Brainstem Circuits Controlling Action Diversification

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ABSTRACT

Neuronal circuits that regulate movement are distributed throughout the nervous system. The brainstem is an important interface between upper motor centers involved in action planning and circuits in the spinal cord ultimately leading to execution of body movements. Here we focus on recent work using genetic and viral entry points to reveal the identity of functionally dedicated and frequently spatially intermingled brainstem populations essential for action diversification, a general principle conserved throughout evolution. Brainstem circuits with distinct organization and function control skilled forelimb behavior, orofacial movements, and locomotion. They convey regulatory parameters to motor output structures and collaborate in the construction of complex natural motor behaviors. Functionally tuned brainstem neurons for different actions serve as important integrators of synaptic inputs from upstream centers, including the basal ganglia and cortex, to regulate and modulate behavioral function in different contexts.

INTRODUCTION

The brainstem is a key structure rostral to the spinal cord and is involved in the regulation of many forms of movement and other physiological functions. Brainstem neurons were inherently difficult to study in the past due to their functional diversity, neuronal intermingling, and complex integration into local, ascending, and descending circuits (Jones 1995; Kuypers 1981; Newman 1985a,b; Orlovsky et al. 1999; Valverde 1961). Consequently, brainstem neurons have often simply been referred to as relay neurons linking upstream and downstream neurons without clear functional assignments. Nevertheless, a series of lesion experiments in different species demonstrated the necessity of the brainstem in controlling movement. In frogs, transection of the neuraxis at progressively more caudal levels allowed researchers to determine the remaining motor abilities after lesion (Roh et al. 2011). Frogs with an intact brainstem but without forebrain performed most behaviors displayed by intact frogs, including jumping, stepping, and swimming. Frogs with transections at the rostral medulla showed partially remaining abilities, whereas all but reflexive behaviors were lost upon transection at the brainstem–spinal cord junction (Roh et al. 2011). Analogous experiments are more challenging in mammals for various reasons, including ethical ones. However, decorticated cats still perform many movements (Bjursten et al. 1976), and cats still locomote after premammillary lesions are introduced rostrally to the superior colliculus (Hinsey et al. 1930, Whelan 1996). These combined studies demonstrate that the brainstem harbors essential neuronal substrates to generate diverse forms of movement and is therefore clearly more than a relay station.

One important question is precisely how the brainstem contributes to movement generation and coordination. The generation of natural behaviors requires selection from competing behaviors and the combination of movements that occur either jointly or in succession, each ultimately implemented by motor neurons located in the brainstem and/or the spinal cord regulating peripheral muscle contractions (Figure 1). During environmental exploration, for example, locomotion and orofacial behaviors are frequently combined, and when animals arrive at a food source, they transport food to their mouth with their forelimbs and begin chewing. The recent implementation of genetic and viral tools, combined with cell type–specific perturbation experiments and refined behavioral analysis, has facilitated the identification of neuronal cell types stratified by different functions.

Here we review work on three large behavioral categories with important brainstem contributions for which there has been significant recent progress in understanding the function and connectivity of involved neuronal cell types—skilled forelimb movement, forms of orofacial and breathing behavior, and full-body locomotion (Figure 1). Recent studies identified specific neuronal populations in the brainstem playing roles in these behaviors, allowing us here to discuss how these circuit elements and their combined usage regulate and coordinate action diversification. The ways in which brainstem circuits regulate functions associated with other behaviors (e.g., eye or head movement) and those not related to movement (e.g., sleep) are not covered here.

BRAINSTEM AND SPINAL CIRCUITS FOR THE CONTROL OF SKILLED FORELIMB BEHAVIORS

Skilled forelimb behaviors rely on the activation of forelimb muscles in diverse sequences to produce an almost infinite number of movement patterns that we and other mammals can perform. Proximal and distal limb muscles represent a constrained spatial continuum along the extremities. The act of moving the arm transports the hand to particular locations (e.g., through the process of reaching), and within these constraints, the hand can carry out a myriad of movements (e.g., grasping, scratching, object manipulation) (Figure 2). The generation of these complex behaviors as well as the monitoring of their execution requires modular, adaptable, and highly organized neuronal circuits. Such circuits are needed to carry out these behaviors with high temporal precision and to allow for adjustments during ongoing movements. The reach-to-grasp task is a common behavioral paradigm that is used to dissect circuits involved in skilled forelimb movement that rodents execute using strategies and behavioral phases similar to humans (Lemon 2008, Sacrey et al. 2009, Whishaw & Pellis 1990). Therefore, although understanding the neuronal circuits controlling skilled forelimb behaviors is a challenging task, it opens the possibility to define and study the function of core circuit elements both in the genetically accessible rodent model and in higher-order species.

Much work in the past has focused on corticospinal connectivity and the role of these pathways in complex forelimb movements, with particular emphasis on direct connections from the cortex to spinal premotor and motor neurons (Dum & Strick 1991, Lemon 2008, Levine et al. 2012, Ueno et al. 2018, Wang et al. 2017). The reason for a high interest in this area was the observation that corticomotoneuronal synapses increase in abundance with advancing evolution from rodents to monkeys to humans (Kuypers 1964, Lemon 2008). This process is paralleled by increasing levels of sophistication in dexterous movements, culminating in the ability to control single digits (Kuypers 1964, Lemon 2008). Early on, it was already clear that circuits in the

brainstem are also involved in controlling skilled forelimb movements, as evidenced by lesion studies and electrophysiological recordings in cats and monkeys (Buford & Davidson 2004, Kuypers & Lawrence 1967, Schepens & Drew 2004, Soteropoulos et al. 2012). Moreover, work with cortical- or spinal cord–injury models suggests that brainstem circuits in the reticular formation and red nucleus gain functional importance under these compromised experimental conditions. Proposed mechanisms contributing to hand function recovery after injury include axonal sprouting by cortical axons at the brainstem level and/or by reticulospinal axons in the spinal cord, thus compensating for the reduction or lack of cortical access to the spinal cord (Baker 2011, Baker et al. 2015, Fregosi et al. 2018, Mosberger et al. 2018). Here, we review progress on the identification, anatomical organization, and function of neuronal circuits connecting the brainstem and spinal cord bidirectionally, with a role in shaping skilled forelimb behaviors in the uninjured nervous system.

A key requirement for the generation of skilled forelimb movements is the ability of spinal circuitry to integrate supraspinal motor instructions, process this information, and send commands to cervical motor neurons innervating forelimb muscles. Classical studies noted a mediolateral division in the lower brainstem, with lateral regions more prominently accessing intermediate and dorsolateral spinal domains proposed to be involved in distal forelimb control (Kuypers 1964, Lemon 2008). Recent work demonstrates that some brainstem populations preferentially communicate with cervical spinal neurons in mice (Esposito et al. 2014) (Figure 2). Of the identified brainstem regions, glutamatergic (vGlut2) neurons in a caudal brainstem area named the medullary reticular formation ventral part (MdV) connect to interneurons and specific cervical motor neuron pools encompassing extensor and flexor subtypes (Esposito et al. 2014). Functional work further demonstrated that MdV-vGlut2 neurons are required for the execution of

skilled forelimb movements. Most notably, in a single food pellet–retrieval task, during which mice carry out the modular sequence of reaching, grasping, and retrieving a food pellet, MdV-vGlut2 neurons are needed for efficient execution of specifically the grasping phase (Figure 2). The work identified additional brainstem regions with distinct connectivity profiles to the cervical spinal cord, but their behavioral role remains to be studied. In addition, the red nucleus located in the midbrain projects to the spinal cord in a dorsolateral tract and has also been implicated in the control of skilled forelimb movement (Jarratt & Hyland 1999, Kuypers & Lawrence 1967, Whishaw et al. 1998) (Figure 2). Specifically, dorsolateral tract lesions in rats lead to defects in the arpeggio phase of the reach-grasp behavior (Morris et al. 2011). Jointly, these observations suggest that distinct brainstem populations control specific aspects or phases of skilled forelimb behaviors by accessing specialized spinal circuits.

How do the descending pathways implicated in skilled forelimb behaviors interact with spinal neurons? Experiments performed in cats identified cervical spinal neurons that receive direct input from cortical, reticular, and rubrospinal neurons and connect intraspinally mostly to neurons within the cervical spinal cord, including motor neurons (Alstermark & Kummel 1986, Alstermark et al. 2007, Illert et al. 1978). Since such neurons were preferentially found at cervical levels C3 and C4, they were named C3-C4 propriospinal neurons. Early experiments in cats using spinal tract lesions of C3-C4 projections suggested an involvement of these neurons in forelimb-specific behaviors such as reaching (Alstermark et al. 1981b). A more recent study performed in monkeys and using a mix of retrograde and anterograde viral tools showed that the silencing of neurons located at C3-C5 and projecting to C6-T1 induces impairments in forelimb reaching and grasping behaviors (Kinoshita et al. 2012). These deficits reversed after a few days, suggesting that compensatory mechanisms developed via unaffected descending pathways such as cortico-,

reticulo-, or rubrospinal projections or other intraspinal relays (Kinoshita et al. 2012). Interestingly, in addition to their direct connections to motor neurons and other spinal interneurons, a fraction of C3-C4 propriospinal neurons also sends ascending projections to the precerebellar lateral reticular nucleus (LRN) in the brainstem, harboring neurons that in turn give rise to cerebellar mossy fibers (Alstermark & Ekerot 2013, Alstermark et al. 1981a). Bifurcating spinal neurons, therefore, serve for both descending motor command integration and the production of ascending efference copy pathways to update and potentially adapt ongoing behavior through cerebellar circuitry.

Recent studies have addressed the identity and functional organization of cervical neurons with supraspinal ascending projections (Azim et al. 2014, Hayashi et al. 2018, Pivetta et al. 2014) (Figure 2). A common entry point for these studies was the finding that, during development, spinal populations with involvement in functionally specific aspects of motor behavior are often derived from distinct progenitor domains (Alaynick et al. 2011, Arber 2012, Goulding 2009, Kiehn 2016). Different spinal populations are characterized by the expression of selective transcription factors, allowing for their genetic targeting. Anatomically mapping bifurcating cervical projection neurons in mice revealed that they distribute much more broadly than to just C3-C4 segments, although they are nevertheless confined to cervical levels (Pivetta et al. 2014). LRN-projecting cervical neurons also fractionate into several genetically distinct populations encompassing excitatory and inhibitory subsets, as demonstrated by intersectional genetic and viral tracing methods that permanently label neurons derived from distinct progenitor domains or neurotransmitter identity (Figure 2). Interestingly, identified populations establish anatomically divergent terminal arborizations within the LRN (Pivetta et al. 2014). The excitatory V2a population contains a fraction of these ascending projection neurons, and targeted ablation of the

overall V2a population at cervical levels in mice elicits defects in reaching but not grasping in a food pellet retrieval task (Azim et al. 2014, Ueno et al. 2018). Furthermore, the optogenetic activation of ascending branches of cervical V2a neurons in the LRN severely perturbs the forelimb reaching trajectory (Figure 2), providing evidence that the ascending V2a branch can affect forelimb behavior (Azim et al. 2014).

The overall V2a population is still a diverse population. In addition to its involvement in forelimb reaching, the V2a population has been functionally linked to left-right alternation in a speed-dependent manner (Crone et al. 2008, 2009). This functional heterogeneity suggests that more distinct subpopulations exist within the V2a population, and indeed two different types (i.e., V2a type I: low Chx10 expression, present throughout the spinal cord; V2a type II: high Chx10 expression, preferentially located at cervical levels and with ascending projections to the brainstem) were recently described (Hayashi et al. 2018). Furthermore, single-cell RNA sequencing of V2a neurons revealed 11 clusters with different fractions of type I and type II V2a neurons, leading to the speculation that specific clusters of type I V2a neurons might be involved in whole-body locomotion, whereas other type II, cervically enriched V2a neuron clusters might be involved in skilled forelimb movements (Hayashi et al. 2018).

Together, functionally diverse subsets of cervical spinal neurons integrate descending motor commands and establish ascending axons to precerebellar neurons in the LRN (Figure 2). This raises the question of whether and how information passing through the cerebellum to deep cerebellar nuclei (DCN) influences skilled (forelimb) behavior to close the loop. Such a looped circuit structure would allow for the comparison of executed to intended movement in order to adjust movement if needed. Integration already seems to occur at the level of granule cells for a

variety of behavioral paradigms, even incorporating learning-related information, including reward and punishment as well as anticipatory movement-related signals (Giovannucci et al. 2017, Huang et al. 2013, Wagner et al. 2017). Purkinje cells (PCs) represent the output channels of the cerebellar cortex, signaling by inhibition to DCN neurons that, as a population, target both ascending and descending structures. It is well established that cerebellar circuitry and the PC-to-DCN pathway are involved in associative forms of learning (Medina 2011). Optogenetic manipulation studies helped determine whether changing the PC firing rate can influence behavior instantaneously (Heiney et al. 2014, Lee et al. 2015). PCs fire spontaneously at high rates (50–100 Hz), and reducing or pausing their firing is predicted to disinhibit downstream DCN neurons and influence movement. Indeed, the transient silencing of PCs by either activation of inhibitory molecular layer interneurons or direct optogenetic inhibition of PCs elicits discrete behaviors, resulting in either eyelid or forelimb movement, according to the inhibited region (Heiney et al. 2014, Lee et al. 2015). Distinct DCN neurons are also accessible genetically. Optogenetic activation and ablation experiments demonstrate that a molecularly defined population in the DCN interposed anterior nucleus (Ucn3+) influences both fore- and hindlimb positioning (Low et al. 2018).

These combined data show that a looped and bidirectionally communicating network between the brainstem and spinal cord plays important roles in the control of skilled forelimb movements. Future work will reveal the identity and connectivity of the circuit components responsible for parsing together the distinct behavioral elements of skilled forelimb movement and how these behaviors can be adjusted. This will increase our understanding of their synaptic and functional interactions with higher motor centers, including cortical, thalamic, and basal ganglia components, and intrabrainstem connectivity between functionally distinct areas.

COORDINATION OF OROFACIAL AND RESPIRATORY MOVEMENTS BY BRAINSTEM CIRCUITS

Another complex set of behaviors coordinated by circuits in the brainstem involves breathing and orofacial movements, including whisking, sniffing, licking, swallowing, and chewing (Figure 3). These behaviors are often temporally tightly coordinated with each other to elicit the desired movement sequence, e.g., to couple jaw and tongue muscles during eating or drinking (Kurnikova et al. 2017, McElvain et al. 2018, Naganuma et al. 2001, Welzl & Bures 1977). They also frequently maintain a strong oscillatory component with rhythmic repetition of the same movement at a specific frequency (Kurnikova et al. 2017, McElvain et al. 2018).

Work on a number of neuronal networks that produce rhythmic outputs has suggested that neurons with intrinsic oscillatory capacity contribute in important ways through their physiological properties even within very simple networks (Marder & Bucher 2001). For breathing, several brainstem regions with oscillatory properties linked to behavior were identified, most notably the rhythmic oscillators within the pre-Bötzinger complex (preBötC), the Bötzinger complex, and the parafacial respiratory groups regulating inspiration and expiration during breathing (Del Negro et al. 2018, Moore et al. 2014) (Figure 3). Several studies, summarized below, have addressed the cellular organization, subpopulation identity, and potential interactions between these circuits and those involved in the regulation of orofacial movements and breathing.

Motor neurons innervating the oral and facial muscles used to produce orofacial movements are clustered into specific brainstem motor nuclei and project to their target muscles through cranial motor nerves (Guthrie 2007). One recent approach to uncovering the organizational principles of networks underlying orofacial behaviors has been to study the organization of premotor neurons to the brainstem motor neurons responsible for driving respective behaviors. The overall, direct synaptic inputs to specific motor neurons were mapped through application of monosynaptic rabies viruses to reveal organizational differences between premotor neurons connecting to motor neuron pools innervating functionally distinct limb muscles (Stepien et al. 2010, Tripodi et al. 2011, Wickersham et al. 2007). In the context of orofacial and respiratory behaviors, studies analyzing the last-order premotor neuron distribution for different oral, facial, and phrenic motor neuron pools also revealed interesting organizational differences (Deschenes et al. 2016, Sreenivasan et al. 2015, Stanek et al. 2014, Takatoh et al. 2013, Wu et al. 2017).

The preBötC, the site of oscillatory rhythmic activity coupled with the inspiratory respiration cycle, has almost no direct connections to diaphragm-innervating phrenic motor neurons (Del Negro et al. 2018, Smith et al. 1991). Instead, the preBötC signals through the rostral ventral respiratory group (rVRG) to access phrenic motor neurons (Del Negro et al. 2018, Feldman et al. 2013). A recent study showed that both structures share the developmental expression of the transcription factor Dbx1 (Wu et al. 2017), demonstrating that the V0 progenitor domain does not only generate preBötC neurons (Cui et al. 2016) within the breathing network. Moreover, Dbx1+ rVRG neurons connect to phrenic motor neurons on both sides (Wu et al. 2017), ensuring tight inspirational control through regulation of the diaphragm muscle across the midline. Neurons in preBötC can also be influenced to produce different breathing behaviors according to motivational and physiological need. To induce a sigh, preBötC neurons are regulated by a

population of only 200 upstream neurons in the retrotrapezoid nucleus/parafacial respiratory group, and these neurons are marked by the expression of bombesin-like neuropeptides (P. Li et al. 2016).

Recent work revealed that premotor neurons connected to different brainstem motor neurons can be in close proximity to each other or even intermingled. For example, neurons premotor to facial motor neurons controlling whisking movements are close to and within the preBötC (Sreenivasan et al. 2015, Takatoh et al. 2013). These premotor neurons show mixed neurotransmitter phenotypes constituting potentially different premotor populations responsible for the protraction and retraction phases of whisking, reinforcing the concept of distinct subpopulations controlling specific motor behaviors (Takatoh et al. 2013). The spatial proximity of vibrissa premotor neurons to the preBötC as well as the rhythmic nature of whisking itself raises the question of whether a potential oscillatory center for rhythmic whisking interacts with the circuits controlling breathing.

Breathing and whisking are functionally tightly coupled, but each can occur in the absence of the other (Moore et al. 2013), which suggests that linked but distinct neuronal circuitry is responsible for respective oscillatory control mechanisms. Additionally, since the breathing rhythm can reset the whisking rhythm but not vice versa, the preBötC seems to act as a master regulator of these behaviors (Kleinfeld et al. 2014, Moore et al. 2013) (Figure 3). Functionally, the intermediate reticular nucleus (IRt), a subregion of the brainstem that is sometimes also referred to as the intermediate band of the reticular formation, is in close proximity to the preBötC and the site of whisker premotor neurons, and it harbors neurons whose activity is tightly locked with rhythmic whisking movements (Deschenes et al. 2016, Moore et al. 2013, Takatoh et al. 2013) (Figure 3).

A combination of activation and lesion experiments provides evidence for the sufficiency and necessity of this region for whisking, demonstrating its role as an oscillatory center under the potential master regulation of the preBötC (Deschenes et al. 2016, Moore et al. 2013). As a further extension of these findings on closely spaced and interacting brainstem networks, oscillatory activity coupled to licking movements as well as the necessity for licking has also been attributed to the IRt (Travers et al. 2000) (Figure 3). The circuits controlling chewing, a behavior that is not phase locked with breathing (McFarland & Lund 1993), also appear to reside within the rather lateral brainstem but rostrally to the breathing and whisking oscillators (Dellow & Lund 1971, Kolta et al. 2007, Morquette & Kolta 2014).

What is the circuit architecture controlling these interrelated behaviors? A common denominator in using anterograde, retrograde, and transsynaptic tracers is that most premotor neurons innervating orofacial and breathing motor neurons reside in intermediate to lateral brainstem areas that occupy partly intermingling or distinct regional hot spots, which are prominently located within the IRt, parvicellular reticular nucleus (PCRt), and preBötC regions (Deschenes et al. 2016, Sreenivasan et al. 2015, Stanek et al. 2014, Takatoh et al. 2013, Wu et al. 2017). Premotor neurons are also molecularly diverse, but common principles are beginning to emerge for some behaviors (Wu et al. 2017). It is currently unclear whether the circuits responsible for different behaviors engage shared neuronal populations. Behavioral and electrophysiological experiments suggest that individual oscillatory centers control distinct movements, including swallowing, licking, and whisking, and that the breathing oscillator can act as a master regulator (Moore et al. 2014) (Figure 3). Taken together, brainstem circuits controlling orofacial and breathing behaviors are made up of specific neuronal subpopulations responsible for individual

motor attributes that are tightly coupled to enable the complex behaviors present during exploration or feeding.

An interesting aspect that has not been addressed yet is the potential interaction between orofacial and breathing circuits with the networks involved in skilled forelimb movements or locomotion. Orofacial behaviors are coordinated with body actions occurring during natural complex movements (Figure 1), for example, reaching for and consuming food, during which the mouth opens to take up food that is subsequently chewed and swallowed. To find food, animals explore the environment; hunt at high speed, requiring an increase in the respiratory rate; and fight with and kill their prey, again requiring tight coordination between the body and orofacial muscles. Now that the specific brainstem subpopulations responsible for orofacial, breathing, and body behaviors are beginning to be identified, studies that clarify the interactions and possible competitions between different neuronal populations and how complex behaviors are coordinated through brainstem motor circuitry at a more global level will be possible.

BRAINSTEM CIRCUITS CONTROLLING FULL-BODY MOVEMENT

Locomotion is a universal behavior in the animal kingdom. This form of full-body movement manifests itself differentially according to the species as walking, running, swimming, crawling, or flying, to mention the most prominent forms (Orlovsky et al. 1999). One common denominator in all species is the need for behavioral coordination throughout the body to move it forward and to optimize speed for controlled interactions with the environment. The brainstem plays important roles in the regulation of locomotion, and the recent work reviewed here begins to

delineate the identity of circuits between the midbrain and more caudally located brainstem regions as instrumental for the control of specific locomotor parameters (Figure 4).

The stimulation of the mesencephalic locomotor region (MLR) in the midbrain elicits coordinated full-body locomotion in a variety of species, including cat, rat, and lamprey (Mori et al. 1989, Ryczko & Dubuc 2013, Shik & Orlovsky 1976, Skinner & Garcia-Rill 1984). Recent studies provide evidence that, despite the spatial intermingling of excitatory (vGlut2), inhibitory, and cholinergic cell types within the MLR, specifically vGlut2-expressing neurons are central for the locomotion-promoting properties of the MLR (Caggiano et al. 2018, Josset et al. 2018, Niell & Stryker 2010, Roseberry et al. 2016). It is also clear that there is further functional diversity within the MLR. Stimulation of vGlut2-expressing neurons within and close to the pedunculopontine nucleus (PPN) in the ventrolaterally located MLR only influences limb muscle activity or elicits low-speed locomotion, while stimulation of vGlut2 neurons in the dorsomedial cuneiform nucleus (CnF) of the MLR induces high-speed locomotion (Caggiano et al. 2018, Josset et al. 2018) (Figure 4). These findings agree with a proposed model in which the CnF is involved in defensive locomotion and the PPN in exploratory forms of locomotion (Jordan 1998). In addition to locomotion-promoting properties, the MLR also seems to house circuits for the attenuation of locomotor behaviors, which was suggested from both electrical (Takakusaki et al. 2016) and neurotransmitter-stratified optogenetic (Josset et al. 2018, Roseberry et al. 2016) stimulation experiments. Yet how these neurons relate to and/or interact with their locomotion-promoting counterparts remains to be defined.

Locomotion-promoting signals from the MLR have been proposed to reach the spinal cord via mostly disynaptic pathways through intermediary neurons in the caudal brainstem, since cooling

experiments in the ventral medulla severely reduce the effects of MLR stimulation on locomotion (Shefchyk et al. 1984). Electrophysiological recordings in the medullary reticular formation in cats and mice revealed patterns of neuronal activity that correlate with locomotor parameters (Drew et al. 1986, Weber et al. 2015). Paired electromyography and neuronal recordings showed highly diverse neuronal discharge patterns linked to the activity of individual or groups of muscles in cats (Drew et al. 1986). Despite these locomotion-correlated activity patterns, electrical stimulation experiments in the caudal brainstem failed to show consistent induction of full-body locomotion, leading to the idea that neuronal diversity might mask the regional properties to bring about such effects (Orlovsky et al. 1999). Indeed, a recent study demonstrated that optogenetic stimulation at different sites within the caudal medulla in mice also cannot induce full-body locomotion (Capelli et al. 2017). However, the specific optogenetic activation of excitatory neurons in the lateral paragigantocellular nucleus (LPGi) elicited reliable and short-latency locomotion (Figure 4). Functional studies further demonstrated that these vGlut2-LPGi neurons were essential for high-speed locomotion and that the MLR locomotion-promoting signal is reduced in the absence of these neurons (Capelli et al. 2017).

Conversely, restricting optogenetic stimulation to intermingled inhibitory neurons within the LPGi and neighboring medullary subregions attenuated locomotor behaviors ranging from simple behavioral stopping to body collapse akin to atonia (Capelli et al. 2017). In addition, another study demonstrated that a more rostrally located excitatory brainstem population marked by the V2a population–specific transcription factor Chx10 also influences the halting of ongoing locomotion, likely through accessing locomotion-inhibiting spinal circuits (Bouvier et al. 2015). Similarly, glycinergic neurons in the pontine reticular formation negatively influence locomotor speed through ascending projections to the thalamus (Giber et al. 2015) (Figure 4). Surprisingly,

V2a neurons in the zebrafish brainstem have opposite behavioral roles in that they promote swimming and, upon silencing, lead to the stopping of this behavior (Kimura et al. 2013). These findings might point to some evolutionary changes in how neurons of similar genetic identity in analogous regions of the nervous system are engaged. Nevertheless, the existence of specific neuronal populations that encode distinct locomotor attributes is conserved across species (Juvin et al. 2016, Kimura et al. 2013).

Together, these findings demonstrate the existence of specific neuronal populations within the brainstem network between the midbrain and more caudal brainstem regions that regulate different attributes of locomotor behavior (Figure 4). The execution of locomotor commands from the brainstem likely occurs through interactions with distinct circuits at the level of the spinal cord. Indeed, it has already become apparent that descending pathways originating from identified neuronal populations access spinal circuits differentially (Bouvier et al. 2015, Capelli et al. 2017).

MODULATORY AND INSTRUCTIVE INPUTS TO BRAINSTEM CIRCUITS

The brainstem is critically involved in many movements including whole-body actions, skilled forelimb behaviors, and orofacial coordination. Mammalian nervous system lesions eliminating the cortex, basal ganglia, and thalamus result in movements with highly reduced complexity (Whelan 1996). Several recent studies have assessed the functional capacity of interacting upstream structures with specific brainstem or midbrain circuits to instruct or modulate specific motor actions.

In the cortex, a fraction of layer 5 neurons, also often referred to as pyramidal tract (PT) neurons, projects to subcortical areas including the colliculi, brainstem, and spinal cord (Shepherd 2013), raising the question of the nature of their influence on behavior. PT neurons located in the anterior lateral motor cortex (ALM) and projecting to the brainstem often showed contralaterally biased, task-related activity before movement onset during a sensorimotor delayed discrimination task involving directional licking (Li et al. 2015) (Figure 5). Interestingly, bilateral ALM silencing during the motor planning phase randomizes licking direction but does not abolish licking in general (N. Li et al. 2016), indicating a modulatory role for these neurons possibly by acting on brainstem targets to orchestrate specifically the licking direction. In a more complex motor task involving the learning of a skilled forelimb movement, learning-related changes in PT neuron activity in the motor cortex provide a possible cellular mechanism for how movement refinement occurs during learning (Peters et al. 2017), but whether this is implemented through interaction with brainstem circuits is currently unclear. Together, these studies suggest a role for cortical neurons that project to the brainstem and spinal cord in modulating the activity of specific circuits in response to behavioral requirements involving fine aspects of motor performance and learning.

It is also interesting to understand interactions between different types of subcortical neurons and the brainstem. The central amygdala (CeA) sends long-range inhibitory projections to distinct centers in the midbrain and brainstem (Tovote et al. 2015). Specifically manipulating CeA projections to the periaqueductal gray (PAG) or the PCRt revealed their differential contribution to hunting or killing behaviors, respectively (Han et al. 2017) (Figure 5). Coincident optogenetic stimulation of axonal terminals in both target areas was sufficient to elicit a complete predatory hunting sequence. Interestingly, however, some effects were only observed in the presence of

natural or artificial prey, suggesting a context-dependent component in the ability to elicit the behavior (Han et al. 2017). The PAG also receives different inputs from the hypothalamus and superior colliculus involved in regulating distinct locomotor modes ranging from freezing to escaping (Evans et al. 2018, Li et al. 2018).

Some basal ganglia regions, including the output structure substantia nigra pars reticulata (SNr), also project to motor-related areas in the brainstem (Arber & Costa 2018, Mena-Segovia & Bolam 2017). Although functional studies linking basal ganglia projections to the brainstem are rare, the revealed neuronal coding within these circuits allows for an interesting hypothesis to be developed. Neurons in the striatum, the major basal ganglia input structure, encode various behavior-related parameters, with specific populations preferentially active during different behaviors such as grooming, locomotion, turning, or rearing (Barbera et al. 2016, Klaus et al. 2017, Parker et al. 2018). Such specific activity patterns are likely transferred and processed between functionally related cell populations within connected basal ganglia circuitry. Indeed, the SNr also harbors action-specific neuronal coding, and this information might be differentially fed toward brainstem circuits (Arber 2012, Jin & Costa 2015, Jin et al. 2014, Mena-Segovia & Bolam 2017, Rossi et al. 2016, Tecuapetla et al. 2016).

These results lead to the hypothesis that subcortical regions contain channels to specific brainstem centers to aid in the selection and execution of certain motor behaviors depending on context. In contrast, direct cortical inputs to the brainstem might rather act as behavioral modulators, allowing adaptation according to behavioral needs, challenges, and motivations.

OUTLOOK AND EVOLUTIONARY CONSERVATION OF BRAINSTEM ORGANIZATIONAL LOGIC

The work reviewed here demonstrates that the brainstem harbors a distributed assembly of neuronal populations that are important for the regulation of diverse motor behaviors. A general principle that emerges is that neurons with different functions are frequently spatially intermingled but connected to precise circuitry ensuring different behavioral roles. Thus, it is critical to isolate neuronal populations based on their neurotransmitter and genetic identity to understand their function. Neuronal populations include dedicated communication channels to the spinal cord that are involved in diverse aspects of controlling bodily movement as well as to networks regulating behaviors steered by motor neurons embedded within the brainstem proper. Although the overall organization of brainstem structures differs between species, the concept of descending pathways communicating specific information for action program execution is evolutionarily conserved.

To illustrate this point, we briefly summarize progress in understanding the organization and function of descending neurons in insects that, with only a few hundred neurons (Gronenberg & Strausfeld 1990, Hsu & Bhandawat 2016), represent simpler models than mammals. Genetic approaches in Drosophila melanogaster were used to systematically assess the organization and function of individual neurons, covering about half the known neurons with projections to the ventral nerve cord (Namiki et al. 2018), the structure analogous to the vertebrate spinal cord. Two groups of descending neurons target nonoverlapping neuropil territories responsible for the control of flight and walking, respectively. A third group projects to the intermediate neuropil and might drive more complex integrative motor behaviors requiring both types of behaviors

such as grooming or takeoff for flying (Namiki et al. 2018). Optogenetic activation in these genetically stratified backgrounds assessed the functional impact of the identified descending neurons (Cande et al. 2018). Notably, the activation of specific descending neurons frequently elicited stereotyped behaviors. Interestingly, however, some induced behaviors depended on the fly's behavioral state before manipulation. These results suggest that the information conveyed by upper centers or feedback mechanisms can be reconfigured in a state-dependent manner and can differentially impact movement regulation. This concept will also be interesting to study in evolutionarily higher species where state dependency might play more prominent roles in behavioral regulation.

Important questions on understanding how brainstem circuits orchestrate the learning and execution of actions remain to be addressed. Although control elements for specific behaviors in the brainstem are beginning to be unraveled, future work will determine how the combination of individual elements for one behavior or the generation of action sequences is achieved. We also need to understand how movement elements occurring in parallel are aligned and coordinated to achieve the overall animal behavior. Moreover, certain action programs that should not occur concurrently most likely rely on inhibitory mechanisms that prevent the unwanted behavior, on the one hand, and enhance the chosen motor program, on the other. Some of these regulatory and interactive mechanisms likely depend on upstream circuits, including the basal ganglia, cortex, and thalamus, as well as the integration of feedback circuits from the periphery. Ultimately, however, integrated information passes through neuronal populations in the brainstem that likely also contribute to all of these processes.

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Due to the broad topical coverage in this review, citations are mostly focused on a selection of recent original literature. We apologize to the authors of the many additional original studies not cited here and the authors of older work for citing review articles instead. We thank Kevin Fidelin, Manuel Ferreira-Pinto, and Rui Costa for their constructive comments on the manuscript. L.R. and S.A. were supported by a European Research Council Advanced Grant (692617), the Swiss National Science Foundation, the Kanton Basel-Stadt, the Novartis Research Foundation, and the Louis Jeantet Prize for Medicine.

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FIGURE LEGENDS

Figure 1. Movement programs regulated by brainstem circuits, and the distribution of motor neurons in the brainstem and the spinal cord responsible for the regulation of skilled forelimb behaviors, orofacial and respiratory movements, and whole-body movements. (a) Schematic (not to scale), top down view of the brainstem. The rostral portion of the scheme contains the cranial motor nuclei 5N, 7N, Amb, 12N, and 10N. The spinal cord (caudal portion of the scheme) contains the cervical, thoracic, and lumbar segments. The LMC innervates limb muscles, the MMC innervates axial muscles, and the HMC innervates hypaxial muscles. Cervical motor neurons innervate FL muscles, and lumbar motor neurons innervate HL muscles. (b) Examples of different behavioral elements of the three categories covered in this review and some ways in which they can be combined during natural behaviors. Abbreviations: 5N, trigeminal nucleus; 7N, facial nucleus; 10N, vagus nucleus; 12N, hypoglossal nucleus; Amb, Amb nucleus; FL forelimb; HL, hindlimb; HMC, hypaxial motor column; LMC, lateral motor column; MMC, medial motor column; Phr, phrenic motor neurons.

Figure 2. Brainstem-centric view of skilled forelimb behaviors. (a) Schematic illustration of the usage of the FLs in skilled behaviors. The arm makes use of the 3D reaching space to bring the hand to a desired location (cone and red spots) in the first phase of the behavior, and the hand then carries out one of the many diverse actions in a second phase. (b) Incomplete scheme of the brainstem/cerebellum (top) and spinal (bottom) circuitry described in this

review and implicated in skilled FL behavior. The left side of the scheme focuses on descending circuit organization for motor execution, and the right side depicts circuits for the computation of motor efference information. Note that bifurcating cervical neurons reside at the boundary between these two categories. They connect to cervical MNs and neurons in the LRN in the brainstem. LRN neurons in turn communicate with cerebellar circuits (GCs, PCs) and DCN. The reticular formation (including MdV) and the midbrain RN are regions implicated in different aspects of skilled GL behavior. Abbreviations: DCN, deep cerebellar nuclei; FL, forelimb; GC, granule cell; LRN, lateral reticular nucleus; MdV, medullary reticular formation ventral part; MN, motor neuron; PC, Purkinje cell; RN, red nucleus.

Figure 3. Schematic diagram illustrating the close spatial proximity of brainstem neurons implicated in orofacial and respiratory behaviors regulated by brainstem circuits. (a) Top-down anatomical depiction of the BötC, preBötC, rVRG, IRt, and PCRt. Excitatory vGlut2 (teal) and inhibitiory vGAT (purple) neurons, as well as developmentally Dbx1-originating (blue neurons), are shown. The rostrocaudal boundary between MRF and PRF is indicated along with relevant cranial motor nuclei (gray). (b) Depiction of licking, breathing, and whisking behaviors; the implicated brainstem structures; and how rhythms between these behaviors can be synchronized. The breathing rhythm can entrain the whisking rhythm, indicating close collaboration between relevant circuit elements. Abbreviations: 5N, fifth motor nucleus; 7N, seventh motor nucleus; 12N, hypoglossal motor nucleus; BötC, Bötzinger complex; Dbx1, developing brain homeobox protein 1; IRt, intermediate reticular nucleus; MN, motor neuron; MRF, medullary reticular formation; PCRt, parvicelluar reticular nucleus; preBötC, pre-Bötzinger complex; PRF, pontine reticular formation; rVRG, rostral ventral

respiratory group; vGAT, vesicular GABA transporter; vGLUT2, vesicular glutamate transporter 2; vlRt, vibrissa zone of the intermediate reticular nucleus.

Figure 4. Brainstem circuits implicated in supraspinal control of locomotion. (a) Prokinetic, locomotion-promoting circuit organization. The MLR in the midbrain contains the PPN and the CnF, which are implicated in low- and high-speed locomotion respectively. Excitatory neurons in the LPGi are implicated in high-speed locomotion. (b) Antikinetic, behavioral arrest-promoting circuits. Different forms of behavioral arrest are induced by the optogenetic stimulation of inhibitory LPGi neurons, rGi Chx10-expressing neurons or rostrally projecting inhibitory neurons in the PRF. The speed versus time plots illustrate that optogenetic stimulation of the respective neuronal populations (blue box) leads to either the induction of the locomotion with increased speed (a) or the decrease of speed with behavioral arrest (b) in mice. Abbreviations: 5N, fifth motor nucleus; 7N, seventh motor nucleus; ChAT, choline acetyltransferase; Chx10, Ceh-10 homeodomain-containing homolog; CnF, cuneiform nucleus; LPGi, lateral paragigantocelluar nucleus; MLR, mesencephalic locomotor region; PPN, pedunculopontine nucleus; PRF, pontine reticular formation; rGi, rostral Gi; vGAT, vesicular GABA transporter; vGlut2, vesicular glutamate transporter 2.

Figure 5. Modulatory and regulators upper motor centers impacting the brainstem. (a) Neurons in the ALM influence the directional bias of licking in a delayed discrimination task. Preparatory cortical activity ramping up during the delay period in right-sided ALM layer 5 pyramidal tract neurons with brainstem projections, which precedes left-directional

licking activity before action is initiated. Note that, during right-directional licks, similar neuronal activity cannot be observed. (b) Predatory hunting behavior composed of pursuit and killing phases regulated by inhibitory neurons in the CeA projecting to the PAG and the PCRt, respectively. Only joint axonal stimulation (blue light) in both target regions elicits full behavior but in a behavioral context-dependent manner. Abbreviations: ALM, anterior lateral motor cortex; CeA, central amygdala; ChR2, channelrhodopsin 2; PAG, periaqueductal gray; PCRt, parvicellular reticular nucleus.

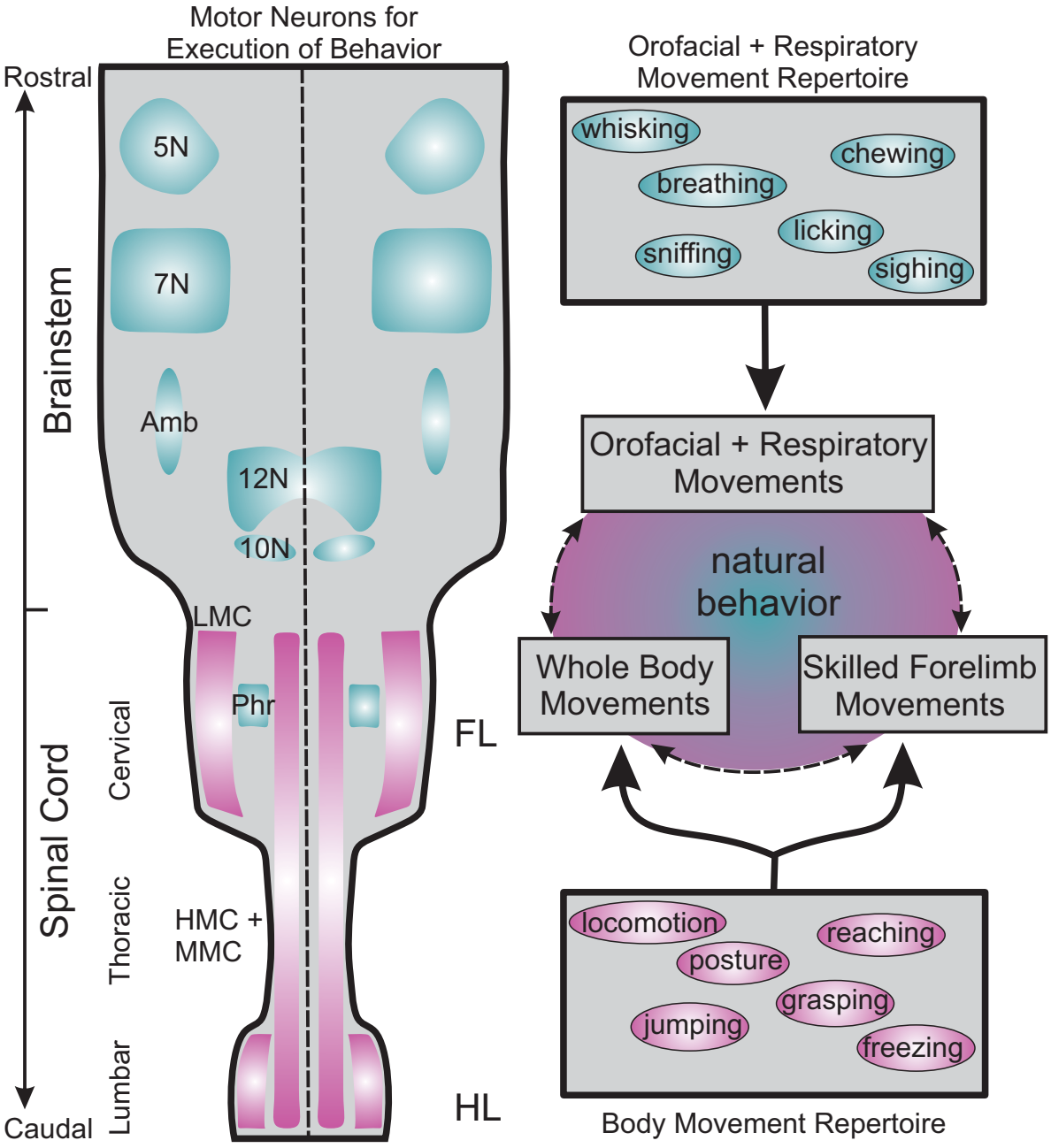

Figure 1 - Movement programs regulated by brainstem circuits

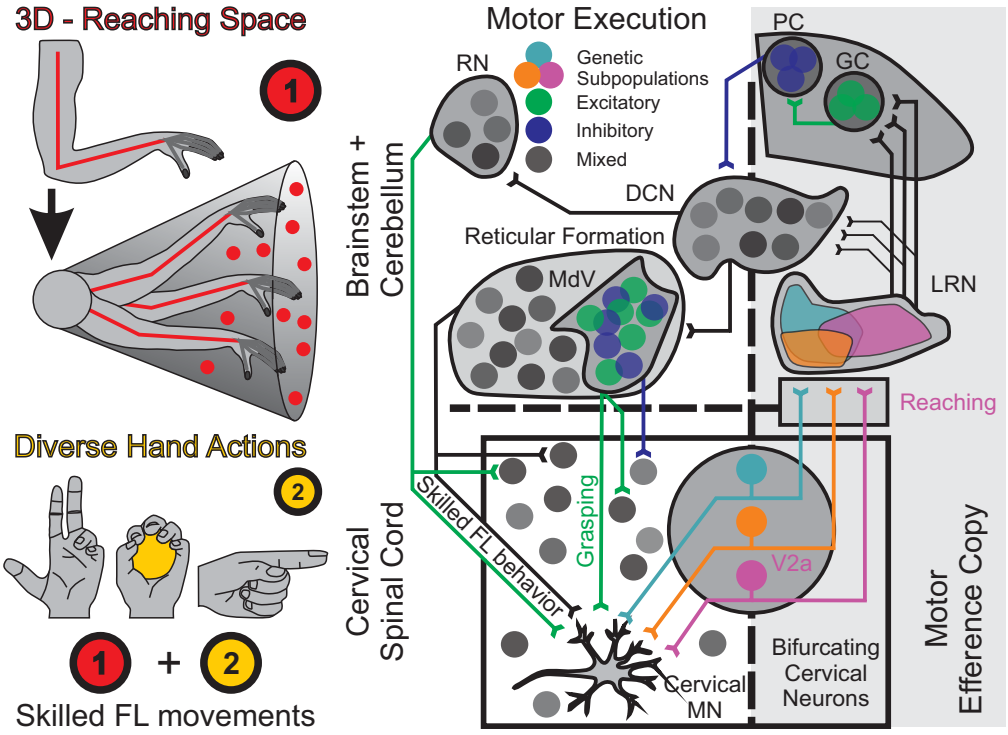

Figure 2 - Brainstem-centric view on skilled forelimb behaviors

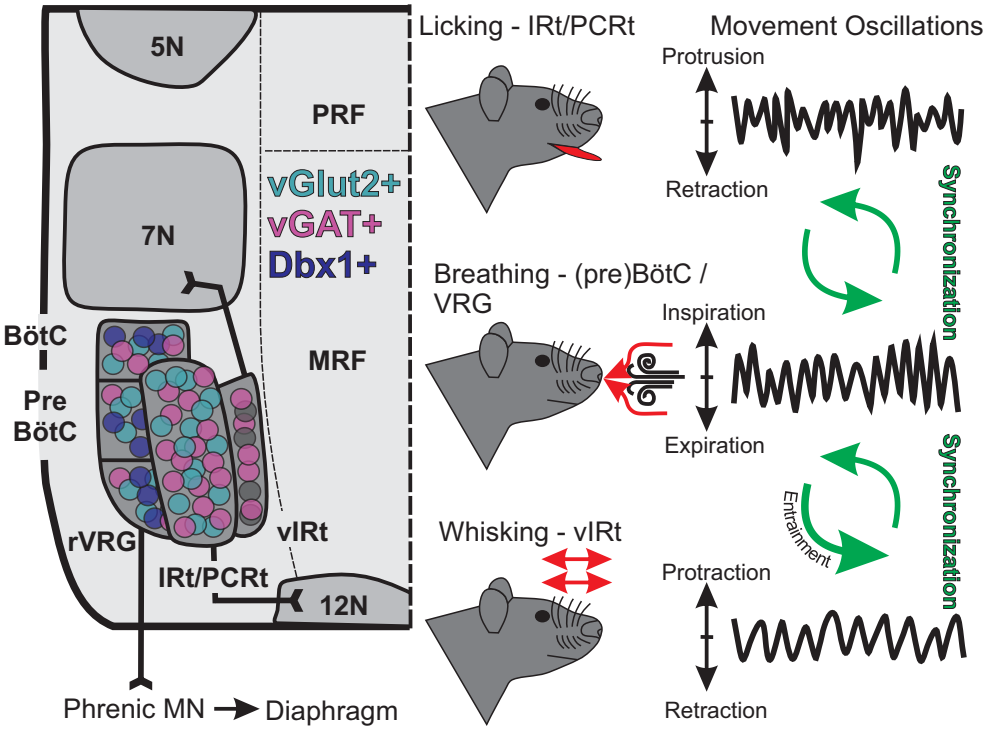

Figure 3 - Generation of orofacial and respiratory behaviors by brainstem circuits

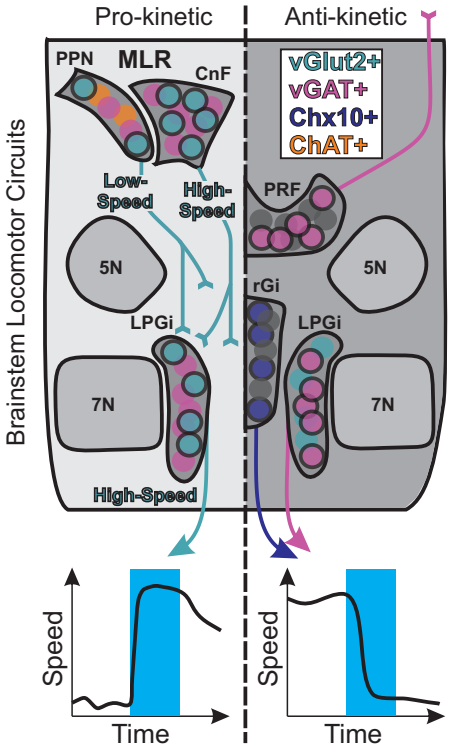

Figure 4 - Brainstem circuits for regulation of locomotion

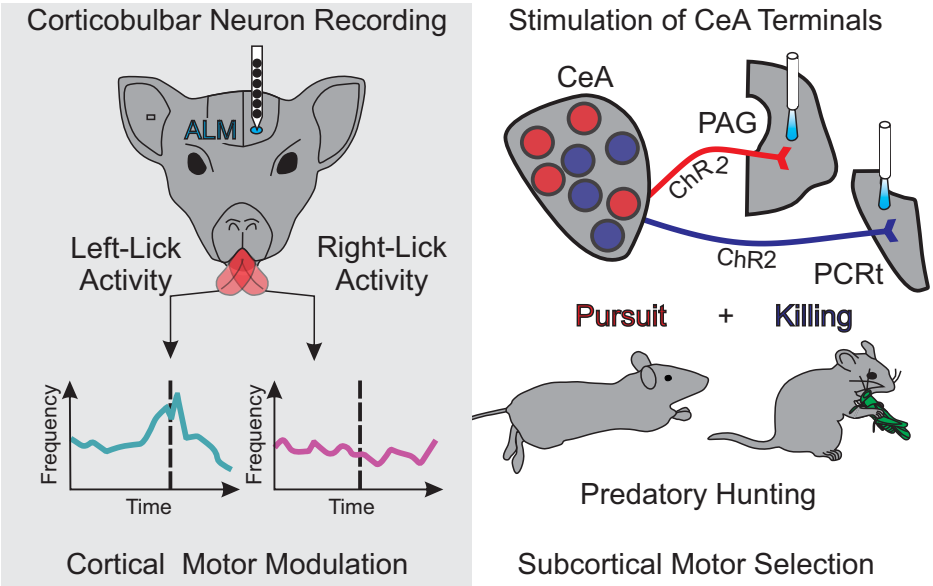

Figure 5 - Modulatory and regulatory circuits impacting on the brainstem