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Structure and function of the mesencephalic locomotor region in normal and parkinsonian primates

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In the past decade, the mesencephalic locomotor region (MLR) has emerged as a new surgical target for alleviating dopamine-resistant gait and balance disorders in Parkinson's disease. Part of the reticular formation, the MLR contains nuclei with diffuse and open boundaries, which are currently difficult or impossible to visualize directly using conventional MRI in humans. Recent experiments have characterized the organization of neuronal populations in the rodent and primate PPN and CuN, and their distinct connectivity profiles. New studies in primates together with cell-type specific optogenetic experiments in mice provide evidence for more-specific roles of the PPN and the CuN in locomotion and arousal. We provide an update on key recent advances on MLR structure and function in normal and parkinsonian primates.

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Introduction

Brainstem circuits involved in locomotion have been the focus of increasingly refined research questions in the past several years. This focus has been paralleled by clinical testing of new deep brain stimulation (DBS) targets for alleviating dopamine-resistant gait and balance disorders in Parkinson's disease (PD) [1–8]. Over the past decade, the main DBS target tested has been the pedunculopontine nucleus (PPN) that, together with the cuneiform nucleus (CuN), comprises the mesencephalic locomotor region (MLR), originally defined by the elicitation of locomotor behavior upon electrical stimulation of this region in the cat [9]. The body of experimental and clinical data collected since highlights a potentially key role for the MLR in the pathophysiology of gait and balance disorders in PD [10], and raised the possibility that activation of this region using low-frequency DBS could alleviate gait and balance disorders in advanced PD. Altogether, the clinical trials suggest that low-frequency stimulation might reduce the frequency of falls in some patients [11], but freezing of gait (FOG) is not consistently improved [6], and predictive factors indicating which patients will show improvements are still lacking [4]. The heterogeneous clinical results of MLR-DBS may in part be explained by differences in the stimulation target chosen [2,12]. This is likely an important factor since the MLR is a challenging surgical target due to its small size, the open boundaries of its constituent nuclei, the difficulty in directly visualizing this region using conventional magnetic resonance imaging (MRI), and the lack of data localizing the MLR within a consistent coordinate system for targeting. Although the PPN is often the stated target, it remains an open question whether the best target within the MLR for alleviating gait and balance disorders in advanced PD is situated in the PPN itself, in the adjacent CuN, or both. New data examining the structure and function of the MLR in non-human primates and humans are important for establishing optimal targets for neuromodulation in the MLR. Here, we highlight recent progress in understanding the structure and function of the MLR in normal and parkinsonian primates, also pointing out recent complementary work in mice using cell-specific viral and optogenetic techniques.

Structural organization of the MLR

Conventional MRI in humans does not yet provide sufficient resolution and contrast to visualize the MLR, which is part of the reticular formation containing many nuclei with diffuse and open boundaries. Neurosurgeons typically rely on indirect visualization based on adjacent structures, delimiting the MLR roughly by the superior and inferior colliculi dorsally, the superior cerebellar peduncle ventrally, the lateral lemniscus laterally and the periaqueductal grey medially, the anterior border of the superior colliculus anteriorly and the posterior border of the inferior colliculus posteriorly. There is significant variability between humans in brainstem size and orientation, and more precise methods of localizing the MLR in individual patients is necessary for reliable and reproducible clinical outcomes. It is possible that specific MRI sequences at ultra-high field-strength (7 T

MP2RAGE sequence at high resolution with enhanced grey–white matter contrast) [13], or registering conventional anatomical MRIs with atlases based on histological data may be used to more reliably target the PPN before surgery [14]. This suggests that detailed data on cell types and their distribution within the nuclei of the human MLR could be useful for surgical targeting as well as for understanding the diverse effects of MLR stimulation.

The PPN, described for the first time by Jacobsohn 1909 [15] as the 'nucleus tegmenti pedunculopontinus', and the CuN were initially differentiated based on detailed cytoarchitectonic criteria in humans by Olszewski and Baxter [16]. These nuclei are composed of neurons with diverse neurotransmitter phenotypes; glutamatergic, cholinergic and GABAergic in the PPN, and glutamatergic and GABAergic in the CuN. The description of the different neuronal types is well-characterized in the rodent PPN [17], where GABAergic neurons are more densely distributed rostrally while glutamatergic and cholinergic neurons are denser caudally. By comparison, fewer papers exist in non-human primates [18,19], and the three-dimensional organization of these neuronal populations in the human PPN and CuN has only recently been characterized [20]. In primates, numerous GABAergic and glutamatergic neurons are intermingled throughout both the PPN and the CuN, and there is no well-defined limit between these nuclei when considering these two neurontransmitter phenotypes. However, since cholinergic neurons are only present in the PPN, their presence has been previously used to delimit the boundaries of the PPN [18,21]. Within the human PPN, the peak densities of cholinergic and GABAergic neurons are similarly located rostrally (Figure 1), and no neurotransmitter co-expression was detected using *in-situ* hybridization [20].

Tracing studies and single-cell labeling experiments show highly collateralized cholinergic PPN axons innervating different components of the basal ganglia, up to the thalamus and down to the reticulospinal pathway, whereas non-cholinergic PPN axons are less extensive [17,21]. In return, the basal ganglia and cortex projects to the PPN, terminating preferentially on its non-cholinergic neurons [22]. Non-invasive diffusion-tensor imaging (DTI) and fiber tractography has been recently used to examine connectivity in primates [23,24], and the connectivity of the PPN has been studied in relation to the anatomo-functional subdivisions of different brain structures [24]. The PPN is a structure where sensorimotor, cognitive and emotional information converge, with a stronger connections from the motor cortex and substantia nigra to the anterior PPN compared its posterior part, suggesting a topographical organization within the PPN itself in the non-human primate brain.

The connectivity of the CuN differs markedly from the PPN. Cortical inputs originate preferentially from limbic cortices (e.g. subgenual cingulate and insular cortex), with no (macaque) or much weaker (human) connectivity between the CuN and motor cortices [24] (Figure 2). Connectivity with the basal ganglia and thalamus are also different, with stronger connectivity between the CuN and the limbic parts of the basal ganglia and thalamus than the PPN. This work in primates agrees with whole brain mono-synaptically restricted trans-synaptic tracing in mice [25••], which further revealed preferential inputs from caudal PPN to the motor nuclei in the medulla and preferential inputs from midbrain limbic nuclei to the CuN. Thus, while electrical stimulation of both the CuN and PPN can elicit locomotion, the presence of distinct connectivity profiles of the two suggests involvement in different aspects of locomotor behavior.

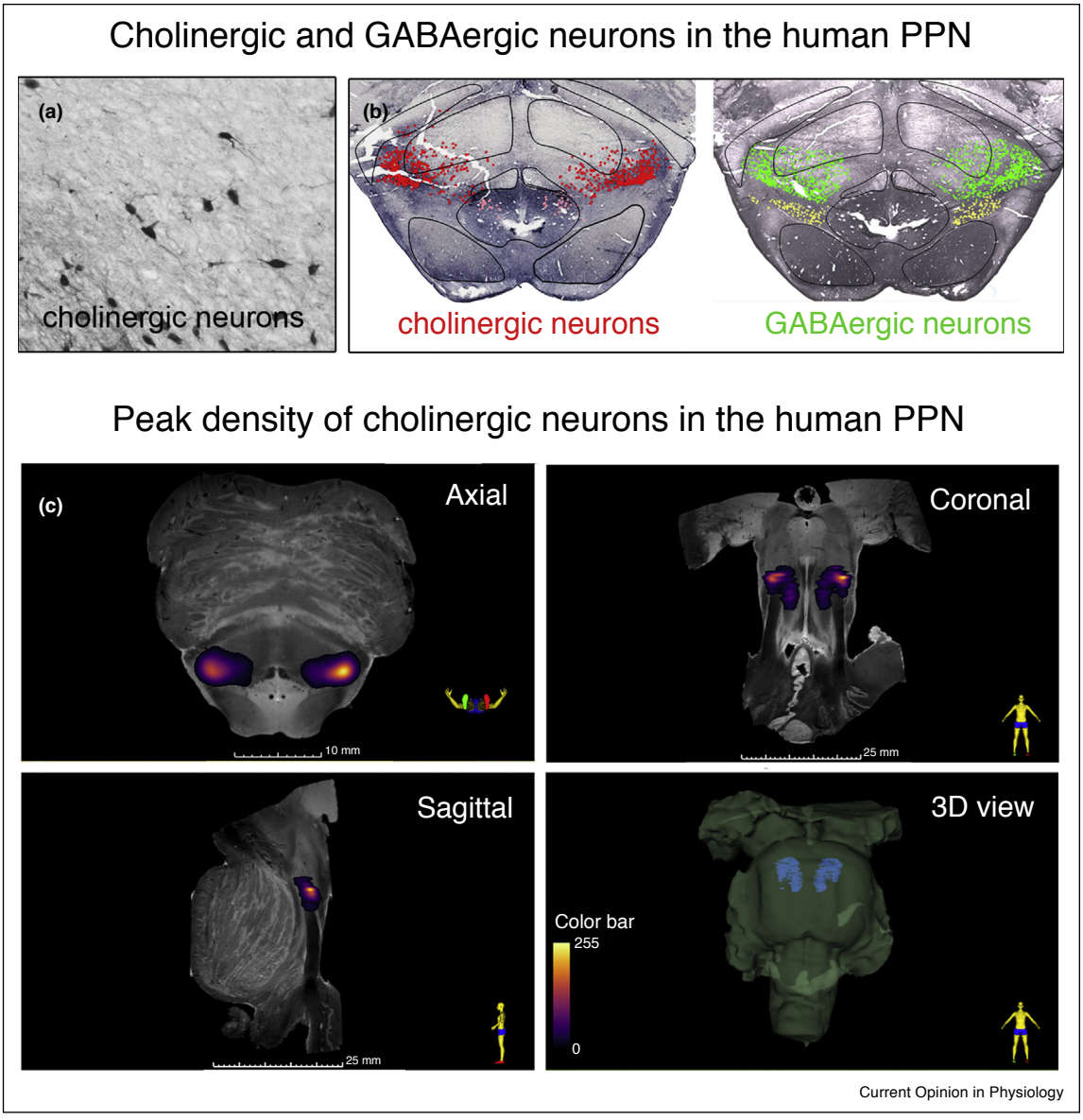
Functional organization of the MLR

The MLR plays a major role in locomotion

While the MLR is so-named based on the effects of stimulation, the more-specific roles of the PPN and the CuN have been strongly debated for many years. Experimental data in cats suggested that CuN stimulation elicits locomotor patterns, whereas PPN stimulation produced changes in muscle tone [26] or site-specific complex movements [27]. Further dissecting specificities has been hindered by the difficulty of selectively modulating intermingled neurons with different neurotransmitter phenotypes as well as the broad and diverse upstream and downstream connections of the MLR. Major advances have recently been made using cell-type specific chemogenetic and optogenetic experiments in mice to show that the activity of glutamatergic neurons in both the PPN and CuN are correlated with the speed of locomotion [25••,28••,29••]. Moreover, optical activation of glutamatergic neurons throughout the MLR is sufficient to initiate and maintain locomotion, while activation of GABAergic neurons can stop it [25••,28••]. Activating cholinergic neurons alone does not initiate gait, but can modulate speed [28••,29••], and increase overall locomotor activity [30•], with at least some locomotor effects mediated by PPN cholinergic projections to midbrain dopamine neurons [31,58]. Glutamatergic together with cholinergic PPN neurons could contribute to slow-walking gait by modulating locomotor pattern and rhythm [29••]. Interestingly, high-speed synchronous locomotion is preferentially elicited by activation of glutamatergic neurons in the CuN [25••]. With its more dominant inputs from limbic brain regions, the CuN may represent a motor-limbic interface of the MLR that is important for driving locomotor behaviors during emotionally significant experiences (e.g. rapid escape from threat).

In monkeys, Goetz *et al.* developed an experimental model of bipedal locomotion to characterize how MLR neurons responded during treadmill walking [32•]. They observed neural responses that were either time-locked to muscular contraction (phasic) during stepping or

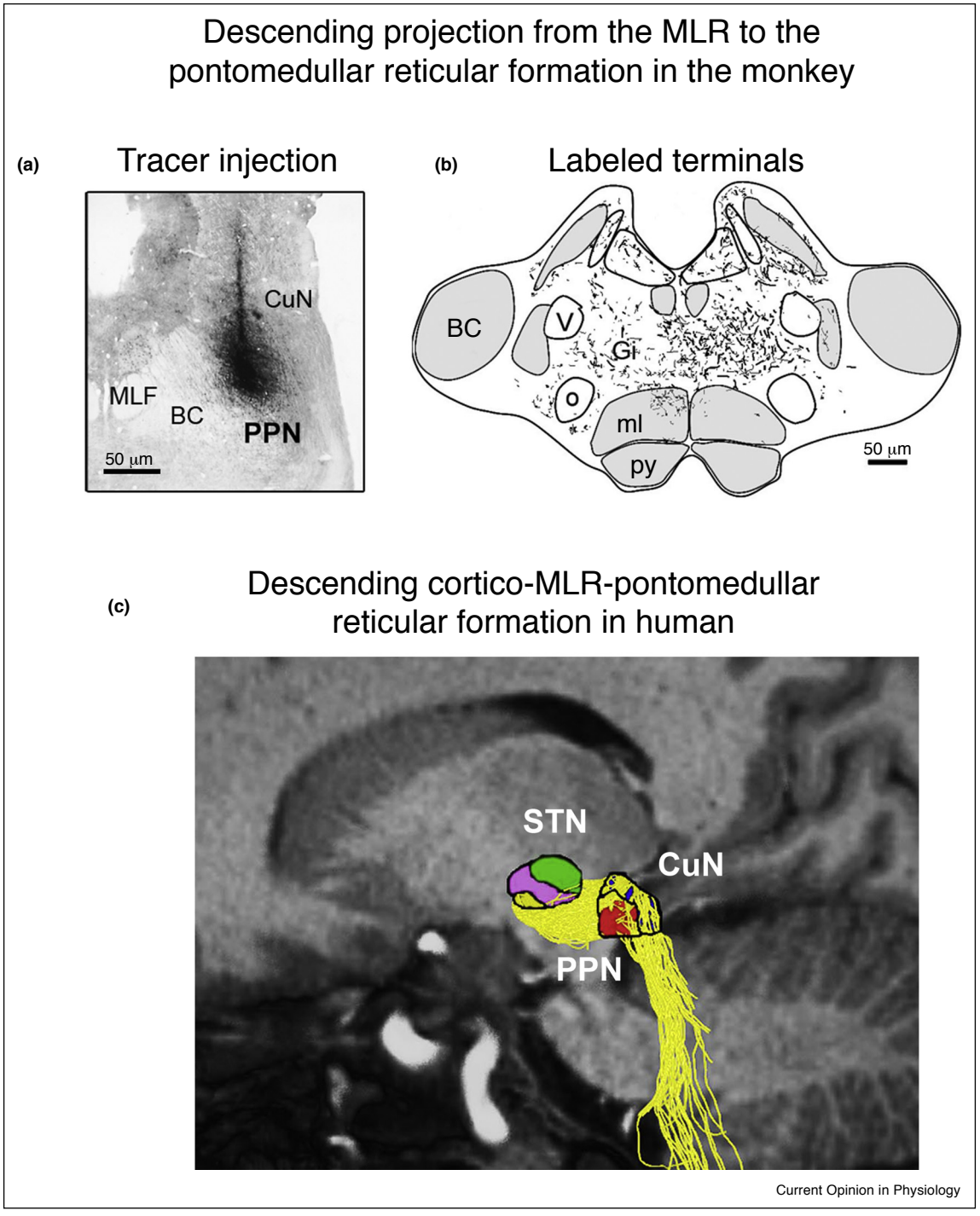
Figure 1



Cholinergic and GABAergic neurons in the human PPN. **(a)** Cholinergic neurons of a control human brainstem stained for choline acetyl transferase immunohistochemistry. **(b)** Cholinergic and GABAergic neurons are mapped in the PPN (red and green, respectively) and in the latero-dorsal tegmental nucleus (pink and yellow, respectively). **(c)** The 3D density maps are shown superimposed on the 11.7T T2-weighted MRI in the axial, sagittal and coronal 2D views and the reconstructed meshes are shown inside the brainstem in a 3D view. The color bar ranges from 0 (dark purple – low neuronal density) to 255 (yellow – high neuronal density). The 2D views were chosen to be located at each highest peak density. The reconstructed meshes were computed from the non-thresholded density maps. Modified from Sébille et al. [20].

sustained for the duration of walking (tonic), the latter putatively corresponding to cholinergic neurons based on electrophysiological characteristics. Neurons with phasic stepping responses localized to the region previously shown to elicit controlled locomotion with low-threshold electrical stimulation in monkeys [33], and there was no clear distinction in neurons localized to the PPN or the CuN, although walking speed was not varied. Integrating phasic neural responses over time may very well produce activity profiles that covary with speed, and varying speed in future experiments may permit differentiating PPN and CuN phasic neurons based on speed as observed in the mouse [25••]. Tonic responses occurred more posteriorly than phasic responses, consistent with the presence of cholinergic neurons in the PPN, and similar tonic modulations have been observed during mimicked stepping [34] or imagined walking [35,36] in PD patients undergoing PPN-DBS.

Figure 2



Descending projections from the MLR in the monkey. A tracer injection into the dorsal pole of the PPN and the ventral portion of the CuN in a monkey (Photomicrograph in **a**) resulted in numerous labeled terminals on a transverse section of the pontomedullar reticular formation (Map in **b**). BC, brachium conjunctivum; Gi, gigantocellular nucleus; ml, medial lemniscus; MLF, medial longitudinal fasciculus; o, olive; Py, pyramidal tract; V, trigeminal nucleus. Modified from Rolland *et al.* [21]. **(c)** Sagittal view of fibers (yellow) connecting the CuN (blue) and the limbic part of the subthalamic nucleus (STN) in a human using diffusion weighted imaging-based tractography. The sensorimotor, associative, and limbic anatomofunctional territories of the STN are represented in green, pink, and yellow, respectively. The PPN is represented in red.

The loss of PPN cholinergic neurons [20,37] as well as decreased cholinergic innervation of the thalamus [38] are correlated with the presence falls in PD. In unrestrained mice, optogenetic activation of cholinergic PPN neurons produces less dramatic locomotor effects compared to activating glutamate neurons, although short-duration excitation prolongs the stance phase during gait, whereas long-duration activation slows locomotion by increasing extensor burst duration [29••]. Cholinergic PPN lesions in monkeys induce postural deficits stemming mainly from axial rigidity [37] and young mice with selective mesopontine cholinergic deficiency are relatively normal in the open field, but exhibit deficits in locomotor learning, coordination and balance using tasks designed to assess-specific gait dysfunctions [39]. These results suggest that cholinergic PPN neurons may have roles in gait and balance disorders in PD patients that are distinct from the initiation and maintenance of locomotor state. This is consistent with recent work showing that PPN-DBS in PD patients with severe gait disorders showed improved control of postural sway [40] and anticipatory postural adjustments [41].

Non-cholinergic neurons, and more specifically GABAergic neurons, also degenerate in the PPN of PD patients, although this loss has not yet been linked with gait disorders [20,42]. Within the PPN, counts of non-cholinergic neurons (presumably primarily GABAergic and glutamatergic neurons) in PD patients are consistent with degeneration also of glutamatergic neurons. In light of the recent work highlighting the major role of glutamatergic neurons in initiating and maintaining locomotion, it would appear that degeneration of neurons with different neurotransmitter phenotypes likely contributes to different aspects of locomotor control underlying gait and balance disorders in advanced PD. No similar anatomical or functional data are available concerning the CuN. Further exploration of this nucleus is warranted in primates considering the marked anatomo-functional differences observed in mice. Such experiments may yield insights into why, for example, certain locomotor programs, such as running, can be preserved in PD patients who have difficulty walking.

The MLR controls arousal

The PPN cholinergic neurons are well known to regulate sleep/wake cycle, a function supported by the PPN-thalamo-cortical projection. Acetylcholine is released during wake and rapid eye movement (REM) sleep when gamma oscillations appear in thalamic and in cortical neurons [43,44]. Optogenetic activation of PPN cholinergic neurons in mice during non-rapid eye movement (NREM) sleep induces REM sleep [45], acting through GABAergic neurons in the thalamic reticular nucleus [46]. Chemogenetic experiments indicate that glutamatergic and GABAergic neurons in the PPN also modulate sleep [59]. Activation of glutamatergic neurons inducing wakefulness, while inhibition reduced wakefulness and increased NREM sleep. Activation of GABAergic neurons slightly reduced REM sleep.

Some research suggests a role of PPN neuronal loss in the sleep abnormalities seen in PD. Low-frequency PPN-DBS improves sleep quality in PD patients with severe gait disorders [47,48]. High-frequency stimulation induces non-rapid eye movement sleep whereas low frequency stimulation increases alertness [48]. Experiments in parkinsonian monkey also reported that lesioning PPN cholinergic neurons improves sleep quality after transient sleep impairment [49].

The MLR integrates multimodal information

Although aspects of walking are highly automated, walking without falling requires a certain amount of attention. This is perhaps unsurprising given the need to adapt muscular activations to environmental conditions that can rapidly change, and one might expect that vigilance and focused attention increase with the complexity of locomotor demands. Indeed, dual tasking while walking reduces gait speed [50], and the tendency to stop walking while talking predicts falls in elderly patients [51]. One recent study showed that some putative cholinergic neurons in the macaque PPN exhibit both increased activity during locomotion and decrease activity during slow-wave sleep, suggesting that individual MLR neurons may participate in controlling levels of arousal [52] presumably via dense ascending projections to the intralaminar thalamic nuclei as well as the basal ganglia [10].

The function of MLR neurons likely extends beyond attention strictly for locomotion, as individual neurons in the primate PPN are active during directed arm or eye movements [53,54] as well as arousing or alerting visual [35], auditory and somatosensory stimulation [36,55], rewards given in the context of correct task performance [55–57]. The reward-related activity may be related to observations that lesioning or inhibiting the PPN alters behavior during nicotine self-administration [54], and that optogenetic inhibition of PPN cholinergic neurons produces place aversion while activation of these neurons reverses it [30•]. Interestingly, while PPN and CuN neurons responding to different task components (reward, attention, sensory, motor) are spatially intermingled, they can be differentiated by their projection targets [30•,55]. In monkeys, PPN neurons with reward-related responses project preferentially to dopamine neurons in the medial substantia nigra pars compacta (SNc), whereas PPN neurons with sensorimotor or arousal signals projected more laterally in the SNc [55]. In mice, optogenetic modulation of PPN cholinergic terminals in the SNc affected locomotion but not place preference, whereas modulation of PPN cholinergic terminals in the ventral tegmental area (VTA) affected place preference but not locomotion [30•]. Thus, examining the nature of

the PPN and CuN activity projecting to a particular target structures will likely continue to be a fruitful strategy in dissecting MLR functions.

Conclusion and future direction

The primate MLR integrates numerous inputs from brain areas encoding cognitive, emotional, and sensorimotor information. This mixture of information may be important for locomotion in complex environments, and adapting locomotor programs to immediate and learned contingencies. Recent breakthroughs have revealed functional specialization of the different nuclei of the MLR, as well as different roles for neurons with different neurotransmitter phenotypes and projection targets. Clever use of optogenetic and chemogenetic techniques in monkeys is still needed to test the role of specific cell types in the primate MLR. Such data will be important for bridging the gap between new understandings of MLR function developed from mouse data and applications of neuromodulation therapies in human patients.

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Conflict of interest statement

Nothing declared.

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