Motor Control: Spinal Circuits and Beyond
University of St Andrews
June 17-20, 2025

https://motor-circuits.wp.st-andrews.ac.uk/

Map of University of St Andrews and Town

Units with staff in Walter Bower House at Eden Campus (84 on inset map)

Accommodation, Conferences & Events
Admissions
Development
Entrepreneurship Centre
Environmental Health and Safety Services
Estates
Finance
Human Resources
IT Services
Library
OSDS (Organisational and Staff Development Services)
Planning
Procurement
Registry
Research & Innovation Services
Residential & Business Services
Student Accommodation Services
Technology Transfer Centre

Key Locations

St Andrews Bus Terminal
https://goo.gl/maps/pJZQr7RoVXpKwVLx5

Medical Sciences Building
https://goo.gl/maps/YPG4nAKVhhBpBxD27

Agnes Blackadder Hall
https://goo.gl/maps/k2qSi4gKsRmwTkfx9

St Salvator's Quadrangle
https://goo.gl/maps/Y8znCXVmHWgcETEk8

West Sands (Bonfire)
https://maps.app.goo.gl/dseuf7WFBcWgcCY29

Kingsbarns Distillery (Whisky Tasting)
https://maps.app.goo.gl/cawHFtyD7W5yNFD88

St Andrews Links Golf Academy (Driving Range)
https://goo.gl/maps/GYUuCFPGSLNxdEWB9

Crail Golfing Society (Golf)
https://maps.app.goo.gl/WbRoZb6TPQYiqwRJA

Fife Coastal Walk (Hiking)
https://goo.gl/maps/DKMfgLo1UuE8hTXdA

St Andrews Guest Information

https://www.st-andrews.ac.uk/accommodation-conferences-events/accommodation/guest-information/

Parking

Free parking outside Agnes Blackadder Hall, close to the medical building.

https://goo.gl/maps/k2qSi4gKsRmwTkfx9

Hiking

Fife Coastal Walk – Recommended route: Starting at East Sands and walking South-East toward Cambo Sands. Alternatively, one could also walk the other direction, however this route is mostly a paved path.

https://fifecoastandcountrysidetrust.co.uk/walks/fife-coastal-path/
https://fifecoastandcountrysidetrust.co.uk/walks/fife-coastal-path/cambo-sands-to-leuchars/

Putting Green

A booking is required, which opens two days prior. It is recommended that bookings are made on June 20 to ensure a time can be secured.

https://www.standrewsputtingclub.com/

Transit

Travel by bus around St Andrews, Fife, and beyond. Fares can be purchased on the official Stagecoach app or on the bus using cash or a contactless debit/credit card. Fares, routes, and schedules can be found below/on the app. Google Maps is also good for routes and bus times.

https://www.stagecoachbus.com/about/east-scotland
https://motor-circuits.wp.st-andrews.ac.uk/travel-housing/

An Undergraduate's Guide to St Andrews

Key places in St Andrews from people who've lived here for 4 years – grocery stores, restaurants, activity recommendations with helpful descriptions.

https://www.google.com/maps/d/edit?mid=1Cm8GijSyBkL1NiTVRJVu4EPL9LjPnow&usp=sharing

More to do in Fife

https://www.welcometofife.com/

Motor Control: Spinal Circuits and Beyond
University of St Andrews, June 17-20, 2025

Day 1: Tuesday, June 17

11:30	**Arrival & Check-in**	**Medical Sciences Building**
16:30	Dave McLean (University of Edinburgh)	**Introduction**
16:45	Keith Sillar (University of St Andrews)	**Opening Lecture**
17:30	**Reception (Drinks & Canapés)**	**Medical Sciences Building Café**

Day 2: Wednesday, June 18

Session # 1: Rhythm & Pattern Generation

9:00	**Chairs** Jessica Ausborn & Muriel Thoby-Brisson	**Introduction**
9:15	Simon Gosgnach (University of Alberta)	**Molecular characterization of neurons involved in locomotor rhythmogenesis**
9:30	Urs Böhm (Institute for Psychiatry and Neurosciences of Paris)	**Optical recording of spinal cord dynamics with voltage imaging**
9:45	Charlotte Le Mouel (Sorbonne Université)	**Decreased spinal inhibition leads to un-diversified locomotor patterns**
10:00	Sufyan Ashhad (National Centre for Biological Sciences)	**PreBötzinger Complex subcritical oscillations underlie instantaneous switches in breathing dynamics**
10:15	Evgeny Bondarenko (University of California, Los Angeles)	**Subthreshold oscillations of preBötzinger Complex VGluT2$^+$ neurons enable instantaneous modulation of inspiratory rhythm generation *in vivo***
10:30	William Smith (University of St Andrews)	**Neuromodulation of motor competition in the *Drosophila* larval locomotor system**
10:45	**Coffee & Tea**	**Medical Sciences Building Café**

Session # 2: Motoneuron Diseases

11:15	**Chairs** Claire Meehan & Santiago Mora Parada	**Introduction**

11:30   Christian Simon
(Leipzig University)

Motor neuron pathology drives spinal circuit defects and motor phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1

11:45   Matthew Broadhead
(University of St Andrews)

The Honeycomb synapse: A story of synaptic diversity and vulnerability

12:00   Florian Gerstner
(Leipzig University)

Cerebellar pathology contributes to motor deficits in spinal muscular atrophy

12:15   Alyssa Corbett
(University of St Andrews)

Gliotransmission is dysfunctional in spinal motor networks of presymptomatic SOD1 mice

12:30   **Lunch**

Medical Sciences Building Café

Session # 3: Spinal Cord Injury

14:00   **Chairs**
Carmelo Bellardita & Tuan Bui

Introduction

14:15   Dimitri Ryczko
(Université de Sherbrooke)

Restoring neural control: regeneration of descending pathways following complete spinal cord injury in salamanders

14:30   Carina Seidl
(Institute of Molecular Biotechnology)

Patterning and functionality of the regenerated nervous system

14:45   Amanda Bernstein
(Burke Neurological Institute)

Enhancing nerve transfer surgery to restore hand and arm movement in chronic tetraplegia

15:00   Nejada Dingu
(Aix-Marseille University)

Na^+/K^+-ATPase dysfunction via calpain-1 fuels motoneuron hyperexcitability and spasticity after spinal cord injury

15:15   D. Leonardo Garcia-Ramirez
(Drexel University)

Effects of spinal activation strategies after spinal cord injury on lumbar locomotor circuit plasticity

15:30   **Coffee & Tea**

Medical Sciences Building Café

Session # 4: Descending Control

16:00   **Chairs**
Eiman Azim & Martha Bagnall

Introduction

16:15   Bella Xu Ying
(University of St Andrews)

Context-dependent coordination of movement in *Tribolium castaneum* larvae

16:30   Nathalie Krauth
(University of Copenhagen)   **A hypothalamus-brainstem circuit governs the prioritization of safety over essential needs**

16:45   Timothy Machado
(University of Pennsylvania)   **From cortex to the premotor network: Descending circuits for orofacial control**

17:00   Andrew Miri
(Northwestern University)   **Neural activity dynamics of descending control across diverse ethological behaviors**

17:15   Vibhu Sahni
(Burke Neurological Institute)   **Development organization and function of cortico-brainstem connectivity**

17:30   Mahalakshmi Dhanasekar
(Paris Brain Institute)   **Sustained noradrenergic neuron activation and the subsequent triggering of a glial calcium wave disrupt motor commands from brain to spinal cord**

17:45   **Reception (Drinks & Canapés)**   **Medical Sciences Building Café**

19:00   **Bonfire (+ Pizza)**   **West Sands**

Day 3: Thursday, June 19

Session # 5: Sensorimotor Integration

9:00   **Chairs**
Ian Duguid & Claire Wyart   **Introduction**

9:15   Alessandro Santuz
(Max Delbrück Center for Molecular Medicine)   **The cost of sensory loss: irreversible motor deficits scale with the degree of proprioceptor ablation**

9:30   Dominic Falardeau
(Université de Montréal)   **Exploring the role of brainstem trigeminal premotor areas in masticatory function**

9:45   Anders Nelson
(New York University)   **Challenging textbook depictions of an ascending spinal circuit**

10:00   Elias Lunsford
(Paris Brain Institute)   **Directional cues relative to center-of-mass shapes somatotopy of lateral line inputs in the brainstem**

10:15   Emily Reader-Harris
(University of Leeds)   **The role of the lateral vestibular nucleus in neural control of walking and balance**

10:30   **Coffee & Tea**   **Medical Sciences Building Café**

11:00	Sahar Zadka (Weizmann Institute of Science)	**Voltage imaging reveals ultrafast plasticity for the error computations in the cerebellum**
11:15	Nicolas Wanaverbecq (Aix-Marseille University)	**In mice, Lumbar CSF-contacting neurons are sensory neurons modulating cardinal motor interneurons**
11:30	Jitka Veldema (Bielefeld University)	**Non-invasive spinal cord stimulation in supporting balance control**
11:45	Antoine Valera (University of Strasbourg)	**Decoding activity patterns across pyramidal cell dendritic trees during spontaneous behaviours using 3D imaging**
12:00-14:45	**Posters (1-35)**	**Medical Sciences Building**
12:30	**Lunch**	**Medical Sciences Building Café**
14:30	**Coffee & Tea**	**Medical Sciences Building Café**
15:00	**Free/Active Time – Hiking/Golf/Driving Range/Whisky Tasting**	**Fife Coastal Path/Crail Golfing Society/St Andrews Links Golf Academy/Kingsbarns Distillery**

Day 4: Friday, June 20
Session # 6: Premotor & Propriospinal

9:00	**Chairs** Graziana Gatto & Laskaro Zagoraiou	**Introduction**
9:15	James Jepson (University College London)	**Coupled *Drosophila* and mouse studies reveal a pharmacological suppressor of intrinsic spinal circuit dysfunction in genetic models of dystonia**
9:30	Léandre Lavenu (Université de Bordeaux)	**Vestibular inhibitory networks orchestrate the vestibulospinal control of posture in *Xenopus laevis* tadpoles**
9:45	Timothy Cope (Georgia Institute of Technology)	**A species-specific spinal circuit for coordinating antagonist motor pools**
10:00	Wen-Chang Li (University of St Andrews)	**Neuromechanics of a vertebrate escape behaviour mediated by axial muscles after capture**

Programme

10:15	**Coffee & Tea**	**Medical Sciences Building Café**
11:00	Jay Bikoff (St. Jude Children's Research Hospital)	**A brain-wide map of descending inputs onto spinal V1 interneurons**
11:15	Alex Adams (New York University)	**Establishing flexible spinal circuits for locomotor and postural control**
11:30	Lora Sweeney (Institute of Science and Technology Austria)	**Innovations in spinal cord cell type heterogeneity during vertebrate evolution**
11:45-14:45	**Posters (36-75)**	**Medical Sciences Building**
12:30	**Lunch**	**Medical Sciences Building Café**
14:30	**Coffee & Tea**	**Medical Sciences Building Café**

Session # 7: Motoneurons

15:00	**Chairs** Marco Beato & Jasper Phelps	**Introduction**
15:15	Ivica Matak (University of Zagreb)	**Effects of botulinum toxin type A on the spinal motor control: not just a peripheral player?**
15:30	Celine Bellegarda (New York University)	**Birth timing predicts tuning and response strength across extraocular motor neuron pools**
15:45	Stephanie Gaudreau (University of Ottawa)	**Developmental changes in the control of primary motoneuron firing properties by multiple ion currents in larval zebrafish**
16:00	Filipe Nascimento (University College London)	**Personalized mapping of inhibitory spinal circuits via neural decoding of high-density electromyography and in silico modelling**
16:15	Merkourios Simos (École Polytechnique Fédérale de Lausanne)	**Reinforcement learning-based motion imitation for physiologically plausible musculoskeletal motor control**
16:30	Samuel Sober (Emory University)	**Motor unit mechanisms of speed control in mouse locomotion**
16:45	Conference Organizers	**Concluding Remarks**
18:00	**Dinner & Ceilidh (Full registration only)**	**Upper/Lower College Hall, St Salvator's Quadrangle**

Motor Control: Spinal Circuits and Beyond
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Talk Abstracts

Session # 1: Rhythm & Pattern Generation

Molecular characterization of neurons involved in locomotor rhythmogenesis

Simon Gosgnach, Vladimir Rancic, Sabrina Haque, Araya Ungkapawa, Toshifumi Yokota

University of Alberta

Locomotor activity in mammals can be generated by neural networks which are located entirely within the spinal cord. While a genetic approach has been used to identify the specific function of several populations of spinal neurons that make up this neural network, there are still large gaps in our knowledge when it comes to: a) identification of the neurons involved in locomotor rhythm generation; and b) our understanding of how these neurons initiate rhythmic activity in the spinal cord. Recently we used a novel approach, involving Ca^{2+} imaging, to visually identify rhythmogenic neurons based on their activity patterns during drug induced fictive locomotor activity. Anatomical analysis of these neurons indicates that they are clustered near the central canal, and project processes primarily ipsilaterally. Next, we used a single cell patch-seq approach to investigate the molecular makeup of these rhythmogenic neurons. While we were unable to find a single marker that can be used to identify this population, the sequencing data allowed us to conclude that rhythmogenic neurons can be divided up into a handful of genetically-defined interneuronal populations. Finally, genetic identification of the ion channels that these cells express provides insight into intrinsic mechanisms that may be involved in locomotor rhythmogenesis. Collectively, the results of these experiments provide key information regarding the organization of mammalian locomotor circuits, and the manner in which they are activated.

Optical recording of spinal cord dynamics with voltage imaging

Urs Böhm, Eva Gomes, Yukiko Kimura, Takashi Kawashima, Misha Ahrens, Shin-Ichi Higashijima, Florian Engert, Adam Cohen, Benjamin Judkewitz

Institute for Psychiatry and Neurosciences of Paris

Spinal cord activity underlies all locomotor output, but due to the difficulty of recording activity in intact, behaving animals, the diversity of both motor and sensory activities during complex behaviors has been largely inaccessible. Voltage-imaging holds great potential to noninvasively record the membrane potential of many spinal cord neurons in parallel and overcome some of these limitations. By combining voltage-imaging and fictive behavior in a virtual environment, we recently demonstrated the potential of this technology. By measuring the activity of all glutamatergic neurons in the larval zebrafish spinal cord we characterized a previously undescribed subpopulation of tonic-spiking ventral V3 neurons and showed that they serve as modulators of swimming strength. We furthermore developed a new approach for fast and light efficient remote focusing that enables high-speed volumetric voltage imaging at 500 volumes/s, enough to image the entire volume of a section of zebrafish spinal cord and record from >100 spontaneously active neurons in parallel. These new approaches allow us to describe the millisecond timing precision of spinal cord neurons during locomotion, and we now apply this technology to investigate the precise timing of motor neurons within and across motor pools.

Decreased spinal inhibition leads to un-diversified locomotor patterns

Charlotte Le Mouel, Myriam de Graaf, Luis Mochizuki, Heiko Wagner

Sorbonne Université

Animals display rich and coordinated motor patterns during walking and running, that are generated and controlled within the central nervous system. Previous computational and experimental results suggest that the balance between excitation and inhibition in neural circuits may be critical for generating such structured motor patterns. In this paper, we explore the influence of this balance on the ability of a reservoir computing artificial neural network to learn human locomotor patterns, using mean-field theory analysis and simulations. We created networks with varying neuron numbers, connection percentages and connection strengths for excitatory and inhibitory neuron populations, and introduced the anatomical imbalance that quantifies their combined overall effect. We trained the networks to reproduce muscle activation patterns derived from human recordings via inverse dynamics, and evaluated their performance Our results indicate that network dynamics and performance depend critically on the anatomical imbalance in the network. Inhibition-dominated networks work well, displaying balanced input to the neurons and sufficient heterogeneity across the neuron firing rate patterns. Excitation-dominated networks, however, lead to saturated firing rates, thereby reducing the firing rate heterogeneity and leading to muscle co-activation and inflexible motor patterns, reminiscent of the motor patterns in cerebral palsy or dystonia. This suggests that motor pattern generation may be robust to increased inhibition but not increased excitation in neural networks.

PreBötzinger Complex subcritical oscillations underlie instantaneous switches in breathing dynamics

Sufyan Ashhad, Evgeny Bondarenko, Omar Ali, Jack Feldman

National Centre for Biological Sciences

Breathing must be flexible to rapidly adapt to evanescent sensory, emotional, cognitive, and motor inputs, crucial for cognitive-motor coupling during vocalization, active sensing, and airway reflexes. Here, we explore the mechanisms underlying the robustness and flexibility of breathing, which has remained enigmatic, with most rhythmogenic frameworks agnostic of the mechanisms underlying its lability. We identify sub-critical network oscillations in the preBötzinger Complex (preBötC) as a fundamental feature of the breathing central pattern generator, providing flexibility to an otherwise robust rhythm. In anesthetized slow-breathing mice, rhythmic bursts of action potentials (I-bursts) driving inspiration were interspersed with low-amplitude burstlets oscillating at harmonics of the breathing frequency (2Hz-10Hz). These oscillations either fail to propagate or trigger inspiratory bursts propagating to inspiratory motoneurons. The propagation of preBötC burstlet rhythm exhibited attractor dynamics, i.e., burstlets arising from pre-inspiratory synchronization must cross a tipping point(s) to drive the next inspiration. The preBötC excitation-inhibition (E-I) balance regulated this tipping point. For instance, chemogenetic inhibition of preBötC glycinergic neurons (with ultrapotent DREADD PSAM4) lowered the tipping point for burstlet propagation, allowing burstlets to propagate as low-amplitude inspiratory efforts. Crucially, the altered E-I balance also enabled spontaneous transitions between high-amplitude low-frequency and low-amplitude high-frequency breaths centered at harmonic frequencies. These findings motivated us to test the hypothesis: subcritical preBötC oscillations underlie the instantaneous switch in breathing frequency necessary for behaviors like sniffing. In awake mice, auditory stimulusinduced sniffs also occurred at higher harmonics of baseline breathing, highlighting the role of burstlet rhythm in the dynamic switching of breathing patterns.

Subthreshold oscillations of preBötzinger Complex VGluT2$^+$ neurons enable instantaneous modulation of inspiratory rhythm generation *in vivo*

Evgeny Bondarenko, Sufyan Ashhad, Jack L. Feldman

University of California, Los Angeles

Breathing must be highly adaptable to meet dynamic physiological and behavioral demands, allowing rapid modulation in response to sensory, emotional, cognitive, and motor inputs. This requires the breathing central pattern generator (bCPG) to operate in multiple modes to support diverse physiological and behavioral needs. The preBötzinger Complex (preBötC) is the bCPG kernel for eupneic homeostatic breathing and its modulated forms, such as sniffing and sighing. Here, we identify the genetic identity of preBötC neurons involved in distinct components of the inspiratory rhythm generation. Recent in vivo electrophysiological recordings (Ashhad et al., companion abstract) reveal low-amplitude, subcritical preBötC oscillations occurring at higher harmonics of the breathing frequency. These oscillations, termed "burstlets", constitute the essential rhythmogenic component of the preBötC bCPG. We observed rhythmic burstlets in excitatory VGluT2 preBötC neurons using fiberoptic calcium imaging in anesthetized mice. Selective inhibition using the ultrapotent DREADD PSAM4 confirmed that these VGluT2 neurons generate burstlets in vivo, while inhibitory GlyT2 neurons provide feedback inhibition to maintain the excitation-inhibition balance - a key determinant of burstlet propagation and transitions between breathing modes. Chemogenetic excitation of the output sigh circuit induced inspiratory doublets and multiplets at higher harmonics of the eupneic breathing frequency, resembling sniffing and sighing patterns. Together, these findings reveal a dynamic framework in which neuromodulatory inputs harness preBötC subcritical oscillations to optimize near instantaneous switching of breathing dynamics necessary for behaviors such as sniffing and sighing.

Neuromodulation of motor competition in the *Drosophila* larval locomotor system

William Smith, Stefan Pulver

University of St Andrews

Dynamic Interactions amongst competing motor programmes shape behavioural output. Here, we explore how adrenergic-like systems modulate competition amongst central pattern generating (CPG) networks controlling locomotion in *Drosophila* larvae. Bath application of octopamine (OA) promoted fictive forwards locomotion, suppressed backwards locomotion, and induced bouts of fictive head sweeps during wash period that were proportional to the promotion of forward waves. In contrast, Tyramine (TA), a co-transmitter also present in OA neurons, promoted collisions and overlap of motor programmes, and bouts of silence during wash periods. Dual-colour calcium imaging of OA/TA neurons together with motor neurons revealed that most OA/TA neurons are recruited phasically, prior to motor neurons. Optogenetic manipulation of activity in OA/TA neurons recapitulated a subset of effects, and further revealed that activity in OA/TA neurons is necessary and sufficient for locomotor CPG activity in the system. Overall, this work provides insights into how adrenergic-like systems can modulate dynamics of motor competition among interacting CPGs within locomotor networks.

Session # 2: Motoneuron Diseases

Motor neuron pathology drives spinal circuit defects and motor phenotype of a mouse model for spinal muscular atrophy with respiratory distress type 1

Christian Simon, Katharina Apel, Margarita Koehler-Sanchez, Florian Gerstner, Aaron Norman, Leonie Sowoidnich, Nathanael Otte, Marie-Luise Stephan, Sibylle Jablonka

Leipzig University

Spinal Muscular Atrophy with Respiratory Distress Type 1 (SMARD1) is a rare neuromuscular disorder caused by mutations in the IGHMBP2 gene, leading to respiratory failure, distal muscle atrophy, and early lethality. The neuromuscular degeneration (NMD) mouse model replicates key pathological features of SMARD1, including motor neuron loss, muscle denervation, and a distally progressing phenotype. However, the impact on spinal motor circuits and the cellular mechanisms underlying disease progression remain largely unknown. To address this, we employed confocal microscopy, whole-cell patch-clamp recordings, a novel inter-sectional viral/genetic approach, and motor behavior assessments to investigate spinal motor circuit dysfunction in the NMD mouse model. Our findings reveal that muscle denervation precedes motor neuron degeneration and is followed by a selective loss of spinal excitatory synapses in distal motor circuits. Notably, this synaptic vulnerability in NMD mice closely parallels observations in spinal cord tissue from a SMARD1 patient. As motor symptoms develop, proprioceptive dysfunction also emerges, characterized by Ia synapse loss, reduced synaptic input, and delayed neurotransmission. Remarkably, genetic restoration of IGHMBP2 in motor neurons fully rescued sensory-motor circuit integrity, prevented muscle atrophy, and restored motor function in NMD mice, demonstrating that motor neurons are the primary drivers of SMARD1 pathology. These findings provide critical mechanistic insights into motor neuron-driven proprioceptive dysfunction and support motor neuron-centered therapeutic strategies as a promising approach for treating this currently incurable disease.

The Honeycomb synapse: A story of synaptic diversity and vulnerability

Matthew Broadhead, Carlotta Löer, Emma Butler, Molly Roberts, Ani Ayvazian-Hancock, Jessica Valli, David Hughes, Edita Boulivaite, Noboru Komiyama, Seth Grant, Claire Meehan, Gareth Miles

University of St Andrews

The diversity of synapses between neurons is critical for the specialization of neural circuits that underlie a broad range of behaviours. One of the simplest neuronal circuits in the mammalian nervous system is the spinal reflex arc, comprising a direct connection between sensory and motor neurons that facilitates proprioceptive feedback. Given its simplicity, one might expect this circuit to rely on a relatively homogeneous population of rudimentary synapses. To investigate this, we used transgenic fluorescent reporter lines, immunohistochemistry, super-resolution fluorescence microscopy, and machine learning-based image analysis. This revealed unexpected synaptic complexity within the reflex arc. We identified a novel subtype of Ia afferent synapse, distinguished by its large size (1–5 μm) and complex morphology, including multiple perforations within the postsynaptic density (PSD), opposed to large VGLUT1-expressing presynaptic boutons. Super-resolution imaging revealed a striking architecture—5–6 molecular scaffolding clusters arranged around each perforation—prompting us to define these structures as Honeycomb Synapses. While the PSD is scaffold-rich, the perforations may act as conduits for electrical coupling via gap junctions. Using a novel multiplexed microscopy approach, we investigated the vulnerability of synapse subtypes in an inducible model of sporadic Amyotrophic Lateral Sclerosis (sALS). While overall VGLUT1 synapses were reduced in number by ~20%, Honeycomb Synapses were entirely lost, indicating a highly selective vulnerability. This study highlights the importance of synaptic diversity within even the simplest circuits. The unique architecture of Honeycomb Synapses may enable mixed electrical-chemical signalling, and their selective loss suggests a critical role in motor circuit stability.

Cerebellar pathology contributes to motor deficits in spinal muscular atrophy

Florian Gerstner, Sandra Wittig, Christian Menedo, Sayan Ruwald, Maria J Carlini, Adela Vankova, Leonie Sowoidnich, Gerardo Martín-López, Vanessa Dreilich, Andrea Alonso Collado, John G Pagiazitis, Francesco Roselli, George Z Mentis, Charlotte J Sumner, Livio Pellizzoni, Christian M Simon

Leipzig University

Spinal muscular atrophy (SMA) is a motor neuron disease caused by SMN deficiency, resulting in muscle weakness and impaired movement due to the degeneration of spinal motor circuits. Recent clinical findings suggest that yet to be identified neuronal circuits in the brain contribute to motor and newly emerging deficits in SMA patients. Here, we discover conserved mechanisms of cerebellar circuit pathology associated with progressive Purkinje cell (PC) degeneration selectively in a severe mouse model and Type I SMA patients. Distinct pathways converge on p53 activation and drive cell-autonomous, non-apoptotic PC death in SMA mice. Cerebellar pathology is further aggravated by synaptic loss and dysfunction of parallel fibers onto PCs, resulting into reduced functional output of the cerebellar cortex. These impairments arise intrinsically within the cerebellum, independent of established spinal motor circuit pathologies, and contribute to motor deficits in SMA mice. Importantly, treatment with different, clinically-relevant SMN inducing therapies including splicing modifiers and gene replacement demonstrate both overlapping and distinct effects on cerebellar pathology in SMA mice. Together, these findings highlight dysfunction of cerebellar circuits and death of PCs as critical yet underappreciated contributors to motor deficits, which should be considered in care of SMA patients receiving current treatments and for development of future therapeutics.

Gliotransmission is dysfunctional in spinal motor networks of presymptomatic SOD1 mice

Alyssa Corbett, Molly Roberts, Matthew Broadhead, Gareth Miles

University of St Andrews

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons (MNs). Prior to MN loss, both synapses and astrocytes show dysfunction. Astrocytes form specialized tripartite synapses with some neurons and modulate spinal motor network output via gliotransmitters: a function known as 'gliotransmission'. In early symptomatic stages of ALS, tripartite synapses have been found to degenerate before MN loss. Thus, my research investigates gliotransmission in the early stages of ALS, as identifying and targeting areas of dysfunction could prevent later neurodegeneration. To begin, astrocytes were exogenously stimulated during in vitro electrophysiological recordings of spinal motor network output in presymptomatic SOD1 mice and wildtype littermates. Astrocytic stimulation significantly reduced motor network output in wildtype mice, but failed to modulate network activity in the SOD1 mice. In further electrophysiological recordings, a specific gliotransmission pathway by which astrocytes modulate spinal motor network output was targeted. The results indicated that this pathway is overactive in SOD1 mice and may thus be unable to respond to additional stimulation. These findings reveal significant dysfunction in a key pathway regulating motor network output, which occurs before MN loss in SOD1 mice. This makes the pathway a promising target for early therapeutic intervention. Future studies will focus on pinpointing the areas of dysfunction within this pathway, at the network and synaptic level, to identify novel therapeutic targets for ALS.

Session # 3: Spinal Cord Injury

Restoring neural control: regeneration of descending pathways following complete spinal cord injury in salamanders

Dimitri Ryczko, Jordan Swiegers, Lea Blanche, Rabelani Negota, Katherine Medina-Ortiz, Ines Khsime, Cornelis Immanuel van der Zouwen, Alberto Joven Araus, Zuzana Tonelli Gombalova, Auke Ijspeert, Andras Simon

Université de Sherbrooke

After a complete spinal cord injury, salamanders regenerate their spinal cord and recover locomotion. However, the neural circuits involved remain unclear. Our Salamandra project investigates the organization of locomotor circuits before and after spinal cord regeneration. Using video recordings and deep learning-based movement analysis, we observed that a complete low thoracic transection caused hindlimb and tail paralysis. These functions gradually recovered over 17 weeks post-injury. We identified brainstem reticular neurons as key players in regaining control of circuits below the injury. Tracer injections revealed the regrowth of reticulospinal neurons, which are controlled by the Mesencephalic Locomotor Region (MLR), a locomotor center present in all vertebrates. Using patch-clamp recordings in the brainstem of intact animals, we found that MLR stimulation evoked synaptic responses and spiking in reticular neurons. In brainstem-spinal cord preparations, MLR stimulation induced spiking in spinal neurons, with rhythmic bursting at frequencies compatible with those recorded during locomotion. To assess the circuit after regeneration, we used two-photon calcium imaging in transgenic salamanders expressing a calcium sensor in neurons, generated by the teams of A Simon (Karolinska Institute) and MA Tosches (Columbia University). At early recovery stages (5–10 weeks), MLR stimulation increased calcium activity in reticular and spinal neurons below the lesion site. Blocking glutamatergic transmission in the reticular formation abolished the spinal response. Altogether, our findings show that brainstem neurons recover control of the spinal cord below the lesion during spinal cord regeneration in salamanders.

Patterning and functionality of the regenerated nervous system

Carina Seidl, Katharina Lust, Stephan Raiders, Elly Tanaka

Institute of Molecular Biotechnology

Regeneration is not only about shape and form but also about function. Few studies have assessed the precision of regeneration of the nervous system from sensory input to activity to motion. Using axolotl tail regeneration, I aim to bring together analysis of tissue patterning with functional circuit neuroscience, to address: How accurate is organ regeneration in terms of function? I am investigating (I) whether projection patterns, connectivity and muscle innervation are comparable in regenerated axolotls, (II) whether swimming behaviours and functionality of neurons are similar within and across muscle segments, and (III) whether sensory input into locomotor central pattern generators (CPGs) is affected by regeneration. Muscle tissue is not well segmented after regeneration, however, even though segmentation is not perfect, regenerated muscle fibers are active when induced with neurotransmitters, and the animals show swimming ability. Detailed comparison of the S-wave swimming motion will reveal changes in rhythm and pattern generation and will guide the path to how regeneration affects sensory-motor integration. Early results show that regenerated tails are less reactive to touch-induced escape responses which implies a discontinuity between sensory and motor function. One neuron subtype, the V2a interneurons, are essential mediators of touch-induced escape response, and intermediaries between the sensory and motor systems. Therefore, I will focus on the v2a interneuron- and motor circuits in regenerating tails. This study will tell us how accurate organ regeneration needs to be to be still functional, and provide a basis for closer investigation of patterning and connectivity in less regenerative species.

Enhancing nerve transfer surgery to restore hand and arm movement in chronic tetraplegia

Amanda Bernstein, Juan Sebastián Jara, Marwa A. Soliman, Paola di Grazia, Adam R. Ferguson, Justin M. Brown, Abel Torres Espin, Edmund Hollis

Burke Neurological Institute

Nerve transfer surgery is a state-of-the-art surgical approach to restore hand and arm motor control in individuals living with tetraplegia, significantly improving their daily lives. The extent of recovery is limited by at least two critical factors: 1) variability in rehabilitation and the ability of the motor system to fully incorporate the new peripheral circuitry created by this surgical procedure, and 2) slow or incomplete regeneration. We took a bedside-to-bench approach to address these factors. First, we evaluated the effects of six weeks of robot-assisted, intensive rehabilitation on the return of hand and arm function as well as on the cortical motor networks in four individuals after nerve transfer (NCT04041063). We found that intensive robotic training resulted in modest improvements in hand function, predominantly in finger extensor strength and upper extremity motor scores. Coincident with these improvements, transcranial magnetic stimulation-mediated motor mapping demonstrated robust cortical reorganization in response to rehabilitation. To address slow or inefficient peripheral regeneration, we performed pre-clinical studies to evaluate the effects of conditioning electrical stimulation (CES) on outcomes in a mouse model of nerve transfer to treat chronic spinal cord injury. In mice, we found that CES of donor nerves one week prior to nerve transfer surgery enhanced anatomical and functional measures of target muscle reinnervation. Furthermore, CES increased the rate of recovery of naturalistic behavior. Taken together, our clinical and pre-clinical results provide an understanding of the current limitations of nerve transfer surgery and guidance for how to improve motor outcomes over current practices.

Na$^+$/K$^+$-ATPase dysfunction via calpain-1 fuels motoneuron hyperexcitability and spasticity after spinal cord injury

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Spasticity, marked by stiffness and involuntary muscle contractions, typically arises weeks to months post-spinal cord injury (SCI), hindering in vitro studies in adults. To overcome this limitation, we developed a neonatal mouse model in which SCI is performed at birth, enabling the in vitro investigation of spasticity mechanisms. Within 4-5 days post-injury, mice exhibited hallmark signs of spasticity including spontaneous involuntary muscle contractions (spasms) and exaggerated reflexes (hypereflexia). Isolated spinal cords displayed heightened excitability below the lesion, amplifying both spontaneous and sensory-driven motor activities consistent with core spasticity manifestations. At the cellular level, motoneurons exhibited marked hyperexcitability, characterized by a reduced rheobase and a depolarized resting membrane potential (RMP). Notably, the RMP depolarization was insensitive to tetrodotoxin (TTX), suggesting that it is independent of both network activity and persistent sodium current. Given the central role of the Na$^+$/K$^+$-ATPase (NKA) in maintaining the RMP, we assessed its function using ouabain, a specific NKA inhibitor. Ouabain induced a greater depolarization in control motoneurons compared to SCI motoneurons, indicating an impaired NKA function following injury. Building on our previous findings that calpain-1 activation contributes to motoneuron hyperexcitability after SCI, we hypothesized that the NKA dysfunction might result from calpain-1 activity. To test this, we employed a gene approach to selectively downregulate calpain-1 in lumbar motoneurons. This intervention preserved the NKA function by normalizing the RMP, thereby reducing spinal network hyperexcitability, and alleviating spasticity in neonatal mice following SCI.

Effects of spinal activation strategies after spinal cord injury on lumbar locomotor circuit plasticity

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Serotonin, produced supraspinally, is crucial for activating and controlling locomotion by potently modulating lumbar circuits in the spinal cord. Spinal cord injury (SCI) disrupts descending control, including serotonergic fibers, leading to paralysis and plasticity in spinal neurons below the injury. Recently, we found that spinal Shox2 neurons, involved in locomotor rhythm generation, are modulated by serotonin in a concentration-dependent manner; low concentrations inhibit, while high concentrations excite Shox2 neurons. However, after a chronic complete T8/9 spinal transection, which eliminates spinal serotonin, both low and high serotonin concentrations excite Shox2 neurons due to the activation of 5-HT receptors normally absent in uninjured conditions. It is assumed that the 5-HT receptor expression compensates for the loss of serotonin. Interestingly, our data demonstrate that the SCI-induced plasticity in receptor activity is prevented by epidural stimulation (ES), indicating that receptor plasticity is not purely dependent on serotonin. We hypothesize that the prevention of SCI-induced serotonergic plasticity by ES is due to increased spinal neuronal activity. To test this, we increased spinal sensorimotor circuit activity with virally-delivered brain-derived neurotrophic factor (AAV-BDNF), injected into lumbar cord of adult Shox2::Cre;R26-lsl-tdTomato mice with complete T8/9 transections. Four weeks post-surgery, we conducted whole-cell patch-clamp recordings and found that lumbar Shox2 neurons from AAV-BDNF mice are generally more excitable and do not exhibit SCI-induced serotonergic plasticity. These findings demonstrate that SCI-induced serotonin plasticity results from altered spinal neuronal activity rather than solely from the loss of descending serotonin inputs, offering crucial insights for therapeutic strategies aimed at locomotor recovery post-SCI.

Session # 4: Descending Control

Context-dependent coordination of movement in *Tribolium castaneum* larvae

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Insect pests, like the red flour beetle *Tribolium castaneum*, destroy up to 20% of stored grain products worldwide, making them a significant threat to food security. Their success hinges upon adapting their movements to unpredictable, heterogeneous environments like flour. *Tribolium* is well developed as a genetic model system; however, little is known about their natural locomotion and how their nervous systems coordinate adaptive movement. Here, we employed videographic whole-animal and leg tracking to assess how *Tribolium* larvae locomote over different substrates and analyze their gait kinematics across speeds. Unlike many hexapods, larvae employed a bilaterally symmetric, posterior-to-anterior wave gait during fast locomotion. At slower speeds, coordination within thoracic segments was disrupted, although intersegmental coordination remained intact. Moreover, larvae used terminal abdominal structures (pygopods) to support challenging movements, such as climbing overhangs. Pygopod placement coincided with leg swing initiation, suggesting a stabilizing role as adaptive anchoring devices. Surgically lesioning the connective between thoracic and abdominal ganglia impaired pygopod engagement and led to escalating impairments in flat-terrain locomotion, climbing and tunnelling. These results suggest that effective movement in *Tribolium* larvae requires thoracic-abdominal coordination, and that larval gait and limb recruitment is context-dependent. Our work provides the first kinematic analysis of *Tribolium* larval locomotion and gives insights into its neural control, creating a foundation for future motor control research in a genetically tractable beetle that jeopardizes global food security.

A hypothalamus-brainstem circuit governs the prioritization of safety over essential needs

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Animals continuously adapt their behavior to balance survival and fulfilling essential needs. This balancing act involves prioritization of safety over the pursuit of other needs. However, the specific deep brain circuits that regulate safety-seeking behaviors in conjunction with motor circuits remain poorly understood. Here we identify a class of glutamatergic neurons in the lateral hypothalamic area (LHA) that target the midbrain locomotor-promoting pedunculopontine nucleus (PPN). Upon activation, this LHA-PPN pathway orchestrates context-dependent locomotion, prioritizing safety-directed movement over other essential needs such as foraging or mating. Remarkably, the neuronal activity of these circuits correlates directly with safety-seeking behavior. These circuits may respond to both intrinsic and external cues, playing a pivotal role in ensuring survival. Our findings uncover a circuit motif within the lateral hypothalamus that when recruited, prioritizes critical needs through the recruitment of an appropriate motor action.

From cortex to the premotor network: Descending circuits for orofacial control

Timothy Machado

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How does the cortex influence its postsynaptic partners in the brainstem to control orofacial movements like licking? And how do these motor commands depend on the state of the animal (e.g. hunger or fear)? Project 1. We investigated cortex-wide representations for movement and threat in mice that are either food deprived or hungry. Using widefield calcium imaging (n=8 mice), we found that neural representations for threatening stimuli across the extent of dorsal cortex are strongly enhanced in hungry mice. This suggests that cortex is likely to send different motor commands to control behavior when animals are in different homeostatic need states. Project 2. To examine context-dependent behavioral control at a more granular level, we made simultaneous neural recordings from the medulla and motor cortex in mice performing a directional licking task (a total of 3,294 single units in our dataset from n=9 mice). We found that cortex exerts differential influence over the medullary network during appetitive licks (made in response to an operant cue) versus consummatory licks (more automatic licks made to consume water). Together, these two projects reveal new insights into how the cortex and the brainstem interact with each other to control movement under different behavioral circumstances.

Neural activity dynamics of descending control across diverse ethological behaviors

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Natural behavior is composed of a wide variety of movements. A hallmark of the motor system is its ability to generate the diverse patterns of muscle activity that drive these different movements. Yet it remains poorly understood how neural activity dynamics coordinated across motor system regions are organized to efficiently control a broad diversity of movement types through descending pathways. Our own recent results have shown that the influence of different motor cortical regions on muscles, and on each other, changes substantially between single-forelimb reaching and naturalistic climbing. This suggests that neural activity dynamics in the motor system are adapted to different behavioral contexts. Building on this, we developed a paradigm that enables investigation of activity dynamics in multiple motor system regions during diverse motor behaviors in mice. In contrast to existing views, we found neither behavior-specific nor behavior-invariant organization in single-neuron activity, population-level covariation, and muscle activity correlation. Instead, pairs of behaviors exhibited activity dynamics that differed to varying degrees, forming a hierarchical organization. This hierarchy was similarly organized in both M1 and striatum, despite M1 activity displaying much less behavior specificity and striatal activity being much less correlated with muscle activity. Network modeling demonstrated that observed striatal activity can induce varying degrees of similarity across behavior pairs in the activity dynamics of muscle pattern-generating circuits, which constitutes a hierarchy. Collectively, our findings reveal a hierarchical organization of motor system activity dynamics across behaviors, which may reflect a balance between behavioral specialization and the efficiency of reusing functional elements.

Development organization and function of cortico-brainstem connectivity

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The brainstem plays a central role in motor control by integrating signals from higher-order forebrain centers and relaying outputs to spinal circuits. However, the development and organization of cortical input to the brainstem is still not well understood. These connections are established by two neuronal populations: 1) cortico-brainstem neurons (CBN) that only innervate brainstem nuclei, and 2) corticospinal neurons (CSN) that project to the spinal cord but additionally collateralize within the brainstem. The absence of molecular tools to distinguish these populations has limited our understanding of their potentially distinct organization and function. We used selective anatomical labeling, FACS purification, and single cell transcriptomics to identify differentially expressed genes between CBN vs. CSN from the earliest stages of their differential axon extension. These molecular delineators also define circuit level distinctions between these populations at maturity. Using these molecular signatures, we establish novel circuit-level substrates for skilled forelimb movement. Finally, to perform in-depth and rigorous anatomical analyses in the brainstem, we have developed StARQ (brainStem Automated Registration and Quantification), a fully automated deep neural network-based method for segmenting brainstem nuclei with minimal human supervision. Using this platform requires no coding experience by the end-user. StARQ can now be broadly applied for all studies requiring high-throughput anatomical analyses of the brainstem in an unbiased manner. Together, our findings and tools provide a comprehensive framework for dissecting cortico-brainstem connectivity with molecular, anatomical, and functional resolution, enabling new insights into motor control and brainstem circuit organization.

Sustained noradrenergic neuron activation and the subsequent triggering of a glial calcium wave disrupt motor commands from brain to spinal cord

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Evading threats relies on deploying complex avoidance strategies such as escape responses or motor arrest. Although studies across vertebrates indicate that the brainstem is critical for motor arrest, the circuit mechanisms are poorly understood. A recent study in larval zebrafish showed that upon sensory-motor mismatch, motor arrest involves noradrenergic signaling eliciting a brain-wide glial calcium wave. However, the precise circuit mechanisms by which motor circuits are disrupted to induce motor arrest remain elusive. Here we leverage the genetic and optical accessibility of larval zebrafish to demonstrate that sustained activation of noradrenergic neurons induces a motor arrest lasting tens of seconds with rolling and reduced heartbeat frequency. Sustained noradrenergic activation is also sufficient to trigger a stereotyped glial calcium wave originating from the rostral spinal cord and propagating both caudally in the spinal cord and rostrally in the brain. Hotspots of sustained glial calcium activity are restricted to regions in the hindbrain lasting for tens of seconds. At circuit level, this sustained noradrenergic neuron activation is also associated with a refractory period during which the electrical stimulation of the caudal hindbrain fails to elicit motor output and the refractory period matches the duration of sustained glial activation in the hindbrain. Consequently, the intrinsic properties of spinal motor neurons remain intact but their synaptic inputs are suppressed. Altogether, our findings reveal that the motor arrest induced by sustained noradrenergic neuron activation and the subsequent induction of glial calcium wave disrupts the communication between the brain and spinal cord to forbid motor output.

Session # 5: Sensorimotor Integration

The cost of sensory loss: irreversible motor deficits scale with the degree of proprioceptor ablation

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To control movement, the central nervous system relies on the integration of sensory information with motor commands. Among the sensory modalities, proprioception is critical for motor control, providing continuous information about body position through muscle spindles and Golgi tendon organs (GTO). However, the extent to which motor function can recover after acute proprioceptive loss and the strategies that support such recovery remain unclear. Here, we used a longitudinal approach to investigate the long-term effects of proprioceptor elimination on mouse locomotion. We used an intersectional genetic approach to specifically target the expression of the human diphtheria toxin (DT) receptor in proprioceptors (PV ;Runx3 ;Mapt ;Rosa). We assessed locomotor performance before and after DT-mediated ablation using high-resolution analysis of kinematic and electromyographic (EMG) data. Results revealed a rapid decline in locomotor function within 72 hours of ablation. Deficits in gait and EMG parameters persisted throughout the 60-day observation period, with no evidence of spontaneous recovery. Principal component analysis of kinematic and EMG data revealed distinct locomotor signatures following proprioceptor ablation, primarily driven by increased paw drag, lower centre of mass and more sustained muscle activation patterns. Histological analysis confirmed a progressive loss of proprioceptive neurons, with motor impairments scaling with the degree of ablation. These findings establish a direct link between proprioceptive loss and irreversible locomotor deficits and highlight the limited potential for sensory reweighting in the absence of proprioception.

Exploring the role of the premotor trigeminal nucleus in masticatory function

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Masticatory movements are part of the more complex behavior of feeding. They are generated by a brainstem network, known as a Central Pattern Generator (CPG), but it is assumed that their coordination with other movements during feeding occurs elsewhere. The boundaries of the CPG are known, but its organisation is less understood. Here we mapped, within these boundaries, areas activated during cortically evoked mastication, using cfos immunostaining. Optogenetic stimulation of the cortical masticatory area (CMA) evoked rhythmic jaw movements (RJMs), and increased activity in the trigeminal main sensory nucleus (NVsnpr), and in regions medial (JuxtV) and ventral (PCRt) to the trigeminal motor nucleus. Optogenetic stimulation of JuxtV and PCRt in head-fixed awake animals produced some uncoordinated jaw movements. In contrast, stimulation of NVsnpr induced robust RJMs, and some hand-to-mouth movements resembling those occurring during natural feeding and food manipulation, raising the intriguing possibility that complex behaviors may be partly encoded at the CPG level. We have previously shown that NVsnpr neurons shift their firing pattern from tonic to rhythmic bursting when the extracellular Ca2+ concentration is decreased by release of S100β, an astrocytic Ca^{2+}-binding protein. Here, we show that S100β levels increase in NVsnpr during CMA-induced RJMs, while injection of an anti-S100β antibody delays these movements. A selective Nav1.6 blocker (4,9-anhydroTTX), acting on the conductance modulated by S100β, also greatly reduced the frequency and amplitude of cortically induced RJMs. These results will help resolve the composition of the masticatory CPG and establish the role of astrocytes in rhythmogenesis.

Challenging textbook depictions of an ascending spinal circuit

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Dexterous movement relies on the seamless transmission of sensory information from the periphery to the brain, conveyed through various neural pathways originating in the spinal cord. Among these circuits, the spinothalamic (ST) system is unique in the breadth of sensory information it encodes, affording it a pivotal role in wide-ranging sensory and motor processes. Yet, our understanding of the synaptic organization of the ST system remains surprisingly incomplete. In this project, we leveraged anatomical and electrophysiological methods to map the input and output organization of ST neurons. We combined transneuronal tracing with anatomical reconstructions to build a quantitative atlas of ST somata positions, revealing a topography that suggests a role for cervical ST neurons in proprioception and motor control. Indeed, intersectional transneuronal tracing showed that cervical ST neurons receive unexpected and selective input from brain regions implicated in motor control. ST neurons may synapse in one or several thalamic nuclei, but existing descriptions of this connectivity matrix are largely qualitative. Focusing on cervical ST neurons, we used intersectional anterograde tracing and optogenetics-assisted electrophysiology to show that ST populations are selective in the thalamic nuclei they target yet are surprisingly promiscuous in their collateral innervation of brainstem structures, suggesting ST neurons broadcast information in their ascendency to the forebrain. Together, these experiments implicate ST neurons in both sensation and action and establish a guiding anatomical framework to test the functions of distinct ST circuits in skilled behavior.

Directional cues relative to center-of-mass shapes somatotopy of lateral line inputs in the brainstem

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Motor command circuits rely on sensory inputs to guide navigation, yet how broadly distributed spatial and directional cues are represented in the brain remain unresolved. This challenge arises from the difficulty of stimulating individual sensors across the entire body while comprehensively recording interneuron responses. The lateral line (LL) system of fishes, which detects fluid flow to mediate essential behaviors, presents an ideal model to address this question. LL neuromasts, located on the head (anterior LL) and body (posterior LL), contain mechanosensitive hair cells that transmit sensory information to the medial octavolateralis nuclei (MON) in the hindbrain. However, how MON neurons are recruited based on sensor position, flow direction, and signal symmetry across body axes remains unclear. To map the topographic organization of flow-responsive MON neurons, we combined microfluidic stimulation, functional calcium imaging, and optical backfills in zebrafish larvae. Systematic neuromast stimulation revealed a spatially organized representation of flow stimuli along the left-right and rostral-caudal axes. MON neurons were primarily activated ipsilateral to the stimulus, with a minor contralateral component. Anterior LL stimulation evoked ventral-rostral MON activity, while posterior LL stimulation additionally activated dorsal-caudal MON neurons. This organization mirrored along the rostral-caudal axis when stimulus direction was reversed. Receptive field overlap increased when flow was directed toward or away from the center of mass. Optical backfills further reveal MON projections forming axosomatic and axodendritic connections with reticulospinal neurons responsible for turning and forward locomotion suggesting MON neurons selectively integrate spatial and directional flow cues to recruit distinct motor command pathways.

The role of the lateral vestibular nucleus in neural control of walking and balance

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The basic mammalian pattern for walking is generated by spinal neuronal circuits which must be extremely adaptable in order to execute a wide range of motor tasks. One key source of sensory feedback is provided by the vestibular system which helps to maintain balance and posture during walking. Humans have a remarkable ability to maintain balance and posture by continually making adjustments to adapt motor output. However, this ability to respond rapidly and effectively reduces with age, with one in three adults over 65 experiencing a fall once a year. In order to generate interventions to prevent falls and reduce injury, it is essential that we understand how balance is controlled. The lateral vestibular nucleus (LVN) is a key structure in balance control as it receives direct input from vestibular sensory apparatus in the inner ear, projects directly to motor neurons and has been shown to be important in gross motor maintenance of balance. Data presented here show that LVN-generated motor corrections can be altered by manipulating the surrounding environment. Furthermore, environmental influence on corrections requires noradrenergic signalling from the locus coeruleus, suggesting a potential link between forebrain structures that convey sensory information about the environment and brainstem circuits that generate motor corrections. Finally, inhibition of the LVN during a variety of behavioural tasks shows how information from the LVN might be integrated into ongoing commands for locomotion.

Voltage imaging reveals ultrafast plasticity for the error computations in the cerebellum

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The cerebellum plays a critical role in motor control by computing sensory errors during movements. The computation of sensory errors is mediated by interactions between granule cells and Purkinje cells, but how such interactions dynamically change in a context-dependent manner remains elusive. Here, we performed voltage imaging in the larval zebrafish cerebellum and identified a subpopulation of Purkinje cells in which membrane potential forms negative images of expected visual reafference at the onset of swimming. This negative image is canceled by visual reafference and also diminished by repeated swimming without visual reference. A granule cell subpopulation that shows similar membrane potential modulation was insensitive to visual reafference, indicating that sensory errors are computed in Purkinje cells. Importantly, such error computation was only observed when the zebrafish chased visual stimuli but not during spontaneous swimming, indicating that a behavioral goal elicits error computations in the cerebellum. The ablation of Purkinje cells caused unstable swim patterns during the mismatch of sensory reafference. These results demonstrate that the cerebellum in the early developmental stage is capable of flexible sensory error computations in a context-dependent manner.

In mice, Lumbar CSF-contacting neurons are sensory neurons modulating cardinal motor interneurons

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Movement is initiated by descending cortical commands activating spinal cord locomotor networks, called central pattern generator (CPG). CPGs control motoneurones and are modulated by supraspinal centres, local spinal interneurones as well as sensory feedback loops to adjust locomotion. In the zebrafish larva, neurones in contact with the cerebrospinal fluid (CSF-cNs) were shown to act as one of these sensory systems capable of modulating swimming behaviour in response to changes in CSF flux and spinal cord bending. CSF-cNs are GABAergic neurons present around the spinal cord central canal of all vertebrates. They exhibit a conserved morphology with the selective expression of chemo/mechanosensory receptors of the polycystin-subtype transient potential receptors (TRP-P), also known as PKD2L1 (Polycystin Kidney Disease 2-like1). We recently confirmed, in quadripeds, that CSF-cNs take part in the modulation of posture and fine movements. Using neuronal tracing approaches combined to ChannelRhodopsin Assisted Circuit Mapping (CRACM), we further indicated their functional connectivity to interneurones within the spinal cord motor circuit. However, the phenotype of these post-synaptic partners and the modulation of this connectivity were not elucidated. By combining high-resolution neuroanatomy, whole-cell patch-clamp recordings with CRACM strategies in lumbar spinal cord acute slices and single-cell RT-PCRs analysis, we reveal for the first time the identity of the cardinal interneurones CSF-cNs are connected to. Our study, extend our previous report and indicate that lumbar CSF-cNs act as a novel intraspinal sensory system to modulate motor interneurones activity. The future challenge will be to identify the physiological stimuli activating this unique neuronal population.

Non-invasive spinal cord stimulation in supporting balance control

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Introduction: Even though available data indicates that the spinal cord plays a crucial role in balance control, existing neuromodulator research focusses almost entirely on the primary motor cortex, the cerebellum, and other brain regions. We present three randomized placebo-controlled trials that investigate the effects of spinal cord modulation on healthy young cohorts. Methods: The first study compares anodal 1.5 mA Direct Current Stimulation (DCS) over (i) the M1, (ii) the cerebellum, (iii) the Th8, and (iv) sham DCS. The second study tests high definition (HD) 1.5 mA (i) anodal DCS, (ii) cathodal DCS, (iii) Alternating Current Stimulation (ACS) and (iv) sham DCS/ACS over Th8. The third study tests repetitive paired pulse magnetic stimulation (r-ppMS) over L2 with the coil's handle oriented (i) superiorly, (ii) inferiorly, (iii) laterally, and (iv) sham stimulation. Results: The first study shows that spinal DCS is comparable to M1 and cerebellar applications in supporting balance control. The second study demonstrates that both anodal HD DCS and HD ACS improve balance control, superficial sensitivity, and deep sensitivity, as compared to sham H DCS/HD ACS and cathodal HD DCS. The third study shows that (i) real r-ppMS supports the balance ability in comparison to sham independently of coil orientation, and (ii) real r-ppMS applied with the coil handle oriented laterally induces greater effects than real r-ppMS applied with the coil handle oriented superiorly and inferiorly. Conclusions: Our data supports further investigation of non-invasive spinal modulation in both healthy and disabled cohorts.

Decoding activity patterns across pyramidal cell dendritic trees during spontaneous behaviours using 3D imaging

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How sensorimotor information is represented across individual pyramidal cell dendritic trees in awake animals remains poorly understood. Recent work has identified activation patterns including global cell-wide, hemi-tree and branch-specific events. However, how distributed tree-wide dendritic activity relates to motor behaviour is not known due to difficulty of recording activity across the entire dendritic arbour. Here we measured dendritic calcium activity in mouse primary motor cortex, a sensorimotor integration hub that plays a key role in modulating primitive movements such as locomotion and whisking. We imaged calcium across dendritic trees of L2/3 cells expressing GCaMP during spontaneous behaviours with 3D acousto-optic lens two-photon microscope equipped with real-time 3D brain motion compensation. We investigated how dendritic local modulations around the cell-wide Ca^{2+} signals were related to behaviour and applied dimensionality reduction and regression approaches. Our analysis revealed highly informative dendritic activity patterns superimposed on global activity, that covaried with uninstructed spontaneous movements. These dendritic modulations (spread over >20 μm compartments) were dynamic across behavioural epochs including quiet-rest, running, active whisker touch, and air-puffs to the whiskers. Remarkably, regression analyses suggest that modulations in activity of dendritic segments are more informative about certain movements than activity at the soma in the majority of cells. We are investigating whether behaviourally informative patterns are stable across days or whether they drift over time as reported for neural populations. Our results indicate that L2/3 pyramidal cell dendritic activity patterns are multidimensional, behaviourally informative and that an individual cell can represent several behavioural features.

Session # 6: Premotor & Propriospinal

Coupled *Drosophila* and mouse studies reveal a pharmacological suppressor of intrinsic spinal circuit dysfunction in genetic models of dystonia

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Dystonia is a debilitating involuntary movement disorder characterised by sustained co-activation of antagonistic muscles. Drug treatments for dystonia are often ineffective or cause severe side effects, and neural circuits that drive or modulate dystonic movements are poorly defined. To address these knowledge gaps, we utilised *Drosophila* and mouse models of early-onset dystonia caused by BK potassium channel gain-of-function (BK GOF) [1,2], which we recently showed perturbs movement through a neurodevelopmental mechanism [3]. By combining an unbiased drug screen with automated movement analyses and GCaMP-based imaging of muscle activity, we identified Rivastigmine – an acetylcholine esterase inhibitor – as an acute suppressor of motor dysfunction and dystonia-like muscle contractions in BK GOF *Drosophila*. In ex vivo spinal cords from BK GOF mouse pups, we used electroneurogram recordings to show for the first time that BK GOF disrupts the rhythmic fictive activity of spinal circuits, and causes co-activation of antagonistic motor units. Importantly, these dystonia-like phenotypes were suppressed by Rivastigmine. Collectively, our data support Rivastigmine as a novel treatment for BK GOF dystonia. By showing that dystonia-like pre-motor circuit activity can be renormalised by enhancing spinal cholinergic tone, our data suggest an unexpected role for spinal cholinergic interneurons as modulators of dystonic movements. Finally, in concert with our recent studies of a distinct mouse model of dystonia [4], our data provide further evidence for intrinsic spinal circuit dysfunction as a unifying feature across genetically diverse forms of dystonia.

Vestibular inhibitory networks orchestrate the vestibulospinal control of posture in *Xenopus laevis* tadpoles

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Brainstem vestibulospinal (VS) nuclei generate excitatory commands in response to multi-modality signal integration to activate specific spinal networks. Comparably organized in bilateral nuclei with both ipsi- and contralateral pathways in all species, excitatory VS projections alone fail to explain the mostly unilateral reflex responses typically observed in postural adjustments. Using an in vitro preparation of *Xenopus laevis* tadpole central nervous system, we show that vestibular galvanic stimulation-evoked spinal responses are shaped by both commissural and local inhibitions. We further show that a complex interaction between vestibular GABAergic and glycinergic inhibitory networks regulate VS neuron excitability and consequently, the expression of spinal responses. Our results clearly demonstrate that while excitatory VS neurons execute the neural score, central inhibitory neurons of the vestibular system coordinate and modulate the overall performance.

A species-specific spinal circuit for coordinating antagonist motor pools

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Everyday motor tasks could not be accomplished efficiently without the coordinated activation of muscles that generate opposing forces at skeletal joints, i.e., muscle antagonists. Current understanding of underlying spinal circuits in mammals has long been driven by detailed examination of felines. However, the neural control of movement must be adapted to allometric, biomechanical, and behavioral differences among species. Here, we explore a short-latency spinal reflex pathway that promotes co-activation of antagonist motor pools in rats but not cats. In anesthetized adult rats, every motoneuron in the TA-EDL or MG motor pools produces an EPSP in response to stretch of the corresponding antagonist muscle. The pathway is oligosynaptic with EPSP latencies that average 5.53 ± 2.19 ms (n=65 motoneurons, MNs) in the extensor to flexor path and 4.06 ± 1.48 ms (n=42 MNs) in the flexor to extensor path. The average amplitudes of the antagonist EPSPs were similar (0.59 ± 0.3 mV, and 0.60 ± 0.4 mV), suggestive of strong pathways, ones in which muscle stretch overcomes anesthesia to drive multiple premotor interneurons. Comparisons of antagonist EPSPs elicited by muscle quick stretch vs vibration, demonstrate pathway activation by IA afferents together with group II and/or IB afferents. Incorporating this novel spinal circuitry into a model of rodent biomechanics preserves locomotor behavior and enhances co-activation of ankle flexors and extensors prior to stance, potentially addressing ankle torque differences between non-cursorial (rats) and cursorial (cats) animals. This approach provides insight into how neural systems adapt to biomechanical and behavioral variations across species.

Neuromechanics of a vertebrate escape behaviour mediated by axial muscles after capture

Wen-Chang Li, Saeed Farjami, Andrey Palyanov, Hong-Yan Zhang, Valentina Saccomanno, Robert Merrison, Andrea Ferrario, Roman Borisyuk, Joel Tabak

University of St Andrews

In contrast with abundant studies on forward locomotion, there has been little research on how aquatic animals like fish or porpoises free themselves from the grip of a predator. We studied how the axial-muscle-powered movements of young *Xenopus* tadpoles help them escape when a predator grips their head. Tracking tadpole movement using video analysis revealed four types of whole body movement when the tadpole was gripped and released: initial coiling, strong tail-to-head travelling body flexions or struggling, transitional coiling and swimming. Recordings of spinal motoneurons and motor nerves during fictive struggling in immobilised tadpoles showed struggling activity propagated caudo-rostrally. Finally, we generated a range of motor commands to drive the movement of a virtual tadpole (VT), which was free or gripped by virtual forceps. The simulations showed that in open water, VT could not generate backward swimming when driven by a reversed sequence of swimming motor nerve commands. In open water the VT could not produce thrust to move itself backwards or forwards when driven by struggling motor nerve rhythms. However, when the VT head was gripped by virtual forceps, it could free itself with the struggling movements. This suggested that the direct interaction between the VT and the gripping forceps allowed the generation of force leading to its escape. We conclude that the natural struggling rhythms, propagating along the body from tail to head with prolonged motoneuron bursts at low frequencies around 4 Hz, allow tadpoles to free themselves from the grip of a predator.

A brain-wide map of descending inputs onto spinal V1 interneurons

Jay Bikoff, Phillip Chapman, Anand Kulkarni, Alexandra Trevisan, Katie Han, Jennifer Hinton, Paulina Deltuvaite, Lief Fenno, Charu Ramakrishnan, Mary Patton, Lindsay Schwarz, Stanislav Zakharenko, Karl Deisseroth

St. Jude Children's Research Hospital

Motor output results from the coordinated activity of neural circuits distributed across multiple brain regions that convey information to the spinal cord via descending motor pathways. Yet the organizational logic through which supraspinal systems target discrete components of spinal motor circuits remains unclear. We have used viral transsynaptic tracing along with serial two-photon tomography to generate a whole-brain map of monosynaptic inputs to spinal V1 interneurons, a major inhibitory population involved in motor control. We identified 26 distinct brain structures that directly innervate V1 interneurons, spanning medullary and pontine regions in the hindbrain as well as cortical, midbrain, cerebellar, and neuromodulatory systems. Moreover, we identified broad but biased input from supraspinal systems onto V1 and V1 neuronal subsets. Collectively, these studies reveal elements of biased connectivity and convergence in descending inputs to molecularly distinct interneuron subsets and provide an anatomical foundation for understanding how supraspinal systems influence spinal motor circuits.

Establishing flexible spinal circuits for locomotor and postural control

Alex Adams, Irene Chen, Anne Cavanagh, Adam Mar, Jeremy Dasen

New York University

The musculature of the body axis is essential for coordinating actions of the limbs and trunk for dynamic control of posture and locomotion. While substantial progress has been made in elucidating circuits that enable selective control of limb muscle, those intrinsic to the axial neuromuscular system have been comparatively understudied. In mice, we have uncovered a rich diversity of axial motor neuron (MN) subtypes that comprise the medial motor column (MMC). We found that the MMC-restricted transcription factor Mecom is essential for subtype diversification of MMC but is dispensable for general MN and MMC features. At postnatal day 7 Mecom MND mice exhibit delayed righting reflex, while adult mice develop gait abnormalities and loss of interlimb coordination.

Innovations in spinal cord cell type heterogeneity during vertebrate evolution

Lora Sweeney, Yuri Ignatyev, Stavros Papadopoulos, Mateja Soretic, Jake Yeung, Tzi-Yang Lin, Leonid Peshkin, Ariel Levine, Elly M Tanaka, Mariano I Gabitto

Institute of Science and Technology Austria

Four-limbed movement is a shared feature across much of the vertebrate clade. Amphibians and mammals, which last shared a common ancestor ~360 million years ago, exhibit largely the same basic limb anatomy and many identical patterns of limb locomotion, including basic sensory escape and coordinated fore/hindlimb movements for feeding and mating. This similarity raises the fundamental question of whether a core, shared motor circuit architecture exists in the spinal cord across such large evolutionary distances and how it may vary in register to vertebrate movement. Recent studies have detailed neural spinal cell-type architecture in mammals, best exemplified in mice and humans. However, a comparable atlas of the non-mammalian, limbed vertebrate spinal cord is lacking. Here, we focus on one of the most primitive amphibians, the frog *Xenopus laevis*, to evaluate conservation in spinal cell-type architecture between frogs, mice and humans. Across species, our analysis defines a core program of cell type specification during development, which segregates spinal neurons into nearly identical cardinal classes and subtypes in both amphibians and mammals. This starkly contrasts with adult stages, when spinal cell-type composition across species is similar at a coarse level but diverges at the level of subpopulations. Using spatial transcriptomics, we localize this species-divergence to the superficial dorsal spinal cord, where variant neuropeptide expression defines mammalian-specific cell types. The dorsal spinal cord thus emerges as a more recently evolved hub for sensory integration in mammals.

Session # 6: Motoneurons

Birth timing predicts tuning and response strength across extraocular motor neuron pools

Celine Bellegarda, Paige Leary, Takuya Kaneko, Cecilia Moens, David Schoppik

New York University

Spinal motor circuit organization relies on developmentally-defined neuron subtypes with distinct anatomical and functional properties. It is unclear to what extent these organizing principles shape motor control outside the spinal cord. Vertebrates stabilize gaze using a conserved circuit that transforms sensed head/body tilts into compensatory counter-rotations of the eyes. This circuit undergoes early developmental changes that drive behavior maturation. Previous work in larval zebrafish showed that the longitudinal responses to body tilts of extraocular motor neurons (EOMNs) innervating the superior oblique (SO) muscle matured early, while development of the downstream neuromuscular junction paralleled improvements in gaze stabilization behavior. To test whether this extended across EOMN pools, we used the transgenic line isl1:H2B-GCaMP6s, labeling all EOMNs, to probe maturation timing for the inferior rectus (IR) and superior rectus (SR) pools. Our results replicated SO findings and revealed similar maturation of response strength and tuning in IR neurons; SR neurons, however, showed delayed directional tuning, consistent with their later neurogenesis and somatic migration. Within and across motor pools, we observed considerable variability in response strength. To investigate whether this heterogeneity reflected circuit organization, we examined the relationship between birth timing and response strength in SO neurons. We developed the transgenic line isl1:GAL4;UAS:nls-Kaedep2a-GCaMP8s to express the photolabile protein Kaede in SO neurons and 'date' them during development. Preliminary results suggest early-born neurons require stronger stimuli to excite—consistent with principles organizing spinal circuits. Future experiments will reveal whether principles of motor circuit organization generalize across vertebrate motor systems or reflect circuit-specific adaptations.

Developmental changes in the control of primary motoneuron firing properties by multiple ion currents in larval zebrafish

Stephanie Gaudreau, Tuan Bui

University of Ottawa

Spinal circuits for locomotion undergo maturation during early development. How intrinsic properties of individual spinal neuron populations change throughout motor maturation is not fully understood. Here, we determine how several ionic currents underlying neuron excitability change in primary motoneurons during early larval zebrafish development (2 – 5 days post-fertilization). We confirm, for the first time, the presence of the persistent outward potassium current, known as the M-current, in primary motoneurons of developing zebrafish. We also reveal the presence of a persistent inward current at 2 to 5 dpf consisting of a riluzole-sensitive persistent inward Na current and a nifedipine-sensitive Ca^{2+} current. An analysis of the amplitude of these currents suggests age-dependent changes at these early developmental time points. For instance, we show that the M-current's control over excitability and repetitive firing of primary motoneurons changes during development such that the magnitude of the M-current transiently increases at 3 days post-fertilization. We also reveal that contributions of the riluzole-sensitive persistent inward Na current and the nifedipine-sensitive Ca^{2+} current to the persistent inward current of primary motoneurons shifts during development. These findings reveal novel mechanisms by which control over excitability and repetitive firing of primary motoneurons in larval zebrafish is ensured, underscoring developmental changes in ion current contributions to intrinsic properties. Broadly, these data support the M-current and persistent inward currents as conserved means to control motoneuron excitability and firing properties across vertebrates.

Effects of botulinum toxin type A on the spinal motor control: not just a peripheral player?

Ivica Matak, Petra Šoštarić, Patrik Meglić

University of Zagreb

Intramuscular botulinum toxin type A (BoNT/A) provides a long-term control of muscular hyperactivity in spasticity and dystonia, presumably due to its local effects on extrafusal and intrafusal fibers. While its canonical actions at the neuromuscular junction are well known, recently we investigated the role of BoNT/A central action on normal and spastic muscle control in rats. By examining the enzymatic effect of intramuscular BoNT/A on its synaptic target synaptosomal-associated protein 25, we discovered that the toxin is axonally transported via peripheral nerve to central cholinergic synapses. By using intrathecal antitoxin-mediated neutralization, we discovered that the duration of its beneficial action in tetanus toxin-induced local spasm is dependent on BoNT/A trans-synaptic traffic within the spinal cord. In addition, in naïve non-spastic animals we found that BoNT/A may trans-synaptically target the premotor circuits involved in reflex digit abduction, gait, coordinated balance, fatigue, and swimming. The central BoNT/A activity did not influence its peripheral muscular actions resulting in neuromuscular blockade or long lasting atrophy, or the exaggerated monosynaptic H-reflex accompanying spastic paralysis. Given that the duration of its beneficial effects may surpass the degree or duration of peripheral muscular paralysis in patients, these experiments suggest that a significant part of BoNT/A-mediated lasting clinical benefits is associated with its specific actions at the level of spinal cord/brainstem motor nuclei. These effects in disinhibited motor circuits are likely to contribute to desirable restoration of muscle control in movement disorders or spasticity.

Personalized mapping of inhibitory spinal circuits via neural decoding of high-density electromyography and in silico modelling

Filipe Nascimento, Alejandro Pascual-Valdunciel, Natalia Cónsul, Robert Brownstone, Marco Beato, Dario Farina, Görkem Özyurt

University College London

A spinal α-motoneuron innervates a group of muscle fibres, collectively forming a motor unit (MU). Muscle contraction depends on the coordinated activity of multiple MUs, modulated by spinal microcircuits that project to motoneurons. Through electromyography (EMG), motoneurons are the most readily recordable cell type in the human CNS, and therefore EMG has been used to study motoneurons and associated spinal microcircuits. Surface EMG records gross muscle activity, while intramuscular EMG can detect individual MU activity. However, surface recordings provide low-resolution data and underestimate circuit temporal properties, while intramuscular EMG is invasive and samples only a few MUs from heterogeneous motor pools. Advancements in high-density surface EMG (HDsEMG) allow non-invasive sampling of multiple MUs simultaneously, with a potential to shed light into mechanisms of motoneuron integration of input and recruitment across a pool. Here we present an optimized framework for investigating inhibitory spinal microcircuits with HDsEMG. We focused on the cutaneous silent period in the first digit interosseous , and reciprocal inhibition in the tibialis anterior . We sampled multiple MUs per individual and demonstrated that inhibition is best interpreted in the context of MU discharge rates. Furthermore, we highlight the need for subject-specific analysis, as most variability was located between subjects. In silico modelling replicated these experimental characteristics and suggested that motoneuron active properties rather than size contribute more to net functional inhibition. Our results show that HDsEMG can highlight distinct control strategies across circuits and motor pools, revealing subject-specific properties of inhibitory spinal microcircuits.

Reinforcement learning-based motion imitation for physiologically plausible musculoskeletal motor control

Merkourios Simos, Alberto Chiappa, Alexander Mathis

École Polytechnique Fédérale de Lausanne

Understanding the neural basis of human movement represents a fundamental challenge in neuroscience. While significant progress has been made in characterizing the control systems underlying basic motor patterns, bridging the gap between neural control and complex, goal-directed movements remains elusive. In this work, we present KINESIS, a reinforcement learning (RL) framework that advances our understanding of muscle-based motor control through motion imitation. Using a physiologically accurate musculoskeletal model of the lower body with 80 muscle actuators and 20 degrees of freedom, we demonstrate that KINESIS achieves robust motion imitation performance on 1.9 hours of locomotion data. The musculoskeletal model can be controlled flexibly through natural-language prompts and high-level commands such as target goal reaching and directional control. Crucially, KINESIS generates muscle activation patterns that correlate significantly with human EMG recordings during walking, suggesting it captures fundamental principles of biological motor control. Our analysis of the learned muscle control policy reveals that, while dimensionality reduction of muscle activity patterns suggests low-dimensional control (supporting muscle synergy hypotheses), task performance is only maintained when preserving most dimensions. This finding challenges simplified neural control models and suggests that seemingly redundant dimensions may serve critical functions in movement execution. KINESIS provides a computational testbed for the deployment and evaluation of motor control hypotheses. The seamless access to all intermediate motor control signals and the ability to intervene in real time highlight the value of KINESIS as a tool for the facilitation of future theoretical and experimental research.

Motor unit mechanisms of speed control in mouse locomotion

Samuel Sober, Kyle Thomas, Rhuna Gibbs, Hugo Marques, Megan Carey

Emory University

During locomotion, the coordinated activity of dozens of muscles shapes the kinematic features of each stride, including systematic changes in limb movement across walking speed. Motor units, each of which consists of a single motor neuron and the muscle fibers it innervates, contribute to the total activation of each muscle through their recruitment and firing rate when active. However, it remains unknown how the nervous system controls locomotor speed by changing the firing of individual motor units. We combined quantitative analysis of mouse locomotion with single motor unit recordings from the lateral and long heads of the triceps brachii, which drive monoarticular extension of the elbow and biarticular movements of the elbow and shoulder, respectively. In contrast to studies employing bulk EMG our recordings revealed the diversity of spike patterning across motor units as well as systematic differences in motor unit activity across muscles and locomotor speeds. First, motor unit activity differed significantly across the lateral and long heads, suggesting differential control of these two closely apposed elbow extensor muscles. Second, we found that individual units were recruited probabilistically during only a subset of strides, showing that bulk EMG signals consistently present in every stride in fact reflect stochastically varying subsets of individual motor units. Finally, the relationship between motor unit firing patterns and limb kinematics suggests that the lateral and long heads of the triceps serve dramatically different biomechanical functions during locomotion. Together, these results reveal how the firing of individual motor units produces flexible locomotor behavior.

Motor Control: Spinal Circuits and Beyond
University of St Andrews, June 17-20, 2025

Poster Sessions & Abstracts

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Mapping the connectivity of spinal cord projection neurons

Sandrina Campos Macas, Quinn Silvermann, Graziana Gatto

University of Cologne

Themes: Ascending control

Motor behavior expression requires the dynamic interactions of neuronal networks across the peripheral and central nervous system. Spinal neurons, by ensuring motor execution and sensory perception, represent a critical hub for the integration and broadcasting of sensorimotor information. Yet, the molecular identity as well as the local and long-range connectivity of spinal projection neurons remain only partially understood. Using viral and intersectional genetic strategies to label all spinal ascending tracts, we observed that spinal projections display a certain degree of topographic organization, differing in innervation density across brain regions, and do not undergo major postnatal refinement. The brain regions receiving more ascending input include already known targets like dorsal column nuclei (DCN), parabrachial nucleus (PBN), cerebellum, and thalamus, but also novel regions, e.g. the dorsal raphe. Within the DCN, direct projections from sensory neurons converge on the external cuneate, while spinal projections target the cuneate and gracile nuclei. The ascending motor-related V2a neurons send direct projections to the cerebellum and the lateral reticular nucleus, while the dorsal sensory-related ascending Calbindin-expressing neurons also innervate the dorsal column nuclei, the thalamus and the PBN. Retroviral injections in the thalamus (PoT nucleus) and the lateral PBN showed that spinothalamic neurons are mostly contralateral and present at cervical and lumbar levels, whereas spinoparabrachial neurons are both ipsilateral and contralateral and distributed across all spinal levels. In sum, our analyses provide an anatomical blueprint of how motor and sensory cues are transmitted to the brain for long-term adaptation and re-planning of behavior.

Probing the functional connectivity of brainstem vsx2 reticulospinal neurons using 3D optogenetics and calcium imaging in vivo

Ninon Peysson, Xinyu Jia, Kevin Fidelin, Claire Wyart

Paris Brain Institute

Themes: Ascending control, Descending control

Electrical or optogenetic stimulations of reticulospinal neurons (RSNs) in the brainstem can elicit locomotion in various vertebrate model organisms. However, how the network of RSNs is structured at the population level to trigger forward locomotion is poorly understood. To tackle this fundamental question, we leverage the transparency of larval zebrafish and combine two-photon population calcium imaging with single cell optogenetic stimulations using digital holography enhanced with temporal focusing. We first tested whether vsx2-expressing RSNs, known to be recruited during forward swimming, can recruit each other to build a network sufficient to drive forward locomotor bouts. Our stimulation paradigm led to the reliable activation of single vsx2$^+$ RSNs, with different response probability reflecting their rostro-caudal location in the brainstem. In addition, we found that a subset of non-stimulated vsx2$^+$ neurons responded to the single cell stimulation demonstrating functional interactions between vsx2 neurons. These recruited vsx2 cells were found to be caudal (~30%), rostral (~70%) ipsilateral (~80%) or contralateral (~20%) to the activated cell. In these experiments, the activation of single vsx2$^+$ neurons failed to elicit forward locomotion, despite the recruitment of vsx2+ neurons in the vicinity. By increasing the number of targeted cells, we will be able to quantify the minimal network required to trigger locomotor bouts. Altogether, our approach demonstrates functional connectivity among vsx2 neurons in the brainstem, and open unprecedented possibilities to dissect brainstem motor circuits organization *in vivo.*

Long ascending projections of dI3 neurons mediate the mouse fly twitch reflex

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University College London

Themes: Ascending control; Premotor & Propriospinal Circuits

The fly twitch reflex (or cutaneous trunci reflex) is a cutaneous reflex, present in most hairy mammals, triggering rapid twitches of their skin in response to a local skin stimulus. It plays an important role as a defense mechanism to ward off insects and remove irritants from their skin. Phenomenologically, the fly twitch reflex results from cutaneous sensory information transmitted locally to the spinal cord and relayed by ascending circuits to motor neurons of the cutaneous maximus in the cervical cord. This reflex is triggered by stimuli ranging from a light touch to a prick in a species-dependent manner. Using virus-mediated tracing combined with mouse genetics, we report that a neural population of lumbar spinal neurons forms bilateral ascending projections from the lumbar and thoracic cord to the cervical enlargement. These projections are formed by a specific subpopulation of dorso-lateral dI3 neurons with a dense and specific innervation of motor neurons controlling the cutaneous maximus. Thus, our work suggests that the fly twitch reflex is mediated by an anatomically distinct subpopulation of dorso-lateral long ascending dI3 neurons.

A two-layer neural circuit controls fast forward locomotion in *Drosophila*

Qianhui Zhao, Xinhang Li, Jun Wen, Yinhui He, Nenggan Zheng, Wen-Chang Li, Albert Cardona, Zhefeng Gong

Zhejiang University

Themes: Ascending control; Rhythm & Pattern Generation

Fast forward locomotion is critical for animal hunting and escaping behaviors. However, how the underlying neural circuit is wired at synaptic resolution to decide locomotion direction and speed remains poorly understood. Here, we identified in the ventral nerve cord (VNC) a set of ascending cholinergic neurons (AcNs) to be command neurons capable of initiating fast forward peristaltic locomotion in *Drosophila* larvae. Targeted manipulations revealed that AcNs are necessary and sufficient for fast forward locomotion. AcNs can activate their postsynaptic partners, A01j and A02j; both are interneurons with locomotory rhythmicity. Activated A01j neurons form a posterior-anteriorly descendent gradient in output activity along the VNC to launch forward locomotion from the tail. Activated A02j neurons exhibit quicker intersegmental transmission in activity that enables fast propagation of motor waves. Our work revealed a global neural mechanism that coordinately controls the launch direction and propagation speed of *Drosophila* locomotion, furthering the understanding of the strategy for locomotion control.

Investigating the dorsal raphe nucleus mediated modulation of motor states and its role in ADHD

Leonard Constien, Gautam Sridhar, João C. Marques, Drew N. Robson, Jennifer M. Li, Antonio C. Costa, Claire Wyart

Institute du Cerveau

Themes: Descending Control

Over the past two decades, the prevalence of attention-deficit/hyperactivity disorder (ADHD) has risen to 10.2% of adults in the United States, with a global estimate of 5%. While dopamine reuptake inhibitors (DRIs) have shown therapeutic success, one third of patients do not respond to treatment, suggesting a more complex pathophysiology beyond dopamine deficiency. Accordingly, an essential ADHD risk gene, cdh13, encoding cadherin-13, is expressed in serotonergic (5-HT) neurons of the dorsal raphe nucleus (DRN), indicating a potential serotonergic contribution. Intriguingly, CDH13 deficient mice show increased DRN cell density and hyperlocomotive-impulsive phenotypes (Forero et al., 2020, Neuropharm., Rivero et al., 2015, Transl Psychiatry) — hallmark symptoms of ADHD. What are the neuronal mechanisms behind DRN-driven modulation of locomotion, and what is the DRN's role in ADHD? To address these questions, I will investigate whether and how long-lived motor strategies in larval zebrafish, previously referred to as *cruising* and *wandering* (Sridhar et al., 2024, PNAS) are shaped by 5-HT DRN activity. My approach combines a high-fidelity behavioral analysis method (Sridhar et al., 2024, PNAS) with calcium imaging and optogenetics. Furthermore, I will employ my methods to obtain a detailed view on changes in motor strategies and neuronal activity in cdh13-/- mutant zebrafish, elucidating how CDH13 deficiency could generate motor symptoms of ADHD. Initial results explore similarities between the 5-HT DRN-correlated states of *exploration* and *exploitation* (Marques et al., 2020, Nature) and the *cruising* and *wandering* motor strategies, and show the expression pattern of cdh13 across developmental stages in larval zebrafish.

Thursday Poster Session (12:00-14:45): Numbers 1-35

Reduced corticospinal output enables flexible input-output scaling in spinal interneurons

Michelle Sanchez Rivera, Constantinos Eleftheriou, Anand Kulkarni, Jay Bikoff, Yuxiao Li, Li Xiangning, Roman Baravalle, Salvador Dura-Bernal, Ian Duguid

University of Edinburgh

Themes: Descending Control

Corticospinal neurons (CSNs) transform intention into action, but while their activation drives movement, the functional role of reduced CSN output, a conserved feature across species, remains unknown. By combining population imaging, projection mapping, and modelling in mice performing a Go/NoGo lever-push task, we reveal that bidirectional (i.e. increased versus decreased) CSN activity is selectively associated with skilled movement execution, observed throughout somatodendritic compartments and dorsoventrally organized, with activation predominantly in upper regions and decreased activity in deeper regions of layer 5B. Single-neuron projection mapping demonstrates that these opposing signals converge onto shared spinal interneurons, while modelling suggests this architecture enables flexible scaling of input-output transformations during movement. These findings redefine corticospinal function, showing that reduced CSN output is critical for shaping spinal cord computations during skilled movement execution.

Anatomical Mapping of the Cortico-Reticulospinal Pathway in Mice

Léa Favier, Matilde Cordero Erausquin

University of Strasbourg

Themes: Descending Control; Premotor & Propriospinal Circuits

Voluntary movement is regulated by the sensorimotor cortex, which generates motor commands and modulates sensory input via the corticospinal tract (CST). However, in the absence of the CST, the cortex is still capable of generating movement, which suggests that parallel pathways are involved in motor control. Specifically The CRS pathway, in which reticulospinal neurons (RSn) receive direct control from the cortex, may serve as an alternative route alternative route. The existence of this pathway has been shown physiologically in primates but its anatomical organization and relevance is yet unexplored in rodents. This challenges the traditional hierarchical view of locomotor control and supports a more integrated system. Using advanced viral tools, including intersectional strategies through retrograde, anterograde, and trans-synaptic infections, our project aims to provide a full anatomical mapping of the CRS pathway. Preliminary results indicate that anterograde trans-synaptic labeling is the most efficient strategy to trace cortical projections. We identified reticulospinal neurons, including a subset with direct cortical input (RSn/CI), confirming the CRS pathway. Ongoing analyses, including synaptophysin labeling, will clarify connection density and specificity.

Peptidergic control of muscle dynamics during behavioral switching in the *Drosophila* larva

Cameron Burton, Maarten Zwart, Michael Pankratz

University of St Andrews

Themes: Descending Control; Neuromodulation and plasticity

The ability to perform different behaviors using a limited pool of muscles is essential for survival. To do this, the nervous system must adjust the output of the motor neurons controlling these muscles to suit the animal's current behavioral needs. In *Drosophila*, a subset of hugin (Hug) neuropeptide-producing interneurons has been shown to modulate the choice between feeding and locomotion behaviors through neuroendocrine pathways. However, how Hug acts on neural circuits to implement this behavioral control is unknown. In this study, we show that a specific group of muscles, the lateral transverse muscles (LTs), have discrete contraction patterns during feeding and locomotion. We use dual-color calcium imaging to show that Hug-VNC neurons are active at the start of fictive forward locomotion, during which the LTs contract. Additionally, we show that the activation of Hug-VNC results in slower crawl speeds and longer wave duration. Together, these results show that Hug-VNC neurons potentially coordinate locomotion and feeding behaviors by modulating the contractions of the LTs. Future work will test whether Hug-VNC neurons modulate LT contractions and will determine the synaptic pathways for this potential modulation using the larval connectome. Combining calcium imaging with optogenetic stimulation will allow for the identification of the sensory modalities that drive the Hug-VNC neuronal modulation of behavior.

Serotonergic neurons of the caudal raphe ensure skilled limb movement during locomotion

Marie Yahia, Andrea Giorgi, Marie-Claude Perreault, Julien Bouvier

Paris-Saclay Institute of Neuroscience

Themes: Descending Control; Premotor & Propriospinal Circuits

A role of serotonin in modulating spinal locomotor circuits has long been inferred, yet its exact contribution to locomotor behavior remains poorly understood. This is due, at least in part, to the lack of studies manipulating the activity of spinally projecting serotonergic neurons cell-specifically.
In this study, we genetically and spatially target the serotonergic neurons of the caudal raphe in the mouse to examine their connectivity, mechanisms of action in the spinal cord, and behavioral function using *ex vivo* electrophysiology, *ex vivo* and *in vivo* optogenetics, and anterograde viral tracing. We demonstrate that caudal raphe serotonergic neurons innervate the locomotor circuits of the ventral lumbar spinal cord already at postnatal stage. Functionally, we show that their photostimulation in the neonatal *ex vivo* brainstem-spinal cord preparation does not recruit lumbar motoneurons, but rather inhibits their sensory-evoked responses, suggesting a role in gating sensory information rather than promoting movement. We tested this hypothesis *in vivo,* in the adult mouse, by manipulating optogenetically the serotonergic neurons of the caudal raphe during different locomotor tasks. We found that, even though we did not observe any alteration in the gross locomotor functions (e.g., speed or orientation) in the open-field test, both photo-activation and photo-inhibition significantly disrupted paw placement accuracy on a horizontal ladder test. Together, these findings reveal a role of the caudal raphe serotonergic neurons in the modulation of sensory information in the spinal cord, which ensures skilled limb movement during locomotion.

Central amygdala inhibition of the preBötzinger complex and breathing

Christopher Del Negro, Jeffrey Gu, Yae Sugimura

William & Mary

Themes: Descending Control; Rhythm & Pattern Generation

Breathing behavior is subject to emotional regulation, but the underlying mechanisms remain unclear. Here, we demonstrate a direct relationship between the central amygdala, a major output hub of the limbic system associated with emotional brain function, and the brainstem preBötzinger complex, which generates the fundamental rhythm and pattern for breathing. The connection between these two sites is monosynaptic and inhibitory, involving GABAergic central amygdala neurons whose axonal projections act predominantly via ionotropic GABAA receptors to produce inhibitory postsynaptic cur rents in preBötzinger neurons. This pathway may provide a mechanism to inhibit breathing in the context of freezing to assess threats and plan defensive action. The existence of this pathway may further explain how epileptic seizures invading the amygdala cause long-lasting apnea, which can be fatal. Althoughtheir ultimate importance awaits further behavioral tests, these results elucidate a link between emotional brain function and breathing, which underlies survival-related behavior in mammals and pertains to human anxiety disorders.

Initiating forward locomotion: From precise activation of vsx2+ reticulospinal subsets to all-optical circuit dissection

Xinyu Jia, Akshey Kumar, Martin Carbó-Tano, Camile Lejeune, Marie Didelot, Ninon Peysson, Moritz Grosse-Wentrup, Claire Wyart

Paris Brain Institute

Themes: Descending Control; Rhythm & Pattern Generation

Reticulospinal neurons (RSNs) integrate sensory and descending signals to drive spinal motor circuits, making them essential for initiating and regulating locomotion. While electrical stimulation of the mesencephalic locomotor region (MLR) can reliably elicit forward locomotion, the specific RSN subsets that relay the forward signal to spinal rhythm-generating circuits remain undefined. Vsx2-expressing RSNs (V2a-RSNs) are reliably recruited by MLR stimulation; however, their anatomical and functional complexity pose a challenge for identifying which subsets drive distinct motor outputs. Taking advantage of transparent larval zebrafish, we optically investigated the recruitment, connectivity, and function of V2a-RSNs. We found putative "start" cells located at the medullary boundaries recruited ~ 300 ms before locomotion starts, with long projection reaching the caudal spinal cords. Optogenetic stimulation of a column of V2a-RSNs identified caudal rhombomere('r') 8 as a reliable trigger site for forward locomotion, whereas activation in the more rostral regions preferentially elicited turning. Furthermore, anatomical reconstructions revealed ipsilateral connections within forward-triggering V2a-RSNs in caudal medulla. Our analysis suggests the caudal medulla could integrate descending inputs from rostral V2a-RSNs and ascending input from spinal V2a neurons. To functionally test the V2a-RSN connectivity, we performed all-optical single-cell experiments using two-photon holographic stimulation. Preliminary results support recurrent connections within V2a-RSNs in the medulla, consistent with a recurrent feedback loop. Here we identify a minimal medullary V2a-RSN subset sufficient to trigger forward locomotion and provide evidence for ipsilateral recurrent excitation. These findings shed light on how modular brainstem networks coordinate movement and could inform general principles of vertebrate motor control.

Lumbar spinal Shox2 interneurons receive monosynaptic excitatory input from the lateral paragigantocellular nucleus in the adult mouse

Shayna Singh, Lihua Yao

Drexel University

Themes: Descending Control; Rhythm & Pattern Generation

Locomotor behavior in vertebrates is generated by signals which traverse the entire nervous system. The direct connection between supraspinal and spinal locomotor-related interneurons is comprised of reticulospinal neurons which are thought to directly contact and provide tonic drive to spinal rhythm generating interneurons. Excitatory neurons in the lateral paragigantocellular nucleus (LPGi) within the medulla have been shown to provide this descending drive in the context of forward locomotor initiation but it is yet unknown which population(s) directly receive such drive. Leveraging transgenic mouse lines has revealed populations of genetically identified locomotor-related spinal neuron populations. Lumbar spinal interneurons expressing the transcription factor Shox2 are thought to be part of the locomotor rhythm generator in mice, including neurons that receive descending input and convert it to a rhythmic output. Here, we performed viral tracing and electrophysiological recordings to examine the anatomical and functional connectivity of the LPGi to Shox2 interneuron connection in adult mice. Using both anterograde AAV and monosynaptic-restricted transsynaptic rabies tracing, we show that excitatory neurons from the LPGi make putative excitatory synaptic contacts onto lumbar spinal Shox2 interneurons. A monosynaptic connection was confirmed by recordings of excitatory postsynaptic potentials in Shox2 interneurons in lumbar spinal slices evoked by optogenetic activation of LPGi terminals. These results suggest that lumbar spinal Shox2 interneurons receive monosynaptic excitatory input from the LPGi in the medulla, a connection which may be important in the initiation of locomotor behavior.

Unravelling corticospinal contributions to locomotion

Charlotte Bichara, Gilles Delbecq, Léa Favier, Philippe Isope, Antoine Valera, Matilde Cordero-Erausquin

University of Strasbourg

Themes: Descending Control; Sensorimotor Integration

Despite decades of research across multiple species, the precise function of the sensorimotor cortex in regulating movement remains under investigation. Neurons of the motor cortex are thought to encode various aspects of movement, such as initiation and finer control of skilled behaviors. However, the specific contributions of corticospinal neurons (CSn), which form the most direct pathway for conveying motor signals, remain unclear. Although our recent data suggest that hindlimb CSn activation, while inducing motor output, primarily serves to gate lumbar sensory information, a key process for movement adaptation and coordination, the precise timing and consequence of their activation *in vivo* in freely moving mice remains unclear. By combining kinematic analysis and virus-mediated circuit perturbations, we aim to pinpoint crucial periods of CSn activity whose disruption leads to task-specific motor impairments. Furthermore, we recorded their activity through in vivo electrophysiological recordings in freely moving mice, identifying CSn through optotagging, to explore their role during incremental challenges in locomotor tasks. Our findings demonstrate that perturbing CSn activity directly impacts locomotor kinematics, even during basic locomotion. Additionally, we identify that a significant proportion of neurons in the somatosensory cortex exhibit phase-dependent activity correlated with several locomotor phases, while others have their activity locked to the beginning of the following stride. Our results highlight the dynamic role of corticospinal neurons in motor control, as they can both encode the ongoing locomotion and anticipate what is coming next, in a task-specific manner.

A dorsal medial prefrontal motor circuits encodes initiation of persistant movement

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Themes: Descending Control; Sensorimotor Integration

Medial prefrontal cortex (mPFC) regulates decision-making by amplifying certain information while suppressing others. Recently we reported a group of mPFC motor projecting (MP) neurons [1], which majorly projects to the primary motor neurons in almost all motor cortices and the striatum, but less to other deep brain regions and local non-MP neurons. Therefore, MP neurons may be involved in the most downstream mPFC circuit, which collects all filtered information to affect subsequent behavior. As such, we asked (Q1) if the MP neurons play a role on instructing subsequent movement in decision-making. MP neurons receive unidirectional inputs from the insular cortex (IC), which encodes valence, and from the basal lateral amygdala (BLA), which is responsible for valence assignment (internal belief), here we asked (Q2) what type of information (contextual or valence) the MP neurons encode during a persistent movement. Using single-unit extracellular recordings and opto-tagging in awake mice, we demonstrated that MP neurons in the dMPFC selectively encode contextual information, rather than natural valence, triggering a persistent movement. Inactivation of dmPFC MP neurons impairs the initiation of persistent movement and reduces neuronal activity in the insular and motor cortex. Finally, a computational model suggests that a successive sensory stimulus acts as an input signal for the dmPFC MP neurons to initiate a persistent movement. These results reveal a neural initiation mechanism on the persistent movement.

Developmental molecular signatures define de novo cortico-brainstem circuit for skilled forelimb movement

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Themes: Development; Descending Control

Skilled movement relies on descending cortical projections to the brainstem and spinal cord. While corticospinal neurons (CSN) have long been recognized for their role in fine motor control, the contribution of cortical projections to the brainstem remains poorly understood. Here, we identify a previously unrecognized direct cortico-brainstem circuit that emerges early in development and persists into adulthood. A subset of subcerebral projection neurons (SCPN) limit their projections to the brainstem from the earliest stages of axon extension without ever extending to the spinal cord. Using FACS purification and single-cell RNA sequencing, we show that these cortico-brainstem neurons (CBN) can be prospectively identified by the expression of Neuropeptide Y (Npy) in development. Functional silencing of Npy+ CBN in adulthood leads to impaired skilled forelimb reaching, demonstrating their essential role in adult motor control. Npy+ CBN project preferentially to rostral brainstem regions, including the midbrain reticular formation. These findings reveal developmental molecular signatures that define cortico-brainstem pathways for adult skilled movement, laying the groundwork for our ongoing investigations into how this circuit adapts following neurological injuries such as stroke or spinal cord damage.

Spinal neurons with a biophysical signature consistent with a gamma motoneuron identity emerge during the third week of postnatal development in mice

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Themes: Development; Motoneurons

Gamma motoneurons are a subtype of spinal motoneuron that innervate intrafusal fibers of muscle spindles and maintain spindle sensitivity during muscle contractions. Their function relies on a distinct biophysical signature characterized by a low recruitment threshold and high firing rate, facilitating rapid fusion of intrafusal fibers at the onset of contraction. However, the intrinsic properties, currents, and ion channels that mediate this signature remain poorly understood. In this study, we identified a cluster of low-threshold, high-firing gain motoneurons from previously published data with intrinsic properties consistent with gamma motoneurons that emerge during the third week of postnatal development in mice. Notably, 92% of putative gamma motoneurons exhibited a sodium pump-mediated ultra-slow afterhyperpolarization, a previously reported marker of gamma motoneurons. We found that their low recruitment threshold could be attributed to a combination of lower capacitance, higher input resistance, and a more hyperpolarized persistent inward current (PIC) activation voltage. In contrast, the higher firing rates observed were not associated with differences in PIC amplitude, but rather with shorter action potential durations and smaller amplitude medium afterhyperpolarizations. These findings provide new insight into the cellular and biophysical mechanisms underlying gamma motoneuron function. By identifying key intrinsic properties that support their characteristic excitability, this study enhances our understanding of how gamma motoneurons contribute to motor control and offers a foundation for future research into their development, regulation, and involvement in neuromuscular disorders.

Activity-dependent assembly and remodeling of spinal sensorimotor circuit for proprioception

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National Institutes of Health

Themes: Development; Premotor & Propriospinal Circuits

Motor behaviors undergo remarkable refinement as mammals mature. Motor improvement is embedded within sensorimotor circuit structure, but the logic that builds functional, plastic motor systems (synaptic architecture, networks) from early hard-wired blueprints (cell types) remains elusive. We hypothesized that the activity-dependent remodeling of a developing spinal circuit might enable motor flexibility. Constitutive proprioceptive afferent (pSN) ablation elicits severe motor ataxia marked by erratic limb posturing and deficient motor improvement, suggesting that early sensory feedback may interact with developing circuit trajectories. Preliminary viral tracing suggests that pre-motor interneurons fail to target appropriate motor pools after pSN ablation, and initial circuit construction is the first point of sensory-dependent divergence from early hard-wired paths. To understand how proprioception guides assembly, we will compare the molecular profiles of pre-motor and motor neurons after pSN ablation and assay changes in the molecular codes for partner matching. Next, to test whether proprioception separately remodels circuits for motor improvement, we are generating a new genetic reagent to ablate pSNs at experimenter-defined stages. We will assay network function and motor improvement using behavioral measurements, acute EMG, and measurements of synaptic development. Future experiments will aim to probe how pre-motor network trajectories emerge along the "fixed-to-flexible" spectrum by imaging correlative, stimulus-driven activity in pre-motor and motor partner populations across development. Together, by defining when and how emerging spinal circuits integrate focused periods of sensory feedback during circuit/network development, our work stands to unlock how precise (fixed) or imprecise (flexible) motor modules are sculpted, remodeled, and plastically recruited during behavior.

Serotoninergic regulation of body axis alignment in the post-embryonic zebrafish

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Themes: Development; Premotor & Propriospinal Circuits

Idiopatic scoliosis is a pathology that affects adolescents worldwide, who suffer from spine deformities of which the aetiology is still not fully understood. Surprisingly, a scoliotic-like phenotype arises in the serotonin-depleted $tph2^{ct817/ct817}$ mutant juvenile zebrafish. Our aim is to unravel how serotoninergic neurons can control the maintenance of a straight body axis during the zebrafish postembryonic life. Firstly, we describe the scoliotic phenotype of the *tph2* mutants at different postembryonic stages. Secondly, we characterise the loss of serotoninergic neurons in the CNS of these fish. All spinal serotoninergic neurons are lost in the *tph2* mutants prior the onset of the scoliotic phenotype. In particular, the ventrally located serotoninergic spinal interneurons, and two sensory neurons populations are lost by 21 dpf. The Reissner fibre, an acellular thread that runs in the central canal from the brain to the tail of the fish, has also been investigated. In fact, it has been previously shown that a defective Reissner fibre leads to a scoliotic phenotype in the embryonic zebrafish. In *tph2* mutants, the Reissner fibre is intact at both embryonic and juvenile stage. Altogether, these results raise the novel hypothesis that the scoliotic phenotype observed in *tph2* mutants could be due to impaired proprioception coupled to a defective fine-tuning of the spinal motor circuits that sustain swimming. In this scenario, *tph2* mutants would be subjected to impaired spinal serotoninergic transmission starting around 6 dpf and lasting into adulthood. This would lead to an unbalanced loading of the spine, ultimately causing spine misalignment.

Characterizing stage-dependent neuromotor patterns in *Drosophila melanogaster* larvae through a graph construction approach

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Themes: Development; Rhythm & Pattern Generation

We investigated developmental changes in neuromotor activity patterns in *Drosophila melanogaster* larvae by combining calcium imaging with a novel graph-based mathematical framework. This allows to perform relevant quantitative comparisons between first (L1) and early third (L3) instars of larvae. We found that L1 larvae exhibit higher frequencies of spontaneous neural activity that fail to propagate, indicating a less mature neuromotor system. In contrast, L3 larvae show efficient initiation and propagation of neural activity along the entire ventral nerve cord (VNC), resulting in longer activity chains. The time of chain propagation along the entire VNC is shorter in L1 than in L3, probably reflecting the increased length of the VNC. On the other hand, the time of peristaltic waves through the whole body during locomotion is much faster in L3 than in L1, so correlating with higher velocities and greater dispersal rates. Hence, the VNC-body interaction determines the characteristics of peristaltic waves propagation in crawling larvae. Further, asymmetrical neuronal activity, predominantly in anterior segments of L3 larvae, was associated with turning behaviours and enhanced navigation. These findings illustrate that the proposed quantitative model provides a systematic method to analyse neuromotor patterns across developmental stages, for instance, helping to uncover the maturation stages of neural circuits and their role in locomotion.

Investigating tripartite synapse pathology in ALS utilizing a hiPSC-derived organoid model

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Themes: Motoneuron Diseases

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by motor neuron (MN) degeneration in the brain and spinal cord. Early-stage synaptic changes and glial dysfunction are though to contribute to MN degeneration. The involvement of tripartite synapses, consisting of presynaptic neurons, postsynaptic neurons, and astrocytes, remains underexplored. Recent work by our lab identified selective vulnerability of tripartite synapses in ALS mouse model spinal cords and human post-mortem tissue. It is hypothesized that tripartite synapse pathology may represent a conserved hallmark of the early stages of ALS. Our goal is to investigate the role of tripartite synapses in ALS using human iPSC-derived organoids. Using immunohistochemistry in cortical organoids we have validated synaptic and astrocytic labelling. Using high-resolution and super-resolution microscopy, and quantitative image analysis, we demonstrate bona fide synapse and tripartite synapse structures in cortical organoids and evidence of synaptic maturation between 60 and 120 days in vitro. From analysis of human iPSC-derived cortical organoid harbouring C9ORF72 mutations (C9) and gene-corrected controls (C9Δ), we find no change in the number of synapses (p= 0.5895), indicating no selective loss of tripartite synapses. However, we observed a significant difference in postsynaptic structure between C9 and C9Δ lines (p=0.01846), irrespective of whether the synapses were tripartite or non-tripartite. Contrary to our hypothesis, our findings indicate no such selective tripartite synapse pathology, despite structural synaptic changes in ALS neurons. We are currently developing a protocol to grow spinal organoids from human iPSCs to investigate structural and functional changes in synapses and astrocytes in ALS.

The role of PERP expression in motor neuron death of SMA mice

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Themes: Motoneuron Diseases

Motor neuron death is a key hallmark of spinal muscular atrophy (SMA) and is caused by a reduction of the survival of motor neuron (SMN) protein. Previous studies have shown that the occurring motor neuron degeneration is executed by the activation of the p53 pathway in SMA mice. However, due to the role of p53 as a tumor suppressor, its inhibition is not a viable SMA treatment. To develop future therapeutic approaches, it is essential to identify the p53 downstream targets associated with cell death that execute motor neuron degeneration. One candidate is p53 apoptosis effector related to PMP-22 (PERP), which was recently shown to be upregulated in vulnerable motor neurons. Here, we applied fluorescence in situ hybridization and immunofluorescence in combination with confocal microscopy to evaluate the role of PERP in motor neuron death of a severe SMA mouse model. First, we provided evidence that a successful virus-mediated knockdown of PERP does not alter p53 nuclear accumulation of vulnerable SMA motor neurons, suggesting that PERP is a downstream transcriptional target of p53. Moreover, immunostaining of a vulnerable spinal segment revealed that the virally mediated knockdown of PERP does not prevent motor neuron death in SMA. Additionally, we were able to demonstrate that PERP knockdown tends to alleviate NMJ pathology in SMA mice. This study shows that PERP inhibition does not rescue p53-dependent motor neuron death in SMA.

Investigating the early FTD phenotypes in a mouse model of TDP-43 proteinopathy

Anna Stuckert, Nicole Johnstone, Mary Everett Toombs, Santiago Mora, Ilary Allodi

University of St Andrews

Themes: Motoneuron Diseases

Frontotemporal dementia (FTD) is a fatal neurodegenerative disease affecting the frontal and temporal lobes, with behavioural variant FTD being characterized by social impairment, executive dysfunctions and personality changes. FTD and amyotrophic lateral sclerosis (ALS) lie on a shared disease spectrum, with 30-50% of patients exhibiting both cognitive and motor symptoms, and the majority of FTD-ALS cases are characterized by TDP-43 pathology. This study uses a tetracycline-repressible TDP-43 mouse model replicating ALS-FTD pathology targeting all neurons, showing motor impairment, brain atrophy, and reversible disease progression. While motor phenotype is well-characterized, other cognitive and behavioural aspects remain less studied. Here, FTD phenotypes are investigated using five cognitive paradigms examining changes in activity, exploration, anxiety, memory and sociability. Mice were tested at three timepoints: preinduction, one week and two weeks postinduction. Conventional behavioural analysis showed hyperactivity and decreased sociability. Unsupervised machine learning was employed to identify subtle changes to behavioural phenotype, revealing decrease in behavioural motifs including standing-still and sniffing, as well as increased locomotion, turning and rearing across behavioural paradigms. These changes indicate hyperactivity and altered exploration, as well as stereotypic behaviour. Moreover, anatomical investigations suggest changes in parvalbumin neurons within the medial prefrontal cortex at two weeks postinduction. In conclusion, the model shows cognitive phenotypes recapitulating FTD symptoms and holds potential to investigate system-level effects of TDP-43 expression.

Spatial transcriptomics reveals early changes in inhibitory synaptic transmission in Amyotrophic Lateral Sclerosis

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Themes: Motoneuron Diseases

Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by somatic motor neurons (MNs) degeneration. Although MNs directly connect to muscles, they are part of a complex network of excitatory and inhibitory interneurons (INs). Previous research in our lab showed that spinal V1 inhibitory INs, positive for Engrailed 1 (En1) transcription factor, are affected in the disease before muscle denervation and MN degeneration, unto which they lose their connections. These dysfunction can trigger maladaptive hyperexcitability, resulting in increased intracellular Ca^{2+} levels, oxidative and endoplasmic reticulum stress, reported in ALS. However, the responsible (MN/IN) of this synaptic inputs loss remains unknown. To elucidate this, spatial transcriptomics was performed on V1 INs and MNs at different timepoints: postnatal days 45, 63 and 83, in the SOD1^{G93A} ALS mouse model, with Wild-type littermates as controls. The novel GeoMX Digital Spatial Profiling technique (Nanostring), coupled with RNAscope, enables to isolate each population and investigate the whole transcriptome. Bioinformatic analysis was performed maintaining spatial resolution at the different timepoints. Differential gene expression and enrichment analysis in control V1 INs and MNs showed a characteristic signature in the overall synaptic machinery of these populations: specifically, transcripts involved in neurosecretion, synaptic vesicle priming, pre and post synaptic element regulation were preferentially expressed in INs. Moreover, *Unc13a*, *Unc13c*, *Snap25* and *Stxbp1* were found among the differentially expressed genes during disease progression. These changes were validated using RNAscope. These results report synaptic signature differences and highlight the contribution of early changes in synaptic transmission of V1 INs in ALS.

Astrocyte transcriptomic and morphological changes in mouse models of DYT1-TOR1A dystonia

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University College London

Themes: Motoneuron Diseases

Astrocytes play important roles in maintaining circuit homeostasis in the CNS. Histological findings of gliosis were reported in post-mortem brain tissue of patients with genetic forms of dystonia and in mouse models of dystonia, raising the question about whether they are involved. Yet, changes in astrocyte transcriptomic signature and protein expression remain unexplored and understanding them may reveal insights into how astrocytes react to dysfunctional spinal circuit and lead to novel treatments. To study astrocyte changes during the progression of DYT1-*TOR1A* dystonia, we used a novel mouse model with spinal restricted conditional knock-out (cKO) of *Tor1a* and a constitutive *Tor1a* knock-out (Tor1a-null) model. While homozygous spinal *Tor1a*-cKO recapitulates the early-onset dystonic phenotype (Pocratsky et al., 2023), heterozygous spinal *Tor1a*-cKO and Tor1a$^{wt/null}$ are phenotypically normal with evident motor circuit dysfunction (unpublished). RNA-sequencing of p7 and p18 *Tor1a*-cKO homozygotes revealed differential regulation in astrocyte enriched genes with upregulation of *Gfap, Cd44, Aqp4,* and downregulation of *Kcnj10 and Slc12a2*, suggesting a shift towards a "reactive astrogliosis" state (Escartin et al., 2021). Interestingly, differential regulation of genes involved in glutamate transport such as *Slc1a1, Slc1a2* were not detected at p7 but were evident at p18, suggesting that astrocyte glutamate transport functions might have become affected at a later stage. Histology showed spatially heterogeneous changes in gross astrocytes morphology with increasing GFAP expression in the gray matter, which further suggests a shift towards reactive astrocytes. In contrast, in p0/p1 homozygotes and non-dystonic heterozygotes, astrocytes remain unaffected in both spinal *Tor1a*-cKO and Tor1a-null model.

Restoration of locomotor function in a chronic Parkinsonian mouse model by selective activation of brainstem command neurons

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Themes: Motoneuron Diseases; Descending Control

Locomotor control is organized hierarchically, with brainstem structures operating downstream of the basal ganglia to modulate locomotion. Among these structures, the pedunculopontine nucleus (PPN) plays a critical role in locomotion initiation and modulation of its speed. Parkinson's disease (PD) causes basal ganglia dysfunction due to the loss of dopaminergic neurons in the nigrostriatal pathway, thereby disrupting the downstream effects of the basal ganglia and resulting in locomotor impairments such as bradykinesia and freezing of gait. Given the hierarchical organization of locomotor control, PPN dysfunction may contribute to parkinsonian motor deficits. Thus, we hypothesized that targeted PPN activation may alleviate motor impairments in chronic PD. To investigate this possibility, we used the MitoPark mouse model of PD. Compared to acute pharmacological and toxin-based PD models, the MitoPark mouse model more closely recapitulates human PD pathology, exhibiting a progressive, adult-onset phenotype that advances to chronic disease stages. MitoPark mice exhibited a progressive, bilateral loss of dopaminergic neurons in the nigrostriatal pathway, accompanied by a progressive development of motor dysfunction. MitoPark mice displayed a gradual decrease in distance traveled and demonstrated impairments in attaining higher speed ranges (10-20 cm/s and >20 cm/s), with these deficits developing over time. Using excitatory DREADDs, we demonstrate that bilateral stimulation of glutamatergic PPN neurons enhances motor function by restoring the speed profile. Activation of the PPN reduced time spent at the lowest speed range (2–5 cm/s) while increasing time in higher speed ranges, suggesting its potential as a therapeutic target for restoring locomotor function in PD.

Thursday Poster Session (12:00-14:45): Numbers 1-35

Criteria for identification and precise quantification of spinal motor neurons in disease mouse models

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Themes: Motoneuron Diseases; Motoneurons

Motor neuron (MN) degeneration is a defining characteristic of MN diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Accurate quantification of MN loss in mouse models is crucial for assessing disease progression and evaluating therapeutic interventions. In this study, we conducted a meta-analysis of 77 publications, revealing inconsistencies in spinal cord dissection specificity, the use of non-specific neuronal markers, and significant variability in reported MN loss within the same mouse models. These findings highlight the need for a standardized approach to MN quantification. To address this, we established key criteria for consistent MN assessment. First, we developed a spinal cord dissection protocol that enables segment-specific MN isolation and counting. Using ex vivo ventral-root backfills and immunohistochemistry in combination with tissue clearing, we identified ChAT and HB9 as the only reliable markers for MN identification and further demonstrated that MN distribution varies across spinal segments. Second, we found that MN loss in SMA mice is confined to specific spinal segments, whereas ALS mice exhibit widespread MN degeneration throughout the spinal cord. Finally, we implemented a workflow that allows visualization of all MNs in a cleared spinal segment, followed by automated quantification using the open-source software Cellpose, ensuring objective and reproducible MN counting. Our study establishes a rigorous framework for MN identification and quantification in mouse models, providing a valuable reference for future research requiring precise MN counts.

Thursday Poster Session (12:00-14:45): Numbers 1-35

Synaptic excitation-transcription uncoupling in motoneurons may play an essential role in amyotrophic lateral sclerosis (ALS) pathophysiology

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Themes: Motoneuron Diseases; Motoneurons

In a previous work, we provided anatomical and functional evidence indicating that Ia synapses to spinal motoneurons are impaired at a presymptomatic age in mSOD1 mice (1). In this study, we explored the consequences of this impairment on the synaptic excitation transcription coupling. Whereas Ia synaptic density and structure of presynaptic boutons are preserved, the post-synaptic side displays multiple alterations: expressions of AMPA andNMDA subunit receptors, metabotropic mGluR5 receptor as well as anchoring proteins are substantially reduced. These anatomical alterations translate into physiological impairment since Ia EPSPs are also reduced in mSOD1 motoneurons compared with WT. We then ask whether these dysfunctions have an impact on the activity-dependent signaling and transcription. To explore this issue, we set up an original protocol combining physiological activation of the Ia synapse and immunostaining assays of phosphorylated transcription factors. We discovered that, in sharp contrast with WT mice, the Ia synaptic excitation is uncoupled from transcription in mSOD1 motoneurons. We further show that enhancing Ia EPSP with Ampakine does not restore the coupling. However, and most interestingly, when the PKA activity is enhanced using Rolipram, a selective inhibitor of cAMP-specific phosphodiesterase-4, the coupling between Ia synaptic activity and transcription is restored. Further experiments are ongoing to investigate the consequences of restoring the synaptic excitation-transcription coupling on ALS cellular disease markers.

Predicting therapeutic windows for synaptic intervention in ALS

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Themes: Motoneuron Diseases; Motoneurons

Amyotrophic Lateral Sclerosis (ALS) is a debilitating and fatal neurodegenerative disease, characterized by the progressive loss of motoneurons throughout the central nervous system, including the spinal cord. Previously, work from our lab found that early stages of ALS are characterized by synaptic loss from V1 inhibitory interneurons, followed by subsequent degeneration of the V1 interneurons themselves. More recently, we showed in the SOD1G93A ALS mouse model that synapses projecting from the V1 interneurons can be rescued by overexpression of a presynaptic organizer. Synaptic stabilization in this context preserves motoneurons and maintains locomotor function. Here, we used a computational model of a spinal central pattern generator to mimic progressive interneuron cell death and synaptic changes. By modeling synaptic stabilization at different stages of disease progression, we predict a time window during which intervention with synaptic stabilizers could be most effective. Our computational model suggests that the untreated disease condition results in flexor biased activity while synaptic stabilization preserves more balanced activity patterns–up to the point of substantial motoneuron loss. We also find that applying synaptic stabilization during degraded synaptic dynamics and synaptic loss, leads to a switch to extensor biased activity which could lead to hyperextension in an animal model. Thereby, our model predicts that excitatory/inhibitory balance must be considered when applying synaptic stabilization. If the balance is degraded, intervention could have unintended consequences.

Hindlimb postnatal motor units are impaired in a mouse model of early onset dystonia

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Themes: Motoneuron Diseases; Motoneurons

DYT-*TOR1A* dystonia is an early onset, generalised movement disorder that typically progresses caudo-rostrally. We previously developed a spinal cord-restricted biallelic *Tor1a* knockout mouse model that phenocopies a severe form of this human condition, with a dystonic phenotype that progresses in severity throughout the postnatal period. Since spinal motoneurons are affected in this model, we aimed to characterise distal hindlimb motor units throughout postnatal development. We find that L4-L5 motoneurons decrease in size and number during development, and this is accompanied by a reduction in sensory afferent input into motor pools suggestive of altered circuit connectivity. Targeted patch-clamp recordings indicate that motoneurons innervating the fast type muscle tibialis anterior (TA) adopt characteristics typical of a slower profile, being predominantly immediate firing with reduced capacitance, conductance and rheobase. In parallel, TA and other distal hindlimb muscles displayed a marked decrease in size, a loss of fast type fibres, and a progressive shift towards a slower fibre type composition. Moreover, we observe persisting expression of the embryonic myosin isoform over time, suggesting a delay in fibre type maturation. Preliminary screening of late postnatal neuromuscular junctions within the TA revealed a striking number of vacant terminals, potentially resulting from axonal retraction due to denervation or incomplete neuromuscular development. These data suggest that postnatal motoneuron to muscle maturation is impaired in this dystonic mouse model. Whether these defects contribute to the progressing phenotype remain to be investigated.

The complex role of interneurons in ALS: from early dysfunction to disease progression

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Themes: Motoneuron Diseases; Premotor & Propriospinal Circuits

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of upper and lower motor neurons (MNs), leading to death within a few years from symptom onset. While MN pathology makes a large contribution to disease onset, increasing evidence suggests that dysfunction of interneurons contributes significantly to disease progression. In the SOD1^{G93A} mouse model, degeneration of V2a excitatory glutamatergic interneurons begins presymptomatically, coinciding with the onset of motor neuron loss. In contrast, V1 inhibitory interneurons degenerate later during disease, with approximately 50% degeneration observed at late stages. Studies have reported alterations in inhibitory and excitatory synaptic inputs in ALS, highlighting circuit-level changes as a key pathological feature. We focused on neuromodulatory systems and especially on the serotonergic and cholinergic inputs on MNs in disease. We demonstrated high 5HT synapse heterogeneity on both MN somata and proximal dendrites in wild type and SOD1^{G93A} mice at all disease stages. We report serotonergic input increase at presymptomatic stage and during disease progression. It is known that V0c interneurons, the source of C-boutons on MNs, remain intact until the late stages of the disease. Work in our laboratory has demonstrated that inactivation of the cholinergic output of C-boutons ameliorates motor performance of SOD1^{G93A} mice in early symptomatic stage. We demonstrated that SOD1^{G93A} mice with inactivated C-bouton cholinergic output have more surviving MNs and more innervated Neuromuscular Junctions (NMJs) at presymptomatic and early stage of the disease in comparison with SOD1^{G93A} mice with intact C-boutons, unravelling the cellular mechanism of such amelioration.

Electrophysiological characterisation of motoneurons controlling urinary function

M Gorkem Ozyurt, Filipe Nascimento, Alejandro Pascual-Valdunciel, Marco Beato, Rob Brownstone

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Themes: Motoneurons

Bladder voiding is controlled by the coordinated actions of supraspinal and local spinal circuits. Voiding is initiated at the brain stem level (Barrington's nucleus) and coordinated primarily by lumbosacral spinal circuits involving autonomic and somatic sphincter motoneurons, and autonomic motoneurons innervating the bladder wall smooth muscles. As the bladder is filled, sphincter motoneurons increase their output to ensure urine storage. Either voluntarily or reflexively in response to bladder pressure, voiding starts with coordinated activity of bladder contraction concomitant with sphincter relaxation often manifesting as bursts. However, we still have minimal knowledge of the physiology of motoneurons involved in urination (sphincter and bladder motoneurons) and the local inputs they receive, largely due to these experimental limitations: 1) the muscles involved in model organisms are small and poorly accessible; 2) spinal circuits are often studied in models at juvenile ages when micturition control is far from fully developed. We optimised a method in mice to inject both the sphincter muscle and intramural ganglia of the bladder, to label autonomic and somatic motoneurons and obtain adult whole-cell patch clamp electrophysiology recordings of identified pelvic motoneurons in vitro. Moreover, we optimised high-density EMG recordings (using myomatrix electrodes) from the sphincter muscles during bladder filling and voiding to study the functional properties of sphincter motor units. Thus, we can now characterise the properties of these motoneurons in normal micturition and the strength of their synaptic connectivity. Ultimately, this knowledge could lead to identifying new cellular and synaptic targets to mitigate urinary dysfunction.

Thursday Poster Session (12:00-14:45): Numbers 1-35

Spinal cholinergic transmission augments focal spasticity induced by tetanus toxin

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Themes: Motoneurons

Recent preclinical studies indicate that botulinum toxin type A (BoNT/A), may exert effects at the level of central cholinergic synapses. However, the role of cholinergic synaptic transmission in sustained or intermittent muscle hyperactivity evoked by disinhibition of lower motor neurons has not yet been characterized. In this study, we investigated the modulatory role of spinal endogenous acetylcholine (ACh) in a rat model of muscle spasm induced by tetanus neurotoxin (TeNT). The rats were injected with varying doses of TeNT to induce moderate or severe calf muscle spasm. The role of spinal cholinergic neurotransmission was examined by low dose lumbar spinal intrathecal (i.t.) injections of acetylcholinesterase (AChE) blocker neostigmine (5 μg/10 μL) or M2 muscarinic receptor antagonist AQ-RA 741 (20 μg/10 μL i.t.). Their motor effects were assessed by measuring the muscle spasm intensity (resistance to passive ankle dorsiflexion), followed by various motor performance and gait tests. Injections of TeNT into the gastrocnemius resulted in dose-dependent moderate to very strong spastic paralysis. Short-term blockage of spinal AChE augmented the severity of TeNT-evoked spasm, while blockage of spinal M2 muscarinic receptors resulted in its amelioration. Present results suggest that the spinal cholinergic transmission, associated with SNARE-dependent vesicular release of ACh and muscarinic M2 receptor signaling, augments the increased muscle tone evoked by lower motor neuron disinhibition. These findings reveal a new role for endogenous spinal ACh, with implications for the possible novel therapeutic approaches in sustained or intermittent muscle hyperactivity.

High resolution tools for electromyography in mouse hindlimb

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Themes: Motoneurons; Premotor & Propriospinal Circuits

Very little is understood about the contribution of pre-motor spinal circuits to motor control during dynamic movements such as locomotion, in large part due to the lack of methods for the minimally invasive interrogation of motor function in awake behaving mice. To address this gap in understanding, we have developed minimally invasive, implantable high-density microelectrode arrays, called Myomatrix, for flexible and high-resolution electromyography (EMG) recordings of single motor units of awake behaving adult mice. These devices allow recording from multiple muscles and collect high-quality bulk EMG recordings that can be leveraged for analysis of single motor unit firing patterns. To record from lumbar spinal cord motor units, we developed customized devices for the optimized recording and analysis of mouse hindlimb muscles during freely moving behaviors. We have applied this technology to interrogate how motor units controlling the hindlimb muscles coordinate dynamic movement during locomotion, by recording populations of single motor units from hindlimb muscles of mice while they engage in treadmill-driven locomotion. Combining simultaneous kinematic tracking from multiple angles with high resolution EMG recordings, we can quantify motor unit recruitment and spike patterning across multiple muscles of the hindlimb and assess the functional coordination of motor units to discrete kinematic variables during behavior. Here, we describe the activity patterns of single motor units across the joints of the hindlimb, and for the first time quantify the firing of individual motor units of the mouse hindlimb during locomotion.

Maintenance of peripheral nerve functionality and circuitry after limb amputation in the axolotl

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Institute of Molecular Biotechnology

Themes: Neuromodulation and plasticity

Limb amputation is one of the most severe consequences of various diseases or traumas. It results in the loss of both sensory feedback and muscle targets, disrupting the spinal circuitry that controls limb function and significantly affecting patients' lives. Mammals, including humans, are unable to regenerate an entire lost limb. However, many amputees experience painful sensations from the missing limb, often due to neuromas. These occur when damaged peripheral nerves fail to reconnect with their original targets and grow into disorganized bundles of connective tissue, forming fibrotic structures. Despite the irreversible nature of amputation, this suggests that mammalian nerves retain some regenerative capacity, although full functionality is not restored. To investigate how damaged nerves can remain viable and functional, I will use the axolotl, a species with extraordinary regenerative abilities, as an in vivo model. Axolotls can regenerate amputated limbs, restoring both structure and function. My aim is to determine whether, and how, they maintain peripheral nerve function after severe injury and prolonged periods without regeneration. To mimic the non-regenerative condition observed in mammals, I will block axolotl limb regeneration by targeting the early steps of this process, including wound epidermis formation and cell dedifferentiation into the blastema. By inhibiting regeneration and labeling specific nerve groups, I will examine nerve behavior in the stump and, after a period of inhibition, assess whether these nerves can reconnect with their original targets upon reinitiated regeneration. This research may offer insights for therapeutic strategies to restore nerve and circuit function in non-regenerative species.

Mechanisms of motor nerve regeneration in the axolotl limb

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Themes: Neuromodulation and plasticity; Motoneurons

Peripheral nerve injury presents a significant challenge to the functionality of sensory-motor circuits in limbs, often resulting in permanent motor deficits due to the limited regenerative capacity of mammalian nerves. In contrast, the axolotl salamander exhibits remarkable regenerative abilities, capable of fully restoring both sensory and motor nerves following nerve injury in the limb. Successful motor nerve regeneration in axolotls involves the reinnervation of target tissues by the original nerve and the reoccupation of lost territories by motor axons, even in cases of severe nerve displacement. However, the precise mechanisms by which regenerating motor neurons accurately reconnect with their specific targets remain unclear. To investigate this process, I will employ live imaging of regenerating motor neurons in axolotl limbs, tracking their trajectories and timing throughout recovery. Additionally, I will assess the formation of nascent neuromuscular junctions (NMJs), determining whether these connections are stable and specific or dynamic and transient. I will determine which cells act to guide motor neurons in the limb tissue by selectively ablating limb resident cell types and look for innervation pattern defects of motor neurons following nerve injury. Further, single cell RNA sequencing of the identified cell types will elucidate potential molecular mechanisms. Finally, I will evaluate functional recovery by quantitatively analyzing limb movement in axolotls placed on a treadmill. These findings will provide critical insights into motor nerve regeneration in axolotls and enhance our broader understanding of regenerative mechanisms in vertebrates.

Exploring the GABAergic and glutamatergic LHA anatomical connectivity to the MLR

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Themes: Neuronal Diversity; Descending Control

It has been suggested that the primary function of the brain is to generate movements that shape behavior. However, the specific contributions of distinct brain regions to different movement patterns remain poorly understood. The lateral hypothalamic area (LHA) is a key integrative center that processes signals related to energy balance, motivation, and reward to regulate locomotor activity. Its diverse neuronal populations and intricate connectivity enable precise modulation of movement, ensuring adaptive behavioral responses essential for survival and well-being. However, the role of GABAergic and glutamatergic LHA projections to locomotor-related brainstem regions remains unclear. The mesencephalic locomotor region (MLR), which includes the pedunculopontine nucleus (PPN) and the cuneiform nucleus (CnF), plays a crucial role in initiating and modulating locomotion. It integrates descending commands from higher brain regions, including the LHA, to coordinate locomotor output. While the LHA is well recognized as a pro-locomotor structure, the specific influence of its excitatory and inhibitory projections on the MLR is poorly understood. Here, we examined the descending connectivity of LHA vGAT+ and vGLUT2+ projections to PPN and CnF. Using an anterograde viral approach, we injected AAV-DJ hSyn FLEX-mGFP 2A Synaptophysin-mRuby into the LHA of vGAT-Cre and vGLUT2-Cre mice (8–12 weeks old, male and female). This approach revealed synaptic boutons from both GABAergic and glutamatergic LHA neurons in close apposition to ChAT+ neurons in the PPN. Our tracing experiments show a direct connection between LHA GABAergic and glutamatergic neurons onto cholinergic PPN neurons, suggesting that LHA modulates locomotor activity through its connection to the MLR.

Brain-wide mapping of descending supraspinal neurons in larval zebrafish

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Themes: Neuronal Diversity; Descending Control

Motor commands originate in the brain and are conveyed to the spinal cord via long-range descending projection neurons that are, for the vast majority, located in the brainstem. However, the wide distribution and complex connectivity of supraspinal neurons make it challenging to fully map the functional organization of motor circuits at the population level *in vivo*. To overcome this challenge, we leverage the transparency, small size, and relative structural simplicity of the larval zebrafish, an ideal model to uncover fundamental principles of neuronal circuit organization at the whole brain level in single animals. Using two-photon microscopy, we optically backfilled spinal-projecting neurons by photoactivating PA-GFP expressed pan-neuronally, targeting descending axonal projections in the spinal cord. We then imaged, registered, and reconstructed the location of retrogradely-labelled neurons in a 3D zebrafish brain atlas, generating a comprehensive whole-brain map of descending projection neurons. Notably, our data revealed the presence of more than 20 anatomically distinct spinal-projecting nuclei distributed across the brain. To further determine the putative molecular identity of supraspinal populations, we performed optical backfills and hybridization chain reaction in situ (HCR) in a library of transgenic lines. Altogether, this novel cellular-resolution framework characterizes the anatomical distribution, projection patterns, and molecular signatures of most descending supraspinal neurons across the entire brain, highlights conserved features of supraspinal nuclei organization across vertebrate species, and sets the stage for future optical and electrophysiological investigation of supraspinal function *in vivo.*

Anatomo-functionnal characterization of non-reticulospinal V2a neurons controlling oculofacial movements

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Themes: Neuronal Diversity; Descending Control

The brainstem reticular formation (RF) coordinates motor behaviours at both the spinal (trunk, limbs,…) and supraspinal (orofacial, ocular,…) levels. Yet, the specific roles of its diverse neuronal populations remain poorly understood. Recent studies showed that V2a neurons, a subset of glutamatergic reticular neurons, within the gigantocellular nucleus (Gi), drive orienting behaviour through reticulospinal projections that impose limb and trunk asymmetries. However, it remains unknown whether it exists, in the Gi and/or in adjacent RF regions, non-reticulospinal V2a neurons that could influence cranial nerve mediated motor actions. We investigate this question here, combining viral tracings and optogenetic manipulations. First, an anterograde mapping study revealed that V2a Gi neurons make abundant synaptic contacts in the brainstem and midbrain, with the most contacted regions being specific sets of cranial motoneurons. Functionally, we confirmed the capacity of V2a neurons to reliably evoke orofacial movements, compatible with a premotor connectivity. Second, we found that these orofacial movements are not mediated by spinally-projecting V2a neurons, arguing for exclusively locally-projecting subtypes. Third, our investigation of the V2a neurons located beyond the Gi revealed that the more rostral ones (pontine RF) can additionally mobilize specific ocular movements, suggesting a rostro-caudal specialization. Fourth, transcriptomic profiling of V2a neurons across the entire RF starts to unveil distinct molecular signatures underlying spinal or supra-spinal projections, as well as rostro-caudal positioning. These results altogether uncover, within the V2a population, a granularity at the connectomics, positional, and gene expression levels that may underlie their implication in diverse types of movements.

Neuronal subtype specification of spinal projection and motor neurons by a common temporal transcription factors sequence

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Themes: Neuronal Diversity; Development

Neuronal diversity emerges from the superposition of spatial and temporal patterning programs in large regions of the developing nervous system. The chronologically ordered generation of the distinct cell types is essential for the establishment of this diversity and the formation of neuronal circuits. While the gene regulatory network (GRN) orchestrating spatial patterning is well-understood, the GRN architecture controlling temporal patterning is just emerging. Here, we show a shared sequence of transcription factors, Onecut2 > Pou2f2 > Pou3f1 partitioning spinal long-range projection neurons based on their timing of neurogenesis. We describe an early emergence of transcriptomic differences between neuron subtypes residing within lamina I and lamina V in the anterolateral system (AS), which relays pain and temperature information from the spinal cord to the brain, and in distinct columnar identities of motor neurons, essential for the correct establishment of their myotopic organization. Moreover, we demonstrate that the loss of Pou2f2 impairs the development of two early-born motor neuron columns and leads to the molecular re-specification of AS projection neurons into a later-born subset. Likewise, the proper development of later-born motor neuron subsets and AS projection neurons depends on the expression of Pou3f1. Together, our results demonstrate that temporal patterning is functionally required for the segregation of long-range projection neurons into molecularly and functionally distinct subtypes in the developing spinal cord.

Regulation the temporal patterning of the developing nervous system by microRNAs

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Themes: Neuronal Diversity; Development

In the developing spinal cord, spatially defined classes of neurons are partitioned into molecularly and functionally distinct subtypes by a shared sequence of temporal transcription factors (tTFs), whose expression in neurons depends on when the neuron has been generated during development. The molecular mechanisms that orchestrate the chronologically ordered expression of these tTFs are still largely unknown. Here, we demonstrate that disruption of microRNA biogenesis by conditional deletion of Dgcr8, a core component of the microprocessor complex, results in the complete loss of late temporal identity neurons, while causing a concomitant increase in early and intermediate identity neurons. Computational analysis suggested that this phenotype is caused by the loss of let-7 and miR-9 family microRNAs, which promote the specification of late neural identities by antagonizing the transcriptional programs of early progenitors and neurons. Consistent with this hypothesis, expression levels of these miRNAs increase during the neurogenic phase in the embryonic mouse spinal cord and in stem cell-based in-vitro differentiations that recapitulate in-vivo temporal patterning. Moreover, ectopic expression of these miRNAs in chicken embryos causes a premature switch from early to late neuronal identities, providing experimental evidence for their functional involvement in this process. Together, our data reveal a critical role for miRNAs in temporal identity specification of spinal cord progenitor and neurons. Such detailed understanding of the role of miRNAs in temporal patterning may contribute to the rational design of differentiation and reprogramming protocols aimed at generating specific neuronal subtypes for disease modeling and regenerative medicine applications.

Heterogeneity in spinal neuron lineages

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Themes: Neuronal Diversity; Development

The complexity and specificity of movement in vertebrates is driven by hundreds of different spinal motor and interneuron types. This neuronal diversity emerges from only eleven progenitor domains in the embryonic neural tube, raising the question of how each progenitor domain gives rise to so many neuron subtypes. Is cell fate specified by intrinsic progenitor programs or shaped by extrinsic signals? Is an individual progenitor restricted to producing only one neuron type or is it multipotent? In the invertebrate nerve cord, cell fate is largely determined by progenitor programs, while in the much larger and more complex vertebrate brain, cell diversity is controlled by a combination of intrinsic and extrinsic mechanisms. Unlike the brain however, in the vertebrate spinal cord, the role of lineage in cell diversification is uncertain. We have thus set out to trace single-cell lineages in the mouse spinal cord using high-resolution mosaic analysis with double markers (MADM). MADM indelibly labels an individual neural tube progenitor and its progeny (i.e. clone), enabling us to resolve the potency and neuro-/gliogenic behaviour of individual progenitors. Our ongoing analysis has revealed variability in progenitor cell division behaviour, clone cell-type composition, and sister neuron morphology, indicating that spinal progenitor output is heterogeneous. We are currently investigating how cell type and number may be regulated at the level of individual progenitor cells. Ultimately, our lineage tracing will propose developmental principles of spinal neuron diversification.

Development and evolution of rostrocaudal spinal networks for limb control

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Themes: Neuronal Diversity; Development

The development of limbs was a major landmark in vertebrate evolution, yet adaptation of spinal interneuron circuits for limb movement remains poorly understood. Amphibians, the first tetrapods to walk on land, transition to limb locomotion during metamorphosis and offer a unique perspective on the underlying spinal cord transformation. Focusing on peak frog limb circuit development, we define novel and conserved spinal motor and interneuron rostrocaudal architecture, uncovering a stereotypical, limb-biased pattern for ventral interneurons but independent dorsal organization profiles. Moreover, using EdU birthdating, we reveal the neurogenesis patterns that produce the architecture differences. Previous studies in mice have demonstrated that Hox genes orchestrate rostrocaudal spinal neuron subtype diversity. Using CRISPR to generate HoxC9 mutant frogs, we find that HoxC9 is a master regulator of both rostrocaudal neuron architecture and neurogenesis itself, with HoxC9 mutation causing a thoracic-to-brachial transformation of neuronal architecture and proliferation. Moreover, the HoxC9 mutation redirects thoracic ventral roots to the limbs. These ventral roots exhibit atypical, bilaterally synchronous activity, which we trace to the HoxC9-mediated transformation of the thoracic premotor circuits. Our study, thus, provides new insight into how spinal neurons differ in their number, type and function along the rostrocaudal axis across species, and pinpoints a Hox-dependent mechanism that orchestrates these differences. It offers a developmental framework for limb circuit specialization, and lays the foundation to relate differences in neuron architecture to limb movement.

Emergence of limb movement requires multifold increase in spinal inhibitory cell types

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Themes: Neuronal Diversity; Development

As vertebrates transitioned from aquatic to terrestrial forms of locomotion during evolution, their motor behaviors adapted from simple undulatory swimming to more complex limb-driven locomotion. How motor circuits, and specifically spinal cord cell types, evolved to support this transition remains unclear. Here, we take advantage of frog metamorphosis, which captures this evolutionary swim-to-limb transition in a single organism, to determine how spinal circuit composition varies to generate aquatic versus terrestrial motor patterns. At swim stages, tadpoles have a relatively simple spinal cord architecture, with transcriptionally and anatomically homogeneous motor and interneuron populations. As limbs emerge and movement complexity increases, these spinal circuits undergo a dramatic expansion in both neural number and subtype heterogeneity. This expansion is most pronounced for V1 inhibitory neurons, which increase ~70-fold in number and acquire dozens of transcriptionally distinct subtypes, mirroring the same broad classes as in mammals but exhibiting species-specific transcriptional codes. Disruption of the transcription factors that define these emergent neuronal types reveals both new and conserved functions in swim versus limb motor control. To functionally map these transitions *in vivo*, we recently established two-photon calcium imaging in behaving tadpoles to visualize spinal circuit dynamics as they shift from coordinating axial to limb-based movement. Our findings thus demonstrate that a multifold increase in inhibitory neuron diversity underlies the transition from tail-based swimming to limb-based locomotion, providing a mechanistic paradigm for how spinal circuits adapted during the analogous water-to-land transition during vertebrate evolution.

Development and differential expression of perineuronal nets in specific spinal motoneuron subpopulations

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Themes: Neuronal Diversity; Motoneurons

The adaptive plasticity of spinal cord motor circuits is regulated in part by the molecular environment surrounding the constituent neurons. Dynamic changes in synaptic plasticity during development and after injury are linked to the presence of a dense network of extracellular matrix molecules known as perineuronal nets (PNNs) that surround certain populations of neurons. Here we have studied the developmental pattern of PNN expression and synaptic stabilization in selected motoneuron (MN) subpopulations in the mouse spinal cord. Using Western blotting, immunohistochemistry, qPCR and RNA-seq we show that PNN expression increases gradually over the first three postnatal weeks and that Aggrecan is a major PNN component in the SC. We find differential expression of Aggrecan by the hindlimb flexor *Tibialis anterior* versus extensor *Gastrocnemius* MNs. We then reduced the expression of Aggrecan and abolished PNNs by *Acan* knockdown in an inducible *Acan* KO mouse model. Looking at changes in the number of synapses in our inducible KO model we found a significant decrease in inhibitory Vgat synapses, while observing no change in excitatory Vglut2 synapses. In ongoing work, we are testing the role of PNNs in the development of selective vestibulospinal inputs to extensor versus flexor MNs.

Monosynaptic Rabies virus reveals distribution of motor and promotor interneurons from peripheral system in adult mouse

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National Institutes of Health

Themes: Premotor & Propriospinal Circuits

Movement is the behavioral output of neuronal activity within the spinal cord. Motor neurons are organized into pools, each serving as a functional unit that innervates a specific muscle. The activity of these motor neurons is regulated by a network of premotor interneurons. Understanding the connectivity between defined premotor interneurons and motor neuron pools is essential for elucidating the organizational structure of the motor output system. However, in adult mice, the muscle-specific inputs to each motor neuron pool remain largely uncharacterized. To address this, we are using two parallel strategies. First, we have characterized the dendritic pattern of numerous hindlimb motor pools. Second, we are adapting monosynaptic retrograde rabies virus tracing in combination with spatial transcriptomics in adult mice hindlimb muscles. This approach will enable us to characterize three-dimensional nature of the pre-motor connectivity matrix, thereby highlighting the organizational principles governing specific motor pool circuit connectivity.

dl3 neurons form sensorimotor circuits across the cervical and lumbar spinal cord for motor control

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Themes: Premotor & Propriospinal Circuits

Neural networks in the spinal cord integrate sensory feedback to adapt motor activity and facilitate complex motor behaviours, such as locomotion or hand grasp, in response to multiple stimulus modalities. This important ability of modulating the activity of motor networks in response to changes in the body or external environment is partly mediated by a population of spinal interneurons, called dl3 neurons. These dl3 neurons receive diverse cutaneous and proprioceptive feedback, which convey information regarding fine touch and the positioning of the limbs, and they provide excitatory inputs to motoneurons that directly control muscle activity. Previous work has demonstrated that sensory drive to dl3s is implicated in recruiting spinal networks involved in locomotion, hand grasp, and motor recovery after spinal cord injury (SCI); however, it is unclear how dl3 neurons are connected to motor networks across the spinal cord to facilitate these complex functions. Through optogenetic activation of dl3s and electrophysiological recordings, we mapped the functional connectivity of dl3s to different circuits throughout and across the lumbar and cervical spinal cord. We demonstrate that lumbar and cervical subpopulations of dl3 neurons form distinct local, commissural, long ascending, and long descending premotor circuits. Furthermore, we found that continuous photoactivation of lumbar dl3 neurons recruited locomotor networks independently from the brain, highlighting their potential as targets for motor recovery after SCI. Our findings suggest that dl3 subpopulations integrate specific sources of sensory information and modulate the activity of distinct motor pools to control complex motor functions such as grasping or locomotion.

Light sheet calcium imaging in adult *Drosophila* links premotor neurons to behavior

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Themes: Premotor & Propriospinal Circuits

Limbs are highly multifunctional, enabling a wide range of behaviors including locomotion, grooming, social behaviors, and object manipulation. Although individual muscles and motor neurons are known to be recruited across multiple behaviors, premotor neurons remain less functionally characterized: Are specific premotor cell types involved in many types of movements, or do they tend to be dedicated to drive specific behaviors? Until now, answering this question has been challenging due to the difficulty of recording from premotor circuits *in vivo* during natural behaviors. To overcome this barrier, we have combined several technologies in the adult fly, *Drosophila melanogaster*: 1) a surgery granting optical access to the fly's ventral nerve cord — analogous to the vertebrate spinal cord – without restricting limb movements, 2) sparse genetic driver lines that reproducibly label individual premotor neurons, 3) genetically encoded calcium sensors, and 4) high-speed single-objective light sheet microscopy. Together, these tools enable recording calcium dynamics of identified premotor neurons at 25+ image volumes per second as the fly freely walks on a spherical treadmill, grooms its body, and reaches for objects. Using this approach, we have found specific neurons in the ventral nerve cord with activity linked to different features of movement. For example, one cell type's activity is most linked to turning direction during walking (a specific, high-level movement pattern), whereas another cell type's activity encodes a specific joint angle (a more general, low-level movement feature). Combining these functional characterizations with the connectome will reveal precise circuit mechanisms for multifunctional limb control.

Relative contributions of ipsilateral and contralateral spinal inhibition on locomotor frequency control

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Themes: Rhythm & Pattern Generation

Locomotor output is generated by a network consisting of ipsilaterally- and contralaterally-projecting spinal interneurons. Inhibitory interneurons in this network coordinate left-right and flexor-extensor alternation, and also influence locomotor frequency. The relative contributions of inhibition from ipsilateral and contralateral interneurons to locomotor frequency control is unknown. However, prior work has shown that drug-evoked locomotor rhythms are lower in frequency in isolated hemicords than in full cords. Here, we tested the hypothesis that contralateral inhibition has a stronger influence on locomotor frequency than ipsilateral inhibition. We recorded from lumbar flexor- and extensor-related ventral roots of isolated intact or hemisected spinal cords from neonatal (P0-4) mice during fictive locomotion over a range of starting frequencies evoked by 5-HT and varied concentrations of NMDA. To enhance inhibition, GABA and glycine reuptake were blocked with nipecotic acid and sarcosine. Locomotor burst parameters were measured in the presence and absence of reuptake blockers. We found that enhancing inhibition had no effect on tested parameters in the intact cord, however the cycle period increases in hemicords with a greater effect at lower starting frequencies. This suggests that the strength of ipsilateral inhibition plays a significant role in setting the pace of the locomotor rhythm. Ongoing experiments are testing whether the lack of effect in the full cord is due to a stronger role of inhibition at lower locomotor starting frequencies achieved only in the hemicord, a ceiling effect of strength of inhibition in the whole cord, or a negligible influence of contralateral inhibition on locomotor rhythm generation.

Differential expression of inward and outward currents in spontaneously oscillating spinal Shox2 interneurons

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Themes: Rhythm & Pattern Generation

The spinal cord contains the neuronal network that generates the rhythmic and patterned basis of locomotion. Within this network, the transcription factor Shox2 marks a subset of putatively rhythmic interneurons in mice. Approximately 1/4 of Shox2 interneurons in the adult spinal slice display spontaneous oscillations, which are not observed in the neonate. An array of rhythmogenic currents shown to shape rhythmic bursting in the CNS are present in Shox2 interneurons and increase in magnitude during postnatal development, suggesting these currents may contribute to rhythmicity observed in the adult spinal slice. In the present study, we tested the hypothesis that rhythmic Shox2 interneurons can be distinguished by specific rhythmogenic currents and/or intrinsic properties. Using whole-cell patch clamp recordings from Shox2 interneurons in lumbar spinal slices from Shox2cre; R26-lsl-tdTomato mice >P20, we found that 21% of Shox2 interneurons displayed rhythmic membrane potential oscillations and/or burst firing in the absence of drugs. Sustained depolarizations, indicative of plateau potentials likely driven by persistent inward currents (PICs), were only observed in rhythmic Shox2 interneurons. The slow afterhyperpolarization (sAHP), an outward current mediated by calcium-activated potassium channels (K_{Ca}), was observed at higher prevalence in rhythmic Shox2 interneurons, although it was also found in a subset of non-rhythmic Shox2 interneurons. Additionally, pharmacological manipulation of either K_{Ca} or PIC channels was sufficient to induce bursting in adult Shox2 interneurons, suggesting that both currents may contribute to rhythmicity in Shox2 interneurons. Future studies will explore the roles of K_{Ca} currents and PICs in rhythmicity of adult Shox2 interneurons.

Dopamine promotes motor programs underlying substrate tunnelling in larval *Drosophila*

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Themes: Rhythm & Pattern Generation; Neuromodulation and plasticity

Dopamine is a conserved biogenic amine with diverse neuromodulatory roles. Here we examine dopamine's role in modulating *Drosophila melanogaster* larval motor programs underlying movement over and through substrates. First, we performed dual-color calcium imaging in tyrosine hydroxylase (TH)-expressing dopaminergic neurons and motorneurons to reveal cell-type specific recruitment patterns during fictive motor programs. Activity of select TH-neurons correlated strongly with fictive headsweeps, forward and backward locomotion. Next, bath applications of dopamine biased the isolated central nervous system towards forward fictive locomotion and inhibited fictive headsweeps, suggesting a behavioral transition away from navigation to direction-oriented locomotion. To probe whether these effects are recapitulated in intact animals, we optogenetically manipulated TH-neurons during crawling and tunnelling. Surprisingly, optogenetic activation of TH-neurons during crawling slowed locomotor rhythms by increasing wave duration and decreasing wave frequency, with no effect on headsweeps. Furthermore, posterior asymmetries, motor sequences characteristic of tunnelling, were enhanced. On the other hand, optogenetically inhibiting TH-neurons had no effects on crawling. Underground, TH-neuron activation enhanced tunnelling activity by increasing wave frequency, instead of duration, to increase overall tunnelling time, whereas inhibition decreased time spent tunnelling. These results suggest that dopaminergic modulation of larval forward locomotion is dependent on sensorimotor context and carry implications toward studies investigating single behavioral contexts. We propose dopamine mediates a coordinated network effort to shift central pattern generators to enhance tunnelling rhythms in *Drosophila* larvae.

Elucidating neural circuits underlying distinct locomotor behaviours in *Drosophila* larvae

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Themes: Rhythm & Pattern Generation; Motoneurons

Drosophila larvae exhibit a wide range of locomotion behaviours including forward and backward locomotion (crawls), turns, head sweeps, hunches and rolls. Crawling consists of coordinated muscle contraction waves that propagate synchronously from tail (A8) to head (T1) during forward locomotion and the opposite direction during backward locomotion. In contrast, turns are generated by asymmetrical muscle contractions in the anterior part of the body (thorax, abdominal segments A1-A4). These behaviours are controlled by interconnected central pattern generators (CPGs) located in each segment along the ventral nerve cord (VNC). In this project, we aim to characterise the anatomical and functional differences underlying distinct behaviours. We focussed on the presynaptic circuits activating the RP3 motoneurons, that are recruited during crawls, turns and hunches. Electron Microscopy (EM) tracing of motoneurons along the VNC shows that some motoneurons, such as the aCCs, exhibit similar morphology across the thoracic and anterior abdominal segments of the VNC, whereas RP3s show regional differences. In addition, their presynaptic connections also show segmental differences that we are further characterising. Activity measurements of the RP3 neurons, using calcium indicators, show several patterns of activity: i) synchronous across thoracic segments (hunches), ii) left-right asymmetrical activity along the thorax (turns) and left-right symmetrical along all the abdominal segments (crawls). Similar patterns of activity are observed in a range of presynaptic interneurons, suggesting their involvement in distinct CPGs. Further experiments will use electrophysiology to determine how the activity of presynaptic interneurons determines the distinct pattern of RP3 activity and the different behaviours.

SK2/3 channels work in tandem with T-type Ca^{2+} channels to gate rhythmogenesis within the central pattern generator for locomotion in mice

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Themes: Rhythm & Pattern Generation

The rhythmogenic core of the spinal locomotor network harbors pacemaker bursting cells driven by the persistent sodium current (INaP), with ionic mechanisms of their emergence still undefined. Here, we show that blocking small-conductance calcium-activated potassium (SK) channels with apamin converts half of tonic spiking interneurons of the central pattern generator (CPG) into INaP-dependent rhythmic bursters. By contrast, enhancing SK channel activity at the CPG level with 1-EBIO abolishes both bursting and locomotor-like rhythmic activity. Immunohistochemistry revealed SK2 and SK3 channel clusters in interneurons within the locomotor CPG region. Consistently, inhibition of SK2 and SK3 with tamapin replicated the effects of apamin. Selective SK2 blockade with Lei-Dab7 or SK3 knockdown with shRNA each induced rhythmic bursting in interneurons, indicating that both subtypes independently constrain pacemaker activity. Interestingly, blocking T-type Ca2+ channels (mibefradil, nickel) promoted bursting, unlike L-type (nifedipine) or P/Q-type (ω-agatoxin IVA) channel inhibition, indicating T-type Ca2+ influx mainly regulates Ca2+-activated SK2 and SK3 to control pacemaker bursting in the CPG. This study identifies a tripartite SK2/SK3/T-type Ca2+ channel partnership as a key gating mechanism for locomotor rhythmogenesis.

Neuroskeletal dynamics during locomotion in mammals

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Themes: Rhythm & Pattern Generation; Biomechanics

Locomotion in mammals involves coordinated activity across the limbs and trunk that is regulated by intricate networks within the central nervous system. Yet, the dynamic oscillations of the trunk and vertebral column during movement remain poorly understood. While much research has focused on limb kinematics and central pattern generation, the role of spinal column motion - specifically how it flexes, extends, and undulates during gait - has been largely overlooked. This is particularly important as the trunk is not merely a passive structure but undergoes rhythmic, coordinated motion that likely plays a critical role in balance, posture, and may provide important sensory feedback for locomotion. To address this gap, we used high-speed X-ray imaging and DeepLabCut, a deep learning-based motion analysis tool, to quantify vertebral column and trunk movements in rats and mice locomoting on a treadmill. Our analyses reveal consistent patterns of vertebral oscillation that are location-specific and phase-locked with limb movement. These findings provide a more integrated view of locomotor biomechanics and suggest that spinal motion itself may contribute to motor coordination. This work lays the foundation for future studies into the neuromechanical coupling between spinal column dynamics and limb control in mammals, and highlights the possibility of unexplored sources of sensory feedback based on spinal column and spinal cord movements.

Neuromodulation of sodium potassium ATPase pumps dynamically regulates mammalian spinal networks

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Themes: Rhythm & Pattern Generation; Premotor & Propriospinal Circuits

Mammalian spinal motor networks are sensitive to descending neuromodulatory input, which can fine tune and adapt motor output. Neuromodulators often act by targeting, either directly or indirectly, ion channels and pumps to change membrane potentials and thus affect the likelihood or rate of action potential generation. One potential target, the sodium/potassium pump, has recently been suggested to be linked to an afterhyperpolarisation termed the ultra-slow after-hyperpolarisation (usAHP). Recent work has also highlighted a potential role for the neuromodulator dopamine in modulating this usAHP. The presented work utilises a combination of in vitro extracellular recordings from isolated spinal cord preparations and single cell patch clamp electrophysiology to interrogate the neural networks underlying spinal motor output and the contribution of the usAHP. Additionally a mutant mouse model carrying a mutation on the ATP1A3 gene was used to investigate if this relationship between dopamine and pump activity was associated with the more dynamically activated alpha3 Na/K pump which may be tied to the usAHP. The presence of the mutation, which caused a 50% reduction in functional ATP1A3 expression, disrupted the rhythmic stabilisation effect observed with application of dopamine suggesting the correct expression of the α3 Na/K pump and the resultant usAHP is required for dopamine induced network stabilisation.

Live brain imaging of a state-dependent sensory response reversal in *Xenopus laevis* tadpoles

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Themes: Sensorimotor Integration

Intro**:** Resting *Xenopus laevis* tadpoles start swimming when stimulated, but stop if the same stimulation is applied during ongoing locomotion. The mechanism underlying this form of neuronal network reconfiguration could be shared by different neuronal networks and across different species. Methods: To identify the brain areas involved in the sensory response reversal (SRR), the brainstem of immobilised transgenic GCaMP6s tadpoles was imaged on an epifluorescence microscope with simultaneous ventral root recordings. Calcium signal traces from regions of interest (ROIs) were clustered and labelled following regression analysis to obtain functional brain maps. Results: At least five different ROI types were identified: motor ROIs, with sustained activity throughout swimming, occupy the ventral brainstem, whereas sensory ROIs, with activity correlated and proportional to sensory stimulation, are more dorsally located - with some overlap with motor ROIs regions; a second type of sensory ROI, with responses independent of stimulus intensity and duration, are found in dorsal rhombomeres (r) 2-5; anti-motor ROIs present activity negatively correlated with swimming episodes and form a narrow ventral stripe close to the midline in r1-r2; stopping ROIs are recruited towards the end of swimming and are mainly identified in rostral midbrain and caudal forebrain. Conclusions: Neurons with different activity profiles were identified in the tadpole brainstem during SRR. In particular, a mixture of sensory and sensory-like ROIs appear to co-exist in the trigeminal nucleus area, some of which are sensitive to motor state. These results will guide further electrophysiology experiments to reveal the neuronal network underlying tadpole SRR.

Sensorimotor integration in the zebrafish inferior olive during motor adaptation

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Themes: Sensorimotor Integration

Neurons in the inferior olive (IO) nucleus of the brainstem are critical for motor control, yet their role in motor control remains contested. One prevailing hypothesis suggests that IO neurons function as comparators, signalling prediction errors to the cerebellum by contrasting expected and actual sensory feedback. To test this idea, we employed two-photon calcium imaging in head-fixed zebrafish during sensory and motor adaptation paradigms. Our objectives were to: (1) characterize sensory and motor representations within the IO, and (2) investigate IO activity during induced motor adaptation to clarify their role in movement correction. Simultaneous recordings of fictive tail motor output and IO calcium dynamics revealed that IO neuron activity is modulated by the magnitude of motor activity but is not elicited by motor output alone in the absence of sensory input. Interestingly, IO neurons selective for forward visual motion exhibited a sigmoidal response curve with increasing swim strength, whereas neurons tuned to backward motion showed a U-shaped relationship. These response patterns correlated with the absolute difference between expected and actual visual feedback given swim strength, consistent with a prediction error signal. Our results support the hypothesis that IO neurons encode sensory-motor mismatch. However, examination of glutamatergic inputs to IO neurons expressing GluSnFr suggests that aspects of this comparative computation occur upstream, rejecting the idea the IO itself performs the error calculation. Instead, we propose that the IO may transform precomputed error signals, for example by selectively amplifying more salient discrepancies to influence cerebellar processing.

Cracking the code of diabetic neuropathy: anatomical and functional changes in spinal and DRG neurons

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Themes: Sensorimotor Integration

Neuropathies are the major cause of disability in diabetes, remaining a therapeutically challenging condition due to the heterogeneity of clinical manifestations and the limited understanding of the underlying pathophysiological mechanisms. Diabetic neuropathies include both sensory and motor impairments, suggesting distinct populations of dorsal root ganglia (DRG) and spinal neurons might underlie specific facets of the disease. We aimed to correlate sensorimotor deficits with anatomical, molecular, and circuit-level changes in $hCdx2::FlpO;R26^{Ai65F\text{-}FSF\text{-}Tomato}$ mice subjected to a high-fat diet followed by streptozotocin injection to induce diabetes. The onset of diabetic neuropathy was confirmed through a multimodal assessment, according to clinical guidelines, including nerve conduction velocity measurements and comprehensive sensorimotor phenotyping. High-fat diet alone led to obesity and hyperglycemia, albeit to a lesser extent than in the diabetic state. Preliminary data indicate that the high-fat diet independently reduced mechanical but not nociceptive sensitivity and induced gait abnormalities. Upon induction of diabetes, mice exhibited pain sensitization—such as mechanical allodynia—and more severe motor impairments, including an increased number of footslips while traversing a narrow beam. These findings suggest that the onset of neuropathy may involve degeneration of specific sensory and motor fibers, whereas the progression and chronification of the neuropathy result from central sensitization, leading to allodynia and further impairment of motor performance. Future work aims to dissect molecular, cellular, and circuit changes in both the central and peripheral nervous systems to determine their causality in the onset and progression of diabetic neuropathy.

Interaction sensory-motor processing between visuo-vestibular and locomotor signals during swimming in larval frog

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Themes: Sensorimotor Integration

Locomotion is accompanied by gaze-stabilizing eye movements that derive from sensory-motor transformations and predictive locomotor efference copies. In larval *Xenopus*, angular vestibulo-ocular reflex (aVOR), derived from horizontal canal, and spino-ocular motor coupling, elicited by CPG efference copy pathways, are integrated at the oculomotor plan through two distinct interactive modalities: gating or summation depending of the swim frequency. So far, how other sensory inputs interact with spino-ocular command and which pathways carry these interactive modes, remain unknown. The aim of this study is to characterize the integrative mechanisms that produce the appropriate neural computation to combine otolith vestibular and visual sensory signal together with spino-ocular command during larval swimming. High speed video recordings of eye and tail movements as well as nerve recordings were used in semi-intact preparations during locomotor activity conjointly enhanced with visual or vestibular stimulation. Like the aVOR, the linear VOR (lVOR), produced by horizontal head translations, was either gated by the spino-ocular command or combined with it, depending on swimming frequency. This first result demonstrated that the locomotor-induced VOR gating affects all horizontal vestibular inputs and not only canal specific. Inversely, preliminary results suggested that visual inputs seem to be conjugated to efference copy signal during combined optokinetic and swimming activity. These sensory-motor interactive processing's could be relayed by a vestibulo-commissural inhibitory pathway activated by the efference copy signal when swimming frequency is sufficiently high. In the future, pharmacological and neuroanatomical approaches should confirm the possible involvement of inhibitory components in gaze-stabilizing multimodal interactions.

Afferent feedback enables robust sit-to-stand transitions in response to V3 stimulation: A neuromechanical modeling study

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Themes: Sensorimotor Integration; Biomechanics

Optogenetic stimulation of lumbar V3 neurons in mice broadly activates hindlimb muscles, eliciting flexor and extensor co-activation, with a bias towards extensor activation. The mouse extends its hindlimbs and transition smoothly from sitting to standing. Yet, how simple activation of a class of interneurons can induce a controlled transition to stable standing is unknown. Here, we present a neuromechanical model of a mouse hindlimb including hip, knee, ankle, and metatarsal-phalange joints actuated by Hill-type muscles representing major flexors, extensors, and bifunctional muscle groups. Computed proprioceptive feedback signals were connected to a simplified neural network model, implementing fundamental spinal reflex circuits. V3 interneurons were modeled to provide excitatory input to contralateral motoneurons. Our simulations demonstrated bistable limb dynamics induced by direct V3-driven extensor motoneuron activation, depending on the gradual increase and decrease of V3 neuron activity. As the limb extends, moment arms of extensor muscles improve. So, once sufficient muscle force is produced to overcome gravity a positive feedback loop is triggered: the resulting extension enhances muscle leverage, further increasing torque production and thus driving a rapid transition from flexion to full extension. Incorporating sensory feedback pathways, including Ia reciprocal inhibition, group II excitation, and Ib disynaptic excitation, alongside flexor-extensor coactivation, reduced the bistable range and enabled a smooth, stable control of limb extension in response to V3 stimulation. These findings suggest that low-level reflex circuits contribute to stabilizing posture and controlling limb responses triggered by broad neural activation, offering insight into mechanisms by which V3 stimulation could induce standing.

Characterizing the neuronal circuit of the fast nociceptive hindlimb withdrawal reflex in mice

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Themes: Sensorimotor Integration; Biomechanics

Potential harmful stimuli are detected at the skin where cutaneous nociceptive sensory neurons activate spinal reflex arcs to drive a withdrawal of an affected limb away from the potential harmful source. While the spinal cord plays a key role in this behavior, the intraspinal mechanisms that underlie the sensorimotor integration and the resultant motor programs that are produced to coordinate muscle firing patterns have yet to be understood. Using optogenetics and multi-electrode array electrophysiology in awake behaving mice, we investigate the neural mechanisms that underlie the patterns of neural activity in the lumbar spinal cord during a stimulus-driven limb withdrawal reflex. Here, we first established methods for high-density *in vivo* electrophysiology recordings from single units in the spinal cords of vertebrally-fixed, awake, and behaving mice. Mice in this preparation can produce clear, repeatable behaviors including self-driven locomotion, grooming, optogenetically-cued limb withdrawal reflexes, and clear responses to different naturalistic stimulations of the hindlimb receptive field. Next, we characterized single unit firing parameters across multiple animals as a function of laminar distribution across the full dorsal-ventral axis. We found that neurons in the medial deep dorsal horn and ventral horn had short latency responses and high firing rates following nociceptive stimulation to the paw. Therefore, we hypothesize that (1) activity from a limb withdrawal response in the ventral horn will be strongly correlated with activity in the deep dorsal horn and (2) that perturbing specific interneuron populations will allow us to investigate the function of those neurons involved in limb withdrawal reflexes.

Malocclusion, mitochondrial disease and 3D reconstruction of the muscle spindle column in mouse deep masseter muscle

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Themes: Sensorimotor Integration; Motoneurons

Muscle spindles are mechanosensors crucial for proprioception and skeletal alignment. Previous studies show a column of >20 muscle spindles within rodent deep masseter muscle, including rats and mice, suggesting a specialised role in sensory feedback during jaw movement. Mitochondrial defects can cause malocclusion, a congenital misalignment of the jaws. Interestingly, we found that muscle spindle sensory terminals are densely packed with mitochondria, occupying >50% of terminal volume. To investigate how the spindle column arises and is affected by mitochondrial defects, we are determining its precise location and structure throughout development, then in a mouse model of malocclusion. This initial study aims to establish a technique to create a 3D model of the spindle column in the adult mouse muscle. The right deep masseter muscle was dissected, fixed in 4% paraformaldehyde overnight, before dehydration and wax embedding. The sample was serially sectioned transversely at 5 μm thickness, then stained with haematoxylin and eosin. Sections were scanned, and images were manually aligned using *GIMP* image processing software. *Reconstruct* software was used to trace manually the muscle and muscle spindle outlines within each section, then compiled to provide the 3D visualisation of their spatial distribution. Preliminary findings confirmed the spindle column's presence near the masseter's anteromedial margin and provided the first comprehensive histological map. Future work will determine the column's location throughout development, and whether malfunctioning mitochondria disrupt its alignment and contribute to malocclusion.

Maturation of abducens motoneurons involved in the angular vestibulo-ocular reflex during larval development

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Themes: Sensorimotor Integration; Motoneurons

The angular vestibulo-ocular reflex (VOR) elicited by the activation of semicircular canals during horizontal head rotations, is supported by a 3 neurons arc circuit where Abducens motoneurons (Abd MN) constitute one of the motor outputs. In larval frog, the angular VOR performance progressively improves before the metamorphosis, suggesting a maturation of the underlying circuitry. We investigated this maturation in Abd MN not relying on a neurogenic activity during this period. Two functional populations of Abd MN have been previously identified based on their discharge dynamics at rest: silent Abd motor units, with low resting activity, and spontaneous unit presenting a sustainable resting firing rate. Spontaneous motor units presented a slow-tonic discharge dynamic during head motion, encoding preferentially the head position, whereas silent motor unit were motion-sensitive only, with a fast-phasic discharge dynamic encoding mainly the velocity. Interestingly, only fast-phasic units showed an increased discharge modulation during the larval development, demonstrating a better response stimulus. In addition, electronic microscopy revealed an improvement of axonal morphology, indicating a better conductivity concomitantly to the increased response of fast-phasic units. Altogether, our results demonstrated that the maturation of the angular VOR was supported by the maturation of fast-phasic Abd MN, responsible for high dynamic eye movements elicited by canal activation. Inversely, slow-tonic Abd MN, also involved in low dynamic gaze-stabilizing responses, did not show any maturation at the same time. However, a maturation in pre-motor relays can't excluded.

Renshaw cell ablation increases monosynaptic H-reflex amplitude in decerebrate mice

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Themes: Sensorimotor Integration; Premotor & Propriospinal Circuits

To identify the functional roles of Renshaw cells (RCs) in motor control, we developed a selective ablation model using diphtheria toxin (DTX) in mature mice based on our previously published genetic targeting approach (Lane *et al.*, 2021; PMID: 34615947). We assessed the effects of RC ablation on the electrical equivalent of the monosynaptic stretch reflex ("H-reflex") to evaluate changes in motoneuron excitability. Immunohistochemical analysis confirmed specific but partial and variable RC ablation throughout the lumbar spinal segments analyzed. Establishing a reliable H-reflex model in mice presented significant technical challenges, including: 1) near-synchronous recruitment of Ia afferents and motor axons during electrical stimulation of peripheral nerves, 2) temporal overlap between H-reflex and M-response waveforms in ankle muscle electromyography (EMG) recordings, and 3) suppression of the reflex by anesthetics like isoflurane. We overcame these limitations by using a decerebrate preparation that eliminated the need for anesthesia and by establishing criteria and time thresholds to differentiate between the H- and M-waves despite partial waveform overlap. Taking advantage of variable RC loss across animals, we observed a strong correlation between the degree of RC loss and H-reflex amplitudes. These results demonstrate that RC elimination significantly increases H-reflex amplitudes in both lateral gastrocnemius and tibialis anterior motor pools, suggesting that RCs exert tonic inhibitory control over monosynaptic reflex circuits in the absence of descending corticospinal input. This finding validates our model for specifically interfering with RC function to study their roles in controlling movement in unrestrained freely-behaving animals in our future experiments.

A spinal sensorimotor circuit for skilled locomotion

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Themes: Sensorimotor Integration; Premotor & Propriospinal Circuits

Production of complex movements requires coordinated muscle activity throughout the body. Propriospinal circuits orchestrate limbs and body movement by linking local circuits across all levels of the spinal cord. Long-projecting propriospinal neurons reciprocally connecting the cervical and lumbar segments are particularly important in quadrupedal locomotion, as they organize the patterns of forelimb and hindlimb activity. Recently, we identified a new class of ascending propriospinal neurons belonging to V0g family (V0g-aNs), located in the lumbar spinal cord and projecting to cervical segments. Surprisingly, perturbation of V0g-aNs function selectively impairs skilled locomotion but does not affect stereotyped movements. The same phenotype is observed after elimination of cerebrospinal fluid-contacting neurons (CSF-cNs), intraspinal sensory neurons that have been show to detect spinal cord bending. Thus, these data suggests that CSF-cNs and V0g-aNs are key components of a sensorimotor circuit required for skilled locomotion. To test this hypothesis, we started by examining the anatomical organization of the CSF-cNs/V0g-aNs circuit. To explore the source of inputs to V0g-aNs, we used rabies monosynaptic tracing approach that revealed input mainly from neurons in dorsal spinal cord and lamina X, including CSF-cNs. Next, we analyzed V0g-aNs output by selective labeling of presynaptic terminals. We found projections to motoneurons and V0c interneurons at both cervical and lumbar levels, as well as supraspinal projections around the central canal in the brainstem. Together, these results highlight a previously unknown circuit involved in adaptive locomotor coordination, linking sensory signals from CSF-cNs with propriospinal pathways mediated by V0g-aNs.

The Honeycomb Synapse: Characterisation of a novel synapse in the mouse lumbar spinal cord

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Themes: Sensorimotor Integration; Premotor & Propriospinal Circuits

Synaptic diversity gives rise to functionally distinct neural circuits. This diversity arises not only from the molecular composition of synapses but also from their nanostructural organisation. Understanding synaptic diversity is crucial as selective synaptic vulnerability is a hallmark of Amyotrophic Lateral Sclerosis and may therefore offer insight into potential therapeutic targets. Recent unpublished findings from the Neural Control of Movement Lab have identified a novel synaptic subtype in the mammalian lumbar spinal cord, termed the Honeycomb Synapse, defined by its distinctively large, perforated postsynaptic density (PSD). The circuit identity and anatomical distribution of this synapse remain unknown. To address this, we employed immunohistochemical labelling, 3-D high-resolution microscopy, and supervised machine learning-based image analysis of Honeycomb Synapses in the ventral horn of the mouse lumbar spinal cord. We report that Honeycomb Synapses are predominantly associated with VGLUT1/Parvalbumin+ presynaptic afferents, located on fast-fatigable α-motoneurons within the lateral motor column of both the upper and lower lumbar spinal cord. We conclude that Honeycomb Synapses are a distinct synapse subtype of the Monosynaptic Stretch Reflex (MSR) pathway and thus may modulate proprioceptive input to motoneurons innervating distal limb muscles. Notably, we identified a subpopulation of Honeycomb Synapses in the medial motor column that are not associated with the MSR pathway and have a smaller PSD. In summary, we have determined the circuit and anatomical distribution of this novel synapse, revealing never-before-seen diversity in the MSR pathway. Our findings provide a framework for future investigations into Honeycomb Synapses in health and disease.

Spinal dI3 circuits modulate skilled and corrective locomotor behaviours

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Themes: Sensorimotor Integration; Premotor & Propriospinal Circuits

Many spinal neurons integrate sensory input to adapt a wide array of movements. Prior research has shown that spinal dorsal interneuron 3 (dI3) neurons receive cutaneous and proprioceptive sensory input and project to motor circuits, most notably through excitation of motoneurons. We sought to better delineate dI3 spinal circuits by testing motor performance of transgenic mice where dI3s could be chemogenetically silenced. Inhibition of dI3s was concomitant with drug treatments that putatively inhibited recurrent motoneuron excitation of central targets such as Renshaw cells (RCs). For performance related to balance and coordination, mice demonstrated a two- to three-fold increase in average foot falls after dI3 inhibition and/or reduced RC excitation during ladder and beam tasks. When RC and dI3 inhibition were combined, we did not observe a compounded effect to motor deficits. The same mice were tested on a treadmill apparatus wherein the hindlimb was perturbed to trigger the stumbling corrective reaction (SCR). dI3 silencing resulted in a significant reduction in SCR step height; once again, reduced RC excitation combined with dI3 inhibition did not induce a compounded reduction to SCR step height. Overall, our findings indicate that dI3s are involved in the activation of the SCR, wherein proprioceptive and cutaneous signals may be used to modulate activation of muscles during locomotion. Our results suggest that a lack of dI3 signaling appears to blunt skilled and corrective behaviours, and further suggests that RCs and dI3s may operate together within a spinal comparator module to monitor sensory signals and adjust motor outputs.

dl3 neurons combine sensory feedback and internal motor copies for corrections of ongoing movements

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Themes: Sensorimotor Integration; Premotor & Propriospinal Circuits

The completion of movements requires continuous adjustments in response to changes in the internal and external environments of organisms. Adaptive circuits function by comparing the actual (instructive) sensory input with that predicted by the intended movement. Numerous spinal cord neuronal populations integrate sensory inputs from multiple modalities. Here, we sought to understand how spinal circuits integrate sensory feedback with motor circuit dynamics to adapt to sensory perturbations. One cardinal class of spinal neurons, dl3 neurons, is known to receive direct sensory feedback as well as inputs from locomotor circuits, and therefore has the potential to combine these two types of information. Here, we use mouse models to demonstrate that a population of dl3 neurons receives spatially segregated multimodal sensory afferents and direct efferent copies of motor commands from both Renshaw cells and motor neurons. We further show that dl3 neurons are wired to mediate flexion, extension and/or co-contraction of antagonist muscles. In mice, reducing the activity of dl3 neurons demonstrates their role in reflexive corrections, while disrupting Renshaw cell activity provides convergent evidence for the role of efferent copies in online corrections. Therefore, by characterizing a comparator module embedded in spinal circuits mediating corrections of ongoing movements, our findings uncover a pivotal function of dl3 neurons in combining internal predictions with external sensory feedback.

Kinematic analysis of motor responses evoked by circumferential electrical stimulation of the lumbar cord

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Themes: Spinal cord injury; Neuromodulation and plasticity

Walking is an essential skill in everyday life but becomes impaired in many movement disorders, such as spinal cord injury (SCI). Neuromodulation approaches, such as electrical stimulation of the spinal cord, show promise in restoring leg movements after SCI. Here, we used a circumferential electrode that wraps around the spinal cord to investigate the effects of electrical epidural stimulation (EES) of the dorsal, lateral, and ventral lumbar cord on the generation of leg movements in anesthetized adult cats. We quantified leg movements evoked by EES at L4 and L5 by measuring kinematics using DeepLabCut™ (Mathis et al., 2018). Our preliminary results show that stimulating dorsally and laterally at L4 did not elicit movements. However, stimulating ventrally at L4 generated small movements, with a decrease (i.e. flexion) in hip, knee, and ankle angles of 2.7˚, 3.9˚, and 4.4˚, respectively. At L5, lateral and ventral stimulation evoked small twitches but no noticeable joint movements. However, dorsal stimulation at L5 produced an increase (i.e. extension) in hip, knee, and ankle angles of 10.5˚, 7.8˚, and 12.1˚, respectively. Our preliminary results suggest that EES of the lumbar cord is highly dependent on the segment and location stimulated. Ultimately, our goal is to find optimal stimulation parameters to reconstruct a functional walking pattern that will be tested in chronic spinal-transected cats before a trial in humans.

The effect of transcutaneous spinal cord stimulation on proprioception

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Themes: Spinal cord injury; Neuromodulation and plasticity

Transcutaneous spinal cord stimulation (tSCS) is a non-invasive neuromodulation technique that shows promise in improving upper-limb function after spinal cord injury (SCI), yet its effect on proprioception remains unclear. We hypothesize that tSCS-related motor gains are linked to improvements in proprioception. Five people with incomplete cervical SCI (AIS C or D) and ten neurologically intact (NI) participants performed two KINARM tasks without visual input: (1) Position Matching— the robot moved the non-dominant arm to nine different positions and the dominant arm matched each position; (2) Movement Matching— the robot moved the non-dominant arm between three targets while the dominant arm simultaneously matched the speed and trajectory of the movement. Each task was performed without tSCS and with tSCS (40 Hz, 1 ms-long pulses with 10 kHz carrier frequency). Stimulation was delivered through electrodes placed over C3–C4 and C6–C7 and return electrodes on the iliac-crests. Stimulation amplitude was set at the level that evoked a spinal potential in the biceps brachii muscle for each participant. Stimulation increased lateral errors in Position Matching ($p=0.02$) in NI participants and vertical errors in participants with SCI ($p=0.02$). However, stimulation improved trajectory smoothness in Movement Matching in participants with SCI ($p=0.02$) and decreased the delay in movement stoppage ($p<0.01$). These preliminary results suggest that tSCS alters proprioception, enhancing the timing and smoothness of dynamic arm movements after SCI. By refining sensory feedback in persons with motor control deficits after SCI, tSCS may fill a critical gap in current rehabilitation strategies.

Reticulospinal plasticity after spinal cord injury and regenerative magnetic stimulation

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Themes: Spinal cord injury; Neuromodulation and plasticity

Neuronal populations in the brainstem are crucial for controlling posture and locomotion. Despite known plasticity in descending reticulospinal tracts after spinal cord injury (SCI), the exact origin, nature, functional implications and modulation of such plasticity by therapeutic approaches remain unclear. We present preliminary data from an ongoing project exploring the comparative plasticity of specific subtypes of reticulospinal neurons anatomically and their contribution to functional locomotor recovery during spontaneous recovery and after repetitive trans-spinal magnetic stimulation. We employ cell-type-specific retrograde and anterograde circuit tracing tools to label discrete, molecularly defined subtypes of reticulospinal neurons in a mouse SCI model of thoracic hemisection. Then, repetitive trans-spinal magnetic stimulation is applied daily for 14 days. Subsequently, various aspects of locomotor recovery are assessed at key time points to examine spontaneous recovery and the potential benefit of the stimulation. Finally, immunohistochemistry is utilized to characterize the anatomical plasticity of the labeled tracts. Our initial findings confirm previous reports that, following a left thoracic hemisection that interrupted all ipsilateral descending connectivity from the brainstem, a strong spontaneous locomotor recovery is seen functionally. Remarkably, some aspects of this recovery, particularly during adaptive stepping tasks, are further enhanced after stimulation therapy. This suggests changes in spinal and/or descending circuitry and their potentiation by the stimulation. To test this, we are currently examining the plasticity of labeled reticulospinal tracts comparatively between controls, lesioned and stimulated groups. This study will provide important insights into the anatomical plasticity of reticulospinal tracts and the benefits of spinal cord stimulation therapy.

dl3 neurons increase excitatory drive to locomotor circuits after spinal cord injury via 5-HT2C-R-dependent mechanism

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Themes: Spinal cord injury; Premotor & Propriospinal Circuits

The dl3 neurons are a class of excitatory premotor spinal interneurons located in the intermediate and deep dorsal horn laminae. Dl3 neurons integrate diverse cutaneous and proprioceptive inputs and project to locomotor circuits. Although dl3 loss-of-function studies have demonstrated relatively minor effects on locomotion in intact mice, dl3 neurons appear to be critical for locomotor activity following spinal cord injury. We hypothesized that the disproportionate involvement of dl3 neurons in locomotor function after spinal injury is due to intrinsic changes in dl3 neuron function following spinal cord injury. To test this, we transiently silenced or activated dl3 neurons using hM4Di or hM3Dq DREADD receptors, respectively, following complete T9–T10 spinal transection (n=7 mice/group). Silencing dl3 neurons led to significant impairments in hindlimb stepping and an increase hindlimb joint extension consistent with a reduction in motor tone. In contrast, activation of dl3 neurons alone did not significantly enhance locomotor function or hindlimb tone. The absence of a clear stimulatory effect suggests that dl3 neurons may already be in a highly excitable state post-injury. Given that spinal cord injury is known to cause constitutive activation of the 5-HT2C receptor, we performed immunohistochemistry at the experimental endpoint and found that despite relatively sparse expression in the lumbar intermediate grey matter, nearly all dl3 neurons in the lumbar spinal cord expressed 5-HT2C-R (92%, n=4 mice, 219 dl3s). This expression of putative constitutively active 5-HT2C-R in dl3 neurons may explain their greater role in locomotion after complete spinal cord injury.

Interrogating cervical spinal circuits using large-scale neural recording for targeted neuromodulation after injury

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Themes: Spinal cord injury; Premotor & Propriospinal Circuits

Loss of hand function following cervical SCI remains a critical challenge with limited therapeutic options. My previous work revealed that V2a interneurons in C3-C4 segments form an important propriospinal network that maintains extensive connectivity to both descending pathways and downstream motor circuits after injury. Histological assessment of this class of interneurons following midline C5 contusion injury in mice revealed two key findings: preservation of C3-C4 V2a interneurons and their projections to the spinal interneurons and motor neurons to the cervical enlargement and while circuits below the injury showed significant neuronal loss (~45% reduction in V2a interneurons) and enhanced synaptic elimination (6-fold increase in complement-mediated pruning). Leveraging these spared circuits, I developed an innovative neuromodulation approach combining ultra-sensitive opsins with a wireless interface. Selective activation of C3-C4 V2a neurons produced real-time improvements in reach-to-grasp performance, increasing success rates by ~40%.To understand the neural basis of these improvements, I have now established and validated methods for large-scale recordings from the cervical spinal cord in awake, behaving animals to capture millisecond-scale activity across diverse neuronal populations. These recordings will help reveal how injury disrupts specific network function and guide the development of novel therapeutic interventions. This research integrates cutting-edge technologies from the Rogers lab (wireless interfaces), Deisseroth lab (optogenetics), and IMEC/UCL/Allen Institute (Neuropixels probes), made possible through the mentorship of Dr. Yoshida and Dr. Miri. These advances lay the groundwork for my independent research aimed at developing circuit-based strategies to enhance motor recovery after injury.

Distinct parametric interactions underlie emergence and robustness in the preBötC Type-1 neuronal physiology

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Themes: Theory and computation

Mammalian inspiratory rhythm arises as putatively rhythmogenic Type-1 neurons in the preBötzinger Complex (preBötC) synchronize to generate inspiratory bursts in every cycle. A comprehensive framework for breathing rhythmogenesis requires understanding Type-1 neuronal physiology and its robustness to various perturbations. Here, we constructed conductance-based biophysical models of Type-1 neurons through a stochastic search spanning 24 model parameters related to voltage-gate ion channels, geometry, and passive electrical properties. We screened them for eleven physiological measurements to ensure they were within their experimental bounds, selecting 135/200000 models as valid. We observed degenerate (weak pairwise correlations) among parameters (Figure 1A), representing the emergence of the Type-1 physiology, encompassing their heterogeneity. Next, we performed perturbation analyses and calculated the Jacobian and the Fisher Information Metric (FIM), revealing the most sensitive (first eigenvector of the FIM) and the most robust direction (last eigenvector of the FIM) to parametric perturbations. We discovered that the pairwise correlation of FIM weights along the sensitive direction (Figure 1B) is structured, where parameters with high/intermediate/low weights correlate negatively/weakly/positively, respectively. This implies that the model's sensitivity to perturbations in the most sensitive parameters (ActV1/2$_{NaT}$, ActV1/2$_{NaP}$, InactV1/2$_{KA}$), is mutually exclusive, shaping the allowed directions for change in this parametric space, which is not completely random. The FIM weight correlation structure shapes the directions for change in the parametric space where changes in physiological measurements are minimal (Figure 1C); hence, directions for homeostatic plasticity. Thus, we present a generalizable framework to evaluate parametric interactions underlying emergence and resilience in neuronal physiology.

Reorganization of spinal neural connectivity following recovery after thoracic spinal cord injury: insights from computational modelling

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Themes: Theory and computation; Spinal cord injury

Rats exhibit significant recovery of locomotor function following incomplete spinal cord injuries, however with altered gait expression, reduced speed, and lower stepping frequency. These changes likely result from and give insight into the reorganization within spared and injured spinal circuits. We previously developed computational models of mouse spinal locomotor circuitry controlling speed-dependent gait expression. Here, we adapted these models to the rat to explore potential circuit-level changes underlying locomotor recovery after two distinct thoracic spinal cord injuries that have roughly comparable locomotor recovery. The model reproduced experimentally observed speed-dependent gait expression before injury and after recovery from contusion and hemisection, suggesting distinct, injury-specific mechanisms of recovery: recovery after asymmetrical (lateral) hemisection required substantial functional restoration of descending drives and long propriospinal connectivity, suggesting compensatory plasticity through formation of detour pathways; conversely, recovery after a moderate mid-line contusion predominantly relied on reorganization of spared sublesional networks and altered control of supralesional cervical circuits, compensating for weakened propriospinal and descending pathways. These modeling results suggest that symmetrical and asymmetrical injuries induce distinct forms of plasticity in different regions of the spinal cord, and corresponding clinical injuries may benefit from tailored therapeutic strategies targeting specific circuits.

Advanced computational tools to define spatial, temporal, and differentiation programs driving neuronal diversity in the central nervous system

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Themes: Theory and computation; Neuronal Diversity

Cell type diversity in the neural tube (NT) emerges from interacting spatial, temporal, and differentiation programs. Although single-cell sequencing data continue to provide deeper insights into developmental processes, scRNA-seq datasets remain difficult to analyse due to their size, noise, and high dimensionality. Standard workflows like Seurat and Scanpy use highly variable gene (HVG) selection for dimensionality reduction, but their weak mechanistic grounding and inconsistent results highlight the need for more principled approaches.I recently introduced Entropy Sorting (EntSort), a mathematical framework that addresses these challenges. As a feature selection algorithm (cESFW), EntSort consistently outperforms HVG methods across diverse datasets. By applying cESFW to mouse NT scRNA-seq data (Delile et al., 2019), I generated a high-resolution embedding that better resolves NT differentiation trajectories.When identifying distinct cell populations or gene expression patterns, determining the optimal clustering resolution remains a core issue. For instance, clustering that captures spatial domains often fails to reveal temporal or differentiation programs. Exhaustive cluster combination analysis is computationally infeasible (~1.45×10^{28} CPU years for 100 clusters). Through EntSort, I have derived a new algorithm that overcomes this bottleneck, enabling detection of gene expression signatures across spatial, temporal, and differentiation axes without prior knowledge.Notably, my method recovers known markers (e.g., Onecut2, Neurod2) and identifies novel temporally regulated genes across progenitors and dorsal/ventral domains. Furthermore, we have experimentally validated that marker genes identified by my EntSort approach are more robust than those identified by typical Seurat or Scanpy workflows.