Cellular-level understanding of supraspinal control: what can be learned from zebrafish?

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Highlights:

- Regions such as the MLR were discovered using electrical stimulation
- Genetic and optogenetic techniques are now revealing functional heterogeneity within brainstem nuclei
- Specific neurotransmitter identity has been linked to distinct functional roles
- We review advances across vertebrate models in understanding supraspinal control of locomotion

Abstract

Electrical stimulation of various brainstem nuclei has revealed sites capable of initiating and modulating locomotion. However, subsequent experiments have revealed that these regions are functionally heterogeneous. Modern techniques including molecular-genetic methods to label cell types and optogenetic approaches to manipulate neural activity are now enabling functional examination at the cellular level. In this review, we will highlight recent work revealing distinct roles for neurons of defined neurotransmitter identity and show how comparisons across species can stimulate new experimental and analytical approaches to further our understanding of how supraspinal populations function at a cellular level to control precise aspects of behaviour.

Introduction

Major brain regions responsible for locomotor control have been discovered by detecting spinal locomotor activity following pharmacological activation or electrical stimulation of different brain sites. One such site is the mesencephalic locomotor region (MLR), first discovered in cats [1]. The MLR is defined as a site at which the intensity of electrical stimulation is proportional to the strength of induced locomotion, but which lacks direct projections to the spinal cord. Such a region has since been identified in numerous vertebrate species including rats [2], salamanders [3], lamprey [4], and carp [5], establishing it as a conserved functional element in vertebrate locomotor control. Over the past few decades, subsequent studies have identified different sub-regions constituting the mammalian MLR, namely the cuneiform nucleus (CnF) and the pedunculopontine nucleus (PPN) [2]. These regions themselves are complex, composed of neurons of different neurotransmitter identity and receiving varied sources of afferent input from different brain regions (Figure 1A; [6,7]). Our growing understanding of these structural intricacies is now motivating research into what motor

instructions these separate neural populations might provide, and how this functional organisation compares across species. Such efforts are now possible due to technical advances that allow researchers to target specific neuronal groups for monitoring or manipulation independently from surrounding regions.

Functional architecture of the MLR

With the advent of viral and genetic techniques, particularly the use of Cre-inducible viruses, it is now possible to examine the functional role of different neurotransmitter populations within the mammalian MLR [8]. A recent study used this technique to express the optogenetic activator channelrhodopsin (ChR2) specifically in either glutamatergic, GABAergic or cholinergic neurons in the mouse MLR (Figure 1A; [9]). Selective photostimulation of glutamatergic MLR neurons could evoke locomotion with speeds similar to those produced by unrestricted electrical MLR stimulation. However, optogenetic stimulation of either GABAergic or cholinergic MLR cells produced no effects when the mouse was stationary, but did cause deceleration or acceleration respectively, when the mouse was running. The role of glutamatergic MLR neurons in locomotion is consistent with other experiments specifically stimulating these neurons [10], however the identification of specific cell types within the MLR that can exert opposing effects on speed was not apparent from earlier region-wide electrical stimulation. This neurotransmitter-level examination using optogenetic targeting indicates how precise motor instructions can be attributed to particular neuronal subtypes.

Building upon the finding that glutamatergic MLR neurons can initiate locomotion [9,10], two studies have recently identified distinct locomotor functions for glutamatergic cells in the CnF and PPN. These cell groups were targeted by localised viral injections and optical fibre placement. Josset et al. used optogenetic stimulation to specifically attribute initiation of locomotion to glutamatergic CnF neurons, but not those in the PPN [11]. In contrast, Caggiano et al. found that optogenetic stimulation of glutamatergic PPN neurons could also elicit locomotion, but only with high frequency stimulation [12]. Notably, the two glutamatergic populations do not show equal effects on locomotion initiation; PPN stimulation induced locomotion with longer latency and reduced speeds as compared to glutamatergic CnF stimulation. These two studies additionally used optogenetic [11] and chemogenetic [12] inhibition to demonstrate a requirement for glutamatergic CnF cells for high speed locomotion and glutamatergic PPN cells for low speed locomotion. Because such direct manipulations may not represent the activity of these regions in the course of normal physiology, Caggiano et al. conducted extracellular recordings from these separate glutamatergic groups, verifying the presence of high-speed selective neurons in the CnF, and low-speed selective neurons in the PPN. Furthermore, by characterising the differing connectivity of the CnF and PPN, Caggiano and colleagues were able to hypothesise how these distinct behaviours could arise from separate neural pathways. These studies illustrate the functional diversity that can be found through progressively focussed investigation of individual cell groups (Figure 1B).

Brainstem structural and functional diversity

Attributing specific functional roles to neuronal subtypes can also be applied to supraspinal circuits downstream of the MLR. In mammals, the gigantocellular nuclei of the medial reticular formation receive inputs from the MLR [13], and within these nuclei there exist multiple neuronal populations with distinct neurotransmitter expression [14]. A recent study found that while optogenetic stimulation of the entire lateral paragigantocellular nucleus (LPGi) failed to induce or modulate locomotion, selective viral targeting revealed dramatically opposing effects of specific cell types [15].

Optogenetic photoactivation of glutamatergic LPGi neurons initiated locomotion in mice, whilst a similar stimulation of glycinergic LPGi neurons produced locomotor arrest. Neurons promoting immobility have also been recently described in the rostral medulla of mice [16]. In this case, optogenetic stimulation of genetically targetable glutamatergic V2a neurons halts ongoing locomotion. Thus, as in the MLR, targeting of distinct cell types is starting to delineate specific and opposing functions within supraspinal control regions. Supraspinal regions containing mixtures of neurons with distinct physiological roles is likely to be a conserved feature across species: recent experiments in lamprey have identified brainstem neurons related to swim cessation located amongst cells associated with initiating and maintaining locomotion [17].

Insights from zebrafish: linking identifiable brainstem neurons to specific locomotor manoeuvres

Larval zebrafish provide a model system where it is possible to come close to understanding the relationship between the single cells within supraspinal populations and precise aspects of behaviour. In this model organism, reticulospinal neurons are highly stereotyped and many are uniquely identifiable across individual animals [18]. In addition, larval zebrafish perform a range of kinematically distinct swim bout types, which are deployed in particular behavioural contexts [19], allowing researchers to investigate a multitude of behaviours and detailed kinematics beyond the initiation and cessation of locomotion. Due to the optical transparency of larval zebrafish, experimenters have also been able to conduct *in vivo* calcium imaging of reticulospinal populations during execution of these behaviours, or during presentation of well-defined sensory stimuli which evoke specific swim types.

In a pioneering study, Gahtan and colleagues performed calcium imaging of the larval zebrafish reticulospinal population during tap-evoked escape responses [20]. Subsequent studies have used calcium imaging to monitor reticulospinal activity in relation to sensory stimuli that reliably induce particular behaviours [21–23], or alongside direct recording of specific swim patterns [24,25]. Furthermore, by rigorously characterising behaviour and testing the role of functionally identified cells using precise cell ablations or stimulation, individual neurons in the larval zebrafish hindbrain have successfully been linked with the generation of specific kinematic features such as turn amplitude, tail posture, and swimming speed [24–26] (Figure 2A).

Targeted electrophysiological recordings from identifiable neurons are a powerful means to examine connectivity within hindbrain motor control circuits. A recent study used whole cell recordings to establish the connectivity of glycinergic feedforward (FF) inhibitory neurons within the Mauthner escape circuit of larval zebrafish [27]. By making use of anatomical neurotransmitter patterning [28] (Figure 2B), circuit modelling, laser ablations and behavioural assays, FF neurons were shown to function within a circuit motif that establishes left-right escape direction and prevents maladaptive bilateral activity in response to ambiguous sensory stimuli.

Larval zebrafish also provide opportunities for minimally biased identification of neural circuits related to specific features of locomotion, throughout the brainstem. Such studies can benefit from detailed characterisation of the molecular phenotypes of hindbrain neurons [29], the creation of specific driver lines [30], and the establishment of open-access community brain atlases [31–33]. A recent study by Severi and colleagues utilised these tools to describe the neurotransmitter phenotype and transcription factor expression of hindbrain neurons active during motor output or in response to visual stimulation [34]. One group of swim-related cells were identified as glycinergic and *engrailed1b* positive, setting the stage for future experiments to determine exactly how these

inhibitory cells influence behaviour. Community brain atlases that allow researchers to aggregate functional and anatomical data across experiments are becoming increasingly powerful tools for multiple model species [35–37].

Concluding remarks

Modern viral and genetic techniques have provided researchers with the opportunity to explore the roles of distinct neuron groups in behavioural control. Examining individual cell groups has allowed researchers to attribute specific functions to these groups that were otherwise masked through non-specific stimulation of brain areas. This has revealed subtle and even contrasting effects on locomotion between separate cell groups within canonical regions such as the MLR, as well as in downstream populations. Research in small model organisms such as larval zebrafish provide a system where it is possible to examine supraspinal control and the single-cell level. Recent zebrafish work illustrates the importance of detailed characterisation of the features of the motor output being examined and the advantages of combining genetic, electrophysiological, and optical techniques.

For future experiments it will be important to consider other cell categories beyond neurotransmitter type, especially as some cells display a change in neurotransmitter identity over time as a form of plasticity in response to variations in physiological conditions [38,39]. Distinct cell types can be characterised based on anatomical position, connectivity, morphology and gene expression patterns. Investigation of supraspinal circuits will benefit from the use of open-access brain atlases, which will allow researchers to directly compare data. Developments in advanced optical techniques such as combined volumetric imaging and optogenetics [40], and online, “closed-loop”, all-optical circuit manipulation [41,42], provide the exciting opportunity to functionally identify and causally test the roles of individual neurons and groups during behaviour in different vertebrate species.

Figure Legends

Figure 1. Cellular-level understanding of supraspinal control, exemplified by functional diversity within the MLR.

A) Sagittal view of the mouse brain highlighting supraspinal regions featured in this review. Inset illustrates the locations and neurotransmitter diversity of nuclei within the MLR. CnF, cuneiform nucleus; PPN, pedunculopontine nucleus; LPGi, lateral paragigantocellular nuclei.

B) Schematic summarising the effects of stimulating different types of MLR neuron on locomotor behaviour.

Figure 2. Summary of larval zebrafish supraspinal organisation.

A) Schematic of a larval zebrafish brain with reticulospinal neurons outlined in black. Individual reticulospinal cells (coloured) have been associated with specific kinematic features including swim speed (nMLF, blue) and turning amplitude (ventromedial cells, green).

B) Cross section at rostro-caudal level indicated in 5A. Neurons are organised in “stripes” with distinct patterns of neurotransmitter and transcription factor expression. Adapted from Kinkhabwala et al., 2011 [29].

Annotated references

**Capelli et al., 2017
In this elegant paper, mammalian brainstem populations with conflicting roles were disentangled by optogenetically isolating excitatory and inhibitory subpopulations.

**Caggiano et al., 2018
Caggiano and colleagues use optogenetics and chemogenetics to show that glutamatergic neurons in the PPN and CnF contribute to slow and high-speed locomotion respectively.

*Josset et al., 2018
In this work, the authors used optogenetic stimulation to determine the initiation of locomotion was localised specifically to glutamatergic CnF neurons, whereas glutamatergic and cholinergic neurons in the PPN contribute to other aspects of locomotion in freely behaving mice.

*Severi et al., 2018
Using large scale calcium imaging and open-access brain atlases, the authors mapped locomotion-related active regions to specific inhibitory neurotransmitter and transcription factor groups in the zebrafish hindbrain.

*Dal Maschio et al., 2017
Using a sophisticated combination of techniques, Dal Maschio and colleagues combine simultaneous 3D stimulation of multiple targeted neurons, volumetric functional imaging, and behavioural tracking to investigate circuit function in larval zebrafish.

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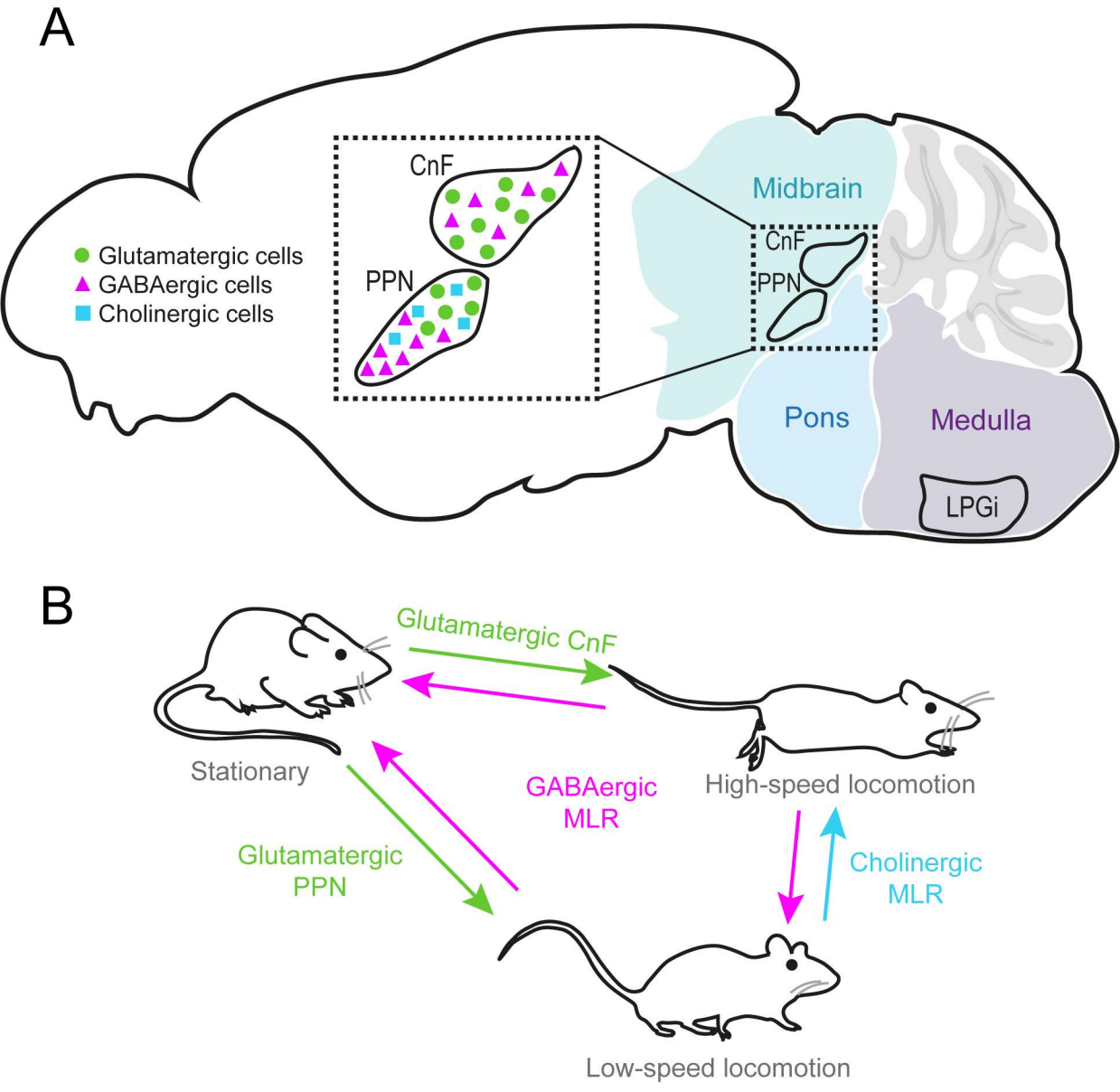
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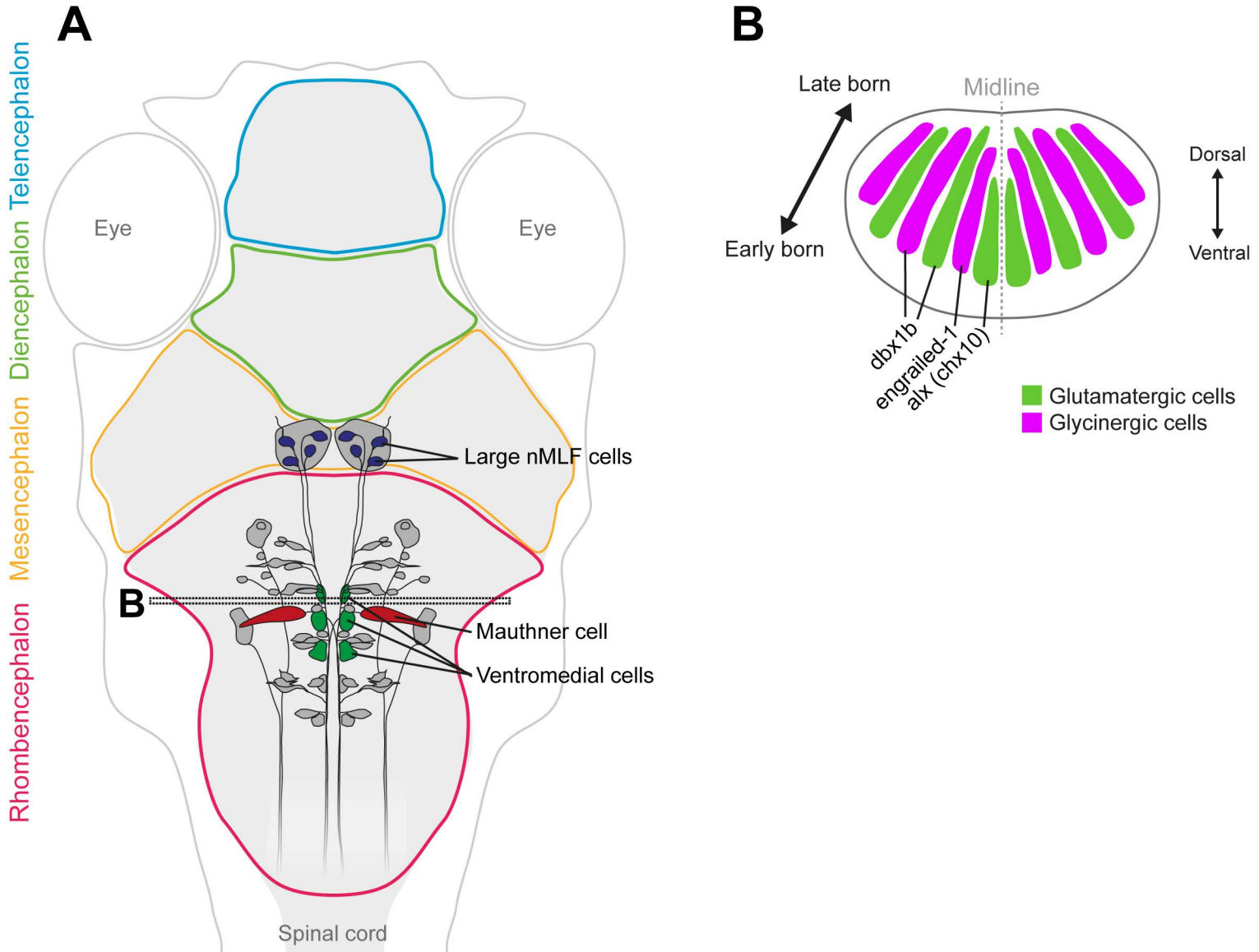
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engrailed-1
alx (chx10)
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Glycinergic cells