Diversity of reticulospinal systems in mammals

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Reticulospinal (RS) neurons provide the spinal cord with the executive signals for a large repertoire of motor and autonomic functions, ensuring at the same time that these functions are adapted to the different behavioral contexts. This requires the coordinated action of many RS neurons. In this mini-review, we examine how the RS neurons that carry out specific functions distribute across the three parts of the brain stem. Extensive overlap between populations suggests a need to explore multi-functionality at the single cell-level. We next contrast functional diversity and homogeneity in transmitter phenotype. Then, we examine the molecular genetic mechanisms that specify brain stem development and likely contribute to RS neurons identities. We advocate that a better knowledge of the developmental lineage of the RS neurons and a better knowledge of RS neuron activity across multiple behaviors will help uncover the fundamental principles behind the diversity of RS systems in mammals.

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Introduction

Reticulospinal (RS) neurons mediate a variety of motor and autonomic functions including escape, micturition, reaching and grasping, sleep, respiration, vomiting, and locomotion. Referring to these RS neurons as one RS system fails to capture this functional diversity. Admittedly, the cellular and circuit organization that lies behind the diversity of the RS systems is still not well understood. Multi-functionality at the single cell-level has been little explored and so has the extent to which the different RS systems are linked to each other. For instance, we have yet to fully understand how the RS neurons that mediate motor functions are linked to the RS neurons that mediate autonomic functions so that our motor actions align to our constantly changing behavioral needs.

The aim of this mini-review is to bring into light the diversity of the RS systems in term of spatial distributions, transmitter phenotypes, and molecular profiles. We focus on the role of RS neurons in motor and autonomic control but it should be kept in mind that another important role of RS neurons is sensory modulation, so that peripheral sensory inputs can ensure the smooth progression of our motor behaviors (McCrea and Perreault [99]; Perreault *et al.* [100]; Rudomin [101]). The mini-review should be placed in the context of the recently renewed interest in the RS systems, partly triggered by technological advances, which are now being applied to the brain stem. Excitingly, some of these advances promise to capture the molecular diversity of the RS neurons with new opportunities for their targeted manipulation.

We have given priority to studies that have identified reticular neurons as spinally projecting neurons, either anatomically with retrograde tracing, or electrophysiologically with antidromic activation and collision test. In keeping with the editorial limits given in term of length and number of citations to older literature, many of the original studies that have shaped our current understanding of the RS systems have been left out. Several of these studies can be found in the recent review by Brownstone and Chopek [1], which has called attention to the lack of information regarding the internal organization of the reticular formation (RF) nuclei and how this information is necessary to understand how RS systems tune descending motor commands.

Spatial distribution throughout the brain stem
Nomenclature
We refer to RS neurons as spinally projecting neurons that reside within the RF of the brain stem (mesencephalon, pons, and medulla), excluding the spinally projecting neurons that have received dedicated names because of their residency in well-delineated RF nuclei (e.g. interstitial nucleus of Cajal, red nucleus, tectum, locus coeruleus, raphe nuclei). RS neurons have axons that enter the spinal cord in the ventral funiculus, the ventral portion of the lateral funiculus, and, to a lesser extent, the dorsal portion of the lateral funiculus. RS axons in the different spinal funiculi have been grouped differently into medial and lateral reticulospinal tracts by classical anatomists and electrophysiologists, which has led to some confusion [2]. For this reason, we have opted not to employ this terminology. Finally, with regard to the cytoarchitecture in the RF, we follow closely the

nomenclature developed by Paxinos and Watson [3] which is the most commonly used for the mouse brain. Occasionally, we also give the alternate nomenclature used for the cat brain. However, a direct correspondence between the two nomenclatures is, sometimes difficult to establish. For instance, the gigantocellular reticular nucleus pars alpha and ventralis (GiA and GiV) in the mouse most likely correspond to the nucleus reticularis magnocellularis in the cat.

Mesencephalon
The mesencephalon is not considered a major source of RS neurons. Mesencephalic RS neurons are found in the ventrolateral periaqueductal gray (PAG), the adjacent mesencephalic reticular tegmentum or pre-cuneiform/cuneiform nucleus (prCnF/CnF), and the pedunculopontine nucleus (PPTg or PPN) [4,5]. RS neurons in the prCnF/CnF project predominantly or exclusively to the cervical and upper thoracic segments whereas the RS neurons in the PAG and PPtg/PPN project also to the lumbar segments [6,7]. Mesencephalic RS neurons in the PAG and the prCnF/CnF have not been recorded yet but mesencephalic RS neurons in the PPTg/PPN have been found to discharge during locomotor activity and also, together with non-RS neurons, during REM sleep [8]. Stimulation of the PAG and prCnF/CnF produces various defensive motor behaviors (e.g. freezing, micturition, fight, and running) [9–14] and cardiovascular and respiratory changes [15]. Stimulation of the PPTg/PPN produces different sleep/wake states [16•] and low-speed locomotion [14,17]. All these functions, however, are believed to be mediated by non-RS mesencephalic neurons and to involve the activation of the RS neurons in the pons and the medulla (see below).

Pons
The pons is the second largest source of RS neurons [4,5,18]. Pontine RS neurons are found dorsally in the Barrington's nucleus (Bar) and the subcoeruleus nucleus (SubC or sublaterodorsal nucleus, SLD), and more ventrally, in the nucleus pontis oralis (PnO) and caudalis (PnC).

The pontine RS neurons in the Bar nucleus project to the sacral cord and have been implicated in the control of micturition and other pelvic motor functions [19,20• ,9,21]. Through their axon collaterals in the thoracolumbar segments, however, Bar RS neurons may also mediate the changes in posture and sympathetic tone that occur during these functions [21]. The pontine RS neurons in the SubC/SLD have been implicated in sleep atonia [22], a task they share with non-RS SubC/SLD neurons that project to the spinal cord via medullary RS neurons [23–28].

The pontine RS neurons in the PnO have not been attributed any specific motor functions yet, while the pontine RS neurons in the PnC have been implicated

in locomotion, reaching, and protective motor reactions [29–31]. Stimulation of the PnO nucleus decreases postural muscle tone whereas stimulation of the PnC increases it [32,33]. Despite the fact that both PnO and PnC RS neurons have axons that branch extensively in the spinal cord [34,35], the effect of PnO stimulation on muscle tone is thought to be mediated mostly through the activation of the RS neurons in the medulla [36]. Independently of the precise type of motor behavior they mediate, pontine RS neurons thus appear to have the capability to produce multi-segmental coordinated actions on motoneurons, a necessary competency for matching postural muscle tone to ongoing motor activity.
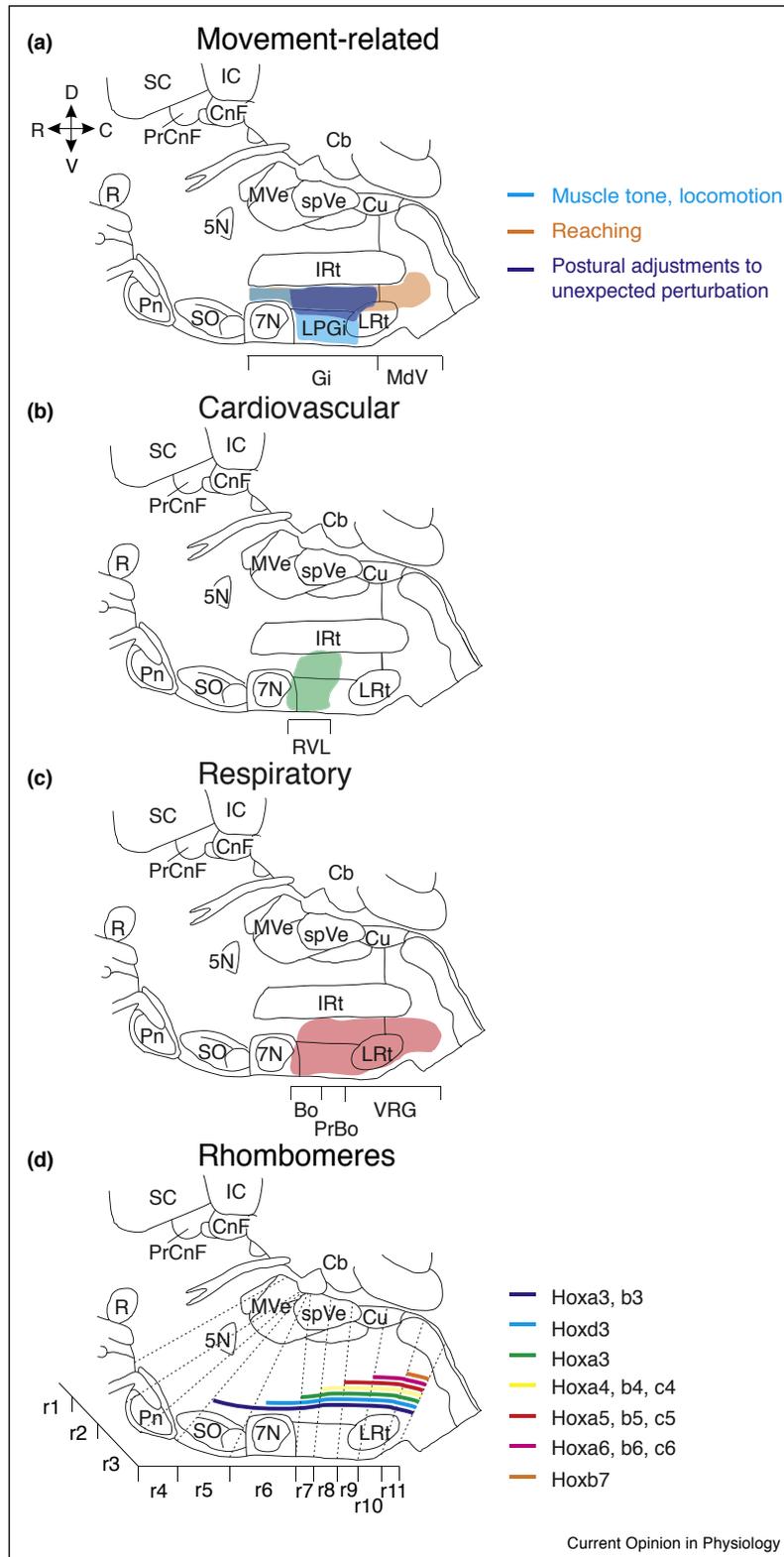
Medulla
Most RS neurons reside in the medulla [4,5,18] whose rostral border we define operationally as the level where raphespinal neurons first appear [18]. Dorsally located medullary RS neurons are found in the dorsal paragigantocellular nucleus (DPGi), parvicellular reticular nucleus (PCRt), and the intermediate reticular nucleus (IRt). Ventrally located medullary RS neurons are found in the gigantocellular reticular nucleus (Gi including its pars alpha (GiA) and ventralis (GiV)), the parapyramidal nucleus (PPy or PPr), the Botzinger (Bo) and pre-Botzinger (PrBo) nuclei, the ventral respiratory group (VRG), and the lateral paragigantocellularis nucleus (LPGi). At the level of the pyramidal decussation, which marks the border between the medulla and the spinal cord, RS neurons are found in the medullary reticular nucleus (or nucleus centralis), a nucleus that is divided by the IRt into a dorsal part (MdD or Cd) and a ventral part (MdV or Cv).

The dorsally located medullary RS neurons in the DPGi, PCRt, IRt, and MdD have been very little studied and their roles in motor control remain obscure, even if the nuclei in which they reside have received much attention. The DPGi, for instance, has been implicated in the regulation of sleep/wake cycle through its reticulo–reticular connections [37,24–28] but the participation of the DPGi RS neurons in this context has not been clarified. Similarly, the MdD, which is often called subnucleus reticularis dorsalis or SRD, is known as a key region for transmission of spinal noxious information to the thalamus. The role of the MdD RS neurons may include a modulation of the ascending noxious information and a transformation of this information into pain-related motor responses through reciprocal connections between MdD and Gi RS neurons [38]. Lastly, the PCRt and IRt contain RS neurons with direct connections to triceps motoneurons, suggesting a role in forelimb extension [39]. However, the behavioral contexts in which these RS neurons would play such a role remain unclear.

In contrast, the ventrally located medullary RS neurons have been implicated in a wide variety of behaviors. The

Figure 1

(a) Movement-related

— Muscle tone, locomotion
— Reaching
— Postural adjustments to unexpected perturbation

(b) Cardiovascular

(c) Respiratory

(d) Rhombomeres

— Hoxa3, b3
— Hoxd3
— Hoxa3
— Hoxa4, b4, c4
— Hoxa5, b5, c5
— Hoxa6, b6, c6
— Hoxb7

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Schematic of parasagittal section through the brain stem at the level of the medulla.
Depiction of spatial overlap in the ventral medulla of RF regions containing RS neurons implicated in **(a)** movement-related, **(b)** cardiovascular, and **(c)** respiratory functions together with **(d)** rhombomeric organization (b–c adapted from Goodchild and Moon [53] and (d) adapted from Toma-Rocas *et al*., [75]). Data from different animal species (*c.f.*, literature cited in text) are mapped on a reference parasagittal section from an adult

Table 1

Transcription factors expressed in progenitors and post-mitotic neurons during brain stem development. Transcription factors set up genetic diversity of brain stem neurons. Progenitor domains dP1–dP6 and p0–p3 give rise to different early-born and late (L)-born cell groups. Partial list of transcription factors expressed within post-mitotic dorsal groups (dA1–dB4) and ventral groups (v0–v3L) (adapted from Gray [98]). Abbreviations: Olig3, oligodendrocyte transcription factor 3; Atoh1, atonal BHLH transcription factor 1; Pax3/6/7, paired box 3/6/7; Msx1, Msh homeobox 1; Ngn1/2, neurogenin 1/2; Ascl1, Achaete–Scute family BHLH transcription factor 1; Gsx2, GS homeobox 2; Ptf1a, pancreas associated transcription factor 1a; Lbx1, ladybird homeobox 1; Dbx1/2, developing brain homeobox 1/2; En1, engrailed 1; GATA3, GATA binding protein 3; NKx2.2, NK2 homeobox 2; Pou4f1, POU class 4 homeobox 1; Barh1, BarH-like 1 homeobox protein; Lhx1/1b/2/5/9, LIM homeobox 1/1b/2/5/9; Evx1, even-skipped homeobox 1; FoxP2/D3, forkhead box P2/D3; Phox2b, paired-like homeobox 2b; Tlx3, T-cell leukemia homeobox 3; Prrxl1, paired related homeobox protein-like 1; bHlHb5, basic helix–loop–helix family, member b5; Wt1, Wilms tumor 1; Dmrt3, double sex and Mab-3 related transcription factor 3; Pitx2, paired-like homeodomain 2; Chx10, visual system homeobox 2

Domains	TFs in progenitors neurons	TFs in post-mitotic neurons
dA1	Olig3, Atoh1, Pax3, Msx1	Pou4f1, Barh1, Lhx2, Lhx9, Evx1
dA2	Olig3, Ngn1, Ngn2, Pax3, Pax7, Msx1	Pou4f1, Lhx1, Lhx5, FoxP2
dA3	Olig3, Ascl1, Ngn2, Pax3, Pax6, Pax7, Gsx2	Pou4f1, Tlx3, Prrxl1, Phox2b, Lmx1b
dA4	Olig3, Ascl1, Ptf1a, Ngn2	FoxP2, Foxd3
dB1	Ascl1, Ptf1a, Ngn2, Pax3, Pax6, Pax7, Gsx1/2	Lbx1, Pax2, Lhx1, Lhx5
dB2	Lbx1	Phox2b, Atoh1
dB3	Ascl1, Pax3, Pax6, Pax7, Gsx1/2, Dbx2	Lbx1, Tlx3, Prrxl1, Pou4f1, FoxP2, Lmx1b
dB4	Ngn1, Ngn2, Pax3, Pax6, Pax7, Dbx2	Lbx1, bHlHb5, Pax2, Lhx1, Lhx5, Wt1, Dmrt3
v0d	Dbx1	Pax2
v0v	Dbx1	Evx1
v0c	Dbx1	Pitx2
v1	En1	Pax2
v2a	GATA3	Chx10
v2b	GATA3	
pMN	NKx2.2	Olig1
v3L	NKx2.2	Lmx1b, Pet1

RS in the Gi and its pars GiA and GiV have been implicated in reaching [40,41,30] and locomotion [42,43,36,29,44–46,30] with subsets of RS neurons controlling specific aspects of movement such as termination [47], muscle atonia [36,48,24,49,33] or adjustments to unexpected perturbations [50]. The RS neurons in the MdV [39] may also participate in forelimb movements and those in the LPGi [51,52] in locomotor activity but their specific contributions await electrophysiologically documentation of their ongoing discharge pattern during these behaviors. This is because these MdV and LPGi RS neurons intermingle with the MdV and LPGi RS neurons that control cardiovascular functions [53–55] and the Bo, PrBo, and VRG RS neurons that control respiratory functions [56,57,53,58–60] (Figure 1a–c). The ventral-most RS neurons may in fact be more concerned with providing the metabolic and homeostatic support required at the onset and during motor activity than the movement itself, especially when this activity is prolonged or becomes intense (e.g. standing up from lying down, start walking, carrying a load, running for escape) [61,62•,63].

Amino acid neurotransmitter phenotypes
As other bulbospinal neurons, RS neurons are by and large glutamatergic neurons and do not exhibit great diversity with regard to amino acid neurotransmitter phenotypes [23,64–67,39,49,47,20•,52,21,28,14]. Still, a small proportion of ventrally located medullary RS neurons [68,52,69•] and MLF-descending pontine RS neurons ([70], however, see Ref. [18]) are gabaergic or glycinergic. This presence of inhibitory neurons is not unique to the RS system. The vestibulospinal system also holds inhibitory spinally projecting neurons [71].

The fact that RS neurons involved in different functions are overwhelmingly glutamatergic and have overlapping spatial distributions (cf. previous section) represents a challenge for optogenetic experiments that use the VGLUT2 promoter, which may lead to apparently conflicting results [14,17]. Another difficulty is that all descending neurons that release glutamate, corticospinal neurons excepted, express VGLUT2, resulting in the activation of en passant glutamatergic fibers of non-RS sources. Although novel tools for intersectional approaches [72] and efficient viral receptor complementation strategies [73] are being developed to address these issues, achieving neurotransmitter-independent specificity would be greatly beneficial. This, however, will likely require determining the molecular identity of the different groups of RS neurons.

(Figure 1 Legend Continued) mouse brain stem (lateral 1 mm, Franklin and Paxinos [97]). Similar functional overlap has been reported in the other regions of the brain stem where RS neurons reside.

Molecular profiles

The embryonic brain stem, under the action of Hox genes, transiently divides into a series of anteroposterior compartments called mesomeres (m1–m2) and rhombomeres (r0–r7 and pr8–pr11) which in the adult give rise to the mesencephalon and the rhombencephalon (cerebellum, pons and medulla) (Figure 1d) [74,75]. These compartments have common dorsoventral divisions (Table 1) whose precise architecture is controlled by the expression of compartment-specific developmental genes [76]. The compartment-specific interactions between transcriptional programs along the anteroposterior and the dorsoventral axes, which govern the production of specific neuronal phenotypes during development, likely give rise to the clustering of the RS neurons into defined anteroposterior and dorsoventral domains [77,78]. Therefore, transcription factors may uniquely identify subsets of RS neurons.

An initial study on transcription factors expressed during RS neurons differentiation has evidenced subsets of RS neurons that differentially express LIM homeodomain transcription factors Lhx1/5 and Lhx3/4, and Chx10 [79]. Further studies on Chx10-positive (Chx10+) RS neurons indicate that they are located primarily in the medullary Gi (incl. GiV and GiA) [80,47] but with a fair number in the medullary IRt and MdV [80] and pontine PnC [47]. Many of the Chx10+ RS neurons project to the cervical spinal cord [80] and many project to the lumbar spinal cord [47]. However, the proportion of Chx10+ RS neurons that project to both cervical and lumbar cord and/or thoracic cord is unknown. Chx10+ RS neurons are thought to be involved in locomotor control. Many Chx10+ RS neurons, especially in the Gi, are activated during MLR-evoked locomotion [80] whereas optogenetic activation of Chx10+ RS and non-RS neurons in the PnC and rostral Gi halts ongoing locomotor activity [47].

Other major groups of RS neurons express other specific transcription factors. For instance, the RS neurons in the Bar involved in micturition express the atonal bHLH transcription factor 1 (Atoh1) [21] whereas the RS neurons in the VRG involved in breathing express either the developing brain homeobox 1 (Dbx1), but not En1 nor Sim1, or the ladybird homeobox 1 (Lbx1), but not Phox2b nor Tlx3 [69•].

While determining the unique molecular signature of all the different subsets of RS neurons will clearly require more work, a molecular basis of their diversity is slowly emerging.

Concluding remarks

As we move toward a clearer understanding of the extent to which the various RS neurons assemble in distinct functional units, it will be important to understand how these functional units coordinate since disruption of this coordination may itself results in pathological conditions [81]. Therefore, the question as to whether RS neurons are dedicated to specific functions or whether most RS neurons can be recruited during most behaviors [82] remains open. In that context, determining the discharge patterns of individual RS neurons in relation to different autonomic and motor behaviors will continue to be essential to gaining insights into how the different RS populations regulate these functions. Molecular genetic approaches that interrogate the developmental origins of RS neurons show great promise although these approaches have not quite reached the level of specificity needed to uniquely define a given RS subset. In this respect, the development of mesomere-restricted or rhombomere-restricted reporter mice might be useful for fate-mapping analysis [83•], enabling gene overexpression or knockdown for transgenic manipulation and physiological investigation that would otherwise not be possible.

The diversity of RS systems underlines their importance for nearly all our essential functions. Understanding how this diversity emerges will not only help understand how we normally perform these essential functions but also how these functions may be recovered after injury or disease. RS neurons are known to be plastic [84–89] and a number of studies have demonstrated that their plasticity can be harnessed to enhance functional recovery [90–96].

Conflict of interest statement

Nothing declared.

Acknowledgements

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