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REVIEW

Evolutionary Conservation of the Early Axon Scaffold in the Vertebrate Brain

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The early axon scaffold is the first axonal structure to appear in the rostral brain of vertebrates, paving the way for later, more complex connections. Several early axon scaffold components are conserved between all vertebrates; most notably two main ventral longitudinal tracts, the tract of the postoptic commissure and the medial longitudinal fascicle. While the overall structure is remarkably similar, differences both in the organization and the development of the early tracts are apparent. This review will bring together extensive data from the last 25 years in different vertebrates and for the first time, the timing and anatomy of these early tracts have been directly compared. Representatives of major vertebrate clades, including cat shark, *Xenopus*, chick, and mouse embryos, will be compared using immunohistochemistry staining based on previous results. There is still confusion over the nomenclature and homology of these tracts which this review will aim to address. The discussion here is relevant both for understanding the evolution of the early axon scaffold and for future studies into the molecular regulation of its formation. *Developmental Dynamics 244:1202–1214, 2015*. © **2015 Wiley Periodicals, Inc.**

Key words: evolution; medial longitudinal fascicle; tract of the postoptic commissure; tract of the posterior commisure; pioneering axons; embryonic

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Introduction

A deeper understanding of many developmental processes during embryogenesis comes from the comparison between different model organisms. For example, this has been important for understanding human development, origin of body structures, and conservation of gene function (Murakami et al., 2001; van den Akker et al., 2008; Parker et al., 2014).

During development of all Bilaterians, nerve connections need to be precisely made to form a fully functional organism. The initial nerve connections within the vertebrate brain were first identified in the early part of the 20th century based on silver staining studies (reviewed in Nieuwenhuys, 1998), but were only termed the early axon scaffold in the early 1990s, following work in zebrafish using antibody staining and axon tracers to reveal neurons and axons (Chitnis and Kuwada, 1990; Wilson et al., 1990). The early axon scaffold is made up of longitudinal, transversal, and commissural tracts that are a basic feature of all vertebrates. Moreover, this array of pioneering axons forms a scaffold for the later, follower axons. Building a scaffold of pioneering axons is an ancient, evolutionary conserved mechanism to ensure these follower axons project along the correct path. This mechanism is also apparent in invertebrates (reviewed by Reichert and Boyan, 1997), but it is unclear whether this simple scaffold evolved independently or was present at the last common ancestor for Bilaterians.

The arrangement of the early axon scaffold in different vertebrates has been reviewed previously (Easter et al., 1993; Nieuwenhuys, 1998; Barreiro-Iglesias et al., 2008); however, a direct comparison of these early neurons and tracts, as well as a comparison of timing in the major model organisms is still lacking. The aim of this review is to bring together the work from the last 25 years describing the anatomy of the early axon scaffold in the vertebrate brain and providing a direct comparison by visualizing the formation of the early axon tracts in different vertebrates using pan-neural antibodies. Many of the early axon tracts have been poorly characterized and there is still confusion over the nomenclature and homology of these tracts, which this review will address. The comparison of these vertebrates, along with key studies in invertebrates highlights that the formation of a pioneering scaffold has remained highly conserved throughout evolution. This comparative review will not only provide a future reference for studies on patterning and axon guidance but also provide assistance for analysis of mutant organisms.

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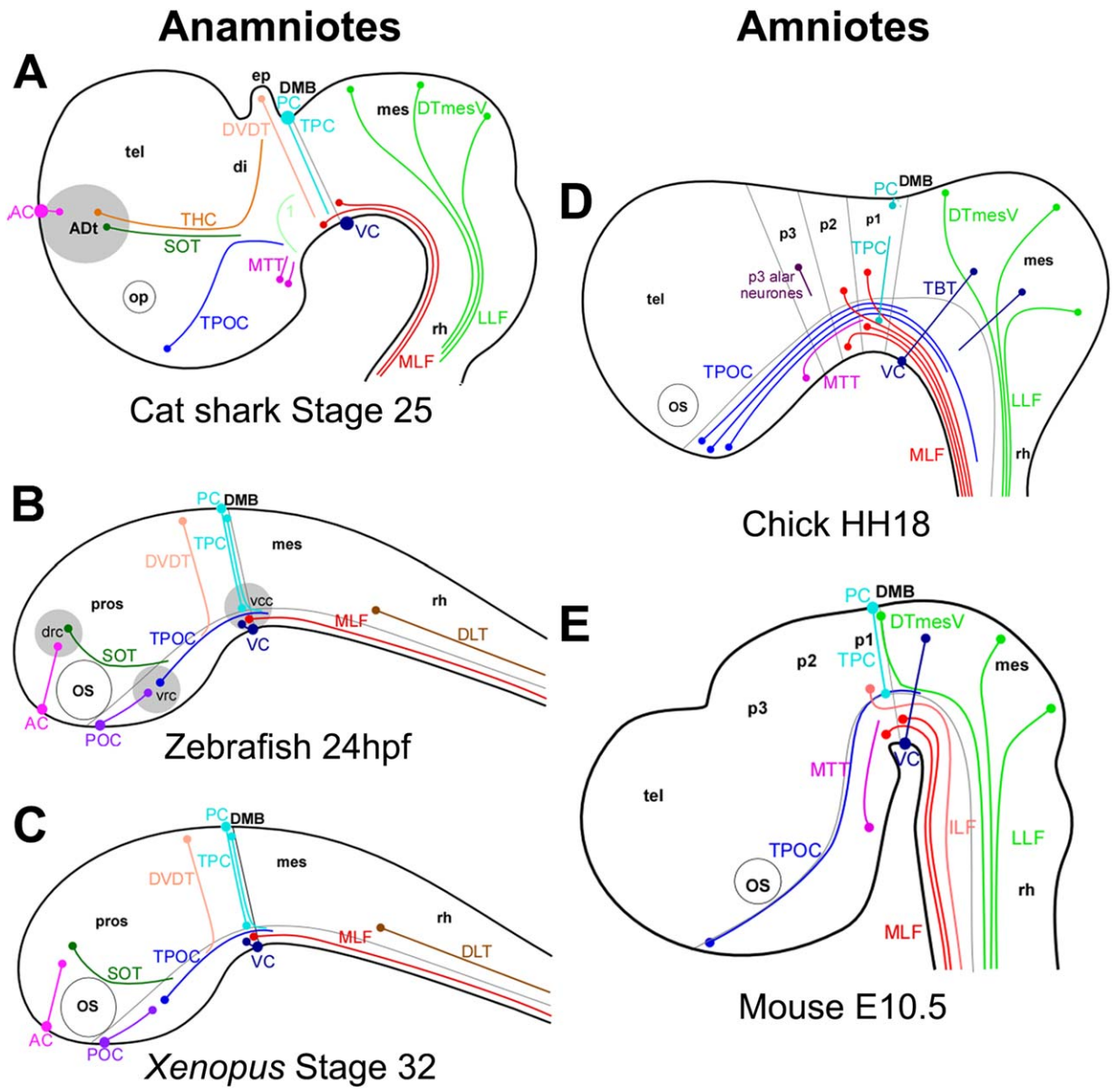

Fig. 1. Schematics of the established early axon scaffold in the vertebrate brain of model organisms. Each tract that forms in the anamniotes or amniotes is colour coded to show where the neurons are located and the projection of their axons. **A**: Cat shark, stage 25 (80 somites) (adapted from Ware et al., 2014b). **B**: Zebrafish, 24 hpf. Gray circles highlight neuronal populations and older terminology for population names. **C**: *Xenopus* stage 32 (26 somites). **D**: Chick HH18 (31 somites) (adapted from Ware and Schubert, 2011). **E**: Mouse E10.5 (33–37 somites). Transversal gray lines mark the prosomeric boundaries. Longitudinal gray line marks the alar/basal boundary. For abbreviations, see Table 1.

The Early Axon Scaffold is an Evolutionary Conserved Structure in the Vertebrate Brain

The early axon scaffold has been well-studied in zebrafish, chick, and mouse (e.g., Chitnis and Kuwada, 1990; Easter et al., 1993; Ware and Schubert, 2011). Descriptions are also available in a variety of other vertebrates such as sea lamprey (Kuratani et al., 1998; Barreiro-Iglesias et al., 2008), cat shark (Ware et al., 2014b), medaka (Ishikawa et al., 2004), turbot (Doldan et al., 2000), axolotl (Eagleson et al., 2001), *Xenopus* (Hartenstein, 1993; Anderson and Key, 1999), and alligator (Pritz, 2010).

Briefly, a common feature of all vertebrates is the ventral longitudinal tract (VLT) system formed of the medial longitudinal fascicle (MLF) and the tract of the postoptic commissure (TPOC). Other common features include, the tract of the posterior commissure (TPC) forming the posterior commissure (PC) and the descending tract of the mesencephalic nucleus of the trigeminal nerve (DTmesV) which is a defining feature of the dorsal mesencephalon. Prominent commissures also include the postoptic commissure (POC), the ventral commissure (VC), and the anterior commissure (AC) (Fig. 1).

In a relatively short developmental time during early axon scaffold formation, the brain increases in complexity and this will continue throughout the development of the embryo. Generally, when the early axon scaffold is established in anamniotes, it is composed of five main tracts: MLF, TPOC, dorsoventral diencephalic tract (DVDT), TPC, and supraoptic tact (SOT), and four commissures: PC,

TABLE 1. Abbreviations Used Throughout the Text and Figures

1	Uncharacterized tract 1
ADt	Anterior dorsal telencephalic neurons
AC	Anterior commissure
di	Diencephalon
DLT	Dorsolateral longitudinal tract
DMB	Diencephalic-mesencephalic boundary
DTmesV	Descending tract of the mesencephalic nucleus of the trigeminal nerve
DVDT	Dorsoventral diencephalic tract
ep	Epiphysis
FR	Fasciculus retroflexus
LLF	Lateral longitudinal fascicle
mes	Mesencephalon
MLF	Medial longitudinal fascicle
MRB	Mesencephalic-rhobencephalic boundary
MTT	Mammilotegmental tract
nMLF	Nucleus of the medial longitudinal fascicle
nMTT	Nucleus of the tract of the mammilotegmental tract
nTPOC	Nucleus of the tract of the postoptic commissure
op	Olfactory pit
os	Optic stalk
p1, p2, p3	Prosomere 1, prosomere 2, prosomere 3
PC	Posterior commissure
POC	Postoptic commissure
pros	Prosencephalon
rh	Rhombencephalon
SM	Stria medullaris
SOT	Supraoptic tract
TBT	Tectobulbar tract
tel	Telencephalon
THC	Tract of the habenular commissure
TPC	Tract of the posterior commissure
TPOC	Tract of the postoptic commissure
VC	Ventral commissure
III	Oculomotor nerve
V	Trigeminal nerve
V1	Ophthalmic profundus nerve

POC, AC, and VC (Fig. 1A–C). The early axon scaffold in amniotes is composed of five main tracts: MLF, TPOC, mammilotegmental tract (MTT), TPC, and DTmesV pioneering the lateral longitudinal fascicle (LLF), with the PC and VC as the main commissures (Fig. 1D,E). In terms of timing, the early axon scaffold in cat shark has been described as being fully established at stage 25 (Fig. 1A; Ware et al., 2014b), in zebrafish at 24 hr post fertilization (hpf) (Fig. 1B; Chitnis and Kuwada, 1990; Wilson et al., 1990), in *Xenopus* at stage 32 (Fig. 1C; Anderson and Key, 1999), in chick at Hamburger and Hamilton stage (HH)18 (Fig. 1D; Ware and Schubert, 2011), and in mouse at embryonic day (E)10.5 (Fig. 1E; Mastick and Easter, 1996). For simplification, a diagram of the established early axon scaffold in each vertebrate has been produced in a box form for easy comparison of each tract and reveals similarities in the formation of each tract (Fig. 2). The box for each vertebrate represents the rostral brain, which has been subdivided into the telencephalon (tel), diencephalon (di), and mesencephalon (mes). Each arrow represents an early tract in the brain and the direction of axon projection. As highlighted previously, the development of the early axon scaffold has remained highly conserved throughout evolution from the divergence between nonjawed and jawed vertebrates to the divergence of mammals. Figure 2 will be used throughout the review to highlight the similarities and subtle differences in tract formation between the vertebrates.

Clustered Organization of the Early Axon Scaffold Neurons

These early tracts are built up from a small set of neuronal clusters developing at distinct positions in the embryonic brain. Previously, in zebrafish, three clusters of neurons are described as differentiating at specific gene expression boundaries in the rostral brain and are termed the ventrocaudal cluster (vcc), ventrorostral cluster (vrc), and dorsorostral cluster (drc) (Fig. 1B; Ross et al., 1992; Macdonald et al., 1994). The same organization of neuronal clusters is present in other vertebrates and also termed, the nucleus of the MLF (nMLF) that corresponds to the vcc, while the nucleus of the TPOC (nTPOC) corresponds to the vrc (Chedotal et al., 1995). For ease of comparison, we use the latter terminology (placing an "n" in front of the tract name) to describe neuronal populations for all vertebrates discussed here. The drc located in the dorsal telencephalon, will be referred to as the Anterior Dorsal telencephalic (ADt) neurons (Gao et al., 2012).

Direct Comparison of the Early Axon Scaffold in Major Vertebrate Models

To highlight similarities and differences during early axon scaffold formation, a side-by-side comparison is shown using immunohistochemistry to label the early tracts in representatives of major vertebrate taxa. These include both anamniotes: cat shark (*Scyliorhinus canicula*) and frog (*Xenopus laevis*); and amniotes: chick (*Gallus gallus*) and mouse (*Mus* musculus). Three stages of each species are shown to highlight the first neurons (Fig. 3), an intermediate stage (Fig. 4) and the fully established scaffold (Fig. 5) based on previous data (Hartenstein, 1993; Mastick and Easter, 1996; Anderson and Key, 1999; Ware and Schubert, 2011; Ware et al., 2014b).

Although the cat shark is not an established model organism, it is included as a representation of cartilaginous fish at the beginning of the phylogenetic tree of jawed vertebrates used for this comparison (Fig. 2). While a brief description of the early axon scaffold has been described in cat shark (Kuratani and Horigome, 2000; Ware et al., 2014b), the direct comparison with other vertebrates will help to further determine the evolutionary conservation of the early axon scaffold.

Previously, many antibodies have been used to label the early axon scaffold neuron populations and axon tracts. Different antibodies/antigens may have selective specificity for certain neurons/tracts, or may label neurons at different stages of differentiation. For simplicity of this direct comparison, the pan-neural antibody, Tuj1 (anti-βIII tubulin; R&D systems MAB1195; 1:1,000) is used to label the differentiating and mature neurons as well as the axons, but not the neuronal precursor cells (Lee et al., 1990) in cat shark, chick, and mouse. As the *Xenopus* embryonic brain expresses βII tubulin instead of βIII tubulin (Moody et al., 1996), Tuj1 does not label any neurons or axons in *Xenopus* (data not shown). Instead,

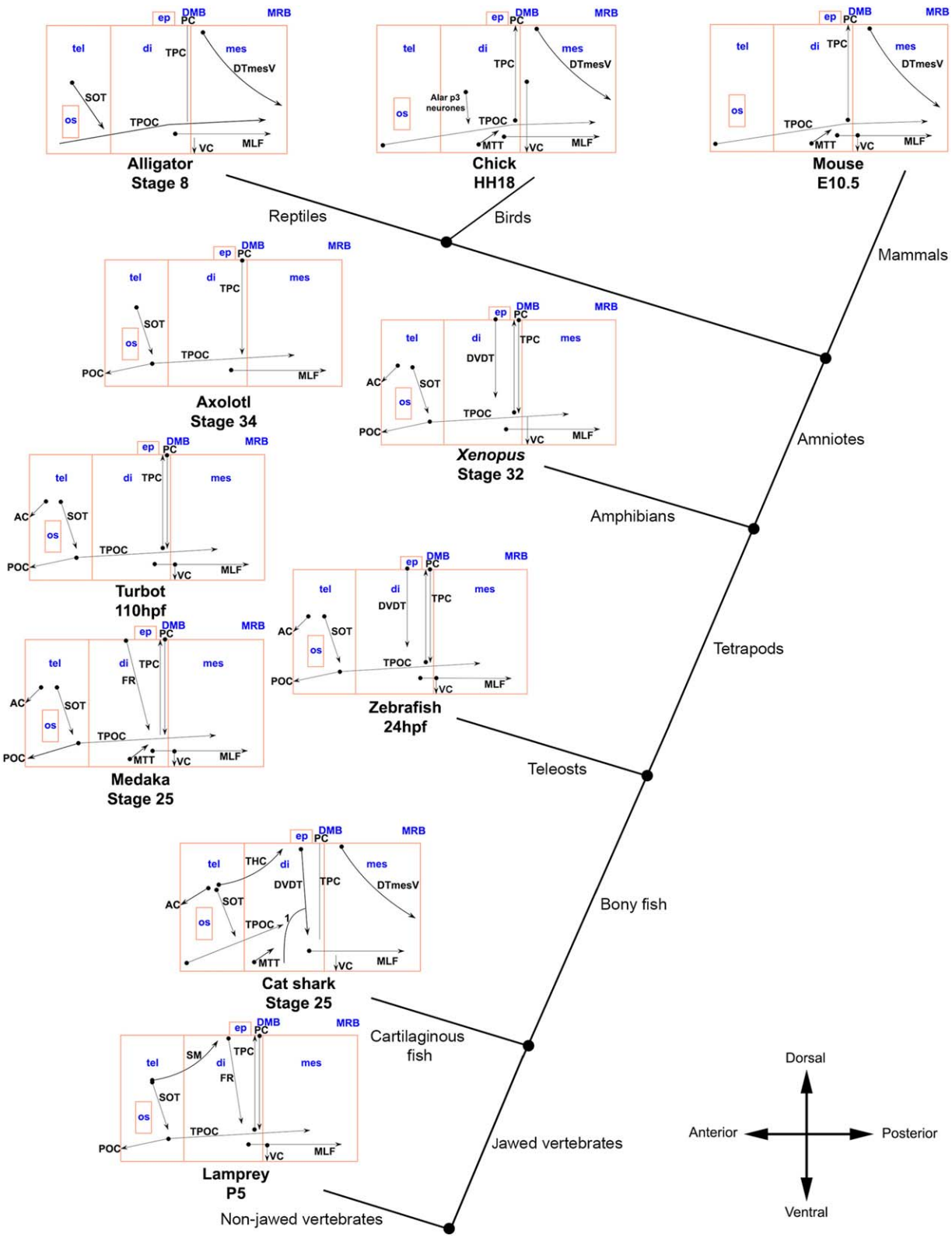

Fig. 2. Schematic representation of the early axon scaffold tracts throughout vertebrate evolution. Lamprey is an example of a nonjawed vertebrate. The first divergence represented for jawed vertebrates is cartilaginous fish with cat shark. The next divergence is for the bony fish with zebrafish, turbot and medaka shown as representatives. The next major divergence is for the tetrapods, with *Xenopus* and axolotl representing amphibians. The next divergence to be made is the amniotes. Chick and alligator are examples for birds and reptiles, respectively. With further divergence for the mammals, mouse is used as an example. The neuron origin is represented by a black circle and the arrowheads indicates the direction of the axon projection from their neurons (where known). For abbreviations see Table 1.

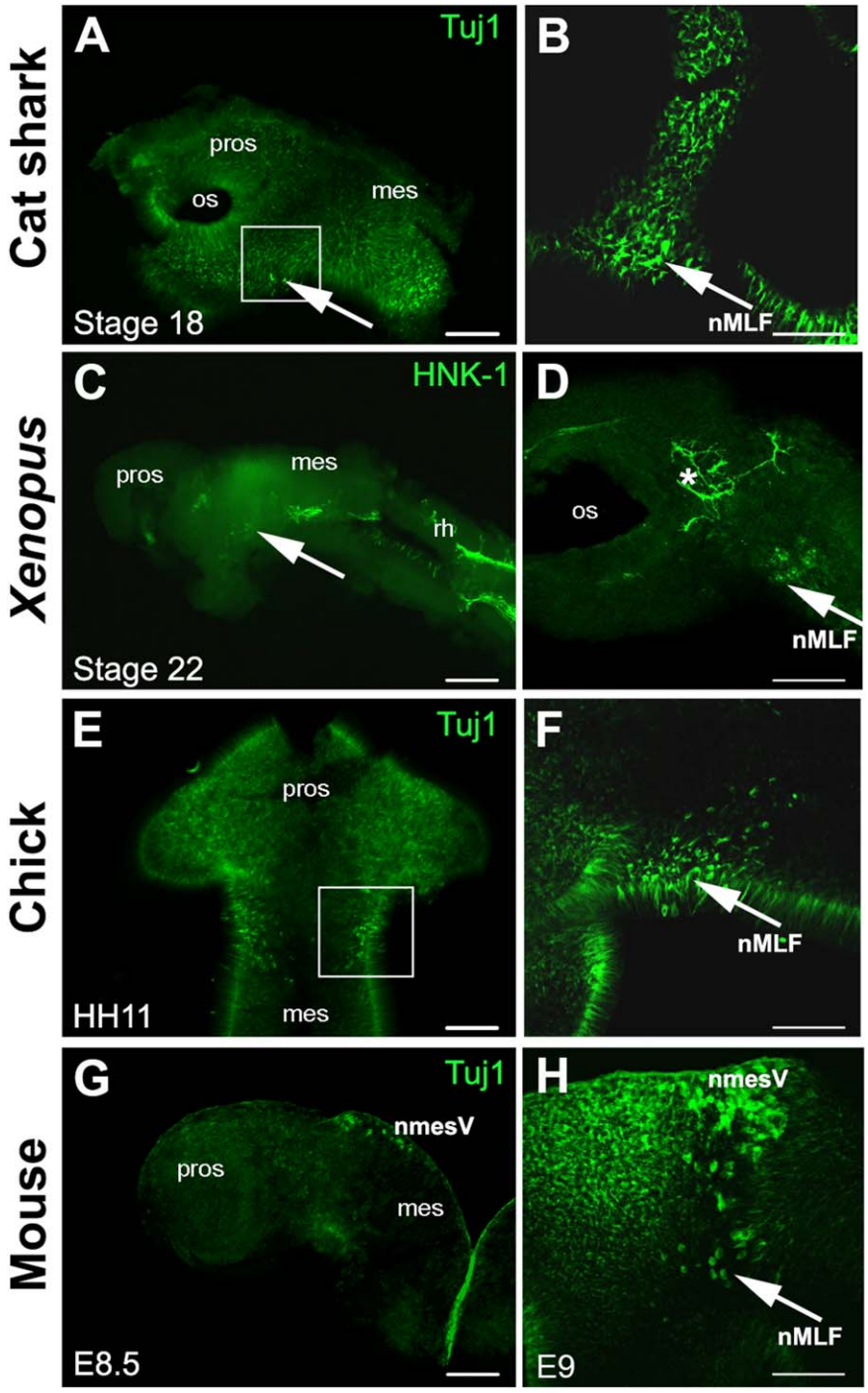

Fig. 3. Comparison of the initial neurons that arise in the cat shark, *Xenopus*, chick, and mouse embryonic brains. **A–F**: The first neurons to arise in the rostral brain are MLF neurons in (A,B) cat shark (Tuj1) at stage 18 (arrow) (adapted from Ware et al., 2014b), (C,D) *Xenopus* (HNK-1) at stage 22 (arrow), asterisk shows peripheral staining, and (E,F) chick (Tuj1) at HH11 (arrow) (adapted from Ware and Schubert, 2011). **G**: In mouse (Tuj1), the first neurons give rise to the DTmesV at E8.5. **H**: The first MLF neurons appear at E9 (arrow). A,E: White box indicates higher magnification from the same embryo in B and F. For abbreviations, see Table 1. Scale bars = 100 μm.

associated surface glycoprotein HNK-1 (Sigma C6680; 1:500) is used to label the axon tracts in *Xenopus*. The pan-neural marker HuC/D (Molecular probes A21271; 1:500) labels the neuronal cell bodies and is used to compare the positioning of the nMLF in cat shark, *Xenopus*, chick and mouse (Fig. 5C,F,I,L). The protocol for immunohistochemistry has been described previously (Lumsden and Keynes, 1989; Ware et al., 2014b).

The Ventral Longitudinal Tract (VLT) has Remained the Most Conserved Tract Throughout Evolution

The VLT forms in the basal plate of the rostral brain, initially consisting of the MLF and TPOC, and is present in all vertebrates

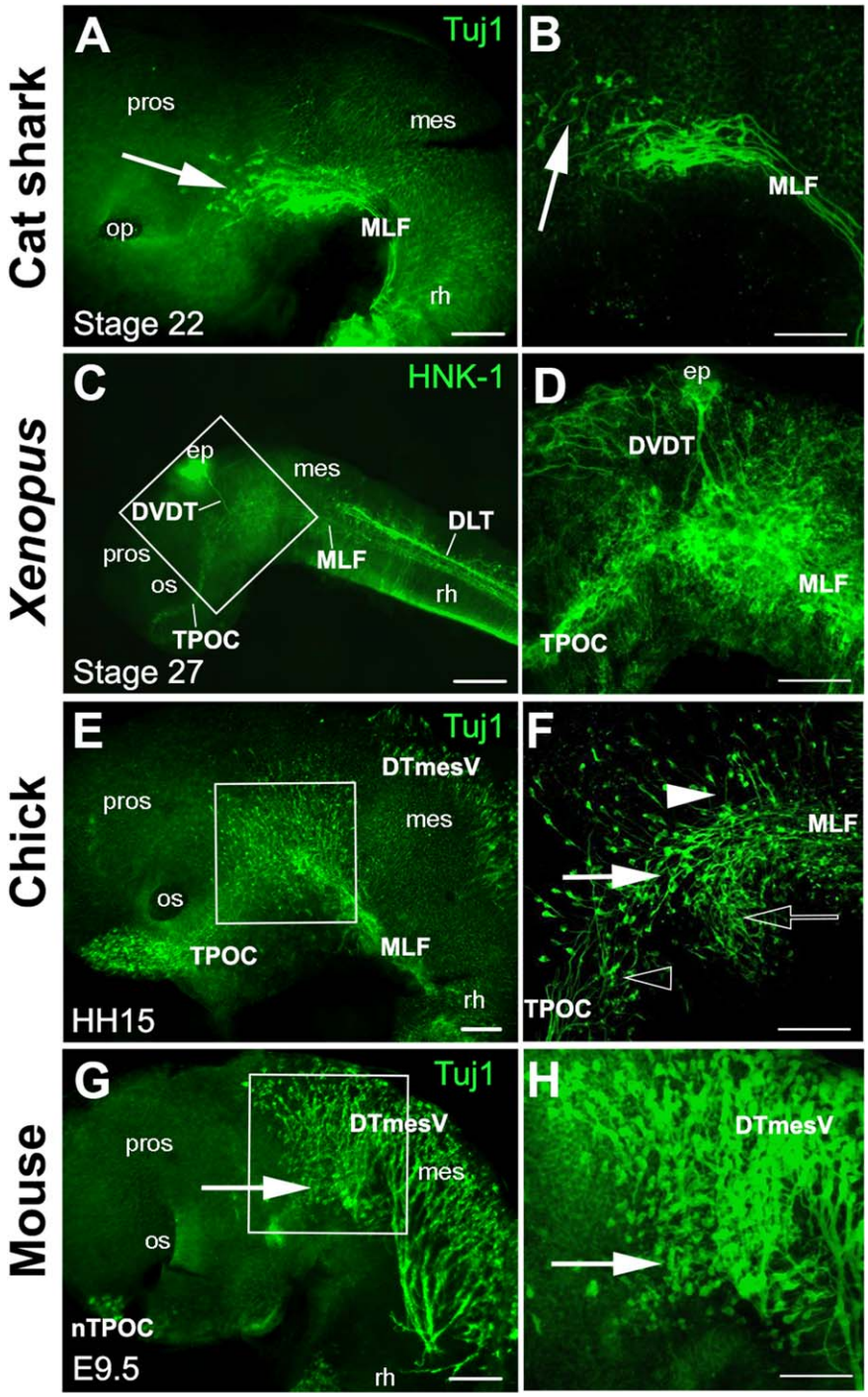

Fig. 4. Comparison of the developing early axon scaffold at an intermediate stage in the cat shark, *Xenopus*, chick, and mouse embryonic brains. All images are in lateral view of whole-mount embryos. More neuron populations are forming and neurons are projecting axons to form tracts. **A,B**: Cat shark (Tuj1), Stage 22 (adapted from Ware et al., 2014b), scattered neurons located rostral to the MLF axon tract (arrow). Scattered neurons are present, rostral to the MLF tract (arrow in B). **C,D**: *Xenopus* (HNK-1), Stage 27. The DVDT, TPOC and DLT have formed tracts. **E,F**: Chick (Tuj1), HH15. The TPOC and DTmesV have started forming tracts. The MLF is formed from three populations of neurons: central (arrow), ventral (unfilled arrow) and dorsal (arrowhead). MTT neurons are located rostrally to the MLF neurons (unfilled arrowhead). **G,H**: Mouse (Tuj1), E9.5. Ventral neurons located in the diencephalon and mesencephalon that belong to the nMLF (arrow). C,E,G: White box indicates higher magnification from the same embryo in D, F, and H. For abbreviations see Table 1. Scale bars = 100 μm.

studied (Figs. 1, 2). In some vertebrates, the MTT also forms part of the VLT (Mastick and Easter, 1996; Ware and Schubert, 2011).

The MLF neurons are the first neurons that appear just rostral to the diencephalic-mesencephalic boundary (DMB), forming the nMLF in cat shark at stage 18 (Fig. 3A,B, arrow), *Xenopus* at stage 22 (Fig. 3C,D, arrow) and chick at HH11 (Fig. 3E,F, arrow). This is the case for all other vertebrates studied, expect mouse, which differs because the DTmesV neurons appear first at E8.5

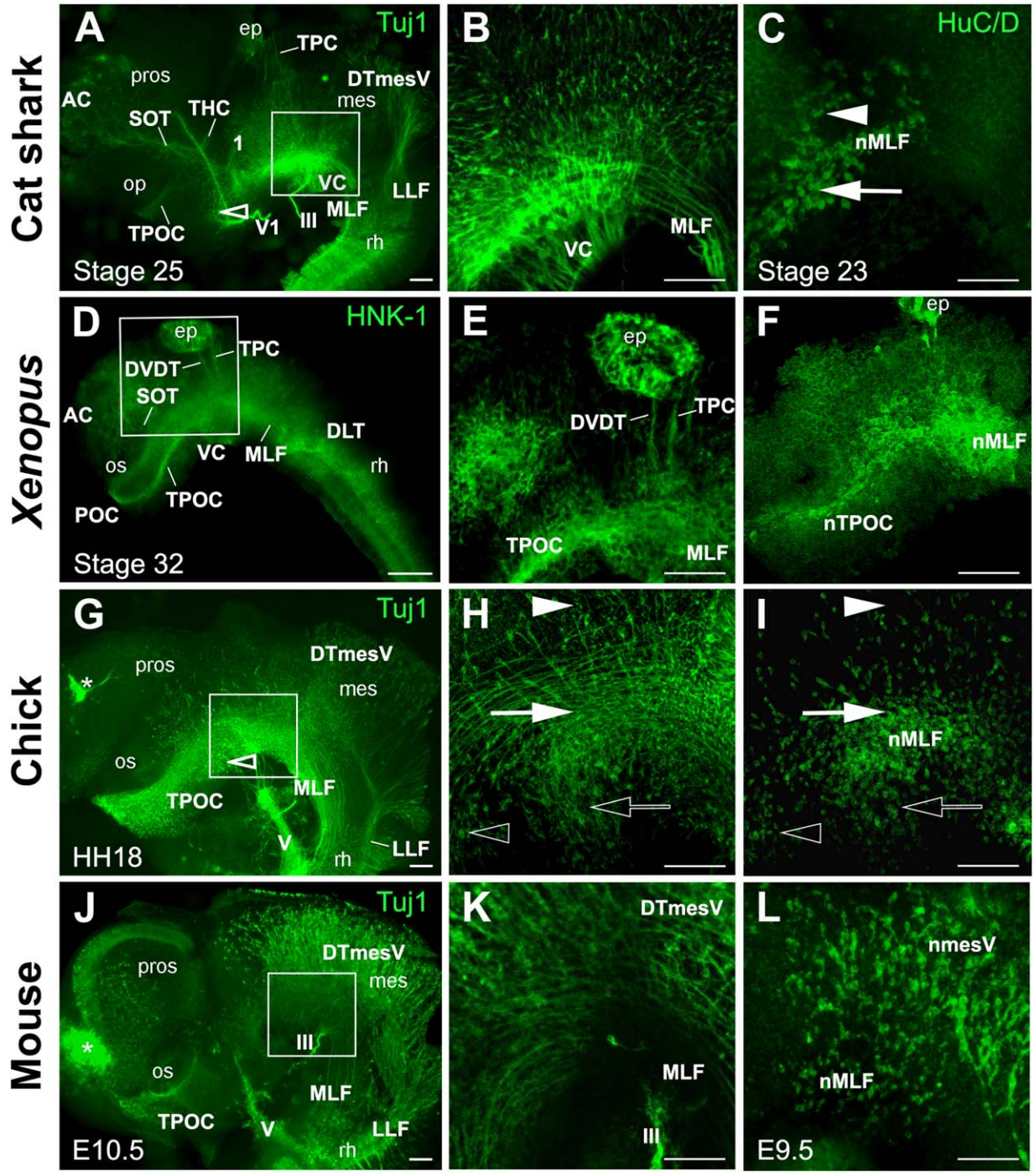

Fig. 5. Comparison of the established early axon scaffold in the vertebrate embryonic brain. All images are in lateral view of whole-mount embryos. The early axon scaffold is well established. **A,B**: Cat shark (Tuj1) at Stage 25 (adapted from Ware et al., 2014b). **D–F**: *Xenopus* (HNK-1) at stage 32. **G–I**: Chick (Tuj1) at HH18 (adapted from Ware and Schubert, 2011). **J,K**: Mouse (Tuj1) at E10.5. G,J: Asterisk: olfactory placode and constitute of the terminal nerve ganglion. Organization of the MLF neuronal populations are highlighted using HuC/D which labels the neuronal cell bodies in all the vertebrates here. **C**: Cat shark, stage 23. Two nMLF populations: ventral (arrow) and dorsal (arrowhead). F: *Xenopus*, stage 32. I: Chick. The MLF is formed from three populations of neurons: central (arrow), ventral (unfilled arrow) and dorsal (arrowhead). MTT neurons are located rostrally to the MLF neurons (unfilled arrowhead). **L**: Mouse, E9.5. MLF neurons are located ventrally (arrow), while DTmesV are present throughout the mesencephalon. A,D,G,F: White box indicates higher magnification from the same embryo in B, E, H and K. For abbreviations, see Table 1. Scale bars = 100 μm.

along the dorsal midline of the mesencephalon (Fig. 3G), while the MLF neurons appear later at the DMB around E9 (Fig. 3H, arrow).

A possible reason for the difference in the nMLF timing between mouse and other vertebrates could be due to the timing in which the embryos are exposed to their surrounding environment. In particular, zebrafish are required to forage for food and avoid predators after only 4–5 days of development (Patterson et al., 2013). Zebrafish and *Xenopus* embryos are exposed to the

TABLE 2. Temporal Appearance of the Early Axon Scaffold Neurons and Axon Tracts in the Rostral Vertebrate Brain[a]

	Sea-lamprey	Cat shark	Zebrafish	*Xenopus*	Chick	Mouse
Axon Tract	Developmental stage of initial neuronal appearance					
MLF	E7-E8	Stage 18	16 hpf	Stage 22	HH11	E9.5
TPOC	E12	Stage 23	17 hpf	Stage 24-25	HH13	E9.5
DTmesV	Later	Stage 23	2-5 dpf	Stage 47	HH14	E8.5
TPC	P1	Present at stage 25	20 hpf	Stage 30	HH18	E10.5
SOT	E12	Present at stage 25	20 hpf	Stage 32	Later	E11.5
POC	P2-P3	Later	22 hpf	Stage 27	Later	Later
AC	Later	Present at stage 25	22 hpf	Stage 28	Later	Later
DVDT	Not known	Stage 23	22 hpf	Stage 26-27	Later	Later
VC	E8-E9	Present at stage 25	18 hpf	Stage 32	HH17	E10.5
MTT	Not known	Present at stage 25	Not known	Not Known	HH15	E10.5
DLT	E7-E8	Later	20 hpf	Stage 25	Not known	Not known
FR	P2-P3	Not known	Later	Not known	Not known	Later
SM	P2-P3	Later	Later	Later	Not known	Later
THC	Later	Present at stage 25	30 hpf	Later	Not known	Later

[a]Where known, the stage when each tract first appears in the lamprey, cat shark, zebrafish, *Xenopus*, chick, and mouse brains is indicated. In some cases, there is evidence the axon tract or commissure forms but the time of appearance is not known (Later). In other cases, it is not known whether the tract appears (Not known).

environment much sooner than mouse embryos that are protected by a uterus until birth. As the MLF has been suggested to be involved in swimming movement and escape mechanisms in zebrafish (Gahtan et al., 2002, 2005; Sankrithi and O'Malley, 2010), it is possible the MLF is required first to set up this pathway to allow the embryo to move.

In cat shark and chick, the MLF forms from three populations of neurons (Figs. 4B,F, 5C; Ware and Schubert, 2011; Ware et al., 2014b), while in other vertebrates the number of MLF neuronal populations is unclear. In mouse, it is especially difficult to distinguish which neurons will form the MLF as there are already so many neurons scattered along the DMB and the neurons located most ventrally have not projected any axons at E9 (Fig. 3H, arrow). The exact location of the nMLF in relation to the neuromeric organization (Puelles and Rubenstein, 2003) has been described in detail in the chick brain (Ware and Schubert, 2011). Using *Pax6* expression that marks the alar diencephalon, the MLF neurons are strictly diencephalic in chick, located in prosomeres 1 and 2 (p1 and p2), unlike in mouse where DiI labeling confirms the MLF neurons differentiate within p1 and the mesencephalon (Mastick and Easter, 1996; Mastick et al., 1997). In zebrafish, the location of the MLF neurons are also located within the *Pax6* expression domain in the diencephalon (Hjorth and Key, 2001). Without analyzing boundary markers, an exact location for the nMLF in cat shark and *Xenopus* cannot be determined. Given the high conservation of the MLF, the nMLF is most likely to differentiate in the diencephalon of the other lower vertebrates, therefore, in Figure 2 the origin of the MLF is positioned in the diencephalon of all vertebrates.

The MLF axons in all these vertebrates project caudally in a tightly fasciculated bundle along the floor plate toward the rhombencephalon (Figs. 4, 5). In cat shark, at stage 22, the MLF is still the only axon tract present in the brain (Fig. 4A), whereas at the intermediate stages of the other vertebrates more neuronal populations have started to differentiate such as the TPOC and DTmesV (Fig. 4C,E,G). In mouse, by E10.5, the MLF axon tract is present but not clearly separated from the DTmesV (Fig. 5J,K). The gap between the MLF and DTmesV axon tracts contains TPOC axons, as well as circumferential descending axons (cda) or intermediate longitudinal fascicle (ILF) axons that cross the midline and turn caudally (Fig. 1E; Mastick and Easter, 1996; Farmer et al., 2008).

The other ventral tract making up the VLT, the TPOC, forms from the nTPOC located in the rostral basal hypothalamus of cat shark at stage 23 (Ware et al., 2014b), *Xenopus* at stage 25 (Anderson et al., 1998), chick at HH13 (Ware and Schubert, 2011), and mouse at E9.5 (Fig. 4G). In contrast to the densely clustered TPOC neurons at the ventral midline in the cat shark, chick, or mouse (Figs. 4E,G, 5A), the TPOC neurons in the *Xenopus* brain are located more caudally in a chain-like manner (Figs. 4D, 5F), similar to zebrafish (Chitnis and Kuwada, 1990). The TPOC axons project ventral to the optic stalk and caudally toward the DMB in all vertebrates. Generally, the axons reach the MLF to form the VLT connecting the prosencephalon with the mesencephalon (Figs. 1, 5D,G), except in cat shark (Fig. 5A) and mouse (Fig. 5J) where the TPOC axons do not project directly with the MLF. In mouse, the TPOC axons project along the alar–basal boundary (Shimamura et al., 1995) and the axons project with the DTmesV once they reach the mesencephalon rather than the MLF (Mastick and Easter, 1996).

The nucleus of the mammilotegmental tract (nMTT) is the second cluster of neurons to form in the hypothalamus just rostral to the MLF neurons in chick (Fig. 4F, open arrowhead) arising at HH14 (Puelles et al., 1987) and E10 in mouse (Easter et al., 1993). In cat shark, the MTT tract appears but development of these neurons is unclear (Ware et al., 2014b). During early development, the MTT is not present in the zebrafish or *Xenopus* brains (Fig. 1; Table 2). Neurons do form later in the mammillary hypothalamus where the MTT neurons differentiate, however, the homology of these tracts has yet to be determined (Wolf and Ryu, 2013). The

only anamniote to form such a tract is in medaka, where the ventral diencephalic tract (VDT) appears to be the MTT homolog (Fig. 2). It is not clear why this population is present in the medaka compared with other anamniotes, further analysis would be required to confirm the MTT homolog.

The positioning and formation of all three neuronal clusters occurs in very specific regions of the ventral brain. The formation of the TPOC and MTT neurons within the hypothalamus has previously been discussed (Ware et al., 2014a).

Formation of the DTMESV and Innervation of the Jaw Muscles

The DTmesV is a large axonal structure that forms in the mesencephalon of cat shark at stage 23 (Ware et al., 2014b), chick at HH14 (Ware and Schubert, 2011), and mouse at E8.5 (Fig. 3G). However, the DTmesV is clearly missing in the *Xenopus* brain at these early stages (Fig. 5D). The DTmesV projects from the mesencephalic nucleus of the trigeminal nerve (nmesV) neuronal population located along the dorsal midline of the mesencephalon. Initially, processes project ventrally before turning caudally to pioneer the LLF (Fig. 1A,D,E). The DTmesV afferents enter the trigeminal nerve and are required for conveying sensory information from the jaw muscles to help determine the positions of the lower and upper jaws to coordinate biting and mastication (Chedotal et al., 1995; Hunter et al., 2001). In mouse, the DTmesV processes enter the trigeminal nerve at E15.5 (Mastick and Easter, 1996).

The DTmesV appearance first in mouse is unusual as the DTmesV forms after the MLF in other mammals, such as, cat, rat, and human (Easter et al., 1993); therefore, a more in-depth analysis is required to understand the difference in timing.

In most anamniotes, the DTmesV forms later in development, for *Xenopus* at around stage 47 (Kollros and Thiesse, 1985) and zebrafish at 3–5 days postfertilization (dpf) (Kimmel et al., 1985). Although, a recent study suggests the DTmesV neurons are specified much earlier by 1 dpf using RNA probes in zebrafish but has yet to be determined using antibodies (Dyer et al., 2014). Among anamniotes, only the cat shark has evidence of this tract during the formation of the early axon scaffold (Figs. 1A, 5A). It remains to be shown why the DTmesV forms early in cat shark, not in zebrafish or *Xenopus* but reappears during early development in chick and mouse.

The dorsolateral longitudinal tract (DLT) forms within the rhombencephalon during early development in zebrafish (Ross et al., 1992) and in the *Xenopus* brain, present at stage 27 (Fig. 4C). The DLT has been suggested to be equivalent to the DTmesV/LLF system in chick and mouse (Barreiro-Iglesias et al., 2008). However, in both mouse and chick the LLF is pioneered by the DTmesV in the mesencephalon (Fig. 5G,J), while the DLT is initially formed by axons from the rhombencephalic Rohan-Beard neurons (Metcalfe et al., 1990). This would suggest the DLT is not homologous to the DTmesV, but is in fact another tract.

The formation and function of the DTmesV has remained conserved throughout vertebrate evolution; however, the timing of tract appearance varies. Generally, these neurons differentiate during early axon scaffold development in amniotes, while appearance is later in the anamniotes (Fig. 2; Table 2).

Timing of Commissural Formation is not Conserved During Vertebrate Evolution

Commissures form from axons that project across the midline of the brain and are essential for communication between the two sides of the brain. In anamniotes, commissures in the rostral brain form during early axon scaffold formation, whereas in amniotes most commissures form later. A recent review has discussed the development of commissures during vertebrate evolution (Suárez et al., 2014). However, their focus remained on the adult brain, although they discuss many commissures that form during the establishment of the early axon scaffold.

There are two highly conserved commissures in the telencephalon, the AC and POC (Fig. 2). The AC is present in both cat shark (Fig. 5A) and *Xenopus* (Fig. 5D), forming from the ADt neurons located in the dorsal telencephalon. The AC axons project rostrally toward the rostral midline, dorsal to the optic stalk. The AC is not yet visible in the chick or mouse (Fig. 5G,J), but does form later (Serafini et al., 1996; Bardet et al., 2010). Surprisingly, the AC does not form during embryonic development in the lamprey (Fig. 2), but is present in the adult (Suárez et al., 2014). The POC forms early in the *Xenopus* telencephalon (Fig. 5D,E), like in most other anamniotes (Fig. 2). The POC forms as axons project rostrally from the nTPOC, ventral to the optic stalk toward the rostral midline where these axons connect the contralateral TPOC axon tracts (Hofmeister and Key, 2013). The POC is not evident in chick and mouse at these early stages (Fig. 5G,J), but does form across the rostral midline later in chick (Ware and Schubert, 2011) and by E14 in mouse (Croizier et al., 2011). In cat shark, a previous study suggests the POC forms early (Kuratani and Horigome, 2000), however, the POC is not clear in a more recent study (Ware et al., 2014b).

The TPC is another highly conserved transversal tract that forms in the caudal diencephalon marking the DMB and pioneering the PC (Fig. 2). The TPC tract is present in cat shark by stage 25 (Fig. 5A), in *Xenopus* by stage 32 (Fig. 5D), in chick by HH18 (Ware and Schubert, 2011) and in mouse by E10.5 (Mastick and Easter, 1996). The TPC axons project from two populations of neurons in anamniotes, one located dorsally and one located ventrally at the DMB (e.g., Ross et al., 1992). In amniotes, the TPC neurons are located ventrally forming the nTPC and project axons dorsally where the axons will cross the midline (mouse; Mastick and Easter, 1996; and chick; Ware and Schubert, 2011). Neurons located dorsally also appear to contribute axons to the TPC in both chick and mouse (Mastick and Easter, 1996; Ware and Schubert, 2011). It is unclear where the TPC neurons are located in the cat shark brain, again due to the high conservation of this tract we would assume the neurons are similarly located in dorsal and ventral populations. This is also likely to be true in medaka; the authors are unclear whether the dorsal mesencephalic tract (DMT) tract is a homolog to the DTmesV or the TPC (Ishikawa et al., 2004). There are several lines of evidence that suggest this tract is part of the TPC: the neurons are located dorsally in close proximity to the PC, the axons project along the MLF and the axons never enter the trigeminal.

The VC is a highly conserved commissure present in all vertebrates (Fig. 2), although the presence of this tract is unclear in the axolotl (Eagleson et al., 2001). In chick, the VC forms at the ventral midline just caudal to the DMB, at HH17 (Ware and Schubert, 2011) and in mouse, cda axons pioneer the VC at E10.5 (Mastick

and Easter, 1996). While, the location of these neurons needs more investigation in other vertebrates, in cat shark, the VC is present by stage 25 (Fig. 5A) and in *Xenopus*, the VC is present at stage 32 (Fig. 5D) forming from the TPOC axons (Anderson et al., 1998).

While all vertebrates form commissures during early development in the brain, the timing of their appearance is varied (Fig. 2). Timing differences may be due to differences in the requirement for sharing information between the two hemispheres or that other longitudinal and transversal tracts are essential for processing information and, therefore, form first.

The Dorsal Prosencephalic Axon Tracts are Less Conserved During Vertebrate Evolution

The anamniote prosencephalon is highly abundant in tracts compared with amniotes during development of the early axon scaffold (Figs. 1–5). The SOT is another tract to arise from the ADt neurons of anamniotes including cat shark, zebrafish, and *Xenopus* (Fig. 1A–C) and is highly conserved among anamniotes (Fig. 2). The SOT axons project from the ADt neurons ventrally toward the TPOC in cat shark and *Xenopus* (Fig. 5A,D), but is not yet present in chick at HH18 (Fig. 5G) and mouse at E10.5 (Fig. 5J). Although the ADt neurons are not present, the SOT arises later in chick (Ichijo and Kawabata, 2001), alligator (Pritz, 2010), and mouse at E11.5 (Nural and Mastick, 2004). This suggests a possible homologous neuronal population may be present later. A structure similar to the ADt is not known in the amniote telencephalon during the formation of the early axon scaffold. The transcription factor *Emx3* is specifically expressed by the ADt neurons and required for differentiation of the ADt neurons in zebrafish and has been identified in *Xenopus tropicalis* (Viktorin et al., 2009). The expression of *Emx3* is only present in some tetrapods and not in amniotes (Derobert et al., 2002). This maybe a possible reason for the lack of ADt neurons in the amniote brain, as *Emx3* expression is not present for the differentiation of these neurons.

The DVDT arises from neurons in the epiphysis that project axons ventrally to join the TPOC, where they turn and project rostrally (Wilson and Easter, 1991). This axon tract is present in both the anamniotes here (cat shark; Ware et al., 2014b and Xenopus; Fig. 5D,E), but is clearly lacking in both the amniotes (chick, Fig. 5G; and mouse, Fig. 5J). In amniotes, such as chick, alligator, and mouse, where the DVDT is not present, loss of this axon tract could be due to a change in function of the pineal gland from a photosensitive role to a hormonal role (Kardong, 2009). Although some authors have suggested the DVDT is present later in both chick and mouse, the data are not hugely convincing (Shimamura et al., 1995; Ichijo and Kawabata, 2001).

Other tracts that form in the telencephalon include the tract of the habenular commissure (THC) in cat shark (Fig. 5A), but this tract is not present at early stages in the other vertebrates here (Fig. 2). The THC does form later in other vertebrates, for example, zebrafish (Ross et al., 1992). The stria medullaris (SM) is present in lamprey only, while the fasciculus retroflexus (FR) is present in lamprey and medaka during early development (Fig. 2). The THC, SM, and FR tracts do form later in some vertebrates (Table 2; Ishikawa et al., 2004; Nural and Mastick, 2004; Suárez et al., 2014). The habenular nuclei along with the SM and FR have previously been described as part of the dorsal diencephalic conduction system involved in a diverse range of cerebral function (Bianco and Wilson, 2009).

There is a clear difference in the early axon scaffold formation between anamniotes and amniotes (Fig. 2). Amniotes have fewer tracts during these initial stages in the telencephalon compared with anamniotes. This difference has not been investigated, but could potentially be due to amniotes forming a far more complex cerebral cortex and undergoing different brain morphology changes compared with anamniotes.

Role of the Early Axon Scaffold as Pioneering Tracts

Several studies, particularly in zebrafish and *Xenopus*, highlight the pioneering function of early tracts like the TPOC. The SOT axons project ventrally from the telencephalon and turn caudally when the axons encounter the TPOC. Ablation of the TPOC (by lesioning the nucleus of the TPOC) in the *Xenopus* embryonic brain, causes some of the SOT axon tracts to overshot ventrally instead of turning caudally (Anderson and Key, 1999). The interaction between pioneering and follower axons is likely to be mediated by cell adhesion molecules, specifically *NOC-1* and *NOC-2*, which are expressed by both the ventral TPOC axons and the SOT axons in *Xenopus* (Anderson and Key, 1996, 1999). In the caudal diencephalon of both zebrafish and *Xenopus*, the TPC axons project from the dorsally located neurons ventrally toward the TPOC. When the TPC axons encounter the TPOC they turn caudally and project with the TPOC. After ablation of the TPOC in zebrafish, the TPC axons often grew along aberrant pathways, particularly using the MLF instead of the missing TPOC for guidance (Chitnis and Kuwada, 1991; Chitnis et al., 1992). *Cyclops* mutant zebrafish where embryos are missing the floor plate confirm the importance of the TPOC for guiding other axon tracts (Patel et al., 1994). As the growth of the TPOC and MLF are affected, the DVDT axons projecting from the epiphysis make aberrant projections once they reach the TPOC, and the TPC axons projecting from the dorsal neurons make errors. In addition, *Pax6* mutants show an axon guidance phenotype, as the TPOC axons are misrouted into the alar plate and fail to cross the p2/p3 boundary (Mastick et al., 1997; Nural and Mastick, 2004), subsequently also affecting the pathfinding of SOT axons (Nural and Mastick, 2004). A possible reason for this could be due to loss of *R-cadherin* expression, a cell adhesion molecule regulated by *Pax6* (Andrews and Mastick, 2003). These results suggest that the TPC and SOT use the TPOC axon tract for guidance, although the presence of some correctly projecting TPC and SOT axons following TPOC ablation point to other axon guidance cues present in the brain to ensure these axons project along the correct path. Live imaging studies have shown the first axons that project to pioneer the axon tract are not only important for the projection of the follower axons but also behave differently (Bak and Fraser, 2003). The function of the TPOC and MTT axon tracts as scaffolds for later axons in amniotes has been reviewed previously (Ware et al., 2014a).

Establishment, Origin, and Evolution of the Early Axon Scaffold

By comparing the early axon scaffold in many vertebrates, it is clear that there is very little divergence of the structure (Fig. 2).

Of interest, there are notable differences in the timing and appearance of some tracts (Table 2). It is evident that in the amniote embryonic brain, fewer axon tracts form during early axon scaffold development than in the anamniote brain, particularly in the rostral prosencephalon (Figs. 1, 2). This could be due to a difference in developmental time of the embryo and the complexity of higher amniote vertebrates. It may be advantageous during evolution to have fewer tracts involved in the guidance of follower axons to provide less room for error or this could also be due to the transition from water to land.

As the early axon scaffold is present in both nonjawed and jawed vertebrates, it would suggest these tracts appear in a common ancestor before the divergence of nonjawed and jawed vertebrates. The axon tracts that are conserved during early development between the nonjawed and jawed vertebrates are the MLF, TPOC, SOT, TPC, POC, and PC (Fig. 2; Table 2). Hagfish is a vertebrate sister group that diverged before the split between nonjawed and jawed vertebrates. Analysis of the adult hagfish brain reveals the presence of the POC, AC, and HC (Suárez et al., 2014) and the MLF (Ronan, 1989). Although there is no available information on the formation of a scaffold in the developing embryo, we would assume that the appearance of these tracts arises during embryogenesis, further confirming these commissures and the MLF are well conserved through vertebrate evolution and suggesting the common ancestor for many of these tracts is further back in evolution.

This review highlights an evolutionary conserved role for the VLT in the guidance of other tracts. In anamniotes, most of the transversal tracts (SOT, DVDT, and TPC) use the VLT for guidance (Chitnis and Kuwada, 1991; Hjorth and Key, 2002). Therefore, it could be that the VLT forms the initial axon scaffold and many of the other axon tracts are simply follower axons. This would explain why the VLT tract has been so well conserved through evolution. Furthermore, a VLT-like structure is present in amphioxus, a small worm-like marine animal that diverged from the last common chordate ancestor (Lacalli et al., 1994). Although the tracts do not appear to be homologous with the vertebrate tracts, the neurons appear to have a similar function (Suzuki et al., 2015). Interestingly, scaffolds of axon tracts are also present in many protostomes. In particular, arthropods such as spider (Linne and Stollewerk, 2011), grasshopper (Boyan et al., 1995), *Drosophila* (Boyan et al., 2003), and centipede (Whitington et al., 1991) are well studied. Similar to vertebrates, an initial scaffold of pioneering tracts, including commissures and descending pathways, is set up during embryogenesis. These tracts act in the same way to guide axons along the correct path (Reichert and Boyan, 1997). There is an ongoing debate whether the central nervous systems of arthropods and vertebrates are homologous, derived from the last common bilaterian ancestor, or evolved in parallel (e.g., Arendt et al., 2008). The molecular organization of the vertebrate dorsal nerve tube and the arthropod and annelid ventral nerve cord, however, is remarkably similar (Denes et al., 2007). It is interesting to note that a ventral descending tract from the brain into the trunk likewise is a shared feature.

Concluding Remarks and Perspectives

Much like we need a road map for directions, later axons also require a "map" of tracts to guide them along the correct path. This is particularly important as some axons have to travel over long distances to reach their target. This "map" or early axon scaffold has remained conserved throughout evolution with many tracts conserved in both nonjawed and jawed vertebrates, with a similar scaffold structure being present much further back in evolution.

The correct formation of the early axon scaffold requires that neurons differentiate at specific positions in the developing brain, and at specific stages. Differentiation of neurons into small clusters is largely controlled by the Notch/proneural network (Ratié et al., 2013). While the differentiation of neurons during spinal cord development is well documented (Matise, 2013), the molecules involved in specifying many of the early axon scaffold neurons is not well known. Studies in zebrafish, *Xenopus*, chick, and mouse have started to reveal some of the molecules governing the formation of the early axon scaffold (Anderson and Key, 1999; Hjorth and Key, 2002; Farmer et al., 2008; Wolman et al., 2008; Riley et al., 2010; Gaudin et al., 2012). Understanding the evolution of the early axon scaffold will help interpret the molecular interactions between signaling molecules, transcription factors and axon guidance molecules that are important for the correct positioning of the developing neurons and their axons.

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