

Review

Brain segmentation and trigeminal projections in the lamprey; with reference to vertebrate brain evolution

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Abstract

Vertebrate brains exhibit remarkable diversity in each animal group, reflecting evolutionary changes at the molecular-level developmental program of the nervous system that vertebrates have experienced. We focused on the developmental morphological plan of the brain to understand the evolutionary scenario that led to the above diversity. By comparing the organization of the brain of non-vertebrate chordates, cyclostomes and gnathostomes, a step-wise modification of brain patterning programs becomes apparent. Furthermore, by labeling the lamprey oral region, the somatotopic projections of the trigeminal nerve that enter into the hindbrain become visible. Finally, by combining the knowledge on rhombomere segments and neuronal projections, the evolutionary relationship between somatotopy and brain segmentation are discussed.

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1. Introduction

The embryonic vertebrate brain is subdivided into a series of segmental structures referred to as neuromeres [2]. In gnathostomes, each neuromere has been shown to represent a center of cell proliferation, early differentiation and migration, and functions as a developmental unit that produces a specific set of neurons [17,21].

Amphioxus is a basal chordate whose nerve cord shows a similar pattern of differentiation along the anteroposterior axis

as vertebrates, and has a similar repertoire of neuronal cell types [5,7]. There are also some similarities in the expression patterns of some regulatory genes in the nerve cord, including the *Hox* genes [20,23]. However, in amphioxus, the anterior, brain-like region of the nerve cord has neither a neuromere-like segmentation nor neuromere-specific gene expression patterns [6,12]. For this reason, neuromeres are thought to have been acquired by the vertebrate lineage after their divergence from the amphioxus.

Living agnathans, i.e., the lampreys and hagfishes, are thought to have diverged early from the remaining vertebrates, and therefore, represent the closest living forms to early vertebrates. They are, therefore, the animals of choice for identifying

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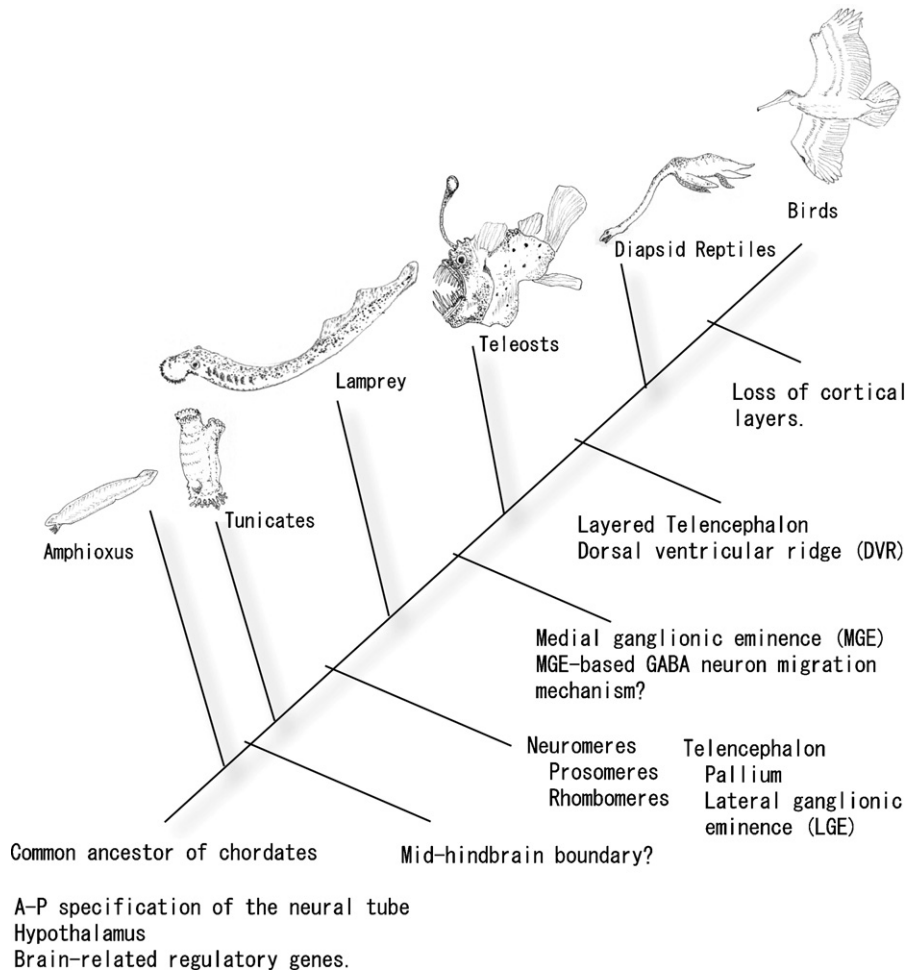


Fig. 1. Presumptive evolutionary process of the vertebrate brain. Through the evolutionary process, a step-wise modification of brain patterning mechanisms becomes apparent. The recent molecular phylogeny has suggested that tunicates are the closest group to vertebrates [4]. In tunicates, *LjPax2/5/8* is expressed adjacent to the *Otx* domain, suggesting that the mid-hindbrain boundary appears to be established in basal chordates.

events in brain evolution that occurred after the divergence of amphioxus but before the radiation of gnathostomes. In this study, we include own data in embryos of the Japanese lamprey, *Lethentelon japonicum*. We summarize the expression patterns of several regulatory genes in the lamprey, present recent data about the trigeminal projections to the hindbrain, and discuss the origin of somatotopy in relation to the brain segments.

2. Evolution of the vertebrate telencephalon

The telencephalon is a defining trait of vertebrates, and it shows a highly diverse morphology in each lineage of animals: its relative size and shape tends to vary in each vertebrate group, more conspicuously than any other parts of the brain, some showing quite unusual specializations. In teleosts, for example, the roof plate of the telencephalon expands laterally and resembles to the fourth ventricle of the hindbrain due to a phenomenon called ‘eversion’ [24]. The amniote telencephalon is characterized by its layered structure, with the most extreme situation in the neocortex of mammals that is differentiated into six layers. Reptiles have a unique structure called the dorsal ventricular

ridge (DVR) that receives inputs from the thalamus, similar to a mammalian cortex (Fig. 1).

Despite the diversified shape and morphology, the telencephalon of vertebrates appears to be based upon a shared developmental program established in the common ancestor from which all the vertebrate species should have originated. According to the analysis by Puelles and colleagues [16,17], the telencephalon can be divided into several segments based on morphology and expression of regional marker genes. The two major telencephalic subdivisions are the pallium and the subpallium. The pallium is organized into four main radial subdivisions: the medial, dorsal, lateral, and ventral pallium. The subpallium is also subdivided further into the striatum and pallidum. The progenitor zone of the striatum is known as the lateral ganglionic eminence (LGE). The pallidal domain, which derives from progenitor cells in the medial ganglionic eminence (MGE), lies below the striatal domain, and includes the globus pallidus, ventral pallidum, and some other nuclei.

Generally, in gnathostomes, genetic markers for these regions are as follows. *Emx* and *Pax6* are expressed in the medial, lateral and dorsal pallium, *Pax6* in the ventral pallium, *Dlx* in the LGE,

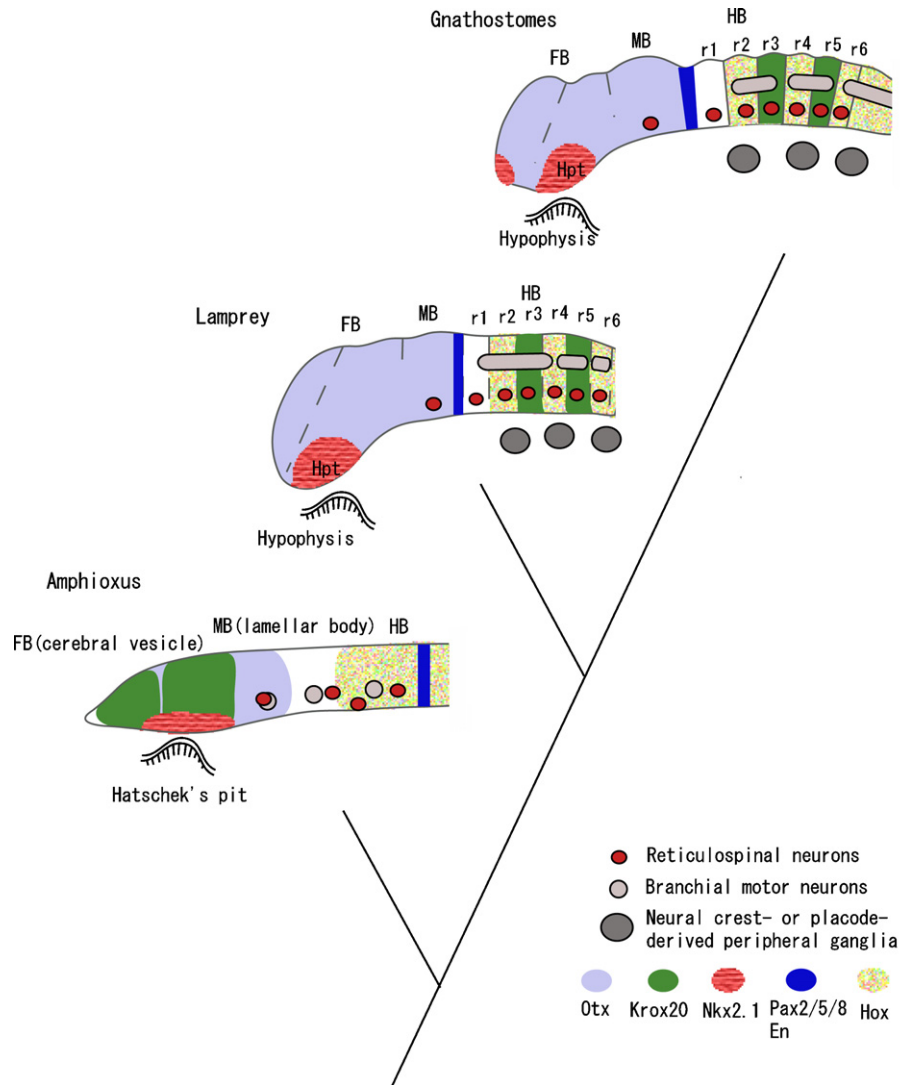


Fig. 2. Comparison of amphioxus, lamprey and gnathostome brains. The amphioxus brain (bottom: compiled from Refs. [6,7]) is basically a simple neural tube with no overt segmental compartments, whereas the lamprey and gnathostome brain (middle and top: compiled from Refs. [12,16]) consists of a series of bulges called rhombomeres (r). In these animals, homologous sets of regulatory genes are developmentally regulated in comparable and non-comparable regions. Anatomically comparable regions have been identified in both the brains, and the position of the hypophysial homologues (Hatschek's pit in amphioxus, hypophysis in gnathostomes) indicates the hypothalamic domain of both the brains. FB, forebrain; HB, hindbrain; MB, midbrain; r1–r6, rhombomeres; Hpt, hypothalamus.

and *Dlx* and *Nkx2.1* in the MGE [16,17]. As is easily imagined, such a conservative embryonic diagram of the brain, as defined by the above-listed gene expression domains, is likely to be derived from a tremendously conserved tissue interactions that takes place at, or precedes the earliest patterning of the rostral neurectoderm [21]. Simultaneously, the so formed embryonic architecture is seemingly not only organized very exquisitely, but is also ready to be modified into various types of brains in later phases of development, into the individual unique brains adaptive for various life styles of animals. In other words, each of the developmental domains (neuromeres) found in the stereotyped brain primordium at the organogenetic period is very likely to represent a unit with morphological identity, with which the expression of a homologous set of genes is almost always associated as has been shown by various comparative studies [10,12,16,17]. The question, therefore, is when and in which sequence in the history of vertebrates, the morphotype of the

brain has become evident as seen in extant gnathostomes. To clarify this, analysis of the embryonic lamprey brain has to be scrutinized.

By way of comparison, in lamprey telencephalon, *Emx* is restricted to the dorsal part [10], to a region probably corresponding to the medial pallium in gnathostomes. In the dorsal telencephalon, a *Pax6*-positive and *Emx*-negative region was observed [10]. It seems most likely that lampreys have pallial structures as in gnathostomes. *Dlx1/6* was expressed in the ventral telencephalon [10], in a region thought to be a counterpart of the striatum and LGE in gnathostomes. However, in contrast to the above, no region expressing *Nkx2.1* was observed in the lamprey telencephalon. This would suggest that lampreys lack the pallidum and MGE (Fig. 1; [12,14]). If so, then the loss of *Nkx2.1* expression in the ventral telencephalon might be related to the absence of the pallidum in this animal. On the other hand, in gnathostomes, establishment of an *Nkx2.1*-

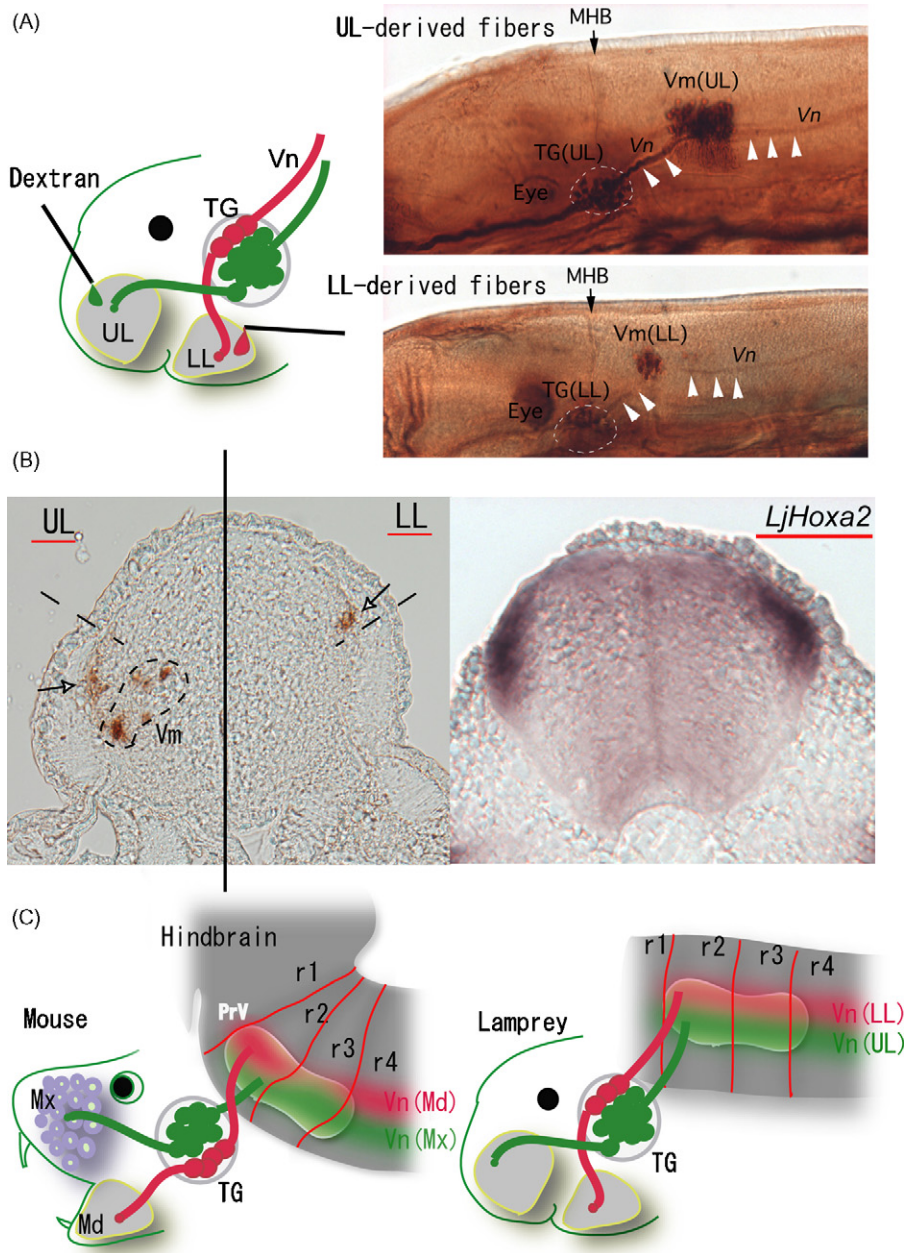


Fig. 3. Comparison of somatosensory inputs invertebrates. In mice, the upper jaw (Mx)- and lower jaw (Md)-derived trigeminal sensory nerves enter via the trigeminal ganglion (TG) into the hindbrain in a somatotopic manner, corresponding to the peripheral branches (left drawing in C: compiled from Ref. [15]). This pattern is related to the rhombomere compartment and *Hoxa2* is involved in the specific connection in mice [15]. In lampreys, dextran biotin-labeled upper lip (UL)- and lower lip (LL)-derived trigeminal fibers (arrowheads) enter into the hindbrain (A), and positioned somatotopically in the hindbrain sensory nuclei (arrows) which express *LjHoxa2* (B). The projections are summarized in (C). Md, mandibular region; Mx, maxillary region; MHB, mid-hindbrain boundary; PrV, primary nucleus of the trigeminal nerve; r1–r4, rhombomeres 1–4; Vm, motor nucleus of the trigeminal nerve; Vn, trigeminal nerve.

expression domain might have facilitated the evolution of the MGE. Thus, the vertebrate brain ‘type’ does not seem to have been obtained at once, but through a history of step-wise elaboration of the developmental program hierarchically associated with the phylogenetic evolution (Fig. 1). The scheme of the developing brain, therefore, consists of plesiomorphic and apomorphic portions.

Interestingly, *Nkx2.1* knockout mice have no MGE [22], and so in a sense are a phenocopy of the lamprey condition. Notably, in gnathostomes, *Nkx2.1* is induced by the *SHH* signal [18],

and in the lamprey telencephalon, no *HH* expression domain has been observed [12,14]. Therefore, MGE-dependent brain function seems to be newly acquired in the gnathostome lineage that first required the establishment of a telencephalic *SHH* domain.

The neurons of the mammalian cortex can be broadly defined as projection neurons and local interneurons. These latter use the inhibitory neurotransmitter GABA, arise in the MGE and reach the cerebral cortex via tangential migration [8,9]. At later stages, cortical GABAergic cells also arise from the LGE [9]. A similar

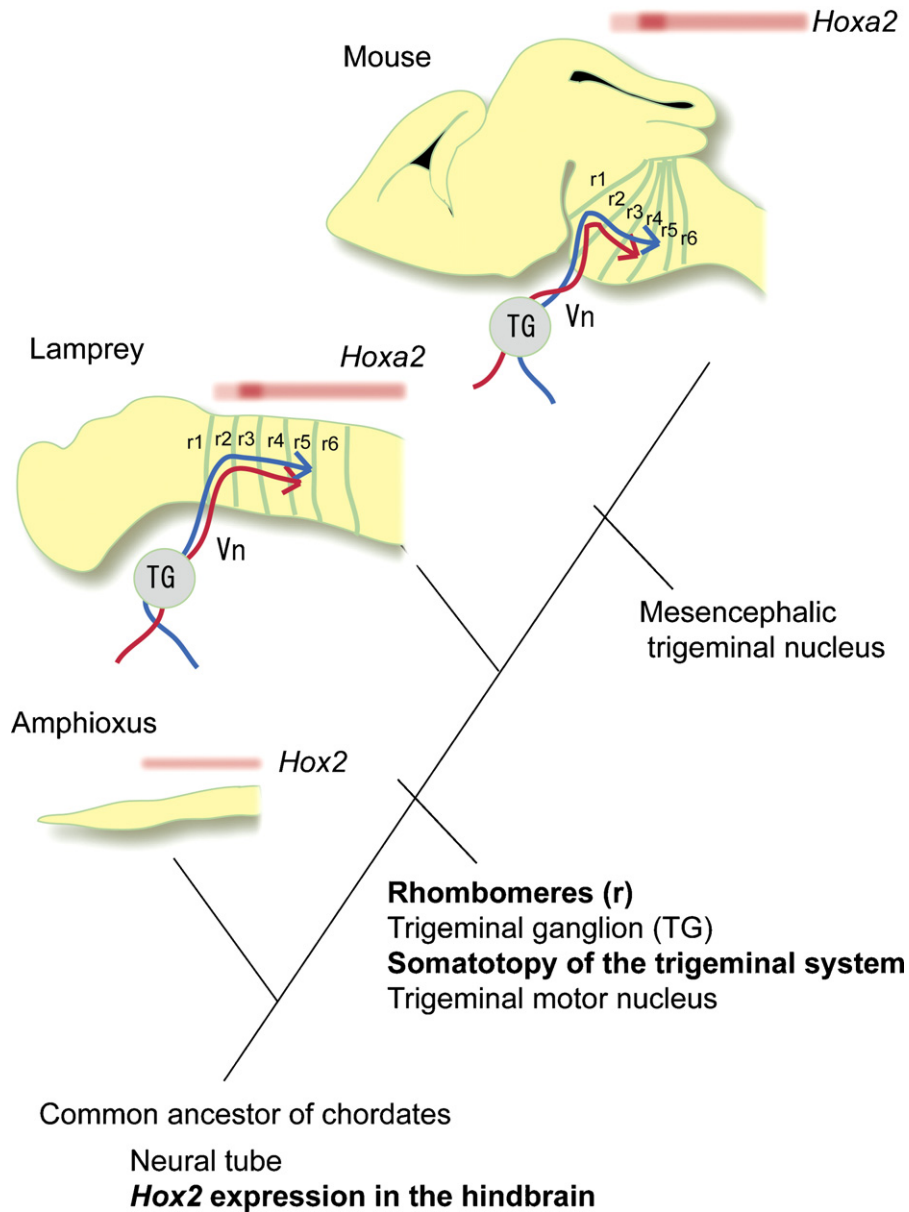


Fig. 4. Summary of the trigeminal system evolution in relation to the brain segment. Amphioxus (bottom) has a *Hox2* expression domain, but lacks brain segments and a trigeminal system. The somatotomy of the trigeminal projection (arrows) and rhombomeres (r) are present in the lamprey (middle). The trigeminal motor nucleus is also observed in this animal. In mice, the mesencephalic trigeminal nucleus becomes apparent. Vn, trigeminal nerve.

migration of GABAergic neurons has been shown in chickens and teleosts [3,24]. In the *Nkx2.1* mutant, which selectively affects the MGE, but not the LGE, there is a severe reduction of the tangential migration of GABAergic interneurons to the neocortex [22].

As described above, lampreys do not have *Nkx2.1*- and *HH*-expression domains in the telencephalon. This suggests the possibility that lampreys lack an MGE, which means it should also lack GABAergic interneurons produced by this region. There are, however, reports of GABAergic neurons in the pallial region of adult lampreys [19], suggesting that these neurons may originate from the LGE or appear without an induction by *HH* or *Nkx2.1*. Their origin needs to be determined before this issue can be fully resolved.

3. The origin of neuromeres

Previous studies have shown that in the developing lamprey brain, a *Krox20* homologue (*LjKrox20*) is expressed in the hindbrain in specific rhombomeres, r3 and r5, and in domains defined by rhombomeric boundaries (Fig. 2; [11]). The reticulospinal neurons in lamprey, which are a kind of interneuron, also develop segmentally in domains corresponding to *LjKrox20* expression (Fig. 2; [11]). Furthermore, the expression domains of *LjPax2/5/8*, *LjOtxA*, *Pax6* and *Dlx1/6* are essentially similar to those in gnathostomes [10,12,16,17]. In addition, Osorio et al., in a study of lamprey forebrain, have shown that lamprey *Lhx15* and *Lhx29* are expressed in the dorsal part of the P3 prosomeric region (P4 in the old nomenclature [14]), as in gnathostomes

[1]. Taken together, these results imply that a significant proportion of the developmental control mechanisms responsible for brain patterning were established before the divergence between cyclostomes and gnathostomes. Notably, however, expression of lamprey *Nkx2.1* is restricted to the ventral diencephalon, i.e., the hypothalamus [10,13], similar to the pattern in amphioxus, a basal chordate (Fig. 2), suggesting that the ventral diencephalon seems very old in origin and dates back to common ancestors of chordates.

4. Evolution of neuronal projections in relation to brain segments

In gnathostomes, sensory information such as the sense of touch, sound and taste are carried through central relay nuclei which in turn project to the dorsal pallium. At all levels of these pathways, the spatial arrangement of neurons faithfully reproduces the physical distribution of peripheral sensory receptors generating somatotopic representations. In mice, the upper jaw- and lower jaw-derived trigeminal sensory neurons enter into the hindbrain in a somatotopic manner, corresponding to the organization of peripheral branches [15]. Oury et al., have shown that the somatotopy is directly linked to the rhombomeric compartment, and that *Hoxa2* is involved in the specific connections [15]. In the lamprey, upper lip (UL)- and lower lip (LL)-derived trigeminal fibers also project somatotopically in a presumptive sensory relay nucleus which expresses *LjHoxa2* (Fig. 3), suggesting that the basic architecture of the topographical projection has already been present in the lamprey. Notably, amphioxus has neither neuromeres nor a trigeminal system, although a *Hox2* expression domain is present in the hindbrain [20]. It seems likely that the link between somatotopy and rhombomeres has been established in the vertebrate ancestor and conserved through the evolutionary process (Fig. 4).

5. Conclusions

According to our analysis, the basic architecture of the vertebrate brain appears to have originated very early. The subdivision into neuromeres is at least as old as the divergence between agnathans and gnathostomes. Some aspects of the anteroposterior organization of the neural tube are even older, dating to the time of an amphioxus-like ancestor but the evolution of cell lineage-restricted compartments seems to have been a later event. The evolution of mechanisms that restrict local cell lineages into a specific region may have been a major step facilitating further brain evolution. However, at least one such region, the medial ganglionic eminence, only evolved in the gnathostome lineages after the divergence of agnathans (Fig. 1). Most of the brain-related genes in vertebrates have homologues in amphioxus [6,20,23], so it is not necessarily the genes that are important for innovation but the way they are deployed in gene control circuits. This evidently led to new mechanisms for controlling cell proliferation, and migration and hence to new brain structures. The present study further suggests that brain segments have provided a basis for correct neuronal projections. Identification and comparison of the developmental plan how

to make various neuronal circuits in relation to brain segments will provide important insights into the field of evolutionary developmental biology.

Acknowledgments

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