Motor Systems Symposium

Friday, November 2, 2018
Salk Institute for Biological Studies
La Jolla, California

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Salk Institute Motor Symposium
November 2, 2018

7:30 am - Bus pickup downtown

8:00 - 9:15 am - Registration and Breakfast

9:15–10:45 am - Short Talk Session 1: Sensorimotor Circuits
9:15–9:30 am: Turgay Akay, Dalhousie University, Canada
Proprioceptive Control of Muscle Synergies during Normal and Cautious Walking
9:30–9:45 am: Aya Takeoka, KU Leuven, Belgium
Functional Local Proprioceptive Feedback Circuit Sustains Locomotor Recovery after Spinal Cord Injury
9:45–10:00 am: Sonia Paixao, Max Planck Institute of Neurobiology, Germany
Identification of Spinal Neurons Contributing to the Dorsal Column Projection and Mediating Fine Touch and Corrective Motor Movements
10:00–10:15 am: Graziana Gatto, Salk Institute for Biological Studies, USA
Dissecting the Organization and Logic of Spinal Circuits for Sensorimotor Coding
10:15–10:30 am: Hendrik Wildner, University of Zurich, Switzerland
The Role of Inhibitory Pvalb Interneurons in Sensory Circuits
10:30–10:45 am: Yuanyuan Liu, Harvard Medical School, USA
Touch and Tactile Neuropathic Pain Sensitivity are set by Corticospinal Projections

10:45–11:15 am - Coffee Break

11:15–12:45 pm Short Talk Session 2: Interneurons, Motor Circuits and Breathing
11:15–11.30 am: Laurence Picton, Karolinska Institute, Sweden
Diversity of Glycinergic V0d Interneurons and their Function in the Zebrafish Locomotor Circuit
11:30–11:45 am: Lora Sweeney, Salk Institute for Biological Studies, USA
Defining Inhibitory Neurons for Limb and Thoracic Movement
11:45–12:00 pm: Ngoc Ha, Drexel University, USA
Intrinsic Properties and Connectivity of Spinal Flexor and Extensor Rhythm Generating Neurons
12:00–12:15 pm: Simon Danner, Drexel University
Interactions between Spinal Circuits and Afferent Feedback to Control Locomotion at Different Speeds: Insights from Computational Modeling
12:15–12:30 pm: Sufyan Ashhad, University of California, Los Angeles, USA
Active Synchronization Underlies Rhythmogenesis in the preBötC Microcircuit
12:30–12:45 pm: Silvia Pagliardini, University of Alberta, Canada
Role of the Parafacial Respiratory Group in the Recruitment of Active Expiration During Sleep

12:45 – 2:15 pm – Lunch and Posters

2:15-2:45 pm: Adam Hantman, Janelia Research Campus, HHMI, USA
Neural Circuits of Dexterity

2:45–4:00 pm Short Talk Session 3: Supraspinal Systems and Behavior
2:45–3:00 pm: Shreyas M. Suryanarayana, Karolinska Institute, Sweden
Connectivity, Cytoarchitecture, Visual, Somatosensory and Motor Maps in the Lamprey Lateral Pallium – A Primordial Vertebrate Cortex
3:00-3:15 pm: Nicole Mercer Lindsay, University of California, San Diego, USA
Motor Cortex Descending Projections Drive Orofacial Behaviors through Specific Brainstem Premotor Networks
3:15-3:30 pm: Spyridon Karadimas, University of Toronto, Canada
Sensory Cortical Control of Movement
3:30–3:45 pm: Matthew Becker, University of Colorado, USA
Control of Reach Kinematics by the Cerebellum
3:45–4:00 pm: Teja Pratap Bollu, Cornell University, USA
Motor Cortex Inactivation Contracts Tongue and Forelimb Trajectories in Mice

4:00-4:30 pm - Coffee Break

4:30–5:15 pm - Past and Future Perspectives

5:15pm–6:30 pm - Drinks and Posters

6:30–8:30 pm - Dinner

8:45pm - Bus departs for downtown

Short-Talk Abstracts

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Proprioceptive Control of Muscle Synergies during Normal and Cautious Walking

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Locomotor behaviour is highly adaptable to the environment providing animals with the flexibility to move in an unpredictable environment. When terrestrial animals locomote, the nervous system has to control the contraction of considerably higher number of muscles than the minimal number that is necessary (redundancy) leading to an enormous number of combinations of muscle contractions to achieve the movement. One concept to address this issue suggests that the CNS controls groups of muscles (muscle synergies -MS), rather than individual muscles. The MS has been investigated during human locomotion under different circumstances but the role of sensory feedback in the adjustments of MSs to the environment has been obscure. We used non-negative matrix factorization to extract MSs from electromyogram (EMG) activity recordings from multiple muscles during normal walking and "cautious" walking. Each MS was divided into a time-invariant "motor module" representing the drive to defined groups of muscle with a certain strength and motor primitives representing the activation of the motor modules over time. We recorded EMG activities from up to seven muscles during walking in wild-type mice (WT) and in mice without proprioceptive feedback from the muscle spindles (*Egr3-KO*). Our data show in both mice, three MS are sufficient to describe the locomotor pattern during walking. Wild-type mouse does adapt the locomotor pattern to compensate for changes in the environment whereas the *Egr3-KO* does not. We show that the locomotor pattern during walking in *Egr3-KO* is less accurate, with limited adaptability, especially when the walking movement is perturbed unexpectedly causing the animal to walk more "cautiously." Proprioceptive feedback from MS is necessary for the precision of the locomotor pattern that is adjustable to perturbations, enabling the animal to walk under different circumstances.

Functional Local Proprioceptive Feedback Circuit Sustains Locomotor Recovery after Spinal Cord Injury

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Somatosensory feedback from proprioceptive afferents is essential for locomotor recovery after spinal cord injury. However, mechanisms of action including how or when proprioception is required remain unknown. Here we establish an intersectional genetic model for proprioceptive afferent ablation with spatial and temporal confinement to reveal requirements of proprioceptive afferents for locomotor control and recovery. Complete or spatially confined proprioceptive afferent ablation in intact mice differentially affects locomotor performance. Following incomplete spinal cord injury, proprioceptive afferent ablation below but not above lesion severely restricts locomotor recovery. Proprioceptive afferents are also essential for maintaining regained locomotor function since their ablation after behavioral recovery permanently reverts functional improvements. In parallel to recovery, proprioceptive afferents below lesion undergo activity-dependent synaptic connectivity reorganization to specific local spinal targets. Together, our study reveals that proprioceptive feedback promotes recovery by interacting with local spinal circuits through synaptic reorganization and serves as a continued driving force to produce locomotor output after injury.

Identification of Spinal Neurons Contributing to the Dorsal Column Projection and Mediating Fine Touch and Corrective Motor Movements

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The dorsal spinal cord is the integrative center that processes and transmits a variety of somatosensory information, including the tactile stimuli that provide us with the capacity for texture discrimination and fine motor control. The molecular identity and function of dorsal horn interneurons (INs) and projection neurons in touch-related spinal cord circuits is still largely unknown.We previously identified a population of INs located in laminae III-V marked by the co-expression of the transcription factor Zic2 and the axon guidance receptor EphA4, which we hypothesized to receive tactile stimuli and to send their axons into an ascending pathway to the brain (Paixão et al., 2013). We generated a Zic2 inducible-cre line and, using a trans-synaptic rabies virus approach, confirmed that Zic2 INs receive sensory inputs from cutaneous afferents. Genetic tracing experiments revealed that at least a subset of Zic2 INs sends projections to the cuneate nucleus, indicating that Zic2 represents a molecular marker of projection neurons forming the postsynaptic dorsal column. Furthermore, Zic2 INs receive monosynaptic inputs from several descending brain centers mainly involved in motor control, like the motor cortex, red nucleus and numerous brainstem nuclei. In turn, Zic2 INs seem to synapse onto spinal motor neurons raising the interesting possibility that Zic2 INs could play a role in the integration of sensory and motor spinal circuits. Using an intersectional genetic approach, we observed that the ablation of Zic2 spinal INs specifically reduces light touch sensitivity and the ability of textural discrimination. Moreover, these mice show an impairment in fine motor control when challenged to cross thin elevated beams. In a complementary approach, chemogenetic activation of Zic2 spinal INs leads to an increased sensitivity to light touch stimuli without affecting nociceptive behaviors. Using retrograde virus, we are currently addressing the functional significance of the Zic2 dorsal column projections. Preliminary behavioral analysis points to the relevance of this pathway in the processing of light touch. Our data suggests a model in which Zic2 INs specifically participate in processing of light touch information, promoting corrective movements and the conveyance of tactile information to the brain.

Dissecting the Organization and Logic of Spinal Circuits for Sensorimotor Coding

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The somatosensory system plays a pivotal role in controlling movement via its reciprocal interactions with the spinal locomotor circuitry. Although rhythmic motor behaviors such as locomotion can be elicited by central pattern generator networks in the absence of sensory input, sensory feedback is required for generating protective reflexes, adapting ongoing movement to environmental perturbations and postural changes, and for shaping complex motor behaviors. Growing evidence support a prominent role of dorsal spinal cord interneurons (dINs) in processing cutaneous and descending inputs. What is not known is how this information is integrated and conveyed to other elements of the motor system to shape both reflexive and corrective movements. By using a holistic approach that includes the combinatorial application of optogenetic, pharmacological and intersectional genetics, we identified a multilayered structure of dorsal spinal dIN populations, with each layer of neurons differentially contributing in generating protective and corrective reflexes. We have characterized multiple molecular markers: CCK, Somatostatin, Calbindin, Calretinin, PKCγ, and RORα that define specific dIN subpopulations that have “layer-enriched” domains of expression in the adult spinal cord. By genetically manipulating the activity of these IN subpopulations we find a striking correlation between laminar localization and behavior. INs localized in superficial dorsal horn laminae contribute to protective itch-induced scratch and noxious mechanical-induced withdrawal reflexes, whereas those localized in the deeper laminae contribute to dynamic touch and corrective motor reflexes. Taken together, our data suggest that laminar location is a key element in defining the functional organization of sensory interneuron cell types, with the dorsal horn being organized in coherent heterogeneous layers that drive stimulus specific motor actions.

The Role of Inhibitory Pvalb Interneurons in Sensory Circuits

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Spinal Glycinergic neurons are essential gate keepers of noxious information such as heat, cold, mechanical pain or puritogen induced itch. Here we study the Pvalb positive subset of Glycinergic interneurons using an intersectional approach. Inhibitory spinal Pvalb+ interneurons constitute about 1/3 of the entire glycinergic dorsal horn population. We find that loss of Pvalb+ glycinergic interneurons specifically evokes touch hypersensitivity but no hypersensitivity to heat or cold stimuli. We also find that loss of Pvalb+ glycinergic interneurons evokes behavior reminiscent of spontaneous itch. We suggest that inhibitory Pvalb+ interneurons of the spinal dorsal horn control *innocuous* sensory input from vGlut1+ primary afferents onto itch relaying Grp+ and deep dorsal horn neurons and that loss of this inhibition results in mechanical hypersensitivity and mechanical itch.

Touch and Tactile Neuropathic Pain Sensitivity are set by Corticospinal Projections

Yuanyuan Liu[1], Alban Latremoliere[1,2], Xinjian Li[3], Zicong Zhang[1], Mengying Chen[1], Xuhua Wang[1], Chao Fang[1], Chloe Alexandre[2], Zhongyang Gao[1], Bo Chen[1], Xin Ding[1], Jin-Yong Zhou[1], Yiming Zhang[1], Chinfei Chen[1], Kuan Hong Wang[3], Clifford J. Woolf[1], and Zhigang He[1]
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Current models of somatosensory perception emphasize transmission from primary sensory neurons to the spinal cord and on to the brain. Mentalinfluence on perception is largely assumed to be acting locally within the brain. We have now examined if there is top-down control of sensory inflow through the spinal cord directly by the cortex. Although traditionally viewed as a primary motor pathway, a subset of corticospinal neurons (CSNs) originating in the S1/S2 somatosensory cortex directly innervate the spinal dorsal horn via corticospinal tract (CST) axons. We show here that either reduction in somatosensory CSN activity or transection of the CST selectively impairs behavioral responses to light touch without altering responses to noxious stimuli. Moreover, such CSN manipulation greatly attenuates tactile allodynia in a peripheral neuropathic pain model. Tactile stimulation activates somatosensory CSNs and their corticospinal projections facilitate light touch-evoked activity of cholecystokinin (CCK+) interneurons in the deep dorsal horn. This represents a touch-driven feed forward spinal-cortical-spinal sensitization loop, which is important for the recruitment of spinal nociceptive neurons under tactile allodynia. These results reveal direct cortical modulation of normal and pathological tactile sensory processing in the spinal cord and open up opportunities for new treatments for neuropathic pain.

Diversity of Glycinergic V0d Interneurons and Their Function in the Zebrafish Locomotor Circuit

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An intrinsic feature of locomotion is the ability to transition between different speeds while maintaining appropriate alternation of limb and axial muscles. While key circuit mechanisms for speed control have been revealed in adult zebrafish, those controlling reciprocal alternation remain unclear. A study in mice has suggested that inhibitory commissural interneurons (V0d) control limb alternation at slow speeds, whilst excitatory commissural interneurons (V0v) take over at faster speeds. In adult zebrafish, most V0v interneurons are recruited only during fast swimming, yet little is known about the adult V0d population. Here, we characterise the glycinergic V0d population in zebrafish. There were ~10 V0d interneurons per spinal hemi-segment, significantly fewer than other interneurons in the swim network. Targeted patch-clamp recordings in larvae revealed a majority of V0d interneurons to be recruited only during fast swimming. By contrast, in adult zebrafish, our analysis revealed three main V0d classes - fast, intermediate, and slow - which are recruited at their respective swim speeds and displayed partially overlapping electrophysiological profiles. However, we found an overall predominance of slow-type V0d interneurons, in contrast to the larva. Furthermore, the axon projections and dendritic trees of adult V0d interneurons were heterogeneous, even within classes, suggesting both functional and anatomical changes during development. To test the overall function of the adult V0d population we performed 2-photon ablations of 20-40 V0d interneurons across 5 spinal segments and recorded contralateral, back-labelled motoneurons (MNs). Preliminary evidence showed that midcycle inhibition was sustained only briefly during a swim episode, before reducing in amplitude such that MN firing became tonic and a coordinated swim pattern collapsed. Finally, our analysis identified a distinct, apparently specialised, V0d subtype in the adult. This subtype occupies a specific dorso-medial position in the spinal cord, and displayed a stereotyped morphology, electrophysiological profile, and activity pattern that all indicate that it may be specialised for escape. Overall, we show that the zebrafish V0d interneuron population undergoes considerable developmental changes in function and morphology, from a relatively homogenous population contributing to fast swimming, to a more heterogeneous population largely involved in slow swimming but supplemented by specialised subtypes. In the adult, the V0d population appears to be critical for the appropriate maintenance of locomotion.

Defining Inhibitory Neurons for Limb and Thoracic Movement

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Motor output varies along the rostro-caudal axis of the spinal cord. At limb levels, diverse pools of motor neurons coordinate paired flexor and extensor muscles about a joint for coarse and fine motion such as running and grasping. At thoracic levels, motor neurons drive abdominal and paravertebral muscle contraction for breathing and posture, and the sympathetic chain for autonomic nervous system function. We have investigated how these differences in motor complexity along the body axis are reflected in the cardinal V1 class of inhibitory neurons.Using a combination of transcription factor profiling, Bayesian statistical analysis, and machine-learning, we have examined whether V1 inhibitory neurons differ in their molecular heterogeneity along the rostro-caudal axis of the spinal cord during mouse development (Sweeney et al., 2018). We find many features of V1 diversity are shared at all levels, including that the same four transcription factors subdivide the majority of V1 inhibitory neurons into four non-overlapping clades. However, we also discover substantial differences in inhibitory cell type heterogeneity at limb and thoracic levels. These differences in V1 subtypes between segments arise, not from the expression of a single transcription factor, but from a combinatorial code—with two or more transcription factors defining level-specific subpopulations of thoracic and limb V1 neurons. Our studies support the idea that molecularly-distinct inhibitory subpopulations exist to subserve limb and thoracic movement. To explore this possibility further, we have analyzed V1 diversity during *Xenopus* metamorphosis. We find that motor and V1 inhibitory neurons increase in number and transcriptional heterogeneity as tadpoles become frogs, with a conserved transcriptional logic associated with thoracic and limb motor output across species.

Sweeney et al. Origin and Segmental Diversity of Spinal Inhibitory Interneurons. Neuron. 2018 Jan17; 97(2):341-355.

Intrinsic Properties and Connectivity of Spinal Flexor and Extensor Rhythm Generating Neurons

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The central pattern generator (CPG) controlling hindlimb locomotion is located in the thoracolumbar region of the spinal cord. Among other interneurons (INs), this CPG includes the rhythm-generating (RG) interneurons, which can convert tonic input received from the brainstem into rhythmic output, providing the necessary drive to downstream neurons in order to generate coordinated locomotor behavior. The mechanisms contributing to rhythmogenesis in these RG interneurons are hypothesized but still largely unknown. Findings in other rhythmic systems suggest that rhythmogenesis can result from activities of individual neurons, from excitatory connections between neurons, or from both. Recently, Shox2 INs have been identified to be one of the cellular components of the rhythm generator for locomotion. During drug-evoked locomotion *in vitro*, Shox2 INs are rhythmically active and can be further divided into flexor- or extensor-RG interneurons based on their phase preferences in correlation to the flexor- or extensor-dominant ventral root activity. The goal of the present study is to determine the underlying mechanisms contributing to the rhythmic activity of the flexor and extensor RG Shox2 INs, with focus on connectivity and intrinsic properties related to excitability and rhythmogenesis. We performed whole cell patch clamp recordings from identified Shox2 INs in reduced isolated spinal cord preparations from neonatal Shox2:Cre; tdTomato mice. We previously showed that excitatory synaptic connections and electrical coupling are present between Shox2 INs. Here, we demonstrate that Shox2 IN interconnectivity is specific to functional populations of flexor and extensor RG populations respectively, with a higher degree of connectivity between flexor-related Shox2 INs. Potential rhythmogenic currents, such as the persistent inward current, are present in both the flexor and extensor Shox2 INs. Together, this suggests that both rhythmogenic currents and the interconnectivity, particularly within the flexor Shox2 IN population, likely contribute to rhythmic activity of Shox2 rhythm generating INs.

Interactions between Spinal Circuits and Afferent Feedback to Control Locomotion at Different Speeds: Insights from Computational Modeling

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To survive, animals need to be able to quickly adapt limb coordination and locomotor speed. Recent optogenetic studies in mice have shown that slow locomotion observed during exploration behavior and fast locomotion observed during escape are differently controlled by distinct supraspinal structures. Yet, how the spinal circuits control slow and fast locomotion are less well understood.We have developed a neuromechanical computational model of hindlimb locomotion in mouse and used it to study the mechanisms of sensorimotor integration and the role of different afferent pathways in the stabilization of locomotion at different speeds and in different environmental conditions. The model includes a neural network model of the spinal circuits controlling and coordinating limb movements, coupled with a 2D musculoskeletal model of the mouse hindlimbs (3 joints and 7 muscles per limb). Each limb is controlled by a two-level central pattern generator (CPG) as well as circuits mediating basic reflexes and motoneurons actuating hindlimb muscles. The muscle velocity- (Ia), force- (Ib) and length-dependent (II) as well as cutaneous feedbacks interact with all levels of the locomotor circuitry. CMA-ES (covariance matrix adaptation evolution strategy) was used to find a pattern of afferent feedback connections to the spinal circuits that provide stable locomotion at different speeds and on level as well as sloped surfaces.The proposed connectome of spinal circuits and the organization of afferent feedback allowed the model to closely reproduce characteristics of mouse locomotion at different speeds and to adapt to changes in the environment. Analysis of locomotor disturbances and failures in the model following selective removal of individual intraspinal, commissural and feedback pathways allowed us to suggest the role of these pathways in different forms of locomotor behavior and in providing stable locomotion under different conditions.

Active Synchronization Underlies Rhythmogenesis in the preBötC Microcircuit

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We tested the hypothesis that preBötzinger Complex (preBötC) rhythmogenesis is an emergent microcircuit property. We recorded synaptic inputs onto inspiratory-modulated (I-M) somatostatin expressing (SST^+) neurons - putative preBötC output neurons - in *in vitro* slices. Under non-rhythmic conditions, these neurons received unsynchronized synaptic inputs that did not produce action potentials (APs). However, with increased neuronal excitability (by increasing bath $[K^+]$), their EPSPs transformed from sporadic to temporally clustered, producing more APs at shorter intervals. This increased activity percolated and grew slowly for ~100 ms, resulting in rhythmic population bursts. Wavelet analysis revealed that the power of these clustered inputs at 5 - 40 Hz significantly increased in the epoch preceding, i.e., preinspiratory period, and during population bursts, but not immediately following bursts. In a biophysically realistic model neuron, an increase in frequency of randomly arriving EPSPs, while resulting in spurious aperiodic synchrony, did not produce any spectrotemporal reorganization; they aligned randomly, with no consequential rhythmic bursting. preBötC SST^+ neurons were not connected through synaptic or electrical coupling, ruling out recurrent excitation amongst them. Yet, membrane potentials of simultaneously recorded I-M SST^+ pairs were strongly correlated at millisecond lags preceding and during inspiratory bursts. This distinct correlation pattern marks the onset and evolution of correlated inputs from an upstream, presumably rhythmogenic, neuronal population. Strikingly, disinhibiting the preBötC network by antagonism of $GABA_A$, but not of glycine, receptors led to emergence of sustained preBötC inspiratory rhythm coincident with synchronous EPSPs in I-M SST^+ neurons. Of interest, under rhythmic conditions I-M SST^+ neurons shifted to a higher conductance state rendering them less excitable but more selective to synchronous inputs. Other observations revealed that active synchronization, not *necessarily* tonic increase in neuronal firing, which can be gated by $GABA_A$ receptors, is critical for rhythmic bursting in preBötC. We posit that EPSP synchrony onto I-M SST^+ neurons in each cycle is a consequence of temporally convergent inputs from rhythmogenic preBötC neurons.

Role of the Parafacial Respiratory Group in the Recruitment of Active Expiration during Sleep

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In resting conditions, breathing is typically characterized by an active inspiratory phase and a passive expiratory phase. Expiration may become active through abdominal (ABD) muscle recruitment during periods of increased inspiratory requirements. This respiratory rhythm is thought to be controlled by three coupled oscillators: preBötzinger complex (preBötC) for generating inspiration, the parafacial respiratory group (pFRG) for generating active expiration, and the post-inspiratory complex (PiCo) which is thought to control the post-inspiratory phase. Although abundant evidence linking preBötC activity to the generation of respiratory rhythm exists, research addressing the role of pFRG in ventilation and rhythm generation across sleep states is limited. Recent work in our laboratory reports the occurrence of ABD recruitment during REM sleep, despite the induction of muscle paralysis during this sleep state. This ABD recruitment was associated with a stabilization of breathing in healthy rats. Because pFRG generates active expiration through the engagement of ABD muscles, we hypothesize that the expiratory oscillator is also responsible for the ABD recruitment observed during REM sleep in healthy rats. To test this hypothesis, we inhibited and activated the pFRG oscillator using a chemogenetic approach (DREADDs) while simultaneously recording EEG, airflow, ABD and neck EMG of transfected rats across sleep/wake cycles. Our results suggest that manipulation of pFRG activity has an effect in the occurrence of ABD recruitment events during REM sleep. Inhibition of pFRG (N=7) significantly reduced the number of REM events with ABD recruitment, whereas activation of this oscillator (N=7) resulted in an increase of the number of REM events in which ABD recruitment was observed. Interestingly, modulation of pFRG activity did not seem to affect the occurrence of ABD recruitment during NREM sleep. These results suggest that the occurrence of ABD recruitment during sleep may be state dependent. Further research investigating the mechanisms behind the recruitment of ABD activity specifically during REM and NREM sleep will be necessary.

Neural Circuits of Dexterity

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Dexterous movements serve the major functions of the brain, perception and manipulation of the world. Considering the range of possible actions and the complexity of musculoskeletal arrangements, control of the hand is an amazing achievement of the nervous system. Dexterous behavior involves understanding objects in the world, developing appropriate plans, converting those plans into appropriate motor commands, and adaptively reacting to feedback. The myriad of these underlying operations is likely performed by a diverse set of neural circuits. By combining anatomy, physiology, and specific (genetic and temporal) manipulations, my lab hopes to identify and understand the neural elements responsible for dexterous motor control. Currently, we focus on the role of the cortico-cerebellar loop in a skilled reach-grab-eat task in the rodent.

Connectivity, Cytoarchitecture, Visual, Somatosensory and Motor Maps in the Lamprey Lateral Pallium – A Primordial Vertebrate Cortex

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The lamprey lateral pallium (LPal), an area corresponding to cortex in mammals, is remarkably conserved in terms of efferent connectivity. Electrical microstimulation of circumscribed regions evokes eye and orienting movements, locomotion and oral movements. Glutamatergic and monosynaptic projection-neurons target all major motor centers in the midbrain, brainstem and rostral spinal cord (Ocaña et al., 2015). The motor area is located in the central and lateral portion of LPal. The LPal has a three-layered laminated cytoarchitecture with a molecular layer an outer and inner cellular layer. GABAergic interneurons represent 22% of the total number of cells in LPal. Pyramidal tract-like (PT-type), intertelencephalic (IT-type) and thalamo-recipient cells (layer 4 equivalent) are located in the cellular layer and extend their dendrites towards the molecular layer. Thalamic input is relayed polysynaptically to PT-type cells, while olfactory input is monosynaptic. The LPal thus, has an overall microcircuit bauplan, similar to reptilian cortici, three-layered mammalian cortici as well as the neocortex (Suryanarayana et al., 2017). Furthermore, we show that primary visual input, relayed via thalamus, is represented in the dorsomedial LPal in a retinotopic fashion. Extracellular multi-unit recordings showed that neurons in the dorsomedial LPal are activated by stimulation of different parts of retina. Whole-cell recordings of LPal neurons during optic nerve stimulation revealed EPSP's followed by inhibition. Local gabazine injections in the visual area resulted in a massive response, with a loss of retinotopy, indicating that GABAergic neurons are important for maintaining specificity. The visual region is separate from the motor areas.Somatosensory information from the dorsal column nuclei is also relayed via thalamus, and represented in a ventromedial area in LPal, distinct from the visual areas. Stimulation of the spinal dorsal column and the trigeminal nerve elicited multi-unit responses in adjacent areas.Olfactory bulb input to LPal is relayed through two routes – directly and via a relay nucleus (dmtn). Both traverse in distinct layers in the LPal molecular layer. Taken together, these findings show that the three-layered LPAL of the lamprey provides a blueprint of the reptilian and mammalian cortici with regard to efferent connectivity, sensory maps and the microcircuit features.

Motor Cortex Descending Projections Drive Orofacial Behaviors through Specific Brainstem Premotor Networks

Nicole Mercer Lindsay, Per M. Knutsen, Adrian F. Lozada, Daniel Gibbs, Harvey J. Karten, and David Kleinfeld
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Focal activation of motor cortex is known to enact behaviorally meaningful motor output, yet the details of how the cortical circuitry interfaces with brainstem premotor circuits is unresolved. We studied the hierarchical nature of this control with respect to motor actions that involve the vibrissae, jaw, and forelimb. The spinal trigeminal nucleus pars oralis (SpVO) and interpolaris rostralis (SpVIr) contain premotor neurons known to directly synapse on vibrissa, jaw, and forelimb motoneurons. In addition to cortical input, SpVO and SpVIr receive direct sensory signals from the periphery. Thus, their ability to integrate descending motor input and peripheral sensory information position them as ideal candidates to determine the specificity of cortex-to-brainstem-to-muscle feed forward networks. Here we show structural labeling and functional activation that determined how two distinct clusters of premotor neurons, one in SpVO and a second in SpVIr, control distinct, yet related, motor actions. A transectional virus strategy preferentially encoded a red-shifted channelrhodopsin in SpVO- versus SpVIr-projecting motor cortex neurons. Activation of the different cortical populations evoked distinct muscle activation. Similar stimulations of localized regions of motor cortex in Thy1-ChR mice show patterns of muscle activity and forelimb, jaw, and nose movements that correspond to behaviorally meaningful movements. All together, our data identifies the functional specificity of motor circuits that originate in cortex and descend onto specific premotor populations. We suggest that this specificity is a major determinate in the coordination of motor actions into behavior.

Sensory Cortical Control of Movement

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Locomotion requires continual higher order integrated spatiotemporal information, which is processed in the somatosensory cortex. Motor cortex does not participate in the generation of regular walking. Here, we demonstrate that direct corticospinal output from the primary somatosensory cortex interfaces with a neural circuit in the lumbar spinal cord to control locomotion. Specifically, we have identified that the primary somatosensory cortex independently of the motor cortex, brain stem and other supraspinal locomotor centers modulates activity in the rhythmogenic area of the locomotor central pattern generator (CPG) via cervical excitatory cells. Activation of this pathway promotes movement, while inhibition disrupts the ability to maintain locomotion and ultimately terminates movement. Our findings reveal a novel neural control of movement whereby the somatosensory cortex is part of the automated neural system generating moving in the environment.

Control of Reach Kinematics by the Cerebellum

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Many of the movement deficits associated with cerebellar dysfunction follow a unique governing principle: the general ability to produce movement remains intact, but its quality is substantially degraded. This is exemplified in the decomposition of reaching movements, termed dysmetria, a tremor-like oscillation of the limb that is a characteristic feature of cerebellar dysfunction. That cerebellar deficits are most prominent during active movement argues for studying the function of cerebellar neural activity specifically during purposive, ongoing movement. Nevertheless, technical limitations in the ability to track movements and manipulate neural activity in real time have thus far limited functional studies of cerebellar contribution to active reaching behavior. Therefore, to test the causal role of cerebellar output in reaching movements, we designed a kinematic closed-loop system that enables brief, scalable, optogenetic manipulation tied directly to ongoing limb kinematics in mice. This technology allowed us to characterize the functional relationship between activity in the anterior Interposed Nucleus (IntA), a cerebellar output structure closely associated with limb control, and ongoing reach kinematics, including its sign, strength, and context-dependence. Bidirectional optogenetic manipulation of IntA activity during reach resulted in monotonically scalable, bidirectional effects on reach velocity, with a relative lack of effect of stimulation during quiescence. IntA stimulation at different phases of reach revealed a consistent directional effect, implying a consistent role of IntA activity throughout reaching movement. Single-unit recordings of IntA neurons during reach show significant firing rate changes, with the direction of modulation aligning with predictions generated from causal manipulations. Taken together, these results describe a continuous, scalable, directional code in IntA for modulating ongoing reach behavior in real time.

Motor Cortex Inactivation Contracts Tongue and Forelimb Trajectories in Mice

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Motor sequences are constructed from primitives, hypothesized building blocks of movement, but mechanisms of primitive generation remain unclear. To dissect the neural circuits underlying generation of movement primitives, we designed two behavioral paradigms for mice. First, using a novel forelimb sensor, we trained freely-moving mice to initiate forelimb sequences with clearly resolved submillimeter-scale micromovements followed by millimeter-scale reaches to learned spatial targets. Hundreds of thousands of trajectories were decomposed into millions of kinematic primitives, while closed-loop photoinhibition was used to test roles of motor cortical areas. Second, we imaged the mouse tongue at 1kHz during a cued directional lick task, and using a novel deep-learning based artificial neural network for semantic segmentation (a custom version of U-net) we resolved tens of thousands of mouse tongue trajectories with a precision of ~60um and a validated accuracy of 0.9971. Photoinhibition of contralateral "forelimb" motor cortex (CFA) during the forelimb task and bilateral "tongue" motor cortex (ALM) in a directional licking task, led to qualitatively identical results. Inactivations reduced peak speed in both tongue and forelimb trajectories but, surprisingly, did not substantially affect primitive direction, termination, or complexity, resulting in isomorphic, spatially contracted trajectories that undershot targets. Our findings identify conditions where loss of cortical drive reduces the gain of motor primitives but does not affect their generation, timing or direction. The combination of high precision forelimb and tongue kinematics with automated training and neural manipulation provides a system for studying how motor sequences are constructed from elemental building blocks.

Poster Abstracts

Motor Systems Symposium
Friday, November 2, 2018

Salk Institute for Biological Studies
La Jolla, California

Identification of a Central Pathway for Mechanical Itch

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The dorsal horn of the spinal cord comprises multiple dedicated networks of interneurons (INs) that process cutaneous sensory information. These networks play key roles in sensorimotor transformation and in generating stimulus-appropriate motor behaviors, including protective reflexes following aversive stimuli. One such protective reflex is scratching in response to itch, which serves to remove irritants from the skin. Itch can be elicited by either chemical or mechanical stimuli, and these activate separate pathways in the periphery and spinal cord. While substantial progress has been made in mapping the transmission pathway for chemical itch, it is unknown how mechanical itch is transmitted centrally. Previously we showed that mechanical but not chemical itch is subject to inhibitory gating by INs that express Neuropeptide Y::Cre (NPY::Cre INs) (Bourane et al. Science. 2015). However, the excitatory targets of NPY::Cre INs and the gating mechanism are unknown. Here, we show that excitatory INs expressing the NPY Y1 receptor (Y1+ INs) are essential for mechanical itch transmission in the dorsal horn. Epistatic diphtheria toxin-mediated ablation of Y1+ INs abolishes the spontaneous scratching that develops in mice following ablation of NPY::Cre INs (Bourane et al. Science. 2015). Furthermore, ablation or chemogenetic silencing of Y1+ INs suppresses scratching in response to mechanical stimulation, whereas activation of Y1+ INs enhances both mechanically evoked and spontaneous scratching independently of the chemical itch pathway. We also show that endogenous NPY acting at Y1 receptors mediates the gating of itch by the NPY::Cre INs. Activation of the NPY::Cre INs suppresses itch responses in a Y1-dependent manner, and pharmacological or genetic disruption of Y1 signaling causes scratching. This study delineates a central circuit for mechanical itch transduction, raising the possibility that the Y1 receptor may provide a therapeutic target for some forms of chronic itch.

Computational Modeling of Brainstem Circuits Controlling Locomotor Speed and Gait

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Locomotion is an essential behavior allowing animals to explore and survive in complex environments. Depending on the environmental context quadruped animals can switch from slow left-right alternating gaits (walk and trot) to higher-speed synchronous gaits (gallop and bound). Recent studies have shown that locomotor gaits and speed are differently controlled by two brainstem nuclei: the cuneiform nucleus (CnF) and the pedunculopontine nucleus (PPN) (Caggiano et al. 2018). Glutamatergic neurons within both nuclei contribute to the control of slow, alternating-gait movements, whereas only stimulation of CnF elicits high-speed, synchronous-gait locomotion. Both regions project to the lateral paragigantocellular nucleus (LPGi) and transsynaptically activate spinal locomotor circuits via the reticulospinal tract (Capelli et al. 2017). To investigate the brainstem control of locomotion, we built upon our previous model of spinal circuits, consisting of four rhythm generators (RGs) interacting via local cervical and lumbar commissural interneurons (CINs) and long propriospinal neurons (LPNs, Danner et al. 2017). We extended this model to incorporate the bilaterally interacting CnF and PPN circuits and their LPGi-mediated descending pathways. We suggest that brainstem control of locomotion is mediated by two pathways, one controlling frequency via connections to the RGs and the other controlling gait expression via connections to CINs and LPNs. Our model reproduces the experimentally observed effects of stimulation of excitatory and inhibitory CnF, PPN, and LPGi neurons. Additionally, the model implicates that the suppression of PPN during CnF stimulation-evoked locomotion leads to a shift of the transition between alternating and synchronous gaits to lower locomotor frequencies.The model provides important insights into brainstem-spinal cord interactions and supraspinal control of locomotion.

* Equal contribution

Sympathetic Overactivity after Chronic Intermittent Hypoxia has Different Mechanisms in Male and Female Rats

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Untreated sleep apnea may lead to hypertension, which is an indicator of early death. Enhanced respiratory modulation in sympathetic nerve activity (SNA) is thought to mediate hypertension in sleep apnea patients. The mechanisms by which this enhancement occurs are not well understood. Rats subjected to chronic intermittent hypoxia (CIH) are an animal model of obstructive sleep apnea. During CIH conditioning periodic excursions in blood-gas balance repetitively activate peripheral chemoreceptors. Long term exposure evokes plasticity in brainstem neuronal circuitry which appears as changes in respiratory and sympathetic motoneuron output. In male rats, a hallmark of CIH conditioning is the appearance of active expiration during restful breathing. It is thought that late-expiratory activity in the SNA contributes to elevated blood pressure in these animals. Female rats subjected to CIH develop hypertension, but they do not experience a change in the threshold for active expiration. Instead, there is a notable facilitation of early-inspiratory activity in the sympathetic motor output. By what mechanism do female rats respond to CIH conditioning? Previously, we investigated the formation of abdominal activity and SNA in male CIH rats in the context of central chemoreflex activation. In our computational model, we showed that sensitization of the central chemoreceptors to CO2 was sufficient to lower the threshold for respiration in hypocapnia and lower the threshold for emergence of active expiration to occur during normocapnia. Here, we use our computational model to recapitulate experimental observations of female rats exposed to CIH. From this data, we assimilate (1) the facilitation of early-inspiratory SNA and (2) evidence that inspiratory neurons recorded in the ventral respiratory group decrease firing rate. Via model simulations we generate the hypothesis that there is decrementing inspiratory inhibition on pre-sympathetic rostral ventrolateral medulla (RVLM) neurons in naive animals that is necessary for SNA pattern formation. Taking into consideration that the caudal VLM (CVLM) is the main source of inhibition to RVLM, we implemented an inhibitory population in CVLM that is modulated by inspiration in the model. In simulation of CIH conditioned female rats, the suppression of this population disinhibits pre-sympathetic neurons in the RVLM which facilitates SNA during early inspiration. These results suggest that male and female rates develop different plastic changes in the medullary respiratory-sympathetic network that both result in enhancement of sympathetic motoneuron output following CIH conditioning.

Mechanisms through which Electrical Spinal Cord Stimulation Restores Locomotion after Spinal Cord Injury

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Epidural electrical stimulation (EES) of the lumbar spinal cord restored locomotion in rodents, nonhuman primates and humans with spinal cord injury (SCI). However, the neural structures through which EES enables motor pattern formation remain poorly understood. Using calcium imaging and chemogenetic inactivation experiments, we demonstrate that the activation of proprioceptive feedback circuits contributes to motor pattern formation during EES. However, EES also recruits cutaneous low-threshold mechanoreceptor feedback circuits. Modeling experiments showed that the activation of these pathways with EES is detrimental to the production of locomotion. To augment the facilitation of movements with EES, we thus reasoned that these two types of circuits should be targeted with opposing neuromodulators. This understanding translated into a circuit-specific electrochemical neuromodulation therapy based on noradrenergic receptor modulation that enabled robust locomotion in paralyzed mice and rats. These findings establish a mechanistic framework for the design of targeted neuromodulation therapies in human patients.

Functional Heterogeneity of Neurons within a Midbrain Nucleus Driving Locomotion in Adult Zebrafish

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Animals navigate their environment with great versatility to find food, escape predators, or find mating partners. While the basic rhythmic locomotor pattern can be generated by circuits in the spinal cord, these circuits are driven by descending projections from the brain. How this descending drive is organized is only beginning to be understood.
In the zebrafish, neurons of the nucleus of the medial longitudinal fasciculus (nMLF) in the midbrain are the most rostral cells projecting to the spinal cord. In the larvae, the nMLF has been implicated in controlling swimming behavior in various ways, e.g. by providing a general excitatory drive or by governing steering movements. Thus far, however, most of these studies have regarded the nMLF as a uniform entity and have examined its role on swimming behavior using imaging or ablation. Here we have taken a single-neuron approach to assess the functional heterogeneity of nMLF neurons. For this, we have used single or dual patch-clamp recordings from identified nMLF neurons during spontaneous swimming using the ex-vivo adult zebrafish preparation.
First, we show that electrical stimulation of the nMLF region induces swimming activity and that nMLF neurons display cell-specific projection profiles along the rostro-caudal level of the spinal cord. Second, we show that nMLF neurons can be subdivided into several subgroups based on their firing properties with individually identified nMLF neurons consistently displaying either a strong spike frequency adaption, tonic firing, or a delayed firing onset with increasing frequency. Finally, we show that nMLF neurons exhibit different activity patterns during spontaneous swimming. While the activity of all neurons was strongly correlated with swimming activity, they show appreciable differences. Some nMLF neurons displayed a rather sustained activity pattern throughout a swimming episode, while in others the activity was tightly linked to the vigor of swimming. These two neuronal types seem to occupy different regions along the medio-lateral aspect of the nMLF. Thus, our results reveal a large degree of heterogeneity in the nMLF that could endow this nucleus with the necessary versatility to control different aspects of swimming behavior.

Inhibitory Interneuron Diversity and Circuitry in the Spinal Motor System

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Animals interact with the world through movement, transforming patterns of neural activity into the orderly contraction of muscles. The circuits directly responsible for movement reside in the spinal cord, where inhibitory interneurons play a crucial role in shaping the output of motor neurons. Yet the identity of these inhibitory interneurons and the organizational logic through which they influence motor output remain obscure. In prior studies, we showed that V1 interneurons can be fractionated into highly diverse subsets on the basis of the expression of 19 transcription factors (1,2). Many of these V1 subsets exhibit distinct physiological signatures and occupy restricted spatial domains. Our findings also revealed the existence of a variant circuit architecture for V1 interneurons that operate on motor pools controlling hip, ankle, and foot muscles. More recently, we have explored how V1 interneuron diversity varies along the rostrocaudal axis of the spinal cord (3). We have identified molecularly distinct subsets of V1 interneurons that exhibit segmental restriction in thoracic versus limb spinal segments. These distinctions appear to emerge through a Hox-dependent regulatory mechanism, independent of motor neurons, revealing a developmental program that organizes inhibitory interneurons associated with differential motor output. In ongoing studies, we are continuing to dissect the diversity and functional organization of spinal interneurons, with an emphasis on how descending systems from the brain interface with distinct interneuron subsets.

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Subpopulations of V2b Neurons in the Zebrafish Spinal Cord

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The V2b (GATA3+) premotor population provides ipsilateral inhibition to motor circuits within the spinal cord and has been implicated in regulating flexor-extensor alternation in mouse hindlimb. However, to date no study has comprehensively described the anatomy, physiology, or connectivity of V2b neurons. We have leveraged the genetic tractability, transparency, and accessibility of the spinal cord in larval zebrafish to understand the basic anatomy and connectivity governing V2b neurons.In doing so we have uncovered anatomical evidence for two distinct V2b subpopulations, defined by differential expression of inhibitory neurotransmitters and accompanying differences in cellular morphology. Specifically, all V2b neurons transmit glycine, as evidenced by transgenic markers and whole cell electrophysiology, while a subset of V2b cells are also capable of releasing GABA. These subpopulations persist into late larval stages. While both populations extend axons caudally in the spinal cord, their axons innervate distinct pathways within the spinal tract. Finally, these populations also demonstrate synaptic specificity. V2b synaptic inhibition onto primary motor neurons, which are recruited for fast swim movements, is mediated exclusively by glycinergic, but not GABAergic, transmission. The anatomical and functional diversity observed in the V2b population suggests the presence of discrete subpopulations, analogous to the genetic and anatomic subpopulations found within other classes of spinal interneurons.

Brainstem Mechanisms of Cardio-respiratory Coupling

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The respiratory and cardiovascular systems work together to oxygenate tissues and remove carbon dioxide and are physiologically integrated. Central neural circuits that control the respiratory and cardiovascular functions are located in brainstem and receive sensory feedback to maintain the gas homeostasis. Respiratory and cardiovascular physiologic outputs are partially synchronized/modulated by each other, and the respective brainstem neuronal networks have reciprocal synaptic connections. However, no quantitative mechanistic description was suggested to explain specific aspects of the cardio-respiratory interactions and their alterations in certain pathophysiological conditions.Two major markers of cardio-respiratory interactions were previously identified: cardio-ventillatory coupling (CVC) and respiratory sinus arrhythmia (RSA). CVC is usually interpreted as a form of partial synchronization between cardiac and respiratory rhythm which is characterized by varying probability of a heartbeat to occur at different phases of the respiratory cycle. RSA is a phenomenon concerned with changes in heartrate at different respiratory phases, which is usually represented by the dependence of the inter-heartbeat interval (R-R interval) on the respiratory phase with R-R interval shortened during inspiration and prolonged during expiration. Due to similar representation, CVC and RSA are often confused. However, there is substantial experimental evidence that independent mechanisms mediate the two phenomena.Here, we introduce a closed loop model of the integrated respiratory and cardiovascular control system to describe mechanisms for both CVC and RSA. The model combines and extends our previous data-driven models that incorporated mechanisms of cardiovascular input to the respiratory system or respiratory input to the cardiovascular system. In this model, CVC is mediated by the pulsatile inputs from arterial baroreceptors to neurons of the respiratory central pattern generator (rCPG) with pulses corresponding to the increases and subsequent relaxations in arterial pressure caused by heart contractions. We implement baroreceptor input to the rCPG as excitatory projections from 2nd order baro-sensitive neurons of the nucleus of solitary tract (NTS) to the expiratory population of the rCPG. This makes the onset of inspiration less likely to occur right after the heartbeat thus reproducing a characteristic structure of the heartbeat probability distribution. Our model explains RSA by modulation of the vagal input to the sinoatrial node of the heart. By fitting the literature data, we suggest that RSA should be driven by respiratory modulation of vagal cardiac neurons from both inspiratory and expiratory rCPG populations to accurately reproduce the experimentally observed dependence of the average R-R interval duration on the respiratory cycle phase.

ER Stress Induced by Tunicamycin can Induce Locomotor Dysfunction: New Parkinson Disease's Model

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive death of dopaminergic neurons of the substantia nigra pars compacta (SNpc), leading to the major clinical abnormalities that characterize this disease. Although PD's etiology is unknown, α-synuclein aggregation plays a pivotal role in PD pathogenesis, which could be associated to some pathological processes such as oxidative stress, endoplasmic reticulum (ER) stress, impaired protein degradation, and mitochondrial dysfunction. Increasing experimental evidence indicates that ER stress is involved in PD, however most of the described results employed cultured cell lines, and genetically modified animal models; models of study without the whole physiological dysfunction complexity. In this study, we analyzed a new rat model of ER stress employing the well-known ER stressor tunicamycin (Tm). The classical 6-OHDA neurotoxin model was used as an established positive control for PD. PD features were induced with an intranigral injection of Tm and motor dysfunction was analyzed 30 and 60 days post injection. We showed that Tm injection induced locomotor impairment, mainly in the swing phase of walking, similar to the results found with 6-OHDA injection. In summary, ER stressor Tm recapitulates some of the phenotypic characteristics observed in rodent models of PD, reinforcing the concept that ER stress could be an important contributor to the pathophysiology of PD. Therefore, we propose the intranigral Tm injection as a new ER stress-based model for the study of PD *in vivo*.

Flight Motor Networks Modulate Primary Olfactory Processing in the Moth Manduca sexta

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Nervous systems must distinguish sensory signals derived from an animal's own movements (reafference) from environmentally derived sources (exafference). To accomplish this, motor networks producing reafference transmit motor information, via a corollary discharge circuit (CDC), to affected sensory networks, modulating sensory function during behavior. While CDCs have been described in most sensory modalities, none have been observed projecting to an olfactory pathway. In moths, two histaminergic neurons (MDHns) project from flight sensorimotor centers in the mesotho- racic neuromere to the antennal lobe (AL) where they provide the sole source of histamine (HA), but whether they represent a CDC is unknown. We demonstrate that MDHn spiking activity is positively correlated with wing motor output and increased prior to bouts of motor activity, suggesting that MDHns communicate global locomotor state, rather than providing a precisely timed motor copy. Within the AL, HA application sharpened entrainment of PN responses to odor stimuli embedded within simulated wing beat induced flows, whereas MDHn axotomy or AL HA receptor (HA-r) blockade reduced entrainment. This finding is consistent with higher order CDCs, as the MDHns enhanced rather than filtered entrainment of AL PNs. Finally, HA-r blockade increased odor detection and discrimination thresholds in behavior assays. These results establish MDHns as a CDC that modulates AL temporal resolution, enhancing odor-guided behavior. MDHns thus appear to represent a novel higher order CDC to an insect olfactory pathway, this CDC's unique nature highlights the importance of motor-to-sensory signaling as a context-specific mechanism that fine tunes sensory function.

Reactivation of Dormant Relay Pathways in Injured Spinal Cord by KCC2 Manipulations

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Many human spinal cord injuries are anatomically incomplete but exhibit complete paralysis. It is unknown why spared axons fail to mediate functional recovery in these cases. To investigate this, we undertook a small-molecule screen in mice with staggered bilateral hemisections in which the lumbarspinal cord is deprived of all direct brain-derived innervation, but dormant relay circuits remain. We discovered that a KCC2 agonist restored stepping ability, which could be mimicked by selective expression of KCC2, or hyperpolarizing DREADDs, in the inhibitory interneurons between and around thestaggered spinal lesions. Mechanistically, these treatments transformed this injury-induced dysfunctional spinal circuit to a functional state, facilitating the relay of brain-derived commands toward the lumbar spinal cord. Thus, our results identify spinal inhibitory interneurons as a roadblock limiting the integration of descending inputs into relay circuits after injury and suggest KCC2 agonists as promising treatments for promoting functional recovery after spinal cord injury.

Age-related Dynamic Changes in Human Motor Network Connectivity

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The interest in ageing-related changes of motor performance and the neural basis thereof are governed by the quest for more detailed insights into the possible reorganization of the key phases of an action. For this reason, it is apt and timely to study ageing-dependent effects on the neural organization of motor performance in more detail. The crucial point of such investigations is the study of both, amplitude and phase synchronization, key mechanisms underlying the coordination of distinct neural populations in shaping complex motor tasks. In earlier EEG-studies on young and older adults, we found that when generating simple finger movements, local oscillations in the δ-θ frequencies over the primary motor (M1), the supplementary motor (SMA) and the pre-motor area (PM) exhibited robust phase locking prior to and during the movement [1,2]. This phase locking represents a trigger of movement preparation and initiation and has no influence on motor performance. We further observed a decrease in post-movement β amplitude synchronization (PMBS) in the medial pre-frontal cortex (mPFC) of older subjects, which may affect the cognitive control of stimulus-induced motor tasks and thereby motor performance [2].To further explore the neural processes underlying age-related dependence of motor performance, we investigate motor network connectivity by means of i) inter-regional phase-locking analysis in which the phase-locking values (PLVs) between the regions of the motor cortex are calculated as well as ii) Dynamic causal modelling (DCM) of induced responses from the EEG records of the two data sets mentioned above. The phase-locking analysis revealed significant PLV in both age groups in the δ-θ frequencies around movement onset. Invariant sub-networks were established by strong PLV between brain areas involved in the motor act, which were different in the two groups. Our data suggest that older subjects compensate for the diminished contralateral M1 - SMA - ipsilateral PM connectivity during movement preparation and execution by establishing additional intra- and inter-hemispheric connections. The DCM analysis revealed that the basic connectivity structure of the motor network during the performance of a motor act is preserved in the aging brain. However, the strength of individual connections within this network significantly changed during aging. We found that during the execution of a visually-guided movement, the core motor regions are under less excitatory control from the prefrontal (conveyed by interactions in the β frequency band) and parietal (conveyed by interactions in α frequency band) regions in the older compared to the younger participants. We further observed decreased modulatory influences from the ipsi- to the contralateral hemisphere in the β frequency band in the older participants, which hint at a mechanism underlying the reduced hemispheric asymmetry well described in older human beings.

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Task-specific V3 Spinal Interneuron Circuit Modules Revealed through Distinct Subpopulation Topographies

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Animals exhibit a wide range of locomotor behaviours that emerge from the coordinated activity of circuits in the spinal cord that direct patterned motor output. While spinal interneurons (INs) are known to play an essential role in establishing precise temporal patterns of muscle contraction, the principles governing recruitment of INs across different behaviours remain elusive. Here, we combine computational models to systematically analyze task-specific c-fos expression within the cardinal V3 IN population in the mouse spinal cord. Our analysis reveals a topographic arrangement of V3 INs into functionally distinct modular domains. Furthermore, we uncover molecularly discrete Nr3b3$^+$, Onecut2$^+$ and Prox1$^+$ V3 IN subpopulation clusters that form unique connectivities, and most notably, differentially assemble within distinct V3 modular domains. Thus, our current work indicates that developmentally and genetically discrete IN subpopulations are the building blocks of topographically clustered spinal circuits engaged in different locomotor tasks.

Remote Control of Neuronal Activity *In Vivo* Using Stable Transgenic Lines: Which Opsin to Choose and Why?

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Thirteen years after the publication of the original Channelrhodopsin-2 proof of principle paper using cultured neurons, we now have available several excitatory and inhibitory opsins with marked improvements in kinetics, efficiency and different spectral properties. Despite these advancements, the field if currently facing several challenges. First there is the issue of generating stable opsin transgenic lines which express high levels of fluorescence. As a second point, there is currently a mismatch between the careful electrophysiological opsin characterisations performed in more accessible *in vitro* models, which are directly followed by opsin-mediated activation or inactivation of neural activity *in vivo*, often deprived of re-calibrations in these new systems. Here we address these issues by taking advantage of the transparent zebrafish larva model to calibrate the performance of the best-expressing opsin transgenic lines available via electrophysiological recordings *in vivo* in motor neurons. We used the Tg(mnx1:gal4) transgenic line to test the functional profile of the following excitatory opsin lines: UAS:ChR2-H134R-YFP, UAS:ReaChR-GFP, UAS:Chronos-tdTomato, UAS:CoChR-tdTomato, UAS:Chrimson-tdTomato, UAS:Cheriff-tdTomato, as well as the neural activity inhibitory drivers: UAS:NphR3-YFP, UAS:Arch3.0-YFP, UAS:GtACR1-tdTomato, UAS:GtACR2-tdTomato. Our preliminary data show that the now “classical” excitatory ChR2-H134R-YFP opsin is able to elicit single spikes in primary and secondary MNs but fails to drive spiking at frequencies higher than 1Hz. In transgenic larvae, ReaChR-GFP on the hand, failed to elicit any spiking activity despite having excellent cellular targeting levels. In terms of inhibitory opsins, both UAS:NphR3-YFP and UAS:Arch3.0-YFP seem to be efficient tools to block neural activity as the lowest light stimulation tested (6mW/mm2) resulted in a ~ 5-10mV hyperpolarisation. The present study is addressing an important gap in current methodology: the need for calibration of optogenetic tools expressed in a stable manner *in vivo*. Our ongoing opsin comparison experiments will shed light on the best available tools for eliciting high fidelity spiking at physiological frequencies *in vivo*.

Motor Primitives are Determined in Early Development and are then Robustly Conserved into Adulthood, and through Rehabilitation after SCI

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Motor patterns in legged vertebrates show modularity in both young and in adult animals, comprising motor synergies or primitives. It is unknown if spinal modules observed in young mammals are conserved into adulthood unaltered. Conceivably, development alters the circuit modules radically through experience and descending pathways' activity. We here analyze lumbar motor patterns of intact adult rats, and the same rats after spinal transection, and compare these with another group of adult rats spinal transected 5 days postnatally, before most motor experience, selecting rats which never developed hindlimb weight bearing. We use Independent Components Analysis (ICA) to evaluate modularity. ICA's information based methods allow identification of both weakly active and strongly active synergies. We compare all spatial synergies and strengths of their drives as proxies of spinal modules and their underlying circuits. Remarkably, we find that spatial primitives/synergies of adult injured and neonatal injured rats are not significantly different from one another, despite different developmental histories. However, intact rats do possess some synergies that differ significantly, though modestly, in their spatial structure from those in the transected rats. Rats injured as adults were more similar in modularity to rats which had neonatal spinal transection, than to themselves before injury. We surmise spinal circuit modules for spatial synergy patterns are determined early, before P5, and are then largely unaltered by subsequent development, or weight bearing experience. Overt expression of modular synergies is modified during intact adulthood, but not fundamental underlying synergy circuitry. This fundamental synergy circuitry, fully determined by P5, persists in intact adults, and is revealed functionally after spinal cord transection. Our data also show that the functional synergies in adults then persist through different rehabilitation regimes.

Phenotypic Variations of Neuronal Activity and Behavior in SK3 and Kv4.3 Potassium Channel Knock-out Mice

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Biological systems are known to be robust to external (environmental) and internal (genetic) perturbations. Numerous studies have demonstrated that neuronal activity can be maintained in the face of chronic pharmacological treatments or genetic deletion of ion channels. Substantia nigra pars compacta (SNc) dopaminergic neurons display a pacemaking activity and release dopamine onto dorsal striatum neurons, thus influencing motor behaviors. Pacemaking relies on many ion channels, including the calcium-sensitive SK3 potassium channels that regulates its regularity and the Kv4.3 potassium channels which controls its frequency.
Electrophysiological and behavioral characterizations of SNc DA neurons in SK3 KO mice show discrepancies at different levels of organization: most studies in single-neurons have suggested an extensive loss of the SK current while, unexpectedly, very little phenotypic variations were observed at the locomotor level. As for the behavioral and electrophysiological outcomes of Kv4.3 channel deletion in SNc DA neurons, they remain to be determined. However, remodelling of electrophysiological features in cortical pyramidal neurons were already observed in Kv4.2 KO mice that provides this cell-type with a maintained phenotype. Despite the preeminent role of these potassium channels in SNc DA neurons, the current knowledge concerning possible homeostatic forms of compensation arising following the genetic deletion of SK3 and Kv4.3 channels have not yeet been fully addressed.
In this study, we first sought to exhaustively define the electrical phenotype of SNc dopaminergic neurons using a multivariate *in vitro* approach. Phenotypic variation of the electrical phenotype was quantified in both SK3 KO and Kv4.3 KO SNc neurons. We eventually delineated the behavioral outcome of these genetic manipulations. At the single-neuron level, SK3 KO SNc DA neuron electrical phenotype displayed small variations from the WT group. In contrast, Kv4.3KO SNc DA neuron electrical phenotype was reminiscent of the effects of acute AmmTX3 application. Consistently, preliminary behavioral experiments in SK3 KO mice showed increased exploratory behavior and intact motor learning abilities while Kv4.3 KO mice seemed to display motor-learning impairments. These results suggest different levels of robustness for SK3 KO and Kv4.3 KO SNc DA neurons: while the loss of SK3 seems to be well compensated for, these neurons appear unable to cope with Kv4.3 genetic deletion.

Endogenously Oscillating Excitatory Motoneurons Produce Undulatory Output in a Model of *C. elegans* without Proprioception

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Neuronal oscillators underlie rhythmic behavior, and particularly locomotion, in all animals in which the neural mechanism has been determined. However, despite the availability of a connectome, the neuronal mechanism underlying undulatory locomotion in the nematode Caenorhabditis elegans is not known. Hypotheses have included sensory feedback and neuronal oscillators. We took a computational approach to find a minimal set of conditions for central pattern generation by a chain of oscillators along the body, in the absence of proprioceptive feedback.
We recently described how the existing *C. elegans* connectivity data can be extrapolated into a complete neuromuscular network by identifying connectivity rules. Here we use an extrapolated network that spans the full length of an animal and includes seven classes of motoneurons, muscle cells, and synaptic connections, both chemical and electrical. We populate our network model with two kinds of motoneurons and muscle cells: leaky (passive) and endogenously oscillating (pacemaker) and systematically screened all 2^7=128 combinations of leaky vs pacemaker motoneuron classes. Within each combination, we screened parameter space and used an evolutionary simulation approach to search for synaptic weights that produce a propagating dorsoventral alternation of muscular activity in forward or backward directions. The opposing directions of locomotion are induced by adding a tonic current to targets of the forward or backward pools of premotor interneurons targets. Successful fictive patterns were fed into a neuromechanical model to interpret the locomotion behavior.
An undulatory pattern in both forward and backward directions was not generated when all motoneuron classes were passive or when all motoneuron classes were endogenous oscillators. Several combinations in which some excitatory motoneurons are oscillators produced undulatory-like motor programs in both forward and backward directions. Notably, a ventral excitatory class of motoneurons and a dorsal excitatory class are represented in all successful combinations.

Chronologically Layered Descending Circuits that Underlie the Development of Locomotor Repertoire after Birth

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The emergence of new and increasingly sophisticated behaviors after birth is accompanied by dramatic increase of newly established synaptic connections in the nervous system. Little is known, however, of how nascent connections are organized to support such new behaviors alongside existing ones. To understand this, in the larval zebrafish we examined the development of spinal pathways from hindbrain V2a neurons and the role of these pathways in the development of locomotion. We found that new projections are continually layered laterally to existing neuropil, and give rise to distinct pathways that function in parallel to existing pathways. Across these chronologically layered pathways, the connectivity patterns and biophysical properties vary systematically to support a behavioral repertoire with a wide range of kinematics and dynamics. Such layering of new parallel circuits equipped with systematically changing properties may be central to the postnatal diversification and increasing sophistication of an animal's behavioral repertoire.

Subtype-specific Integration of Isolated, Immature Spinal Interneurons into the Mature Spinal Cord

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Progress from the spinal cord injury field has made transplantations in the adult spinal cord yielding neurons capable of survival and a reality. But because the specific identity and exact fate of the engrafted neurons is rarely established, these studies have done little to help us understand the capacity of immature neurons to integrate into the mature spinal circuity. Instead, I am isolating then transplanting murine embryonic stem (ES) cell-derived, immature neurons of established spinal neuron subtypes into the uninjured adult mouse spinal cord. By differentiating these neurons of a known identity to an early post-mitotic point where subtype-specific physiological properties have been established, I hope to promote functionally relevant and identity-specific integration of the transplanted neurons.Through histological characterizations of the engrafted cells anatomy and growth, I've begun to assess if the *in vitro* derived spinal neurons can recapitulate their expected *in vivo* growth patterns and diversity starting with the V3 and V1 spinal interneuron subtypes. Like their *in vivo* counterparts, the engrafted V3 interneurons interneurons formed long, multi-segment processes and some contralateral projections, while the engrafted V1 interneurons grew shorter processes to nearby laminae in the ventral spinal cord. Finally, to determine if the transplanted neurons achieve functional integration into the existing circuitry, therefore, causing changes in motor output, engrafted mice completed a highly sensitive locomotor assay before and after transplantation. Preliminary data from these trials suggest the addition of the transplanted neurons to the already functional motor circuit perturbs normal gait in a manner specific to the spinal neuron transplanted. If functional integration is truly occurring, isolating and transplanting these easily producible ES-derived spinal neurons with an established identity could be a viable new approach to modulating spinal circuitry.

Inhibitory Pathway Specifically Modulated in a Selection of Muscles in the Contralateral Leg During Walking

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Sensory afferents play an important role in coordinating the movement of multiple joints within and between legs during locomotion, as removal of sensory feedback severely impairs locomotion. It has been established in cats that sensory information from one leg influences the motor neuron (MN) activity of the contralateral leg (crossed reflex) through spinal commissural interneurons (CINs). Experiments in humans have shown that similar pathways exist since sensory stimulation can evoke motor responses in the contralateral leg. However, the neural circuits transmitting sensory information to the contralateral side of the spinal cord remain poorly understood. Experiments with mice revealed that left-right motor coordination is controlled by at least two genetically distinct classes of CINs (V0 and V3). Despite the insights regarding V0 and V3 involvement in locomotor activity, the role of these CINs in crossed reflexes remains unknown. Recently, crossed reflex pathways have been shown in mice *in vitro* (Jiang et al, 1999, Brain Res, 816:493) and *in vivo* (Nakanishi and Whelan, 2012, J Neurophysiol, 107:500) by applying a moderately strong toe pinch suggesting that crossed reflex pathways are also present in mice. Our goal was to characterize crossed reflex pathways in fully awake mice *in vivo*. Here, we describe crossed reflex motor output after the contralateral stimulation of cutaneous and proprioceptive afferents (tibial nerve stimulation), or only cutaneous afferents (sural nerve stimulation). The electromyogram (EMG) activity of different flexor and extensor muscles in the contralateral leg was recorded simultaneously while adult mice were resting or walking *in vivo*. In summary, our data shows 1) that crossed reflex responses can be evoked in freely behaving mice in both flexor and extensor muscles following proprioceptive and cutaneous afferent stimulation; 2) that sensory information is transmitted to contralateral motor neurons through an inhibitory and excitatory pathway, where the inhibitory influence is most likely mediated by cutaneous afferents; and 3) that crossed reflexes in mice are subject to modulation depending to the activity of the muscle prior to stimulation during walking. These experiments will serve as ground work in the mouse model to identify specific CIN pathways involved in crossed reflexes and the role of these crossed reflex pathways during motor behaviour. In the future we aim to assess the involvement of V3 and V0 CINs in crossed reflex.

$Chx10^+$ Pedunculopontine Neurons: A Dedicated Glutamatergic Subpopulation that Arrests Motor Behavior

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The pedunculopontine nucleus (PPN) is located in the midbrain and is one of the structures that together with the cuneiform nucleus comprises the mesencephalic locomotor region, known to promote locomotion (Caggiano et al., 2018). The PPN has three main neuronal populations (glutamatergic, cholinergic and GABAergic) which are differentially segregated along the rostro-caudal axis of the nucleus. Cholinergic and glutamatergic neurons are more abundant in the caudal part, while the rostral part is occupied mainly by GABAergic and fewer glutamatergic neurons. In this work, we demonstrate the presence of $Chx10^+$ (V2a) neurons in the PPN and confirm their glutamatergic nature with *in situ* hybridization by using the RNAscope technology. Moreover, we show that the Chx10 subpopulation of glutamatergic neurons is more abundant in the rostral part of the PPN. Optogenetic activation of Chx10-expressing neurons in the rostral PPN causes a momentary arrest of all ongoing motor behaviors for the duration of the optical stimulation. The arrest not only affects locomotion but also other spontaneous motor behaviors such as rearing or grooming. The motor arrest is terminated directly upon completion of the stimulation and mice quickly resume the activity they were previously engaged on. In order to further characterize the evoked behavior, we have performed electromyographic (EMG) recordings and measured physiological parameters. EMG recordings of limb muscles in freely-moving animals show the muscular signature of the evoked motor arrest. A reduction of vibrissae movements is also observed upon optical stimulation together with a reduction of the respiratory frequency. This characteristic motor arrest with a similar autonomic signature seems to also occurs spontaneously when the animal is in an attentive state. We conclude that the activation of the glutamatergic V2a neurons in PPN causes a behaviorally relevant motor arrest probably used while evaluating novel stimuli.

Postsynaptic Response Patterns to Stimulation of Low-threshold Hindlimb Afferents in Flexor- and Extensor-aligned Rhythm-generating Neurons

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The basic hindlimb locomotor pattern is generated in the thoracolumbar spinal cord by neural circuits known as central pattern generators (CPGs). The locomotor CPG integrates descending commands and ascending sensory information to activate, modulate and halt the rhythmic program. In spinal cord injury and stroke, descending control is impaired but afferent pathways to the CPG remain comparatively intact. Thus, understanding the specific structure of CPG afferent processing circuits may have therapeutic relevance for gait recovery in these disease states. Several recently identified genetically-labeled neuronal populations are thought to participate in CPG circuits on the basis of locomotor alterations following ablation or inhibition. The mammalian CPG structure has not been directly tested, but modeling experiments suggest a two-layer architecture in which rhythm-generating (RG) neurons produce the basic motor program and indirectly recruit motoneurons through an intermediate pattern-forming (PF) neuron population. Using intersectional genetics, a subpopulation of RG neurons has been identified that express Shox2 during development and lack expression of Chx10. Hip angle and ankle load strongly regulate stance/swing phase duration and transition, and these effects are predicted to be mediated at the RG level. To directly test this, we developed a lumbar-hemisected isolated spinal cord preparation with preserved peripheral nerves to study RG neuron operation in Shox2::cre;Rosa26-lsl-tdTomato;Chx10GFP mice. In this preparation, RG neuron response to activation of functionally-specific afferent pathways can be measured using whole-cell patch clamp of visually identified RG neurons. Drug-evoked fictive locomotion is possible in the preparation and allows further specification of RG neurons to flexor- and extensor-aligned populations. Under this paradigm, we show that Shox2 flexor- and extensor-aligned neurons display postsynaptic currents following activation of specific hindlimb afferents, consistent with predictions from experimental and modeling studies. RG neuron behavior during afferent perturbation of ongoing locomotion and phase-specific changes in afferent responses will yield new insights into operation and feedback control of CPG circuits.

Early Commencement and Prolonged Interference with Persistent L-type Calcium Channels Prevent Development of Dystonia and Spasms after Spinal Cord Injury

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Spinal cord injury (SCI) often results in spasticity, involving increased muscle tone – dystonia – and the occurrence of muscle spasms. Despite major progress in pharmacological approaches, no preventive treatment exists for spasticity. Here, we investigate the possibility that an early onset and long-term blocking of the persistent L-type calcium channels, Ca_v 1.3, may interfere with the development of dystonia and muscle spasms over time. We used a mouse model with a sacral lesion where spasticity is developed 6 weeks after SCI. Using a genetic approach we show that the Ca_v 1.3 channels are expressed in motor neurons as well as in excitatory and inhibitory interneurons. Complete silencing of the Ca_v 1.3 channels (Ca_v 1.3 KO) prevents the development of dystonia and muscle spasms as well as conditional silencing of the channels in just the inhibitory or excitatory neurons even thought to a lower extend than the complete silencing. Next, we applied a pharmacological interference protocol using daily subcutaneous injection of nimodipine (10mg/kg) to target the Ca_v 1.3 channels. When nimodipine was given from day 1 after the lesion and for the first 6 weeks lesioned wild-type mice neither developed dystonia nor spasms. After the end of nimodipine treatment, spasticity did not return for the rest of the observation period (6 weeks). In contrast, when nimodipine was given for the first time 6 weeks after the lesion (when spasticity was fully developed), it just partially reduced dystonia and muscle spasms. Lastly, early application of the nimodipine treatment to the Ca_v 1.3 KO mice confirmed that the main effect of nimodipine on spasticity was mediated by blocking the Ca_v 1.3 channels. In conclusion, an early commencement and prolonged treatment with nimodipine permanently prevent the development of dystonia and muscle spasms after SCI. The study outlines a new treatment protocol in humans for treatment of hyperexcitability of spinal circuitries after brain injury.

Motor Cortex Descending Projections Drive Orofacial Behaviors through Specific Brainstem Premotor Networks

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Focal activation of motor cortex is known to enact behaviorally meaningful motor output, yet the details of how the cortical circuitry interfaces with brainstem premotor circuits is unresolved. We studied the hierarchical nature of this control with respect to motor actions that involve the vibrissae, jaw, and forelimb. The spinal trigeminal nucleus pars oralis (SpVO) and interpolaris rostralis (SpVIr) contain premotor neurons known to directly synapse on vibrissa, jaw, and forelimb motoneurons. In addition to cortical input, SpVO and SpVIr receive direct sensory signals from the periphery. Thus, their ability to integrate descending motor input and peripheral sensory information position them as ideal candidates to determine the specificity of cortex-to-brainstem-to-muscle feed forward networks. Here we show structural labeling and functional activation that determined how two distinct clusters of premotor neurons, one in SpVO and a second in SpVIr, control distinct, yet related, motor actions. A transectional virus strategy preferentially encoded a red-shifted channelrhodopsin in SpVO- versus SpVIr-projecting motor cortex neurons. Activation of the different cortical populations evoked distinct muscle activation. Similar stimulations of localized regions of motor cortex in Thy1-ChR mice show patterns of muscle activity and forelimb, jaw, and nose movements that correspond to behaviorally meaningful movements. All together, our data identifies the functional specificity of motor circuits that originate in cortex and descend onto specific premotor populations. We suggest that this specificity is a major determinate in the coordination of motor actions into behavior.

Activation of D_2-*like* Receptors Depresses Synaptic Transmission in Deep Dorsal Horn Interneurons and DRPs Produced by Stimulation of Low-threshold Afferent Fibers

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It has been shown that dopamine (DA) depresses low-threshold afferent stimulation-evoked primary afferent depolarization (PAD) with no effect on afferent synaptic transmission. These effects are mediated by the activation of D_2, D_3 and probably D_4 receptor subtypes in the *in vitro* mouse spinal cord (SFN Abstracts 422.04/Q5, 2015; 232.02/EE29, 2017). In the present work we further examine the actions of DA and the D_2-*like* receptor agonist, quinpirole, on afferent synaptic transmission in deep dorsal horn interneurons along with effects on PAD.Experiments were carried out on P6 sagittally-hemisected mouse spinal cord with dorsal roots and peripheral nerves attached for afferent stimulation. Stimulus strength was based on multiples of threshold (xT) of the most excitable fibers recorded from the incoming afferent volley, with strengths ≤ 2 xT recruiting only myelinated afferents. PAD was inferred from dorsal root potentials (DRPs) recorded at L_3-L_4 dorsal roots, whereas monosynaptic component of excitatory postsynaptic currents (EPSCs) or excitatory postsynaptic potentials (EPSPs) were recorded from deep dorsal horn interneurons with micropipettes filled with potassium gluconate with QX314. We found that the endogenous ligand DA (10 μM) depressed EPSPs and EPSCs to 67.1±6.0% (n=17), 61.9±7.4% (n=17) of control, respectively. Whereas the D_2-*like* receptor agonist, quinpirole (1 μM), depressed EPSPs and EPSCs to 64.2±8.8% (n=10) and 59.4±8.0% (n=10) of control, respectively. These results were correlated with depression of DRPs by DA (44.2±2.9%, n=17) and quinpirole (53.8±5.4%, n=10). When the monosynaptic components were isolated with mephenesin (1 mM), DA depressed EPSPs, EPSCs and DRPs to 49.37±7.71% (n=4), 42.87±10.13% (n=4) and 34.82±5.05% (n=4) of control, respectively. These results suggest that depression of monosynaptic transmission by DA occurs at a postsynaptic level and support the hypothesis that the modulating inhibitory effects by DA on PAD take place at the interneuronal level by the activation of D_2-*like* receptors (D_2, D_3 and probably D_4 receptor subtypes, as shown previously). We are exploring the effect of the D_2-*like* agonist, quinpirole, on the isolated monosynaptic component of afferent-evoked responses from deep dorsal horn interneurons and on their membrane properties.

Mechanisms for Saliency Coding and Transmission from the Lamprey Tectum to the SNc/VTA

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The dopaminergic system was already well developed at the dawn of vertebrate evolution, according to data in lampreys. The SNc/VTA modulation of the basal ganglia through the direct and indirect pathways in the striatum is already present in these animals, and its connectivity is virtually identical to that of mammals. This includes SNc/VTA direct projections to different motor centers, and the same neurons that project to the striatum also send collaterals to the optic tectum (superior colliculus), which controls orienting/evasive movements. The SNc/VTA direct modulation of tectum has profound effects on its motor commands, acting on two subpopulations of tectal neurons expressing either D1 or D2 receptors, which are able to enhance or suppress neuronal activity respectively. Dopaminergic modulation of the striatum and tectum is therefore performed in parallel. Direct projections from the SNc/VTA allow a fast modulation of tectum to better react to salient stimuli (meaning here the ability to stand over other stimuli), which evoke a dopaminergic activation. Here, we investigate the mechanisms coding saliency in the SNc/VTA. Using a preparation maintaining the eyes together with the brain, we applied visual stimuli to analyze the impact in the SNc/VTA of different aspects involved in saliency (including speed, intensity and size), showing that activity in the SNc/VTA increases in parallel with the saliency of the applied stimulus. Given that visual information to the SNc/VTA comes from tectum, we explored how this last region codes and transmits saliency to the SNc/VTA. Our results show that tectum alone is sufficient to transmit saliency information to the SNc/VTA. The increase in activity parallel to saliency persists in the SNc/VTA after inactivation of other brain regions influencing tectum and/or SNc/VTA, including the parabigeminal nucleus, pallium (cortex), or the habenula. However, responses to visual stimuli are abolished in the SNc/VTA when locally injecting kynurenic acid in tectum, confirming that saliency coding arises in this last region. Saliency information is transmitted to the SNc/VTA from a group of tectal neurons that form a defined population in the anterior part of the visual map, rather than being spread throughout tectum. Performing patch-clamp recordings of these neurons, we have also analyzed the synaptic mechanisms for saliency coding in tectum. Our results show that the tectal inhibitory interneurons have a key role in how saliency is transmitted to the SNc/VTA. Given the high degree of conservation of the dopaminergic system and the optic tectum, this study provides the evolutionary bases for saliency detection in the SNc/VTA.

Role of the Parafacial Respiratory Group in the Recruitment of Active Expiration During Sleep

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In resting conditions, breathing is typically characterized by an active inspiratory phase and a passive expiratory phase. Expiration may become active through abdominal (ABD) muscle recruitment during periods of increased inspiratory requirements. This respiratory rhythm is thought to be controlled by three coupled oscillators: preBötzinger complex (preBötC) for generating inspiration, the parafacial respiratory group (pFRG) for generating active expiration, and the post-inspiratory complex (PiCo) which is thought to control the post-inspiratory phase. Although abundant evidence linking preBötC activity to the generation of respiratory rhythm exists, research addressing the role of pFRG in ventilation and rhythm generation across sleep states is limited. Recent work in our laboratory reports the occurrence of ABD recruitment during REM sleep, despite the induction of muscle paralysis during this sleep state. This ABD recruitment was associated with a stabilization of breathing in healthy rats. Because pFRG generates active expiration through the engagement of ABD muscles, we hypothesize that the expiratory oscillator is also responsible for the ABD recruitment observed during REM sleep in healthy rats. To test this hypothesis, we inhibited and activated the pFRG oscillator using a chemogenetic approach (DREADDs) while simultaneously recording EEG, airflow, ABD and neck EMG of transfected rats across sleep/wake cycles. Our results suggest that manipulation of pFRG activity has an effect in the occurrence of ABD recruitment events during REM sleep. Inhibition of pFRG (N=7) significantly reduced the number of REM events with ABD recruitment, whereas activation of this oscillator (N=7) resulted in an increase of the number of REM events in which ABD recruitment was observed. Interestingly, modulation of pFRG activity did not seem to affect the occurrence of ABD recruitment during NREM sleep. These results suggest that the occurrence of ABD recruitment during sleep may be state dependent. Further research investigating the mechanisms behind the recruitment of ABD activity specifically during REM and NREM sleep will be necessary.

Large Ventrolateral Glycinergic Interneurons are Less Excitable in a SOD1 Mouse Model of ALS

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While a great deal of attention has been devoted to determining the intrinsic excitability of vulnerable populations of motoneurons in amyotrophic lateral sclerosis (ALS), few studies have investigated synaptic drive to motoneurons. This study assessed excitability of ventrolateral lumbar inhibitory interneurons at an early time point (postnatal day 6-12) using whole cell patch clamp in transverse spinal cord slices from SOD1G93A x GlyT2 eGFP mice. Large (input resistance <325 MOhms) GFP+ neurons were targeted, as this group includes inhibitory interneurons which are presynaptic to motoneurons (including Renshaw cells and Ia inhibitory interneurons). Active and passive properties were measured. SOD1 glycinergic interneurons (n = 16 from 10 mice) show significant dampening of intrinsic excitability compared to controls (n = 25 interneurons from 15 mice), including higher threshold voltage for action potential firing (control threshold = -41mV, standard deviation (SD) 4mV; SOD1 threshold -37mV, SD 6mV; p = 0.022) and altered voltage sensitivity of persistent inward currents, including more depolarized onset and peak voltages in SOD1 interneurons (control onset = -51mV, SD 7mV; SOD1 onset = -45mV, SD 5mV; p = 0.006; control peak = -34mV, SD 6mV; SOD1 peak = -30mV, SD 5mV; p = 0.017). In conclusion, glycinergic inhibitory interneurons in SOD1 mice are less excitable and therefore are likely to spike less often and inhibit motoneurons less *in vivo*.

Alterations in Spinal Cord Injury-induced Plasticity of Spinal Rhythm Generating Interneurons Following Treadmill Training with Epidural Stimulation in Mouse

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Neuronal circuitry generating locomotion is located in the thoracolumbar spinal cord. Spinal rhythm generating interneurons (INs) convert descending inputs into rhythmic outputs. Rhythm generating INs are strongly influenced by afferent feedback and supraspinal control, including serotonergic modulation. Spinal cord injury (SCI) disrupts the descending control of spinal locomotor circuits but this circuitry is located below the level of most SCIs and is relatively intact; however, plasticity occurs. Current clinical therapies to recover motor control after SCI include treadmill training and epidural stimulation (ES), targeting the locomotor circuitry. However, the state of the spinal circuits targeted after SCI and rehabilitation is poorly understood. Rhythm generating INs should be a prime access point for these treatments. Previously we found that rhythm generating INs expressing the transcription factor Shox2 are modulated by serotonin (5-HT) in a dose-dependent manner, producing inhibitory actions at low concentrations and excitatory actions at high concentrations. Further, Shox2 INs received mainly polysynaptic afferent input mediated by both excitatory and inhibitory pathways. After SCI, 5-HT only increased the excitability of Shox2 INs, regardless of concentration, and Shox2 INs received only excitatory inputs from afferent pathways. The main objective of the present study was to identify how the combination of treadmill training and ES then modifies the SCI-induced plastic changes in afferent-evoked inputs to and 5-HT modulation of Shox2 INs. Complete thoracic spinal transections were performed on adult Shox2::Cre;Rosa26-lsl-tdTomato mice. ES wires were implanted at lumbar level L2 for ES during treadmill training (SCI+ES) for 5 weeks after SCI. Whole cell patch clamp recordings targeted Shox2 INs in lumbar spinal slices, with dorsal roots attached for afferent stimulation, from SCI and SCI+ES mice. After treadmill traning with ES, 5-HT hyperpolarized Shox2 INs and there was a return of afferent-evoked inhibitory inputs to Shox2 INs. This suggests that treadmill training with ES shifts the balance of excitatory/inhibitory afferent pathways to Shox2 INs and the serotonergic control of Shox2 INs back towards that observed in the uninjured state.

Etonogestrel: Effect on Ventilation in Adult Female Rats

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It is well known that progesterone acts both centrally and peripherally as a respiratory stimulant, as women who are in the luteal phase of their menstrual cycle or during pregnancy experience periods of hyperventilation, and post-menopausal women display an increased frequency of sleep disordered breathing compared to pre-menopausal women. Congenital central hypoventilation syndrome (CCHS) is a disorder caused by a genetic mutation of the transcription factor PHOX2B, which is essential for neural development of several classes of neurons. CCHS is associated with the inability to maintain proper ventilation and blood gas levels during sleep. There is no effective cure for the disease and the only option for treatment is mechanical ventilation or diaphragm pacing. Respiratory stimulants have proven ineffective, with the exception of a recent report that indicated a 2-3 fold increase in the ventilatory response to hypercapnia in female CCHS patients with the onset of desogestrel, a potent progestin into their daily regimen. In this study we hypothesized that etonogestrel (ETO) stimulates progesterone receptor expressing neurons to increase ventilation. Adult female rats were instrumented with implants to chronically deliver ETO (or sham rats) over a four-week period. An additional group of rats was also treated with 17β estradiol (E2) to increase progesterone receptor expression in presence of ETO or sham rats. Rats were then tested weekly in whole-body plethysmographs to determine changes in respiratory parameters during normoxic , hypoxic and hypercapnic conditions. At the end of the experiment, response to hypoxia and hypercapnia was also tested under isofluorane anesthesia.Our results indicate that ETO does not affect the hypercapnic or hypoxic ventilatory response in freely behaving healthy female rats. However, ETO or E2 induce potentiation in the second phase of the hypoxic ventilatory response under isofluorane anesthesia.

Cervical Glutamatergic Interneurons Modulate Breathing

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Cervical interneurons that form synapses on phrenic motor neurons, which control the main inspiratory muscle, can modulate phrenic motor output and diaphragmatic function. However their role in breathing has not yet been defined. Here, using a combination of pharmacogenetics and respiratory physiology assays, we show that mid-cervical excitatory interneurons are not necessary for breathing under normal conditions, however their stimulation enhances inspiratory amplitude. Thus we hypothesized that these neurons are essential for breathing when the respiratory system is under challenge. To prove this we silenced this population after chronic traumatic and non-traumatic cervical spinal cord injury. Indeed, the animals' respiratory motor output was significantly impaired while the cervical excitatory neurons were silenced.Respiratory insufficiency in the acute phase of traumatic cervical spinal cord injury often necessitates mechanical ventilation, and can ultimately lead to life-threatening respiratory complications. Effective treatments to restore breathing during this crucial phase are currently lacking. Because of the above presented data mid-cervical eINs represented an ideal target to rescue breathing at the acute phase after SCI. We show that pharmacogenetic stimulation of cervical excitatory interneurons restored respiratory motor function immediately after traumatic cervical spinal cord injury.

Astrocytes Modulate Brainstem Respiratory Rhythm-generating Circuits

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Astrocytes, the electrically silent CNS cells, have been proposed to modulate neuronal network activity, including vital brainstem respiratory rhythm-generating circuits of the preBötzinger complex (preBötC), although such a modulatory function at the level of preBötC circuits has not been directly demonstrated. Previously, in conscious adult rats and using viral vector technologies, we have shown that interfering with vesicular release mechanisms of preBötC astrocytes significantly reduced the resting respiratory rate and frequency of periodic sighs, decreased rhythm variability, impairs respiratory responses to hypoxia and hypercapnia, and dramatically reduces the exercise capacity (Nat. Commun. 2018 Jan 25;9(1):370). Here, we employed transgenic mice expressing Cre recombinase under control of the human glial fibrillary acidic protein (hGFAP-cre) promoter for astrocyte-specific expression of Channelrhodopsin-2 (ChR2) or archaerhodopsin (Arch) and applied optogenetic techniques to manipulate activation of preBötC astrocytes in rhythmically active neonatal medullary slice (*in vitro*) and in adult perfused brainstem-spinal cord preparations (*in situ*). Activation of Arch in preBötC astrocytes via laser (593 nm, 2-10 mW, *in vitro*), decreased and eventually stoped the frequency and amplitude of hypoglossal nerve activity, whereas laser activation of ChR2 (473 nm, 0.5-5 mW) in preBötC astrocytes increased frequency of hypoglossal (*in vitro*) and phrenic nerve (*in situ*) activities. Furthermore, we obtained evidence that L-lactate released from optogenetically-stimulated astrocytes acted as a novel signalling molecule exciting preBötC circuits *in vitro*. Our results have confirmed the capability of astrocytes to modulate activity of preBötC rhythm-generating circuits.

Claustrum Relays Limbic Information to Motor Cortex to Guide Decision-making

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The claustrum is a forebrain, subcortical structure whose function remains unknown. Here, we dissect the precise input-output organization of a subset of claustrum neurons that project to secondary motor cortex (M2). By employing AAVretro.Cre injections into M2, we can precisely target claustrum projection neurons (CPNs) with Cre-dependent AAV injections into the claustrum. Our results reveal that CPNs target numerous cortical areas including sensory, association, and limbic cortices. Cre-dependent, monosynaptic rabies tracing from CPNs found the majority of inputs originating from limbic and associative regions of frontal cortex. The second major input to CPNs was from the claustrum itself, consisting of other CPNs and an array of claustrum interneurons (PV, SOM, NPY, VIP). Subcortical labeling included intralaminar and limbic nuclei of the thalamus, as well as reciprocal connections between the claustrum and the basolateral amygdala. We next used the same AAVretro.Cre strategy to target the claustrum for optogenetic manipulation and molecular lesions (using a Cre-dependent diphtheria cell ablation method). Our optogenetic experiments revealed the claustrum is associated with negative valences, exhibiting a strong aversion to activation of M2 projecting CPNs. Following molecular lesions of the claustrum, we observed anxiolytic effects and dysregulation of behavioral responses in assays involving emotional processing. By contrast, a host of other behavioral assays that test sensorimotor functions were not affected. Together these data indicate the claustrum is involved in the emotional regulation of action control by acting as a node connecting limbic information to higher order cortical motor areas to guide decision making.

Mechanisms Underlying Modulation of Posture and Locomotor Speed by Sensory Neurons Contacting the Cerebrospinal Fluid in Vertebrates

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The cerebrospinal fluid (CSF) is a complex solution circulating around the brain and spinal cord. Although development and function of the nervous system are influenced by the content and flow of the CSF, the underlying mechanisms are not well understood. CSF-contacting neurons (CSF-cN) by their location at the interface between the CSF and the nervous system are in ideal position to sense CSF cues and to relay information to local networks. We previously demonstrated that the CSF-cNs detect local bending of the spinal cord and in turn, feedback GABAergic inhibition to excitatory interneurons driving slow locomotion and motor neurons controlling posture in the ventral spinal cord. Here we performed high throughput quantitative behavior analysis in animals where the output of these cells is silenced to investigate the contribution of CSF-cNs to locomotor speed and postural control during swimming. While these sensory cells decrease locomotor frequency and speed in the slow regime during exploration, they increase locomotor frequency and speed in the fast regime during acoustic escapes. Furthermore, the analysis of escape swimming revealed a higher roll over ratio in transgenic larvae where CSF-cNs are silenced, suggesting a role in active postural control for these cells. The neuronal targets we previously identified in the spinal cord cannot explain such effects on locomotor speed. We therefore investigate whether CSF-cNs synapse on other neurons in hindbrain and spinal cord. In the ventral spinal cord, we identify that CSF-cNs form axo-axonic varicosities on descending axons from V2a interneurons and somatic varicosities on vglut2+ V3 interneurons. Using ChR-assisted connectivity mapping, we did not observe functional monosynaptic inputs from CSF-cNs in glutamatergic V2a and V3 interneurons. However, we did measure in vglut2+ V3 neurons polysynaptic inputs subsequent to CSF-cN activation. Interestingly, vglut2+ V3 interneurons systematically show delayed spiking following CSF-cN activation, suggesting an indirect projection from CSF-cNs onto V3 interneurons. In the hindbrain, we found that the ascending axon of rostrolmost CSF-cNs arborize onto pectoral and occipital motor neurons, putatively involved in the control of fin movement and posture. To investigate the physiology of these putative connections, we are now combining optogenetics, cell-targeted photoablation, and *in vivo* whole cell recording of putative CSF-cN targets.

A Sensory-motor Pathway Detecting Pathogens Invading the Cerebrospinal Fluid Confers Survival Benefit during Meningitis

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Cerebrospinal fluid (CSF) is a complex fluid circulating around the nervous system whose composition dramatically changes as a function of an organism's physiological state and health status. A singular class of spinal sensory neurons known as CSF-contacting neurons (CSF-cNs) surrounds the central canal in vertebrate species. Recent evidence show these neurons project a mixed microvilliary/ciliary extension into the CSF and detect mechanical and chemical cues associated with changes in CSF flow, pH, and osmolarity. Here, we investigated the relevance of CSF-cN chemosensation during pathogen invasion in the CSF. We performed a transcriptome analysis of CSF-cNs, which revealed a number of receptors and secreted factors associated with innate immunity. To test whether CSF-cNs could detect pathogen invasion in the CSF, we developed a zebrafish model of bacterial meningitis where Streptococcus pneumoniae invades the CSF, including the central canal, within 24 hours. We show that in response to a bacterial infection, animals reduce massively their spontaneous locomotion. Concomittantly, CSF-cNs undergo drastic changes in activity during pathogen invasion: while basal activity is globally reduced, a subset exhibits massive calcium transients lasting for tens of seconds. Such massive CSF-cN activation was mimicked by injection of the bitter compound denatonium but not by the bacterial toxin pneumolysin nor by lipopolysaccharides and was not seen in other spinal cells such as motor neurons and ependymal radial glia. Interestingly, we found that CSF-cNs secreted factors include proinflammatory cues, proteins attracting macrophages and antimicrobial "defensin" peptides, suggesting that the activation of CSF-cNs *in vivo* could enable peptidergic release involved in innate immunity for fighting pathogen in the CSF. Using ablation and silencing of vesicular release in genetically-targeted CSF-cNs, we find that CSF-cN release actively contributes to survival and recovery during bacterial meningitis. Altogether, we discover that spinal sensory neurons modulating locomotion are actively detecting pathogen invasion in the CSF and contribute to recovery following a bacterial infection leading to meningitis.

The Potential Roles of Mouse Lumbar v3 Interneurons for Fore Hind Limb Coordination

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Trotting is the major and most stable running gaits for quadruped animals. However, the understanding of neural circuits that regulate trotting behaviors is still lacking. V3 interneurons are a major group of glutamatergic commissural neurons in the spinal cord. They directly innervate motor neurons as well as other ventral interneurons. When we specifically deleted the expression of Vesicular Glutamate Transporter 2 (VGLUT2) in V3 INs in Sim1cre/+; VGluT2 flox/flox (V3OFF)mice, we found that these mutant mice couldn't run faster than 40 cm/s on the treadmill, at which speed wildtype mice trot constantly. However, V3OFF mice couldn't trot properly due to the lack of the capacity to precisely synchronize their diagonal limbs while running. Optical stimulation of V3 INs in the lumbar segments of the isolated spinal cord of the neonatal Sim1cre/+; Rosa26 ChR2/+ mouse could simultaneously evoke activities at lumbar and cervical ventral roots. Blocking glutamatergic synapses exclusively in the lumbar region didn't prevent this light-induced cervical activity, suggesting that lumbar V3 interneurons could directly innervate cervical motor circuits. Using retrograde tracer, cholera toxin subunit B, injected in the cervical motor region, we then identified clusters of V3 INs within dorsal and intermediate spinal laminae in higher lumbar segments that had long ascending projections to the cervical region. Next, we observed that the number of c-Fos protein expressing lumbo-cervical projecting V3 INs significantly increased after running at 40cm/s on the treadmill, compared to that after walking at low speeds, indicating that these long projecting V3 INs were highly active during trotting. Taken together, we propose that lumbo-cervical projecting V3 interneurons provide a direct excitatory drive to cervical locomotor networks during medium locomotor speeds essential for the transition from a walking to a trotting gait.

Understanding a Flexible Locomotor Pattern in *Xenopus* Larvae

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Spinal neural circuits that control rhythmic locomotor activity gradually mature during development and gain the ability to produce flexible movements. Although this ability is essential for all animals, including humans, very little is known about how flexible locomotor patterns develop. The spinal locomotor network of *Xenopus* larvae, unlike its embryonic counterpart, has developed the ability to produce a flexible pattern that randomly appears during fictive swimming, displaying variability in frequency and intensity of motor bursts [1]. Similar rhythmic changes can be induced by application of NMDA, while 5-HT released from hindbrain neurons is also likely involved [2, 3]. To address how such a flexible pattern can be naturally produced and modulated, we first systematically examined the spontaneous flexible motor output in young *Xenopus* larvae. Secondly, a hindbrain lesion at the otic level abolished the pattern in the majority of larvae, indicating that descending inputs play a critical role in initiating the pattern. Thirdly, using spinalised and intact tadpoles, we further tested the effects of different substances that target at NMDA, glycine and acetylcholine receptors on the generation of the flexible motor pattern. Finally, we explored the electrical properties of individual locomotor neurons to reveal their contribution to the flexible swim pattern. Our results show that the NMDA receptor-mediated excitation plays a more important role than the glycine receptor-mediated inhibition in the generation of the flexible pattern, even though both excitatory and inhibitory interneurons are more excited during the frequency increase. Data obtained from this study will elucidate the functional development of the spinal locomotor circuit and in particular, the locomotor speed change.

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