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Mesencephalic locomotor region stimulation—cuneiform or pedunculopontine?

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Roussel et al.[1] provide new insight into mecencephalic locomotor region (MLR) stimulation to treat spinal cord injury in mice. Previously, it was unclear which part of the MLR to target. Now, evidence converges on cuneiform nucleus activation.

Experiments in the mid-1960s identified the mecencephalic locomotor region (MLR), a midbrain structure that, when stimulated, elicits stepping and even running in the decerebrated cat.[2] The MLR and its function are evolutionarily conserved across species, including lamprey, rodents, and primates.[3] The effects of MLR stimulation on locomotion have been studied across health and disease. Here, the authors investigate the effects of MLR stimulation in a murine model of chronic spinal cord injury as a therapeutic avenue to promote recovery. In technically demanding experiments, multiple behavioral, kinematic, and electrophysiological readouts are used to refine our understanding of MLR stimulation in the context of optogenetic and genetic manipulations.

Spinal cord injury disrupts descending input from supraspinal centers, resulting in paralysis. However, below the lesion, circuitry that generates many aspects of hindlimb locomotion is often intact. Spinal cord motor networks themselves are neuronal assemblies capable of producing different patterns and rhythms of locomotion,[4] but even though the majority of spinal cord injuries are incomplete, remaining spared supraspinal input fails to engage these networks. The MLR projects to the medial medullary reticular formation and, via spared reticulospinal axons, could serve to activate intact locomotory circuitry below the level of the lesion. This makes it an attractive supraspinal candidate to target using deep brain stimulation (DBS).

While early stimulation studies defined an anatomical region able to initiate movement and modulate speed, the molecular and cellular heterogeneity of the MLR was underappreciated until quite recently. There are differential roles for neuronal populations within the cuneiform (CnF) and pedunculopontine nuclei (PPN) within the MLR. Groundbreaking studies using genetic and viral tools dissected glutamatergic and cholinergic function in the CnF and PPN. These broadly converged on a model where CnF orchestrates fast-escape responses from stationary and change speed, whereas PPN acts mostly downstream of basal ganglia during slower exploratory behavior.[5–7]

But what does this mean for stimulation strategies to promote recovery from spinal cord injury? In the context of a current clinical trial that aims to use MLS-DBS following spinal cord injury,[8] this is important to define. Indeed, in rats, both CnF stimulation[9] and PPN stimulation[10] have been proposed to promote recovery, the latter when combined with local lumbar epidural stimulation. Now, combining mouse genetics and activation and ablation strategies alongside sensitive outcome measures, Roussel et al.[1] aim to probe and pinpoint any distinction with greater accuracy.

Mice exhibit capacity for some spontaneous recovery following spinal injury. Roussel et al.[1] first focused on this spontaneous recovery following a thoracic lateral hemisection model of spinal cord injury. This is a one-sided transection lesion of the spinal cord, abolishing the MLR input on that same (left, ipsilesional) side but largely sparing that coming from the contralesional MLR. Consequently, these mice have paralysis in the left hindlimb, which gradually improves over days and weeks: they eventually step but retain deficits. They first found that the anatomical organization of glutamatergic and cholinergic MLR neurons that project to brainstem locomotor circuits, which subsequently project to spinal cord, is maintained following this injury model. Second, they characterized the contribution of the contralesional CnF and PPN to spontaneous recovery. These are axons spared by the injury and still projecting to the lumbar spinal cord, which could mediate spontaneous improvements in limb function. They did this by diptheria toxin (DTX) ablation of each population after spontaneous recovery occurred. In both overground locomotion and swimming performance measured by hindlimb kinematics, deletion of CnF had a more profound impact than deletion of PPN. Next, before injury and at various timepoints following spontaneous recovery, they placed the mice on a treadmill and optogenetically stimulated the contralesional (spared, projecting) CnF or PPN while simultaneously recording flexor-extensor electromyography (EMG) to analyze the degree of muscle movement in response (flexor and extensor muscles work antagonistically during optimal stepping). They found that excitatory CnF, but not PNN, stimulation correlated with spontaneous improvement in locomotor score over time. It looks like CnF could mediate aspects of spontaneous recovery.

So could stimulation be used as a therapeutic strategy to promote recovery beyond that which occurs spontaneously? To test this, they took mice with a chronic spinal cord injury who already recovered spontaneous function to the function's limits: these mice step, but

not as effectively as an uninjured mouse. They then stimulated the CnF during overground locomotion while measuring EMGs and analyzing walking performance using kinematics. CnF simulation itself initiated walking behavior. EMGs and kinematic analysis of stepping performance improved. Taken together, CnF stimulation modulates spatiotemporal muscle recruitment and improves coordination and walking speed overground.

Next, in the chronically injured mice, they tested locomotor performance in a swimming task and directly compared CnF stimulation with PNN stimulation. Here, PNN stimulation decreased swimming speed, whereas CnF stimulation improved performance. Thus, mechanistically, these results suggest that the CnF is important for spontaneous locomotor recovery, and its stimulation can modulate muscle firing to improve locomotion farther than occurs spontaneously in chronic spinal injured mice.

Whether the reasonably extensive sparing in this particular lateral hemisection model could influence relative contribution of CnF and PNN could still be questioned. However, this is not the first study to evidence that CnF should be the target for DBS.[9] Indeed, using a chronic severe bilateral contusion injury in rats, which closely models the type of injury most commonly found in the clinic, electrophysiological CnF stimulation was shown to promote recovery.[9] Thus, the convergence of these two studies provides evidence that CnF stimulation could prove an effective therapeutic target for improvement in lower limb function, especially in the population of individuals recruited for the current clinical trial[8] who have some intact motor function below the level of lesion.

REFERENCES

1. Rousell, M.L.-Z.,D., Josset, N., Lemieux, M., and Bretzner, F. (2023). Functional Contribution of Mesencephalic Locomotor Region Nuclei to Locomotor Recovery after Spinal Cord Injury. Cell Rep. Med. *4*, 100946.
2. Shik, M.L., Severin, F.V., and Orlovskiĭ, G.N. (1966). Control of walking and running by means of electric stimulation of the midbrain. Biofizika *11*, 659–666.
3. Orlovsky, G., Deliagina, T., and Grillner, S. (1999). Neuronal Control of Locomotion: From Mollusc to Man.
4. Kiehn, O. (2016). Decoding the organization of spinal circuits that control locomotion. Nat. Rev. Neurosci. *17*, 224–238. https://doi.org/10.1038/nrn.2016.9.
5. Roseberry, T.K., Lee, A.M., Lalive, A.L., Wilbrecht, L., Bonci, A., and Kreitzer, A.C. (2016). Cell-type-specific control of brainstem locomotor circuits by basal ganglia. Cell *164*, 526–537. https://doi.org/10.1016/j.cell.2015.12.037.
6. Josset, N., Roussel, M., Lemieux, M., Lafrance-Zoubga, D., Rastqar, A., and Bretzner, F. (2018). Distinct contributions of Mesencephalic locomotor region nuclei to locomotor control in the Freely behaving mouse. Curr. Biol. *28*, 884–901.e3. https://doi.org/10.1016/j.cub.2018.02.007.
7. Caggiano, V., Leiras, R., Goñi-Erro, H., Masini, D., Bellardita, C., Bouvier, J., Caldeira, V., Fisone, G., and Kiehn, O. (2018). Midbrain circuits that set locomotor speed and gait selection. Nature *553*, 455–460. https://doi.org/10.1038/nature25448.
8. Stieglitz, L.H., Hofer, A.S., Bolliger, M., Oertel, M.F., Filli, L., Willi, R., Cathomen, A., Meyer, C., Schubert, M., Hubli, M., et al. (2021). Deep brain stimulation for locomotion in incomplete human spinal cord injury (DBS-SCI): protocol of a prospective one-armed multi-centre study. BMJ Open *11*, e047670. https://doi.org/10.1136/bmjopen-2020-047670.
9. Hofer, A.-S., Scheuber, M.I., Sartori, A.M., Good, N., Stalder, S.A., Hammer, N., Fricke, K., Schalbetter, S.M., Engmann, A.K., Weber, R.Z., et al. (2022). Stimulation of the cuneiform nucleus enables training and boosts recovery after spinal cord injury. Brain *145*, 3681–3697. https://doi.org/10.1093/brain/awac184.
10. Bonizzato, M., James, N.D., Pidpruzhnykova, G., Pavlova, N., Shkorbatova, P., Baud, L., Martinez-Gonzalez, C., Squair, J.W., DiGiovanna, J., Barraud, Q., et al. (2021). Multipronged neuromodulation intervention engages the residual motor circuitry to facilitate walking in a rat model of spinal cord injury. Nat. Commun. *12*, 1925. https://doi.org/10.1038/s41467-021-22137-9.