Organization and function of neuronal circuits controlling movement

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Movement is essential for survival and represents the final behavioral output of many computations in the nervous system. One of the most striking characteristics associated with movement is the seemingly endless repertoire of distinct actions and motor programs that our bodies can generate, raising the important question of the underlying neuronal circuit mechanisms that are at the core of regulating different forms of movement. Motor control-relevant parameters can be measured throughout the nervous system, indicating that information about movement is broadly distributed. This feature is also underscored by the fact that many diseases affecting the nervous system lead to perturbation in movement, often severely disabling affected patients.

To understand how the various sensory systems functionally assemble and process incoming information, it has been very fruitful in the past to study the organization of neuronal circuits at their first steps into the nervous system. This collective work has unraveled the high precision with which information is processed as well as the identity of involved neuronal subpopulations and their functions. In contrast, much less was known about whether and how motor output pathways at the opposite end of the nervous system follow an organizational logic at the level of neuronal circuits, and how such a circuit logic might translate into different functions in the regulation of movement. This commentary summarizes some of the work my laboratory has contributed recently to the understanding of circuit-level organizational principles within the final output pathways of the motor system, and how this anatomical work relates to functional parameters in the execution of movement.

The spinal cord as a highly organized final executive center for body movement

Motor neurons in the spinal cord are spatially organized into motor neuron pools, each innervating a distinct skeletal muscle in the periphery. Since body movement is a result of coordinated muscle contractions, it is essential to understand how different motor neuron pools are recruited in line with the biomechanical ability of the innervated muscles, a property largely determined by the identity of synaptic inputs to these motor neurons. An important question was therefore whether studying the distribution and identity of neuronal populations premotor (i.e., with direct synaptic connections to motor neurons) to functionally distinct groups of motor neurons could be leveraged to visualize and understand the organization of functionally distinct connectivity matrices in the spinal cord. Such approaches were made possible by recent technological advances on genetically modified versions of rabies viruses for trans-synaptic tracing, restricting their labeling potential to directly connected (monosynaptic) neuronal populations.

We applied this emerging technology to reveal the spatial distribution of spinal premotor neurons connected to motor neuron pools of different function (Stepien et al., 2010). In this first study, we found that premotor interneurons distribute over many segments of the spinal cord and that patterns are highly reproducible across individuals, but distinct for different motor neuron pools. After this proof-of-principle study, we asked whether distinct premotor populations regulate motor neurons responsible for control of stance (extension) and swing (flexion) phases in the locomotor sequence (Tripodi et al., 2011). We found that in the overall neuronal distribution pattern, extensor premotor interneurons in the spinal cord are located more medially than their flexor counterparts (Fig 1A), illustrating the existence of an anatomical trace correlating with motor function even at a circuit level only one step away from actual execution. Moreover, the basis for these spatial and connectivity differences is laid down during development, when postmitotic neurons giving rise to extensor and flexor premotor neurons are generated from the same progenitor domain territory but at different developmental time points (Fig 1A; Tripodi et al., 2011). In more recent work, we demonstrated that interneurons premotor to motor neurons regulating postural muscles involved in trunk stability, and with very distinct function from limb muscles, show a bilaterally symmetrical distribution in the spinal cord, while counterparts associated with the control of limb muscles are biased toward the ipsilateral spinal cord (Goetz et al., 2015). We also found that alternation of axon guidance molecules on genetically defined interneurons downstream of transcription factors can alter premotor connectivity patterns and lead to behavioral abnormalities (Satoh et al., 2016). Lastly, the majority of studies on spinal interneurons in the past and in particular the ones linking developmental transcriptional identity to locomotor function focused mostly on local interneuron circuitry (Arber, 2012). In recent work, we describe the diverse genetic identity, synaptic organization, and function of spinal neurons with long axonal projections (Ruder et al., 2016). We found that cervical neurons with long descending projections

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to the lumbar spinal cord subdivide into genetically tractable neuronal subpopulations with distinct connections, in part based on developmental origin (Fig 1B), and that the overall neuronal population plays an important role in the regulation of whole-body locomotor parameters to ensure stability of locomotion, including regulation of postural stability and speed-dependent interlimb coordination (Ruder et al., 2016). In summary, our work on the organization of motor circuits in the spinal cord reveals that understanding precise connectivity patterns and genetic identities can provide important insight into functional circuit properties in the motor output system within the spinal cord. They raise the question of whether similar principles might apply to descending pathways from the brain, a line of studies we have recently carried out and that is described in the next section.

The brainstem as modular switchboard for regulation of diverse action programs

While the spinal cord is clearly an essential component for the execution of body movements, it is well established that it cannot generate movement without external input. The most striking demonstration of this point is the observation that patients with complete spinal cord injuries exhibit paralysis of body parts regulated by spinal segments below injury. External input sources to the spinal cord include most importantly descending pathways from the brain and sensory feedback from the periphery. This commentary will solely focus on communication between supraspinal centers in the brainstem and the spinal cord and in particular address the organization of connectivity in relation to behavioral function.

Species with four limbs including humans make extensive use of their extremities, but rostral and caudal extremities show important functional differences, and these are conserved across evolution. The most striking difference is the use of forelimbs for precise manipulation tasks, a functional property almost lacking for hindlimbs in most species. Our experimental approaches to understand whether there are anatomical correlates to these behavioral differences was to uncover sites in the brain in which neurons reside with direct connections to spinal motor neurons innervating forelimb or hindlimb muscles, using transsynaptic rabies virus tracing experiments with monosynaptic restriction (Esposito et al., 2014; Fig 2A). Strikingly, we identified more brainstem subregions with neurons establishing direct connections to forelimb than hindlimb-innervating motor neurons (Fig 2A). In particular, there were three regions that showed almost exclusively connections to fore- but not hindlimb-innervating motor neurons, named MdV, PCrT, and SpV (Esposito et al., 2014). Moreover, we identified three regions with indiscriminate connectivity profiles to both kinds of motor neurons and these neurons were found in a bilaterally distributed pattern (Mc, Pn, Gi). And lastly, two brainstem regions showed higher connectivity to hindlimb-innervating motor neuron populations compared with forelimb counterparts (Ve, SpVe).

To follow up on these anatomy-based connectivity findings, we started to dissect how subpopulations of brainstem neurons are embedded in motor output pathways and what their functions in the regulation of motor behavior are. We found that within the caudally located MdV brainstem nucleus, only glutamatergic neurons establish synaptic connections to very specific forelimb muscle-innervating motor neuron pools. In functional studies, we revealed that these excitatory MdV neurons do not have a role in regulating locomotion, but that in the functional absence of these neurons either by specific ablation or by transient pharmacogenetic silencing approaches, mice were significantly impaired in carrying out a forelimb-reaching and retrieval task for single food pellets. Most notably, we traced the
Circuits for movement

essential for accurate motor behavior. In this
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2014). A second topic we studied is the
phase back to the mouth (Esposito
and no deficiency was found in the reaching
the unilateral forelimb behavior (Fig 2A),
defect specifically to the grasping phase of
the unilaterial forelimb behavior (Fig 2A),
and no deficiency was found in the reaching
phase toward the food pellet or the retrieval
phase back to the mouth (Esposito et al,
2014). A second topic we studied is the
connectivity from the vestibular nucleus to
hindlimb-innervating motor neurons (Basaldella et al,
2015). As previously found
in cats, these synaptic inputs are directed
preferentially to extensor over flexor
hindlimb-innervating motor neurons also in
mice, but within extensor motor neuron
pools, synaptic inputs are targeted in high
number to slow- over fast type motor
neurons (Fig 2B). Since this motor neuron
subclass is recruited for postural tasks, the
revealed specific connectivity matrix from
brainstem vestibular neurons thus matches
perfectly this behavioral requirement. We
found that the precision of these synaptic
conections is established during development
and requires multisensory signaling from
both vestibular and proprioceptive
sources (Basaldella et al, 2015). A third
example of a functionally dedicated pathway
was described in a recent collaborative
study, in which we identified specific
neurons in the periaqueductal gray in the
midbrain involved in the regulation of defen-
sive behavior, signaling through the caudal
brainstem nucleus Mc (Tovote et al, 2016).
Finally, ascending communication from the
spinal cord to supraspinal centers is also
essential for accurate motor behavior. In this
context, we have recently identified a
complex connectivity matrix between spinal
neurons and the brainstem nucleus LRN,
composed of distinct genetically identifiable
subpopulations with specific connectivity patterns (Fig 2C; Pivetta et al, 2014).

In summary, current evidence from our
work and recent studies by other investiga-
tors that cannot be described here due to
space limitations begin to suggest that the
identification of functionally dedicated
subpopulations in the brainstem, defined by
position, genetic identity, and connectivity,
is instrumental to understand the function of
these neurons in the regulation of motor
behavior. The emerging theme is that these
subpopulations and their associated circuitry
represent dedicated modules that are at the
core of regulating diverse motor actions
and programs. Future work will reveal how
many of these modules exist, how they
interact with each other, and how circuits
involved in competing motor programs
decide on actual behavior to be carried out.

Outlook: specific neuronal
subpopulations at the core of healthy
and diseased nervous system

The theme that anatomical connectivity
patterns prefigure behavioral function can
be used as an entry point to gain a deeper
understanding of the neuronal circuits
underlying the regulation of motor behavior,
and in particular action diversification. It
will be important to understand how motor
centers in the brainstem interact with final
executive circuits in the spinal cord to imple-
ment motor programs for body control, and
how higher motor centers involved in deci-
sion-making and action sequence generation
interact with neuronal circuits in the brain-
stem. In the long run, understanding the
healthy configuration of these circuits and
how they function will likely be very useful
for developing strategies to interfere with
movement disorders, often affecting higher
motor centers, but for which considerable
amelioration might be achieved by interfer-
ence at a level of neuronal circuits closer to
execution, including the spinal cord and the
brainstem. From our own recent work, we
found that a specific source of sensory feed-
back derived from muscle spindles is abso-
lutely essential to drive functional recovery
after incomplete spinal cord injury (Takeoka
et al, 2014). Notably, the observed func-
tional recovery processes were paralleled by
circuit reorganization in the spinal cord
establishing detour circuits across the lesion
site, and these circuit adjustments were also
impaired in the absence of muscle spindle
feedback. Together, these findings suggest
that not only for the healthy nervous
system, but also in disease or after injury, it
is crucial to understand the response proper-
ties of specific neuronal subpopulations to
gain access to key circuit mechanisms to
interfere with nervous system dysfunction. I
am convinced that future discoveries in this
direction will drive the process of develop-
ing medicines for the impaired nervous
system.
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Conflict of interest
The author declares that she has no conflict of interest.

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