

Poster Abstracts

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Re-routing Cortical Drive through Residual Spinal Tissue Mediates Motor Function Recovery after Severe Spinal Cord Injuries

Leonie Asboth, Quentin Barraud, Lucia Friedli, Janine Beauparlant, Cristina Martinez-Gonzalez, Selin Anil, Galyna Pidpruzhnykova, Elodie Rey, Laetitia Baud, Mark Anderson, Joachim von Zitzewitz, and Grégoire Courtine
International Paraplegic Foundation Chair in Spinal Cord Repair,
Center for Neuroprosthetics and Brain Mind Institute, School of Life Sciences,
Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

A severe contusion of thoracic segments disrupts the motor-circuit communication matrix linking the brain and the spinal cord. Electrochemical stimulation applied over lumbar segments restored this communication, which enabled volitional control of leg movements in rodents and humans with motor complete paralysis. However, the circuit-level mechanisms through which the cortical drive regains functional access to the spinal circuits controlling leg movements during electrochemical stimulation remain poorly understood. Using mice expressing light-sensitive channels in cortical projection neurons, we first showed that electrochemical stimulation enabled the hindlimb motor cortex to regain a graded control over hindlimb locomotor movements in otherwise paralyzed animals. Using virus-mediated tract tracing and circuit-specific inactivation techniques, we found that after injury the cortical drive is rerouted through glutamatergic reticular neurons with residual projections below the injury. Robot-assisted gait training enabled by electrochemical stimulation promoted an extensive reorganization of these pathways. We found a robust growth of motor cortex projections into the reticular formation, and a substantial sprouting of residual reticulospinal axons into specific regions of the spinal cord below the injury. We established causal relationships between this anatomical reorganization and the recovery of voluntary leg motor control in response to gait rehabilitation. These results illustrate the remarkable capability of neural pathways to reorganize in order to mediate motor recovery, even after the most severe types of spinal cord injury.

Circuit Organization in the Ventral Respiratory Column: Insights from Site-specific Optogenetic Stimulations and Computational Modeling

Jessica Ausborn¹, Hidehiko Koizumi², William H. Barnett³, Tibin John², Yaroslav I. Molkov³, Jeffrey C. Smith², and Ilya A. Rybak¹

¹Department of Neurobiology and Anatomy, Drexel University, Philadelphia, PA 19129

²Cellular and Systems Neurobiology Section, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, MD 20892

³Department of Mathematics and Statistics, Georgia State University, Atlanta, GA 30303

The organization of neuronal interactions within the mammalian ventral respiratory column (VRC), specifically within and between the pre-Bötzinger (pre-BötC) and Bötzinger (BötC) complexes - is not fully understood and is under continuous debate. To shed light on VRC circuit organization, we used selective optogenetic stimulation of inhibitory neurons in the pre-BötC or the BötC to evoke perturbations of respiratory neural activity. The results were then incorporated into a computational model to explore the underlying network mechanisms and generate predictions that may guide further experiments. We employed a transgenic mouse line expressing Cre-recombinase under the promoter for the vesicular GABA transporter (VGAT) to express Channelrhodopsin-2 (ChR2) in both GABAergic and glycinergic inhibitory neurons. In the adult transgenic mouse in situ perfused brainstem-spinal cord preparation, we used laser (473 nm) stimulations of inhibitory neurons in pre-BötC or BötC regions, while recording activity of the phrenic and vagus nerves. We used single pulses of light (0.1 - 0.5 s, delivered at different phases of the cycle) and continuous light stimulations (20 Hz pulse train for 60 s) applied to either the pre-BötC or BötC. The effects of stimulations were application site-specific and depended on stimulus intensity and duration. Specifically: (1) Low intensity (< 2 mW) single pulse stimuli to the pre-BötC during the inspiratory phase did not terminate inspiration, whereas stronger stimulations (≥ 2 mW) terminated inspiratory activity for the stimulus duration. When the stimulation pulse ended in or was applied during expiration, rebound initiation of inspiration occurred; (2) Continuous stimulation (0.5 - 3 mW) of the pre-BötC increased respiratory frequency, whereas a further increase of stimulus intensity (> 3 mW) reduced the frequency and eventually terminated respiratory oscillations (apnea); (3) Single pulses (0.1 - 3 sec) or continuous 20 Hz optical stimulations of low or high intensity applied to the BötC inhibited output activity producing apnea for the duration of the stimulation. We have revised our previous computational model of pre-BötC and BötC microcircuits by incorporating an additional population of post-inspiratory inhibitory neurons in the pre-BötC. This population inhibits all other populations in the network and receives reciprocal inhibition from early-inspiratory neurons of the pre-BötC and from augmenting-expiratory neurons of the BötC. In our model, we assumed that the early-inspiratory neurons are less sensitive to light stimulation than the other inhibitory neurons. The new model was able to reproduce the above experimental findings based on the proposed network architecture and the different sensitivity of the populations to light stimulations. The proposed organization of inhibitory interactions between and within the pre-BötC and BötC leads to a number of testable predictions about their specific role in respiratory pattern generation.

Computational Modeling and Analysis of Half-center CPGs

Jessica Ausborn¹, Abigail C. Snyder², Bartholomew J. Bacak¹,
Natalia A. Shevtsova¹, Jonathan E. Rubin², and Ilya A. Rybak¹

¹Department of Neurobiology and Anatomy,
Drexel University College of Medicine, Philadelphia, PA 19129

²Department of Mathematics, University of Pittsburgh, Pittsburgh, PA 15260

The spinal locomotor central pattern generator (CPG) can autonomously generate rhythmic activity with alternating flexion and extension phases over a wide range of frequencies. The organization of this CPG and the neural mechanisms involved in rhythmogenesis remain largely unknown. It is commonly accepted that the rhythmic pattern results from, or at least involves, inhibitory interactions between two neural populations representing flexor and extensor half-centers. The classical half-center concept assumes a symmetrical organization in regard to cellular properties, operational regimes and synaptic interactions between the half-centers (Graham Brown, 1914; McCrea & Rybak, 2008). Another approach suggests an asymmetric, flexor-dominated CPG organization in which only the flexor half-center has intrinsic rhythmic capabilities (Pearson & Duysens, 1976; Zhong et al. 2012; Shevtsova et al. 2015). There is also a possibility that both half-centers can autonomously generate rhythmic activity (Hägglund et al. 2013). In this theoretical study, we have suggested that each of the three mechanisms can operate in the same CPG depending on conditions, such as preparation type or methods used to produce the rhythm. Two distinct models were considered: (i) a large-scale model with flexor and extensor populations (each consisting of 200 neurons modeled in the Hodgkin-Huxley style with sparse excitatory interactions) mutually inhibiting each other via two inhibitory populations and (ii) a reduced model, in which both half-centers were represented by single non-spiking neurons coupled with mutual inhibition. The second model was used for qualitative analysis of the system dynamics. In both cases, neuronal oscillations were based on the slowly inactivating persistent sodium current. Due to this intrinsic rhythmogenic mechanism, an increase of excitatory drive to each isolated half-center caused a transition from silence to rhythmic bursting and then to tonic activity. Therefore, by manipulating drives to each half-center we could induce systematic transitions between all three half-center mechanisms and analyze the bifurcations involved in transitions between them, the emergence of bistability, and the control of oscillation frequency in each regime. Stable rhythmic activity occurred in both symmetric and asymmetric network structures. However, frequency modulation with changing drive to one or both half-centers was dependent on the underlying rhythm-generating mechanisms and flexor- extensor symmetry. Our study suggests different regimes in locomotor CPG operation and proposes explanations for some seemingly contradictory experimental data.

Kolliker Fuse Orchestrates Timing of Abdominal Nerve Bursting

William Barnett¹, Ana Paula Abdala², Julian F.R. Paton², Daniel Zoccal³, and Yaroslav Molkov¹

¹Department of Mathematics and Statistics, Georgia State University, Atlanta, GA 30303

²School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom BS8 1TD

³Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, Brazil 14801-903

A hallmark of the respiratory reflex response to hypercapnia is the emergence of active expiratory pattern in the abdominal motor output (AbN). This pattern consists of late-expiratory (late-E) bursts attributed to the recruitment of expiratory neurons in the parafacial respiratory group (pFRG) putatively driven by chemosensitive cells in the retrotrapezoid nucleus (RTN). Evidence suggests participation of other cellular groups in control of AbN late-E activity generation. Late-E activity is abolished by systemic application of riluzole, indicating potential involvement of the persistent sodium current. However, there is no direct experimental evidence for the presence of persistent sodium channels in pFRG late-E neurons. In this scenario, we suggest that riluzole dependence of late-E activity is mediated by excitation from pre-inspiratory/inspiratory (pre-I/I) neurons of pre-Bötzinger complex (pre-BötC), which are known to contain persistent sodium channels. It has been previously suggested that the pFRG late-E population is suppressed by inhibition from post-inspiratory (post-I) neurons of the Böttinger complex under eupneic conditions. Therefore, areas that control post-I activity, such as the Kölliker-Fuse (KF) nucleus, may be involved in the control of hypercapnia-induced late-E activity. Here, we explore the role of the KF nucleus and the pre-BötC pre-I/I neurons in modulating late-E respiratory activity. Based on model simulations, we propose that pre-I/I neurons in the pre-BötC are stimulated by RTN chemosensitive neurons during hypercapnia and which then drive late-E activity in pFRG neurons. Assuming that the KF area provides excitatory drive to BötC post-I inhibitory neurons, our model predicted that during hypercapnia: 1) KF inhibition lower the recruitment threshold of late-E activity; 2) KF excitation prevents emergence of late-E activity. These modeling predictions were tested experimentally in the in situ arterially perfused rat. We found that: i) inhibition of KF with isoguvacine (10mM) advanced by 1s the onset of late-E bursts in AbN after exposure to hypercapnia (8% CO₂); and ii) disinhibition of KF with gabazine (100μM) greatly attenuated AbN late-E activity. The model suggests that the pre-BötC pre-I/I neurons are an important source of excitatory synaptic inputs to the pFRG expiratory neurons. Moreover, KF-driven inhibitory inputs to the pFRG, possibly through the post-I BötC neurons, may determine the presence and/or onset timing of AbN late-E bursts during hypercapnia. Supported by: NIH/NCCAM R01 AT008632

Persistent Neural Activity in Excitatory Spinal Circuitries Drives Muscle Spasms after Spinal Cord Injury

Carmelo Bellardita, Vittorio Caggiano, Roberto Leiras, Vanessa Caldeira, Andrea Fuchs, Julien Bouvier, Peter Low, and Ole Kiehn
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden 171 77

Spasms after spinal cord injury (SCI) are debilitating involuntary muscle contractions associated with increased motor neuron plateau properties and decreased inhibition. However, spinal excitatory interneurons involvement and their functional role in muscle spasm after SCI are largely unknown. Here we use mouse genetics, electrophysiology, imaging and optogenetics to directly target all major classes of spinal interneurons as well as motor neurons during spasms in a new animal model of chronic SCI. We found that assemblies of excitatory spinal interneurons are recruited by sensory input into functional circuits to generate persistent neural activity which drives both motor neurons to generate spasms and inhibitory interneurons to curtail them. Our study reveals hitherto unrecognized spinal mechanisms for spasms generation and maintenance, shifting the wide-held view of muscle spasms as passive result of inhibition-induced excitation in motor neuron and expression of plateau potentials. The study opens new perspectives to understand and to treat muscle spasms after SCI.

Spinal Cholinergic Interneurons Differentially Control the Excitability of Motoneurons and Alter the Operational Range of the Locomotor Network

Maria Bertuzzi and Konstantinos Ampatzis

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden 171 77

While cholinergic modulation is important for the locomotor circuit operation, the specific neuronal mechanisms that acetylcholine employs to regulate and fine-tune the speed of locomotion are largely unknown. Here, we show that cholinergic interneurons (ChAT-INs) are present in the zebrafish spinal cord, and differentially control the excitability of distinct classes of motoneurons (MNs) in a muscarinic dependent manner. Moreover, activation of muscarinic acetylcholine receptors (mAChRs) serves as a plasticity mechanism to alter the operational range of MN modules. These unexpected findings provide novel insights into the functional flexibility of MNs and their involvement in the execution of locomotion at different speeds.

Synapses between Encoders and Decoders: Understanding a Coordinating Network

Felix Blumenthal and Carmen Smarandache-Wellmann
Institute of Zoology, Animal Physiology; Emmy-Noether Group;
University of Cologne, Cologne, Germany 50674

The swimmeret system is an excellent model to understand neural mechanisms of coordination of distributed central pattern generators (CPG). During swimming the four paired swimmerets of the crayfish's abdomen are coordinated in an anteriorly proceeding metachronal wave. Each swimmeret is innervated by neurons from its own module, containing a CPG, these have to be coordinated among each other. The intersegmental coordination of four ipsilateral, anatomically separated CPGs is achieved by three neurons located in each hemisegment forming a coordinating circuit. One ascending (ASCE) and one descending (DSC) coordinating neuron encode the information about the status of their home module and project it to the other neighbors. A nonspiking neuron, Commissural Interneuron 1 (ComInt1), decodes this information transmitted by three neighboring coordinating neurons and integrates it into its own CPG. ComInt1 receives these inputs with a gradient of synaptic strength, where the strongest excitatory postsynaptic potential (EPSP) is always elicited by the ASCE from the posterior neighbor. The anterior DSC elicits a weaker EPSP and the most distant coordinating neuron has the weakest input. Here we want to investigate if the gradient of synaptic strength is due to the morphology of synaptic contacts. For these experiments, ComInt1 and coordinating neurons were filled with fluorescent dye and the presynaptic boutons of the coordinating neurons on ComInt1 were marked immunohistochemically with Anti-Synapsin. An immunohistochemical labeling of the postsynapses of ComInt1 with PSD-95 was so far not successful. The axons of the coordinating neurons run dorsally, parallel to the midline through each segment. ComInt1 has its soma in one hemisegment, sends its primary neurite dorsally over the midline to the lateral neuropil on the contralateral side where it forms an electrical synapse with one of the CPG neurons. ComInt1 has one ascending and one descending dendritic branch parallel to the midline and to the axons of the coordinating neurons. At the midline we identified synapses of the coordinating neurons by colocalized presynaptic boutons with an intracellular stained axon. The colocalizations were dorsally all along and strictly parallel to the midline of the ganglion. These colocalizations do not yet explain the three distinct sizes of EPSPs in ComInt1 but it is a first approach to investigate this morphologically. We will perform more double stainings of two different coordinating neurons and presynaptic boutons as well as triple stainings with ComInt1 to investigate if the areas of colocalization and the number of synapses vary in accordance to the synaptic inputs. DFG SM206/3-1

The Role of Amygdala-striatal Circuitry in Exploration

Paolo Botta

Champalimaud Foundation, Lisbon, Portugal

In a world varying in time and space, exploration of new territories can increase inclusive fitness. Exploratory activity is achieved through the progression of a stochastic selection of motor actions essential to explore and become familiarized with novelty. Such actions are modulated by emotional states. The inability to cope with variable novel situations and environments is symptomatic of serious emotional disorders such as anxiety and depression in humans. Given that the initiation of specific exploratory actions is sensitive to contextual and emotional states, we decided to explore the contribution of limbic brain areas to exploratory activity. The amygdala is a well-known limbic structure important for emotional processing that projects to the medial parts of striatum involved in action selection. Therefore, we investigated the role of amygdala-striatal circuits in action selection computation during exploration. We developed a dimensionality emergence assay to investigate differences in exploratory activity in a habitual and novel environment, and during the process of habituation. In order to specifically target the basolateral portion of amygdala, we characterize a transgenic mouse line specifically expressing CRE recombinase in basolateral amygdala. We found that it expresses in pyramidal calmodulin kinase II expressing neurons. Using this line, we imaged the Cre defined amygdala neuronal ensembles in freely-exploring mice using conditional viral expression of calcium-sensors in the paradigm described above. Principal cells of basolateral amygdala massively project to dorsomedial striatum. The effect of amygdala-striatal projections on locomotor activity and the functional connectivity with dopamine receptor 1 and 2 expressing neurons is currently being tested using optogenetic tools combined with slice electrophysiology. These experiments will increase our understanding of the role of a key limbic circuit on processing different contextual and emotional information to favor exploratory motor actions.

Evaluation of Sensory Function and Thermal Pain Withdrawal Reflexes in MEIS1 Knockout Mice, a Possible Animal Model for Restless Legs Syndrome (RLS)

Samantha Meneely¹, Mai-Lynne Dinkins¹, Yuqing Li², and Stefan Clemens¹

¹Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC 28734

²Department of Neurology, University of Florida, Gainesville, FL 32610

Restless Legs Syndrome (RLS) is a chronic sensorimotor disorder characterized by uncomfortable sensation and a strong urge to move the legs. Symptoms occur most often in the evening or at night and can severely disrupt sleep. Genome-Wide Association studies (GWAS) point to a role of genetic factors surrounding the MEIS1 (MEIS homeobox 1) gene, which plays a role during neural development. RLS patients show a reduced mRNA and protein expression of MEIS1. Currently, the first line of drug therapy for RLS uses dopaminergic agents, but no behavioral data are available that have assessed thermal pain withdrawal latencies in the MEIS1 knockout (MEIS1 KO) animal model, or their modulation by dopaminergics. We compared pain responses (tail-flick and Hargreaves) in MEIS1 KO mice and their appropriate wild type controls (WT). Animals were i.p. injected with either sham (control), levodopa (L-dopa, 10 mg/kg), pramipexole (a D3R agonist, 0.5 mg/kg), SKF 38393 (D1R agonist, 1 mg/kg), SCH 39166 (D1R antagonist, 0.1 mg/kg), or morphine (2 mg/kg). Following the behavioral experiments, spinal cords were harvested and processed for detection of D1R expression (abcam 78021). Under baseline conditions, thermal withdrawal latencies were similar between WT and MEIS1 KO animals in both Hargreaves and tail-flick conditions. L- dopa, pramipexole and morphine significantly increased withdrawal reflexes in both WT and MEIS1 KO, while block of the D1R pathway increased thermal reflexes significantly in MEIS1 KO only. Further, preliminary WB analyses suggest an up-regulation of spinal D1R expression levels over that of WT animals. Together, the data from this behavioral study indicate that sensory responsiveness in MEIS1 KO animals is largely unaltered over WT controls, with the possible exception of D1R-mediated responses. As D1R pathways in the spinal cord are predominantly associated with motor functions, our data suggest that the MEIS1 gene may only play a minor functional role in the sensory dysfunctions observed in RLS patients, but rather be associated with the associated motor symptoms and Periodic Leg Movements (PLMs).

Descending Input and its Effect on a Coordinating Network

Felix Clotten and Carmen Smarandache-Wellmann

Zoological Institute, Animal Physiology, Emmy Noether Group,
University of Cologne, Cologne, Germany, 50647

The swimmeret system of the crayfish is an easily accessible model for studying locomotion. The segmental organization of the central nervous system provides the opportunity to elucidate the mechanisms of both, the intersegmental coordination of central pattern generators (CPGs) and the coordination of coupled CPGs within one segment (left-right coordination). The general network properties of the swimmeret system and the coordination of CPGs that underlies this coupled activity were previously investigated in detail. Consequently it is of great interest to understand the effect of descending input from the brain on the coordination of the swimmeret CPGs. Both excitatory and inhibitory command neurons of the swimmeret system were described but no information is available about the input these neurons receive or their neural targets within the swimmeret system. In this comparative study separated axon bundles in the connectives of the abdominal nerve cord were stimulated electrically. Stimulation induced and terminated rhythmic activity in inactive and active preparations, respectively. Histological identification of the stimulation sides revealed that the locations of the stimulated axon bundles are consistent with previously described locations of excitatory and inhibitory command neurons. In the signal crayfish, *Pacifastacus leniusculus*, electrical stimulations affected both sides of the nervous system in the same manner. Rhythmic activity was initiated or terminated bilaterally to the same extent. In contrast, asymmetric rhythmic activity (i.e. rhythmic activity solely ipsilateral to the stimulated axon bundles) could be induced in the galician crayfish, *Astacus leptodactylus*. In intact crustaceans this behavior is known as a righting response of the swimmeret system due to spatial movements of the animal. Bath application of carbachol, a nicotinic and muscarinic analog of acetylcholine that increases the excitation of the swimmeret system, increased the stimulation effect on the side of the abdominal nerve cord that was contralateral to the stimulation side. This suggests that the ipsilateral or bilateral initiation of swimmeret movements depends on the system's excitation level. With increasing stimulation frequencies the period of the evoked rhythmic activity decreased and more motor neurons were recruited. These results, in addition with intracellular recordings of motor neurons during sub-threshold stimulations, give evidence that both the swimmeret motor neurons and presynaptic interneurons of the pattern-generating micro-circuits are possible targets of the command neurons within the swimmeret system.

Spinal V3 Subpopulations Exhibit Distinct Neurogenesis Profiles and Subsequent Downstream Functional Separation Regulated by the Sim1 Transcription Factor

Dylan Deska-Gauthier, Jeremy Chopek, and Ying Zhang
Medical Neuroscience, Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4R2

V3 interneurons (INs) in the spinal cord are a major group of excitatory commissural INs that are essential in establishing a robust and balanced locomotor rhythm during walking. In the early embryonic mouse spinal cord, V3 INs arise from the most ventral progenitor domain (P3) as marked by the expression of the basic helix-loop-helix transcription factor, Sim1. Subsequently, V3 INs form both dorsal and lateral migratory streams establishing separate subpopulations by postnatal day (P) 0 with unique laminar distributions, axon projection profiles and membrane properties. In order to better understand the developmental mechanisms establishing different V3 subpopulations from the P3 progenitor domain we investigated their respective temporal neurogenesis profiles. V3 INs were visualized using a Sim1Cre;Rosa26TdTomato mouse line. Distinct dorsa-ventral subpopulations were further divided into ascending and descending groups via biotin-dextran-amine retrograde labeling. The birthdates of P0 V3 INs were determined by preempted 5-Ethynyl-2'-deoxyuridine (EdU) pulses between embryonic day (E) 9.5 and E12.5, respectively. Dorsal V3 INs are born between E9.5 and E10.5 while ventral V3 INs are born later between E10.5 to E12.5. In addition, preliminary data suggests early born ventral V3 INs are predominately ascending while later born ventral V3 INs are descending. Following this, the role of Sim1 in establishing the V3 neurogenesis profile and the subsequent physiological differentiation between dorsal and ventral groups was investigated. In Sim1 complete knockout mice V3 INs demonstrate delayed progenitor domain exit and therefore shifted neurogenesis profiles to later embryonic stages. Following this, dorsal and ventral V3 subpopulations are no longer physiologically distinguishable at P0 in the Sim1 mutant. These results suggest embryonic Sim1 expression establishes a patterned V3 neurogenesis profile and downstream functional separation of V3 subpopulations.

In Search for Interneurons Implicated in the Resetting of the Locomotor Rhythm by Extensor Group I Afferents

Lucia Esther Dominguez-Rodriguez¹, Katinka Stecina²,

David Leonardo García-Ramírez¹, Lourdes Martínez-Silva¹, Elvia Mena-Ávila¹, Jonathan Jair Milla-Cruz¹, Hans Hultborn^{1,3}, and Jorge Noel Quevedo¹

¹Physiology, Biophysics and Neuroscience, CINVESTAV, Mexico City, Mexico 07360

²Spinal Cord Research Centre, University of Manitoba, Winnipeg, Canada R3E0J9

³Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark 2200

Sensory information arising from limb movements is able to control the spinal locomotor circuitry for locomotion to adapt the motor pattern to demands of the environment. Stimulation of extensor group I afferents during the flexion phase of fictive locomotion produces a suppression of the ongoing flexion and initiation of the subsequent extension (resetting to extension; Conway et al Exp Brain Res 1987, 68: 643-56; Hultborn et al Ann N Y Acad Sci 1998; 860:70-82). During fictive locomotion, instead of the classical Ib non-reciprocal inhibition, stimulation of extensor group I afferents produced an oligosynaptic excitation in extensor motoneurons with latencies (~3 ms) compatible with 3 interneurons interposed (see Gossard et al, Exp Brain Res 1994, 98:213-28). We assume that some interneurons in this pathway actually belong to the rhythm-generating layer of the Central Pattern Generator (CPG), since it causes a resetting of the rhythm. The present work was aimed to identify the interneurons causing the resetting to extension and mediating the polysynaptic group I excitation during *L-DOPA*-induced fictive locomotion in the cat (and was a continuation of previous work; Stecina et al 2007, SfN poster 78.11). Peripheral nerve dissection, laminectomy and decerebration were made under isoflurane anesthesia. Subsequently spinalization at C1 was performed. Fictive locomotion was induced by the injection of *Nialamide* (50 mg/kg) followed by *L-DOPA* (100 mg/kg). Ankle extensor motoneurons were recorded with *K+-acetate*-filled micropipettes. Orthodromic extracellular field potentials (EFPs) and interneuron spikes were recorded in the intermediate nucleus and the ventral horn with *Na+-glutamate*-filled micropipettes (to be able to cause tonic firing of interneurons). We recorded candidate interneurons to mediate the resetting to extension based on the following criteria: i) rhythmic activation during the extension phase, ii) mono-, di-, or trisynaptic activation by extensor group I afferents, iv) polysynaptic activation by stimulation of contralateral flexor reflex afferents. Some interneurons were antidromically activated from extensor motor nuclei and their spikes associated to negative EFPs in the same motor nucleus, suggesting they were last-order excitatory interneurons. We conclude that interneurons recorded fulfill the characteristics to belong to the neuronal pathway activated by extensor group I afferents during locomotion. As the activity of interneurons exhibit the same pattern as the 'resetting to extension', they may belong to the rhythm generating layer of the CPG for locomotion.

Decrease of Rate Dependent Depression of H-reflex in Newborns with Muscle Hypertonia After Antenatal Hypoxia-ischemia in Rabbit Cerebral Palsy Model

Alexander Drobyshevsky¹ and Katharina Quinlan²

¹Pediatrics, NorthShore University HealthSystems, Evanston, IL 60201

²Physiology, Northwestern University, Chicago, IL 60611

Newborn rabbit kits after global antenatal hypoxic-ischemic (H-I) injury exhibit motor deficits similar to human infants with cerebral palsy, including muscle hypertonia. Loss of supraspinal presynaptic inhibition has been implicated as a mechanism leading to the development of hypertonia and spasticity in cerebral palsy. We hypothesized that a decrease of descending corticospinal motor projections, observed in this rabbit model, will affect the rate dependent depression (RDD) of H-reflex as a measure of supraspinal inhibitory control. The secondary objective was to examine developmental changes in H-reflex during postnatal maturation. H-reflex was elicited in lightly anesthetized P1, P5, P11, P18 and adult rabbits using needle electrodes for stimulation and thin wire electrodes placed in m. flexor carpi radialis in forelimbs and m. gastrocnemius in hind limbs for recording. In some experiments H-reflex was measured on exposed nerve preparation with subsequent nerve cutting to confirm the origin of H-wave. RDD was measured as a ratio of H-wave magnitudes in control and test stimuli pairs, presented every 10 sec with 5, 2, 1, 0.5, 0.15, 0.08, 0.03 sec inter-stimulus intervals (ISI). In addition, maxH/maxM amplitude ratio was measured by varying stimulation amplitude. Newborns of naïve control and rabbit dams that underwent global fetal H-I at E25 for 40 min were used. We were able to elicit H-reflex 85% in forelimbs and 65% hind limbs at P1, and in almost all kits at later ages. The magnitude of the test pulse decreased from 82% to 53% to 21%, at 5, 0.5, 0.03 sec ISI respectively. There was no significant difference in RDD during postnatal development between P1 and P11 in either forelimb or hind limbs. There was also no significant difference in RDD between forelimbs and hind limbs at corresponding ages. Significant reduction of RDD was found in hypertonic kits after H-I, both in fore- and hind limbs at P1 and P11, at 0.3, 0.15, 0.08 sec ISI: Significant increase in hypertonic kits in maxH/maxM from 0.24 to 0.42 was found only in hind limbs at P1 but not at P11. We conclude that the significant reduction of RDD in hypertonic kits suggests a decreased supraspinal inhibitory control that might be mediated by the loss of descending motor projections after fetal H-I. An increase of maxH/maxM may indicate that stronger afferent synaptic connection is retained in hypertonic kits due to delayed arrival of corticospinal projections. Subsequent decrease of maxH/maxM at later ages may indicate continued degeneration of the spinal circuit in hypertonic kits.

Exploring the Synaptic Fate of Injured Spinal Cord Axons

David Elliott, Andreas Husch, and Frank Bradke
Axon Growth and Regeneration Group,
German Center for Neurodegenerative Diseases,
Bonn, North Rhine-Westphalia, Germany 53175

Central nervous system axons are typically unable to regenerate following injury. Several strategies have been developed to promote axon regeneration, however, little is known about the potential of these axons to form new synapses and the identity of their potential post-synaptic partners. To address this issue we are pursuing two avenues of investigation: i) Interaction of injured dorsal column (DC) axons with non-neuronal NG2⁺ cells, ii) Development of an anterograde trans-synaptic tracing strategy to assist in the identification of post-synaptic partner cells. NG2⁺ cells proliferate after spinal cord injury and are abundant in the injury site. NG2 expressing oligodendrocyte precursor cells (OPCs) in uninjured tissue are known to receive synaptic input from neurons, thus raising the possibility that some NG2⁺ cells within the scar site may also synaptically interact with injured axons. Furthermore, previous studies suggest that injured axons form synaptic-like contacts with non-neuronal cells and this is speculated to play a role in preventing axon regeneration. In this study, the NG2-dsRed mouse line was used to identify NG2⁺ cells and AAV-GFP was used to identify DC axons. Following DC lesion, axons entering the injury site are tightly associated with NG2⁺ cells with axonal trajectories intertwined alongside NG2⁺ cell processes. Pre-synaptic proteins were present at these contact locations in some but not all of these axons. Staining with the OPC marker Olig2 revealed that the majority of these cells are not OPCs. To better understand the nature of these heterogeneous NG2⁺ cells present specifically after injury, we performed patch clamp electrophysiological recordings in ex vivo spinal cord slices. The intrinsic electrophysiological profiles were heterogeneous with some NG2⁺ cells displaying more complex electrophysiological properties. We have started to further elucidate a potential synaptic interaction between injured DC axons and these potential postsynaptic cells. In a parallel line of investigation we aim to identify synaptic interaction with all potential post-synaptic partners, not just those expressing NG2. The anterograde trans-synaptic tracer Wheat Germ Agglutinin was fused with Cre and expressed in a floxed Td-Tomato reporter mouse line. To increase tracing efficiency, we created an AAV expressing solely WGA-Cre and injected it directly into the dorsal root ganglion. After 4 weeks a sufficient level of trans-synaptic labeling was observed in most mice. Grey matter neurons known to receive innervation from DC axons were labeled in the uninjured spine. After injury, tracing revealed cells with heterogeneous and unique morphologies within the injury site. We are now further elucidating the identity and electrophysiological properties of these cells.

Optogenetic Analysis of the Role of Motoneurons in the Regulation of Locomotor-like Activity in the Neonatal Mouse Spinal Cord

M. Falcgairolle¹, J.G. Puhl², A. Pujala³, and M.J. O'Donovan¹

¹Developmental Neurobiology Section, National Institutes of Health, Bethesda, MD 20892

²Department of Neuroscience, University of Minnesota, Saint Paul, MN 55108

³Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147

Stimulation of motoneuron axons in an isolated preparation of the neonatal (P0-4) mouse spinal cord can activate the central pattern generator (CPG) for locomotion. To establish if motoneuron firing can influence the CPG during locomotor-like activity evoked by drugs, we generated mice in which either Channelrhodopsin-2 (ChR2) or Archaeorhodopsin-3 (Arch) was expressed in ChAT⁺ neurons. We used an LED system to illuminate the cord over the lumbar (L1- L6) segments. In ChAT-ChR2 mice, a train of light pulses at 1-4 Hz for 10 sec activated locomotor-like activity in the absence of drugs confirming our earlier experiments with electrical stimulation of the ventral roots. ChAT-Arch mice were used to examine the effect of silencing motoneuron firing on the locomotor rhythm recorded from slow ventral root potentials (after low pass filtering the neurograms) and from whole cell recordings from motoneurons. In the locomotor cocktail, individual motoneurons were hyperpolarized by 10-20 mV during illumination. Firing was easier to suppress in extensor compared to flexor motoneurons, and in the extensor- dominated L5/6 roots compared to the flexor-dominated L1/2 roots. This appeared to be because the primary rhythmic synaptic drive to flexors is predominantly excitatory and that to the extensors is mainly inhibitory. Illumination frequently hyperpolarized extensor motoneurons below the chloride equilibrium potential rendering their inhibitory synaptic drive depolarizing and in phase with flexor activity. We found that the frequency of the locomotor-like rhythm was transiently depressed in ChAT-Arch animals and was transiently increased in ChAT-ChR2 mice. In the ChAT-Arch cords, when the light was turned off, the amplitude of the locomotor bursts and sometimes their frequency was transiently increased for tens of seconds before returning to control levels. Similar results were obtained in mice expressing Halorhodopsin in ChAT⁺ neurons suggesting that the effects are not due to a change in extracellular pH. Finally, we were unable to abrogate these effects with either cholinergic or gap junction blockers. We conclude that motoneurons provide feedback to the CPG during drug-induced locomotor-like activity that acts to stabilize the locomotor rhythm.

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Partially Shared Inhibitory and Excitatory Spinal Circuits for Withdrawal Reflex, Scratch and Locomotion

Graziana Gatto and Martyn Goulding

Molecular Neurobiology Laboratory, Salk Institute for Biological Studies,
La Jolla, CA 92037

Animals display a highly varied repertoire of motor behaviors that reflect the patterned activity of a limited number of muscles and motor neurons. Rhythmic behaviors, such as walking or breathing, are generated by the activity of neuronal networks, known as central pattern generators (CPGs). Work in turtles has shown a partial overlap of the CPG neurons that are active during locomotion and scratching. To date, these neurons have not been molecularly identified. Using a tripartite intersectional genetic approach we have investigated the role of specific interneuron populations during reflex responses and locomotion. Spinal ablation of V1 (En1-Cre) and V2a (Chx10-Cre), but not V2b (Gata3-Cre) interneurons in adult mice leads to impaired responses to chloroquine-induced scratch. Among the analyzed mice, ablation of V1 or V2a interneurons causes a strong reduction in the speed of scratching, and in the number of scratch bouts, suggesting that these interneuron populations have an essential role in enabling fast scratch movements. Acute silencing of these neurons, using CNO-activated hM4D receptor, recapitulates the slower velocity during scratching. Interestingly, ablation of V1 or V2a interneurons has already been shown to impair mice performance at high locomotory speeds, implying that scratch and locomotion share the CPG neurons responsible for facilitating fast movements. We also find an impaired response to mechanical stimuli but not noxious-induced paw withdrawal in V1 and V2b ablated mice, suggesting that the withdrawal reflex may recruit different interneuron pathways according to the applied stimulus. Taken together, these data identify two key CPG populations as common elements in the CPG circuits for rhythmic scratch and locomotion. Our data also infer that V2a interneurons might be part of the scratch- rhythm generator, while the V1 interneurons are required for the fine-tuning of the speed. In the future, it will be important to address how these two populations are connected and interact during scratch (a unilateral rhythmic behavior) and locomotion (a bilateral coordination of rhythm).

Analysis of Multielectrode Data from CPG Networks using Stochastic Dynamic Operators

Maryam Abolfat-Beygi¹, Terence D. Sanger¹, and Simon F. Giszter²

¹Department of Bioengineering, University of Southern California,
Los Angeles, CA 90089

²Department of Neurobiology, Drexel University College of Medicine,
Philadelphia, PA 19104

We seek to develop a new stochastic framework using stochastic dynamic operators (SDO) in order to quantify the inter-relations of neural dynamics and neural connectivity in motor control, and to develop predictive models suitable for neuroprostheses. We use real neural data collected in spinal frogs, and artificial data from simulated spinal networks. While most current methods are usually based on linear mappings between neural recordings and movement variables or EMG signals, SDO frameworks instead use linear mappings in the probability domain, which simultaneously embed nonlinear relationships between high-level neural activity and output movement variables. In the SDO framework each spike triggers a differential equation operator mapping probability of current state of movement variables to the probability of next state in a continuous-time Markov model. An SDO thus represents both sensory (current state) and motor (mapping to the next state) effects in the neuron. In spike triggered averaging the temporal stimulus vector is averaged across the spiking events, whereas SDO methods attribute to each value of the stimulus or output state a probability that is obtained using sampling methods. Investigating neural dynamics and connectivity, SDOs describe nonlinear pair-wise interactions among neurons. At the same time, multiple-to-single neuron interactions can be modeled by linear superposition of these SDOs, which is one of the appeals of this new framework. The SDO model can be best compared with likelihood point process models such as the generalized linear model, since it describes the probability of firing rate of one neuron in terms of history of firing of itself and other neurons. The difference is that the SDO model can represent the pure nonlinear interactions. One of our goals is investigate the potential of this framework in extracting neural connectivity. We have used the Rybak, Shevtsova, Markin spinal cord simulation of populations of pattern generation neurons. The network simulator is used to generate many realizations of spike trains from 'ground truth' networks of known connectivity and hierarchy, driven with Hodgkin Huxley dynamics. These spike trains are employed as sampled subsets of neurons for SDO analysis. The 'fictive' simulated network generates rhythms, deletions, and motor pool spiking which can be integrated to simulate electromyographic (EMG) recordings. First tests have built SDO-based predictive models of EMG, validating SDO use in predictive controls. Simulations also allow us to test how SDOs can best be used to understand hierarchy, rhythm generation and pattern formation using analysis of spike train recordings, and to predict deletion behaviors. Deletions and corrections are network behaviors present in spinal cords of frogs, turtles, and mammals such as cat. We seek to predict deletions from spike train recordings using appropriate SDO analyses, and use SDO analysis to inform a deeper understanding of network dynamics. Our data show SDO analysis can capture deletions and must account for role of hierarchy in their accurate prediction. Tests in real frogs show SDO estimation from neural recordings is also relatively straightforward in multielectrode spike data from unparalyzed spinal frogs.

A Stop Signal for Swimming Originates from the Mesencephalic Locomotor Region

Swantje Grätsch^{1,2,3}, Francois Auclair², Danielle Veilleux²,
Ansgar Büschges³, and Réjean Dubuc^{1,2}

¹Groupe de Recherche en Activité Physique Adaptée,
Université du Québec à Montréal, Montréal, Canada H3C 3P8

²Groupe de Recherche sur le Système Nerveux Central,
Université de Montréal, Montréal, Canada H3T 1J4

³Biocenter Cologne, Department of Neurobiology, University of Cologne,
Cologne, Germany 50674

In vertebrates, the mesencephalic locomotor region (MLR) plays a crucial role in controlling locomotor activity. Via a descending pathway it activates reticulospinal (RS) cells in the brainstem, which in turn project to central pattern generators (CPGs) in the spinal cord. The neural mechanisms underlying the initiation and maintenance of locomotion are partly understood, whereas the termination mechanisms are far more elusive. In the lamprey, distinct RS cell populations were found to control locomotor initiation (“Start Cells”), maintenance (“Maintain Cells”), and termination (“Stop Cells”) [Grätsch et al., 2015; SfN 421.20]. During swimming, “Stop Cells” were found to generate a burst of spikes right before the end of locomotion (termination burst). Pharmacological activation and inactivation experiments demonstrated that “Stop Cells” play a crucial role in locomotor termination. We have shown that membrane properties are unlikely involved in generating the stop signal, suggesting the presence of specific synaptic inputs from other brain regions which still need to be identified. Here, we investigate the origin of the stop signal and show that the MLR is a very likely candidate. We performed experiments in the lamprey semi-intact preparation, in which swimming movements of the intact body can be recorded and correlated to the cellular activity of brainstem RS neurons. In this preparation, electrical stimulation of the MLR initiates locomotion that often outlasts MLR stimulation (28.02 ± 14.18 s; $n=5$). During the swimming period exceeding the MLR stimulation, we found that a stimulation pulse delivered in the MLR at a lower intensity (50 % of control) halted locomotion (within 6.83 ± 3.09 s; $n=5$). On the other hand, a stimulation pulse delivered at a higher intensity (100 % of control) prolonged the swimming bout. The MLR had similar effects during spontaneous or sensory-evoked locomotion. Local ejection of D-glutamate in the MLR lead to the same results: a small quantity of D- glutamate terminated ongoing swimming (within 8.41 ± 4.34 s; $n=3$), whereas a large quantity prolonged it. When “Stop Cells” were recorded intracellularly a termination burst was seen as locomotion was stopped by MLR stimulation. “Maintain Cells”, however, repolarized in this situation. We propose that the MLR provides a synaptic input to “Stop Cells”, which is the source of the termination burst. Funding: Funded by CIHR, NSERC and GLFC. SG received studentships from DAAD, UzK.

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

Ngoc Ha, Lihua Yao, Natalia Shevtsova, and Kimberly J. Dougherty
Departments of Neurobiology and Anatomy,
Drexel University College of Medicine, Philadelphia, PA 19104

The hindlimb locomotor central pattern generator (CPG) consists of ventrally located interneurons (INs) within the lumbar region of the spinal cord. Each limb contains a CPG with at least two layers, the rhythm generating (RG) layer and the pattern formation layer. The rhythm generating layer includes flexor RG INs, extensor RG INs, and inhibitory INs that connect and coordinate flexor- extensor RG INs ipsilaterally. Previous studies have proposed a flexor/extensor asymmetry where locomotor rhythmicity results from the activity of the flexor RG INs. The extensor RG INs fire tonically and are driven into bursting mode by the phasic inhibition from the rhythmically active flexor RG INs via inhibitory INs. This then suggests that there are differences in intrinsic excitability, burst generating mechanisms, and/or connectivity between flexor and extensor RG INs. Recently, Shox2 INs have been identified to be one of the cellular components of the rhythm generator for locomotion. Ablating these cells led to a reduction of the locomotor rhythm and no change in the flexor and extensor alternation. The goal of the present study is to determine potential factors contributing to a flexor/extensor asymmetry, focusing on connectivity and intrinsic properties related to excitability and rhythmogenesis. We performed whole cell patch clamp recordings from identified Shox2 INs in reduced isolated spinal cord preparations from neonatal Shox2:Cre; tdTomato mice. Flexor and extensor RG INs were identified based on fluorescence and their preferred firing during drug-evoked locomotion in vitro. Voltage clamp and current clamp protocols were run to study the intrinsic passive and active properties of these cells, including ionic currents linked to cellular rhythmicity. Our data suggest that there are no differences in the cellular excitability between flexor- related Shox2 INs and extensor-related Shox2 INs. Potential rhythmogenic currents including I_h , persistent inward current, and T-type Ca^{2+} current are present in some of these cells. A differential distribution of these currents in the flexor/extensor Shox2 INs likely plays a role in establishing cell rhythmicity during locomotion. In addition to intrinsic properties, dual Shox2 IN recordings show that interconnectivity is scarce but may be preferential within flexor or extensor RG populations. Therefore, connectivity and expression of particular ionic currents in rhythm generating neurons may contribute to flexor-extensor asymmetry.

Motor Activity in the Three Major Leg Joints of the Turning Stick Insect is Modified in a Joint- and Context-specific Way

Elzbieta Hammel, Ansgar Büschges, and Matthias Gruhn
Department of Neurobiology, Animal Physiology, University of Cologne,
Zùlpicher StraÙe 47b, Cologne, Germany 50674

Locomotion depends on neuronal activity, muscle contractions, and sensory feedback. The generation of straight walking has already been well studied and much is known about the neural activity underlying basic locomotor patterns in the single leg (Büschges 2005). However, we only begin to understand the neuronal mechanisms underlying behavioral flexibility, and the knowledge on local segmental activity during adaptive locomotion and the role of descending influences on it is scarce (Hellekes et al. 2012; Martin et al. 2015; Gruhn et al. 2016). Here we investigate the neural mechanisms underlying curve walking in the stick insect (*Carausius morosus*). Turning of the tethered animals on the slippery surface was induced using an optical stimulus, and walking sequences were monitored by video and by EMG recordings of the *Flexor tibiae* muscle in both front legs. We studied the influence of optomotor induced turning on motor activity in all three major leg joints by recording extracellularly from leg nerves nl2 (containing protractor motoneurons (MNs)), nl5 (retractor MNs), C1 (levator MNs), C2 (depressor MNs), nl3 (extensor MNs), and branches of the nervus cruris (flexor MNs) of the deafferented meso- and metathoracic ganglia in a reduced preparation with all legs cut off except for the two front legs. The motor activity of the three joints showed three types of responses: 1) a context-dependent change in activity in the subcoxal joint: meso- and metathoracic protractor MNs showed an increased activity during inside over outside turns (mesothorax (Ms) N = 4, metathorax (Mt) N = 12), while retractor MNs showed an increased activity during outside vs. inside turns (Ms N = 4, Mt N = 8). 2) a context-independent activity: the meso- and metathoracic levator and depressor MNs mostly did not show changes depending on the turning direction. The levator MN activity increased as soon as the front legs started stepping in either direction (Ms N = 8, Mt N = 6), whereas depressor MN activity ceased (Ms N = 4, Mt N = 6). 3) no characteristic pattern for either inside or outside turns: extensor (Ms N = 8, Mt N = 9) and flexor (Ms N = 13, Mt N = 12) MNs showed either increase or decrease in activity upon inside or outside turning with high variability in neuronal activity, without an obvious context-dependent pattern. Our results indicate that changes in meso- and metathoracic motor activity during curve walking of the front legs are not thorax-segment specific but occur on the level of each leg joint. Further studies will clarify in how far the activity of meso- and metathoracic central pattern generators are affected by descending influence from curve walking front legs.

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Investigating the Role Terminal Schwann Cells Play in Muscle Type Specific Recovery Following Partial Denervation in G93A SOD1 mice

Julia M. Harrison and Victor F. Rafuse
Medical Neuroscience, Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4R2

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron (MN) disease, with a prevalence of 3.9 cases in 100,000. ALS is characterized, in part, by the progressive death of MNs innervating skeletal muscles fibers. As MNs die, muscles become increasingly weaker due to progressive muscle fiber denervation. This ultimately leads to death due to respiratory failure. If muscles are partially denervated in healthy individuals due to injury, uninjured MNs sprout axons to reinnervate neighboring denervated muscle fibers. This process, known as collateral reinnervation, can restore muscle force to pre-injury values and is mediated, in part, by terminal Schwann cells (TSCs). Collateral reinnervation is compromised in ALS, which contributes to the rate of progressive weakness. Interestingly, paralysis of fast contracting muscles occurs significantly earlier in ALS compared to slow muscles. To examine whether this is due to impaired collateral reinnervation, we partially denervated soleus (slow) and plantaris (fast) muscles in SOD1^{G93A} (ALS) mice and wild type (WT) littermates by ligating and cutting the 5th lumbar spinal nerve (L5) at P30 to examine the effects of partial denervation (PD) pre disease-onset. Mice were allowed to recover for thirty days to determine innervation recovery, or for three days to examine the TSC response to denervation. Our results to date show that the proportion of innervated muscle fibers in partially denervated plantaris muscles was less than similarly denervated soleus muscles in ALS mice, but not WT mice following thirty days of recovery. Furthermore, we found that a significant proportion of denervated muscle fibers in the ALS PD plantaris muscle lacked TSCs. Additionally, early results suggest that numbers of TSCs at denervated endplates decrease over time during this “pre-symptomatic” stage of disease. Interestingly, at partially innervated synapses in ALS mice, TSCs were present but often only in the region of the end plate with the axon present. Also of note, denervation in the uninjured leg was seen by P60 in the ALS mice, supporting previous findings that pathologies occur in this model before obvious motor deficits. We are currently exploring potential genomic and proteomic differences between TSCs associated with fast and slow muscles as well as between ALS and WT mice. Together, these results suggest that the sprouting capacity of fast MNs in ALS is compromised even before disease onset and that this pathology may be related to abnormalities in TSCs.

V2a Neurons Drive Accessory Respiratory Muscle Activity and Degenerate in Mouse Models of ALS

Victoria N. Jensen¹, Shannon H. Romer², Kari Seedle²,
Sarah M. Turner², and
Steven A. Crone²

¹Neuroscience Graduate Program, University of Cincinnati, OH 45221

²Neurosurgery/Developmental Biology, Cincinnati Children's Hospital,
Cincinnati, OH 45221

Respiratory failure is the leading cause of death in amyotrophic lateral sclerosis (ALS) and the only current treatment to improve breathing is mechanical ventilation. Despite progressive degeneration of phrenic motor neurons innervating the diaphragm, ALS patients and rodent models are able to maintain ventilation at early stages of disease, but experience a sharp decline at late disease stages. The mechanisms that maintain ventilation at early stages or lead to ventilation failure at late disease stages are unknown. We propose that compensatory mechanisms including recruitment of accessory respiratory muscles (ARMs) help to maintain ventilation at early stages of disease. Using a customized system for simultaneous non-invasive measurement of ARM electromyograph (EMG) activity and breathing (whole body plethysmography), we demonstrate that ARMs are recruited in early stage ALS model mice and increase ventilation. Surprisingly, ARMs are not used for breathing at late disease stages, even though they are used to perform other motor functions. In addition, we have identified a glutamatergic neuron class in the spinal cord and brainstem, the V2a class, that is able to drive accessory respiratory muscle activity and that degenerates in mouse models of ALS. These results support the hypothesis that a central deficit in respiratory circuitry underlies the failure to activate ARMs at late stages of disease. Our studies suggest that therapies to protect, replace, or improve the function of V2a neurons could help to restore or maintain ventilation in patients with ALS, other neuromuscular diseases, or spinal cord injury.

Spinal Neurons from Adult Mice Do Not Show Enhanced Excitability after Spinal Cord Injury in Reduced Calcium Saline and Elevated Temperature

Bruce Johnson¹, Kawasi Lett¹, Shelby Dietz¹, Andreas Husch², and
Ronald Harris-Warrick¹

¹Department of Neurobiology and Behavior, Cornell University,
Ithaca, NY 14853

²German Center for Neurodegenerative Diseases, Ludwig-Erhard-Allee 2,
Bonn, Germany 53175

Spinal motor neurons and interneurons from neonatal rodents show enhanced excitability and bistable firing properties when recorded in temperatures above 30°C and using saline with 1.2 mM calcium, approximating normal *in vivo* conditions (Bouhadfane et al 2013. J Neuroscience, 33:15626; Brocard et al 2013. Neuron 77:1047). This increased excitability is thought to contribute to locomotor rhythm generation and plateau properties of spinal neurons. We are investigating the intrinsic firing properties of lumbar motor neurons (MNs), V1 interneurons (INs), and V2a INs from intact and spinal cord injured (SCI) adult (at least 8 weeks old) mice, comparing 2.5 to 1.2 mM Ca and 20 to 30°C, in order to optimize experimental conditions to detect excitability differences between spinal neurons recorded from intact and SCI mice. Perforated and whole cell patch recordings were made from identified lumbar MNs in longitudinal spinal cord slices from ChAT-GFP transgenic mice; V1 and V2a INs were recorded in transverse slices from En1-RFP and Chx10-CFP mice. Most neurons were pharmacologically isolated from fast synaptic input. Excitability was measured with depolarizing current steps and ramps, and bistability was detected by negative hysteresis on ramps, or prolonged after-discharges following a current step. At 30° C and in 1.2 mM Ca saline, action potential firing in MNs and INs from intact adult mice was generally limited to the depolarizing phase of a symmetrical current ramp current; bistability was not detectable. After SCI, we have only examined excitability in MNs, 4 to 6 weeks after transection of the thoracic spinal cord, with the higher temperature and low calcium saline. MNs from SCI mice did not show signs of enhanced excitability or bistability, confirming our earlier work on MN properties at room temperature and higher calcium concentration. Some SCI MNs even expressed reduced excitability when shifted to higher temperature and 1.2 mM Ca, compared to room temperature and 2.5 mM Ca saline. SCI mice showed rear leg paralysis and other behavioral deficits consistent with complete SCI. Motor neurons from SCI mice were 100-1000-fold more sensitive to serotonin than control MNs, as found previously for V2a INs. These results suggest that the enhanced excitability seen under higher temperature and reduced Ca conditions in MNs and INs from neonatal mice may be lost during postnatal development, and SCI does not restore it, at least in MNs.

Functional Contribution of the Mesencephalic Locomotor Region to Locomotor Control

Nicolas Josset, Marie Roussel, and Frédéric Bretzner
Neurosciences, CHUL de Québec Research Center, Université Laval, Canada

Recently, electrical stimulation of the Mesencephalic Locomotor Region (MLR) has been shown to improve locomotor recovery in hemi-lesioned rodents. Although the anatomical correlates of the MLR has been initially identified as the cuneiform nucleus (CnF), a cluster of glutamatergic neurons, and the pedunculopontine nucleus (PPN), a cluster of glutamatergic and cholinergic neurons, there is still an on-going debate about the exact anatomical correlate of this supraspinal locomotor center. Using adult VGluT2+ or ChAT+cre transgenic mice expressing Channelrhodopsin 2, optical cannulas were implanted chronically above the CnF or PPN, and wires were implanted in hindlimb flexor and extensor muscles for electromyographic (EMG) recordings. Kinematic and EMG recordings were performed at rest and during treadmill locomotion upon photostimulations. Photostimulations of glutamatergic CnF or PPN neurons initiated episodes of locomotion at rest. During on-going locomotion, photostimulations of glutamatergic CnF or PPN evoked short-latency excitatory responses in hindlimb flexor and extensor muscles during the swing phase, and inhibitory responses in extensor muscles during the stance phase. Interestingly, photostimulations of glutamatergic CnF neurons applied within a step cycle shortened the duration of the step cycle and extensor burst, thus resetting the locomotor rhythm and switching gaits from a slow trot to a fast gallop or full-bound. In contrast to glutamatergic populations, photostimulations of cholinergic PPN neurons failed to evoke episodes of locomotion in mice kept at rest. However, these photostimulations evoked excitatory motor responses in extensor muscles at rest and during treadmill locomotion. They also increased the duration of the step cycle and extensor burst, thus slowing-down the locomotor rhythm and consequently switching the gait from a trot to slow-walking gaits, such as lateral or out-of-phase walks. In summary, glutamatergic CnF and PPN neurons initiate and modulate the locomotor pattern, and accelerate the rhythm, while cholinergic PPN neurons decelerate it.

Planar Covariation of Hindlimb Elevation Angles is Present during Walking of Intact and Spinal Cats and in Simulated Locomotion of a Neuromechanical Model

Alexander N. Klishko¹, Michel A. Lemay², Irina N. Beloozerova³,
Sergey N. Markin⁴, Ilya A. Rybak⁴, and Boris I. Prilutsky¹

¹School of Biology Sciences, Georgia Institute of Technology,
Atlanta, GA 30318

²Department of Bioengineering, Temple University, Philadelphia, PA 19122

³Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

⁴Neurobiology & Anatomy, Drexel University, Philadelphia, PA 19129

Planar covariation of leg elevation angles has been demonstrated during various locomotor behaviors (Borghese et al. 1996) and suggested to reflect neural constraints that simplify the control of kinematically redundant leg. These constraints might be related to kinematic synergies stabilizing leg length and leg orientation during locomotion (Ivanenko et al. 2007; Klishko et al. 2014), although their mechanisms are not known. Here, we investigated planar covariation of hindlimb elevation angles in cats during locomotor behaviors that require substantial contribution of the motor cortex (precise stepping on horizontal ladder), exclusive contribution of the spinal cord (hindlimb locomotor movements after spinal cord transection at the thoracic level) and normal level and slope ($\pm 50\%$) walking in intact cats with presumably greater contribution of the spinal cord. In addition, we examined planar covariation of hindlimb elevation angles in level and slope walking simulated using a comprehensive neuromechanical computational model of hindlimb locomotion (Markin et al. 2016). Planar covariation of hindlimb segments was evaluated using principal component analysis (Borghese et al. 1996). The results demonstrated a high degree of planar covariation of hindlimb segment angles for all locomotor behaviors studied, including simulated level and slope walking. The orientation of the covariance plane in the space of tarsal, shank and thigh elevation angles differed however among locomotor behaviors. Time profile of the first principal component (PC1) was highly correlated with the hindlimb orientation ($r > 0.9$), whereas that of PC2 showed the highest correlation with hindlimb length although the correlation was generally much weaker ($r > 0.4-0.6$). A variety of stable locomotor patterns were generated by computer simulations of level and slope walking by varying the values of the sensory feedback gains and supraspinal inputs to interneurons of the CPG. All generated locomotor patterns demonstrated a high level of planar covariation of hindlimb segment angles. We suggest that potential neural constraints responsible for the kinematic synergy of planar covariation of elevation angles during locomotion are inherent in the spinal locomotor circuitry.

Structural and Functional Correlates of Variation in Neuronal Numbers in the Locus Coeruleus of Zebrafish Imaged with Multiphoton and High-speed Light Sheet Microscopy

Matthew J. Farrar^{1,2}, Kristine E. Kolkman¹, and Joseph R. Fetcho¹

¹Department of Neurobiology and Behavior, Cornell University,
Ithaca, NY 14853

²Department of Mathematics, Physics and Statistics,
Messiah College, Mechanicsburg, PA 17055

Zebrafish are an ideal model organism in which to study the role of the LC in physiology and pathology due to the ease of creating transgenic models, transparency in the larval stage, and small size of the LC nucleus. Using a Tg:ET-VMAT GFP line confirmed by antibody staining, we examined zebrafish larvae at 5 days post fertilization using 2PEF microscopy. In agreement with previous studies, LC neuron cell counts ranged between 10 and 20 bilaterally, with a mean of 15. This variation— as much as 100%— is not unique to zebrafish. To study the correlation between LC number and neurite projections, we performed single-cell electroporations in the Tg:ET-VMAT GFP line. Using 3-D Sholl analysis, we found no systematic differences in projection density proximal to the soma of individual neurons between fish with few and many LC neurons. To explore whether neurite density in target areas distal to the soma is correlated with LC neuron count, we used CRISPR/Cas9-mediated knock-in to create a transgenic line (NET:mCFP) with a membrane-targeted CFP under the endogenous NE transporter (NET) promoter, allowing us to reveal all NE projections with high fidelity. We examined the optic tectum and the spinal cord since NE projections in these areas are predominantly from the LC. We found a positive correlation ($n = 5$ fish) between LC number and neurite density. We investigated the activity of LC neurons by creating a transgenic line expressing nuclear localized GCamp6f (NET: H2B-GCamp6f). To achieve high-speed bilateral imaging of neuronal activity, we custom-built a high-speed light sheet microscope (based on Ahrens et al., 2013) that allowed for whole brain imaging at several brains a second. We imaged spontaneous activity in the LC neurons in intact larvae as well as ones with hindbrain spinal-projecting neurons backfilled with Calcium Green dextran to allow correlation of LC activity with activity of identified descending neuronal populations. Correlated activity was observed between LC neurons and cells associated with swimming behavior including putative MiV2 and 3 neurons as well as neurons in the nucleus of the MLF. The average activity of individual neurons in the LC was negatively correlated with LC neuron count ($n = 9$ fish). These findings suggest that fish with fewer LC neurons may maintain physiological NE levels at lower projection density by a compensatory increase in the activity of individual cells.

Crossed Reflex Pathways in Freely Walking Mice

Olivier D. Laflamme^{1,2} and Turgay Akay^{1,2}

¹Department of Medical Neuroscience, Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4R2

²Brain Repair Centre, Atlantic Mobility Action Project,
Halifax, Nova Scotia, Canada B3H 4R2

The commissural neuronal circuitry, involved in left-right coordination, has been investigated at a cellular and functional level by combining *in vivo* experiments in cats and *in vitro* electrophysiological experiments with mouse genetics. These experiments identified several genetically distinctive commissural pathways and their possible role in transmitting the activity of central pattern generators (CPG) underlying locomotion from one side of the body to the contralateral side. Investigations with the cat unraveled several crossed reflex pathways, but the role of these pathways during walking and their relation to the commissural pathways identified in mice remained unknown. The overall aim of our study is to characterize crossed reflex pathways in mice that would enable connections to be formed between the crossed reflex data from cat experiments and the more recent commissural CPG pathways from mouse experiments. Here, we describe crossed reflex pathways in mice induced either by proprioceptive or cutaneous afferent signals. We chronically implanted nerve stimulation electrodes to activate cutaneous (Sural nerve) and proprioceptive feedback from flexor (Peroneal nerve) and extensor (Tibial nerve) muscles in fully awake mice during resting and walking on a treadmill. In parallel, electromyogram activities from multiple flexor and extensor muscles of the contralateral leg were recorded to document reflex responses transmitted to the contralateral side of the spinal cord. Electrical stimulation of all three nerves evoked motor responses on the contralateral side of the body in every muscle investigated. All muscles were activated approximately at the same latency, except for the tibialis anterior, which was slightly faster. Moreover, the temporal aspect of this pattern remained similar during resting and walking. The amplitude of flexor and extensor muscle activation in response to sural nerve stimulation was consistently stronger during walking than during resting. However, during proprioceptive nerve stimulation, regardless of whether from flexor or extensor muscle, the extensor muscle response decreased, while flexor activity either slightly increased or remained the same during walking compared to resting. We describe the crossed reflex in mice and show that the amplitude of the flexor and extensor muscle activities, but not the temporal structure of muscle response is modulated during walking compared to resting.

Functional Contribution of Glutamatergic Neurons of the Medullary Reticular Formation to the Motor Control in Mice

Maxime Lemieux and Frédéric Bretzner

Centre de recherche du CHU de Quebec, Department of Psychiatry and
Neurosciences, Universite Laval, Quebec City, Quebec, Canada G1V 4G2

The motor activity of limbs is generated within the spinal cord network but is initiated and modulated by supraspinal centers. Due to its intermediate position between the mesencephalic locomotor region (MLR) and the spinal interneuronal circuit, the medullary reticular formation (MRF) is bound to play a crucial role in the locomotor control. However, the identity of MRF neurons involved in motor control is still unclear. Using optogenetic tools accessible in the mouse, our goal was to study the functional contribution of MRF glutamatergic neurons to motor and locomotor control. Optical probes were inserted chronically into one side of the MRF of VGluT2-cre mice expressing Channelrhodopsin-2, a photo-activated cationic channel. The EMG activity was recorded from both flexors (Tibialis anterior, TA) and extensors (Gastrocnemius lateralis, GL) of the ankle at rest and during locomotion upon a 10 or 80 ms photostimulation. At rest, a 10 ms photostimulation evoked excitatory responses in TA and GL on both sides. As the intensity of photostimulation increased, the failure rate and the latency decreased, whereas the duration and amplitude of the EMG responses increased. Responses were evoked at the lowest threshold and the shortest latency in the ipsilateral TA. During locomotion, a 10 ms photostimulation evoked excitatory responses in TA and inhibitory ones in GL during the stance phase, while it evoked excitatory responses in the GL and either excitatory or no responses in the ipsilateral TA during the swing phase. Inhibitory responses were sometime observed in the contralateral TA. An 80 ms photostimulation increased the duration of the ongoing step cycle, but without influencing the subsequent step cycles. We conclude that glutamatergic neurons from the MRF act on flexor and extensor muscles at rest and influence the locomotor pattern but not the rhythm.

Motor Cortex-directed Movement of the Mystacial Vibrissae through Pre-motor Neurons in the Spinal Trigeminal Nuclei

Nicole Mercer Lindsay¹, Per M. Knutsen², Daniel Gibbs³,
Harvey J. Karten³, and David Kleinfeld⁴

¹Section of Neurobiology, Division of Biological Sciences,
University of California, San Diego, La Jolla, CA 92093

²Section of Physiology, Department of Molecular Medicine,
University of Oslo, Oslo, Norway NO-0317

³Department of Neuroscience, University of California, San Diego,
La Jolla, CA 92093

⁴Department of Physics, University of California, San Diego,
La Jolla, CA 92093

The coordination of orofacial motor actions into a context dependent behavior requires a convergence of reflexive circuits, sensory feedback, and motor commands in brainstem pre-motor circuits. The spinal trigeminal nuclei (SpV) receive primary sensory input and contain pre-motor neurons that project onto facial motor neurons (Takato et al. Neuron 2013; Matthews et al. J Comp Neurol 2015). Here, we use a combination of modern viral techniques to investigate descending projections from vibrissa motor cortex (vMCx) onto SpV pre-motor neurons and their role in controlling movement of the mystacial vibrissae. We have three preliminary results. First, we utilized glycoprotein (G)-deleted rabies virus in combination with cre dependent G in protractive pre-motor populations and found that vMCx projects directly to SpV pre-motor neurons. Second, we used a retrograde lentivirus that expressed ReaChR (Lin et al. Nat Neurosci 2013) together with a scanning laser system to selectively activate SpV-projecting vMCx pyramidal neurons and create a spatial map of induced protractive and retractive vibrissa movement. Third, small, focal injections in vMCx of an anterograde synapse-labeling virus indicate that this cortical innervation is somatotopic, reflecting the sensory organization of SpV. The results of these three experiments illustrate the fine motor map present across trigeminal projecting vMCx neurons. We conclude that SpV pre-motor neurons integrate inputs from vMCx and primary trigeminal afferents, along with those from the extensively studied vibrissa primary somatosensory cortex (Matyas et al. Science 2010). Descending projections from vibrissa motor cortex to SpV likely modulate whisking behavior through control of the set-point of vibrissa position. Grants: NIH/NINDS NS090595 and NS058668; NSF EAGER 201490

Invariant Phase Locking but Decreasing Power in Motor Network Activity in Normal Aging

L. Liu^{1,2}, N. Rosjat^{1,2}, S. Popovych^{1,2}, B.A. Wang², A. Yeldesbay^{1,2}, T.I. Tóth¹, S. Viswanathan^{2,3}, C. Grefkes^{2,3}, G.R. Fink^{2,3}, and S. Daun^{1,2}

¹Heisenberg Research Group of Computational Biology, Department of Animal Physiology, Institute of Zoology, University of Cologne, Germany

²Cognitive Neuroscience, Institute of Neuroscience and Medicine (INM-3), Research Centre Jülich

³Department of Neurology, University Hospital Cologne, Germany

Motor actions are generated by complex interactions of various brain regions. The same brain regions can build various functional networks depending on the action. Identifying the neural signals that encode an action's component (selection, preparation, execution) remains a difficult task. In the current study, we seek to identify common neural markers of movement execution within individual cortical motor regions. Therefore we recorded EEG data from 18 young (22-35 years) and 24 elderly (60-78 years) right-handed healthy subjects as they performed a simple motor task. The task required participants to execute a left or right index finger button press triggered by a visual cue or by an uncued voluntary choice. In both age groups, we found significant phase locking in the delta-theta frequency band (2-7 Hz) in motor areas contralateral to the moving hand prior to movement execution. This phase locking occurred irrespective of how the action was initiated (by a visual cue or noncued) and was not significantly different in strength between the two age groups. In addition, we could show that differences between young and old healthy subjects exist in the amplitude of sensorimotor post movement beta synchronization and that this post-movement beta amplitude is positively correlated with the motor performance, i.e. the accuracy of the motor act, meaning the higher the post-movement beta amplitude, the higher the accuracy of the performance. In summary, our study suggests that phase and amplitude play different roles in the control of a motor act, i.e. during movement preparation and initiation and movement execution and motor performance: Phase locking in the delta-theta frequency band is a trigger of movement initiation but not an indicator of movement performance and it is invariant of age while decreased post-movement beta amplitude seems to be related to deficits in motor performance of old adults.

Role of Muscle Spindle Feedback in the Swing Movement Dynamics and Foot Placement during Walking

William P. Mayer¹, Warren G. Tourtellotte², and Turgay Akay¹

¹Department of Medical Neuroscience, Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4R2

²Department of Pathology and Neurology, Northwestern University,
Chicago, IL 60611

During walking, each step consists of stance and swing phases. During stance, the foot is on the surface and moves from the anterior extreme position (AEP) to the posterior extreme position (PEP). During swing, the foot is in the air and moves from PEP to AEP, where it touches down on the surface and starts the next stance phase. Previous experiments (Akay et al, 2014; PNAS 111:16877) demonstrated that Egr3 knock out mice (Egr3-KO), in which muscle spindles (MSs) are absent, can walk on a treadmill at 0.2m/s with subtle abnormalities. Specifically, it was observed that the activity of the ankle flexor muscle (tibialis anterior, TA) is active throughout the swing phase in contrast to wild types in which TA activity is terminated mid-swing. The prolonged TA activity is expressed in exaggerated lifting of the foot during swing phase. Additionally, Egr3-KO mice are severely compromised when challenged to walk on a horizontal ladder that would require precise foot placement from rung to rung. Here we hypothesize: “the MS feedback is necessary for the precisely timed TA offset required to place the foot in a predefined AEP location at the end of swing phase during walking.” We first measured the average distance of hind leg AEP (HLAEP) distance relative to fore leg PEP (FLPEP) as 4 mm with a standard deviation (SD) of 2 mm in wild type (WT) mice during treadmill walking at 0.2 m/s. This distance did not change, even when the swing was perturbed mechanically (5 mm, SD: 3 mm, $p=0.09$ after t-test) or through electrical stimulation of the saphenous nerve (3 mm, SD: 1 mm, $p=0.21$), both causing stumbling corrective response (SCR). The average HLAEP-FLPEP distance in Egr3-KO mice was not only significantly larger (14 mm, $p<0.001$) but it was also more variable (SD: 5 mm, $p<0.001$ after f-test) than in WT mice during unperturbed walking. When swing phase was perturbed by mechanical stimulation in Egr3-KO mice, the HLAEP-FLPEP distance did not change significantly from unperturbed swing movements in Egr3-KOs, however trajectory of foot movement became more variable. We are currently analyzing the EMG data from SCR experiments with wild type and Egr3-KO mice, to address the question of how the muscle activation pattern is related to the targeting phenomenon. Nevertheless, our data suggest that normal swing movement trajectory during walking and precise foot placement at AEP requires MS feedback.

The Spatial Topography of Cranial Motor Neuron Activity Patterns in Larval Zebrafish Revealed by Confocal and Whole Brain Light-sheet Calcium Imaging

Kimberly L. McArthur and Joseph R. Fetcho
Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

A neuron's location in the developing neuroepithelium both reflects its ontogenetic history and predicts its functional future. Spatial signaling gradients drive differential gene expression, such that specific neuronal subtypes reliably arise in particular brain locations. Spatial location is further refined by neuronal migrations - including some dramatic repositioning of entire populations and more subtle relative positioning within each population. Ultimately, the location of a neuron's cell body and extensions will determine its access to presynaptic and neuromodulatory inputs - though the relationship between location and connectivity may be more or less flexible for specific synaptic partners. To understand the relationship between location and function in the developing hindbrain, we focus on the facial branchiomotor neurons (FBMNs) in larval zebrafish, a group of cranial motor neurons driving respiration and feeding. These neurons adopt a clear age topography early in development and serve as a general model for the relationship between dorsoventral location, age, and function in cranial motor pools. Further, because these neurons execute an early and dramatic caudal migration in wild type animals (but not in specific mutant lines), we have the opportunity to study the impact of abnormal population positioning on circuit architecture throughout the hindbrain. We mapped FBMN activity patterns in three-dimensional space, using confocal and light sheet imaging of genetically encoding calcium indicators. We found a loose functional topography, where FBMNs exhibiting higher-frequency respiratory bursts (in addition to swim-related activity) are concentrated in the ventrolateral portion of the motor nuclei in wild type larvae. These results are consistent with previous backfill data regarding the location of motor pools known to participate in respiratory behaviors. We also probed for neurons that have correlated activity with FBMNs. This revealed neurons with potential functional relationships to the FBMNs, including other motor neurons and potential presynaptic interneurons. This wild type data provides a basis for comparison to whole-brain activity in mutants, to discover to what extent the spatial structure of hindbrain neuronal activity is altered when a critical motor nucleus is abnormally positioned. Leveraging whole-brain calcium imaging in this way, in this specific group of neurons - when combined with single-cell morphology and electrophysiology - will finally allow us to test hypotheses about the degree to which large-scale circuit architecture depends on proper positioning of neuronal populations.

Neuronal Diversity in Substantia Nigra Pars Reticulata Delineates Specialized Basal Ganglia Output Pathways

Lauren McElvain^{1,2,3}, Byungkook Lim¹, David Kleinfeld^{1,2}, and Rui Costa³

¹Neurobiology Section, University of California, San Diego,
La Jolla, CA 92093

²Physics Department, University of California, San Diego,
La Jolla, CA 92093

³Champalimaud Neuroscience Programme, Champalimaud Foundation,
Lisbon, Portugal 1400-38

The basal ganglia play a central role in motor control, but the circuit mechanisms that mediate their effects on the broader motor system remain poorly understood. We interrogated the largest basal ganglia output, the substantia nigra pars reticulata (SNr), to delineate the organization of basal ganglia efferent signaling. Viral tracing methods were combined with electrophysiological recordings to define the unique projection populations in SNr and their downstream targets in the brainstem and thalamus. Anterograde AAV tracing in mice from GABAergic and parvalbumin-positive SNr neurons revealed major projections to the superior colliculus; inferior colliculus; midbrain, pontine, and medullary reticular formations; pedunculopontine nucleus; dorsal raphe; and VM, VA, MD, and Pf thalamic nuclei. To determine whether these regions receive inputs from segregated or overlapping SNr populations, we retrogradely labeled SNr neurons projecting to each structure and assayed their intrinsic electrophysiological properties in vitro. All SNr projection neurons shared several intrinsic features, including sustained fast-firing (> 50 Hz) capabilities and linear firing rate responses to depolarizing currents. However, SNr neurons that projected to brainstem regions exhibited specialized intrinsic characteristics and differed significantly from each other. Brainstem-projecting SNr neurons were topographically organized and, in some cases, morphologically distinct. In contrast, thalamus-projecting SNr neurons exhibited heterogeneous electrophysiological and morphological properties and were distributed throughout the nucleus. In a final set of experiments, intersectional virus strategies mapped the axonal collateralizations from SNr neurons to determine whether projections to thalamus arose from brainstem-projecting SNr neurons. Our experiments delineate specialized populations of SNr projection neurons and demonstrate their specific and extensive axonal collateralization. The results establish a framework for future investigations to link SNr output populations to their motor functions.

Serotonin Reduces Synaptic Efficacy of Myelinated Afferents and Depresses PAD by the Activation of 5-HT_{1B} Receptors in the *In Vitro* Mouse Spinal Cord

David Leonardo Garcia-Ramírez¹, Jonathan Jair Milla-Cruz¹,
Jorge Ramón Calvo¹, Carlos Miguel Villalón², Shawn Hochman³, and
Jorge Noel Quevedo¹

¹Physiology, Biophysics and Neuroscience, CINVESTAV,
Mexico City, Mexico 07360

²Pharmacobiology, CINVESTAV, Mexico City, Mexico 14330

³Department of Physiology, Emory University, Atlanta, GA 30322

We reported previously that *serotonin* (5- hydroxytryptamine; 5-HT) markedly depressed low-threshold afferent stimulation-evoked primary afferent depolarization (PAD) as well as monosynaptic transmission by presynaptic mechanisms in the *in vitro* mouse spinal cord (García-Ramírez et al., PLoS One, 2014). We also reported that the non- selective 5-HT_{1B/1D} receptor agonist, *zolmitriptan*, depressed PAD and monosynaptic transmission evoked by stimulation of myelinated afferents (SFN Abstract 828.11). The aim of the present study was to investigate whether 5-HT_{1B}, 5-HT_{1D} or both receptor subtypes are involved in these actions. Experiments were carried out on the P6-7 sagittally-hemisected mouse lumbar spinal cord with intact nerves for afferent stimulation. Stimulus strength was based on multiples of threshold (xT) of the most excitable fibers recorded from the incoming afferent volley. Peripheral nerves were stimulated at strengths that preferentially recruited myelinated afferents (2 xT). PAD was inferred from dorsal root potentials (DRPs) recorded at L3-L5 dorsal roots while monosynaptic responses were recorded in the deep dorsal horn as intraspinal extracellular field potentials (EFPs), as intracellular excitatory postsynaptic currents (EPSCs), or as excitatory postsynaptic potentials (EPSPs). A paired- pulse protocol assessed homosynaptic EFP depression with conditioning - test intervals between 25 ms – 10 sec. The effects produced by selective agonists for 5-HT_{1B} and 5-HT_{1D} receptors (1 µM) were analyzed. In this respect, the selective rodent 5-HT_{1B} receptor agonist *CP-93,129*, significantly depressed DRPs and EFPs (by 42 and 62% of control, respectively) whereas the 5-HT_{1D} agonist *PNU142633* did not (13 and 4% of control, respectively). *CP-93,129* also depressed EPSCs and EPSPs (by 24 and 62% of control, respectively) and reduced the magnitude of homosynaptic depression (n=5) similar to that seen with *serotonin* and *zolmitriptan*. These results lead us to conclude that 5-HT depresses synaptic efficacy of myelinated afferents via activation of 5-HT_{1B} receptors. Depression of DRPs may be due to a decrease in synaptic transmission of afferent fibers giving PAD, but we cannot exclude effects downstream on the interneuronal pathways mediating PAD.

Modulation of Input from Paw Cutaneous Afferents and Quadriceps-sartorius Stretch Afferents Differentially Affects Lateral Static and Dynamic Stability during Cat Split-belt Locomotion

Hangue Park¹, Ricky Mehta², Stephen P. DeWeerth¹, and Boris I. Prilutsky²

¹School of Electrical and Computer Engineering and

²School of Biological Sciences, Georgia Institute of Technology,
Atlanta, GA 30332

Motion dependent sensory input from paw cutaneous afferents and muscle proprioceptors allow animals to maintain stable locomotion in the changing environment (Bouyer, Rossignol 2003; Akay et al. 2014). The goal of this study is to investigate how modulation of paw cutaneous input and input from stretch sensitive afferents from quadriceps and sartorius muscles affect static and dynamic stability of walking in the cat. Four cats performed 80-s bouts of locomotion on a split-belt treadmill (Berotec, USA) with different speed ratios between left and right belts – 0.4:0.4 m/s, 0.4:0.6 m/s, 0.4:0.8 m/s. These locomotor conditions were repeated while tactile cutaneous input from paw pads of right fore- and hindlimbs was changed by anesthetic injections or/and electrical stimulations of distal tibial or/and sural nerves ($\leq 1.2T$) during right hindpaw contact. In a separate set of experiments, the above experimental conditions were repeated after stretch reflex was removed from the right quadriceps and sartorius by muscle self-reinnervation (Cope et al. 1994). Center of mass (CoM) position of the cat was determined from 3D recordings of 28 markers on the cat body and known inertial body segment parameters (Hoy, Zernicke 1985). Center of pressure (CoP) was derived from paw positions on the treadmill and measured ground reaction forces. Static stability was determined as the shortest distance between the vertical projection of CoM and the boundary of the support area in the lateral direction, whereas the margin of dynamic stability was defined as the distance between CoP and the extrapolated center of mass (xCoP, Hof et al. 2007). With the equal belt speeds, paw anesthesia increased margins of static and dynamic lateral stability due to more lateral placements of the affected paws and shifting CoM in the same direction. Combining paw anesthesia with electrical stimulations of distal tibial or sural nerves tended to reduce these effects. During split-belt locomotion with unequal belt speeds, margins of static lateral stability decreased due to shifting CoM towards the slow moving belt, while margins of dynamic stability did not change. Paw anesthesia and quadriceps-sartorius self-reinnervation increased margins of dynamic lateral stability at all speed ratios owing to more lateral paw placements of the affected limbs. Thus in more challenging locomotor conditions (unequal belt speeds or reduced sensory input), cats adopt motor strategies that allow for increased dynamic lateral stability.

Dopamine Modulates Sensorimotor Transformation in the Optic Tectum

Juan Pérez-Fernández, Andreas A. Kardamakis, Brita Robertson, and Sten Grillner

Department of Neuroscience, Nobel Institute for Neurophysiology,
Karolinska Institutet, Stockholm, Sweden SE-171 77

The optic tectum (superior colliculus in mammals) shows well conserved features through vertebrate evolution, and controls gaze movements through excitatory output neurons projecting to the brainstem. In all vertebrates the optic tectum integrates different sensory modalities (which are species dependent), and when two stimuli from different senses coincide in space and time a response enhancement occurs increasing event detection reliability. In lamprey, visual and electrosensory information is integrated at the level of single output cells of the deep layer, where direct excitatory inputs are combined to reinforce the motor command. Sensory inputs also evoke strong inhibition following the excitatory input, which arises in the superficial layer interneurons and continually resets the output cells allowing for spatial and temporal stimuli discrimination. This sensorimotor transformation is also modulated by dopamine from the nucleus of the posterior tuberculum, the homologous region of the mammalian substantia nigra *pars compacta* (SNc). The dopaminergic system was already well developed at the dawn of vertebrate evolution, and the basal ganglia in general, and the SNc connectivity in particular, are very similar from lamprey to mammals. One striking feature is that the SNc in lamprey, as in mammals, sends direct dopaminergic projections to motor command centers, including the diencephalic and mesencephalic motor regions and the output layer of the optic tectum. Here, we explore how dopamine modulates motor responses in the optic tectum. D1 and D2 receptor expressing cells are two different subpopulations, and dopamine has a differential modulation of their excitability, enhancing the excitability of D1 expressing cells, and decreasing the excitability of those expressing the D2 receptor. This differential modulation affects their responsiveness to the multisensory inputs regulating their motor responses. We have developed a novel preparation keeping the eyes with the brain and rostral segments of the spinal cord, allowing us to apply different visual paradigms coupled to electrophysiological recordings. Local injections of dopamine agonists in the optic tectum have a strong effect in tectal motor responses, including eye movements, but also escape and orienting movements, as reflected in ventral root activity. Our results show that dopamine performs a complex modulation in the optic tectum and, given the high degree of conservation of the basal ganglia and the presence of direct dopaminergic projections from the SNc to the superior colliculus in rats, this previously unexplored mechanism is likely to be present also in mammals.

Serotonin Sensitivity of Spinal Motor Neurons from Hypoxia-Ischemia Rabbit Model of Cerebral Palsy

Katharina Quinlan

Physiology, Northwestern Feinberg School of Medicine, Chicago, IL 60611

Spinal motor neurons show profoundly increased excitability in the presence of 5HT. This excitability has been shown in previously published studies to underlie muscle spasms after spinal cord injury. More recently, an increased presence of serotonin in the spinal cord has been shown in the rabbit hypoxia-ischemia (H-I) model of cerebral palsy as well. Increased hypertonicity of the limb muscles was correlated with increases in serotonin immuno-positive fibers and serotonin concentration (determined by HPLC). Blocking serotonin receptors with intrathecal methysergide *in vivo* was shown to decrease the muscle stiffness of the rabbit kits affected by H-I. However, changes in gene expression levels of 5HT₂ receptors and the 5HT transporter suggest H-I affected spinal cords could be less responsive to serotonin. Thus, this work aimed to determine whether spinal motor neurons are equally responsive to serotonin (specifically 5HT₂ receptor agonists) in control and H-I affected rabbit kits. Spinal motoneurons were targeted for whole cell patch clamp in transverse spinal cord slices from control (sham operated and unaffected kits) and H-I affected rabbit kits between birth and 5 days of age. Lumbar motor neurons from H-I affected kits have higher input resistance (suggestive of smaller cell size), a larger amplitude after-spike after hyperpolarization (AHP), and a smaller current amplitude at firing onset (I-on) than the lumbar motor neurons of control rabbits. Motor neurons typically respond to bath perfusion of 0.3 μ M alpha-methyl 5HT (a 5HT₂ receptor agonist) and 10 μ M citalopram (a selective serotonin reuptake inhibitor) with an increase in input resistance, a reduction of I-on and an increased AHP duration. Preliminary results show H-I affected motor neurons respond differently to 5HT₂ receptor activation than controls. Specifically, H-I motoneurons do not show a change in AHP duration or an increased frequency of spontaneous psp's after 5HT application like control MNs. While H-I motoneurons appear less sensitive to 5HT based on those parameters, other parameters show the opposite: action potentials from H-I motoneurons are dramatically altered by 5HT (including spike overshoot, duration, and rates of rise and fall), while action potentials of control motoneurons are unaffected. In conclusion, lumbar motoneurons from H-I affected rabbits show some baseline properties that align with increased serotonergic tone, and respond differently to exogenous 5HT₂ agonist application. Future work will verify current results and test sensitivity of H-I affected motoneurons to inhibition of serotonergic receptors.

The Modulatory Effects of Caffeine on the Intrinsic Properties of Spinal Lateral Motoneurons: Evidence for its Dependence on Adenosine A1-dopamine D1 Receptor Heteromers

Marla S. Rivera-Oliver, Yocasta Alvarez-Bagnarol, Christian Ayala-Santiago, Estefania Moreno, Garrett Seale, Luis Perez-Portocarrero, Omar Acevedo-Arus, Vicent Casadp, Sergi Ferre, and Manuel Diaz-Rios

¹Department of Biology, Rio Piedras Campus, University of Puerto Rico

²Department of Anatomy and Neurobiology, Medical Sciences Campus, University of Puerto Rico

³Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Spain

⁴Integrative Neurobiology Section, Intramural Research Program, National Institute of Drug Abuse, National Institutes of Health, Bethesda, MD

Caffeine is a known non-selective adenosine receptor antagonist whose actions include multiple sites within the brain, mostly by binding to adenosine A1 and A2A receptors (A1R and A2AR). It produces similar behavioral effects as other classical psychostimulants including increased motor activation, arousal, and having reinforcing effects related to indirect dopaminergic mechanisms that depend on heteromerization of A1R and A2AR receptors with dopamine D1 and D2 receptors (D1R and D2R) in the striatum, respectively. We recently performed extracellular recordings of ventral nerves from the lumbar cord of mice in the presence of serotonin (5-HT), NMDA and dopamine (DA), which are known to elicit locomotor activity in mammals, and showed that caffeine stimulates motor activity by blocking A1R, by potentiating the ability of dopamine to activate D1R. Also, perforated patch clamp recordings in lumbar cord slices showed that the effects of caffeine are specifically targeted at modulating the intrinsic membrane properties of spinal lateral motoneurons (MNs). We then investigated if these properties of caffeine depended on the existence of A1R-D1R heteromers within spinal MNs. Thus, we proceeded to assess the presence of A1R-D1R complexes within spinal lateral MNs using electrophysiological and histological techniques. Basal concentrations of DA or NMDA perfusion in the presence of synaptic blockers of inhibitory and excitatory neurotransmission depolarized the membrane potential of most MNs reversibly. The addition of caffeine, in the presence of DA or NMDA, significantly depolarized the membrane potential, decreased the action potential after-hyperpolarization (AHP) and increased the firing frequency by 90% of the recorded MNs. Also, we were able to support our theory that caffeine exerts its neuromodulatory effects on the spinal lateral MNs via A1R-D1R heteromers when the perfusion of an A1R agonist before a D1R agonist completely blocked the modulatory effects of the D1R agonist. Finally, potential A1R-D1R heteromer in MNs were localized anatomically through immunohistochemistry and with the use of a proximity ligation assay using antibodies directed toward the A1R and the D1R. Our experiments suggest that the primary target for the neuromodulatory effects of caffeine in the lumbar region of the spinal cord are the lateral MNs and that the excitatory effects produced by caffeine onto this neuronal population is dependent on A1R-D1R heteromers.

Differences in Movement-related, Inter-regional Phase-locking in Young and Elderly Healthy Subjects

Nils Rosjat^{1,2}, Svitlana Popovych^{1,2}, Liqing Liu^{1,2}, Bin A. Wang¹, Tibor I. Tóth²,
Christian Grefkes^{1,3}, Gereon R. Fink^{1,3}, and Silvia Daun^{1,2}

¹Cognitive Neuroscience, Institute of Neuroscience and Medicine (INM-3),
Research Center Jülich, Germany 52428

²Heisenberg Research Group of Computational Biology,
Department of Animal Physiology, University of Cologne, Germany 50923

³Department of Neurology, University Hospital Cologne, Germany 50937

The vast majority of motor actions, including their preparation and execution, is the result of a complex interplay of various brain regions. Novel methods in computational neuroscience allow us to assess interregional interactions from time series acquired with *in vivo* techniques like electro-encephalography (EEG). These methods provide different neuronal representations of movement (e.g. ERD, ERS, PLI). However, our knowledge of the functional changes in neural networks during non-pathological aging is relatively poor.

To advance our knowledge on this topic, we recorded EEG (64 channel system) from 18 right-handed healthy young participants (22-35 years, 10 female) and 24 right-handed healthy old participants (60-79 years, 12 female) during a simple motor task. The participants had to execute voluntary low frequency left or right index finger tapping movements.

We used the relative phase-locking value (rPLV) computed from the phases obtained by Morlet wavelet transformation of the Laplacian-referenced EEG data to identify the functional coupling of brain regions during the motor task. We analyzed the connectivity for electrodes lying above the left and right premotor areas (IPM: F3, FC3 and rPM: F4, FC4), supplementary motor area (SMA: Cz, FCz) and the left and right primary motor cortex (IM1: C3, CP3 and rM1: C4, CP4). We compared the resulting networks of significant phase-locking increase in time-intervals prior, during and after the movement.

Our analysis revealed an underlying coupling structure around the movement onset in the δ - θ frequency band (2-7 Hz), only. For young subjects, the connection from SMA to M1 contralateral to the moving hand showed a significant rPLV increase already in the preparatory phase of the movement. This synchronization remained significant during the movement and in a time interval after it. In elderly subjects, however, the change in rPLV between SMA and contralateral M1 was significant only during the execution of the movement. We furthermore monitored the behavioral performance of the two age groups and observed a lower movement speed in the elderly subjects. We therefore suggest that a lateralized rPLV between SMA and M1 prior the movement is needed to accurately initiate and perform the finger movements.

Ionic Currents and their Influence on Rhythm Generation and Coordination

Laura Schläger and Carmen Smarandache-Wellmann

Institute of Zoology, Animal Physiology, University of Cologne,
Cologne, Germany 50674

The function of nervous systems is based on the interaction of neuronal networks. These networks are built of neurons with individual activity patterns that together regulate and coordinate complex movements and behavior. To better understand the properties of such networks, we investigate the crayfish swimmeret system. Swimmerets are four pairs of abdominal limbs that move in alternating power- and return strokes in a metachronal wave from posterior to anterior. In each hemiganglion a similar subset of neurons can be found that drives this movement. Each microcircuit is composed of five interneurons forming the rhythm generating circuit, three coordinating neurons, and 70 motor neurons (MN). When the system is active, all of these neurons show membrane potential oscillations but with distinct activity patterns. Despite our good understanding of the cellular components and synaptic contacts among them, the intrinsic mechanisms that enable the individual activity pattern still remain unknown. Therefore we are interested in the ionic currents underlying the similar yet different activity patterns of the neurons and how they influence rhythm generation and coordination between segments. We performed current clamp recordings with sharp electrodes from dendritic arborizations in the isolated abdominal nervous system of the crayfish, *Pacifastacus leniusculus*. To identify and reveal different ionic currents we bath applied selective ion- channel blockers. After application of channel blockers against the hyperpolarizing activated cation current I_{H} (ZD7288), the high voltage activated calcium current I_{L} (Nifedipine) and the transient potassium current I_{A} (4-AP) we could observe an altered ability of the entire system to produce a steady and coordinated motor rhythm. This led to the conclusion, that the activities of the pattern generating, as well as the coordinating neurons, might be dependent on these currents. To verify this hypothesis cellular properties were started being investigated in a synaptically isolated condition. We detected that some neurons showed the ability to produce a post-inhibitory rebound (PIR). Since PIR has often been shown to be a key mechanism of cells to depolarize upon a phase of inhibition, we wanted to know the ionic basis of the PIR. Despite that none of the investigated neurons showed a sag-potential, likely being induced by I_{H} , the application of the I_{H} current blocker ZD7288 reduced the PIR. The additional application of the I_{L} current blocker Nifedipine completely abolished the PIR. These results suggest a special importance of I_{H} and I_{L} in generating the well-coordinated rhythmic activity of the system by enabling the neurons to produce a post-inhibitory rebound after receiving inhibitory synaptic input.

Movement Feedback Signal Processing in a Curve Walking Stick Insect is Task- and Segment-Specific

Joscha Schmitz, Matthias Gruhn, and Ansgar Büschges

Department of Animal Physiology, University of Cologne, Biocenter Cologne,
Zùlpicher Strasse 47b, Köln, Germany, 50674

Walking animals constantly have to adjust their leg movements to a given motor task. Changes during curve walking are generated by specific modifications in the kinematics of each leg on both sides of the animal: a middle outside leg generates large amplitude, longitudinally directed stance movements, whereas the inside leg generates small amplitude stance movements with marked tibial flexion (Gruhn et al., 2009). In a previous study, Hellekes et al. (2012) showed that such task-specificity in leg stepping kinematics is accompanied by differences in the processing of movement-related feedback on both sides of the curve walking animal. Flexion signals from the Femur-Tibia (FTi-) joint, reported by the femoral chordotonal organ (fCO), induce reinforcement of the *Flexor tibiae* activity more often on the inside than on the outside. In the present study, we asked, if 1) different parameters of tibial movement are processed differently between inside or outside steps, and if 2) the same parameters of tibial movement are processed differently during directional stepping. To answer this, we stimulated the middle leg fCO with a large range of stimulus velocities (150-750deg/s), varying amplitudes of FTi-joint movement (40-100deg), and at varying starting angles (70-150deg) while recording tibial motoneuron and muscle activity in curve walking animals. The frequency of occurrence of reinforcement of tibial motoneuron activity increased with increasing starting angles and decreasing stimulus velocities (cf. Bässler, 1988) for both, the inside and outside leg, while it was unaffected by the amplitude of the FTi-joint excursion. The likelihood for the generation of reinforcement of movement for all three modalities was significantly higher during inside compared to outside steps. The highest probability was found to be 70% for the inside leg condition with an FTi-joint movement amplitude of 100deg, a movement velocity of 150deg/s and a starting angle of 150deg (N=11, n=132). Our results show that the occurrence of movement reinforcement during inside and outside steps caused by fCO flexion signals is in both cases mostly dependent on starting angle and the velocity of the angular movement. However, the thresholds for eliciting the response are drastically lower for the inside leg. It is quite conceivable that during curve stepping such differences in processing of tibial movement signals can support leg kinematics generated on each side. To explore the mechanism behind this difference, we currently perform intracellular recordings from tibial motoneurons and premotor interneurons (cf. Driesang and Büschges, 1996). This work was supported by DFG grant Bu857/14.

Investigation of Neural Circuits in the Zebrafish Hindbrain during Active Locomotion.

Kristen Severi, Urs Boehm, Mario Chavez, and Claire Wyart
Institut du Cerveau et de la Moelle épinière, Paris, France 75013

Locomotion is a complex process relying on motor circuits in the spinal cord that integrate dynamic sensory feedback as well as descending commands from the brain. The hindbrain is a major control center to drive locomotor central pattern generators and perform sensory integration across modalities. In the larval zebrafish, activity patterns in the hindbrain apart from the reticulospinal neurons have been vastly underexplored. To characterize the hindbrain population driving motor output and integrating sensory signals, we perform *in vivo* two-photon calcium imaging in both paralyzed and actively swimming larval zebrafish expressing genetically-encoded calcium indicators under panneuronal drivers. By inducing locomotion with whole-field visual motion and simultaneously recording high-speed video of the moving tail during calcium imaging, we can link tail kinematics to neural signals. These experiments contribute to a functional map of the hindbrain and by comparing active and paralyzed conditions, to explore activity associated with either locomotor drive or mechanosensory feedback mechanisms.

Modulation of Rhythmic Activity in Mammalian Spinal Networks is Dependent on Excitability State

Simon A. Sharples¹ and Patrick J. Whelan^{1,2}

¹Hotchkiss Brain Institute, University of Calgary, AB, Canada T2N 1N4

²Department of Comparative Biology and Experimental Medicine,
University of Calgary, AB, Canada T2N 1N4

Neuromodulators play an important role in activating rhythmically-active motor networks; however, what remains unclear are the network interactions whereby neuromodulators recruit spinal motor networks to produce rhythmic patterns of activity. Evidence from invertebrate systems has demonstrated that the effect of neuromodulators is dependent on the pre-existing state of the network. We explored the role of network excitation state in the generation of rhythmic locomotor activity evoked by dopamine in the neonatal mouse isolated spinal cord. We found that dopamine can evoke unique patterns of motor activity that are dependent on the excitability state of motor networks. Different patterns of rhythmic motor activity ranging from tonic, non-rhythmic activity to multi-rhythmic, non-locomotor activity to locomotor activity were produced by altering global motor network excitability through manipulations of the extracellular potassium and bath NMDA concentration. A similar effect was observed when network excitation was manipulated during an unstable multi-rhythm evoked by a low concentration (15 μ M) of 5-HT – suggesting our results are not neuromodulator specific. We therefore suggest that neuromodulators are capable of evoking locomotor-like patterns of activity, by moving the network through an excitation-based state space. The level of network excitation has important implications and could account for a great deal of variability between preparations and is an additional factor that must be considered when circuit elements are removed from a network to infer cellular function.

Effects of Neural Progenitor Transplantation Following Cervical Spinal Cord Contusion Injury

Victoria Spruance, Kristiina Hormigo, Lyandysha Zholudeva,
Tatiana Bezdudnaya, and Michael Lane
Departments of Neurobiology and Anatomy, Drexel University,
Philadelphia, PA 19104

Impaired breathing is a devastating consequence of cervical spinal cord injury (SCI) that increases morbidity and the risk of mortality. Injuries at high-to-mid cervical levels (C1-4) result in the most severe deficits as the phrenic motor circuitry – controlling the diaphragm – is directly compromised, typically resulting in dependence on assisted-ventilation. While there is mounting evidence for spontaneous respiratory improvement, the extent of recovery – or functional plasticity – remains limited. Thus, there is a need to develop therapeutic strategies for enhancing repair and recovery of respiratory pathways. Our ongoing research aims to elucidate spinal and supraspinal changes that may influence respiration post- SCI, and assess whether treatments can harness ongoing neuroplasticity to improve function post-injury. These studies have identified that spinal interneurons represent a potential therapeutic target for enhancing plasticity and recovery of phrenic motor function. With a particular focus on the phrenic motor system, the goal of the present work is to assess whether transplantation of neural precursor and stem cells (NPCs/NSCs) can facilitate repair of the injured adult rat cervical spinal cord and promote lasting, functional recovery. We hypothesize that spinally derived NPCs, rich in interneuronal precursors, will provide a source of neurons that facilitate a novel neuronal relay capable of restoring input to phrenic motoneurons. Adult, female Sprague-Dawley rats (~250g) received lateralized C3/4 contusions (200 kilodynes, Infinite Horizons Pneumatic Impactor). One week post-injury, NPCs derived from developing rat spinal cord (E13.5 Sprague Dawley or E13.5 Fisher rat, expressing green fluorescent protein) were injected directly into the injury cavity (~1 million cells). Transplanted animals are compared against injured, untreated animals. Four weeks or one year later, a transsynaptic, retrograde tracer (pseudorabies virus) was delivered to the ipsilateral hemidiaphragm or directly into the transplant. Tracing revealed synaptic integration between donor neurons and host phrenic circuitry at one month following transplantation. However, evidence for this connectivity is lost at one year following transplantation. Terminal electrophysiology analysis revealed variable phrenic and diaphragm recovery at both time points in those animals that received NPC transplants following cervical contusion injury. These ongoing studies are providing insight into the therapeutic potential for NPC therapy in the injured spinal cord.

Pkd2l1 Underlies Spontaneous Activity in Intraspinal Sensory Neurons

Jenna Sternberg¹, Lydia Djenoune¹, Andrew Prendergast¹,
Johnathan McDearmid², Hugues Pascal-Mousellard¹, and Claire Wyart¹
¹Sorbonne Universités, Université Pierre and Marie Curie - Paris 06, Inserm,
CNRS, Institut du cerveau et la moelle (ICM) - Hôpital Pitié-Salpêtrière,
Boulevard de l'hôpital, Paris, France 75013
²Department of Biology, College of Medicine,
Biological Sciences and Psychology, University of Leicester,
Leicester, United Kingdom, LE1 7RH

Cerebrospinal fluid-contacting neurons (CSF-cNs) are intraspinal sensory neurons conserved in vertebrates and recently shown to relay mechanical and chemical sensory information locally to motor circuits. CSF-cNs in diverse species express GABA and the transient receptor potential channel TRPP3 or PKD2L1, involved in sour taste. Here we use genetic targeting, calcium imaging, pharmacology, and electrophysiology in the zebrafish to investigate the role of PKD channels in spontaneous activity and sensory function in these neurons. We show that Pkd2l1 forms a complex with Pkd1l2 in CSF-cNs in zebrafish. Calcium imaging showed large long-lasting calcium transients in a subpopulation of CSF-cNs as sensory function begins to appear. These calcium transients reflect high frequency firing and were abolished in a pkd2l1 mutant. We used whole-cell patch clamp recordings to investigate whether differences in frequency of spontaneous firing between the two subpopulations were due to differences in subcellular localization of Pkd2l1 or intrinsic properties. We found that Pkd2l1 opens spontaneously and that current from a single channel opening is sufficient to generate an action potential in all CSF-cNs. Differences in intrinsic and extrinsic properties alter the probability of channel opening in these two subpopulations, thereby conditioning high frequency spiking. Altogether, these data show that a single channel opening can generate activity in GABAergic intraspinal sensory neurons.

Descending Modulation of Thoracic Motor Activity in the Stick Insect

Thomas Stolz, Max Diesner, Susanne Neurpert, and Joachim Schmidt
Department of Neurobiology/Animal Physiology, Biocenter,
University of Cologne, Germany D-50674

Neuromodulators are instrumental in the selection of task-specific motor output in animals. The biogenic amine octopamine is a key modulator of insect thoracic locomotor networks. In inactive stick insects, for example, octopamine alters the response properties of a leg-proprioceptive feedback system towards those that characterize the active state of animals (Büschges et al. 1993). Furthermore, octopamine increases a tonic depolarization, ubiquitous in mesothoracic leg motoneurons during walking (Westmark et al. 2009). Until now, the identity of octopaminergic neurons modulating thoracic motor activity has remained elusive. In insects, octopamine can be released from dorsal unpaired median (DUM) neurons. Six DUM neurons with somata located in the posterior part of the locust subesophageal ganglion have axons that are bilaterally descending (abbreviated DUM-SD) to thoracic ganglia (Bräunig and Burrows, 2004). We hypothesize that presumably homologous neurons in the stick insect might be candidates for the modulation of thoracic motor activity. Using semi-intact preparations and intracellular recordings, we observed the generation of action potentials in DUM-SD neurons during stance phases, when animals were stepping with a single middle leg (N=33) and during restrained six-legged walking (N=6). Mechanical stimulation by passive movement of legs was excitatory to DUM-SD neurons (N=40). In contrast, pharmacologically evoked activity of central pattern generating neurons (CPGs) had no effect on DUM-SD neuron activity (N=14). Thus, the excitatory input to DUM-SD neurons during walking most likely arises from leg sensory organs rather than from coupling to CPG activity. In order to test a possible role of DUM-SD neurons in the modulation of thoracic motor activity, we studied the effect of DUM-SD neuron activity on reflex responses evoked by stimulation of the mesothoracic femoral chordotonal organ (fCO). We observed two major effects: 1. Stimulation of some DUM-SD neurons decreased resistance reflex responses in middle leg extensor tibiae motoneurons (N=10). 2. Spike activity in other DUM-SD neurons induced an increase in extensor tibiae motoneuron activity (N=15). Additionally, it increased the likelihood for the occurrence of assistance reflex responses during fCO stimulation (N=10). Preliminary results of recent experiments using MALDI-TOF MS indicate that the somata of both DUM-SD neurons mediating excitatory as well as inhibitory effects on extensor tibiae motoneuron activity contain octopamine. Thus, individual octopaminergic neurons appear to differentially modulate a specific motor behavior, rather than promoting a general state of arousal. The project is supported by DFG Grant Schm 1084/3-1.

Developmental Origins of Inhibitory Interneuron Diversity in Limb and Thoracic Motor Circuits

L. B. Sweeney¹, J. B. Bikoff², M.I. Gabitto², M. Baek³, S. Brenner-Morton², J. Yang¹, C. Diaz², J. S. Dasen³, T. M. Jessell², and C. R. Kintner¹

¹Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037

²Department of Neuroscience, Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Kavli Institute for Brain Science, Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY 10032

³Department of Neuroscience and Physiology, NYU Neuroscience Institute, New York University School of Medicine, New York, NY 10016

Motor output varies along the rostro-caudal axis of the tetrapod spinal cord. At limb levels, a large number of motor pools are needed to control the alternation of flexor and extensor muscles and produce movement about a joint. At thoracic levels, a smaller number of pools supply muscle groups that support posture, inspiration and expiration. We have examined whether the difference in motor neuron and muscle number at limb and thoracic levels is associated with a similar distinction in interneuron diversity. We used V1 spinal inhibitory interneurons as a means to explore interneuron diversity along the rostro-caudal axis of the mouse spinal cord. V1 interneurons regulate locomotor rhythm, and with V2b interneurons, control many aspects of flexor/extensor alternation. Differential expression of 19 cell type specific transcription factors divides the lumbar V1 population into ~50 distinct subpopulations (Bikoff et al 2016, Gabitto et al 2016). These diverse V1 subpopulations fall into four major clades, with differential synaptic connectivity and electrophysiological properties, suggesting they define distinct microcircuits. We have now examined the expression of these 19 transcription factors at thoracic levels, to define distinctions in V1 subset representation at thoracic and limb levels. Using a Bayesian framework to infer cell type identity, our analysis indicates that the four lumbar V1 clades and many of their subpopulations also exist at thoracic levels. This analysis also detects V1 interneuron subpopulations that are restricted to limb versus thoracic levels, and vice versa. The identification of such restricted V1 subpopulations provides an initial step in defining segment-specific spinal microcircuits. The existence of rostro-caudally restricted V1 subpopulations led us to explore how V1 diversity is generated along an organism's rostro-caudal axis. Is V1 diversity influenced by cell-intrinsic transcriptional programs, cell-extrinsic cues from the surrounding cellular environment, or both? Using the transcription factors that define limb- and thoracic-specific V1 populations as markers, we find that limb- and thoracic-specific differences in V1 interneurons, like motor neurons, require the Hox gene, HoxC9. But in motor neuron deficient Olig2 mutant mice, the rostro-caudal distribution of limb-specific V1s is largely unaffected, supporting a model in which early Hox patterning of the spinal cord specifies the rostro-caudal identity of V1 interneurons, independent of motor neurons. Future work aims to explore the connectivity and function of limb- and thoracic-specific V1 interneuron subpopulations.

Vglut2-expressing Ventral Spinal Cord Neurons Are Essential for Purposeful Motor Activity

Adolf Talpalar, Vanessa Ribeiro-Caldeira, and Ole Kiehn

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden 171 77

Motor functions like reaching, grasping, keeping the upright position, or moving in space are essential for animal behaviour and survival. Descending inputs from the brain interact with spinal circuits to perform purposeful behaviour, like generation of locomotor rhythm and pattern or keeping a correct body posture. Drug-induced rhythmic locomotor-like activity can be produced in excitation-proficient as well as in global glutamatergic deficient (i.e. Vglut2-KO) isolated spinal cord preparations, suggesting that both pure excitatory and inhibitory spinal networks may perform selective forms of motor activity. To further address this issue we used electrophysiology, cellular and molecular methods in perinatal and adult mice with targeted inactivation of glutamatergic cells in the spinal cord. For this we used *Hoxb8::cre* crossed with *Vglut2^{flox/flox}* (*Hoxb8::cre; Vglut2-KO*) mice to produce pups that were devoid of Vglut2 in the spinal cord caudal to cervical segment C4. *Hoxb8::cre; Vglut2-KO* mice are able to breath and survive postnatal life for several days, move their forelimbs but do not show either spontaneous or sensory-evoked motor activity in their hindlimbs (n=22). Like in the global KOs the *in vitro* spinal preparation of perinatal *Hoxb8::cre; Vglut2-KO* mice was able to produce rhythmic activity in the presence of neuro-active drugs. This activity shared properties with global Vglut2-KO mice including higher drugs' concentration threshold for eliciting rhythmic-activity, preserved flexor-extensor and left-right alternation and asynchronous activity in presence of inhibitory blockers (n=12). Adult animals with expression of inhibitory DREADD in spinal Vglut2-positive neurons only showed normal behaviour (n=9). Application of saturating doses of clozapine-N-oxide (CNO) ligand left these animals completely paralysed and unable to keep the upright posture. Partial doses of CNO decreased the spontaneous locomotor-frequency, reduced the distance travelled in an open field, and reduced the maximal reachable speed on a treadmill (n=9). Animals with expression of inhibitory DREADD only in dorsal PAX7-derived spinal neurons showed some signs of ataxia but roughly conserved locomotor properties after a supra-maximal dose of CNO, suggesting that the activity of dorsally located neurons is not essential for locomotor function and that the paralysis is mediated by inhibition of ventral Vglut2-positive spinal neurons. This study shows that ventral Vglut2-expressing spinal neurons are essential for purposeful motor activities including locomotion and postural control.

Dynamic Phase-amplitude Coupling of theta and low-gamma Oscillations in the EEG is Related to Gait Phase

Johanna Wagner, Ramon Martinez-Cancino, Christa Neuper,
Gernot Mueller-Putz, and Scott Makeig
Department of Psychology, University of California, San Diego,
La Jolla, CA 92093

Successfully performing rhythmic movements including walking synchronized to a rhythmic cue sequence, exact temporal prediction and motor execution are essential. It has been proposed that synchronous entrainment of brain activity to external pacing events may play a role in supporting temporal motor regularity. This entrainment has been suggested to optimize timing of action execution by controlling excitability in sensory cortices. In previous work (Wagner et al., 2012, 2014) we have shown that low gamma band activity in the Supplementary Motor Area (SMA, a critical motor planning node) entrains to the gait cycle during steady-state treadmill walking, suggesting a role of these oscillations in the planning of rhythmic movements. To investigate the role of cortical field oscillations in the temporal planning of gait, we performed a study in which we examined the high-density EEG dynamics of participants attempting to step in time to an auditory pacing tone sequence. After periods of steady-state walking, participants had to adapt their step length and rate to shifts in tempo of the pacing stimulus (e.g., following unforeseen shifts to a faster or slower pacing tempo) (Wagner et al., 2016). Analysis revealed that during steady-state walking, cortical field activity projecting to the scalp from one or more sources isolated in or near the SMA entrain to the rhythm of walking in both the (25-40 Hz) low gamma and (4-8 Hz) theta frequency bands, suggesting cross-frequency coupling between these rhythms. To assess this, we tested for coupling between low gamma and theta band activities by testing for coupling between all combinations of phase and amplitude in the two frequency bands. We found that during steady-state walking, ~35-Hz low-gamma band power amplitude is coupled to ~4-Hz theta phase. This coupling is maximal during stance and swing gait cycle phases, but is absent during transitions between these movement phases. We propose that this entrainment and coupling of theta and low gamma oscillations may help generate temporal predictions required for maintaining the temporally precise synchronization of gait to external pacing events.

Investigation of a Mechanosensory Interface Relaying Information from Cerebrospinal Fluid to Motor Circuits

Urs Böhm, Lydia Djenoune, Kevin Fidelin, Andrew Prendergast, Jeff Hubbard,
Jenna Sternberg, Steven Knafo, Laura Desban, Pierre-Luc Bardet, and
Claire Wyart

Department of Physiology, Institut du Cerveau et de la Moelle épinière (ICM),
Paris, France 75013

The cerebrospinal fluid (CSF) is a complex solution circulating around the brain and spinal cord. Behavior has long been known to be influenced by the content and flow of the CSF, but the underlying mechanisms are elusive. CSF-contacting neurons by their location at the interface with the CSF are in ideal position to sense CSF cues and to relay information to the nervous system. By combining electrophysiology, optogenetics and calcium imaging *in vivo* in zebrafish larvae, we demonstrate that neurons contacting the CSF detect local bending of the spinal cord and in turn feed back GABAergic inhibition to multiple interneurons driving locomotion in the ventral spinal cord. This GABAergic feedback modulates target in a state-dependent manner, depending on the fact that the animal is at rest or locomoting. Behaviour analysis of animals deprived of this mechano-sensory pathway reveals a differential contribution to slow and fast locomotions, as well as a role in the control of posture during active locomotion. Altogether, this body of work sheds light on the cellular and network mechanisms enabling sensory-motor integration of cues from the CSF onto motor circuits controlling locomotion and posture in the spinal cord.

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Phase Reduction of an Inter-segmental Network Model of Stick Insect Locomotion

Azamat Yeldesbay^{1,3}, Philip Holmes², Tibor Tóth¹, and Silvia Daun^{1,3}

¹Heisenberg Group of Computational Biology, Department of Animal Physiology, Institute of Zoology, University of Cologne, Cologne, Germany 50674

²Program in Applied and Computational Mathematics, Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544-100

³Institute of Neuroscience and Medicine, Cognitive Neuroscience, Research Center Jülich, Germany 52425

Detailed neuronal network models of animal locomotion are important means to understand the underlying mechanisms that control the coordinated movement of individual limbs. However, the analysis of such systems is a formidable task if they have a large number of variables and parameters. Thus, the complex behavior of the neural network in question can much better be explained by means of a reduced simplified model. In a previous work [Daun-Gruhn & Tóth, J Comp Neuroscience 2011], an inter-segmental network model of stick insect locomotion was constructed based on experimental results. It consists of three segments that correspond to the front, middle and hind leg. This model could reproduce the basic locomotion coordination patterns, such as tri- and tetrapod, and the transitions between them. In this study, we employ phase reduction and averaging theory to this large network model, to reduce the local networks that include the central pattern generators (CPG) and are associated with the protractor-retractor muscle activity of the stick insect. This enables us to analyze of the behavior of the system in a reduced parameters space (3D compared to 60 dimensional). We show that the reduced model reproduces the results of the original model including the transitions between the coordination patterns. By analyzing the interaction of just two coupled phase oscillators, we found that the neighboring segmental CPGs can operate within two distinct regimes – synchronously and asynchronously, depending on the phase shift between the sensory inputs from the extremities and the phases of the individual CPGs. We demonstrate that this is essential to produce different coordination patterns and the transition between them in the reduced model. Additionally, applying averaging theory to the system of phase oscillators we calculated stable fix points – that correspond to stable coordination patterns. We are now going to use these results to build a model based on the same principles for the investigation of 6-legged walking in different animals. We will then be able to compare stick insect and cockroach locomotion.