Networking brainstem and basal ganglia circuits for movement

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Abstract

Execution and learning of diverse movements involve neuronal networks distributed throughout the nervous system. Brainstem and basal ganglia are key for processing motor information. Both harbor functionally-specialized populations stratified based on axonal projections, synaptic inputs and gene expression, revealing a correspondence between circuit anatomy and function at a high level of granularity. Neuronal populations within both structures form multi-step processing chains dedicated to the execution of specific movements. However, the connectivity and communication between these two structures is only just beginning to be revealed. Brainstem and basal ganglia are also embedded into wider networks, and systems-level loops. Important networking components include broadcasting neurons in cortex, cerebellar output neurons, and midbrain dopaminergic neurons. Action-specific circuits can be enhanced, vetoed, work in synergy or competition with others or undergo plasticity to allow for adaptive behavior. We propose that this highly specific organization of circuits in the motor system is a core ingredient for supporting behavioral specificity, and at the same time providing an adequate substrate for behavioral flexibility.

Introduction

Behavior is a stream of movement events bound in time. The nervous system needs to select and execute the appropriate movement in a given context at a given time, track it and adjust it instantaneously, and learn how to execute it better in the future. Critical insight into these processes will come with the identification of neuronal circuits needed to produce a particular movement with precision, as well as understanding the circuit mechanisms to commit to a movement over other possible movements, and the implementation of learning.

The brainstem is a large center involved in many physiological functions including movement spanning the hind- and midbrain. The hindbrain is the most caudal extension of the brainstem immediately upstream of the spinal cord, further divided into pons and medulla (Fig. 1a). The hindbrain transitions rostrally into the midbrain encompassing many subregions including the colliculi (Fig. 1a). To regulate body movements engaging limbs through spinal motor neurons, the brainstem is conveniently located at the junction between higher-level motor centers, spinal circuits involved in their execution and sensory feedback. The brainstem also engages in the control of other behaviors such as orofacial movements (e.g. whisking, licking) or coordination of head and eye movements, enacted through cranial motor neurons located within the brainstem proper[1-3].

One important structure providing input to many regions of the brainstem in the context of movement control is the multi-layered basal ganglia system. It spans fore- and midbrain regions and has been implicated in learning, selection and commitment of movements. Recent work primarily carried out in rodents begins to provide insight into the specific neuronal circuits that process information for the generation of different forms of movement within the brainstem and basal ganglia. The use of genetically modified animals expressing recombinases combined with application of intersectional viral tools has led to

huge progress in our understanding of the principles by which brainstem and basal ganglia are organized and function. These studies, mostly carried out in mice, reveal a high degree of cellular granularity with precise neuronal subcircuits processing information entailed with specific forms of movements and learning of these actions. They lead us to propose how basal ganglia and brainstem might be networked to process information related to movement and are embedded into systems level networks.

In this review, we will first present emerging organizational principles for brainstem and basal ganglia circuits, focusing on the elucidation of specific neuronal subpopulations and their roles in controlling different forms of body movements. We then ask how these two subsystems communicate with each other and are integrated with other networks related to movement, namely output neurons of motor cortex and cerebellum, as well as dopamine neurons, to influence movement control, selection and learning. We conclude by synthesizing emerging principles on how neuronal circuits are organized and function to support the generation of diverse body movements and phrase pertinent questions arising from these insights.

Motor functions of the brainstem

The brainstem has long been recognized as critically involved in many body functions including the control of movement. However, the spatial intermingling of brainstem neuronal populations involved in different functions has made it difficult to understand the roles and information flow in specific brainstem circuits[3-6]. Inherent to this problem is the difficulty to understand the organization and function of synaptic input and output of different brainstem neurons, and their embedding into system-wide networks. In this section, we will review recent literature on the identification of populations of brainstem neurons with different roles in the control of body movements. We will mostly focus on recent literature elucidating the circuit organization and function of brainstem neurons involved in two behaviors engaging limbs, i.e. (1) the regulation of speed and directionality of locomotion, and (2) the control of diverse movements engaging forelimbs without full body translocation.

Locomotion: speed and direction

Speed and direction are two important parameters for the regulation of locomotion[7]. Together, they determine from where to where, by which trajectory and how fast an animal translocates. Recent experiments reviewed below demonstrate that speed and direction of locomotion are regulated by distinct brainstem circuits in part residing in close proximity (Fig. 1a-c). These findings suggest that a behavior perceived as one overall action is constructed by collaboration between different information streams carried by distinct neuronal populations influencing the exact manifestation of the chosen behavior. Information from these distinct brainstem circuits is likely combined in the spinal cord, where local circuits and sensory feedback also contribute further processing towards execution.

One of the earliest insights in brain regions involved in the control of locomotion came from electrical stimulation experiments. These studies were aimed at the identification of sites

the stimulation of which elicits locomotion in cats[8]. They revealed a region in the midbrain fulfilling these criteria, since then referred to as mesencephalic locomotor region (MLR), which turned out to be evolutionarily highly conserved[9]. A series of studies provided convincing evidence that glutamatergic MLR neurons expressing the vesicular glutamate transporter vGlut2 are responsible for the locomotion-promoting activity[10-14]. Glutamatergic MLR neurons reside in three subdivisions, namely the pedunculopontine nucleus (PPN), cuneiform nucleus (CnF), and mesencephalic reticular formation (mRT) (Fig. 1b). While CnF glutamatergic neurons are required for high-speed locomotion, the role of PPN/mRT glutamatergic neurons is less clear at the overall population level[11-13] (Fig. 1b). In line with a role in the regulation of locomotion, a fraction of MLR neurons encode locomotor state and/or speed[10,11,13,15]; yet, others are preferentially recruited during distinct spontaneous behaviors including rearing, grooming or food handling[15], suggesting a more diversified role of excitatory MLR neurons in movement regulation. Recent work demonstrates that the stratification of excitatory MLR neurons by projection target helps to disentangle their functional properties[15]. While spinally-projecting PPN/mRT neurons are required for body extension such as rearing or occurring during initiation of locomotion[15] (Fig. 1b), an intermingled but anatomically segregated population projecting to basal ganglia output structures exhibits a distinct role[15], discussed later in this review. Excitatory neurons projecting to the medulla are most widely distributed in the MLR and their input to caudal brainstem circuits serves as important intermediary signaling step in the regulation of locomotion. Specifically glutamatergic neurons in the caudal medulla subregion lateral paragigantocellular nucleus (LPGi) but not neighboring magnocellular nucleus subregions are required for high-speed locomotion[14] (Fig. 1b). In addition, optogenetic stimulation of glutamatergic but not all LPGi neurons can elicit locomotion[14]. Together, these findings demonstrate that high-speed locomotion is brought about by excitatory interactions between the MLR subregion CnF and the medulla subregion LPGi, giving rise to a CnF>LPGi module needed for high-speed locomotion. Still, the identity of an analogous brainstem circuit module for the implementation of exploratory locomotion, which occurs usually at lower speeds, is less clear at present (Fig. 1b). Connectivity between brainstem modules and circuits in the spinal cord remains to be uncovered in rodents, but interestingly, work in zebrafish revealed separate spinal premotor networks for fast (escape) and slow swimming[16]. These findings suggest that interactive and possibly connected modules for speed processing might be found in brainstem and spinal cord.

Optogenetic stimulation of excitatory MLR or LPGi neurons has the striking behavioral property that one-sided stimulation elicits completely bi-lateralized, straight forward locomotion without directionality changes, unless natural barriers such as walls are encountered[10-14,17]. Therefore, unilateral locomotion signals are likely distributed symmetrically, leading to non-distinguishable motor program execution of both body halves. Furthermore, the observations that natural barriers can impede locomotion even when locomotion is induced optogenetically suggest that either parallel circuits enact this additional information to avoid barriers, or that circuits downstream of the MLR-LPGi network (e.g. in the spinal cord) can still integrate signals to influence behavior. Thus, ongoing motor programs can be stopped or “vetoed” depending on circumstances, even when movements are induced optogenetically and relatively close to motor execution.

Recent findings on other brainstem populations begin to provide insight in the elements implementing directionality of locomotion. Glutamatergic brainstem neurons expressing the transcription factor Chx10 take center stage[18,19] (Fig. 1c). Chx10 is expressed by a subset of medially located excitatory brainstem neurons in the gigantocellular nucleus (Gi)[20]. Through bilateral functional interrogation experiments, Chx10-Gi brainstem neurons were implicated in locomotion stopping behavior[20]. A later study proposed a role for these neurons in ipsilateral turning behavior based on Chx10-Gi neuron unilateral perturbation experiments[18]. A model suggests that turning towards the ipsilateral body side is achieved by unilateral activation of a stopping program, while the unperturbed side continues to move, thereby resulting in orienting curvature towards the ipsilateral side during translocation. Chx10-Gi neurons segregate into at least two populations based on their spinal targets[19]. Whereas optogenetic stimulation of cervically-projecting neurons induces ipsilateral head turning likely by direct excitation of motor neurons, lumbar-projecting Chx10-Gi neuron stimulation induces locomotion deceleration but no orienting movement[19] (Fig. 1c). Head turning was also observed upon unilateral optogenetic stimulation of vGlut2-Gi neurons[14]. Thus, an alternative model for changes in locomotion directionality could be that short-latency head turning combined with a following speed decrease allows body reorientation to align with head position. Moreover, a recent study showed the existence of locally projecting Chx10-Gi neurons with distinct *in vitro* physiological properties and connected to the long-range projecting Chx10-Gi population[21]. The *in vivo* function of these neurons is however not known. Whether or not different Chx10 populations are coactive during natural behaviors as they are during optogenetics is currently unknown. Sampling small numbers of Chx10-Gi neurons revealed that they exhibit diverse activity profiles[22]. Disentangling the proposed models for ipsiversive orienting will require a deeper understanding of Chx10-Gi neuron behavioral recruitment dynamics. Moreover, the brainstem neuronal substrates for contraversive turning also remains to be elucidated. It is likely that different forms of orienting engage distinct neuronal populations, allowing the fine adjustments during such behaviors. To achieve a broader understanding of regulation of brainstem neurons involved in orienting movements and associated postural adjustments, it will be informative to know the sources of synaptic input to these neurons. Chx10-Gi neurons, and more generally neurons in the reticular formation, receive synaptic input from deep layer neurons of the contralateral superior colliculus[18,19,23] that are a likely source to influence orientation-regulating brainstem neurons (Fig. 1c). Of note, superior colliculus neurons expressing the transcription factor Pitx2 are engaged during head turning in mice[24]. Input by midbrain sources signaling postural states may also play a role in this process[15].

Forelimb movements

While the regulation of locomotion entails the entire body and all four limbs, behaviors that preferentially engage fore- but not hindlimbs are also very common. These encompass behaviors during which an animal uses one or both forelimbs to target a source it aims to touch, manipulate, grasp and/or retrieve and can serve the purpose of interactive exploration/manipulation, food intake or body cleaning (grooming). Frequently, these forelimb movements are also coordinated with orofacial behaviors[3]. Recent work demonstrates that these forelimb-engaging behaviors are regulated by a separate set of brainstem neurons than locomotion. The lateral rostral medulla (latRM), first identified as

possible neuronal substrate for the regulation of forelimbs through its preferential connectivity to forelimb- but not hindlimb innervating motor neurons[25], plays an important role in different forelimb movements including reaching and food handling[26]. Notably, although forelimbs are used during locomotion, latRM neurons are recruited in a task-specific manner during forelimb behaviors but not during locomotion, and display a diversity of firing patterns across sampled neurons. While some latRM neurons are mostly recruited during forelimb reaching, others are activated during digit engaging food handling behaviors (Fig. 2a). At least four essentially distinct anatomical populations of excitatory latRM neurons exist, projecting to different downstream targets (Fig. 2b). The most ventrally located latRM population projects to the spinal cord and is recruited during reaching, whereas a more dorsally located latRM population is active during handling and terminates in the caudal medulla MdD region[26]. This knowledge was subsequently used to probe function through optogenetic stimulation of the anatomically defined subpopulations. Elicited behaviors were stably expressed by individual mice, suggesting that access to a specific latRM population allows the reliable expression of a defined behavior upon artificial recruitment of these neuronal ensembles. Specifically, optogenetic stimulation of spinally-projecting latRM neurons elicited unilateral forelimb reaching movements. More complex forelimb movements (reach-to-grasp, hand-to-mouth, grooming, tapping) were only observed upon stimulation of latRM populations terminating in the caudal medulla (Fig. 2b). In contrast, optogenetic stimulation of a randomly targeted subset of excitatory latRM neurons irrespective of projection target did not elicit such complex behaviors. Together, these findings suggest that recruitment of caudal medulla neurons is an important component in processing information for the generation of complex forelimb movements. Earlier work showed that excitatory caudal medulla neurons in MdV are required for grasping during a food retrieval task[25], supporting the view that downstream brainstem neurons are needed for complex forelimb movements. These studies suggest that synaptic connectivity between latRM neuron populations and specific caudal brainstem neurons represents a likely prerequisite to construct precise complex forelimb movements.

Circuit principles for body movements

The elucidation of brainstem circuits involved in the regulation of locomotion and forelimb movements showcases the involvement of intra-brainstem processing by connected neuronal populations. Neuronal stratification based on anatomy and function to a sufficient level of granularity was essential since within one small brainstem region, functionally diverse populations are often intermingled. For example, MLR neurons projecting to medulla, spinal cord or basal ganglia output structures are distinct populations, and at least four latRM populations exist based on output and function. Intra-brainstem interactions between specific neuronal populations are required to control specific attributes or parameters of the regulated behavior. Based on these findings, we propose a model in which key steps for action control occur in the brainstem with processing by dedicated brainstem populations (Fig. 2c). How much granularity exists within these intra-brainstem modules will have to be determined, but we hypothesize that a high degree of specificity and selectivity will be found.

The brainstem and most notably its hindbrain part is the most caudal region of the brain, with many populations establishing direct projections to the spinal cord[4,27]. It therefore

represents a key gateway for the regulation of body movements, and in the descending motor system the last opportunity for more rostral brain structures without direct access to the spinal cord to influence the behavioral content for execution and possibly selection of body movements. The functionally separate information streams in connected brainstem circuits both with respect to broad behavior (e.g. locomotion MLR>LPGi vs forelimb movement latRM>caudal medulla) or finer aspects of one overall behavior (e.g. speed vs direction of locomotion; different variants or phases of similar behaviors, e.g. reach and retract) generates the opportunity for upstream information streams to influence brainstem neurons differentially, by choosing some but avoiding others (Fig. 2c). Such signals likely contribute to enhancing one program over another, or in the most extreme case might even veto or block the execution of a behavior. Brainstem channels must ultimately be combined to generate output signals for behavioral execution of the body through interaction with spinal circuits (Fig. 2c). These themselves process descending and local information within highly specific interneuronal circuits before motor neurons ultimately combine all information to regulate execution through eliciting muscle contractions. This organizational principle makes brainstem circuits versatile for their use in different settings and contexts. We propose that they can contribute to behavioral flexibility by crosstalk between different behavioral programs before the signal reaches the spinal cord (Fig. 2c).

Most recent studies identifying brainstem populations relevant for movement have focused on neurons with glutamatergic neurotransmitter identity. Despite its established importance, only few studies have addressed the diversity and role of inhibition within brainstem circuits. As opposed to rostral brain areas, many glycinergic neurons exist in the brainstem, which can mediate long range communication to spinal motor neurons[28]. There is also evidence for important roles of intra-brainstem excitation-inhibition balance for orofacial behaviors[29]. When asking the question of how brainstem circuits work together and how they are influenced by upstream inputs, it will be important to consider the nature of neurotransmitter identity as well as the magnitude, specificity and spread of communication. Whether and how brainstem circuitry communicates between or within functional circuit units and via which neurotransmitters will reveal the extent to which computation and information processing occurs within the brainstem or is mostly a reflection of synaptic input to brainstem neurons (Fig. 2c). Answers to these questions will reveal whether brainstem circuits engaged in the regulation of one behavior also repress/veto and/or modulate alternative behaviors thereby contributing to action selection and/or commitment to an action, or whether these decisions occur in the upstream circuits, presynaptic to the brainstem, reviewed below.

Basal ganglia circuit organization

Work reviewed in the previous section provides evidence that specific populations of brainstem neurons are involved in different movements. These populations receive excitatory input from upstream structures including cortex and superior colliculus, likely contributing to their activation[14,18,19,25]. However, brainstem neurons also receive direct and/or indirect output from the basal ganglia, most notably from GABAergic neurons of the Substantia Nigra pars reticulata (SNr)[30,31], which itself is part of the midbrain (Fig. 3a). Basal ganglia modulation of brainstem populations has been proposed to be critical for the selection and/or commitment to different movements. A classical model suggests that for

selection and execution of specific movements, brainstem populations need coincident activation from excitatory inputs and disinhibition from basal ganglia output structures[32]. In agreement with this model, execution of saccadic eye movements in a well-trained and rewarded context is accompanied by inhibition of globus pallidus internus (GPi) and SNr inputs to superior colliculus[33] (Fig. 3b). However, this model of SNr neurons providing tonic inhibition with a pausing during movement in order to disinhibit downstream brainstem populations has not been widely tested.

Strikingly, while some SNr neurons are indeed inhibited during movement, others are excited[34-36]. This could be explained by the fact that SNr neurons can in principle target both glutamatergic and GABAergic brainstem neurons, and thereby excited neurons might still disinhibit downstream motor centers. Another possibility is that this phenomenon reflects a center-surround circuit mechanism (Fig. 3c). In this center-surround model, inhibited SNr neurons target specifically brainstem populations required for execution of a selected/desired movement, while activated surrounding SNr neurons project to other regions to inhibit competing/non-desired movements[37] (Fig. 3c). Indeed, at the population level, SNr neurons inhibited during locomotion are predictive of locomotion initiation, whereas excited neurons are not[35]. Several conditions should be met for this model to be generally applicable to SNr output signaling. The first is that to convey action specificity, different SNr populations should project to distinct brainstem areas associated with the control of these movements, and not broadcast generally to all downstream areas. The second is that inputs to SNr neurons should also exhibit specificity. For example, SNr neurons inhibited during a particular movement should get specific inputs from the striatum related to the execution of that particular movement, but surrounding SNr neurons should get different input and not be inhibited. We will next describe experimental progress on both topics.

SNr output channel organization

Recent work demonstrates that indeed distinct SNr populations target different regions within the brainstem (Fig. 3d). SNr neurons labeled retrogradely from each of seven major brainstem areas (medullary and pontine reticular formation, three medio-laterally distinct regions of the superior colliculus, the inferior colliculus and the dorsal Raphe) exhibit sparse collaterals between each other (Fig. 3d), suggesting that parallel SNr channels targeting different brainstem regions[38]. Within the SNr, these different populations are found in striking spatially organized patterns. While more anterior-lateral SNr areas project to different areas of the superior colliculus, more posterior-medial areas project to caudally located brainstem regions in the pons and medulla (Fig. 3d). SNr subpopulations also exhibit different electrophysiological properties, depending on projection target. More lateral SNr neurons are large, fire fast and have fast membrane time constants, whereas more medial SNr neurons have slow firing rates and decay constants[38]. Lateral and medial SNr neurons also have been proposed to exhibit different functions, with more lateral neurons involved in movement and more medial neurons in states of low motor activity and low arousal, such as sleep[39]. Although different SNr populations preferentially target some brainstem regions, they all send collaterals to the thalamus and the midbrain areas including the PPN and midbrain reticular formation, in part contained within the MLR (Fig. 3d). However, different SNr channels exhibit spatial segregation within the thalamus, suggesting their integration

into spatially close but distinct neuronal loops. Superior colliculus projecting SNr neurons terminate in more lateral domains of the thalamus, while pons and medulla-projecting SNr neurons target medial Pf and VM domains[38]. These findings demonstrate that SNr subpopulations targeting distinct brainstem regions collateralize to specific thalamic areas. This likely leads to concomitant regulation of brainstem and thalamo-cortical circuits involved in the same movements, thus making these output channels specific to brainstem targets and specific regions of the thalamus. In addition, collaterals targeting thalamus might also serve to convey efference copy signals of to-be performed movements to thalamus and cortex, and this information is used for learning[40]. It is possible that SNr populations also target functionally distinct neurons in the MLR region. Previous work suggested that SNr inputs to MLR can influence postural adjustments and locomotion parameters[41]. Taken together with the recent findings on MLR subpopulations[15], it is conceivable that this might be achieved through specific interactions with spinally projecting MLR neurons implicated in posture, and medulla-projecting neurons functionally linked to locomotion. This uncovered specificity of SNr output channels will allow for testing the model of how SNr interacts with its different targets to regulate shifts in movement, for example how SNr would regulate specific brainstem modules involved in locomotion (speed and direction) versus skilled forelimb movements described above.

Input organization to SNr

To address how different SNr subpopulations are recruited during behavior, understanding identity and organization of synaptic inputs to the SNr is of key importance (Fig. 3d), since careful balancing of excitatory and inhibitory inputs to SNr neurons determines their functional properties. Major inhibitory inputs to SNr neurons are derived from the striatum and globus pallidus externus (GPe), while excitatory inputs are provided by the subthalamic nucleus (STN) and Rbp4-transgene positive neurons of the MLR (Fig. 3d). Rbp4-transgene positive MLR neurons are located in the mRT medially adjacent to the PPN and are distinct from the descending excitatory MLR population[15] (Fig. 1b). They are preferentially active during the forelimb-behaviors like handling and grooming, but not during rearing and locomotion. Optogenetic interference experiments suggest an important modulatory role for these neurons in a variety of body movements[15]. It is currently not known whether Rbp4-MLR neurons target specific subpopulations of SNr neurons, but such information is already available for other synaptic inputs to the SNr.

Notably, striatal inputs to basal ganglia output populations are spatially segregated and carry distinct functional information (Fig. 3e). Different striatal regions target distinct SNr domains[42], with dorsolateral areas of the striatum targeting more lateral SNr, and ventromedial striatal areas terminating in the more medial SNr. Extending this view, two recent studies found that the striatum connects to basal ganglia structures through a highly organized matrix using highly localized injection strategies[43,44]. Specifically, dorsomedial (DMS), dorsolateral (DLS) and ventrolateral (VLS) striatum target different SNr and GPe subregions, and through this specific input pattern, maintains fine topography with respect to brainstem and thalamic areas targeted by striatal-input SNr recipient neurons[43] (Fig. 3e). A finer map was revealed by the second study [44], also demonstrating organizational differences between how the striatum communicates with SNr and GPe. While a highly spatially segregated map seems to exist in GPe, convergence of some input channels to SNr

was observed [44]; the functional implications of this are currently unknown. Perhaps related to this, even when DMS and DLS provide input to SNr neurons projecting to the same area of the superior colliculus, these populations target different layers, suggesting functional differences in the targeted downstream network[43]. To probe the model of differential function carried by specific pathways, optogenetic stimulation experiments provide first insights. Stimulation of striatum-SNr projection neurons in VLS, a region previously implicated in orofacial movements[45,46] induced licking, while stimulation of analogous neurons in DLS, previously implicated in body movements[47,48], modulated body turning[43] (Fig. 3e). These observations are consistent with the fact that DLS recipient SNr neurons include projections to the brainstem region Gi implicated in directional control[18,19], and VLS recipient SNr neurons also target medulla centers implicated in orofacial movements[2]. How similar functional linkage for other body movements including locomotion and skilled forelimb movement between striatum, SNr and downstream motor centers is implemented remains to be determined (see also below) but the known anatomical roadmap provides valuable entry points. Interestingly, segregated basal ganglia circuits also form segregated closed-loops with thalamus and cortex[43,49], indicating that basal ganglia output pathways can modulate their own input and suggesting that specific movement-relevant information is propagated within wider networks and not just transmitted to brainstem centers for execution.

Insight into the overall anatomical organization of the striatal projectome raises the question of whether within one striatal region, additional diversity exists, which might contribute to understanding the functionality of the mapped pathways in the future. Interestingly indeed, even within a confined striatal region, there can be a high degree of granularity in terms of somatotopy and movement representation[47], which might not be reflected in optogenetic stimulation experiments. Recent work imaging hundreds of striatal projection neurons in DLS while mice performed different body movements including locomotion, directional changes, and rearing revealed that distinct neuronal ensembles were active during different movement patterns, and that single striatal neurons exhibit a rather high degree of movement specificity[50] (Fig. 4a). Furthermore, neurons in DLS encode detailed task-related movement kinematics[51]. GABAergic striatal projection neurons (SPN) also divide into two major groups based on their targets (Fig. 4b, c). The striatum can modulate the activity of SNr via direct (dSPN) and indirect (iSPN) projection neurons. dSPNs have direct synaptic connections with SNr neurons, and express D1-type dopamine receptors (Fig. 4b), which positively modulate neuronal excitability. In contrast, iSPNs can only influence SNr neurons indirectly through GPe and STN, and express D2-type receptors (Fig. 4c), which negatively modulate neuronal excitability[52]. These findings led to the classical postulation that dSPNs are active during movement to directly inhibit basal ganglia output neurons and hence disinhibit downstream motor centers, while iSPNs are inactive during movement[53,54]. In line with the center-surround model of SNr function posited above, it has also been proposed that dSPNs disinhibit downstream circuits important for the execution of the desired movement, while iSPNs would actively inhibit unwanted movements[37,55]. Interestingly, both dSPNs and iSPNs in DLS are active during movement[56,57] and their activities are movement-specific[50,58]. Imaging of single neurons revealed that most dSPN or iSPN neurons are active during a specific behavioral cluster, and show similar movement-specificity[50] (Fig. 4b, c). Simultaneous fiber-photometry recordings of both populations also revealed specific dSPN versus iSPN activity patterns during the execution of

particular behavioral motifs[58]. The finding that iSPNs are movement-specific may seem contradictory to the idea of center-surround where their function would be to suppress competing movements. However, the timing of their activation may be important since *in vivo* patch-clamp membrane potential recordings from dSPN versus iSPN revealed that these neuron types have different timings of depolarization at millisecond timescale[59]. Furthermore, one should consider that while dSPN activity reaches SNr directly (Fig. 4b), additional information can be integrated within the indirect pathway in the intermediate basal ganglia processing stations GPe and STN before it reaches the SNr (Fig. 4c). These additional processing steps can add heterogeneity to the information streams of the indirect pathway. Moreover, recent studies suggested that fine topography between striatal regions and GPe appears to be maintained, whereas more convergence seems to be evident in direct striatal input to SNr[44]. Therefore, to better understand how action-specific striatal information is propagated within basal ganglia circuits, it will be fundamental to characterize neuronal activity in different regions of the indirect pathway. Interestingly, different domains and/or cell types in GPe and STN exhibit different motor functions, even in humans[60-63]. Furthermore, GPe, but not SNr, sends projections back to striatum[64-67] (Fig. 4c), allowing for further processing. Finally, GPe is itself an output region, directly projecting to cortex[68] and thalamus[69] (Fig. 4c). Thus, iSPNs have the opportunity to disinhibit circuits outside the basal ganglia by inhibiting GPe projections to downstream circuits outside basal ganglia, just like dSPNs. Furthermore, it should be considered that even in the classical case where activity of iSPNs leads to excitation of SNr neurons, SNr inhibitory output neurons can also target inhibitory neurons in the motor centers, leading to disinhibition that way. These recent studies therefore call for careful characterization of the so-called indirect pathway, and also of the activity of basal ganglia projection populations to different motor centers. Several studies attempted to determine whether the functional specificity of dSPNs and iSPNs is also reflected at the gene expression level by analyzing gene expression profiles. Interestingly, the core molecular features of striatal projection neuron identity seem to be conserved across all of striatum, but in addition, the expression of some genes varies across striatal areas or residence in the subcompartments striosome or matrix[70].

The circuit organization described above suggests a model in which neurons in different processing stations of the basal ganglia exhibit specific anatomical organization patterns to communicate with downstream components with high selectivity. To what extent these pathways themselves exhibit further granularity aligned with their function in behavior, collaborate during execution, and are malleable during learning is currently unknown. In many situations, it is important to simultaneously select or disinhibit two or more movement components to perform an action, for example locomoting and re-orienting at the same time. Therefore, the same circuit organization must accommodate synergistic movements to co-occur, by not only separating but also mixing different motor program components, depending on the context.

Systems-wide processing for movement

A common principle to bind subsystems into nervous system wide networks is the elaboration of axon collaterals to communicate information to more than one target. These systems-wide processing channels permit the integration and adjustment of movement plans contained in the different information steams, and also allow the nervous system to

learn to repeat and refine movements. Accordingly, the brainstem and basal ganglia are embedded in communication and integration pathways able to coherently and constantly distribute relevant information throughout the nervous system. We will therefore next review circuit mechanisms by which subsystems are bound into systems-wide motor networks to control ongoing movement, movement planning and learning.

Circuit elements in motor output pathways frequently establish collaterals to other regions thereby distributing and broadcasting the intent of upcoming plans and the outcome of past actions. These communication channels can connect to movement and/or sensory information processing centers. The best understood example of this kind are cortical neurons, with corticospinal neurons collateralizing to many regions as they project to the spinal cord[71,72] (Fig. 5a). Sensory pathways assemble information about already executed movements that may contain the need to update motor plans and they also convey external information that can deviate motor plans towards other actions. The cerebellum plays a key role in the processing and integration of these different information sources[73], conveyed to the rest of the nervous system by cerebellar output channels. Furthermore, dopaminergic neurons project widely to different nodes of the system to modulate movement and learning. In this section, we will focus on recent literature unraveling the principles by which these three highly-communicative systems collaborate with brainstem and basal ganglia circuits to compute systems-wide information for execution and learning of movements.

Motor cortex output organization

Motor cortex is a large structure communicating widely within the central nervous system, and includes cortical regions involved in motor execution and/or planning. The precise motor cortex region under study matters tremendously since the subcortical projection territory can differ significantly between cortical sites. This in turn has important implications for cortical function in motor cortex subregions. The striatum is a major target of cortical projection neurons. Early work using transsynaptic viruses provided evidence that different cortical areas project to distinct striatal regions[42] (Fig. 5b). A systematic study with many different injection sites demonstrates the high spatial precision with which different cortical subregions communicate with the striatum[74]. Studies performing simultaneous recordings from cortex and striatum reveal that striatal activity topographically reflects deep layer cortical activity[75]. But how granular is this connectivity? Do motor cortex neurons that encode a particular movement preferentially contact striatal neurons that encode that same movement? Striatal neurons active during the same movement (i.e. belonging to the same movement-related ensemble) have higher zero-lag cross-correlated activity between them than neurons not active during the same movement, even in periods where that movement is not performed[50]. This suggests that there is more functional connectivity between neurons that belong to the same movement-related ensemble than across ensembles (Fig. 5b). However, although striatal projection neurons are interconnected, positive cross-correlated activity cannot emerge from lateral connectivity given that SPNs are GABAergic. SPNs are relatively hyperpolarized and require substantial glutamatergic inputs to be activated. It is therefore likely that the observed correlation between SPNs in the same movement-related ensemble emerges from common input, i.e. that they receive glutamatergic input from cortical or thalamic ensembles active during that same movement[76]. Therefore, even within a defined striatal region, different striatal

subcircuits/ensembles likely receive specific glutamatergic inputs that encodes particular movements at a high level of granularity (Fig. 5b). This input can be cortical, but also from other sources[51], like for example, thalamus. Based on these studies, we posit that striatal action-specificity reflects highly organized excitatory input into the striatum.

Information on the extent to which different motor cortical regions organize their synaptic output to target regions closer to motor execution including spinal cord and brainstem is currently less well understood. Recent work demonstrates the differential use of motor cortex areas in behavioral phases of a forelimb-guided food pellet retrieval task[77]. Whereas a caudal motor cortex area terminates preferentially in a dorsal domain of anterior cervical spinal segments and is engaged in the forelimb reaching phase, a more rostral motor cortex area targets a shifted ventral domain all the way into caudal cervical spinal segments and is engaged during food pellet grasping[77] (Fig. 5c). Premotor interneurons connected to extensor or flexor motor neurons respectively are specially segregated along the medio-lateral axis (Fig. 5c) [77,78]. Moreover, neighboring orofacial motor cortex areas communicate through distinct pathways to brainstem premotor neurons that regulate motor neurons involved in different aspects of orofacial behavior[79]. Anterior lateral motor cortex (ALM) signals submovements of the tongue, and is critical for proper tongue control[80]. In addition, primary tongue-jaw motor cortex (tjM1) is also essential for contralateral licking and its pre-stimulus neuronal activity predicts lick direction[81]. These cortical pathways regulating orofacial behaviors likely work through brainstem circuits, as has been demonstrated for whisker motor and somatosensory cortex[82]. However, *in vivo* recordings from spinal or brainstem neurons has been technically challenging, making it difficult to measure the impact of cortical activity on neuronal tuning to the extent we know for SPNs. Future work will also reveal the full extent to which a mapping between cortical location and subcortical targeting specificity exists. It will need to address the functional impact of cortical neurons on targeted neurons and the contribution of these inputs to the generation, modulation, or learning of movements.

Recent molecular and single neuron reconstruction approaches have contributed tremendously to revealing the enormous diversity of motor cortex neurons with subcortical projections, providing an additional dimension of insight. Early work already demonstrated that single motor cortex neurons can establish axon collaterals to many parallel target regions[72] (Fig. 5a), establishing the principle that long-range descending motor cortex neurons can in parallel target basal ganglia, superior colliculus and brainstem. Two-photon block-face imaging and semiautomatic neuron reconstruction took this approach to a different level by revealing the morphology of over 1000 neurons including in the motor cortex[83]. Intratelencephalic (IT) neurons reside in upper layer 5 and represent a very diverse group of neurons. While most project bilaterally to the striatum, their targeting and axon elaboration in other cortical regions is highly variable. Neurons with projections beyond the telencephalon are frequently referred to pyramidal tract (PT) or extra-telencephalic (ET) neurons, and these divide into populations as well. Two molecularly distinct layer 5b neurons[84] are upper layer 5b PT neurons projecting to thalamic targets[83,84] in addition to some extra-thalamic targets[83], and lower layer 5b PT neurons with projections to brainstem and spinal cord but not to thalamus[83,84]. Layer 6 thalamus-projecting motor cortex neurons project almost exclusively to the thalamus but in a more widespread fashion than layer 5b PT neurons[83]. Interestingly, the neuronal activity profiles of the anatomically distinct

neurons also differs[84], suggesting that they likely exhibit distinct functions in controlling behavior. Interestingly, recent work also suggests that subpopulations of corticospinal PT neurons that target different spinal neurons also send preferential collaterals to different forebrain neurons, namely striatal dSPNs versus iSPNs[85]. In a series of recent collaborative papers from the Brain Initiative Cell Census Network (BICCN), a wealth of information on motor cortical cell types has been published, data beyond the scope of this review, and interested readers are referred to synopses of these studies[86,87]. In summary, while motor cortex neurons with subcortical projections as an entire population communicate with many targets, division into anatomically distinct subpopulations based on differential targets and/or molecular entry points is now becoming possible, and this knowledge will be instrumental to understand control and learning of diverse movements.

Cerebellar output pathways

The cerebellum integrates many information sources needed for the control of movement[73]. To understand how the cerebellum impacts motor function through interactions with system-wide networks, studies on deep cerebellar nuclei (DCN) have provided important information. These studies begin to determine the organization of DCN with respect to neuronal diversity by assessing cell body location, axonal projections, gene expression and electrophysiological properties of neurons[88,89]. Mammals contain three DCN arranged bilaterally along the medio-lateral axis, with the medial (Med) DCN representing the evolutionarily oldest structure, followed by the interposed (Int) and lateral (Lat) DCN positioned progressively more laterally (Fig. 5d). Additional subdivisions along the three anatomical axis are discernable within each of these regions, making anatomical and functional studies challenging.

To determine the extent of direct interactions between DCN and the rest of the central nervous system, systematic anterograde injections were carried out across all three[88] or Med[89] DCN respectively. For all DCN, 125 ipsilateral and 140 contralateral CNS-targets were identified[88] (Fig. 5d), while Med DCN projects to overall 60 targets[89]. In line with the evolutionary divergence, Int and Lat DCN targets were more similar to each other than Med DCN counterparts. In addition, the evolutionarily youngest Lat DCN does not provide input to the spinal cord, while Med and Int DCN do[88,89]. Gene expression analysis from databases or transcriptome profiling provided molecular entry points to stratify DCN neurons and these data also suggest a high degree of organizational specificity. While three inhibitory neuron subtypes are conserved across the three DCN divisions, glutamatergic neurons making up the majority of widely-projecting DCN neurons exhibit a high degree of molecular diversity[88] (Fig. 5d). Interestingly, 15 molecularly distinct glutamatergic neuron subtypes map onto specific DCN subregions[88]. Moreover, these 15 subtypes segregate into large Class B neurons with higher spontaneous firing rates than smaller Class A neurons (Fig. 5d), categories that were found in all three DCN[88] and also identified specifically in the Med DCN[89].

To address how DCN output influences motor control, we will next review some recent exemplary functional studies in mice beginning to dissect subtype function in Int DCN. *In vivo* recordings from IntA DCN neurons during a food pellet reaching and retrieval task showed preferential recruitment of some neurons during the phase approaching the

reaching endpoint[90]. In agreement with a role of IntA neurons in defining reaching endpoint precision, closed-loop optogenetic stimulation experiments resulted in opposite phenotypes, with endpoint positions premature for activation and overextended for inhibition[90]. Functional perturbations of a large-diameter IntA subset defined by a BAC transgenic line also points to a role of these neurons in reaching endpoint targeting[91]. Together, these findings suggest an adaptive impact of IntA DCN neuron activity on achieving the targeting of a stable endpoint during reaching.

Another study used retrograde targeting to stratify DCN neurons and found that subpopulations of ipsilateral IntA and contralateral IntP/Med neurons project to the cervical spinal cord[92]. The two groups of excitatory DCN neurons exhibit different roles in movement control as determined by chemogenetic silencing. While the ipsilaterally-projecting population is needed for successful food pellet retrieval through functioning in accurately engaging with the target, the contralaterally-projecting population functions in learning but not execution of the accelerating rotarod task. To what extent the small spinally-projecting IntA subpopulation overlaps with neurons targeted in the above-mentioned studies[90,91] is currently unclear. Spinally-projecting DCN populations can also establish axonal collaterals to the thalamus and brainstem[92], demonstrating that even a small DCN population can broadcast information to multiple downstream regions. The neuronal substrates through which the behavioral functions described in the here reviewed studies[90-92] are achieved are currently unknown.

Are there general principles that can be discerned based on these data? Although we understand far less about DCN than cortical subpopulations, the reviewed data begins to suggest that DCN neurons fractionate into many different subtypes that also have distinct behavioral roles. It is also likely that DCN neurons broadcast information content to multiple target structures simultaneously (Fig. 5d), but anatomical data at single cell resolution is lacking. One important question for the future is in how far different DCN subregions, or neuronal subpopulations within DCN that cannot be dissociated by location, project to overlapping versus separate target regions within a broader target structure or across different output regions. Previous analyses revealed that axonal targeting territories are likely differential within a broad structure, with shifted termination zones elaborated by different DCN, subregions or subpopulations thereof[88,89] (Fig. 5d). Interestingly, one study suggests that the motor effects elicited by DCN signaling might depend on appropriate receptivity of downstream targets according to behavioral context[90]. In a model with varying receptivity of target regions as gate keepers, the broadcasted information might therefore be heard and acted upon only by a subset of targets. This in turn could result in highly selective functional readouts postsynaptically during different motor behaviors or contexts from one population reaching out to multiple distant targets. Therefore, understanding how diverse DCN inputs within their target structures are integrated during movement, also in collaboration with other intersecting information streams, is an important avenue to pursue.

Dopaminergic modulation of movement

Neuromodulatory systems also project widely in the brain and modulate the activity of motor circuits. Dopamine is notably involved in both movement and motor learning.

Dopamine neurons (DANs) in the substantia nigra pars compacta (SNc) project mainly to dorsal and lateral striatum, while dopamine neurons in the ventral tegmental area (VTA) project mainly to medial parts of striatum and also to cortex (Fig. 5e). Dopamine released from these terminals modulates excitability of SPNs and the plasticity of their cortical inputs[93]. Interestingly, although DANs mainly in VTA and medial SNc encode a reward-prediction error, many DANs in lateral SNc are transiently active during movement initiation[36,94,95] (Fig. 5e). Furthermore, even with SNc, movement- and reward-related SNc DANs appear to be different subpopulations. The activity of lateral SNc DANs is critical for movement initiation[94,95], and modulates the vigor, for example the speed, of upcoming movements[94-96]. However, distinct from what is observed for motor cortex and striatum, the activity of movement-related DANs is not movement-specific[95], and most single neurons are significantly active during initiation of a variety of different movements (Fig 5e).

What does this mean for the role of SNc neurons in movement control and choice? DANs do not seem to convey information about which movement to perform to striatal projection neurons, but rather whether or not movement should be performed at a particular moment in time, and how vigorous this movement should be (Fig. 5e). Information about which movement to perform would arrive to striatal neurons via specific glutamatergic input including the cortex or thalamus. Coincident specific glutamatergic and generic dopaminergic inputs into striatal projection neurons would result in the selection of a specific movement-related ensemble and consequential disinhibition of the appropriate downstream motor circuits. Accordingly, depletion or elevation dopaminergic tone across all cells dramatically affects the size of movement-related striatal ensembles that respond during a particular movement[97]. Dopamine signaling can also change the movement-correlation and the functional connectivity of striatal ensembles[98], suggesting that dopamine influences both magnitude and specificity with which the striatum communicates to its targets.

Systems-level network collaborations

These findings on the organization and function of widely-projecting neuronal populations in the motor system raise the question of how they integrate with subsystems and generate linkage with specific synaptic subcircuits. It is also interesting to ask how these systems-level networks are used during execution and learning of movements. Work using polysynaptic rabies viruses in monkeys suggests that indeed highly organized loops exist that bind subsystems into coordinated networks in very specific ways[99]. Recent work implicates the DCN-thalamus-cortex axis in motor preparation. For learned dexterous forelimb movements, continued thalamic input is required for cortical dynamics to unfold during execution[100]. In a whisker-based tactile discrimination task linked to a delayed directional licking motor response for reward, not only implicated cortical neurons within ALM are recruited and needed, but also the thalamic regions linked to ALM (ThalALM) show analogous properties. Strikingly, bidirectional connectivity between ThalALM and ALM is instrumental for the emergence of preparatory activity including spike rates and neuronal selectivity[101] (Fig. 6a). ThalALM receives input from multiple brain regions, including DCN (Fig. 6a) and midbrain motor centers, making it possible that these inputs contribute to the activity of the recurrent ALM-ThalALM network. Indeed, Med DCN neurons also exhibit preparatory activity and are required for correct behavioral choice from the late sample

throughout the delay period[102]. Consistently, perturbation of ALM activity also affected Med DCN activity[102]. Another study demonstrates that Lat DCN neurons are instrumental for the generation of preparatory activity in ALM neurons in a visually-guided virtual reality task[103]. Moreover, Lat and Int DCN input to ventral anterior-lateral thalamus subdivisions passing to CFA motor cortex convey a movement initiation signal for a specific trained lever pull task[104]. Stimulation of this pathway can substitute for the task go signal. Interestingly, when stimulated outside the precise behavioral context, the trained lever push movement cannot be evoked reliably, but instead variable forelimb movements are generated[104]. Also, the precise DCN and interacting recipient thalamic neurons are key for the type of movement that can be generated since no tongue movements could be elicited by this manipulation. Overall, the impact of this pathway critically depends on the context of the particular task, whereby the movement initiation timing signal can only correctly engage cortical circuits jointly with other inputs. In line with the idea that looped systems are involved in learning different aspects of motor tasks, coherence of neuronal activity between motor cortex layer 5 neurons and cerebellar granule cells in a learned forelimb choice task crystallizes only over learning[105], also suggesting that synaptic plasticity is involved in shaping the specificity of engaged circuits (Fig. 6a).

Dopamine input to striatum and cortex also modulates learning. Learning is accompanied by an increase in synaptic strength at cortical-striatal synapses[106], and by a scaling of corticostriatal interactions[75,107,108] (Fig. 6b). Plasticity at cortico-striatal synapses is necessary for mice to learn to select the particular behavioral pattern that leads to reward[109], and for the brain to select which neuronal pattern to repeat to obtain rewards[107,110]. The granularity of the organization between cortex, striatum, SNr and brainstem discussed above suggests that dopamine-dependent plasticity at cortico-striatal synapses permits the brain to learn which output populations in the brainstem and the cortex, - i.e. which motor behaviors - should be disinhibited or selected in particular contexts. We hypothesize that a change in synaptic strength from a particular cortical movement-specific ensemble onto the corresponding striatal movement-specific ensemble would increase or decrease the probability of disinhibiting the corresponding circuit in the brainstem or cortex (through re-entrant loops via thalamus), and hence the probability of executing a particular movement in a given context. But although learning usually starts with a fast increase in the probability of performing the same gross movement, it is followed by a slower phase of refinement of the movement[111,112]. The time course of emergence of coordinated activity between cortical and striatal circuits matches the learning of the gross movements[111] (Fig. 6b). Inactivation of both motor cortex and DLS impaired the performance of gross movements. However fine movements seemed to rely more heavily on motor cortex[111]. Accordingly, spine remodeling and synaptic scaling in motor cortex accompanies skill learning[113,114], and dopamine-dependent plasticity in motor cortex is necessary for learning fine movements[115] (Fig. 6c). These findings are consistent with models proposing that dopamine-dependent cortico-striatal plasticity rapidly increases the probability of re-entering a specific neuronal pattern in brainstem or cortex via thalamic loops (Fig. 6b), but that the gradual refinement of the pattern selected also requires cortical plasticity[112](Fig. 6c). It will be interesting to consider a role of cerebellar-motor cortex loops, via cerebellar recipient thalamus, in this gradual cortical plasticity (Fig. 6c). Loops via basal ganglia or cerebellar recipient thalamic regions[116] could mediate different aspects of learning.

In summary, the activity perpetuated within the cerebellar-thalamo-cortico-basal ganglia network and its influence through dopamine appears to be critical for preparation and learning of motor tasks, but loops are likely highly specific depending on the task. It will be interesting to determine whether frontal networks are employed preferentially during learned and cognitive tasks involving periods of preparatory activity, and perhaps more proximal and faster DCN output pathways are used for the regulation of more spontaneous movements and online adjustments.

Conclusions and Outlook

The reviewed work provides evidence that circuits of the motor system are connected into highly organized systems-wide networks. These networks support the execution and learning of motor behaviors, and have the ability to generate many different movements, react flexibly to adjust to feedback, contexts or changed circumstances. We conclude our review by summarizing emerging principles based on these results and we formulate perspectives and open questions arising.

An important finding is that functionally distinct neuronal populations can reside in close proximity to each other yet be embedded into different and highly specific circuit modules (Fig. 7a). These spatially close neuronal populations can however frequently be stratified based on gene expression, axonal projections and/or connectivity into circuits. This knowledge allows to study functionally distinct populations and generates a better understanding of motor circuit organization and function. Previous data provide evidence that big structures (e.g. cortex, thalamus, striatum) contain subregions that are highly organized with respect to synaptic output. Based on new insights, we have to consider that within smaller structures (e.g. subregions of thalamus, DCN or brainstem), functionally diverse neurons contribute to the generation of precisely connected circuits as well. We propose that employing a strategy of information processing encompassing multiple steps contributes to the generation of behavioral diversity and flexibility (see also Fig. 2c). In this view, every processing step in a chain is not a simple relay station but allows the integration of specific information flowing into an interconnected circuit at variable steps. Such connectivity chains can be found at relatively short distance (e.g. within the brainstem), but also at longer distance (e.g. striatum to SNr to brainstem), sometimes even leading to the generation of loops (see Fig. 6).

Information about movement is processed through systems-wide networks. We have reviewed work demonstrating that some brain regions projecting to highly diverse targets can still employ a high degree of target specificity, with different neuronal populations projecting to separate target structures (Fig. 7b). This strategy theoretically allows for different information to be distributed to different targeted structures. In contrast, some neurons use the principle of broad axon collateralization to many targets (Fig. 7b). This strategy can allow connected targets to receive the same information, which is useful to simultaneously inform distant regions. However, even if a neuron with many targets broadcasts the same information to these targets, not all connected neurons are necessarily equally receptive to the emitted signals. Therefore, the broadcasted information might impact on distinct targets differentially. Neuronal recruitment depends on identity and biophysical properties of all inputs (including neuromodulators) and intrinsic neuronal

excitability. These features are not restricted to the motor system - in the visual cortex, stimulus-specific response amplification occurs through preferentially connected microcircuits[117] and in the hippocampus, excitability is an important contributor to representation[118,119]. Thus, communication by axon collaterals of the same population can promote both synchronous or asymmetric information flow, which is more difficult to achieve with many separate neuronal populations.

Another important point to consider is input integration at the target site, for which the precise location and functional identity of connected neurons must be considered. As we begin to understand the details of nervous system organization, we must characterize convergence and divergence of different inputs in the target structures at the fine level (Fig. 7c). Some regions receive convergent input from two or more sources, while neighboring regions only process a subset of these inputs. This is exemplified within the thalamus, where cerebellar and SNr input form specific zones and these domains even vary across species [5,116,120,121]. Even within a more circumspect region of thalamus, there seems to be a topographic organization of SNr inputs[38]. Thus, thalamic neurons have the potential to listen to one or more conversations, which has important consequences for function and flexibility. To what degree such circuit organization also exists in other regions is beginning to be elucidated. This organizational principle, however, comprises a high potential to support movement diversity and flexibility, to adjust circuit function during both execution and learning of movements. Using this strategy, different information sources can be precisely mixed and matched (see also Fig. 2c), and this may vary depending on behavioral context or state of learning.

What has neuronal activity analysis during different behaviors taught us? Some neurons are strongly tuned to one movement, while others are more promiscuous and can contribute to several conversations (Fig. 7d). Similarly, some neurons exhibit high reliability in terms of being consistently recruited during a movement, while others “tune in” only sometimes. This organization allows the ensemble of neurons active during a particular movement to be different from one instantiation to another. It permits the conditional and flexible execution of a movement, and also the expansion, change or refinement of ensembles through learning. The combination of fine tuning of movement parameters within precisely connected circuits and broadcasting through systems-wide networks allows the nervous system to exploit precision and flexibility at the same time. The here reviewed repertoire of circuit and network motifs is being gradually unraveled throughout evolution, with certain subsystems gaining more importance in some species while others becoming less prominent[71,122,123]. Understanding these principles across different species will lead to a deep understanding of how movement execution and learning is regulated within the motor system.

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Figure Legends

Fig. 1 Brainstem neurons regulating speed and direction of locomotion.
(a) Schemes of transverse sections through the mouse brainstem at different rostro-caudal levels to illustrate the anatomical arrangements (from top to bottom: midbrain, pons, medulla and spinal cord).
(b) Neuronal subpopulations in the brainstem implicated in the regulation of locomotor speed. Within the MLR, glutamatergic (vGlut2; green), inhibitory (vGAT; blue) and cholinergic (ChAT; yellow) neurons are found, with the latter defining the boundaries of the pedunculo-pontine nucleus (PPN). Excitatory (vGlut2) neurons in the cuneiform (CnF) subregion of MLR regulate high speed locomotion, by their synaptic interactions with vGlut2-expressing neurons in the lateral paragigantocellular nucleus (LPGi) in the medulla. The LPGi also contains intermingled inhibitory (vGAT) neurons, optogenetic activation of which elicits stopping. The role of excitatory neurons in the PPN is currently less clear (question mark).
(c) Regulation of locomotion directionality by brainstem populations. Neurons in the medially located Gigantocellular nucleus (Gi) encompass different populations. Neurons marked by the transcription factor Chx10 represent a subpopulation of mostly ipsilaterally projecting vGlut2-expressing neurons, which again subdivide into different populations. Lumbar projecting Chx10 neurons in the Gi elicit stopping and connect to spinal interneurons, while cervically projecting neurons elicit ipsilateral head turning and connect directly to MNs. Their joint unilateral activation leads to ipsilaterally turning locomotion behavior.
Abbreviations: 7N: facial motor nucleus; 5N: trigeminal motor nucleus; MLR: mesencephalic locomotor region; CnF: cuneiform nucleus; PPN: pedunculo-pontine nucleus; mRT: mesencephalic reticular formation; SC: superior colliculus; IC: inferior colliculus; vGlut2: vesicular glutamate transporter 2; ChAT: choline acetyltransferase; vGAT: vesicular GABA transporter; LPGi: the lateral paragigantocellular nucleus; contra: contralateral; Gi: gigantocellular nucleus; L: lumbar; C: cervical; MN: motor neuron.

Fig. 2 Brainstem neurons for the construction of forelimb movements
(a) Brainstem neurons in the lateral rostral medulla (latRM) located dorsally to the facial nucleus (7N) are engaged during different phases of forelimb-specific behaviors. Neurons tuned to ipsilateral unilateral forelimb reaching (during a pellet retrieval or lever pressing task) are preferentially located in the ventral domain of latRM and are not recruited during food handling. In contrast, neurons tuned to food handling are located preferentially in the dorsal domain of the latRM. These neurons are not recruited during forelimb reaching, but are engaged in food pellet handling following the retrieval of food pellets in the pellet task. Excitatory latRM neurons are required for precision of reaching and handling. Neuronal recording plots are modified after Ruder et al. 2021.
(b) LatRM excitatory (vGlut2) neurons stratify into at least four different populations based on their axonal projection targets. When optogenetically stimulated, spinally-projecting vGlut2-latRM neurons (green) are located ventrally and elicit unilateral forelimb reaching, caudal medulla-projecting vGlut2-latRM neurons stratify into neurons terminating in the medullary reticular formation dorsal (MdD) and ventral (MdV) part, with MdD-projecting neurons located most dorsally. These two populations can elicit more complex digit involving forelimb movements when stimulated optogenetically, including hand-to-mouth

and grooming (MdD) and reach-to-grasp, tap (MdV) movements. The fourth population projects contralaterally and its stimulation does not elicit obvious behavioral phenotypes.
(c) Summary of concepts for neuronal circuit organization in the brainstem engaged in motor control. Figure displays two hypothetical pure motor programs A and B and how these might be processed in passing the brainstem. Specialized neuronal populations can receive additional information through brainstem external inputs or information processed within brainstem circuits (e.g. enhance, veto, or cross communication). Together, these processing steps can modify an action plan signal towards its output from the brainstem to the spinal cord (outputs). In the spinal cord, these descending signals are mixed and matched by recipient circuits, and further processed to finally lead to execution/body movement. We hypothesize that this strategy enables behavioral flexibility and can result in execution of pure programs A or B or mixtures thereof (right).

Fig. 3 Interactions between basal ganglia and brainstem circuits
(a) Substantia nigra reticulata (SNr) neurons are gate keepers between basal ganglia and brainstem motor centers. SNr neurons integrate information from the striatum, subthalamic nucleus (STN) and globus pallidum externum (GPe) from within basal ganglia. How their inhibitory output (minus sign) interacts with the brainstem has remained unclear.
(b) The classical model proposes that pausing of tonically firing in SNr neurons leads to disinhibition of downstream neurons in the superior colliculus (SC). As a consequence, excitatory SC neurons upregulate firing and are involved in the execution of a saccade.
(c) A select/suppress model suggests that SNr neurons are highly organized with respect to their downstream targets, and only SNr neurons relevant for a desired behavior (red) and projecting selectively to a specific downstream target (SC) should be inhibited, but not surrounding SNr neurons (blue) that continue active thereby inhibiting non-desired movements. This has however not been tested experimentally to date.
(d) Different SNr neuron populations project to particular brainstem targets (illustrated by different colors), but in addition all of these neurons generate axonal collaterals to pedunculo pontine nucleus (PPN), midbrain reticular formation (RF) and motor/intralaminar thalamus. Excitatory inputs to SNr neurons are shown by plus signs, inhibitory inputs by minus signs.
(e) Topographical projection from different striatal regions (DMS: yellow; DLS: blue; VLS: red) into different regions of SNr. These distinct SNr subregions also target different downstream targets in the caudal brainstem, as shown by anterograde transsynaptic virus transfer experiments from the striatum into the SNr. Stimulation of SPNs in DLS and VLS elicits licking or turning respectively, in agreement with the functional specializations downstream of respective SNr neurons in the brainstem.
Abbreviations: DMS: dorso-medial striatum; DLS: dorso-lateral striatum; VLS: ventro-lateral striatum.

Fig. 4 Action specificity in striatal circuits
(a) Monitoring striatal projection neuron (SPN) activity using Calcium imaging in the dorso-lateral striatum (DLS; left) shows that distinct, action-specific neuronal ensembles are recruited during different behaviors such as left turn, right turn and rearing. Recruited neurons associated with these behaviors are indicated by colored shading and grey indicates neurons not recruited by shown behaviors.

(b) SPNs encompassing the direct pathway (dSPN, expressing D1 receptor) project directly to substantia nigra reticulata (SNr), with minor collaterals to globus pallidus externus (GPe). (c) SPNs encompassing the indirect pathway (iSPN, expressing D2 receptor) terminate in GPe. Many regions are targeted by GPe neurons, in part by distinct neuronal populations. These include the striatum itself, the subthalamic nucleus (STN), SNr, cortex (CTX) and thalamus (Th). Furthermore, the STN, which is also part of the indirect pathway, targets GPe and SNr, and itself receives cortical input (hyperdirect pathway). Excitatory projections are shown in green, inhibitory projections in red.
Abbreviations: dSPN: direct striatal projection neurons; iSPN: indirect striatal projection neurons; GPe: globus pallidus externus; SNr: substantia nigra reticulata; STN: subthalamic nucleus, Ctx: cortex, Th: thalamus; DLS: dorso-lateral striatum.

Fig. 5 Widely projecting neuronal populations in motor system
(a) Motor cortex neurons are amongst the most widely projecting neurons in the nervous system. Neuronal reconstruction redrawn from Kita and Kita, 2012, showing that a single traced motor cortex neuron from the lateral section of agranular cortex (AGl) projects to and arborizes in many target regions and can thereby contribute in the simultaneous distribution of the emitted signal to many downstream circuits.
(b) Cortical neurons target distinct regions in the striatum, following an anatomical and functional topography (left). Recordings demonstrate that there is a fine level of functional granularity with respect to the content recorded in cortical neurons and recipient neurons in the striatum (right, where different colors indicate functional granularity), suggesting that cortical input represents a major driver input to the building of striatal ensemble activity.
(c) Cortico-spinal neurons located in different cortical regions (RFA: rostral forelimb area; CFA: caudal forelimb area; AM: antero-medial; posterior-lateral) terminate in different dorsal-ventral domains of the cervical spinal cord (cervical levels C2-C4 and C6-C7) and exhibit different recruitment profiles during skilled forelimb behaviors. Different terminations of cortical neurons are matched with a positional matrix of premotor neurons in the spinal cord, where extensor premotor neurons are located more medial and flexor premotor neurons more lateral. Data shown summarizes work from Wang et al., 2017.
(d) Organization of cerebellar output signals from deep cerebellar nuclei (DCN; left). Lateral, interposed and medial are the three main divisions of DCN. Recent work identified 125 ipsilateral and 140 contralateral targets for all DCN neurons jointly. Using molecular profiling, three main inhibitory neuron types were identified (i1-3), which occur in all three DCN divisions. In contrast, excitatory DCN neurons can be separated into 15 subtypes, but these subtypes are found in distinct DCN. Moreover, there is an overarching functional division of excitatory neurons into Class A and B types based on electrophysiological characteristics. Regarding axon collateralization, DCN populations (IntA) are likely to arborize broadly (left). Scheme on the right illustrates that understanding the interactions of DCN output in targeting regions will be important to reveal the extent to which output information from different DCN populations is convergent (domain overlapping between two colored circles) or divergent (domains non-overlapping between two colored circles).
(e) Dopaminergic neurons (DANs) in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) exhibit divergent axonal projections and functional properties. SNc neurons project mostly to the dorsal striatum, while VTA neurons preferentially target medial and ventral striatum as well as cortex (left). Center: SNc DANs are frequently recruited during action initiation, but there is no action specificity with respect to the

precise action executed – that is, similar patterns of activity are observed regardless of behavior (left turn, right turn, rear shown as examples). This is in contrast to SPNs which are action specific (see Fig. 4a), and suggests that action content cannot be decoded from SNc neurons (depicted as same green color of SNc neurons during action initiation). DANs can also encode action intensity or vigor (illustrated by high, medium and low in different green colors in the graph, far right), with bigger signals leading to longer and/or more vigorous actions and smaller signals leading to weaker and/or shorter actions.

Fig 6. Different brain networks mediate different aspects of motor learning
(a) Scheme summarizing current understanding of the processing of movement and learning related information in exemplary small and big loop networks. Bidirectional communication during task preparation between cortex and corresponding thalamic regions is needed for correct task execution (small loop; loop shown generally for cortex, but discussed in the text specifically for anterior lateral motor ALM and two-directional licking task). In addition, input from DCN to thalamus is also required for this and other learned motor tasks, for which likely different DCN subpopulations are recruited and needed during different tasks and contexts. In the course of learning a forelimb skilled task, increased coherence in the recruitment of a big loop between cortex, and cerebellum, most likely through processing of the brainstem's pontine nucleus emerges over the course of learning. Top scheme illustrates that ensemble coherence (red and blue colors for different ensembles) across brain wide networks emerge and increase during learning.
(b) Plasticity at specific cortico-striatal synapses through influence of dopamine (DA) is critical for motor reinforcement. For example, increased synaptic strength between cortical and striatal neurons involved in the execution of the blue motor program can make it more likely to disinhibit the blue brainstem neurons than the red neurons, and hence rapidly change the probability of doing the movement. Plasticity at cortico-striatal synapses also makes it more likely to disinhibit particular thalamo-cortical ensembles.
(c) The gradual refinement of fine movements takes longer and likely requires synaptic plasticity in the cortex (shown here by blue connecting lines between neurons of an ensemble, under the influence of DA, shown in green). This gradual cortical plasticity is likely differentially shaped by basal ganglia-recipient thalamus versus cerebellum-recipient thalamus.

Fig 7. Principles for network processing for action diversification and learning
(a) Neuronal subpopulations (depicted here in four different colors) are frequently found in spatially intermingled configurations within many regions of the nervous system. Different parameters can be used to disentangle diversity to understand different functions (A-D). Useful arbiters for this purpose can be neurotransmitter identity, gene expression profiles, as well connectivity, that is, axonal projections and input patterns.
(b) A brain region can output to many targets via distinct neuronal populations projecting to different targets (left). In this case, the functional impact on targets reflects the population activity as well as the receptivity of target neurons. Alternatively, neurons in a region can establish collaterals to different targets (right). In this case, the same signal is broadcasted to all targets; however, not all axon collaterals have identical impact on postsynaptic target cells (indicated by the number of pluses or crosses, which indicate high or low impact on postsynaptic neuron, respectively) due to e.g. differences in excitability of postsynaptic neurons or the concomitant processing of other inputs to these target regions (not shown).

Functional impact of a given neuron (black) on postsynaptic targets during two different states (blue and red) can lead to very different outcomes in the processing at various downstream targets.
(c) Processing of information derived from two distinct neuronal populations (A and B) within a common target region can use fine axonal arborization differences to result in downstream domains in which these two inputs converge and are integrated, or separation of targeting domains leading to divergent input processing. Using this strategy for different neuronal populations, as occurs in most situations in the nervous system, allows to mix and match different inputs for integration within downstream regions.
(d) Many regions in the motor system contain neurons encoding movement parameters related to specific actions (depicted here as Action A and B). These action-specific neurons exhibit different degrees of action specificity (indicated by transparency of colors for A, B, A+B). In addition, action specific neurons show different levels of reliability in terms of being recruited during an action (indicated at the bottom), with highly reliable neurons recruited almost every time an action is executed to less reliable neurons only recruited occasionally or rarely during the same action. Neuronal ensemble recruitment profiles are subject to changes during different states or across learning.

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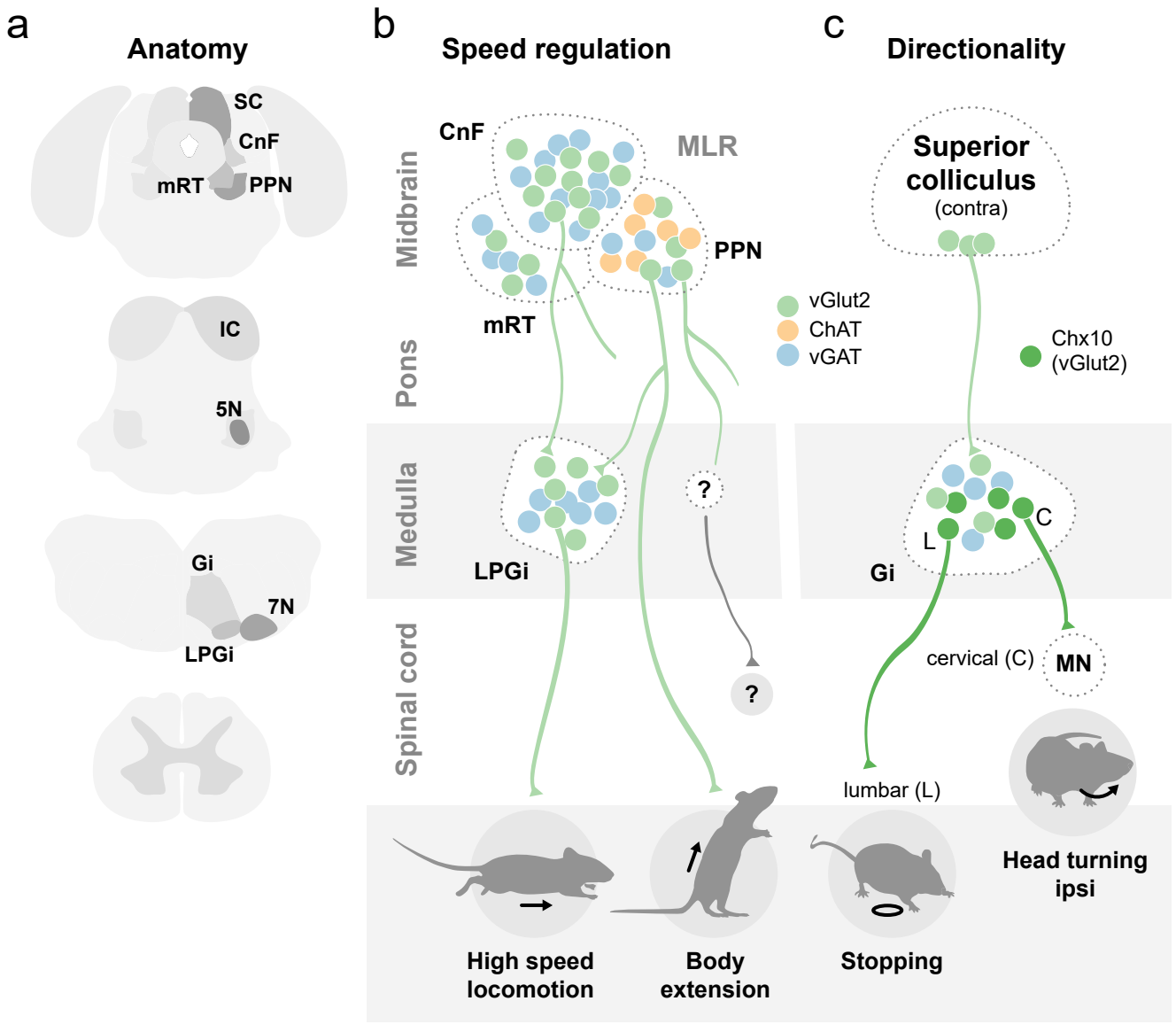
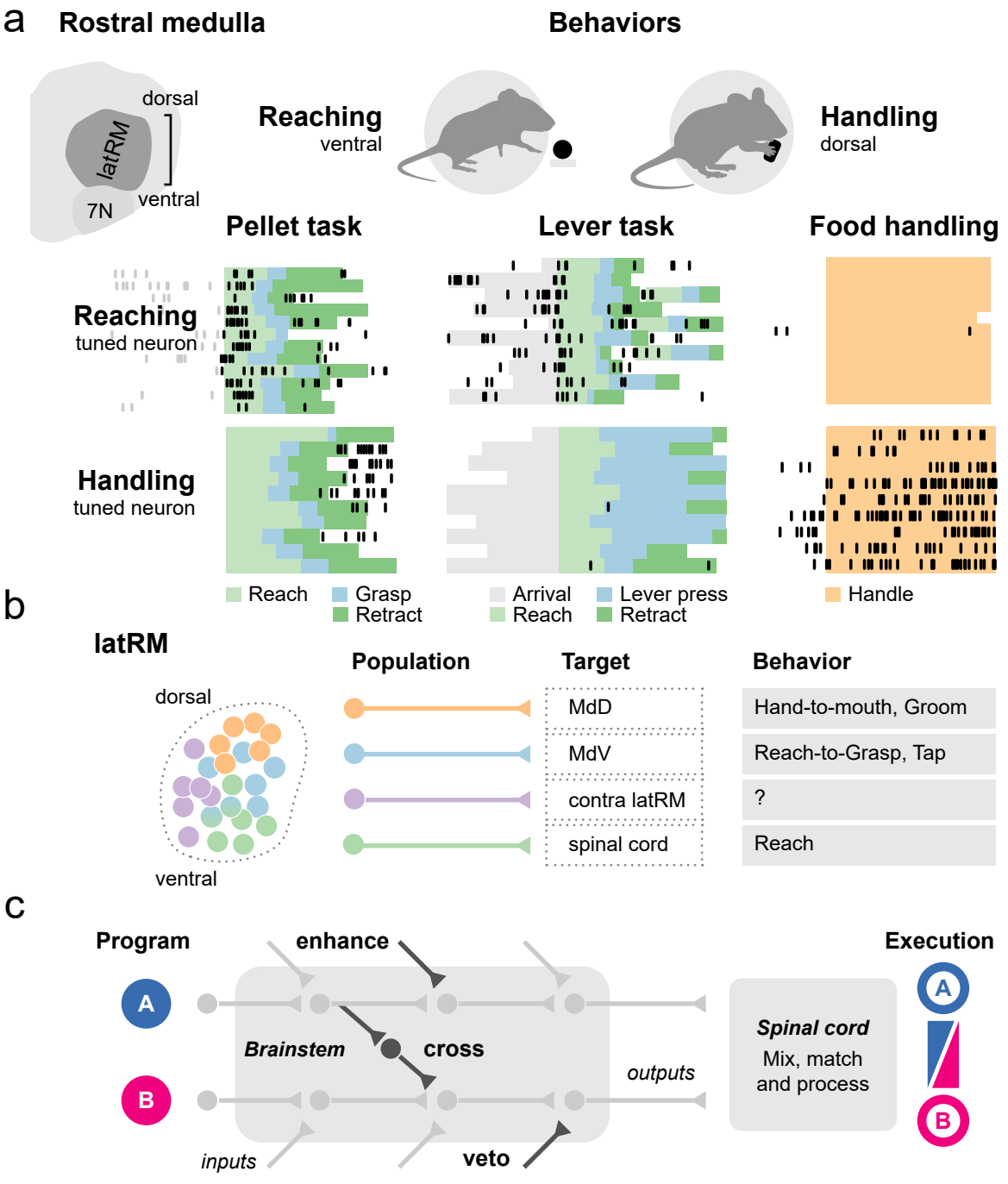
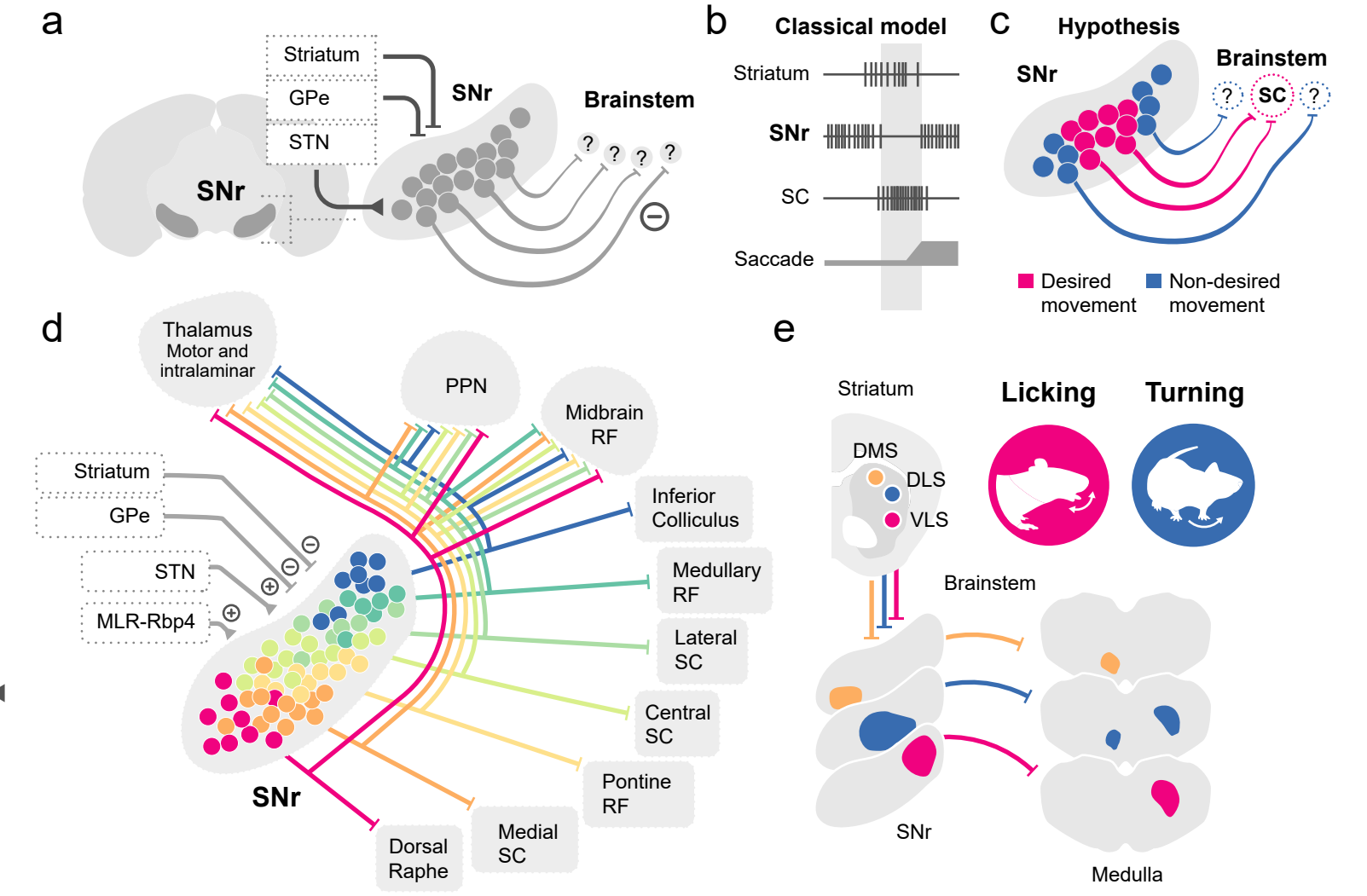
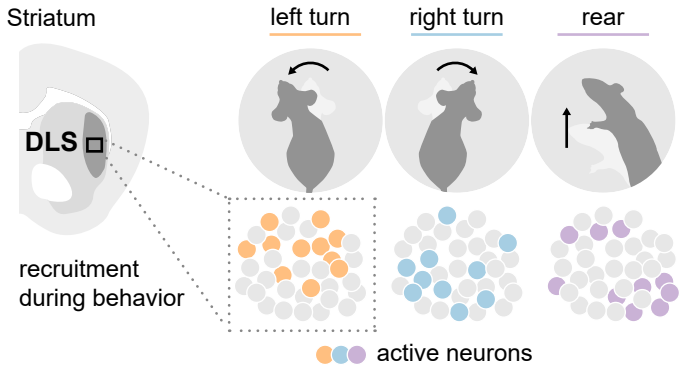

a
Anatomy
SC
CnF
mRT
PPN
IC
5N
Gi
7N
LPGi
b
Speed regulation
Midbrain
Pons
Medulla
Spinal cord
CnF
MLR
PPN
mRT
vGlut2
ChAT
vGAT
LPGi
?
?
High speed locomotion
Body extension
c
Directionality
Superior colliculus
(contra)
Chx10
(vGlut2)
Gi
L
C
cervical (C)
MN
lumbar (L)
Stopping
Head turning ipsi


a
Rostral medulla
Behaviors
dorsal
latRM
ventral
7N
Reaching
ventral
Handling
dorsal
Pellet task
Lever task
Food handling
Reaching
tuned neuron
Handling
tuned neuron
Reach
Grasp
Retract
Arrival
Reach
Lever press
Retract
Handle
b
latRM
dorsal
ventral
Population
Target
Behavior
MdD
MdV
contra latRM
spinal cord
Hand-to-mouth, Groom
Reach-to-Grasp, Tap
?
Reach
c
Program
enhance
Execution
A
B
Brainstem
cross
outputs
inputs
veto
Spinal cord
Mix, match
and process
A
B

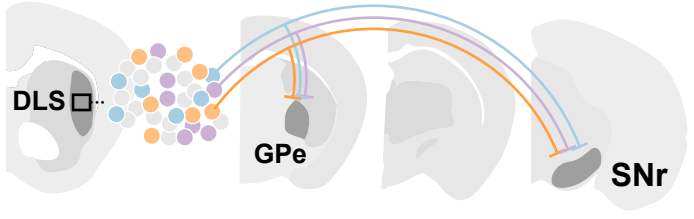

a
Striatum
GPe
STN
SNr
SNr
Brainstem
b
Classical model
Striatum
SNr
SC
Saccade
c
Hypothesis
SNr
Brainstem
SC
Desired movement
Non-desired movement
d
Thalamus Motor and intralaminar
PPN
Midbrain RF
Striatum
GPe
STN
MLR-Rbp4
Inferior Colliculus
Medullary RF
Lateral SC
Central SC
Pontine RF
Medial SC
Dorsal Raphe
SNr
e
Striatum
DMS
DLS
VLS
Licking
Turning
Brainstem
SNr
Medulla

a



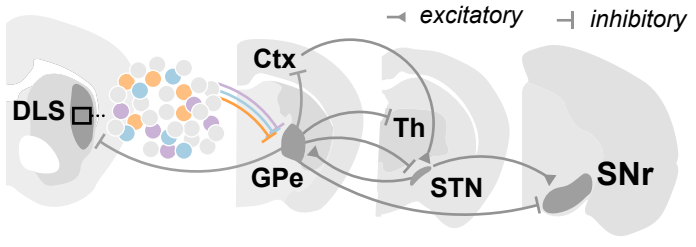
b

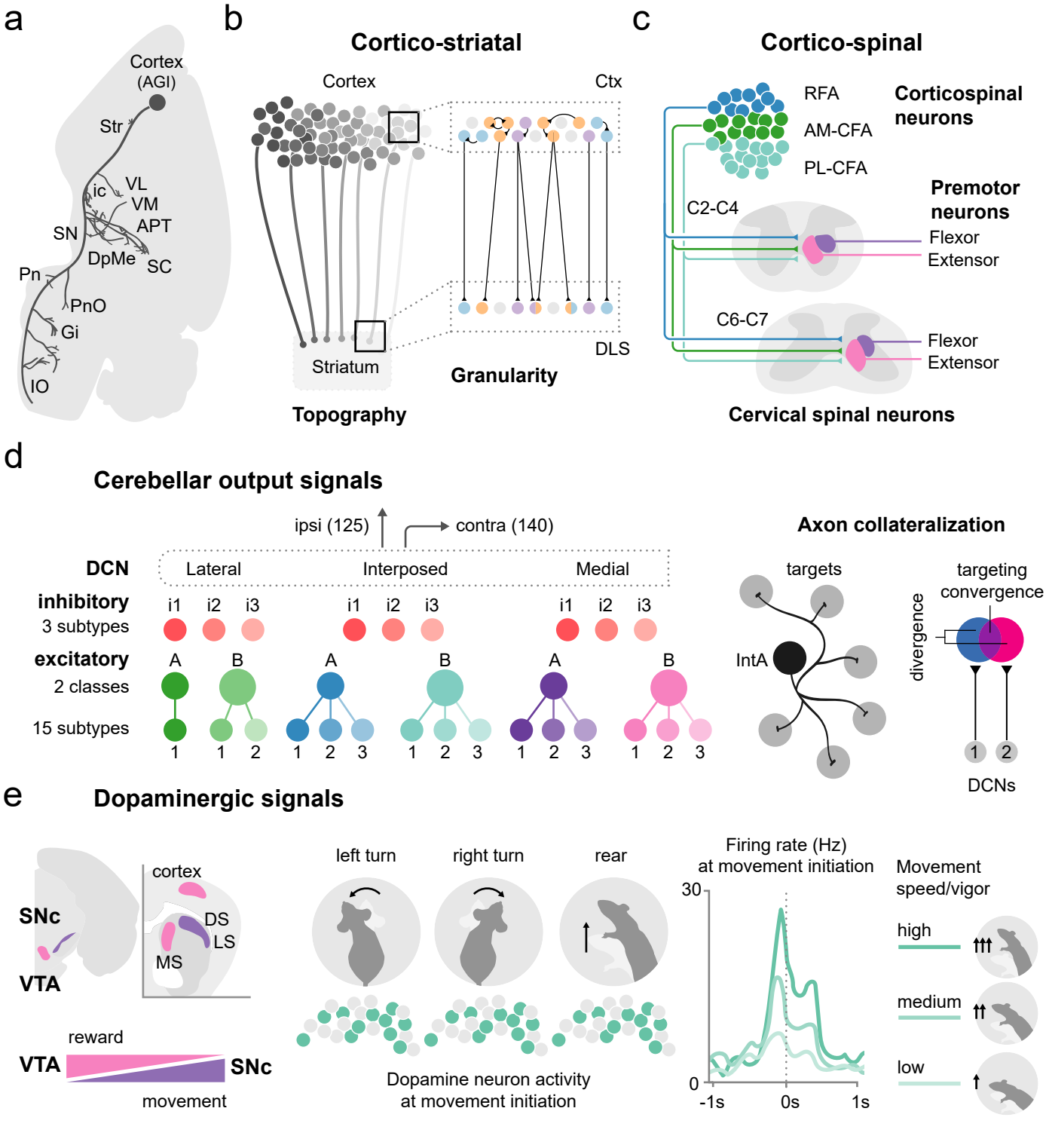
Direct pathway (D1 receptor)

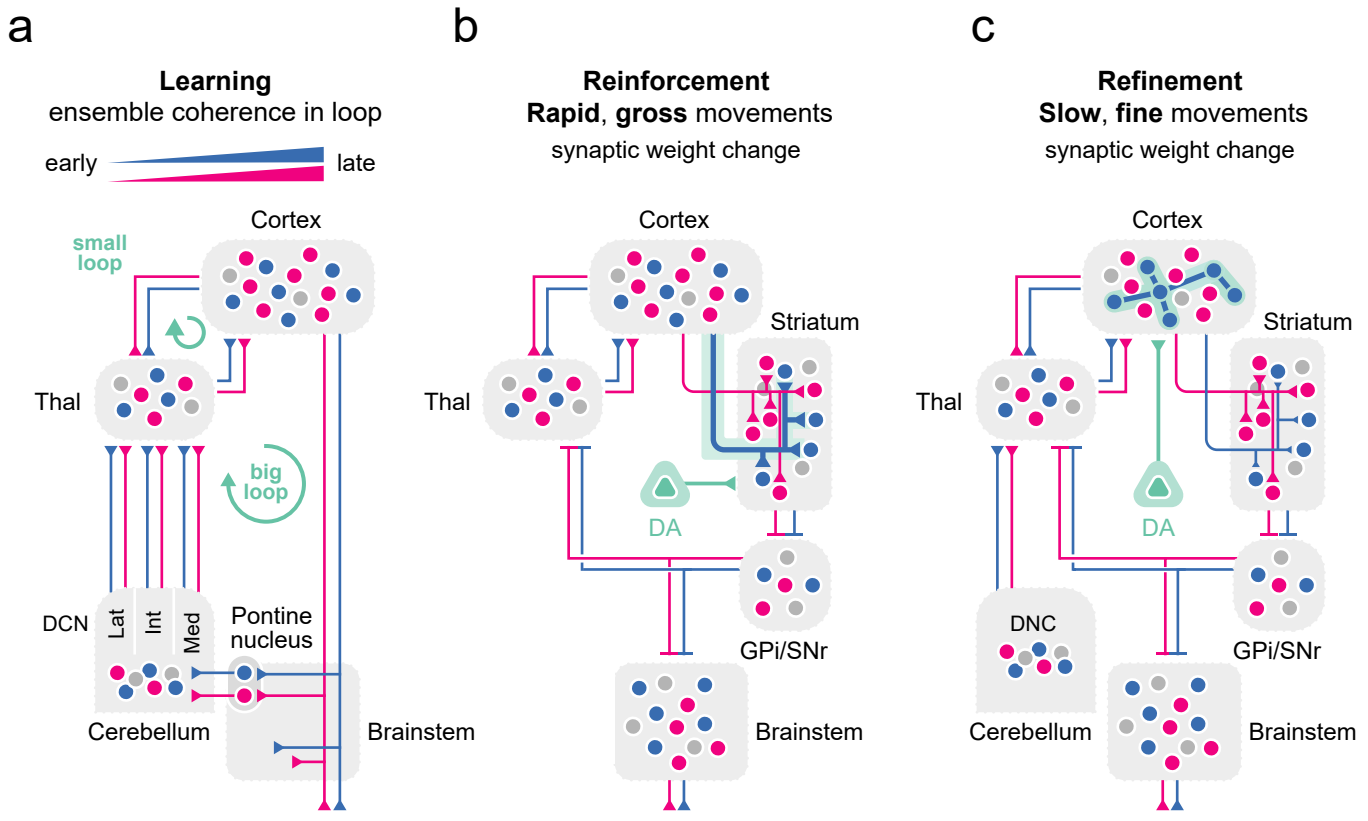


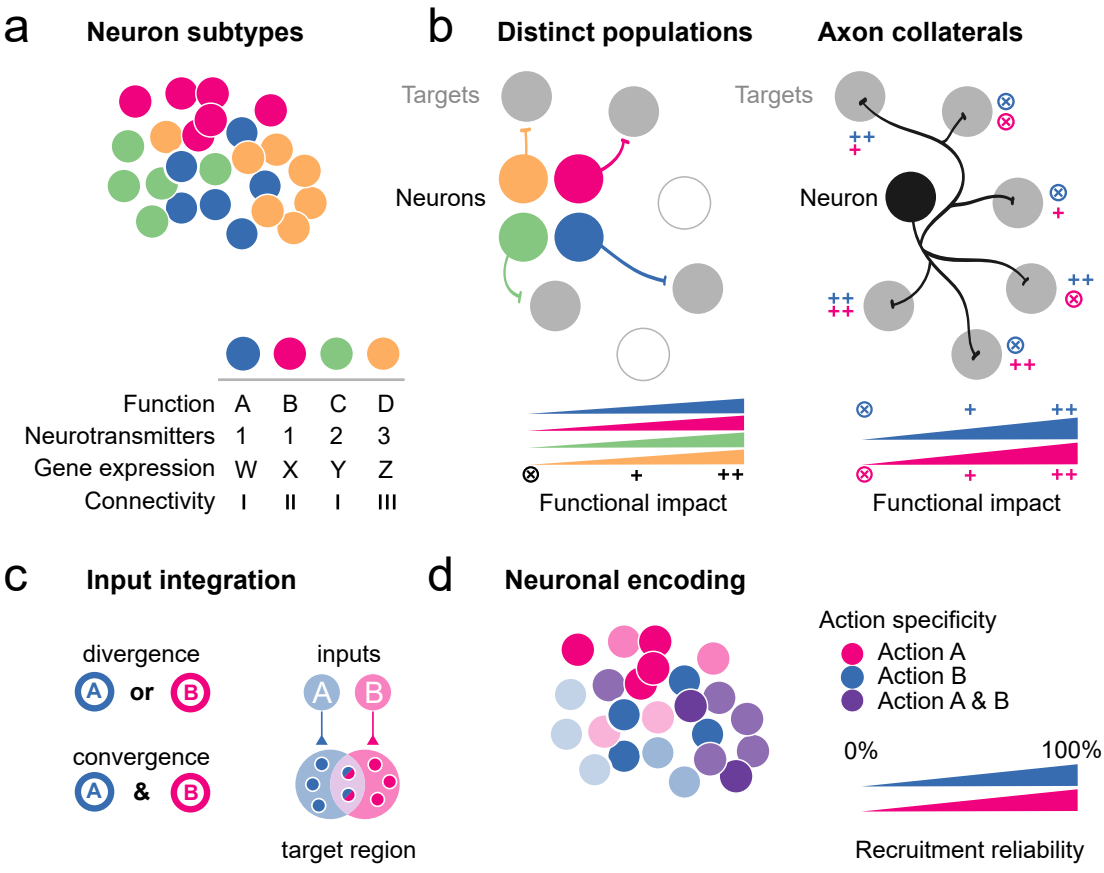
c

Indirect pathway (D2 receptor)




a
Cortex
(AGI)
Str
ic
VL
VM
APT
SN
DpMe
SC
Pn
PnO
Gi
IO
b
Cortico-striatal
Cortex
Ctx
Striatum
DLS
Granularity
Topography
c
Cortico-spinal
RFA
AM-CFA
PL-CFA
Corticospinal
neurons
Premotor
neurons
C2-C4
Flexor
Extensor
C6-C7
Flexor
Extensor
Cervical spinal neurons
d
Cerebellar output signals
ipsi (125)
contra (140)
DCN
Lateral
Interposed
Medial
inhibitory
3 subtypes
i1 i2 i3
excitatory
2 classes
A B
15 subtypes
1 1 2 1 2 3 1 2 3 1 2 3
Axon collateralization
targets
IntA
targeting
convergence
divergence
1 2
DCNs
e
Dopaminergic signals
SNc
VTA
cortex
DS
LS
MS
reward
VTA
SNc
movement
left turn
right turn
rear
Dopamine neuron activity
at movement initiation
Firing rate (Hz)
at movement initiation
30
0
-1s
0s
1s
Movement
speed/vigor
high
medium
low


a
Learning
ensemble coherence in loop
early
late
Cortex
small loop
Thal
big loop
DCN
Lat
Int
Med
Pontine nucleus
Cerebellum
Brainstem
b
Reinforcement
Rapid, gross movements
synaptic weight change
Cortex
Striatum
Thal
DA
GPi/SNr
Brainstem
c
Refinement
Slow, fine movements
synaptic weight change
Cortex
Striatum
Thal
DA
DNC
GPi/SNr
Cerebellum
Brainstem


a
Neuron subtypes
Function A B C D
Neurotransmitters 1 1 2 3
Gene expression W X Y Z
Connectivity I II I III
b
Distinct populations
Targets
Neurons
Functional impact
Axon collaterals
Targets
Neuron
Functional impact
c
Input integration
divergence
A or B
convergence
A & B
inputs
A B
target region
d
Neuronal encoding
Action specificity
Action A
Action B
Action A & B
0%
100%
Recruitment reliability