Brain, Behavior and Evolution

Brain Behav Evol 2005;66:234–254
DOI: 10.1159/000088128

Published online: October 25, 2005

Evolutionary Patterns of Cranial Nerve Efferent Nuclei in Vertebrates

Edwin Gilland Robert Baker

Department of Physiology and Neuroscience, New York University Medical Center, New York, N.Y., USA

Key Words
Hindbrain · Evolution · Segmentation · Cranial nerves · Rhombomere · Motoneuron · Migration · Lamprey · Shark

Abstract
All vertebrates have a similar series of rhombomeric hindbrain segments within which cranial nerve efferent nuclei are distributed in a similar rostrocaudal sequence. The registration between these two morphological patterns is reviewed here to highlight the conserved vs. variable aspects of hindbrain organization contributing to diversification of efferent sub-nuclei. Recent studies of segmental origins and migrations of branchiomotor, visceromotor and octavolateral efferent neurons revealed more segmental similarities than differences among vertebrates. Nonetheless, discrete variations exist in the origins of trigeminal, abducens and glossopharyngeal efferent nuclei. Segmental variation of the abducens nucleus remains the sole example of efferent neuronal homeosis during vertebrate hindbrain evolution. Comparison of cranial efferent segmental variations with surrounding intrinsic neurons will distinguish evolutionary changes in segment identity from lesser transformations in expression of unique neuronal types. The diversification of motoneuronal subgroups serving new muscles and functions appears to occur primarily by elaboration within and migration from already established segmental efferent pools rather than by de novo specification in different segmental locations. Identifying subtle variations in segment-specific neuronal phenotypes requires studies of cranial efferent organization within highly diverse groups such as teleosts and mammals.

Copyright © 2005 S. Karger AG, Basel

Introduction

The neuronal systems and peripheral relations of the hindbrain are some of the most highly conserved vertebrate characteristics. Many central nuclei crucial for orientation, respiration, vocalization and cardiovisceral control reside in similar hindbrain locations in most vertebrates [Baker and Gilland, 1996; Nieuwenhuys et al., 1998; Taylor et al., 1999]. This conserved pattern contrasts with the diversity of sensorimotor anatomy that is served by hindbrain circuitry. A critical role for neuroepithelial segmentation in the generation of hindbrain neuronal organization was long supported by comparative morphological data [Gilland and Baker, 1993] and is now well established, in large part based on functional analysis of Hox genes [reviewed in Lumsden and Krumlauf, 1996; Cordes, 2001; Moens and Prince, 2002]. Mechanisms underlying dorsoventral specification of neuronal types [Briscoe et al., 2000] are now being meshed with Hox regulation of hindbrain axial patterning, allowing the first glimpses of how segment-specific and dorsoventral transcriptional networks interact to organize both spatial and temporal aspects of specific neuronal pheno-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2005 S. Karger AG, Basel
0006–8977/05/0664–0234$22.00/0

Accessible online at:
www.karger.com/bbe

Dr. Edwin Gilland
Marine Biological Laboratory
7 MBL Street
Woods Hole, MA 02543 (USA)
Tel. +1 508 289 7335, Fax +1 508 495 0751, E-Mail egilland@mbl.edu

types [Gaufo et al., 2003; Pattyn et al., 2003; Samad et al., 2004]. The columnar functional organization arising from dorsoventral and temporal partitioning within neuromeric segments provides a genetic and structural framework for documenting the diversification of behavioral circuits during hindbrain evolution.

The best-known aspect of neuroepithelial segmentation in vertebrates is the partitioning of the hindbrain into a morphologically distinct series of rhombomeres [Cordes, 2001; Moens and Prince, 2002]. Many neuronal types appear to be patterned according to the rhombomeric framework [Gilland and Baker, 1993; Cambronero and Puelles, 2000; Straka et al., 2001; Chandrasekhar, 2004] and the presence or locations of some nuclei are directly influenced by spatial expression patterns of individual transcription factors [reviewed in Cordes, 2001]. However, studies on hindbrains that develop without rhombomeric borders due to either genetic or signaling intervention [Nittenberg et al., 1997; Waskiewicz et al., 2002], and on retinoid induced alterations in reticulospinal and cranial motoneuron organization [Linville et al., 2004; Murakami et al., 2004] have raised important issues regarding the extent to which rostrocaudal neuronal patterning is generated by versus merely modulated by, segmentation of the hindbrain neuroepithelium. It is possible that axial signaling gradients (e.g., FGFs and retinoids) may be sufficient to specify a general rostrocaudal sequence of neuronal phenotypes independent of segmental patterning. Segmentally restricted gene expression might act largely to fine-tune this general pattern by either supporting or repressing specific neuronal types within individual segments. If so, there may be varying degrees of registration between rhombomeres and different classes of neurons, e.g. reticular versus motor. The segmental locations of specific hindbrain nuclei in different vertebrate groups might be expected to show considerable variation under such a scenario. A survey of earlier data on vertebrate hindbrain organization showed a number of possible differences in segmental locations of cranial nerve efferent nuclei [Gilland and Baker, 1993]. To further test these possibilities the present review brings together more recent published and unpublished data on hindbrain neuroepithelial anatomy and cranial efferent neuronal patterns in a wide taxonomic range of vertebrates: lamprey, shark, cyprinid, frog, chick, and mouse. Because the goal is to identify taxonomically invariant and variable aspects of hindbrain neuronal organization, studies that establish segmental origins and specific targets of cranial efferent neurons will be highlighted.

The Vertebrate Hindbrain Neuroepithelium

Molecular subdivision of the hindbrain begins during gastrulation, followed by sequential appearance of visible rhombomeric borders [Moens and Prince, 2002]. The precise order of border formation varies among species, but generally r4 is the earliest recognizable definitive segment. Although rhombomeres are usually no longer discernable at later stages of embryogenesis, surgical fate mapping in birds shows that individual rhombomeres give rise to essentially intact segmental portions of the hindbrain [Cambronero and Puelles, 2000]. Some neuronal populations, including rhombic lip derivatives and cranial nerve efferents, move between segmental territories, but the ventricular neuroepithelium, along with most of the constituent neurons and glia of the brain wall, appear to retain the early segmental pattern throughout morphogenesis. Vertebrates with prolonged larval periods such as lampreys and frogs retain visible segmental patterning through much of their postembryonic development [Straka et al., 2005]. The conservation of overall hindbrain structure across vertebrates can be appreciated by comparing flatmount preparations of larval lamprey (fig. 1A) and embryonic spiny dogfish (fig. 1E), quail (fig. 2A) and mouse (fig.2B). In these, as in all vertebrate embryos or larvae described so far, the hindbrain neuroepithelium comprises a distinctly 'rhombomeric' region spanning rhombomeres (r) 2–6, bracketed by equally highly conserved rostral (r0–1) and caudal (r7–8) regions (r8 not fully shown for the quail and mouse). The nomenclature for the latter two regions is quite variable as they lack morphologically recognizable inter-rhombomeric borders such as those that bound r2–6.

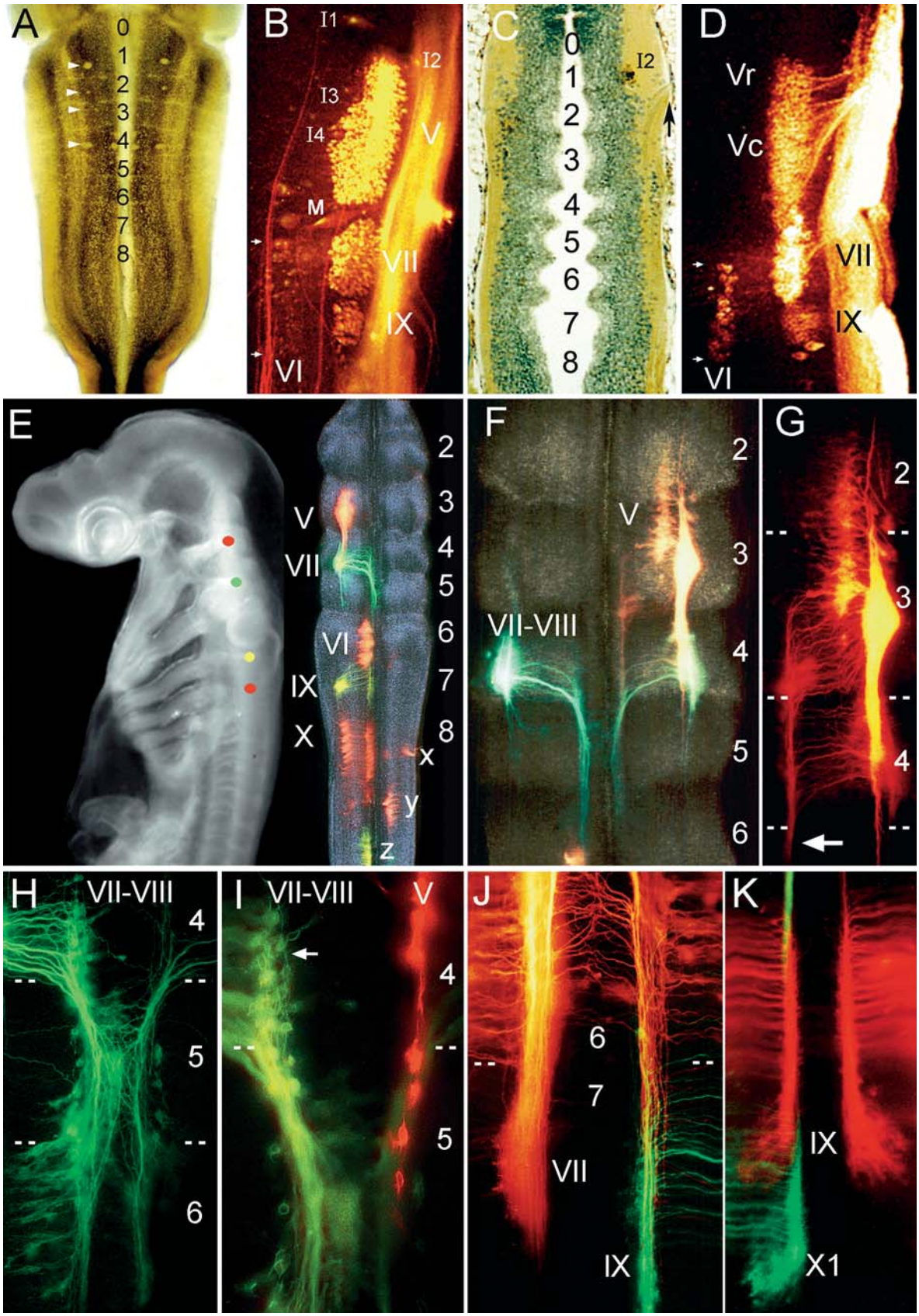
The midbrain-hindbrain border (MHB) corresponds in early stages to the interface of Otx2 and Gbx2 expression [Zervas et al., 2004], and is usually visible at later stages as a thin cell-free zone extending from near the floor plate at the front edge of the trochlear nucleus up to the roof of the neuroepithelium at the anterior medullary velum. The region between the r1–2 border and the MHB is often referred to simply as r1, but because it is much longer than individual segments 2–6 and has rostrocaudal differences, some studies distinguish the rostral part as an isthmic or r0 division, and the caudal as r1 [Vaage, 1969; Gilland and Baker, 1993; Moens and Prince, 2002]. In the absence of distinct morphological boundaries, the trochlear nucleus serves as a rough indicator of the extent of r0. The combined r0–r1 region is distinguished from caudal midbrain and r2 by expression of Gbx2, but neither Otx2 or Hoxa2. Gene expression patterns point to

distinct identities of r0 and r1. In zebrafish, Ziro3, Fgfr3 and EphA4 are differentially expressed in r0 and r1 [Waskiewicz et al., 2002]. Rhombomere 1, but not r0, shows restricted expression of Nkx1.2 in mouse [Gavalas et al., 1997] and a wedge-shaped zone of strong Pax6 expression medially in chick [Eddison et al., 2004]. Further subdivision of this region may eventually be warranted when the details of cerebellar and isthmo-pontine development are better known in different species [Eddison et al., 2004; Zervas et al., 2004].

Rhombomeres 2–6 contain most of the roots and efferent neuron origin zones for nerves V, VI, VII, and IX, with the latter located also in r7 in some species. Curiously, certain characteristics of the shapes and sizes of r2–6 are conserved widely across species. Most notable are the oblique and transverse orientations of the r3–4 and r4–5 borders, respectively; and the consequent greater length of r4 ventrally than dorsally, with r3 showing the reverse, having a greater length dorsally (figs. 1A, lamprey; 2A, G, bird; 2B, J, K, mouse).

The r2-r6 rhombomeric borders contain distinctive glia-like cells, extracellular matrix components and localized expression of signaling proteins [Moens and Prince, 2002; Riley et al., 2004]. Although no specific border cell type or lineage has yet been isolated, neuroepithelial cells in the borders appear to have larger apical ends [Ojeda and Piedra, 1998] and differential junctional coupling relative to adjacent cells [Martinez et al., 1992]. Many early commissural axons project through these zones, resulting in a ladder-like segmental framework visible in hindbrains visualized with neurofilament proteins. In chick embryos, the inter-rhombomeric zones contain fan-shaped arrays of cells that down-regulate expression of genes seen in neighboring rhombomeric zones. These cells take on the appearance of radial glia and show elevated expression of vimentin, follistatin, and Fgf3 [Heyman et al., 1995; Nittenberg et al., 1997]. Rhombomeric borders in embryonic alligators are similar to those in chicks and appear as narrow bands in sectioned material immunostained for calretinin, peanut agglutinin, vimentin and acetylcholine [Pritz, 1999]. Rhombomere border regions in *Xenopus* develop over a much more protracted time period. Patterns of distinct radial glia cells and elevated levels of vimentin and proteolipid proteins continue to be refined through nearly the whole larval period [Yoshida and Colman, 2000]. Proliferating cells appear to be concentrated near the borders in *Xenopus*, whereas cells expressing neuronal and astroglial markers predominate in the central rhombomeric areas [Katbamna et al., 2004]. In zebrafish a double palisade of glial cells are present at the rhombomeric borders [Moens and Prince, 2002], matching the expression patterns of multiple *wnt* genes in these regions [Riley et al., 2004]. Knockdown of *wnt* expression suggests that localized control of cell proliferation is one of the functions of cells in the boundary zones [Riley et al., 2004]. The role of rhombomere border regions as signaling centers and pathways for early axon tracts, and their presence late in larval development, implicates a subset of hind-

Fig. 1. Rhombomeres and cranial nerve efferent nuclei in larval lamprey *(Petromyzon marinus)* (**A–D**), and spiny dogfish *(Squalus acanthias)* (**E–K**). Rhombomeres are numbered 0–8 with cranial nerve roots and dye labeled neurons indicated in roman numerals. **A–D** Larval lamprey hindbrains retain the rhombomeric (**A**, **C**) and neuronal (**B**, **D**) patterns established at embryonic stages. **A** Flatmount of a 5.5-cm larval hindbrain treated with a dilute solution of osmium tetroxide to visualize the ventricular neuroepithelial surface. Rhombomere borders appear as thin, pale lines traversing the neuroepithelium and giant reticular neurons as pale dots (I1, I3, I4 and M; arrowheads). **B** Cranial motor and reticulospinal neurons retrogradely labeled with Rhodamine Dextran Amine (RDA) in an 8-cm larva flatmount (midline is at left border of frame). Rostral and caudal limits of abducens motoneurons indicated by arrows. **C** Horizontal paraffin section through the hindbrain of a 2-cm larva showing prominent rhombomeres and the Vth root (arrow). **D** Confocal reconstruction of cranial motoneurons labeled with RDA in a 10.5-mm larva showing the rostrocaudal limits of the VI nucleus relative to VII and IX and two large dorsal cells caudal to the IX nucleus. **E** Lateral view of head region and dorsal view of hindbrain flatmount with lipophilic dye labeled cells in Scammon stage 24 *S. acanthias* embryo. Nerve roots V, VII–VIII, IX and X_1 in the lateral view are shown by colored dots matched to the wholemount dye labels. Occipital roots x, y and z are shown in the wholemount. **F** Stage 25 hindbrain after application of diI to root V and diO to VII–VIII. **G** Labeled V efferent neurons in caudal r2 and r3. Migrating V neurons have already reached r6 by this stage (not shown) via the medial fiber tract in r5 (arrow). **H**, **I** Stage 24 embryo in which the left VII–VIII root was labeled with diO (yellow-green) and the right Vth root with diI (red; both colors in I). Large numbers of VII–VIII neurons form a continuous ipsilateral column from r4 to 6, including presumed VIII efferents oriented laterally in r6. Crossing fibers densely fill the floorplate in r5–6 and a few VIII cells are in contralateral r5. The leading end of the caudally migrating V neurons in r5 and the VII–VIII neurons in medial r4 are shown in **I**. VII–VIII neurons are shown in r4. **J** Stage 26 embryo in which diI (red) was applied to left VII–VIII and diO (green) to the right IXth root. Fibers and presumed migrating VII neurons have reached the middle of r7. Trailing processes of laterally migrated VIII efferents in r6 fill the upper left and right of the frame. **K** Stage 27 embryo in which diI was applied to the IXth roots bilaterally and diO to the left X_1 root. Migrating branchiomotor neurons that were adjacent to the floorplate in earlier stages are turning laterally.


A
0
1
2
3
4
5
6
7
8
B
I1
I2
I3
I4
V
M
VII
IX
VI
C
0
1
2
3
4
5
6
7
8
I2
D
Vr
Vc
VII
IX
VI
E
V
VII
VI
IX
X
2
3
4
5
6
7
8
x
y
z
F
V
VII-VIII
2
3
4
5
6
G
2
3
4
H
VII-VIII
4
5
6
I
VII-VIII
V
4
5
J
6
7
VII
IX
K
IX
X1

brain glia in providing a permanent framework of segmental positional cues.

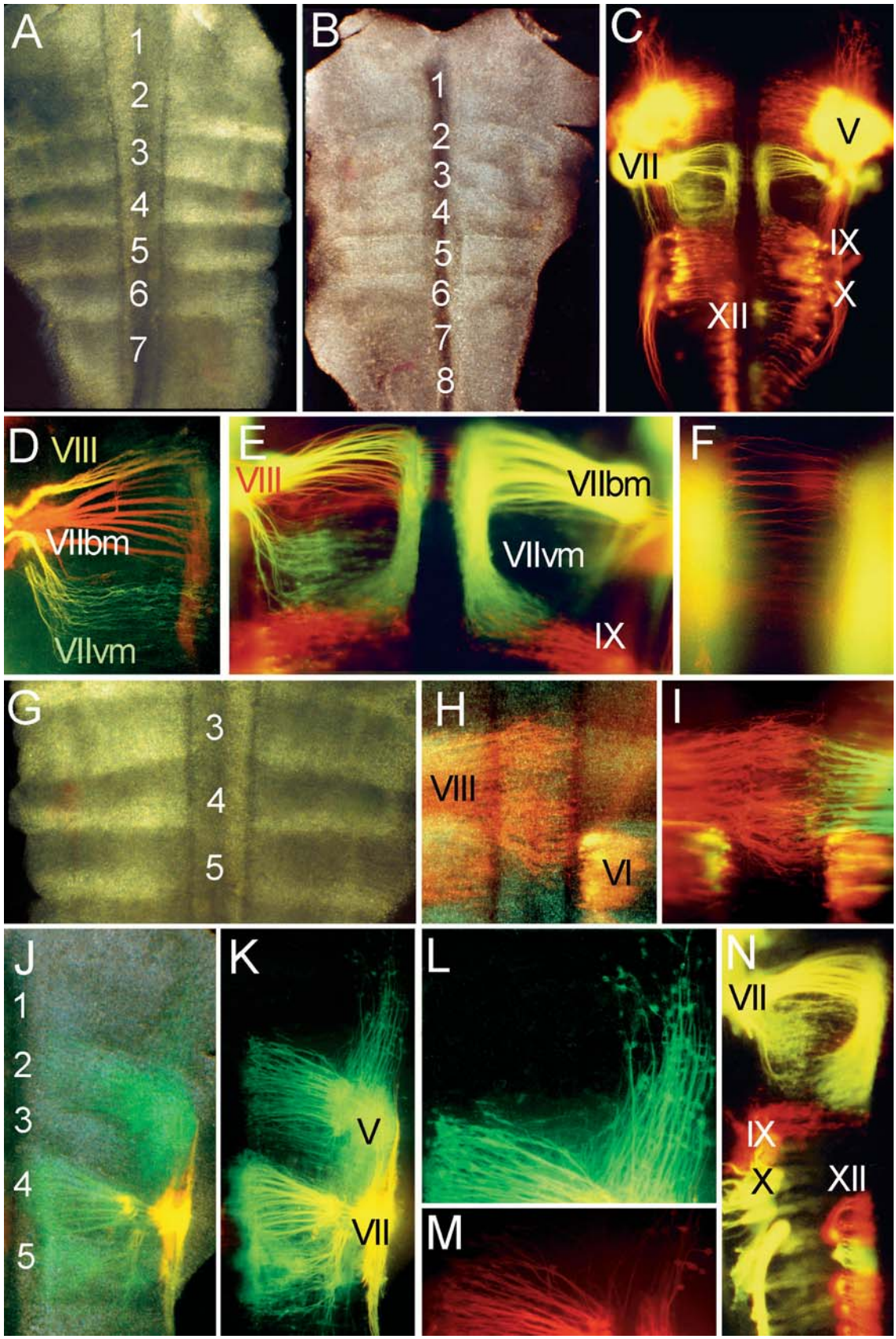
The hindbrain caudal to r6 comprises a generally recognizable r7, that is continuous with a much longer unsegmented region, often referred to simply as r8. This region gives rise to the vagal nuclei, the hypoglossal/spino-occipital motor nuclei, inferior olive, inferior reticular formation and many nuclei that regulate posture, respiration and cardiac function [Nieuwenhuys et al., 1998]. Possibly because of the lack of visible landmarks, the caudal hindbrain has received much less developmental attention than r2–6. Vaage [1969] described the caudal hindbrain in the chick as developing from the merging of r7 with the first few myelomeres (intersomitic dilations of the neural tube) adjacent to the rostral somites. Cambronero and Puelles [2000] mapped this region surgically in avian embryos and found that the hindbrain-spinal cord junction, defined by traditional central landmarks, mapped to the embryonic neural tube at the mid-point of somite 5. The limits of many hindbrain nuclei and intranuclear subdivisions were found to be in register at intervals along the fate map, thus providing evidence for an underlying segmental pattern in the caudal hindbrain, referred to as 'pseudorhombomeres' 7–11 [Cambronero and Puelles, 2000]. Although this region has not been developmentally mapped in other species, the widespread topographic similarity of caudal hindbrain nuclei [Nieuwenhuys et al., 1998] suggests that the avian segmental pattern may be typical. In terms strictly of length relative to rhombomeres, the caudal hindbrain of frogs and zebrafish seem to include a roughly similar amount of territory. Quantitative mapping of cranial efferent nuclei in larval and adult frogs showed that the region between the r6–7 border and the spinal cord was about five segments long [Straka et al., 2005]. The caudal hindbrain in zebrafish extends back to the commissura infima at the level of the third myotome [Myers, 1985] and thus contains not only the vagal nuclei and inferior reticular formation, but also what are often called the first two spinal cord segments. Although just a relative measure of length, the region encompassing r7–Sp2 in zebrafish is likewise about 5 segments long [Hanneman et al., 1988]. Fate mapping the caudal hindbrain nuclei in these species would test the generality of the pattern found in birds.

The embryonic hindbrain-spinal cord junction at mid-somite 5 in chicks matches the mesodermal occipito-cervical junction [Huang et al., 2000]. Based on the similar number of occipital somites and comparable patterns of embryonic hypoglossal and cervical nerves in mammals, birds and reptiles [de Beer, 1937; Müller and O'Rahilly, 2003], the hindbrain-spinal cord and cranio-vertebral interfaces may be closely linked throughout amniotes. This may also be the case in zebrafish, as the craniovertebral junction, like the end of the hindbrain appears to be around somite 3 [Morin-Kensicki et al., 2002]. Such a relationship may seem self-evident, especially as somitic influences on neuronal specification seem to include both regional [Ensini et al., 1998] and fine-grained effects [Lewis and Eisen, 2004]. However, the offsets between neural and mesodermal Hox gene expression patterns and phenotypic effects suggest that the establishment of coincident anatomical borders in brain and skeleton involve regulatory integration of axial patterns between, as well as within, both tissues.

Classes of Cranial Nerves and Efferent Neurons

The cranial nerves that carry peripheral efferent axons are generally classified into somatic, branchiomeric and octavolateral groups. Octavolateral efferents share close developmental relationships with branchiomotor efferents of nerves VII and IX, thus the VIIIth and lateral line

Fig. 2. Flatmounted hindbrains of embryonic quail (**A**, **G–I**) and mouse (**B–F**, **J–N**) labeled with lipophilic dyes to highlight cranial nerves V and VII–VIII. Rhombomeres are numbered 1–8 with cranial nerve roots and dye-labeled neurons indicated in roman numerals. Facial branchiomotor (VIIbm) and visceromotor (VIIvm) are distinguished in **D** and **F**. **A**, **B** Hindbrains of H–H stage 19 quail and 11.5 day mouse showing identical organization of rhombomeres including individual segment shapes and dimensions. **C** Overview of a 12.5-day mouse embryo showing the distribution of all efferent neurons in rhombomeres 2–8, except for abducens. **D–F** Mouse embryos at 11.5 (**D**) and 12.5 (**E**, **F**) days showing the separate roots and migration paths of VIIbm, VIIvm and VIIIth nerve efferents in r4–r5. Dye combinations used in **D** versus **E–F** were different so VIII efferents are yellow vs. red, respectively. In **E**, the VIIvm area is shown on the right and the labeled neurons on the left. **F** High magnification of VIII efferent processes crossing the midline in r4. **G–I** The contrasting pattern of VIII efferent neuronal migration in birds showing migration of neurons, not processes, across the midline in r4/5. **G** Rhombomeres 3–5 at H–H stage 19. **H–I** Bilateral VI, right VII and left VIII labeled at H-H stage 23–24. **J–M** Trigeminal root label in mouse at 11.5 (**J–L**) and 10.5 days (**M**) showing the lateral group of neurons in r1 with greater number and more rostral location in the older embryo. **N** Location of the rostral hypoglossal neurons (orange) in a 12.5-day mouse just caudal to the X neurons in r7. VI neurons in r5 are obscured by the mass of migrating VIIbm neurons.


A
1
2
3
4
5
6
7
B
1
2
3
4
5
6
7
8
C
V
VII
IX
X
XII
D
VIII
VIIbm
VIIvm
E
VIII
VIIbm
VIIvm
IX
F
G
3
4
5
H
VIII
VI
I
J
1
2
3
4
5
K
V
VII
L
M
N
VII
IX
X
XII

nerves proper do not need to be considered separately. The oculomotor (III), trochlear (IV), abducens (VI) and hypoglossal/hypobranchial (XII) nerves are considered somatic motor nerves that share basic similarities with vertebrate spinal ventral roots. Inclusion of the IIIrd and IVth nerves in this group has a long and contentious history intertwined with theories of head segmentation and possible serial homology of head and trunk structures [Neal, 1914]. The trigeminal (V), facial (VII), glossopharyngeal (IX), vagus (X) and accessory (XI) nerves are grouped together as the branchiomeric series based on a number of shared features correlated with their presumed origins as serially repeated elements of the primitive vertebrate pharynx. Attempts to explain the phylogentic origins of these two classes of cranial nerves invoke elaborate theories of nervous system organization within chordates and raise problems of serial and taxonomic homology that remain largely unresolved [Gaskell, 1889; Neal, 1914; Goodrich, 1930; Fritzsch and Northcutt, 1993; Kuratani et al., 1999]. Those topics are beyond the scope of the present review, so the somatic and branchiomeric nerve groups will only be surveyed to see which efferent neuronal types they contain and how well the individual cranial nerves fit such a classification.

Brainstem efferent neurons can be sorted into different types based on their target cells and developmental origins. Somatomotor and branchiomotor neurons project to striated muscle, visceromotor neurons to parasympatheic ganglia and octavolateral efferents to mechanosensory hair cells. All of these cell types originate in the paramedian portion of the ventral basal plate in what was often termed the 'primitive motor column'. A lateral origin of somatomotor neurons within this column, versus a medial origin for the other three types (and their subsequent migration away from the ventral region) was originally demonstrated using silver stains [see Windle, 1970 for earlier references]. Similar results were obtained using phosphatase histochemistry, which also showed the III and IV nuclei to share certain features with branchiomotor rather than somatomotor nuclei [McAlpine, 1959]. Genetic and molecular analysis has confirmed the different origins of these groups and shown that the ventral region near the floorplate also gives rise to serotonergic neurons, other ventral neuronal types as well as oligodendrocytes [Ericson et al., 1997; Briscoe et al., 2000; Cordes, 2001; Pattyn et al., 2003; Samad et al., 2004]. Formation of distinct columns of ventral neuronal progenitor domains is initiated by a gradient of sonic hedgehog protein secreted by the floorplate and notochord. This gradient is interpreted on a concentration dependent basis by two classes of mutually repressive homeodomain proteins, that in turn regulate combinatorial expression of other transcription factors to uniquely define different ventral neuronal classes [Ericson et al., 1997; Briscoe et al., 2000]. As a result, cranial efferent neuronal types can now be defined not only by their targets, origin zones and migration paths, but also by the expression of specific subsets of the genetic regulatory elements responsible for their specification. All of the hindbrain efferent types arise within a ventral region near the floorplate expressing Nkx6.1 and Nkx6.2 that subsequently partitions into two domains. Branchiomotor, visceromotor and octavolateral efferents develop from a ventro-medial domain of cells expressing Nkx2.2 and Nkx2.9, followed by Phox2b and then Phox 2a [Pattyn et al., 2003]. These efferent neurons extend axons to reach the dorsally located sensory-motor root zones and then migrate laterally within the axonal processes or caudally within secondary neurites.

In contrast, precursors of abducens, hypoglossal and spinal motoneurons emerge in a dorso-lateral domain, farther from the floorplate, from cells expressing Pax6 and Olig2 [Ericson et al., 1997; Gaufo et al., 2003]. Axons project directly to ventral root exits whereas the motoneurons remain just lateral to the early medial longitudinal fasciculus, or, in some cases, translocate laterally within secondary neurites (e.g., accessory abducens). Oculomotor and trochlear neurons develop differently than VI and XII neurons. Instead of $Pax6^{+}$ precursors, III and IV neurons express Phox2a and Phox2b in reverse order compared to the branchiomotor group. In animals lacking Phox2a, III and IV neurons fail to form, but neither of these genes are required for development of VI and XII motoneurons. Differential expression of LIM-class homedomain proteins specify further distinctions within and between the major efferent types [Briscoe et al., 2000; Cordes, 2001], but for the present purpose the distinct progenitor zones, axonal projections and targets are sufficient to examine the validity of classifying cranial nerves into somatic and branchiomeric series.

Branchiomeric cranial nerves are mixed sensory-motor nerves with proximal and distal sensory ganglia arising from neural crest and epibranchial placodes, lateral rather than ventral motor roots and laterally located motor nuclei in adults. Efferent innervation is to striated muscles of the pharyngeal wall and parasympathetic ganglia in the head and thoraco-abdominal viscera. The VIIth, IXth and rostral divisions of the Xth nerve generally possess all these features, including gustatory sensory components. In both fish and tetrapods, caudal vagal divisions differ considerably from the branchial arch nerves

proper and primarily innervate the post-branchial region of the pharynx and cardio-intestinal targets. In amniotes and some amphibians the parasympathetic components of nerves VII–X originate from nuclei that are anatomically distinct from the branchiomotor nuclei. Fish gills have extensive autonomic innervation [Sundin and Nilsson, 2002], but other than cardio-intestinal subdivisions of the vagal nuclei [Taylor et al., 1999], distinct central preganglionic visceromotor nuclei projecting through the branchiomeric nerves do not appear to have been described in either elasmobranchs or bony fish.

The trigeminal nerve has a unique identity that is not easily explained by derivation from a common branchiomeric pattern. Unlike other branchial nerves, the Vth lacks visceromotor and gustatory components and primitively has two sensory ganglia, neither of which corresponds precisely to typical branchial nerve ganglia in either origin or function. Despite these unusual features, trigeminal motoneurons appear to match expectations of proper branchiomotor neuron development. The final nerve traditionally associated with the branchiomeric series is the accessory nerve (XI), which innervates apparently homologous cucullaris/trapezius muscles in elasmobranchs and tetrapods (unclear in bony fish). In amniotes, XI axons exit the rostral spinal cord through laterally positioned roots that appear to form a continuous series with the caudal vagal rootlets. Although small, essentially ectopic sensory ganglia are often associated with XI roots; this nerve has no intrinsic ganglia or dorsal roots. Interpretations of the XIth nerve based on a proposed evolutionary origin of the target muscles from caudal branchial constrictors or levators [e.g., Straus and Howell, 1936] view the XI nucleus as a specialized part of the vagal branchiomotor nucleus that migrated caudally during phylogeny [Székely and Matesz, 1993]. Although the XI nucleus is usually restricted to the spinal cord, extension into the caudal hindbrain is known from skates and some amphibians [Sperry and Boord, 1992; Székely and Matesz, 1993]. Because developing XI neuron precursors share topographic and gene expression features with branchiomotor neurons [Pabst et al., 2003], a branchiomeric derivation of the XIth nerve remains plausible, despite serious doubts stemming from the uncertain evolutionary origins of the target muscles and the strictly spinal location of the efferent neurons in most species [Wake, 1993].

Somatic classification makes clear sense for the tetrapod XIIth nerve and hindbrain 'occipitospinal' nerves in fish, as they form the rostral end of the series of ventral roots that develop in relation with somites. In fact, these nerves are more purely somatic than spinal ventral nerves, most of which contain autonomic efferents in many vertebrates [see Fritzsch and Northcutt, 1993 for difficulties in defining primitive spinal nerve organization]. In addition to the unique genetic specification of the IIIrd and IVth nuclei (above), the nerves innervating extraocular muscles have numerous morphological features that raise issues about their status as phylogenetically modified members of a primitive somatic nerve class. In gnathostomes, the main abducens motoneurons innervate mesoderm rostral to the nucleus and root instead of immediately adjacent, but otherwise fit the somatic profile. In many groups an accessory VI nucleus forms by lateral migration from the main nucleus [Evinger, 1988], but because this migration is through secondary neurites rather than through the primary axon, it is quite unlike the early lateral migration seen in many branchiomotor and visceromotor neurons. The main peculiarity of the trochlear nerve is projection of axons dorsally to innervate contralateral muscles, an accomplishment unlike any other efferent neurons [Irving et al., 2002]. The oculomotor nerve exits ventrally, and projects bilaterally, resulting from somal translocation across the midline [Evinger, 1988; Pombal et al., 1994]. Although this is an unusual feature for somatomotor neurons, it is perhaps not entirely unique, as sonic motor nuclei in some teleosts fuse across the midline and project bilaterally. The IIIrd nerve also has a parasympathetic component projecting to the ciliary ganglion, a feature that is unique with regard to cranial somatic nerves, but typical for sacral ventral roots in many groups.

The abducens and trochlear nerves of lampreys add further unusual features. The axons of VI neurons in lampreys do not exit ventrally like those of somatomotor neurons, rather they ascend rostrally to exit in close association with the V root, acting more like branchiomotor axons. Likewise, the lateral abducens subdivision present in lampreys may arise by somal translocation through the primary axon instead of through secondary neurites [Fritzsch et al., 1990; Fritzsch, 1998a]. Lamprey trochlear axons share the gnathostome feature of exiting the brain dorsally but, in addition, the nucleus is located far dorsally in the alar plate. Whether the cells originate dorsally or, instead, originate ventrally and migrate dorsally, is still a debatable issue [Pombal et al., 1994; Fritzsch, 1998a]. The lamprey IVth nucleus projects bilaterally to the same muscle (caudal oblique) in both orbits [Fritzsch et al., 1990], although it is unsettled to what degree this depends on axon routing or somal midline crossing [Pombal et al., 1994].

The minimal assumption for embracing the extraocular nerves within the somatomotor group has traditionally been the idea that the somites in early vertebrates originally extended to the front of the head as in cephalochordates, and that nerves III, IV and VI were motor nerves to three such somites [Neal, 1914; Goodrich, 1930]. Various explanations were then devised to account for the peculiar features of individual extraocular nerves as specializations related to the early elaboration of ocular motility [Neal, 1914]. Although the broader metameric theories that produced these interpretations of extraocular nerve origins are no longer tenable, the similarities between abducens and somatic nerves such as XII, suggest that primitively segmented cranial mesoderm might still be a viable hypothesis. Models of early vertebrates without segmented cranial mesoderm must assume the extraocular nerves originated without any relation to somites. Accordingly, the unique features of nerves III and IV would not be seen as problematic since these nerves can be viewed as unique cranial innovations, basically unrelated to other cranial or spinal nerves. The many somatomotor features of the abducens nerve are instead the issue that requires special explanation.

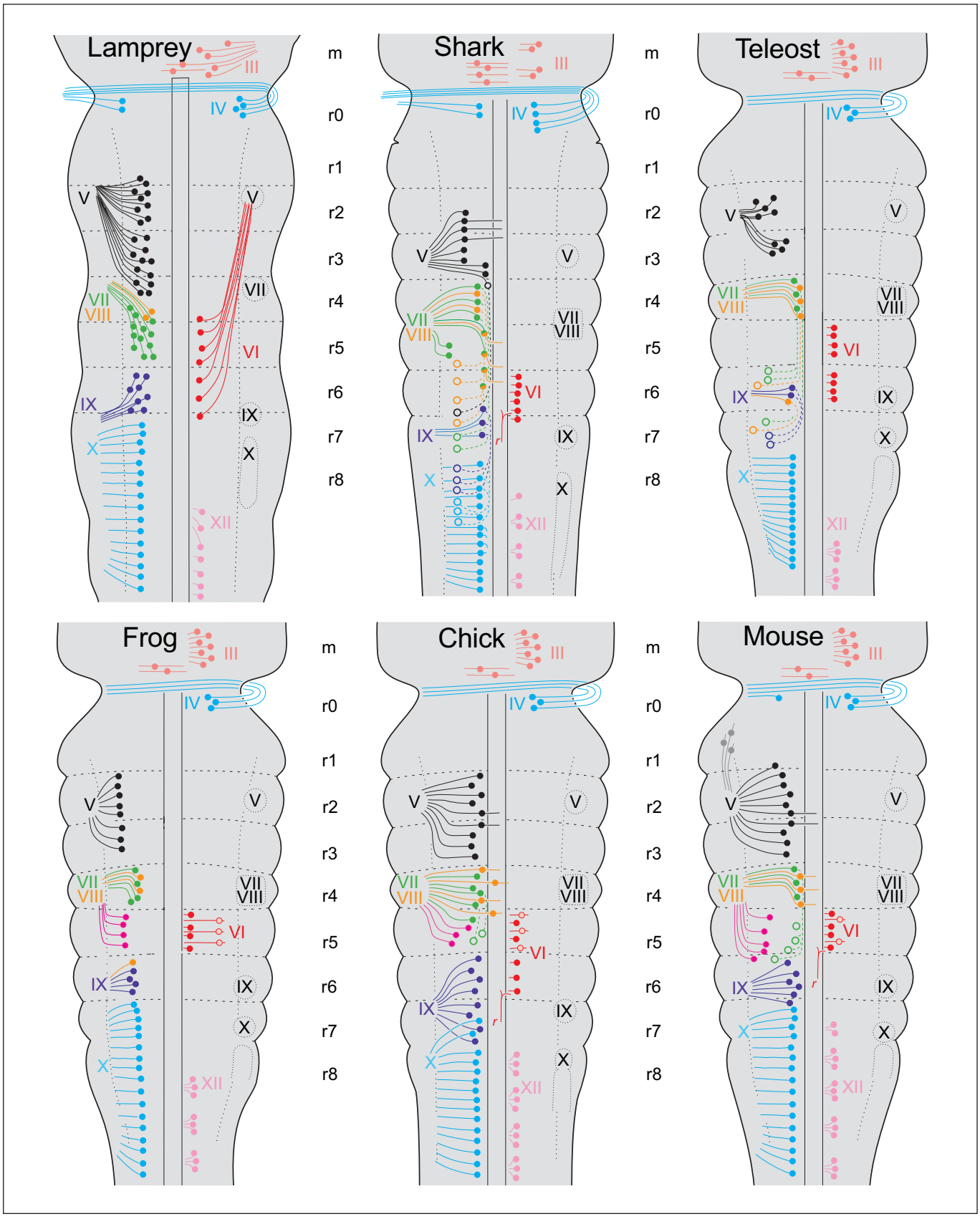
In both classical [Gaskell, 1889] and recent studies [Fritzsch and Northcutt, 1993] the origins of different classes of cranial nerves and efferent neuronal types were sought in comparisons with spinal nerve organization, with the view that spinal nerve patterns in various chordates would show the primitive antecedents from which cranial nerves were assembled. Recent studies on amphioxus cranial somatomotor neurons provide a more direct approach to these problems and may eliminate the need for head-trunk serial comparisons. Ultrastructural reconstruction demonstrated two classes of somatomotor neurons innervating the dorsal (DC) and ventral (VC) components of rostral myotomes of larval amphioxus [Lacalli and Kelly, 1999]. The DC neurons showed a more regular repeating pattern and closer association with visceral motoneurons, whereas the VC neurons were less clearly segmented and extended farther rostrally than the DC group. The proposal that vertebrate branchiomotor neurons might have evolved from a DC-like system and cranial somatomotor neurons from a VC-like system [Lacalli and Kelly, 1999] could greatly simplify analysis of hindbrain and cranial nerve evolution. Initial correlations between DC neurons and segmentally expressed genes [Bardet et al., 2005] are shifting the focus to direct comparison of hindbrain organization within chordates, rather than on cranial versus spinal patterns.

Branchiomeric and Octavolateral Efferent Nuclei

Trigeminal Motor Nuclei

The trigeminal-innervated musculoskeletal system in adult cyclostomes is structurally complex and differs greatly from that of gnathostomes [Hardisty and Rovainen, 1982]. Lampreys have an apparent homolog of the mandibular nerve, but in addition, a larger 'maxillary' division that innervates rostral muscles via the apical and basilar nerves [Hardisty and Rovainen, 1982]. Accordingly, the trigeminal motor nucleus in larval and adult lamprey is organized quite differently than in gnathostomes [Homma, 1978; Fritzsch, 1998b; Kuratani et al., 2004; Murakami et al., 2004]. The V motor nucleus in a late larval lamprey (fig. 1B) occupies a large area in the

Fig. 3. The segmental locations of efferent neurons of cranial nerves III–XII are schematically depicted for lamprey *(Petromyzon)*, shark *(Squalus)*, teleost *(Danio)*, frog *(Rana)*, chick *(Gallus* and *Coturnix*) and mouse *(Mus)* with respect to axial origin and migration, and secondarily, to mediolateral position. Efferents of branchiomeric and octavolateral nerves are shown on the left side of each diagram with symbols indicating segmental cell origins (solid circles), primary axonal pathways (solid lines), migratory paths (dashed lines) and late migratory segmental locations (open circles). Somatomotor efferent neurons and branchiomotor root entry/exit points are shown on the right; colors of all elements are matched across species. Tetrapod VII visceromotor neurons are shown separately (magenta), but those of III, IX and X are not distinguished from either somatomotor or branchiomotor components. Ontogenetic stages represented in the schematic: Lamprey, outline and most neurons (see below) from 10.5-mm pro-ammocoete, rhombomeres from horizontal sections of 12-mm ammocoete; Shark, Scammon Stages 24–27; Zebrafish, 24–48 h post fertilization; Frog, Gosner Stage 27 larva with hypoglossal elements added from adult; Chick, Hamilton-Hamburger Stages 19–20, except VIII in floorplate (H–H 23) and migrating VIIbm (H–H 25); Mouse, E9.5–11.5, except ipsilateral IV, from adult mammal. All oculomotor (III) neurons originate ipsilateral; however, the superior rectus subdivision migrates to the contralateral side in all species as does the medial rectus subdivision in shark [Evinger, 1988; Fritzsch et al., 1990]. Contralateral V dendrites are shown in embryonic shark, chick and mouse, but contralateral projections are omitted for octavolateral efferents of shark and zebrafish. All data are from our studies except: lamprey III, IV and VIII, adult locations [Fritzsch et al., 1989; Fritzsch, 1998a]; Zebrafish [Chandrasekhar et al., 1997; Chandrasekhar, 2004; Sapède et al., 2005]; Frog [Straka et al., 2001, 2005]; Chick VIIbm, VIIvm [Jacob and Guthrie, 2000]. Abbreviations: r0–r8, rhombomeres; m, midbrain; III, oculomotor; IV, trochlear; V, trigeminal; VI, abducens and accessory abducens; VII, facial; VIII, lateral line, vestibular and auditory; IX, glossopharyngeal; X, vagus; XII, hypoglossal-hypobranchial.


Lamprey
Shark
Teleost
Frog
Chick
Mouse
m
r0
r1
r2
r3
r4
r5
r6
r7
r8
III
IV
V
VI
VII
VIII
IX
X
XII
r

lateral part of the anterior hindbrain, bounded on three sides by giant reticular neurons; rostrally by I1 and I2, medially by I3 and I4, caudally by the Mauthner cell. Based on the rhombomere borders and giant neurons visible in figure 1A and B, the nucleus extends through all of r2-r3, the rostral half of r4 and slightly into r1. Physiologically-identified neurons in the rostral V nucleus innervate ventrolateral muscles by way of the mandibular nerve, whereas neurons in the caudal part of the nucleus mostly innervate buccal muscles served by the apical and basilar nerves [Homma, 1978]. This pattern has been shown to derive directly from the initial embryonic condition [Kuratani et al., 2004]. In a 2 cm larva exhibiting well defined rhombomeres, the Vth root can be seen to exit the brain at the r1-r2 border, just caudal to the I2 neuron (fig. 1C, arrow). Confocal reconstruction of dye labeled cranial efferent neurons in a 10–11 mm larva shows the rostral part of the nucleus and separate intramedullary root to be distinct (fig. 1D, Vr). By examining genes with segmentally restricted expression borders (e.g., Krox20, EphC, Pax6 and Hox3) in combination with retrogradely labeled branchiomotor or reticulospinal neurons, Murakami et al. [2004] determined rhombomeric origins for giant reticular and trigeminal motoneurons in late embryonic stages. Other than the rostral limit of V neurons, which they place farther into r1, the segmental locations of neurons in large ammocoetes (fig. 3) is essentially the same as in late embryonic stages [Murakami et al., 2004].

In contrast to lampreys, trigeminal motoneurons appear to originate largely or solely from r2 and r3 throughout gnathostomes (fig. 3). Song and Boord [1993] proposed that trigeminal motor nuclei and muscles conformed to a general pattern in which a rostral motor subnucleus (r2) innervated jaw closers, and a caudal subnucleus (r3) innervated jaw openers. This pattern has now been directly demonstrated in zebrafish [Higashijima et al., 2000] and chick [Prin et al., 2005] using genetic markers combined with retrograde nerve labeling to identify the segmental origins and peripheral targets of early Vth nerve subnuclei. Frogs also appear to match the general pattern, based on segmental locations in larvae and innervation patterns in adults [Straka et al., 2005].

Trigeminal motoneurons in elasmobranchs originate in r2–3 and form separate rostral and caudal motor nuclei in adults, but both embryonic and adult features exhibit unique aspects not seen in other species. Unlike other vertebrates, the main trigeminal root in elasmobranchs lies in r3, with only a small rostral part of the motor root originating in r2 (fig. 1E). This unusual location develops secondarily due to a caudal shift of neural crest that arises initially from r2 [Neal, 1898; Kuratani and Horigome, 2000]. The V motoneurons lie in a lateral column extending from the middle of r2 to the middle of r3 (fig. 1F, G). Axons of motoneurons in caudal r2 and rostral r3 project laterally and turn caudal, forming a longitudinal collecting trunk that joins the main root in the middle of r3 (figs. 1G and 3). The caudal half of r3 contains few laterally located neurons and r4 none, yet both rhombomeres are filled with transversely running processes that form a dense longitudinal descending tract adjacent to the floorplate (fig. 1G, arrow). A thin, continuous chain of neuronal cell bodies extends along the dorsal (ventricular) aspect of the medial tract from caudal r3 to caudal r6 at stage 25 (leading cells are shown in r5 in fig. 1I). The migrating Vth nerve neurons likely form the nucleus 'A' comprised of large branchiomotor-like neurons located adjacent to the abducens nucleus [Smeets et al., 1983], which appears to match the caudal Vth nucleus in *Raja* that innervates the levator maxillaris, levator labialis and spiracularis muscles [Song and Boord, 1993].

The greatest reorganization of the trigeminal motor system in gnathostomes is associated with the development of the dentary-squamosal joint in mammals [Székely and Matesz, 1993]. Surprisingly, early stages of V motor development give no hint of the subsequent profound changes in trigeminal nuclear organization and motoneuronal morphology. Trigeminal motoneurons in mice, as in chicks, originate as a medially located cell column extending through r2 and r3, with neurons appearing earlier in r2 (fig. 2J, K). Occasional cells are seen in caudal r1 and rostral r4 (not shown). The adult trigeminal motor nuclei include a main dorsolateral subdivision largely innervating muscles homologous to jaw adductors, and a ventromedial subdivision projecting to the mylohyoid and anterior digastric, classic jaw openers [Székely and Matesz, 1993]. Evidence that the dorsolateral Vth nucleus derives from r2 and ventrolateral from r3 would confirm that the developmental pattern proposed by Song and Boord, as shown for zebrafish and chick, holds generally for gnathostomes.

An unusual feature seen in mouse (and ferret) embryos is the presence of large numbers of retrogradely labeled trigeminal neurons in the lateral parts of r1 (fig. 2J–M). These neurons are first seen between days 10.5 and 11 in a small cluster immediately rostral to the root entry point (fig. 2L). The cells have thick peripheral, and much thinner central, processes oriented, not mediolaterally as with the main group of trigeminal neurons, but rostrocaudally.

By day 11.5 more lateral neurons are present, with somas located farther rostral from the root (fig. 2K). These neurons are probably a portion of the mesencephalic trigeminal (MesV) system, but their initial appearance near the V root is difficult to reconcile with a mesencephalic origin as proposed in birds [Narayanan and Narayanan, 1978]. These cells were previously identified as mesencephalic trigeminal neurons from Vth nerve labeling [Easter et al., 1993] and, at slightly later stages, from label applied near the mesencephalic/rhombencephalic border [Widmer et al., 1998]. In human embryos, an isolated group of monopolar putative MesV neurons were described in r1 [Windle, 1970]. Although caudal migration of midbrain neurons without long leading processes is possible, direct demonstration in mammals is lacking. Unlike many vertebrates, the major portion of the mesencephalic trigeminal nucleus in mammals is located in the hindbrain [Terashima, 1996], including the MesV pontine nucleus just rostral to V motor. Thus an origin of part of this sensory system either from the alar part of r1, or from neural crest cells migrating centrally via the Vth nerve root zone, cannot yet be ruled out.

Facial and Rostral Octavolateral Efferent Nuclei

Facial (VII) and octavolateral efferent (VIIIeff) nuclei in adult vertebrates are remarkably diverse in terms of topographic positions and peripheral targets. However, because the VII branchiomotor (VIIbm) and VIIIeff neurons in most species appear to originate primarily in r4, the various adult locations result mainly from differing degrees of caudal and lateral migration (see overview in fig. 3). In lampreys, the VIIbm nucleus extends from the Mauthner cell in caudal r4 to just behind the accessory Mauthner cell in caudal r5 (fig. 1B, D) at all stages from early larvae [Murakami et al., 2004] through post-metamorphic adults. The VIIIeff neurons in adult lampreys are located ipsilaterally near the Mauthner cell and VIIbm neurons [Fritzsch et al., 1989], thus likely arising in r4.

In elasmobranch embryos, the VIIth and VIIIth nerves join the brain in a common root located in the caudal part of r4. The VII–VIII efferent neurons migrate caudally at nearly the same time as axons reach the root. They initially form a dense column of medially located neurons extending through all of r4, r5 and slightly into r6, with transversely running axons restricted to r4 (fig. 1E; stage 23). Slightly later, neurons are still present medially in r4–5, but large numbers of transversely oriented neurons are in ipsilateral r6 and a few in contralateral r5–6 (fig.1H, I; stage 24). A dense network of crossing fibers covers the midline especially in r5, and a large axonal trunk extends to the contralateral VII–VIII root (fig.1F, H, I). A small group of neurons in the lateral part of r5 extend axons laterally and anteriorly to the VII–VIII root (fig. 1F). By stage 26 a large group of VIIbm cells has reached the r7 region originally occupied by IX neurons (fig. 1J) and few cells, if any, remain in medial r4 (not shown). The caudal edges of the transversely oriented VIIIeff neurons that fill caudal r5 and all of r6 are visible in the upper part of figure 1J [see Gilland and Baker, 1992]. Thus, it is likely that VII motoneurons and VIII efferents originate principally in r4, possibly also in r5, and migrate caudally adjacent to the floorplate. They then take separate paths, giving rise to a large octaval efferent nucleus located bilaterally in r5–6, as well as a VII branchiomotor nucleus in r7, just behind the migrated V neurons (see summary in fig. 3).

The early VII and VIII efferents in zebrafish show many similarities to those of elasmobranchs as a combined VII–VIII efferent cell group originates in r4, migrates caudally along the edge of the floorplate and turns laterally to form a large cell group in r6 along with a smaller one in r7 [Chandrasekhar et al., 1997; Higashijima et al., 2000; Sapède et al., 2005]. The r6 group appears to give rise to the rostral VII branchiomotor and octavolateral efferent nuclei, while the smaller r7 group contributes to the caudal VII branchiomotor and octavolateral efferent nuclei [Luiten, 1976; Bricaud et al., 2001].

Frogs exhibit a simplified pattern in which VIIbm and VIIIeff neurons originate in r4 and VIIvm in r5, locations retained in adults [Straka et al., 2001, 2005]. The VIIIth nerve efferents project only ipsilaterally, as in lampreys. A rostral group of lateral line efferent neurons in larval frogs is located close to the VIIIeff neurons in r4 (fig. 3). In chicks, the early pattern is similar [Jacob and Guthrie, 2000], with most VIIbm and VIIIeff neurons located in r4 and VIIvm in r5; however, many of the branchiomotor neurons in r4 migrate to r5 where visceromotor and a few VIIbm neurons appear to originate [Jacob and Guthrie, 2000]. The VIIIth nerve efferents in r4 extend processes across the floorplate to the contralateral VIIIth nerve, and some of these cells migrate across the midline to settle in contralateral r4 and r5 (fig. 2H, I) [Simon and Lumsden, 1993].

By far, the most elaborate VII and VIII efferent organization is found in mammals, in which early events in r4–5 are correspondingly diverse. The facial-vestibulocochlear nerve complex in mice has three major efferent rootlets that when teased apart and labeled separately (fig. 2D, E) roughly outline the main efferent groups.

VIIbm and VIIIth nerve neurons form a large column of medial neurons that contribute to forming the internal facial genu in r4. VIIvm neurons in r5 project axons laterally and rostrally to reach the root. By labeling separate peripheral branches and different central locations within r4–5, Bruce et al. [1997] worked out the segregation and migration of these groups as illustrated in fig. 2. Visceromotor neurons that project through the greater petrosal nerve and chorda tympani originate solely from r5. They translocate laterally within axonal processes between days 10.5 and 13.5 (cf. fig. 2D, E, N), and then radially within secondary neurites to form the superior salivatory nucleus. Branchiomotor neurons (day 10) located medially only in r4, begin migrating caudally through r5 on day 11 (fig. 2D) and turn laterally to settle in r5–r6, caudal to the abducens and caudomedial to the superior salivatory nuclei (fig. 2D, N and fig. 3 summary). Efferents projecting through the vestibulocochlear nerve are found in the medial part of ipsilateral r4 on days 11–12, but both ipsi- and contralaterally in r4 by days 12–13. The contralateral otic projections in mice do not involve somal translocations across the midline (fig. 2F) as occurs in chick (fig. 2I). The crossing zone in mice is limited to r4 and contains only axon collaterals extended by cells that remain ipsilateral (fig. 2E, F). Migrations of VIII efferent neurons differ from the VIIbm neurons by involving projection of secondary processes laterally in r4 followed by caudal migration within r4 and into r5 (Summary in fig. 3).

Glossopharyngeal and Caudal Octavolateral Efferent Nuclei

In most species, IXth nerve efferents appear to arise mainly from r6. The IX motoneurons in embryonic, and larval lampreys form a compact nucleus in r6, with axons running caudally and laterally to a root near the r6–r7 border (fig. 1B, D) [Murakami et al., 2004]. An origin of glossopharyngeal neurons either chiefly or solely from r6 is found also in teleosts, frogs and mammals (fig. 3). In zebrafish, IX neurons originate in r6 and migrate caudally into r7 [Chandrasekhar et al., 1997], a pattern shared with some of the neurons contributing to the caudal lateral line efferent nucleus [Sapède et al., 2005]. The roots and efferent neurons of the IXth and posterior lateral line nerves in larval frogs are located in r6, but unlike zebrafish, do not migrate [Straka et al., 2005]. A parasympathetic nucleus of unknown segmental origin associated with the IXth nerve has been reported in frogs [Matesz and Székely, 1996]. In mouse and ferret embryos, the IXth nerve motor root is located in the middle of r6 originating mainly from neurons in that segment, but a few cells are formed also in rostral r7 (fig. 2E, N). The adult position of IXth efferents in the inferior salivatory nucleus and rostral pole of the nucleus ambiguus imply that ventrolateral, but not much caudal, migration occur at later stages.

Elasmobranchs and non-mammalian amniotes seem to provide the greatest departure from a simple r6 origin of glossopharyngeal neurons. In *Squalus* the IXth nerve root emerges in the middle of r7 (fig. 1E; stage 24). The motor root is formed by convergence of a ladder-like array of axons that span the posterior half of r6 and much of r7. The IX neurons form a medial column of cells, displaced slightly caudal relative to the array of axons, that extends from the caudal edge of r6 back to the presumed rostral edge of r8. At later stages the IX neurons appear as a dense cell cluster extending laterally in the region caudal to the transverse axons (fig. 1K). This sequence suggests that glossopharyngeal neurons originate in both r6 and r7 and migrate caudally to settle in rostral r8 (fig. 3).

The IXth nerve efferent neurons in chick embryos are exceptionally numerous in both r6 and r7, and the motor root emerges at the r6-r7 border [Lumsden and Keynes, 1989] (fig. 3). The distribution of branchiomotor and visceromotor neurons within this pool has not been directly demonstrated in embryos, but each subgroup might arise from separate locations as is the case for the VIIth nerve. Quail-chick surgical mapping indicated that the retrofacial nucleus of IX originated from r6 and a more dorsally located periventricular IX nucleus, primarily from r7/rostral r8 [Cambronero and Puelles, 2000]. Because the retrofacial IX innervates the branchiomandibularis muscle in birds [Wild, 1981; Dubbeldam and Bout, 1990] and the dorsal IX nucleus is likely visceromotor, it seems possible that the r4-branchiomotor r5-visceromotor pattern seen in VII is repeated among IX efferents. Closer study of IX efferent development in frog, chick and mouse could test whether the inferior salivatory nucleus is typically an r7 component in tetrapods.

Vagal Nuclei

The embryonic vagal nerve complex in most species arises from a series of many small lateral rootlets extending through the whole length of the caudal hindbrain (fig. 1E, 2C; subsets of vagal roots shown). Typically, the more rostral X rootlets are larger and emerge from the brain as separate motor and sensory root fascicles, whereas the caudal rootlets are smaller and emerge as individual motor fascicles. The most rostral vagal roots are lo-

cated not far caudal to the IXth nerve roots, and thus vary in location together with the latter. The X roots in lamprey, frog and mouse (fig. 2N) appear to start in r7 whereas those in shark (fig. 1E), zebrafish and chick begin in r8. The IX and X efferent neurons overlap considerably in r7 in chick [Lumsden and Keynes, 1989], but less so in the other species. The caudal limit of vagal neurons in embryonic stages is difficult to determine as the X and XI rootlets form a continuous series and the immature neurons within the cell column look wholly similar. Vagal rootlets fasciculate peripherally and collect into a few major trunks in the vicinity of the rostral rootlets, with fibers from the caudal rootlets forming the long 'descending' tract. In amniotes roots of the XIth nerve emerge in a similar pattern from the rostral spinal cord and in most species also take part in the tract, making separate X and XI nerve root labeling unfeasible in the caudal vagal region.

In adults of many groups the vagal nuclei extend into the rostral spinal cord [Sperry and Boord, 1993; Székely and Matesz, 1993; Matesz and Székely, 1996; Nieuwenhuys et al., 1998; Taylor et al., 1999], but whether cell migration is involved in any of these cases is not clear. A general rostrocaudal sequence of branchiomotor and visceromotor subdivisions are evident in the vagal column of fish and tetrapods, with efferent pools to obvious branchial muscle homologs located rostrally, intestinal visceromotor efferents concentrated caudally and cardiac efferents distributed in intermediate and caudal regions [Taylor et al., 1999]. Motoneurons to the intrinsic laryngeal muscles in tetrapods are generally located near the caudal end of the vagal nuclei, far removed from the classic branchiomotor neurons of the rostral nucleus ambiguus [Székely and Matesz, 1993; Matesz and Székely, 1996]. The fate map of vagal nuclei in birds suggests that an orderly rostrocaudal pattern is preserved during caudal hindbrain development [Cambronero and Puelles, 2000]. The fine-grained pattern within the vagal column is likely established by some combination of 5′ Hox gene expression [Oosterveen et al., 2004] along with local influences from occipital somites [Ensini et al., 1998; Lewis and Eisen, 2004].

The Spinal Accessory Nucleus

Precise delineation of rostral XI and caudal X neurons in early embryos would help clarify the possible evolutionary relations between these neurons and provide further indicators of axial specification at the hindbrain-spinal cord junction. An antibody that recognizes the dm-grasp membrane protein has been reported to transiently mark XI neurons [Schubert and Kaprielian, 2001], and loss of function of the nkx2.9 homedomain protein [Pabst et al., 2003] has been shown to reduce the number of neurons contributing to the XI and probably caudal X nuclei. Nerve labeling combined with molecular markers or gene expression patterns [e.g., Ericson et al., 1997] should thus help to define X and XI neurons as either a single type with shared history or two distinct types that simply share a path to the periphery.

Cranial Somatic Efferent Nuclei

Trochlear Nuclei

Aside from the dorsal location in lampreys mentioned earlier, the organization and function of the trochlear (IV) nucleus is largely invariant in living vertebrates. In gnathostomes, trochlear motoneurons originate and remain medially in the most rostral hindbrain (r0), extend axons circumferentially to exit dorsally near the MHB and innervate the contralateral superior oblique eye muscle [Evinger, 1988]. A few ipsilaterally projecting neurons are often present in adult mammals, but these seem likely to result from misrouting of axons in the trochlear commissure. Many more ipsilaterally projecting neurons are present in embryonic sharks (pers. observation; fig. 3). In chick embryos the rostral part of the trochlear nucleus develops in the basal part of the narrow Fgf 8-positive isthmic zone, whereas the caudal part of the nucleus extends almost half way to the r1–r2 border [Irving et al., 2002]. Expression of Fgf 8 does not extend all the way ventrally in the isthmus, and the IV motoneurons originate in the part lacking Fgf 8. Chemotaxis experiments suggested that Fgf 8 guides pathfinding of trochlear axons, thus providing a mechanism for the dorsalward circumferential projection [Irving et al., 2002]. A curious anomaly likely related to this is the occasional appearance of labeled neurons in the caudal part of the IVth nucleus in adult mammals following dye application to trigeminal innervated muscles such as the tensor tympani [Shaw and Baker, 1983]. Because the number of these neurons increased in cases where the trochlear nerve was severed some months before the tensor tympani labeling, they likely represent trochlear neurons whose axons misroute down the mlf and then navigate to the nearest root exit, namely the Vth nerve.

Abducens Nuclei

The abducens nuclei in lampreys and gnathostomes exhibit an intriguing pattern of variation with regard to segmental origins, nuclear subdivisions, muscle targets

and axonal paths (mentioned above). The mosaic distribution of these features among different taxa and the conserved functional roles of the target muscles provide unique opportunities to test the effects of specific genetic regulatory elements across vertebrates. In 9–11 mm lamprey larvae, retrogradely labeled VI motoneurons form a loose column of cells lying medial and ventral to the branchiomotor neurons, with axons that extend laterally, dorsally and then rostrally to exit in association with the Vth root (fig. 1D). The early abducens nucleus extends from near the level of the rostral end of the VII nucleus back to a level slightly caudal to the IX nucleus. In late larval stages (fig. 1B), VI neurons have similar rostrocaudal and medial limits as in the early larva, but extend much farther laterally. The rostral limit of VI neurons thus lies slightly in front of the r4–5 border, and the caudal limit slightly behind the r6–7 border [see also Fritzsch, 1998a]. Abducens neurons in gnathostomes are likewise found primarily in r5–6, but with the notable difference that in many groups only one of those segments is occupied. Abducens motoneurons originate in both r5 and r6 in zebrafish and chick, only in r5 in frog and mouse, and mainly in r6, but with a few cells often present in rostral r7, in sharks (fig. 3). These locations seem typical for the taxonomic groups containing these species (e.g., teleosts, mammals, anurans), but variations certainly might exist. Extension into caudal r4 as in lamprey has not been reported in gnathostomes. The variation in axial location of VI neurons correlates with the rostral expression domains of Hox3 paralog group genes in lamprey [cf. fig. 1B, D and Murakami et al., 2004], zebrafish and mouse [Cordes, 2001; Moens and Prince, 2002]. Direct regulation of abducens phenotype involving Hoxb3 has been demonstrated in mice [Gaufo et al., 2003], although transposition of the nucleus by misexpression has not yet been shown.

In all vertebrates with eye muscles, motoneurons in a primary abducens nucleus innervate an 'abductor' of the eye; lateral rectus in gnathostomes, ventral rectus in lampreys [Evinger, 1988; Fritzsch et al., 1990]. An accessory abducens nucleus, probably arising by lateral migration of neurons from the main nucleus, innervates ocular retractor muscles in tetrapods (fig. 3) [Evinger, 1988; Székely and Matesz, 1993]. Lampreys have a similarly located VI nucleus that innervates the caudal rectus muscle, raising the possibility that accessory VI nuclei and related muscles might be primitive features for vertebrates [Fritzsch et al., 1990; Fritzsch, 1998a]. The segmental origin of motoneurons and the presence or absence of accessory VI nuclei do not seem to be correlated, as most of the possible different combinations of these features occur in the species reviewed here (fig. 3).

Occipitospinal/Hypoglossal Nuclei

In all vertebrate embryos a more or less continuous column of somatomotor neurons projecting through ventral roots commences in the caudal hindbrain and continues down the spinal cord (fig. 1E, 2C,N). In adult fish this is often called the spino-occipital motor column [Nieuwenhuys et al., 1998], and the hindbrain portion contributes to innervation of hypobranchial and/or rostral epaxial muscles [Neal, 1897; Sperry and Boord, 1997]. In tetrapods it forms the main part of the hypoglossal nucleus and probably the supraspinal nucleus of birds [Székely and Matesz, 1993; Cambronero and Puelles, 2000]. The ventral motor roots generally emerge in register with somites, beginning with somite 1, which in amniotes is located adjacent to caudal r7 and/or rostral r8 [Vaage, 1969; Müller and O'Rahilly, 2003]. The exact segmental location of the rostral end of the column can vary for a number of reasons. The location of the first somite relative to the rhombomeres varies between species, for example, lying quite far caudal to the r7–r8 border in zebrafish [Hanneman et al., 1988]. Furthermore, it is not clear whether a ventral root always forms adjacent to the most anterior somite in different species and many reports suggest that one or more rostral ventral roots disappear later in development. Because somatomotor neurons show no tendency to migrate longitudinally in the brain, the rostral limit of these cells in embryos and of the hypobranchial or hypoglossal nuclei at later stages should reflect the formation and subsequent retention or loss of efferent neurons originally established in developmental relation to the occipital somites.

In chick, quail, mouse and ferret embryos examined by dissection, the most rostral roots tended to be smaller than succeeding caudal ones and generally they emerged from the neuroepithelium about one segment caudal to the IX root, thus lying in caudal r7 or rostral r8 (fig. 2N; 3E, F). Although the rostralmost rootlets were difficult to visualize, the general impression from directly comparing preparations with the VIth, IXth and XIIth nerve roots intact was that the XII rootlets in mammals extended slightly farther rostral than in the birds. A continuous series of small ventral rootlets emerging between the first large occipital root and the caudal abducens roots has been reported for embryos of various mammal and bird species [Bremer, 1908; Kuratani et al., 1988]. A possible explanation in chicks is that the rootlets present in rostral

r8 develop in relationship to somite 1, but the inconstant r7 rootlets relate to the immediately rostral mesoderm, the often described 'incomplete' somite. A number of dye marking studies showed that this mesoderm is present at slightly later stages lying immediately caudal to the otic vesicle between the roots of nerves IX and X [Hinsch and Hamilton, 1956], precisely the span of r7 where the inconstant rostral occipital rootlets are observed. The rostral limit of the hypoglossal nucleus in chicks maps to the r7–r8 boundary [Cambronero and Puelles, 2000], suggesting that the more usual rostral r8 neurons forming the first main occipital root in early embryos persist through development. The slightly more rostral locations of XII neurons and the IX root in mammals likely reflect subtle, but definite, differences in the relations of anterior somites, neuromeres, neural crest and otic vesicle among different species [Müller and O'Rahilly, 2003]. The occasional presence of rootlets between XII and VI in mammals [Bremer, 1908] further implies that variability in somatomotor neuron production can involve not just r7, but also r6. Fully developed abducens and hypoglossal motoneurons are much different from one another, thus deciphering the early stages of their genetic and phenotypic divergence could throw substantial light on the precise control of neuronal identity in r5–r7 [Gaufo et al., 2003].

The occipital region and hypoglossal roots in frogs and other amphibians are substantially different from amniotes and at first glance seem somewhat incomparable. Frogs, other than aglossal types such as *Xenopus*, have a hypoglossal nerve and central nucleus that innervate tongue-related structures as in amniotes [Matesz et al., 1999]. In adults, nerves are not present between the roots of the IX-X-XI complex, originating in larvae at r6–r7, and the second spinal nerve (Sp2) which emerges behind the first vertebra. The roots of the hypoglossal nerve are wholly incorporated within the ventral roots of the Sp2 complex, which appears as a series of 2–5 rootlets centered at the level of the obex [Straka et al., 2005]. A small first spinal nerve likely contributes to innervation of the anteriormost myotomes in larval frogs. Transitory rootlets to two occipital myotomes have been described at earlier stages in a number of studies [Schlosser and Roth, 1997]. The main dorsolateral hypoglossal nucleus (XIIdl) in frogs is directly comparable with the hypoglossal nucleus of amniotes both in terms of muscle targets and dendritic morphology of the motoneurons [Matesz et al., 1999]. Individual rootlet labeling showed that all but the most caudal XII-Sp2 rootlets contained axons solely from XIIdl neurons, and that the rostrocaudal order of neurons in the nucleus matched their organization within the rootlets. When compared with the segmental locations of VI, IX and X neurons, the rostral end of the frog XIIdl nucleus mapped to a position that corresponded to the 'r8'–'r9' border of birds [cf., Cambronero and Puelles, 2000; Straka et al., 2005]. Because this border is one segment caudal to that of the bird XII nucleus, it implies a motoneuron source related to somites 2–4. The adult frog pattern could arise by either the generation of motoneurons and peripheral target muscles at metamorphosis or development of central descending axon collaterals by neurons present since early stages [Schlosser and Roth, 1997]. Comparison of early hindbrain somatomotor neuron production and the later fates of these cells in *Xenopus* and ranid frogs would distinguish between these two mechanisms.

The hindbrain somatomotor neurons in zebrafish are most often referred to as the first two segments of spinal motoneurons. Other than the anterior two myotomes, specific motor innervation from this location has not been reported, but it likely includes the sternohyoid muscle, which is the only hypobranchial muscle in zebrafish [Schilling and Kimmel, 1997]. The sternohyoid in eel is innervated by spino-occipital neurons extending on either side of the obex [Mukuda and Ando, 2003], thus fitting this location. As in amphibians, the presence of free occipital nerves apparently collected together into the first spinal nerve appears to be highly variable in teleosts.

In dogfish embryos, beginning at 25–30 somites, small ventral roots can be identified in sectioned material adjacent to the anterior somites [not shown, but see Neal, 1898, 1914]. At slightly later stages the most rostral of these roots lies at the level of the anterior vagal rootlets, thus not far behind the putative r7–r8 border. This root does not persist, and by stage 23–24, only three occipital roots are usually present, with the most anterior lying well behind the r7–8 border (x in fig. 1E) [Gilland and Baker, 1993]. Older embryos and adults often have only two occipital roots, and because the hypobranchial motor column in adult *Squalus* [Smeets et al., 1983] and *Raja* [Sperry and Boord, 1997] only begins near the obex, a genuine loss of the rostral somatomotor column likely occurs during elasmobranch ontogeny.

In lamprey larvae slightly younger than shown in figure 1D, the most rostral ventral roots and motoneurons labeled from dye applied to the anterior myotomes were located at about the same distance behind the abducens nucleus as root 'x' in Squalus (fig. 1E; lamprey data not shown). This would place the neurons quite far caudal relative to somite 1 [Kuratani et al., 1999], so it seems

possible that either more rostral roots were present but not labeled, or ventral roots do not form in association with somite 1 in lamprey. Because of the long branchial region in lampreys, somitic and motor nerve contributions to the hypobranchial system begin 4–5 somites farther caudally than in gnathostomes [Neal, 1897].

Discussion and Conclusions

The data summarized in figure 3 suggests that the overall segmental pattern of cranial efferent nuclei is widely conserved in vertebrates and that most variations involve relatively minor differences in the rostral and caudal limits of homologous nuclei. Two major segmental variations stand out; the different caudal limits of trigeminal motoneurons in lampreys (in r4) versus gnathostomes (r3–4 border), and the shifting location of abducens motoneurons within the region spanning from caudal r4 (lamprey) to rostral r7 (shark). The generally conserved features and minor variations will be discussed first, with an emphasis on opportunities for further testing of possible systematic patterns. The small differences, reported here and elsewhere, are difficult to evaluate as they may result from normal 'spillover' of cells originating near borders, discrepancy in estimating precise locations of the borders at depth in the neuroepithelium and, in some species, ambiguity in identifying the rhombomeric borders.

Branchial and Octavolateral Efferents

Studies in the past decade on the origins, migrations and subdivisions of branchiomotor, visceromotor and octavolateral efferents in zebrafish and tetrapods have refined the general picture of vertebrate branchial nerve development [e.g., Bruce et al., 1997; Chandrasekhar et al., 1997; Jacob and Guthrie, 2000; Straka et al., 2005]. The common pattern in these species for VIIbm and octavolateral efferent production in r4 likely includes lampreys and elasmobranchs (fig. 3). Although the precise origins of VII and octavolateral neurons in the elasmobranch need to be determined at earlier stages, the overall similarity of r4 origin and caudal migration in zebrafish and shark points to a likely broad generality of this pattern in jawed fish. Branchiomotor neuron production in r5 needs to be determined in more taxa to distinguish whether the lack of these neurons in zebrafish, frogs and mice is a widespread feature, or is instead merely a variation on a more general r4–r5 origin as in lampreys and chick (fig. 3). Similar questions apply to the origins of IX branchiomotor and visceromotor efferents in either r6 or r7 (fig. 3). Two related problems are ascertaining the identities and origins of cranial visceromotor neurons serving branchial arches in fish and establishing the distinctions between caudal X and rostral XI neurons at early embryonic stages. Examining these issues in salamanders, turtles, alligators and marsupials would likely clarify the basic patterns of branchial nerve efferents in tetrapods, and information from non-cyprinid teleosts or any of the more basal actinopterygians would at least begin revealing the general patterns in bony fish.

The largely conserved origins of branchiomotor and octavolateral efferent nuclei in species described here, along with the widespread occurrence of ascending axonal paths for these neurons in adults of many groups [Nieuwenhuys et al., 1998] point to caudal neuronal migration as the main mechanism producing different relative neuronal locations in adults. The extremes of this process are seen by comparing lamprey and frog with shark (fig. 3). In the former, the adult efferent nuclei retain the same axial positions as in early larva, indicating an absence of longitudinal migration. In *Squalus*, virtually the entire system of respiratory-related neurons relocates farther caudally, producing a continuous column of V-VII-IX-X branchiomotor neurons extending from r6 back to the caudal end of the hindbrain (fig. 3). Other than the Vth component, zebrafish exhibit a similar migration pattern that is probably typical among both cartilaginous and ray-fined fish. Limiting the observations to frogs, chicks and mice undoubtedly gives an incorrect impression of the role of efferent migration in tetrapods. Lack of efferent migration in frogs is ostensibly secondary and likely due in part to paedomorphosis [Straka et al., 2005]. The extensive overlap of cranial efferent nuclei in adult salamanders, including some highly paedomorphic species [Roth et al., 1988], suggests that caudal efferent migration occurs in amphibians, but that paedomorphosis alone cannot explain lack of migration in frogs. Likewise, the adult locations of the VII and IX nuclei in reptiles suggest that birds might exhibit less caudal relocation of efferent neurons than many other amniotes [Nieuwenhuys et al., 1998]. The common occurrence of caudal, but not rostral, efferent migration begs the question of why these movements happen at all. Candidate molecular mechanisms are emerging [Chandrasekhar, 2004], but few clues are yet available for the underlying physiological or evolutionary rationale [see Straka et al., 2005].

The Caudal Hindbrain

Comparisons of segmental organization in the embryonic vagal nuclei between species are not available. Nev-

ertheless, it seems clear that the hindbrain caudal to the r6 border is a highly conserved and precisely patterned region of the brain. Initial fate mapping [Cambronero and Puelles, 2000] combined with the demonstration of retinoid-mediated nesting of multiple hox gene expression limits [Oosterveen et al., 2004] indicate that the caudal hindbrain comprises a patterning system on the same scale as the r2–r6 region. The extensive anatomical and physiological data available for this region and the presence of unique and highly conserved pre-cerebellar, reticular, branchiomotor and visceromotor cell groups [Baker and Gilland, 1996; Taylor et al., 1999] make comparison between the rhombomeric and non-rhombomeric parts of the hindbrain an ideal test of the underlying principle for the elaborate boundary mechanisms defining the former. If the occipital somites turn out to contribute to the fine-grained axial patterning of the caudal hindbrain, then a possible reason for 'inventing' rhombomeres could have been to replace the loss of primitive somitic patterning of cranial mesoderm adjacent to the rostral half of the hindbrain (see below).

XII/Occipital

Disparities in the rostral limits of the XII/occipital somatomotor neurons (fig. 3) probably arise from a number of factors, some of which may relate to the caudal hindbrain Hox code and/or early interactions with occipital somites. These effects would presumably also influence the axial locations and axonal routes of motoneurons serving specific epaxial, hypobranchial/hypoglossal and pectoral muscles. The differing patterns of occipital and rostral spinal nerve roots within elasmobranchs, amphibians and actinopterygians may turn out to be largely inconsequential as far as specific motoneuron populations and muscle targets are concerned. The large first spinal nerve in many teleosts and second spinal nerve in frogs appear to be collector trunks that numerous, highly specific efferent pools choose as points of common egress, likely due to impediments posed by skeletogenic processes occurring during formation of the occipital and craniovertebral elements. Ontogenetic studies in a few species of amphibians and teleosts with special attention to the precise origins and fates of the spino-occipital motoneurons might clarify these long-standing issues.

V Nuclei in Lamprey and Gnathostomes

The extension of the Vth nucleus in lampreys well into r4 and slightly into r1 [further according to Murakami et al., 2004] contrasts with the tighter restriction of trigeminal efferents to r2–3 in zebrafish and tetrapods (fig. 3). Dye labeling results from *Squalus* shown here cannot exclude an origin of some migratory caudal Vth neurons from r4. The fibers running transversely in r4 are interpreted as afferents because, unlike in r3, cells were never seen in r4 either medially or laterally other than those directly within the migratory stream that extended caudally from r3. Trigeminal afferent fibers joining the migrating caudal V motoneurons makes physiological sense, but verification will require double labeling of motor and sensory roots or cell type-specific immunohistochemical markers.

The caudal trigeminal nucleus of lampreys that originates in r3–r4 and innervates the rostral muscles is generally considered to have no clear parallels in gnathostomes [Hardisty and Rovainen, 1982; Kuratani et al., 2004]. In contrast, the rostral Vth subnucleus located mainly in r2 (fig. 1B, D) and the corresponding mandibular arch/velar targets of lampreys correspond to the r2-mandibular adductor system in gnathostomes. One possibility is that the shared r2-mandibular pattern might be primitive for vertebrates, whereas the caudal Vth nuclei and muscle targets were subsequently and independently derived within lineages leading to cyclostomes and gnathostomes. This hypothetical primitive absence of branchiomotor neuron production in r3 along with the near (or complete) absence of r5 branchiomotor neuron production seen in some gnathostomes (fig. 3) suggests a proto-vertebrate with an alternating set of branchiomotor nuclei in r2, r4 and r6 serving the three rostral branchial arches. Alternatively, parts of the caudal Vth nuclei and buccal muscles in lampreys may be directly homologous to those in sharks [Song and Boord, 1993; Mallatt, 1996]. If so, then the question still remains whether the caudal V and rostral V systems are of equal age in vertebrates, or represent two stages in branchial arch evolution. In cases like the Vr/Vc and VI (below) of lampreys and gnathostomes where amphioxus may not supply sufficient neuronal features for out-group analysis, the main hope for resolving stages of character evolution is the chance that historical traces can be found either in the pattern of regulatory genes that produce the features, or at a finer level, in the genomic organization of non-coding sequences associated with those genes.

Abducens and Primitive Somatomotor Column

The differences separating the oculomotor and trochlear nerves from both somatic and branchial series suggest that they have a very different history than the abducens nucleus. Other than the branchiomotor-like axonal

path in lampreys, abducens motoneurons appear to be specialized but genuine members of the somatomotor group. This raises the issue of whether somatomotor neurons existed primitively in the r4–r6 region of proto-vertebrates or were a later addition, possibly generated by Hox-mediated transposition of the somatomotor phenotype. Given the segmental variation in r5–6 in gnathostomes, the occasional appearance of ventral roots between XII and VI [Bremer, 1908; Kuratani et al., 1988] and the extension of abducens neurons into r4 in lampreys, two alternative scenarios for the origins of the vertebrate abducens can be proposed. In the first, a somatomotor phenotype that never existed rostral to r8 was transposed forward to create abducens neurons in the r5–r6 region. This implies likely substitution of Hox4 regulatory control by Hox3, and is well suited to a model in which somites primitively did not exist rostral to the current location of the otic vesicle [Kuratani et al., 1999]. Alternatively, somatomotor neurons could have primitively extended to the rostral limits of Hox3 influence (or even farther forward) and the abducens nucleus represents a remnant of that distribution carved out by suppression in r7 (and possibly r2–4). This would fit with a model in which head mesoderm formerly was organized into somites at least through the hyoid region (lateral rectus origin in gnathostomes) just anterior to the present ear. This scheme also offers an explanation for the existence of rhombomeres, as loss of distinct somitic patterning might have necessitated a new means of stabilizing precise axial spatial pattern in the rostral hindbrain neuroepithelium [Bardet et al., 2005].

Determining the primitive status of vertebrate cranial mesoderm as somitic or not largely depends on comparisons with the rostral somites of amphioxus. Demonstration of similar patterns of early axial patterning genes would support a model in which suppression of somitogenesis occurred in early vertebrate evolution, whereas lack of similarity would point to non-homology, suggesting de novo generation of a new class of rostral mesoderm [e.g., Kuratani et al., 1999; Mazet and Shimeld, 2002]. The similarities in gastrulation, mesoderm formation and basic antero-posterior patterning within chordates make such a radical repatterning difficult to envision, as it implies major changes in mesoderm-neural plate interactions during axial specification. That would appear to be a much more fundamental change than the emergence of a new dorsal class of neuropeithelium in proto-vertebrates, the neural crest. Retaining the underlying antero-posterior pattern represented in somites 1–5 in amphioxus and just suppressing aspects of somite/myotome formation to make appropriately patterned mesoderm available for extraocular and branchial muscle specialization seems a more parsimonious mechanism for incorporating the eyes, otic capsules and muscular pharyngeal arches into the head.

References

Baker R, Gilland E (1996) The evolution of hindbrain visual and vestibular innovations responsible for oculomotor function. In: Acquisition of Motor Behavior in Vertebrates (Bloedel JR, Ebner TJ, Wise SP, eds), pp 29–55. Boston: MIT Press.

Bardet PL, Schubert M, Horard B, Holland LZ, Laudet V, Holland ND, Vanacker JM (2005) Expression of estrogen-receptor related receptors in amphioxus and zebrafish: implications for the evolution of posterior brain segmentation at the invertebrate-to-vertebrate transition. Evol Dev 7:223–233.

Bremer JL (1908) Aberrant roots and branches of the abducent and hypoglossal nerves. J Comp Neurol 18:619–639.

Bricaud O, Chaar V, Dambly-Chaudière C, Ghysen A (2001) Early efferent innervation of the zebrafish lateral line. J Comp Neurol 434:253–261.

Briscoe J, Pierani A, Jessell TM, Ericson J (2000) A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell 101:435–445.

Bruce LL, Kingsley J, Nichols DH, Fritzsch B (1997) The development of vestibulocochlear efferents and cochlear afferents in mice. Int J Dev Neurosci 15:671–692.

Cambronero F, Puelles L (2000) Rostrocaudal nuclear relationships in the avian medulla oblongata: a fate map with quail chick chimeras. J Comp Neurol 427:522–545.

Chandrasekhar A (2004) Turning heads: development of vertebrate branchiomotor neurons. Dev Dyn 229:143–161.

Chandrasekhar A, Moens CB, Warren JT, Jr, Kimmel CB, Kuwada JY (1997) Development of branchiomotor neurons in zebrafish. Development 124:2633–2644.

Cordes SP (2001) Molecular genetics of cranial nerve development in mouse. Nat Rev Neurosci 2:611–623.

de Beer GR (1937) The Development of the Vertebrate Skull. Oxford UK: Oxford University Press.

Dubbeldam JL, Bout RG (1990) The identification of the motor nuclei innervating the tongue muscles in the mallard *(Anas platyrhynchos)*; an HRP study. Neurosci Lett 119:223–227.

Easter SS, Jr., Ross LS, Frankfurter A (1993) Initial tract formation in the mouse brain. J Neurosci 13:285–299.

Eddison M, Toole L, Bell E, Wingate RJ (2004) Segmental identity and cerebellar granule cell induction in rhombomere 1. BMC Biol 2:1–14.

Ensini M, Tsuchida TN, Belting HG, Jessell TM (1998) The control of rostrocaudal pattern in the developing spinal cord: specification of motor neuron subtype identity is initiated by signals from paraxial mesoderm. Development 125:969–982.

Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J (1997) Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90:169–180.

Evinger C (1988) Extraocular motor nuclei: location, morphology and afferents. Rev Oculomot Res 2:81–117.

Fritzsch B (1998a) Evolution of the vestibulo-ocular system. Otolaryngol Head Neck Surg 119:182–192.

Fritzsch B (1998b) Of mice and genes: evolution of vertebrate brain development. Brain Behav Evol 52:207–217.
Fritzsch B, Dubuc R, Ohta Y, Grillner S (1989) Efferents to the labyrinth of the river lamprey *(Lampetra fluviatilis)* as revealed with retrograde tracing techniques. Neurosci Lett 96: 241–246.
Fritzsch B, Northcutt RG (1993) Cranial and spinal nerve organization in amphioxus and lampreys: evidence for an ancestral craniate pattern. Acta Anat 148:96–109.
Fritzsch B, Sonntag R, Dubuc R, Ohta Y, Grillner S (1990) Organization of the six motor nuclei innervating the ocular muscles in lamprey. J Comp Neurol 294:491–506.
Gaskell WH (1889) On the relation between the structure, function, distribution and origin of the cranial nerves; together with a theory of the origin of the nervous system of vertebrates. J Physiol 10:153–211.
Gaufo GO, Thomas KR, Capecchi MR (2003) Hox3 genes coordinate mechanisms of genetic suppression and activation in the generation of branchial and somatic motoneurons. Development 130:5191–5201.
Gavalas A, Davenne M, Lumsden A, Chambon P, Rijli FM (1997) Role of Hoxa-2 in axon pathfinding and rostral hindbrain patterning. Development 124:3693–3702.
Gilland E, Baker R (1992) Longitudinal and tangential migration of cranial nerve efferent neurons in the developing hindbrain of *Squalus acanthias*. Biol Bull 183:356–358.
Gilland E, Baker R (1993) Conservation of neuroepithelial and mesodermal segments in the embryonic vertebrate head. Acta Anat 148:110–123.
Goodrich ES (1930) Studies on the structure and development of vertebrates. London: Macmillan and Co., Ltd.
Hanneman E, Trevarrow B, Metcalfe WK, Kimmel CB, Westerfield M (1988) Segmental pattern of development of the hindbrain and spinal cord of the zebrafish embryo. Development 103:49–58.
Hardisty MW, Rovainen CM (1982) Morphological and functional aspects of the muscular system. In: The Biology of Lampreys. Vol 4A (Hardisty MW, Potter IC, eds), pp 137–231. London: Academic Press.
Heyman I, Faissner A, Lumsden A (1995) Cell and matrix specialisations of rhombomere boundaries. Dev Dyn 204:301–315.
Higashijima S, Hotta Y, Okamoto H (2000) Visualization of cranial motor neurons in live transgenic zebrafish expressing green fluorescent protein under the control of the islet-1 promoter/enhancer. J Neurosci 20:206–218.
Hinsch GW, Hamilton HL (1956) The developmental fate of the first somite of the chick. Anat Rec 125:225–245.
Homma S (1978) Organization of the trigeminal motor nucleus before and after metamorphosis in lampreys. Brain Res 140:33–42.
Huang R, Zhi Q, Patel K, Wilting J, Christ B (2000) Contribution of single somites to the skeleton and muscles of the occipital and cervical regions in avian embryos. Anat Embryol 202: 375–383.
Irving C, Malhas A, Guthrie S, Mason I (2002) Establishing the trochlear motor axon trajectory: role of the isthmic organiser and Fgf8. Development 129:5389–5398.
Jacob J, Guthrie S (2000) Facial visceral motor neurons display specific rhombomere origin and axon pathfinding behavior in the chick. J Neurosci 20:7664–7671.
Katbamna B, Jelaso AM, Ide CF (2004) Connexin 43 expression in glial cells of developing rhombomeres of *Xenopus laevis*. Int J Dev Neurosci 22:47–55.
Krammer EB, Lischka MF, Egger TP, Riedl M, Gruber H (1987) The motoneuronal organization of the spinal accessory nuclear complex. Adv Anat Embryol Cell Biol 103:1–62.
Kuratani S, Horigome N (2000) Developmental morphology of branchiomeric nerves in a cat shark, *Scyliorhinus torazame*, with special reference to rhombomeres, cephalic mesoderm and distribution patterns of cephalic crest cells. Zool Sci 17:893–909.
Kuratani S, Horigome N, Hirano S (1999) Developmental morphology of the head mesoderm and reevaluation of segmental theories of the vertebrate head: evidence from embryos of an agnathan vertebrate, *Lampetra japonica*. Dev Biol 210:381–400.
Kuratani S, Murakami Y, Nobusada Y, Kusakabe R, Hirano S (2004) Developmental fate of the mandibular mesoderm in the lamprey, *Lethenteron japonicum*: Comparative morphology and development of the gnathostome jaw with special reference to the nature of the trabecula cranii. J Exp Zoolog B Mol Dev Evol 302:458–468.
Kuratani S, Tanaka S, Ishikawa Y, Zukeran C (1988) Early development of the hypoglossal nerve in the chick embryo as observed by the whole-mount nerve staining method. Am J Anat 182:155–168.
Lacalli TC, Kelly SJ (1999) Somatic motoneurones in amphioxus larvae: cell types, cell position and innervation patterns. Acta Zool 80:113–124.
Lewis KE, Eisen JS (2004) Paraxial mesoderm specifies zebrafish primary motoneuron subtype identity. Development 131:891–902.
Linville A, Gumusaneli E, Chandraratna RA, Schilling TF (2004) Independent roles for retinoic acid in segmentation and neuronal differentiation in the zebrafish hindbrain. Dev Biol 270:186–199.
Luiten PG (1976) A somatotopic and functional representation of the respiratory muscles in the trigeminal and facial motor nuclei of the carp (*Cyprinus carpio* L.). J Comp Neurol 166:191–200.
Lumsden A, Keynes R (1989) Segmental patterns of neuronal development in the chick hindbrain. Nature 337:424–428.
Lumsden A, Krumlauf R (1996) Patterning the vertebrate neuraxis. Science 274:1109–1115.
Mallatt J (1996) Ventilation and the origin of jawed vertebrates: a new mouth. Zool J Linn Soc 117:329–404.
Martinez S, Geijo E, Sanchez-Vives MV, Puelles L, Gallego R (1992) Reduced junctional permeability at interrhombomeric boundaries. Development 116:1069–1076.
Matesz C, Schmidt I, Szabo L, Birinyi A, Székely G (1999) Organization of the motor centres for the innervation of different muscles of the tongue: a neuromorphological study in the frog. Eur J Morphol 37:190–194.
Matesz C, Székely G (1996) Organization of the ambiguus nucleus in the frog *(Rana esculenta)*. J Comp Neurol 371:258–269.
Mazet F, Shimeld SM (2002) The evolution of chordate neural segmentation. Dev Biol 251: 258–270.
McAlpine RJ (1959) Selected observations on the early development of the motor neurons in the brain stem and spinal cord of the white rat as revealed by the alkaline phosphatase technique. J Comp Neurol 113:211–243.
Moens CB, Prince VE (2002) Constructing the hindbrain: insights from the zebrafish. Dev Dyn 224:1–17.
Morin-Kensicki EM, Melancon E, Eisen JS (2002) Segmental relationship between somites and vertebral column in zebrafish. Development 129:3851–3860.
Mukuda T, Ando M (2003) Medullary motor neurones associated with drinking behaviour of Japanese eels. J Fish Biol 62:1–12.
Müller F, O'Rahilly R (2003) Segmentation in staged human embryos: the occipitocervical region revisited. J Anat 203:297–315.
Murakami Y, Pasqualetti M, Takio Y, Hirano S, Rijli FM, Kuratani S (2004) Segmental development of reticulospinal and branchiomotor neurons in lamprey: insights into the evolution of the vertebrate hindbrain. Development 131: 983–995.
Myers PZ (1985) Spinal motoneurons of the larval zebrafish. J Comp Neurol 236:555–561.
Narayanan CH, Narayanan Y (1978) Determination of the embryonic origin of the mesencephalic nucleus of the trigeminal nerve in birds. J Embryol Exp Morphol 43:85–105.
Neal HV (1897) The devlopment of the hypoglossus musculature in *Petromyzon* and *Squalus*. Anat Anz 13:441–463.
Neal HV (1898) The segmentation of the nervous system in *Squalus acanthias*. Bull Mus Comp Zool Harvard 31:147–294.
Neal HV (1914) The morphology of the eye-muscle nerves. J Morph 25:1–188.
Nieuwenhuys R, ten Donkelaar HJ, Nicholson C (1998) The central nervous system of vertebrates. Berlin: Springer.
Nittenberg R, Patel K, Joshi Y, Krumlauf R, Wilkinson DG, Brickell PM, Tickle C, Clarke JD (1997) Cell movements, neuronal organisation and gene expression in hindbrains lacking morphological boundaries. Development 124: 2297–2306.

Ojeda J, Piedra S (1998) Supraependymal cells and fibers during the early stages of chick rhombencephalic development. Anat Embryol 198: 237–244.

Oosterveen T, Meijlink F, Deschamps J (2004) Expression of retinaldehyde dehydrogenase II and sequential activation of 5′ Hoxb genes in the mouse caudal hindbrain. Gene Expr Patterns 4:243–247.

Pabst O, Rummelies J, Winter B, Arnold HH (2003) Targeted disruption of the homeobox gene Nkx2.9 reveals a role in development of the spinal accessory nerve. Development 130: 1193–1202.

Pattyn A, Vallstedt A, Dias JM, Samad OA, Krumlauf R, Rijli FM, Brunet JF, Ericson J (2003) Coordinated temporal and spatial control of motor neuron and serotonergic neuron generation from a common pool of CNS progenitors. Genes Dev 17:729–737.

Pombal MA, Rodicio MC, Anadón R (1994) Development and organization of the ocular motor nuclei in the larval sea lamprey, *Petromyzon marinus* L.: an HRP study. J Comp Neurol 341:393–406.

Prin F, Ng KE, Thaker U, Drescher U, Guthrie S (2005) Ephrin-As play a rhombomere-specific role in trigeminal motor axon projections in the chick embryo. Dev Biol 279:402–419.

Pritz MB (1999) Rhombomere development in a reptilian embryo. J Comp Neurol 411:317–326.

Riley BB, Chiang MY, Storch EM, Heck R, Buckles GR, Lekven AC (2004) Rhombomere boundaries are Wnt signaling centers that regulate metameric patterning in the zebrafish hindbrain. Dev Dyn 231:278–291.

Roth G, Nishikawa K, Dicke U, Wake DB (1988) Topography and cytoarchitecture of the motor nuclei in the brainstem of salamanders. J Comp Neurol 278:181–194.

Samad OA, Geisen MJ, Caronia G, Varlet I, Zappavigna V, Ericson J, Goridis C, Rijli FM (2004) Integration of anteroposterior and dorsoventral regulation of Phox2b transcription in cranial motoneuron progenitors by homeodomain proteins. Development 131:4071–4083.

Sapède D, Rossel M, Dambly-Chaudiere C, Ghysen A (2005) Role of SDF1 chemokine in the development of lateral line efferent and facial motor neurons. Proc Natl Acad Sci USA 102: 1714–1718.

Schilling TF, Kimmel CB (1997) Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. Development 124:2945–2960.

Schlosser G, Roth G (1997) Evolution of nerve development in frogs. I. The development of the peripheral nervous system in *Discoglossus pictus* (Discoglossidae). Brain Behav Evol 50:61–93.

Schubert W, Kaprielian Z (2001) Identification and characterization of a cell surface marker for embryonic rat spinal accessory motor neurons. J Comp Neurol 439:368–383.

Shaw MD, Baker R (1983) The locations of stapedius and tensor tympani motoneurons in the cat. J Comp Neurol 216:10–19.

Simon H, Lumsden A (1993) Rhombomere-specific origin of the contralateral vestibulo-acoustic efferent neurons and their migration across the embryonic midline. Neuron 11:209–220.

Smeets WJAJ, Nieuwenhuys R, Roberts BL (1983) The central nervous system of cartilaginous fishes. Berlin: Springer-Verlag.

Song J, Boord RL (1993) Motor components of the trigeminal nerve and organization of the mandibular arch muscles in vertebrates. Phylogenetically conservative patterns and their ontogenetic basis. Acta Anat 148:139–149.

Sperry D, Boord R (1997) Organization of the hypobranchial motor column of the clearnose skate, *Raja eglanteria*, with comparisons to tetrapods. Acta Anat 160:21–32.

Sperry DG, Boord RL (1992) Central location of the motoneurons that supply the cucullaris (trapezius) of the clearnose skate, *Raja eglanteria*. Brain Res 582:312–319.

Sperry DG, Boord RL (1993) Organization of the vagus in elasmobranchs: its bearing on a primitive gnathostome condition. Acta Anat 148: 150–159.

Straka H, Baker R, Gilland E (2001) Rhombomeric organization of vestibular pathways in larval frogs. J Comp Neurol 437:42–55.

Straka H, Baker R, Gilland E (2005) Preservation of segmental hindbrain organization in adult frogs. J Comp Neurol, in press.

Straus WL, Howell AB (1936) The spinal accessory nerve and its musculature. Quart Rev Biol 11: 387–405.

Sundin L, Nilsson S (2002) Branchial innervation. J Exp Zool 293:232–248.

Székely G, Matesz C (1993) The efferent system of cranial nerve nuclei: a comparative neuromorphological study. Adv Anat Embryol Cell Biol 128:1–92.

Taylor EW, Jordan D, Coote JH (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol Rev 79:855–916.

Terashima T (1996) Distribution of mesencephalic trigeminal nucleus neurons in the reeler mutant mouse. Anat Rec 244:563–571.

Vaage S (1969) The segmentation of the primitive neural tube in chick embryos *(Gallus domesticus)*. A morphological, histochemical and autoradiographical investigation. Ergeb Anat Entwicklungsgesch 41:3–87.

Wake DB (1993) Brainstem organization and branchiomeric nerves. Acta Anat 148:124–131.

Waskiewicz AJ, Rikhof HA, Moens CB (2002) Eliminating zebrafish pbx proteins reveals a hindbrain ground state. Dev Cell 3:723–733.

Widmer CG, Morris-Wiman JA, Calhoun JC (1998) Development of trigeminal mesencephalic and motor nuclei in relation to masseter muscle innervation in mice. Brain Res Dev Brain Res 108:1–11.

Wild JM (1981) Identification and localization of the motor nuclei and sensory projections of the glossopharyngeal, vagus, and hypoglossal nerves of the cockatoo *(Cacatua roseicapilla)*, Cacatuidae. J Comp Neurol 203:351–377.

Windle WF (1970) Development of neural elements in human embryos of four to seven weeks gestation. Exper Neurol 29:44–83.

Yoshida M, Colman DR (2000) Glial-defined rhombomere boundaries in developing *Xenopus* hindbrain. J Comp Neurol 424:47–57.

Zervas M, Millet S, Ahn S, Joyner AL (2004) Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. Neuron 43:345–357.