphology, embryology and paleontology. Of these two, one bears resemblance to the evolution of extant flatfishes, whereas the

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3This can be understood if one imagines an ancestor which had its eyes above the midline. In that case, the shortest distance up for the left eye would be to migrate clockwise like the mouth and no twist would have evolved.
Figure 6: Possible evolutionary scenarios. Panels show schematic frontal views displaying eyes, nostrils, mouth, and fin rays. One eye and
one nostril are dotted to show that they do flip bodysides. (A) A deep bodied, free-swimming, early vertebrate (1) turned on its left side
(2), for example to hide on the sea floor like a flatfish. The direction of compensation (3) of the mouth and external body parts (e.g. tail fin
and dorsal and anal fins) is clockwise (perspective of the animal), as indicated with filled arrows. The eyes and the nostrils migrate in the
opposite, anti-clockwise, direction (open arrows). (B) A benthic animal (1) might have turned on its left side to swim stretches or to capture
prey (2). A more active form specialised on the locomotory orientation compensated the turn, again with the eyes and nostrils compensating
anti-clockwise (open arrows), and the mouth and fins compensating clockwise (closed arrows, panel 2-3).

latter is backed by an interesting proposal of Dzik (1995) and might provide a link to the asymmetric development
of lancelets, as explained above.

7.3. Outlook

The evolution and development of body asymmetries and left-right and dorso-ventral patterning in the body
has long intrigued biologists. This interest has strongly re-
vived since the development of molecular techniques. Since
the early works (Levin, 1998; Brown and Wolpert, 1990)
a large number of transcription factors have been linked
with early dorso-ventral and left-right patterning, and it
has become clear that these processes are guided by com-
plex hierarchical interactions which begin very early in de-
velopment (cf. fig. 5A) and in which the organiser plays
a central role (Hamada et al., 2002; Rodríguez-Gallardo
et al., 2005; Aldworth et al., 2011; Levin, 2004; Lee and
Anderson, 2008; Raya and Izpisua Belmonte, 2004; Raya
and Belmonte, 2006).

At present, no study has viewed these results from a
perspective that the pattern in the anterior region is op-
posite to more caudal regions, as suggested by the present
work. However there are results to suggest that the molec-
ular patterning may agrees with the axial twist hypothesis.
The homeobox transcription factor \( Gsh2 \) (which regulates
\( Pax5 \) and \( Dbx1 \)) is expressed in the vertebrate dorsal neu-
ral tube and the ventral forebrain (Wilson and Maden,
2005). Hibino et al. (2006) mention that \( Pitx \) genes play
a central role in left-right patterning. \( Pitx2 \) is located
on the left bodyside in birds and mammals, but on the
right rostral from Hensen’s node (Schlueter and Brand,
2007). These results suggest that the axial twist model
may present a useful model for analysing and interpreting
the developmental patterning in vertebrates.

The axial twist hypothesis also presents a model that ex-
plains why some developmental anomalies, such as a \( situs inversus viscerum \) originate in the asymmetric formation
of the organiser (cf. fig. 5A) long before development of
the heart. Also, it explains why chicks that turn to the
right always develop \( situs inversus \) (von Baer, 1828).

Holoprosencephaly is a common and dramatic devel-
opmental malformation of the forebrain (Matsumaga and
Shiota, 1977; Fernandes and Hébert, 2008). In severe
forms, the forebrain remains severely underdeveloped. In
so-called interhemispheric holoprosencephaly the cerebral
hemispheres have a left-right orientation instead of the
normal antero-dorsal orientation (Simon et al., 2002). This
orientation is consistent with a failure of the anti-clockwise
compensations in the forebrain region. These examples
show that the axial twist hypothesis may provide helpful
in shedding new light on the mechanisms underlying com-
mon developmental malformations.

Asymmetric development and twisting movements are
common in the animal kingdom. Among the deu-
terostomes, vertebrates (this manuscript), echinoderms
(de Lussanet, 2011), cephalochordates (see above), but
also tunicates develop strongly asymmetric, whereas hemi-
chordates seem to be the only major clade to develop sym-
metrically. Among other bilaterians, many molluscs twist
strongly and obtain so a looped gut. In this light, the axial
twist hypothesis for vertebrates is not extraordinary.
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Appendix A. Additional Files

The additional movies can be viewed and downloaded from FigShare.com, doi: 10.6084/m9.figshare.24104085.

Appendix A.1. Additional movie 1: low.mov

Supplementary movie A.1 is based on the supplementary movie S2 of Keller et al. (2008), (with permission) © Keller www.embl.de/digitalembryo. For this we extracted a number of movie frames from their supplementary Movie S2. During this period (865-1000 min) the cells that will form the prospective eye cell masses, migrate clockwise (encircled blue and red respectively), whereas the future mid- and hindbrain rotate anti-clockwise (green arrows). The prospective right eye cell mass (blue) is initially invisible because it is hidden by the embryo. Starting from 865 min, every 15-min a frame was marked. For accurate playback, please select “Play All Frames” in the QuickTime Player. Note that the beginning and end of the movie are sped-up.

Appendix A.2. Additional movie 2: selected.mov

Movie A.2 is a sped-up version of Additional movie 1, to show more clearly the axial compensatory movements. Advice for repeated playing: select “Loop” in your Quick-time player.