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Gaskell revisited: new insights into spinal autonomics necessitate a revised motor neuron nomenclature

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Abstract

Several concepts developed in the 19th century have formed the basis of much of our neuroanatomical teaching today. Not all of these were based on solid evidence nor have withstood the test of time. Recent evidence on the evolution and development of the autonomic nervous system, combined with molecular insights into the development and diversification of motor neurons, challenges some of the ideas held for over 100 years about the organization of autonomic motor outflow. This review provides an overview of the original ideas and quality of supporting data and contrasts this with more accurate and in depth insight provided by studies using modern techniques. Several lines of data demonstrate that branchial motor neurons are a distinct motor neuron population within the vertebrate brainstem, from which parasympathetic visceral motor neurons of the brainstem evolved. The lack of an autonomic nervous system in jawless vertebrates implies that spinal visceral motor neurons evolved out of spinal somatic motor neurons. Consistent with the evolutionary origin of brainstem parasympathetic motor neurons out of branchial motor neurons and spinal sympathetic motor neurons out of spinal motor neurons is the recent revision of the organization of the autonomic nervous system into a cranial parasympathetic and a spinal sympathetic division (that is, there is no sacral parasympathetic division). We propose a new nomenclature that takes all of these new insights into account and avoids the conceptual misunderstandings and incorrect interpretation of limited and technically inferior data inherent in the old nomenclature.

Introduction

Gaskell's research breakthrough was his 1880 paper revealing the dual innervation of the heart by what we now refer to as myelinated preganglionic fibers coming from the vagus nerve and unmyelinated postganglionic fibers coming from the sympathetic chain of ganglia

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(Gaskell, 1880). This insight allowed him to show later (Gaskell, 1886) that the sympathetic chain is connected to the spinal nerves by two branches, which he referred to respectively as the grey ramus (formed by unmyelinated nerve fibers associated with each sympathetic ganglion) and the white ramus (formed by myelinated nerve fibers) and that the white rami were absent in certain areas of the spinal cord, notably in the lumbar and cervical segments innervating the limbs. In 1889 Gaskell (Gaskell, 1889) expanded on these important and accurate findings to develop a new theory of cranial and spinal nerve organization based on his analyses of vertebrate nerves and their components. He included some unusual speculations on the evolution of the vertebrate brain that were later summarized in a 1906 volume suggesting how an invertebrate-like brain and sensory organs could be transformed into a vertebrate brain. Gaskell's studies, as well as his understanding and interpretation of the pertinent literature of the time, led him to formulate a novel definition of what he termed the 'complete segmental nerve', which comprised a posterior (dorsal) root component containing afferent fibers [both somatic and splanchnic (=visceral)] and an anterior (ventral) root component containing '(1) efferent non-ganglionated splanchnic and somatic fibers and (2) efferent ganglionated splanchnic fibers. He also believed that some spinal efferent fibers may pass through the dorsal root as claimed much later by others in lampreys, turtles and some spinal nerves of dogs (Gaskell, 1920).

With this framework in mind, he also attempted to define the cranial nerves as iterative homologs of the spinal segmental nerves (Gaskell, 1889). After excluding the olfactory and optic nerves as nonconformant in this segmental scheme, he concluded that oculomotor and trochlear nerves are 'purely efferent in existing animals, whatever [they] may have been originally'. He claimed that the ciliary ganglion is 'wholly sympathetic' and not a displaced dorsal root ganglion as claimed by some. However, he also claimed that the trochlear nerve was composed of large and small caliber fibers with the identity of the small fibers as yet undetermined. He specifically asked the question: 'Where then are the afferent fibers of these two nerves?' and answered this question in the affirmative: 'for both these nerves possess within themselves structures which appear to me to have been originally the nerve cells...corresponding to the cells and the nerve fibers of...posterior root of a spinal nerve.' Following similar claims made by others, Gaskell concluded that 'in the IIIrd and IVth nerves we have the remnants of nerve fibers and nerve cells which in by-gone animals were once of functional importance, but which have become useless and so lost their living protoplasmic contents, leaving the mere husk to show us still that these two nerves originally possessed...ganglion cells corresponding...to the afferent root of a spinal nerve.' (Gaskell, 1889). Essentially, he agreed with Balfour (Balfour, 1880) that in the original spinal nerve type, dorsal roots of mixed motor and sensory composition were the only roots present, and that the purely motor ventral roots were a later evolutionary elaboration. Gaskell went on to speculate that the trigeminal nerve had a visceral component targeting the lachrymal gland and, based on observations of Ahlborn in lampreys (Ahlborn, 1884), that its somatic motor component was the (displaced) abducens nerve. However, Gaskell also associated the abducens nerve with the facial nerve as the latter's somatic motor component and concluded that cranial nerves III–VII comprised at least 'four fully formed segmental nerves, which for some reason or other have lost a certain portion of their original components.' He continued to conclude that cranial nerves IX to XII comprised 'at least five segmental nerves that have

not lost any particular component.’ Gaskell went on to interpret the origin of the spinal and cranial nerves to be ‘a chain of bilateral ganglia which give origin to a series of segmental nerves and are bound together by connecting fibers.’ His idea was that ganglia form outside a non-nervous tube and that the lumen of the central nervous system was the lumen of the ancestral prevertebrate gut that was ‘utilized as the supporting tissue’ when the ventral ganglia expanded to become the central nervous system (Gaskell, 1908).

Gaskell’s 1889 paper was accompanied by excellent images (see his most important plate replicated as Fig. 1) that perfectly illustrated his ideas to the point that they still influence neuroanatomy and neurology textbooks today, despite the fact that many of his ideas were based on flimsy evidence and some have since been shown to be wrong. In the present article we describe some critical steps in the history of our understanding of cranial and spinal nerves, and we provide newer evolutionary, developmental, and molecular insights that refine and replace some aspects of Gaskell’s organizational framework. We also provide figures that better explain the complex neuroanatomy rather than creating the frustration of having to learn concepts and names that go unexplained, since their original root in Gaskell’s writing has long since been forgotten in modern textbooks.

The impact of Gaskell on Langley and the physiological definition of autonomic nervous system divisions

To fully understand the lasting impact of Gaskell’s work one needs to appreciate the revolution he initiated when he for the first time described explicitly the organization of visceral nerve fibers as they are associated with the heart and the sympathetic ganglia (Gaskell, 1880). Gaskell was the first to accurately describe that many, but not all, sympathetic ganglia had white rami and that all had grey rami (Gaskell, 1920). Gaskell came to that conclusion by distinguishing between large and small diameter fibers in his description (Gaskell, 1889). Gaskell thus established an anatomical feature of the autonomic nervous system that was until that time unclear. Most researchers at that time accepted ideas concerning the autonomic nervous system that were proposed by Winslow (Winslow, 1732). Winslow called the sympathetic chain of ganglia (in his nomenclature, the ‘intercostal nerve’) the ‘great sympathetic’, and believed that it provided the means by which one part of the body affected another part to integrate functions or, in the language of the time, to bring about ‘sympathies of the body’. Winslow also felt that the 7th and 10th cranial nerves had sympathetic functions and called them the ‘small sympathetic’ and the ‘medium sympathetic,’ respectively. The basic idea was that two distinct parts of the nervous system existed, one composed of the brain and spinal cord, the other of the sympathetics, and that these were interconnected by the peripheral nerves thus facilitating integration (‘sympathies’) between visceral and somatic functions. These ideas influenced greatly even 19th century comparative anatomists, who described the evolutionary development of visceral and somatic integration in these terms (Romer, 1970, Romer, 1972).

The confusion during the 18th and 19th centuries concerning the ‘visceral’ or ‘splanchnic’ innervation (Fig. 2) is well summarized by Langley (Langley, 1921) and Gaskell (Gaskell, 1920) and the reader is referred to those summaries of previously held ideas for details

beyond the scope of the current review. On this backdrop it is easy to understand the impact Gaskell's work had on the field when he published his 1889 paper. He essentially established a new concept, based on quantitative anatomical material, regarding the connections of the sympathetic chain of ganglia with the central and peripheral nervous systems (Gaskell, 1889). He conjectured that all visceral (splanchnic) nerves arose from a single column of efferent neurons in the central nervous system, interrupted only at the levels of the brachial and lumbar plexi. He thus identified cranial, thoraco-lumbar, and sacral sets of visceral nerves, in line with the concept that all nerves were reiterations of the same basic plan that had variably lost during evolution one or more of the basic components related to the four central columns (visceral versus somatic and afferent versus efferent columns). Gaskell also defined two different types of visceral ganglia:

- A. proximal or vertebral ganglia starting with the inferior cervical (first thoracic) ganglion down to the last sacral ganglion;
- B. distal ganglia, among which he included the superior and middle cervical ganglia and the mesenteric and other ganglia located near the tissues they innervate.

Gaskell's observations coincided with the first pharmacological experiments using purified substances such as nicotine and adrenaline. The physiological differences in responses led Langley to introduce the term 'autonomic' nervous system with the chief components being the parasympathetic (with tectal and bulbo-sacral subdivisions) and the sympathetic (thoraco-lumbar) (Langley, 1900, Langley, 1905, Langley, 1921). Langley based this distinction mainly on the differential effects of drugs. Adrenaline mimicked the stimulation of sympathetic, thoraco-lumbar nerves only, whereas nicotine, because it 'caused effects more or less confined to those produced by stimulating tectal and bulbo-sacral nerves', led Langley to state that 'it was convenient to use a common name for these (latter) nerves and I place them together as the parasympathetic system' (Langley, 1905, Langley, 1921). The work of Langley together with results of related physiological studies into the pharmacology and physiology of each nerve component (obtained in large part through selective surgical ablation) consolidated Gaskell's ideas. Gaskell's simplified diagram, which provided an eminently teachable scheme of the autonomic peripheral nervous system (Gaskell, 1920), was thus assimilated into the physiology of Langley and others and has become a fundamental component of medical education. Despite the eloquence of Langley's writing, Gaskell's earlier nomenclature was over the years variably retained and integrated into Langley's nomenclature, and was thus never fully replaced by Langley's more nuanced terminology, in which an 'ocular' component was distinguished from a 'bulbo-sacral (oro-anal)' component within the parasympathetic division (Langley, 1921) (see Figure 2).

In conclusion, Gaskell's fundamental anatomical insight, amplified by the subsequent work of Langley, defined the basic divisions of the autonomic nervous system as the cranio-sacral parasympathetic and the thoraco-lumbar sympathetic systems. This organizational scheme is still maintained today in current neuroanatomical textbooks.

Herrick and the American School of comparative neuroanatomists

Gaskell's seminal work took into account the fundamental separation of the developing neural tube into the alar plate (dorsal, sensory) and basal plate (ventral, motor) proposed by His (His, 1889), and also by the much earlier work of Bell (Bell, 1811) who suggested separate dorsal sensory and ventral motor components of spinal nerves. Superimposing his ideas on the anatomically accurate drawings of German neuroanatomists such as Edinger [(Edinger, 1885) see Fig. 1], Gaskell combined variably his own and other accurate observations with speculations that he eventually fully explored in his summary treatise on the evolution of the vertebrates (Gaskell, 1908). Subsequently, the American School of neuroanatomists (such as Herrick, Johnston, and Strong) adopted some of the ideas proposed by Gaskell and his scheme of functional longitudinal columns. We focus here only on the motor components as eloquently summarized by Herrick (Herrick, 1918) and Nieuwenhuys (Nieuwenhuys, et al., 1998). Herrick, noting that many aspects of the four principal functional columns employed by Gaskell are transformed in the hindbrain, called these hindbrain novelties "neomorphs" (Herrick, 1918). He believed the neomorphs could be derived from the organization of the functional columns in the spinal cord. In his Introduction to Neurology (Herrick, 1918), he developed a simple scheme and nomenclature that even today reflects in current medical teaching the ideas developed in large part by Gaskell. According to Herrick (Herrick, 1918), the four primary motor divisions of cranial nerves is 'present in the same primitive unspecialized form as seen in the spinals, and also by specialized systems found only in one or more cranial nerves':

- a. General somatic efferent nerves, supplying the general skeletal muscles of the body (XI)
- b. Special somatic efferent nerves supplying the two groups of highly specialized somatic muscles, namely the external eye muscles and a part of the tongue muscles (III, IV, VI, XII).
- c. General visceral efferent nerves, for unstriated involuntary muscles, heart muscles, glands etc, distributing through the sympathetic nervous system (III, VII, IX, X, XI)
- d. Special visceral efferent nerves supplying highly specialized striated muscles of a different origin from the striated trunk muscles. These muscles are connected with the visceral or facial skeleton of the head and are derived from gill muscles of fishes (V, VII, IX, X, XI). (Fig. 3)

Herrick based some of his claims on the same drawings of Edinger used by Gaskell to support his claims, merely changing the labeling of the nuclei already identified by Edinger (Edinger, 1885). It is also important to note that the use of the terms "special somatic" and "special visceral" was meant to imply that these were evolutionary elaborations of the general somatic and visceral phenotypes. As we shall see below, this supposition does not hold in the face of more recent findings.

In his final reviews and books, Herrick slightly modified his earlier perception of the elements of cranial and spinal nerves. In his final book on the Brain of the Tiger Salamander (Herrick, 1948), he only used three motor columns (p 99):

1. The somatic motor fibers (cranial nerves III, IV, VI; Herrick moved cranial nerve XII into the spinal somatic motor system in his final writings).
2. Special visceral motor fibers of cranial nerves supplying striated muscles related to the visceral skeleton (V, VII, IX, X).
3. Preganglionic fibers of the general visceral (autonomic) system terminating in sympathetic and parasympathetic ganglia, where they activate postganglionic fibers distributed to unstriated and cardiac muscles and glands (III, VII, IX, X).

Much of the writing of Langley, Gaskell and Herrick has remained unchallenged and has driven textbook interpretations that sometimes have become so distorted as to be fully inaccurate, like the organization of brainstem motor nuclei depicted in the well-known Gray's Anatomy textbook (e.g. Fig. 2.65 (Williams, et al., 1989)). It is only with the onset of modern tracing techniques and the molecular details made possible by the revolution in developmental and comparative studies during the last 30 years that the proposals of Gaskell, Langley, and Herrick have been revisited and partially corrected. Yet, despite accumulating evidence disproving certain key aspects, the hypothetical framework proposed by Gaskell and adopted by most of his followers remains with us today. What we know now to be incorrect features are presented in most textbooks and learned by medical and comparative anatomists without an understanding of their historical origins.

Among the ideas in Gaskell's original work now known to have no basis in modern neuroanatomical fact is that visceral motor neurons evolutionarily predate branchial motor neurons in the brainstem. This has led to the expansion of the four columns of the spinal cord (two motor and two sensory) into three motor columns (somatic, general visceral, special visceral) and four sensory columns (general and special somatic afferents, general and special visceral afferents), a scheme that can be found in many textbooks of on human embryonic development and anatomy.

With this background of concepts and ideas provided by the most influential researchers of their times (Gaskell and Langley for the organization of the autonomic nervous system, Herrick for the comparative neuroanatomical considerations) we can now begin to consider their proposals in the context of modern molecular biological and comparative insights. Specifically, we will address the following:

1. The molecular basis of motor neuron induction and patterning in the spinal cord and brainstem.
2. The evolution of Phox2-dependent brainstem motor neurons.
3. The evolutionary history of neural crest-derived sympathetic and parasympathetic ganglia as targets of sympathetic motor neurons of the preganglionic (spinal) visceral motor column (PGC) and of parasympathetic visceral motor neurons (PVMs), respectively.

4. An integrated perspective of chordate motor neuron diversification highlighting open questions.

The molecular basis of motor neuron induction and patterning in the spinal cord and brainstem

The first insights into the molecular basis for motor neuron induction began with experimental embryological studies involving transplantation of pieces of notochord to ectopic locations around the neural tube (Glover, 1991, Schoenwolf and Alvarez, 1991, Yamada, et al., 1991). These studies led to the discovery that a gradient of Sonic hedgehog (Shh) initiates the induction of motor neuron differentiation at a specific, ventral level of the gradient (Echelard, et al., 1993, Ericson, et al., 1997, Litingtung and Chiang, 2000, Oh, et al., 2009). Specific transcription factors including the Homeobox genes *Nkx6.1/6.2* (Müller, et al., 2003, Sander, et al., 2000) and the bHLH gene *Olig2* (Lu, et al., 2002, Zhou and Anderson, 2002) were soon discovered to play critical roles in translating graded Shh signaling into the specification of neural progenitors to generate motor neurons. Additional studies demonstrated that combinatorial expression of the Lim-Homeodomain gene *Isl1* and other Lim genes specifies the different spinal motor neuron columns (Pfaff, et al., 1996, Tsuchida, et al., 1994). Further work extended the basic concept of sequential and combinatorial expression of different transcription factors to add antero-posterior diversification driven primarily by specific members of the Hox-c gene cluster, which are involved not only in specifying different motor neuron columns, but also in specifying muscle-specific motor neuron pools within those columns (Dasen, et al., 2003, Dasen, et al., 2005). The picture that has emerged is that a combination of longitudinally modulated Hox gene expression and nested expression of early- and late-acting transcription factors generates the various motor neuron populations identified by their generic targets (limb muscles: lateral motor column or LMC; axial muscles: medial motor column or MMC; body wall muscles: hypaxial motor column or HMC; autonomic ganglia: preganglionic (spinal) visceral motor column or PGC) as well as by their specific muscle targets (individual motor neuron pools). These studies also provide a molecular explanation for the cervical and lumbar gaps in the PGC that baffled so many early students, including Gaskell and Langley: the transcription factors involved in specifying PGCs are not expressed in those segments (Dasen, Tice, Brenner-Morton and Jessell, 2005, Golden and Dasen, 2012). Indeed, our understanding of the molecular logic of spinal motor neuron specification has advanced to the stage that it is now possible to experimentally change one type of motor neuron into another using targeted gene misexpression (Golden and Dasen, 2012, Hinckley, et al., 2015, Machado, et al., 2015), manipulate motor neuron development *in vitro* to study motor neuron-specific diseases in the dish (Davis-Dusenbery, et al., 2014) or generate synthetic circuits *in vitro* composed of specific types of spinal neurons (Sternfield et al 2017).

This molecular framework for spinal motor neuron specification, including the specification of the visceral motor neurons in the PGC, surprisingly does not fully apply to the motor neurons in the brainstem. Whereas a central role of some features, such as Shh signaling and *Isl1* expression, is conserved, the specification of brainstem motor neurons is in addition critically dependent on the expression of the *Phox2* transcription factors (*Phox2a* and

Phox2b). These are essential for the development of nearly all brainstem motor neurons, including those making up the spinal component of the accessory (XI) cranial nerve, but not the abducens (VI) or hypoglossal (XII) motor neurons (Pattyn, et al., 1997). Phox2-dependence is irrespective of the type of brainstem motor neuron involved; all are critically dependent on either Phox2a or Phox2b. By contrast, neither Phox2a or Phox2b are expressed by spinal motor neurons. Interestingly, when Phox2b expression is experimentally forced in the developing spinal cord (Hirsch, et al., 2007) it induces an unusual feature only seen in some brainstem motor neurons (including the spinal accessory motor neurons), namely an exit of the axon from the alar plate of the neural tube rather than the basal plate. Phox2b thus contributes to defining a population of brainstem motor neurons with a unique property: an efferent axon trajectory that leaves the CNS at the same point where the sensory afferents enter. The brainstem motor neurons that project in this way and which are dependent on Phox2b expression are known as **branchial motor neurons (BM)**; projecting via cranial nerves V, VII, IX, X, XI to muscle fibers associated with branchial arches) and preganglionic **parasympathetic visceral motor neurons (PVM)**; projecting via cranial nerves VII, IX, X to cranial and cervical (and in the case of X, also truncal) autonomic ganglia; with respect to PVMs projecting in cranial nerve III, see below).

The most anterior nuclei of the motor column (the oculomotor and trochlear, III and IV) contain the most unusual motor neurons. These brainstem motor neurons express Phox2a prior to expressing Phox2b, and are dependent on Phox2a but not Phox2b expression (Pattyn et al 2000). They include the only Phox2-dependent motor neurons that deviate from the expected axon trajectory by having either ventrally exiting axons (oculomotor somatic motor neurons and PVMs in the Edinger-Westphal portion of the nucleus), or axons that decussate dorsally before exiting (trochlear somatic motor neurons). Thus, while the somatic motor neurons in these two nuclei are superficially similar to spinal somatic motor neurons with respect to the type of muscle innervated, and in the case of the oculomotor nerve in having a ventral axon exit point, their dependence on Phox2a expression and lack of expression of other somatic motor neuron-specific transcription factors (Pattyn, Morin, Cremer, Goridis and Brunet, 1997) puts them into a class of their own. We propose therefore that the somatic motor neurons in the oculomotor and trochlear nuclei should be referred to as **special somatic motor neurons (SSM)**. It should be noted that this terminology was initially proposed by Herrick (Herrick, 1922) based on his knowledge of certain unusual features (bilateral distribution of oculomotor motor neurons, dorsal exit of decussating trochlear motor neuron axons). Setting aside these two cranial nerve nuclei also accords with Langley's division of the parasympathetic system into ocular and bulbo-sacral parts, whereby the PVMs of the Edinger-Westphal portion of the oculomotor nucleus correspond to the ocular part.

More recent developmental work has added to the unusual features of the oculomotor and trochlear motor nuclei the migration of a specific pool of oculomotor motor neurons (the superior rectus motor neurons) across the midline (Fritzsche and Northcutt, 1993b, Glover, 2003, Puelles and Privat, 1977), and the fact that both motor nuclei depend on Wnt1 signaling at the midbrain/hindbrain boundary (Fritzsche, et al., 1995), which otherwise acts only along the dorsalmost edge of the brainstem and spinal cord with no effect on other

motor neurons. Further evidence for the peculiar nature of oculomotor and trochlear motor neurons is their unique dependence on certain transport proteins leading to innervation defects not seen in other motor neuron populations (Cheng, et al., 2014), and their insensitivity to certain motor neuron diseases (Nijssen, et al., 2017). Interestingly, experimental work in mice and clinical work in humans indicates that the abducens motor neurons, which have similar functions in driving extraocular musculature, are less sensitive than oculomotor and trochlear motor neurons to pathogenetic mutations in *Phox2* genes (Bosley, et al., 2006).

Specification of BMs, PVMs and somatic MNs along the longitudinal axis of the brainstem evidently arises at least in part through the Hox-code that identifies distinct rhombomeres (hindbrain segments) in all jawed and jawless vertebrates except hagfish (Parker, et al., 2014). It should be noted that a widely disseminated proposal of a two-rhombomere origin of cranial motor neuron nuclei, derived from studies in the chicken embryo (Lumsden and Keynes, 1989), is not applicable in many species including the mouse [reviewed in (Glover, 2001)]. In the mouse, trochlear (IV) motor neurons originate only from the most anterior part of the large rhombomere 1 (Fritzsche, Nichols, Echelard and McMahon, 1995), held by some authors to represent a special 'isthmus rhombomere' (Watson, et al., 2017) and trigeminal (V) motor neurons originate from rhombomere 2 and the rostral 2/3 of rhombomere 3 (Müller, Jabs, Lork, Fritzsche and Sander, 2003). Abducens (VI) motor neurons originate only from rhombomere 5 in the mouse as opposed to rhombomeres 5 and 6 in the chicken (Fritzsche, 1998, Fritzsche, et al., 1993, Gilland and Baker, 1993). Facial (VII) branchial motor neurons in the mouse and chick originate from the caudal 1/3 of rhombomere 3 and all of rhombomere 4, and also include inner ear efferents (Karis, et al., 2001, Marín and Puelles, 1995, Simmons, et al., 2011, Tiveron, et al., 2003). Considering the more posterior cranial nerves in the mouse, glossopharyngeal (IX) motor neurons seem to originate only from rhombomere 5, vagal motor neurons originate from rhombomeres 6 and 7, spinal accessory (XI) motor neurons originate from rostral cervical segments of the spinal cord and hypoglossal motor neurons (XII) originate from rhombomeres 5–7 based on tracing data (Gilland and Baker, 1993). However, experimental work in chick and mice suggests that IX motor neurons arise in chick in r7, vagal MNs arise in r8–r11 (Cambronero and Puelles, 2000) and hypoglossal motor neurons arise from cyrtorhombomeres r10–r11 (Cambronero and Puelles, 2000, Marín, et al., 2008, Tomás-Roca, et al., 2016).

Phylogenetic differences in the rhombomeric origins of brainstem motor neurons may be related to phylogenetic differences in the rhombomeric expression patterns of Hox genes. Although the basic pattern of Hox gene expression in the brainstem is governed by a gene regulatory network that is highly conserved throughout the vertebrate radiation, it exhibits species-specific differences in anterior expression limits and in expression intensity among rhombomeres (Fritzsche, 1998, Glover, 2001, Marín, Aroca and Puelles, 2008, Murakami, et al., 2004, Parker, Bronner and Krumlauf, 2014, Parker, et al., 2016, Tomás-Roca, Corral-San-Miguel, Aroca, Puelles and Marín, 2016). However, the rhombomeric origins of cranial nerve motor nuclei have not been exhaustively assessed (origins have not been confirmed in all reports through bona fide fate-mapping and could be convoluted by migration and cell death, see Glover 2001 for discussion). Thus, the link between brainstem motor neuron

specification and the Hox-code, and its evolutionary diversification, remains only partially characterized.

In summary, induction of both spinal and brainstem motor neurons is triggered by a comparable molecular mechanism based on an initial dorsoventral gradient of Shh signaling that is parcellated into specific dorsoventral progenitor domains, one of the most ventral of which gives rise to motor neurons. Clear molecular divergence occurs during subsequent steps of specification, however, exemplified particularly by the dependence of brainstem motor neurons on the expression of Phox2a or Phox2b, neither of which are expressed in spinal somatic or PGC visceral motor neurons. Phox2b expression is required for the formation of all brainstem BMs and PVMs (with the possible exception of the PVMs in the Edinger-Westphal nucleus), and evidently confers a unique trait to these motor neurons, namely an axonal projection with an alar plate (embryonic dorsal) exit point, adjacent to where the associated sensory afferent axons exit (the only exception being the Edinger-Westphal PVMs, which have ventrally exiting axons). A second common trait is that the cell bodies of BMs and PVMs secondarily translocate into the liminal alar plate (Ju, et al., 2004, Nieuwenhuys and Puelles, 2016). Brainstem somatic motoneurons (abducens and hypoglossal motor neurons) do not depend on Phox2 expression, but the SSMs (oculomotor and trochlear motor neurons) are dependent on Phox2a expression. Some brainstem motor neuron populations migrate variable distances, both longitudinally and in the transverse plane (Fritzsche and Northcutt, 1993b, Glasco, et al., 2016, Heaton and Moody, 1980, Ju, Aroca, Luo, Puelles and Redies, 2004, Kim, et al., 2015b, Nieuwenhuys and Puelles, 2016, Puelles and Privat, 1977, Windle and Austin, 1936), to settle in their distinct cranial nuclei, well known by their century-old neuroanatomical names. The PGC motor column in the spinal cord also engages in a migration from its ventral origin (Levi-Montalcini, 1964). Its eventual intermediolateral (alar) location is continuous with the accessory/dorsal vagus PVM motor nuclei in the brainstem, superficially suggesting a segmental iteration as envisioned by Gaskell (Gaskell, 1889). However, the topological similarity of spinal PGC visceral motor neurons to brainstem PVMs is an independently derived feature. The ventral axon exit point and lack of Phox2 expression by the PGC visceral motor neurons make them distinct from the brainstem PVMs.

Evolution of Phox2-dependent brainstem motor neurons

Phox2 is highly-conserved across all chordates (Dufour, et al., 2006), consistent with the fact that all chordates studied to date have some efferent neurons whose peripheral axons exit the CNS at the same point as the sensory afferents enter (Fritzsche and Glover, 2006, Fritzsche and Northcutt, 1993a). Phox2 binding to the CREST1 enhancer of *Isl1*, which encodes the pan-motor neuronal transcription factor Islet1, is necessary for Islet1 expression in brainstem BMs and PVMs, and the Phox2 binding motif in CREST1 is highly conserved from lampreys to mammals (Kim, et al., 2015a). Phox2 expression specifically related to visceral neurons is already present in protostomes [represented by molluscs, (Nomaksteinsky, et al., 2013)]. In another major clade of deuterostomes, the echinoderms (represented by sea urchins) it is expressed in the developing CNS together with Nkx2.1 (Wygoda, et al., 2014), although it is unclear if this expression presages the differentiation of any specific type of motor neuron. Given these early origins, it seems logical to assume that chordate motor

neuron evolution included the early recruitment of Phox2 transcription factors into the gene regulatory network dedicated to motor neuron induction and specification.

The distribution patterns of different motor neuron types in cephalochordates (represented by the lancelets, or *Amphioxus*), urochordates (represented by the ascidian *Ciona*) and cyclostomes (jawless vertebrates, represented by the lamprey) provide clues regarding the role of Phox2 in specifying brainstem BMs and PVMs. Although Phox2 expression has yet to be characterized in the lancelets, they have what appear to be branchial motor neurons in the rostral nervous system that project through dorsal roots to a muscle associated with the branchial slits (Fritsch and Northcutt, 1993a, Nieuwenhuys, ten Donkelaar and C, 1998). Lancelets also have somatic motor neurons located more caudally that innervate the segmental body wall musculature, but unlike in vertebrates these do not project axons outside the CNS but rather synapse on processes from somatic muscle fibers and from the contractile notochord cells that abut the CNS. In ascidians, Phox2 expression is limited at larval stages to neurons in the region proposed to be homologous with the vertebrate brainstem, and in the sessile adult is present in neurons associated with the branchial arches (Dufour, Chettouh, Deyts, De Rosa, Goridis, Joly and Brunet, 2006). In lampreys, Phox2 expression is limited to the brainstem, and although its expression in motor neurons has not yet been demonstrated directly, it likely overlaps with motor neurons associated with cranial nerves III–X (Häming, et al., 2011) (lampreys have neither a hypoglossal nerve (they are jawless and tongue-less) nor an accessory nerve (associated with muscles of anterior appendages, absent in lamprey) (Fritsch and Northcutt, 1993a).

In summary, Phox2 transcription factors appear to be associated with the specification of brainstem motor neurons in all craniates and are likely to have evolved this function at the base of the chordate lineage. Peripherally, the molecular basis for branchial slit formation apparently is conserved with hemichordates (Lowe, et al., 2015, Simakov, et al., 2015) and, like the central nervous system patterning (Albuixech-Crespo, et al., 2017), is thus likely ancestral for deuterostomes. This strongly suggests that BMs and the transcription factors driving their specification evolved in the prechordate bilaterian lineage, and already in cephalochordates and urochordates were utilized specifically to innervate branchial musculature (Dufour, Chettouh, Deyts, De Rosa, Goridis, Joly and Brunet, 2006, Fritsch and Northcutt, 1993a). It therefore appears likely that the branchial motor system formed at the base of the deuterostomes, possibly through modification of gene regulatory networks shared with protostome motor neuron development (Nomaksteinsky, Kassabov, Chettouh, Stoeklé, Bonnaud, Fortin, Kandel and Brunet, 2013).

Whereas branchial motor neurons and musculature appear to have evolved very early, extraocular muscles and the motor neurons innervating them evolved only in craniates and show distinct differences in their pattern of innervation and distribution between cyclostomes (jawless vertebrates) and gnathostomes (jawed vertebrates) (Fritsch, et al., 1990). It has not yet been demonstrated whether Phox2 gene(s) are expressed in lamprey extraocular muscle motor neurons [(Häming, Simoes-Costa, Uy, Valencia, Sauka-Spengler and Bronner-Fraser, 2011) III, IV, VI] and if those motor neurons are dependent on Phox2 expression, as they are on Phox2a in mice (Pattyn, Morin, Cremer, Goridis and Brunet, 1997). It would also be interesting to determine whether hagfish (another cyclostome that

separated from a common ancestor with the lampreys about 350 million years ago, (Simakov, Kawashima, Marlétaz, Jenkins, Koyanagi, Mitros, Hisata, Bredeson, Shoguchi and Gyoja, 2015)), which lack extraocular muscles and their innervating cranial nerves (III, IV and VI), exhibit any transient expression of *Phox2* in the developing midbrain/hindbrain region where the III and IV nuclei normally develop. Absence of *Phox2* expression might be associated with changes in for example *Wnt1* signaling, on which extraocular motor neuron development depends (Fritzscht, Nichols, Echelard and McMahon, 1995).

In summary, the evolutionary history of brainstem motor neurons suggests that BMs and their innervation of branchial muscle fibers stretches back to the base of the deuterostome lineage. It also suggests a much later evolution within the craniate lineage of a population of extraocular muscle-innervating motor neurons associated with the evolution of mobile eyes.

Evolutionary history of neural crest-derived sympathetic and parasympathetic ganglia as targets of sympathetic motor neurons of the PGC and of PVMs

The bulk of phylogenetic evidence indicates that brainstem BMs were already in place at the base of the chordate tree and that somatic motor neurons in their vertebrate form (with efferent axons that extend out of the CNS to their peripheral striated muscle targets) arose no later than the common ancestor of urochordates and vertebrates. By contrast, brainstem PVMs and preganglionic sympathetic visceral motor neurons (SVMs) in the spinal PGC appear to have evolved later, appearing first in the gnathostome lineage, with a full-blown PGC appearing first in the tetrapod lineage (Jung and Dasen, 2015); bony and cartilaginous fish probably have preganglionic visceral neurons, since they possess sympathetic ganglia, but their numbers are evidently too low to create an obvious column). This raises the question of the molecular evolution that elaborated these preganglionic motor neuron populations from existing motor neuron precursors and the co-evolution of their target ganglion cells.

Sympathetic visceral motor neurons of the PGC are distinguished from other motor neuron columns by the selective expression of *Hoxc9*, which represses the expression of more anteriorly expressed *Hoxc4–8* genes that specify the LMC (lateral motor column) fate in the brachial region. Moreover, the expression of *Hoxc9* restricted to the thoracic region (and not the lumbar region) is regulated postmitotically by epigenetic marks and the repressive actions of specific genes of the polycomb gene locus (Golden and Dasen, 2012). Thus, the PGC column (and other segmentally restricted columns like the limb-innervating LMC) likely arose in evolution through a layering of regulatory element expression onto a somatic motor neuron differentiation program that initially was continuous along the spinal cord (Jung and Dasen, 2015).

Neural crest precursors have been claimed to exist in lancelets and ascidians based on molecular and neuroanatomical evidence (Abitua, et al., 2015, Abitua, et al., 2012, Fritzscht and Northcutt, 1993a, Häming, Simoes-Costa, Uy, Valencia, Sauka-Spengler and Bronner-Fraser, 2011), but in these taxa they do not form peripheral ganglia per se but rather other

neural crest-typical derivatives such as (scattered) peripheral sensory neurons and mesenchymal cell types (Pasini et al 2012, Häming et al 2011). The evidence for the presence of bona fide autonomic ganglia in cyclostomes is scanty and then only in association with the vagus nerve. Gaskell (Gaskell, 1920) made it clear in his own studies that lampreys have only a few ganglia associated with the vagus nerve, lack the sympathetic chain of ganglia and lack the ciliary ganglion innervating the intrinsic musculature of the eye. At best, in cyclostomes only rudimentary precursors of autonomic ganglia are associated with cranial nerves and only a few dispersed autonomic ganglia are associated with spinal nerves (Fritzsche and Northcutt, 1993a). Consistent with the absence of peripheral ganglion targets is the absence of any distinct visceral motor neurons in either the cranial or spinal region suggesting that brainstem PVMs and spinal SVMs evolved in temporal register with the evolution of neural crest-derived autonomic ganglia. Neural crest-derived autonomic ganglia are found in all gnathostomes (Bronner and LeDouarin, 2012), but tend to be small and often irregularly spaced in bony and cartilaginous fish (Nilsson, 2011), which could explain why a PGC is not evident in these species.

It is important to note that the development of parasympathetic ganglia innervated by brainstem PVMs is quite distinct from the development of spinal sympathetic ganglia innervated by the SVMs of the PGC. Molecular developmental evidence indicates that parasympathetic ganglia transdifferentiate from emigrating Schwann cells and that this is dependent on the presence of PVM axons, although the molecular signals inducing this transdifferentiation have not yet been identified (Chen, et al., 2011, Coppola, et al., 2010, Dyachuk, et al., 2014, Espinosa-Medina, et al., 2014). Sympathetic ganglia differentiate directly from emigrating neural crest precursors without a Schwann cell intermediate and are not dependent on innervation for their differentiation (Espinosa-Medina, et al., 2016).

An integrated perspective of chordate motor neuron diversification and its implication for the origin and diversification of the autonomic nervous system

Recent research has shown that branchial motor neurons are ancestral to chordates (Fig. 5a) whereas somatic motor neurons with peripheral axons to muscle fibers arose in the chordate lineage after the split between cephalochordates on the one hand and urochordates and craniates on the other (Dufour, Chettouh, Deyts, De Rosa, Goridis, Joly and Brunet, 2006, Fritzsche and Northcutt, 1993a, SØviknes, et al., 2007). Among craniates, jawless vertebrates (cyclostomes) lack both visceral motor neurons and neural crest-derived autonomic ganglia (Fritzsche and Northcutt, 1993a), but express the branchial motor neuron-determining gene *Phox2* in the brainstem (Häming, Simoes-Costa, Uy, Valencia, Sauka-Spengler and Bronner-Fraser, 2011). Visceral motor neurons innervating neural crest-derived autonomic ganglia arose first in the jawed vertebrates (gnathostomes). Moreover, molecular data clearly distinguish between *Phox2*-expressing brainstem branchial and visceral motor neurons (BMs and PVMs) and *Olig2*-expressing spinal somatic and visceral motor neurons (SMs and SVMs) (Häming, Simoes-Costa, Uy, Valencia, Sauka-Spengler and Bronner-Fraser, 2011, Pattyn, et al., 1999). The evolutionary sequence and molecular distinction that have diversified motor neurons contradict Gaskell's (Gaskell, 1886, Gaskell, 1889) assumption

that identical visceral motor neurons extend from spinal cord to brainstem and that the branchial motor neurons evolved from the brainstem visceral motor neurons, hence the term ‘special visceral motor neurons’. Available evolutionary and developmental data suggest the opposite: that brainstem visceral motor neurons innervating the cranial parasympathetic ganglia evolved from the branchial motor neurons (Pattyn, Morin, Cremer, Goridis and Brunet, 1999).

The attempts to extend functional longitudinal columns from the spinal cord into the brainstem by Gaskell and his followers are historically important and still applicable for certain neuron categories. For example, the longitudinal expression of specific transcription factors in the dorsal spinal cord and the brainstem (Bermingham, et al., 2001) define nuclei that receive proprioceptive input, and all motor neurons express Islet1. Phox2b, by contrast, is expressed in brainstem but not spinal cord motor neurons (Häming, Simoes-Costa, Uy, Valencia, Sauka-Spengler and Bronner-Fraser, 2011, Pattyn, Morin, Cremer, Goridis and Brunet, 1999). Thus, neither molecular nor evolutionary data support Gaskell’s original scheme (Fig. 6). We therefore propose to abandon the nomenclature introduced by Gaskell and Herrick and their contemporaries and alter text books to reflect the ancestry and uniqueness of branchial motor neurons. In particular, using the term ‘special visceral motor neurons’ for these motor neurons misleadingly implies that visceral motor neurons predate branchial motor neurons whereas the evolutionary evidence suggests the opposite.

Necessary revision of the autonomic nomenclature extends beyond this. Very recent data from mice (Espinosa-Medina, Saha, Boismoreau, Chettouh, Rossi, Richardson and Brunet, 2016) debunks the long held idea, again based on the limited evidence obtained by Gaskell and Langley more than 100 years ago, that there is a spinal (sacral) component of the parasympathetic autonomic nervous system. Across a panel of molecular and developmental characteristics that distinguish sympathetic and parasympathetic neurons and fibers, the sacral autonomic outflow fulfills only the sympathetic criteria. Thus, the so-called cranial and sacral divisions of the autonomic nervous system, found in all text books related to human neuroanatomy, are misleading. The data provided by Espinosa-Medina et al (Espinosa-Medina, Saha, Boismoreau, Chettouh, Rossi, Richardson and Brunet, 2016) unify features of the autonomic motor system into a more consistent and cohesive organization, simplifying the outflow into cranial parasympathetic and spinal sympathetic divisions (Fig. 4).

Summary

We have here reviewed modern comparative, developmental, and molecular studies into the specification of different types of brainstem and spinal cord motor neurons. We feel that the accumulated evidence provides new insight that should be used as the impetus to rename motor neurons along the entire neuraxis according to more consistent principles. This new scheme (Table 1) not only conveys an accurate picture of the development and evolution of vertebrate motor neurons as revealed by contemporary research, but also serendipitously provides a scheme that is easier to learn by those studying human anatomy.

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Fig. 1. Reproduction of PLATE XVIII (From Gaskell, 1889) (*Authors' note: the legend that follows is taken verbatim from Gaskell, 1889*).

Fig. 1. Section of the medulla oblongata (after Edinger). The letters A, B, C, D, E have the same meanings as in the diagram of the spinal cord on p. 155 (*Authors' note: this refers to p. 155 of Gaskell, 1889*). Fig. 2. Diagrammatic representation of the arrangement of the supposed segmental cranial nerves. The diagram represents roughly a longitudinal section through the brain of a young dog-fish, upon which the general plan of arrangement of the cranial nerves has been drawn as explained in the text. Fig. 3. These three diagrams illustrate the possible origin of the vertebrate nervous system as suggested in the text. 3 A. Diagram of the relation between the Crustacean alimentary and nervous systems. 3 B. Diagram of relation between the nervous and non-nervous parts of the central nervous system in the Ammocoete. It illustrates also the formation of the three cerebral vesicles from the simple stage represented in Fig. 3 A. 3 C. Diagram of relation between the nervous and non-nervous parts of the central nervous system in the Elasmobranch and in the higher vertebrates generally. It illustrates the formation of the choroid plexuses and the increasing invasion of the walls of the original cephalic stomach by the increase in the amount and complexity of the nervous material.

Fig. 2.

Text figure from Langley (1921) showing his proposed organization of the peripheral nervous system. Note that within the parasympathetic system Langley considered the ocular/tectal subsystem to be distinct from the bulbo-sacral (oro-anal) subsystem, with the latter segregated further into bulbar (IX + X cranial nerves) and sacral portions.

Fig. 74.—Diagrammatic cross-section through the adult human medulla oblongata at the same level as shown in Fig. 73, for comparison of the arrangement of the principal functional columns with that of Figs. 69 and 70.

Fig. 3.

The concept of motor columns envisioned by Herrick (1918) that directly follows the ideas of Gaskell (1889) based on the data of Edinger (1885) as depicted in Figure 1 (above).

Fig. 4.

Recent molecular and developmental characterization of autonomic nerves and ganglia indicate that the autonomic nervous system comprises only two components: the spinal sympathetic outflow and the cranial parasympathetic outflow. Note that pelvic ganglia do not receive a dual sympathetic/parasympathetic innervation with this revised scheme, but do receive dual innervation from lumbar and sacral preganglionic sympathetic fibers with opposing effects. Taken from (Espinosa-Medina, Saha, Boismoreau, Chettouh, Rossi, Richardson and Brunet, 2016).

Fig. 5.

Chordates evolved branchial motor neurons (BM) before somatic motor neurons (SMs); both types of motor neuron project axons to peripheral muscle fibers (MF). Phox2 genes are expressed in the BMs but not the SMs. The SSMs (ocular and trochlear motor neurons) appeared first in the jawed vertebrates and share many attributes with SMs, but differ importantly in being critically dependent on the expression of Phox2a. Jawless vertebrates (represented here by the lamprey) have no neural crest-derived autonomic ganglia and no parasympathetic or sympathetic visceral motor neurons. Once neural crest evolved to generate molecularly distinct autonomic (sympathetic and parasympathetic) ganglia, some BMs co-evolved into the parasympathetic visceral motor neurons (PVMs) in the brainstem to innervate the parasympathetic ganglia, and some SMs co-evolved into the sympathetic visceral motor neurons (SVMs) to innervate the sympathetic ganglia.

Fig. 6.

This scheme shows the molecular governance of motor neuron development in the motor column of mammalian embryos and the differential distribution of Hox and Phox2 genes that dictates regional variation within the motor column. Note that all motor neurons are Isl1/2 positive (red), whereas Phox2 gene-positive motor neurons are restricted to the cranial region (red and blue checked). The adult mammal has a single somatic motor column (SM, red) that extends throughout the spinal cord and into the brainstem to include the hypoglossus (XII) and abducens (VI) nuclei. Preganglionic sympathetic motor neurons (SVM, green) are present only in the spinal cord in a column interrupted only in the segments that innervate the lumbar plexus [although developmentally there is also a cervical portion that degenerates at fetal stages (Levi-Montalcini, 1997)]. Parasympathetic visceral motor neurons (PVMs, purple) are present only cranially, in the dorsal motor nucleus of the vagus (DMV), the superior and inferior salivatory nucleus (SS, IS) and the Edinger-Westphal

nucleus of the oculomotor system. Branchial motor neurons (blue) are present cranially in the nucleus ambiguus (NA) and the trigeminal (V) and facial (VII) nuclei, but also extend slightly into the cervical spinal cord, within the accessory (XI) nucleus. The oculomotor (III) and trochlear (IV) nuclei have a unique dependence on Phox2a and on Wnt1 expression at the midbrain/hindbrain boundary, which sets them apart from other somatic motor neurons, hence their designation as special somatic motor neurons (SSM, pink). Modified after (Burke, et al., 1995, Davis-Dusenbery, Williams, Klim and Eggan, 2014, Fritzscht, 1998, Haines, 2004, Parker, Bronner and Krumlauf, 2016)

Table 1
Functional Classes of Motor Neurons

To accommodate modern evolutionary, developmental and molecular insights into the true nature of functional longitudinal motor columns, we propose the following revised motor neuron nomenclature.

Conventional Classification	Proposed New Classification	Critical Genes Expressed	Structures Innervated	Nerves
<i>Cranial Motor</i>				
Special Visceral		Phox2b	Muscles of face, jaw, neck,	V, VII, IX, X, XI
General Visceral		Phox2b	Parasympathetic ganglia	III, VII, IX, X
General Somatic		Phox2a	Extraocular muscles	III, IV
<i>Spinal Motor</i>				
(Spinal) Somatic		Olig2	Muscles of body and tongue	VI, XII, spinal
(Spinal) Visceral		Olig2	Sympathetic ganglia	Spinal preganglionic