Abstracts invited speakers

FRIDAY, AUGUST 17th

Rhythm and Pattern Generation

08:30h - 09:00h

I01- **Inhibitory and excitatory synaptic interactions in the pre-Bötzinger and Bötzinger complexes in situ**

Jeffrey Smith[1], Yaroslav Molkov[2], Anke Borgmann[1], Hidehiko Koizumi[1], Ruli Zhang[1], Ilya Rybak[3]
[1]NINDS, NIH, BETHESDA, United States of America
[2]Indiana University - Purdue University, INDIANAPOLIS, United States of America
[3]Drexel University College of Medicine, PHILADELPHIA, PA, United States of America

The brainstem pre-Bötzinger (pre-BötC) and Bötzinger (BötC) complexes contain microcircuits of interneurons that have long been hypothesized to interact and play fundamental roles in generating the three-phase rhythmic pattern of respiratory activity. Phasic excitatory and inhibitory synaptic inputs to these interneurons during the respiratory cycle should reflect interactions between the neuron populations involved in rhythm and inspiratory-expiratory pattern generation. Reconstruction of these synaptic inputs is therefore necessary to understand how these microcircuits operate, and also allows experimental testing of computational models suggesting specific excitatory/inhibitory network mechanisms for rhythm and pattern generation. We applied techniques that allow quantitative reconstruction of postsynaptic conductances at high temporal resolution from sharp microelectrode current-clamp intracellular recordings. Recordings from the main types of pre-BötC (pre-I/I, early-I, aug-I, post-I) and BötC (post-I, aug-E) interneurons were obtained from *in situ* perfused brainstem-spinal cord preparations of juvenile rats, which generate a three-phase respiratory pattern and provide mechanically stable conditions for intracellular recordings in functionally intact brainstem circuits. Our approach provides nearly continuous readouts of inhibitory (Gi) and excitatory (Ge) conductances throughout the respiratory cycle. We will describe the functional circuit architectures of interacting interneuron populations and mechanisms of rhythm/pattern generation suggested by the reconstructed patterns of Ge and Gi in different types of respiratory neurons and discuss them in the context of current conceptual/theoretical models of the microcircuit organization in the pre-BötC and BötC.

09:00h - 09:30h

I02- Respiratory rhythm regularity and central apneas depend on inhibitory mechanisms within the pontine Kölliker-Fuse nucleus

Ana Paula Abdala Sheikh[1], Marie Toward[1], Mathias Dutschmann[2], John Bissonnette[3], Julian Paton[1]
[1]*University of Bristol, BRISTOL, United Kingdom*
[2]*Florey Neurosciences Institutes, University of Melbourne, MELBOURNE, VICTORIA, Australia*
[3]*Ob/Gyn & Cell & Develop Biol, Oregon Health & Science University, PORTLAND, OREGON, United States of America*

Central apneas and respiratory irregularity are a common feature in Rett syndrome (RTT). We used a mouse model of RTT as a strategy to obtain insights into mechanisms essential for respiratory rhythmicity during normal breathing. Previously we showed that, augmenting endogenous GABA in the brainstem of RTT mice markedly reduced the occurrence of central apnoeas (PNAS 107:18208, 2010). Further, we found that, during central apneas, post-inspiratory drive to the upper airways was enhanced in amplitude and duration in RTT females. Since pontine Kölliker-Fuse (KF) drives post-inspiration, suppresses inspiration, and can reset the respiratory oscillator phase, we hypothesized that synaptic inhibition in this nucleus is essential for respiratory rhythm regularity. We found that: (i) RTT mice show a deficiency of GABA synaptic boutons in the KF; (ii) blockade of GABA reuptake in the KF of RTT mice reduced breathing irregularity; (iii) conversely, blockade of $GABA_A$ receptors in the KF of healthy animals mimicked the RTT respiratory phenotype of recurrent central apneas and prolonged post-inspiratory activity; (iv) after blockade of $GABA_A$ receptors in the KF, the correlation between post-inspiratory activity and expiratory cycle length increased from 62% to 99%, indicating enhanced KF control over duration of expiration. Our results imply that reductions in synaptic inhibition within the KF induce rhythm irregularity whereas boosting GABA transmission reduces respiratory arrhythmia in a murine model of Rett syndrome. Our data suggests that manipulation synaptic inhibition in KF may be a clinically important strategy for alleviating the life threatening respiratory disorders in Rett syndrome.

09:30h - 10:00h

I03 - Respiratory rhythm generation: From ball-and-sticks to clouds: 'The Seattle Perspective'

Jan Ramirez, Alfredo Garcia III, Tatiana Malaschenko, Atsushi Doi
University of Washington, Seattle Children's Research Institute, SEATTLE, United States of America

The respiratory network within the pre-Bötzinger complex is sparsely connected. During each cycle different neurons assume the lead: Neurons can discharge early during some and late during other cycles. In this configuration a relatively large number of neurons is required to maintain regularity. Conditions that alter synaptic transmission have significant consequences on the number of active neurons, the regularity, breathing, and the responsiveness to neuromodulation. Another important characteristic is the discharge pattern of neurons in the pre-Bötzinger complex. The discharge patterns could be classified by visual inspection into "Early-I, Ramp-I, Late-I, Post-I, and Expiratory neurons". However, multiarray recordings reveal that there is a gradual transition from one to the other discharge pattern. These types cannot be regarded as distinct classes of neurons. Instead there is a "cloud"of discharge patterns that covers all types of neurons without obvious statistically distinct neuron classes. This presentation will discuss several conditions that significantly alter the configuration and modulatory response of the respiratory network. We conclude that the respiratory network is highly plastic, which allows the organism to adapt to environmental, metabolic and behavioral changes. We propose that existing ball-and stick models do not adequately capture the degree of plasticity that characterizes this network.

10:00h - 10:30h

I04 - Prebotzinger complex: Something new!

Jack Feldman, Wiktor Janczewski, Kaiwen Kam, David Sherman, Jason Worrell
David Geffen School of Medicine at UCLA, LOS ANGELES, United States of America

We will present several new perspectives on the preBötC and generation of respiratory rhythm:

1. Inspiratory drive is broadcast from the preBötC with little or no information specific to the (ultimate) targets.
2. $GABA_A$ergic and glycinergic inhibition in the preBötC (and BötC) is not necessary for normal breathing in adult rats.
3. Microcircuitry in the preBötC plays a critical role in generation of respiratory rhythm. Supported by the National Institutes of Health.

Spinal cord mechanisms

11:00h - 11:30h

I05 - Multiple pathways to spinal respiratory motor facilitation: functional implications

Gordon Mitchell, Erica Dale, Nicole Nichols, Michael Hoffman
University of Wisconsin, MADISON, United States of America

Multiple distinct cellular cascades give rise to phenotypically similar long-lasting phrenic motor facilitation (pMF). Although we do not yet fully understand the functional significance of mechanistic heterogeneity in pMF, diverse mechanisms to reach a similar goal may contribute to robustness in this critical homeostatic system, while still preserving its flexibility. Five distinct pathways to pMF will be discussed. Phrenic long-term facilitation (pLTF) refers to pMF following acute intermittent hypoxia (AIH). pLTF elicited by moderate AIH (~40 mmHg) requires episodic spinal 5-HT2 receptor activation, PKC activity, new BDNF synthesis, TrkB activation and ERK MAP kinase signaling. Since multiple Gq protein coupled metabotropic receptors (5-HT2, a1 receptors) elicit similar pMF, we suggest a common mechanism referred to as the 'Q pathway.' An alternate pMF mechanism ('S pathway') is initiated by spinal Gs protein coupled receptors (A2A, 5-HT7 receptors), and requires PKA activity, new TrkB synthesis and Akt signaling. The S and Q pathways to pMF interact via cross-talk inhibition since spinal A2A receptor inhibition amplifies pLTF following moderate AIH. With severe AIH (PaO_2 ~25 mmHg), the S pathway becomes dominant and spinal A2A receptor inhibition blocks pLTF. Other pMF mechanisms are induced by: 1) VEGF (ERK and Akt dependent); 2) erythropoetin (ERK and Akt dependent); and 3) respiratory inactivity (atypical PKC and TNFa dependent). Regardless of the specific biological role played by each mechanism, we have come to appreciate their implications in the development of novel therapies for disorders of respiratory (and non-respiratory) motor function, including spinal injury and motor neuron disease.

11:30h - 12:00h

I06 - Spinal mechanisms of plasticity following reduced phrenic neural activity

Tracy Baker-Herman
University of Wisconsin, MADISON, United States of America

Phrenic motor neurons must faithfully transmit the brainstem-generated neural drive to breathe to the diaphragm to trigger inspiration. The fundamental question guiding work in our laboratory is: Does a reduction in synaptic inputs to phrenic motor neurons elicit compensatory mechanisms of plasticity to preserve inspiratory motor output? Here, we discuss evidence that withdrawing synaptic inputs to phrenic motor neurons by either global inhibition of respiratory drive (central neural apnea) or by local inhibition of descending fibers to a portion of the phrenic motor pool (unilateral axon conduction block) in anesthetized, ventilated rats elicits a form of spinal plasticity we term inactivity-induced phrenic motor facilitation (iPMF). iPMF is manifested as a rebound increase in phrenic burst amplitude and consists of at least two mechanistically distinct phases: 1) an early, labile phase that requires TNFa-induced atypical PKC (PKCz and/or PKCi/l) activity to transition to a 2) late, stable phase that does not require TNFa or PKCz/i activity to maintain. Collectively, our data suggest that local mechanisms sense and respond to reduced synaptic inputs to phrenic motor neurons. Although the physiological role of iPMF is unknown, we suspect that iPMF represents a compensatory mechanism, assuring adequate motor output in a physiological system where prolonged inactivity ends life.

12:00h - 12:30h

I07 - Chondroitinase ABC and light stimulated recovery of breathing in cervically injured rats also reveals dramatic plasticity of spinal cord circuitry

Warren Alilain[1], Xiang Li[2], Thomas Dick[3], Stefan Herlitze[4], Jerry Silver[3]
[1]MetroHealth Medical Center/Case Western Reserve University, CLEVELAND, United States of America
[2]Columbia University, NEW YORK, United States of America
[3]Case Western Reserve University, CLEVELAND, United States of America
[4]University of Bochum, BOCHUM, Germany

Spinal cord injury is often at the cervical level and can lead to breathing complications and death. Diminished respiratory capacity is due to impairment of the diaphragm. To study these complications and potential therapeutic strategies we utilize the C2 hemisection model of SCI, which disrupts bulbospinal inputs to the phrenic motor nucleus and results in paralysis of the ipsilateral hemidiaphragm. While most of the SCI community is at chronic post-injury states, there are few reparative strategies that have been successful at chronic timepoints. We have shown that expression and photostimulation of the light-sensitive cation channel channelrhodopsin-2 can induce long-lasting recovery of the paralyzed hemidiaphragm at acute stages after injury. Additionally, there is a rapid increase in the expression of the inhibitory chondroitin sulfate proteoglycan containing perineuronal net surrounding phrenic motor neurons. We hypothesized that after infecting spinal neurons in and around the phrenic motor pool to express ChR2, photostimulation would restore respiratory motor function in chronic C2H rats. Indeed, we show that expression of ChR2 and subsequent photostimulation leads to fragmented and chaotic inspiratory activity of the diaphragm. After combining photostimulation with chondroitinase ABC (ChABC) treatment to remove CSPGs and the PNN, there is a profound restoration of rhythmic hemidiaphragmatic activity. These results suggest significant remodeling of respiratory circuitry in the chronically injured animal. Overall, these sets of experiments demonstrate that optogenetics and ChABC treatment can induce recovery in the chronically C2H animal; as well as reveal the changes which take place after injury and the capacity for SC plasticity.

12:30h - 13:00h

I08 - Inflammation impairs spinal respiratory plasticity following acute intermittent hypoxia

Jyoti Watters, Stephanie Smith, Adrianne Huxtable, Gordon Mitchell
University of Wisconsin, MADISON, United States of America

Although inflammation is associated with many clinical disorders that challenge ventilatory control, little is known concerning the impact of inflammation on any component of ventilatory control, such as rhythm generation, chemoreception or respiratory plasticity. We are using two diverse inflammation models to investigate their impact on phrenic long-term facilitation (pLTF), an important model of spinal respiratory plasticity induced by acute intermittent hypoxia: 1) lipopolysaccharide (LPS) and 2) severe intermittent hypoxia (sIH, 2 min episodes, 8 hrs). LPS and sIH elicit distinct inflammatory gene expression profiles in spinal homogenates and isolated microglia, indicating differential cellular responses; in general, microglial effects were more sustained. Although LPS and sIH promote neuroinflammation via independent mechanisms, the non-steroidal anti-inflammatory drug (NSAID) ketoprofen restored pLTF after both inflammatory stimuli, suggesting that common downstream mediators underlie both responses. We are currently testing the hypothesis that p38 MAP kinase activity undermines pLTF because it: 1) is activated by multiple inflammatory stimuli, 2) inactivates key molecules necessary for pLTF, and 3) is targeted by NSAIDs. p38 may represent a convergence point whereby multiple inflammatory stimuli, each with distinct initiating mechanisms, interferes with this form of respiratory plasticity. Ongoing studies are aimed at identifying key inflammatory molecules, their cellular sources and the signaling pathways that impair respiratory plasticity after varied stimuli to systemic and/or CNS inflammation.

Supported by HL111598, NS049033, HL08029, T32 HL007654

Pontine mechanisms

16:00h - 16:30h

I09 - The pontine Kölliker-Fuse nucleus: A bridge for the sensory and behavioural adaptation of a stereotyped respiratory motor pattern

Mathias Dutschmann
Florey Neuroscience Institutes, MELBOURNE, Australia

Breathing is determined by a sequential motor pattern that is generated by a distributed ponto-medullary neural network. Synaptic interactions between and within specific sub-compartments of this central pattern generator (CPG) generate the breathing movements. The Kölliker-Fuse nucleus (KF) mediated gating of postinspiratory (stage 1 expiration) activity during the respiratory cycle is an essential determinant of inspiratory/expiratory phase duration and transition. In my presentation I will reflect on the current understanding of the role of the KF as vital part of the CPG and how its cellular activity linked inspiratory/expiratory phase transition may provide a key pathway for sensory and behavioural adaptation of the respiratory CPG.

16:30h - 17:00h

I10 - Role of the Parabrachial Complex in Respiratory Arousal

Nancy Chamberlin[1], Satvinder Kaur[2], Nigel Pedersen[2], Shigefumi Yokota[2], Clif Saper[2]
[1]BIDMC Harvard Medical School, BOSTON, United States of America
[2]BIDMC, BOSTON, United States of America

The parabrachial complex (PB) has a well established, albeit incompletely characterized, role in respiratory control. Recent evidence suggests that an area that includes the PB is a key component of the ascending reticular activating system (Fuller et al., 2011). We therefore wondered if the PB plays a role in arousal responses to respiratory stimuli. A clinically relevant example of the latter is the arousal that occurs following airway obstruction occurring during sleep in OSA patients. We developed a murine model for OSA arousals that comprises repetitive acute hypercapnic challenges. Mice were chronically instrumented for sleep/wake determination with EEG and EMG electrodes and placed in a plethysmograph chamber ventilated by a gas-mixer. During the light period (mouse sleep period) the gas in the plethysmograph was transiently and repetitively switched from normal air to normoxic hypercapnia (10% CO2) for 30 seconds every 5 minutes. When a hypercapnia trial occurred during sleep, the mice showed EEG desynchronization and acute rises in ventilation. To study the role of glutamatergic PB neurons in these events we focally knocked out the vescicular glutamate transporter, vGluT2, that is expressed in the PB. We used mice with loxP sites surrounding exon 2 (critical for function) of vGluT2 and achieved focal deletion by injection of an AAV vector that induced expression of cre recombinase. We found site-specific attenuation of EEG and ventilatory arousals. Our data are consistent with a role of subsets of PB neurons in both respiratory and EEG arousals.

17:00h - 17:30h

I11 - Site-specific effects on respiratory rhythm and pattern of ibotenic acid injections in the pontine respiratory group of goats

Bert Forster
Medical College of Wisconsin, MILWAUKEE, WISCONSIN, United States of America

We tested the hypothesis that ibotenic acid (IA) injections into the pontine respiratory group (PRG) would disrupt eupneic respiratory rhythm and pattern. In adult goats, cannulas were bilaterally implanted into the rostral pontine tegmental nuclei (RPTN, n=3), the lateral (LPBN, n=4) and medial (MPBN, n=4) parabrachial nuclei, or the Kölliker-Fuse nucleus (KFN, n=4). In the awake state after recovery from surgery, 1 and 10 Âµl injections (one week apart) of IA were made bilaterally through the cannulas. During the first five hours after the injections, breathing frequency increased ($P<0.05$) in RPTN injected goats, pulmonary ventilation increased ($P<0.05$) in LPBN goats, there were not effects on breathing in MPBN goats, and there was a biphasic ventilatory response in KFN injected goats. This biphasic response consisted of a hyperpnea for 30 minutes followed by a prolonged hypopnea and hypoventilation with marked apneas, apneusis-like breathing, and shifts in the temporal relationship between inspiratory flow and diaphragm activity. In the awake state 10-15 hours after the 1 Âµl injection, the number of apneas was greater ($P<0.05$) than during other studies at night. However there were no incidences of terminal apnea. Breathing rhythm and pattern were normal 22 hours after the injections. Subsequent histological analysis revealed that for goats with cannulas implanted into the KFN, there was nearly 50% fewer ($P<0.05$) neurons in all 3 PRG nuclei than in control goats. We conclude that PRG neurons particularly those within the KFN are important determinates of respiratory rhythm and pattern in awake and sleeping goats

17:30h - 18:00h

I12 - Mechanism of bidirectional short-term plasticity of pneumotaxic post-inspiratory drive under vagal and peripheral chemoreceptor inputs

Chi-Sang Poon, Gang Song
Massachusetts Institute of Technology, CAMBRIDGE, United States of America

Recent evidence indicates that the post-inspiratory (post-I) phase of the three-phase respiratory rhythm is dependent not only on the respiratory pattern generator in the ventrolateral medulla but also descending excitation from the Kölliker-Fuse nucleus (KFN) in the dorsolateral pons. A critical question arising is whether such pneumotaxic drive is tonic (null hypothesis) or rhythmic with post-I phase modulation and/or malleable with activity-dependent neural plasticity (alternative hypotheses). Here, we show that the well-known post-hypoxic frequency decline (characterized by abrupt rebound prolongation of expiratory duration following acute hypoxia) is mediated by NMDA receptor-dependent short-term *potentiation* (sensitization) in the KFN that selectively facilitates the post-I phase. This is in contrast to the demonstrated disfacilitation of the expiratory phase by NMDA receptor-dependent short-term *depression* (desensitization) in the KFN that acts to counteract the Hering-Breuer reflex facilitation of the post-I phase during low-intensity high-frequency vagal stimulation. Further, we show that early-expiratory neurons in the KFN are linked to the post-I phase and exhibit NMDA receptor-dependent short-term plasticity consistent with the phase selectivity and plasticity properties of the pneumotaxic drive. These findings shed new light on the organization of the pontomedullary microcircuit that provides a self-adaptive fail-safe mechanism facilitating the inspiratory off-switch independent from vagal feedback.

Plenary lecture 1 by Sten Grillner

18:30h - 19:30h

I13 - Networks in Motion

Sten Grillner
Nobel Institute for Neurophysiology, STOCKHOLM, Sweden

The vertebrate brain controls a great variety of movements through dedicated networks like those controlling respiration, locomotion and eye movements. These networks are to a large degree conserved through the vertebrate phylum. The neural mechanisms underlying the control of goal directed locomotion will be in focus. The propulsive locomotor synergy is controlled from command regions in mesencephalon, which in turn control central pattern generating (CPG) networks in the spinal cord. The presentation, based on the lamprey CNS, will address the intrinsic function of the adaptable CPG that can generate different motor patterns. In addition, I will discuss the tectal mechanisms underlying steering movements, and the control from the lamprey basal ganglia. The mechanism by which different motor programs are selected will be in focus. Our recent findings establish that the structure and function of the basal ganglia are conserved to a surprising degree from the ancient lamprey version to primates. This applies to the input to striatum (pallium, thalamus, dopamine, 5-HT, histamine input), the pallidal structures (GPi, substantia nigra reticulata (SNr), GPe and the subthalamic nucleus) and their output targets, the cellular properties of striatal and pallidal neurons and the effects of an MPTP induced dopamine denervation.

SATURDAY, AUGUST 18th

Modelling

08:30h - 09:00h

I14 - Sodium and calcium mechanisms of rhythmic bursting in the pre-Bötzinger complex

Ilya Rybak[1], Patrick Jasinski[1], Yaroslav Molkov[2], Natalia Shevtsova[1], Jeffrey Smith[3]
[1]*Drexel University College of Medicine, PHILADELPHIA, United States of America*
[2]*Indiana University - Purdue University Indianapolis, INDIANAPOLIS, United States of America*
[3]*NINDS/NIH, BETHESDA, United States of America*

The neural mechanisms generating rhythmic bursting activity in the the pre-Bötzinger complex (pre-BötC) involved in respiratory rhythm generation that persist after blockade of synaptic inhibition remain poorly understood. Experimental studies in medullary slices from neonatal rodents containing the pre-BötC identified two mechanisms that may contribute to generation of rhythmic bursting: one based on the persistent sodium current (*INaP*), and the other involving the voltage-gated calcium current (*ICa*) and/or the calcium-activated nonspecific cation current (*ICAN*), activated by intracellular Ca^{2+} accumulated from extra- and/or intracellular sources. However, the involvement and relative roles of these mechanisms in rhythmic bursting are still under debate. In this theoretical/modelling study we investigated Na^{+}- and Ca^{2+}-dependent bursting generated in single cells and heterogeneous populations of synaptically interconnected excitatory neurons with *INaP* and *ICa* randomly distributed within populations. We analyzed the possible roles of network connections, ionotropic and metabotropic synaptic mechanisms, intracellular Ca^{2+} release, and the Na^{+}/K^{+} pump in rhythmic bursting generated under different conditions. We show that a heterogeneous population of excitatory neurons can operate in different oscillatory regimes with bursting dependent on *INaP* and/or *ICAN*, or independent of both. The oscillatory regime and operating bursting mechanism may depend on the general neuronal excitation, synaptic interactions within the network, and relative expression of particular ionic currents. The existence of multiple oscillatory regimes and their state-dependence may explain different rhythmic activities observed in the pre-BötC circuits under different conditions.

09:00h - 09:30h

I15 - 5-HT receptor-mediated reorganization of the brainstem respiratory network: insights from computational modeling

Natalia Shevtsova[1], Till Manzke[2], Yaroslav Molkov[3], Jeffrey Smith[4], Anne Bischoff[2], Ilya Rybak[1], Diethelm Richter[2]
[1]*Drexel University College of Medicine, PHILADELPHIA, PA, United States of America*
[2]*University of Göttingen, GÖTTINGEN, Germany*
[3]*Indiana University - Purdue University, INDIANAPOLIS, IN, United States of America*
[4]*NINDS/NIH, BETHESDA, MD, United States of America*

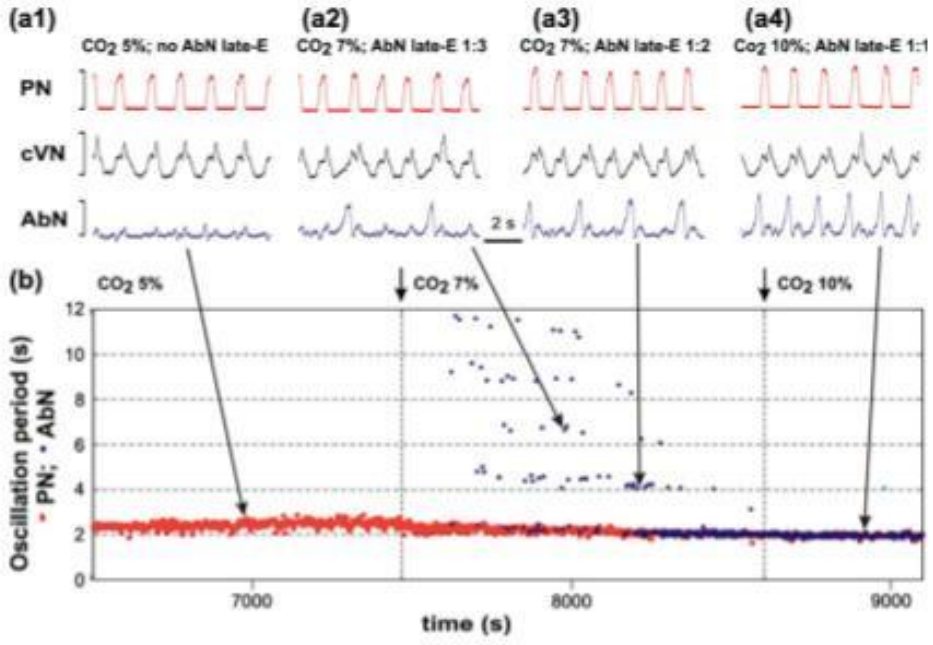
Brainstem respiratory neurons express the glycine α_3 receptor (Glyα_3R), which is a target of several serotonin (5-HT) receptor agonists. Application of the 5-HT_{1A} receptor (5-HT_{1A}R) agonist 8-OH-DPAT was shown to cause a decline of cellular cAMP, leading to dephosphorylation of the Glyα_3R that induces (1) an augmentation of postsynaptic inhibition of neurons expressing Glyα_3R, and (2) a 8-OH-DPAT dose-dependent hyperpolarization of respiratory neurons via increased conductances of serotonin-activated potassium leak channels (Manzke et al. 2010). These processes protect breathing during opioid pharmacotherapy of pain leading to opioid-induced apneas. This counter effect seems to rely on the enhanced Glyα_3R-mediated inhibition of inhibitory neurons causing disinhibition of their target neurons. To evaluate this proposal and investigate neural mechanisms involved, an established computational model of the brainstem respiratory network (Smith et al. 2007) was extended by (1) incorporating distinct subpopulations of inhibitory neurons (glycinergic and GABAergic) and their network synaptic connections within the Bötzinger and pre-Bötzinger complexes, and (2) assigning the 5-HT_{1A}R-Glyα_3R complex to some of these inhibitory neuron types in the network. The model was used to simulate the effects of 8-OH-DPAT on the respiratory pattern and could realistically reproduce a number of experimentally observed responses, including the shift in the onset of post-inspiratory activity to inspiration and the conversion of the eupneic three-phase rhythmic pattern into a two-phase pattern lacking the post-inspiratory phase. The model also proposes a mechanistic explanation for the 5-HT_{1A}R-induced disinhibition of the respiratory network and associated recovery of the respiratory rhythm from opiate-induced apneas.

09:30h - 10:00h

I16 - Fundamental principles can be extracted from reduced models

Jonathan Rubin
University of Pittsburgh, PITTSBURGH, PA, United States of America

A computational model representing a particular biological system can be used to simulate experiments on that system. While this approach allows for systematic manipulation of elements that may be difficult to access experimentally and for rapid performance of a large number of simulated experiments, its utility depends on capturing sufficient biological detail within a model. However, the complexity of a model need not approach that of a biological system for the model to provide useful insights about that system. In this talk, I will illustrate this idea, starting with an example based on the analysis of some general half-center oscillator models. I will next discuss how ideas emerging from the half-center oscillator analysis can offer an explanation for results on quantal recruitment of abdominal nerve discharges with progressive hypercapnia in the *in situ* arterially perfused brainstem-spinal cord of juvenile rat. Interestingly, the model used to provide this explanation is sufficiently reduced to allow for analysis yet also produces biphasic abdominal nerve activity with simulated hypercapnic hypoxia and quantal slowing of inspiratory activity with decreased excitability of preBötzinger complex neurons. Finally, if time permits, I will briefly comment on using simplified models to study synchronized bursting in a network of preBötzinger complex neurons featuring heterogeneity in their intrinsic burst-generation capabilities.



A simple model can explain the invariance of phrenic nerve period despite recruitment of AbN late-E activity with hypercapnia in an in situ prep.

10:00h - 10:30h

I17 - Recent advances in simulations of brainstem respiratory networks modeled from multineuron recordings

Kendall Morris[1], Russell O'Connor[1], Teresa Pitts[2], Donald Bolser[2], Lauren Segers[1], Sarah Nuding[1], Bruce Lindsey[1]
[1]University of South Florida, TAMPA, FLORIDA, United States of America
[2]University of Florida, GAINESVILLE, FLORIDA, United States of America

The long range goal of our research is to delineate brainstem mechanisms for the production and regulation of breathing and airway protective reflexes, such as cough. Our central hypothesis is that the core respiratory network is controlled by neuronal assemblies dynamically organized into regulatory elements required for breathing and the expression of airway defensive behaviors. These behavioral control assemblies are composed of neurons that operate in transiently-configured circuits to process and store information related to the regulation of a given behavior. Our overall approach is to expand and test models for known regulatory features of breathing and the cough reflex, as well as to predict features via simulation. Using arrays of individually-adjustable microelectrodes, we record the extracellular activities of many distinct brainstem neurons together with electromyograms and whole nerve activity in decerebrated or spontaneously breathing, anesthetized cats. Spike train analysis methods are used to identify correlations among the neurons. We iteratively incorporate the inferred functional interactions among the brainstem neuronal populations into computational models. Recent progress includes identification of the spatiotemporal determinants of the cough motor pattern and the effects of baroreceptor input. We have also produced simulations of feed-forward oscillatory networks based on cross-correlation evidence from *in vivo* experiments involving modulation of afferent input. We hypothesize that such feed-forward oscillatory brainstem neural networks act as filters and are involved in both behavior selection and appropriate timing of behaviors in coordination with breathing.

Support: HL89104, NS019814 & HL89071.

Cardio-respiratory coupling
11:00h - 11:30h

I18 - Modeling interactions of respiratory CPG with autonomic nervous system

Yaroslav Molkov[1], Daniel Zoccal[2], David Baekey[3], Benedito Machado[4], Thomas Dick[5], Ilya Rybak[6]
[1]*Indiana University - Purdue University Indianapolis, INDIANAPOLIS, United States of America*
[2]*Federal University of Santa Catarina, FLORIANÓPOLIS, Brazil*
[3]*University of Florida, GAINESVILLE, United States of America*
[4]*Universidade de SÃ£o Paulo, RIBEIRÃO PRETO, Brazil*
[5]*Case Western Reserve University, CLEVELAND, United States of America*
[6]*Drexel University College of Medicine, PHILADELPHIA, United States of America*

Sympathetic nerve activity (SNA) normally exhibits respiratory modulation that suggests the existence of central interactions between the respiratory and sympathetic networks within the brainstem. Hypertension elicited by chronic intermittent hypoxia (CIH) is associated with elevated SNA that displays an enhanced respiratory modulation reflecting a strengthened interaction between the networks. We have developed a computational model of interacting respiratory and sympathetic circuits to investigate the possible mechanisms of sympatho-respiratory interactions and their role in the baroreceptor reflex control of sympathetic activity and in the elevated sympathetic activity following CIH.
We speculate that baroreceptor activation during expiration results in its prolongation via transient activation of post-inspiratory and inhibition of augmenting expiratory neurons of the Bötzinger Complex (BötC). We propose that these BötC neurones are also involved in the respiratory modulation of SNA, and contribute to the respiratory modulation of the sympathetic baroreceptor reflex.
Under hypercapnia expiration becomes active through phasic excitation of abdominal motor nerves (AbN) in late expiration. In rats exposed to CIH, such AbN discharges emerge in normocapnia suggesting that CIH conditioning increases the CO2 sensitivity of central chemoreceptors. We have confirmed experimentally that CIH conditioned rats under normocapnia exhibit synchronized discharges in AbN and tSN similar to those observed in control rats during hypercapnia. Moreover, the hypocapnic threshold for apnea was significantly lowered in CIH-conditioned rats relative to that in control rats. We conclude that CIH may sensitize central chemoreception and that this significantly contributes to the neural impetus for generation of sympathetic activity and hypertension.

11:30h - 12:00h

I19 - Late-expiration and sympathetic overactivity in rats submitted to chronic intermittent hypoxia

Daniel Zoccal[1], Davi Moraes[2], Benedito Machado[3]
[1]*Federal University of Santa Catarina, FLORIANÓPOLIS, Brazil*
[2]*School of Medicine of RibeirÃ£o Preto, University of SÃO Paulo, RIBEIRÃO PRETO, Brazil*
[3]*Department of Physiology, University of SÃO Paulo, RIBEIRÃO PRETO, Brazil*

It is known that chronic intermittent hypoxia (CIH) introduces neural plasticity in the respiratory and sympathetic networks. As a consequence, changes in the mechanisms controlling baseline respiratory and sympathetic activities can be observed after CIH exposure, including exaggerated respiratory and sympathetic chemoreflex responses, enhanced respiratory long-term facilitation and baseline sympathetic overactivity. The latter has been pointed out as a critical factor contributing to the development of arterial hypertension associated with CIH. In our studies, we have been exploring the hypothesis that the enhanced sympathetic drive following CIH exposure is, at least in part, dependent on alterations in the respiratory network and its interaction with the sympathetic nervous system. We verified that juvenile rats exposed to CIH for 10 days exhibited, at baseline conditions, a pattern of active expiration with recruitment of abdominal muscles during late expiratory phase (late-E). Correlated with the emergence of late-E bursts in abdominal activity, we found that sympathetic nerve of CIH rats also displayed additional bursts of activity during late-E, which were eliminated after the reduction of the central respiratory drive with hypocapnia. These abdominal and sympathetic late-E activities of CIH rats were recorded in the absence of peripheral feedback information and were associated with changes in the neuronal activity of respiratory and sympathetic neurons of the ventral medullary surface. Altogether, our data suggest that strengthened central interactions between respiratory and sympathetic neurons contribute to elevate baseline sympathetic activity of CIH rats.

12:00h - 12:30h

I20 - Cardiorespiratory coupling in brainstem parasympathetic cardiac neurons and spinally-projecting RVLM neurons: What we can, and cannot, learn from in-vitro studies

David Mendelowitz, Carie Boychuk, Amanda Woerman, Olga Dergacheva, Xin Wang
George Washington University, WASHINGTON, DC, United States of America

The respiratory system influences autonomic function in a multitude of ways including modulation of cardiovascular parasympathetic and sympathetic activity. This work tests the hypothesis that respiratory modulation of cardiovascular function occurs within the brainstem, and examines how the brainstem cardiorespiratory network responds to challenges such as hypoxia and hypercapnia. To test these hypotheses, an in-vitro brainstem slice that generates fictive rhythmic respiratory motor discharge was utilized, and pre-motor parasympathetic cardioinhibitory vagal neurons (CVNs), and spinally-projecting rostral ventrolateral medulla (RVLM) neurons were identified by retrograde tracers, and their activity and synaptic inputs were studied. The frequency of both GABAergic and glycinergic synaptic events in CVNs significantly increase with each inspiratory burst. This activity was changed in a biphasic manner in which an initial increase was followed by a depression of inhibitory activity with hypoxia/hypercapnia. These mechanisms are most likely responsible for the increase in heart rate that occurs with each inspiration, and the transient tachycardia that is followed by a parasympathetically-mediated bradycardia in response to hypoxia/hypercapnia. In contrast to the homogenous population of CVNs, there are two populations of RVLM-bulbospinal neurons distinguished by spontaneous firing activity. The firing frequency of low discharging RVLM neurons was facilitated by hypoxia/hypercapnia, which depended upon reduced inhibitory neurotransmission. The firing frequency in RVLM neurons with high discharge rates was inhibited, independent of synaptic input, by hypoxia/hypercapnia. In addition RVLM bulbospinal neurons did not have any respiratory modulation under control conditions. In summary, many important, but not all, cardiorespiratory interactions are contained within the brainstem.

12:30h - 13:00h

I21 - Control of the cardiorespiratory system by neuropeptides: echoes and memories

Paul Pilowsky, Melissa Farnham, Angelina Fong
Macquarie University, SYDNEY, Australia

All biological systems require a series of mechanisms that permit short-term adjustment to stimuli, long-term adjustment to stimuli and permanent adjustment. A possible name for this sequence of adaptations could be the 'temporalome' the most familiar of which is immunisation which may be short-lasting or permanent depending on the antigen. In respiration, responses to brief hypoxia normally return to control levels within seconds, but repetitive activation leads to an adaptation known as long-term facilitation that may last for hours. We now report that activation of afferent mechanisms can also evoke long term facilitation in sympathetic pathways. Long term facilitation can also be evoked by intermittent application of peptides that are present in presympathetic pathways, and blocked with their antagonists. Underlying mechanisms will be discussed.

Respiratory control in humans

16:00h - 16:30h

I22 - Emotional Breathing in Humans

Ikuo Homma, Yuri Masaoka
Showa Univ. School of Med., TOKYO, Japan

Breathing pattern is not only generated by metabolic demand, but also generated by various emotional changes. Many respiratory studies showed that emotions can alter respiratory patterns (Boiten et al. 1994, Homma and Masaoka 2008). We showed that the respiratory rhythm increased during anticipatory anxiety and this increased respiratory frequency was correlated with the trait anxiety scores in human. The localization of the brain activity was examined by the EEG dipole tracing method. EEG was simultaneously recorded with respiratory movement. As a result, brain activity was observed in the amygdala and was synchronized with the respiratory rhythm. Respiratory related activity was also observed in the amygdala in the limbic-brainstem-spinal cord preparation of new born rat. These relationships between emotions and respiratory rhythms were examined in various situations. For example, both anxiety scores and respiratory rhythms of subjects decreased after performing 'ikebana', a Japanese traditional flower arrangement. Another example is when breathlessness occurred in subjects who were asked to observe breathlessness in another person. The observers with high trait anxiety felt more breathlessness accompanied by an increase in respiratory rate. This type of empathy may be explained through the close relationship between emotion and breathing.

16:30h - 17:00h

I23 - Discharge properties of upper airway dilator muscle motor units during wakefulness and sleep

John Trinder
University of Melbourne, MELBOURNE, Australia

Obstructive Sleep Apnea is partly due to insufficient activity in upper airway muscles (UAMs) during sleep. Thus understanding the motor control of these muscles is of considerable importance. The control system for upper airway motor neurons is complex, as motor neurons in human UAMs have a variety of discharge patterns. Within a muscle, motor neurons vary as to whether they are tonic, phasic or both, and if phasic, whether they are inspiratory or expiratory phasic. Further, within the respiratory system pre-motor inputs to motor nuclei vary according to sleep-wake state, chemical and mechanical stimuli and voluntary manipulations. We have studied upper airway motor neurons by measuring motor unit activity in Genioglossus (GG) and Tensor Palatini (TP) muscles using mono-polar, thin wire, intra-muscular electrodes, while manipulating sleep-wake state, arterial CO2 and inspiratory loading. Our findings indicate that both muscles have the full range of motor unit discharge patterns, with GG and TP having a slight preference for inspiratory modulated and tonic units respectively. At sleep onset approximately 50% of inspiratory GG units become silent, while in TP both inspiratory and expiratory units tend to cease activity. Arousal from sleep, hypercapnia and inspiratory loading all result in the recruitment of inspiratory modulated units, with subtle modifications of tonic units. Changes in muscle activity are associated with recruitment-derecruitment rather than rate coding. Finally, studies of common drive in both GG and TP identified an inspiratory pre-motor component that is common to the two muscles and tonic components that are idiosyncratic to each muscle.

17:00h - 17:30h

I24 - What determines the output of human inspiratory motoneurones?

Jane Butler
Neuroscience Research Australia, RANDWICK, Australia

During eupnoea neural drive to inspiratory 'pump' motoneurone pools is via direct and indirect bulbospinal pathways. However, the timing and level of activation of these pools differs, which suggests a complex organisation of premotoneuronal drive. For the intercostal muscles, there appears to be a principle of 'neuromechanical' matching in which muscles with a high mechanical advantage for the development of inspiratory pressure are activated preferentially. Evidence for this principle derives from both animal and human studies (for review De Troyer *et al* [2005] Physiol Rev 85: 717-56; Butler & Gandevia [2008] 586: 1257-64). This 'neuromechanical' principle of recruitment is presumably superimposed on Henneman's size principle of motoneurone recruitment. Our recent work indicates the pattern of differential activation of inspiratory intercostal motoneurones is similar in both voluntary and eupnoeic breathing. The underlying mechanism for this pattern of recruitment is not known but it may involve a pre-motoneuronal network at the level of the spinal cord. The inspiratory muscles are also capable of performing non-respiratory voluntary tasks such as trunk rotation. In such tasks, integration of voluntary and involuntary neural drives onto the motoneurones may also occur at a spinal cord.

17:30h - 18:00h

I25 - Manipulation of Cardiorespiratory Performance in Humans by Neurosurgery

Jonathan Hyam, David Paterson, Tipu Aziz, Alexander Green
University of Oxford, OXFORD, United Kingdom

Introduction: Deep brain stimulation (DBS) of subcortical brain areas such as the periaqueductal grey (PAG) and subthalamic nucleus (STN) has been shown to alter cardiovascular autonomic performance. The supramedullary circuitry controlling respiratory airways is not well defined and has not been tested in humans.
Methods: Patients with in-dwelling deep brain electrodes for movement disorders or chronic pain underwent spirometry as per the European Respiratory Society guidelines. Testing was performed three times On and three times Off stimulation, randomly, with patients blinded. Ten PAG and ten STN patients were tested. To control for confounding pain and movement disorder relief, the sensory thalamus (seven patients) and globus pallidus interna (ten patients), respectively, were also tested. Nine patients with PPN stimulation were also studied and local field potential recordings made simultaneously.
Results: Peak expiratory flow rate (PEFR) significantly increased with PAG and STN stimulation by up to 14% ($p=0.021$ and 0.005, respectively, using paired samples Student's t-tests). Stimulation of control nuclei produced no significant PEFR change. Similarly, changes in thoracic diameter reflecting skeletal activity rather than airway calibre did not correlate with the improvement in PEFR. Forced expiratory volume in 1 second was unchanged by stimulation. PPN stimulation not only increased PEFR by 14% but alpha oscillations were associated with spirometry manoeuvres.
Conclusions: DBS can improve PEFR in chronic pain and movement disorder patients. A neurophysiological signal is associated with these manoeuvres. This finding provides insights into the neural modulation of respiratory performance and may explain some of the subjective benefits of DBS.

Plenary lecture 2 by Diethelm Richter

18:30h - 19:30h

I26 - Significance of a glycinergic connectome in respiratory control: An access to translational medicine?

Diethlem Richter
University of Goettingen, GOETTINGEN, Germany

The lecture will present evidence that under in vivo conditions it is the synaptic interaction within the respiratory network that produces various forms of stable rhythm. The underlying processes depend on the molecular machinery determining the organization and operation of its connectomes. These processes provide a good rationale for translational application in medicine.

SUNDAY, AUGUST 19th

Development & Genetics

08:30h - 09:00h

I27 - Development of respiratory rhythm generating circuits in the mouse
Gilles Fortin
CNRS, GIF SUR YVETTE, France

We breathe roughly half a billion times in a lifetime, generally in an effortless and even unconscious manner owing to activity of a respiratory central pattern generator (CPG) located in the hindbrain. The respiratory CPG relies on the coupling of two prominent rhythmogenic sites located in the medulla, the pre-Bötzinger Complex (preBötC) and the para-Facial Respiratory Group (pFRG). Working in the mouse embryo, we have identified the emergence of forerunning versions of these two oscillators using developmental genetics tools, electrophysiological and optical recordings. We have defined molecular and functional signatures for cells composing each oscillator. More precisely, data will be presented showing the independent developments of (i) an Egr2- (also known as Krox20-) derived, Phox2b/Lbx1/Atoh1-expressing embryonic parafacial oscillator and (ii) a Dbx1-derived population of glutamatergic interneurons required for both preBötC rhythm generation and bilateral synchrony through Robo3-dependent axonal commissural pathfinding. These results indicate that each oscillator is not assembled from cells of disparate origins. Rather, each oscillator is made of cells with selective built-in functional properties that derive from a discrete transcriptionally defined domain of the neuroepithelium. Hence, the dual organisation of the respiratory CPG seems to reflect the modular origin of its composing cells. Linking cellular identity to network assembly and function requires an inspection of neural connectivity. I will present preliminary data suggesting that two novel techniques, holographic laser stimulation and viral transynaptic tracing, may help in this endeavour.

09:00h - 09:30h

I28 - Genetic control of coordination between respiratory unit burst oscillators

Paul Gray
Washington University School of Medicine, ST. LOUIS, MO, United States of America

Breathing needs to coordinate the timing and activity of multiple muscle groups. It is proposed simple rhythms are produced by segmentally organized "unit burst oscillators"that generate coordinated rhythms. Whether this organization is conserved in breathing is unknown. Isolated perinatal mouse hindbrain/spinal cord preparations generate independent, but strongly coupled, inspiratory(I) and expiratory(E) output from cervical and lumbar motor roots. It is hypothesized expiratory rhythm is generated by pontine Atoh1-dependent neurons. Here we show that I/E temporal coordination requires Atoh1 dependent neurons, but that they are not necessary for rhythmogenesis. We tested the obligatory role of Dbx1 and Atoh1 dependent neurons in the generation and coupling of coordinated respiratory output. Dbx1, but not Atoh1, dependent neurons are necessary for both inspiratory and expiratory rhythm, *in vitro*. Without Atoh1, however, normal I/E temporal lags were lost. Further, I/E oscillators partially decouple after peptidergic modulation. We propose the respiratory network underlying complex respiratory behaviors consists of independent oscillators whose coupling is controlled by Atoh1-dependent excitatory neurons that play no role in rhythm generation. We propose these data are consistent with proposed segmental oscillator models of simple behavior but provide new insights into the neural mechanisms for the generation of complex behaviors.

09:30h - 10:00h

I29 - Breathing with and without Phox2b

Jean-François Brunet
Ecole normale supérieure, PARIS, France

Mutations in the neuron type-specific homeogene *Phox2b* are causative for central congenital hypoventilation syndrome (CCHS). We have shown that introducing one of the most common human mutations in mouse leads to a CCHS-like respiratory syndrome, including apneas, arythmia, and unresponsiveness to a hypercapnic challenge "'and neonatal death. While the mutation preserves many *Phox2b*-positive neural structures, it destroys the retrotrapezoid nucleus (RTN), a group of neurons at the ventral surface of the facial motor nucleus. Electrophysiological studies have implicated the RTN in central CO2 sensitivity and perinatal entrainment of the main respiratory pacemaker. Altogether, these data point to the RTN as a major culprit in CCHS pathogenesis. However, we recently found that spatially limited expression of the *Phox2b27Ala* allele can be compatible with life, while nevertheless destroying the RTN and abolishing the perinatal hypercapnic response. Thus, the central chemoreflex is dispensable for neonatal life and defects in *Phox2b*-expressing neurons outside the RTN contribute to the full CCHS-like syndrome in mouse.

Upper airway reflexes

11:00h - 11:30h

I30 - Short-term potentiation of cranial motor outputs during rhythmic swallowing: evidence for a modulation by nitric oxide

Christian Gestreau[1], Théodora De la Pöeze d'Harambure[1], Stéphane Obled[2], Michelle Bévengut[1], Sébastien Zanella[1]
[1]CRN2M CNRS 7286 Aix-Marseille University, MARSEILLE, France
[2]CHU Nîmes, NÃŽMES, France

Swallowing propels the food from the mouth to the stomach according to a stereotyped motor sequence under control of the brainstem swallowing central pattern generator. Repetitive stimulation of sensory afferent fibers from the pharyngeal cavity can trigger rhythmic swallowing, but qualitative changes that eventually occur in motor output to tongue, pharyngeal or laryngeal muscles and which may reflect neural plasticity have not been studied. Here, we report various experimental conditions in the rat where repetitive stimulation induced a marked and progressive increase in motor output to upper airways (UA) during rhythmic swallowing. We identified several factors contributing to this augmentation of neural drive to UA muscles. Among them, sensory feedback from UA muscles plays an important but not exclusive role, as the augmentation is not fully eliminated in paralyzed and ventilated rats. We also compared the strength of swallowing-related muscle or nerve activity when two series of stimulation were separated by a variable period. Stronger swallows could be evoked by the second series more than three minutes upon completion of the first series of stimulation, revealing a form of memory corresponding to short-term potentiation. I will end my presentation with preliminary pharmacological data suggesting Nitric Oxide modulates this neural plasticity.

11:30h - 12:00h

I31 - Breathing on the edge: harnessing noise to promote stability

David Paydarfar
University of Massachusetts Medical School, WORCESTER, United States of America

Abnormal neural oscillations are implicated in certain disease states, for example repetitive firing of injured axons evoking painful paresthesia, and rhythmic discharges of cortical neurons in patients with epilepsy. In other clinical conditions, the pathological state manifests as a vulnerability of an oscillator to switch off, for example prolonged pauses in automatic breathing commonly observed in preterm infants. I will present theory and experimental observations on the initiation and termination of neural rhythms at the cellular, tissue and organismal levels. The findings suggest how small appropriately tuned noisy inputs could silence a neural oscillator or, conversely, could promote rhythmic activity. Noise-sensitive neurons have intrinsic properties that yield interesting physiological properties on the edge of a bifurcation, affording remarkable adaptive capacities to circuits that require rapid and efficient on-off switching; between multiple modes of activity (e.g., quiescence, repetitive firing, bursting) and between multiple functions (e.g., breathing, swallowing, coughing, and vocalization). I will illustrate the therapeutic potential of stochastic stimulation for promoting stability of breathing and preventing central apnea in preterm infants.

12:00h - 12:30h

I32 - Central regulation of airway protection

Donald Bolser[1], Teresa Pitts[1], Ivan Poliacek[2], Paul Davenport[1], Bruce Lindsey[3], Kendall Morris[3]
[1]*University of Florida, GAINESVILLE, United States of America*
[2]*Institute for Medical Biophysics, Comenius University, MARTIN, Slovakia*
[3]*University of South Florida, TAMPA, United States of America*

A variety of neuromuscular diseases result in impaired airway protective behaviors, such as disordered cough (dystussia) and/or swallow (dysphagia), resulting in increased risk of aspiration. Our research has evolved from a focus on the pharmacology of cough to investigation of the fundamental mechanisms of brainstem circuits responsible for the production and coordination of airway protective behaviors in mammals. The long term goal is understand the operational features, identity, and specific neural mechanisms that regulate and coordinate the occurrence of cough and swallow to optimize airway protection. Our central hypothesis is that the 'core' cough/respiratory network is reconfigured by neuronal assemblies dynamically organized into regulatory elements necessary for the expression of airway defensive behaviors (behavioral control assemblies-BCAs). We have identified several functional brainstem mechanisms that regulate coordination of cough and swallow to reduce the risk of aspiration. These control mechanisms include a) *phase restriction* of the occurrence of swallow during repetitive coughing, and b) prolongation of the cough E_2 phase to allow the execution of swallow. These mechanisms represent cooperation between different pattern generators to reduce co-expression of airway protective behaviors. We also speculate that neuronal assemblies (BCAs) in the medial reticular formation and midline raphÃ© nuclei participate in these regulatory functions. Predictions regarding coordination of cough and swallow by the cough/respiratory control network are being generated by computational models. Simulations of a revised brainstem respiratory control network show the feasibility of developing a unified computational model of breathing, swallowing, and coughing.

Support: NIH NS19814, HL89104, HL89071, and HL103415.

12:30h - 13:00h

I33 - Swallow reconfiguration of respiratory neural pattern.

Paul Davenport[1], Donald Bolser[1], Teresa Pitts[1], Ivan Poliacek[1], Bruce Lindsey[2], Kendall Morris[2]
[1]University of Florida, GAINESVILLE, FLORIDA, United States of America
[2]University of South Florida, TAMPA, FLORIDA, United States of America

The function of swallow is to move a bolus from the oral cavity through the pharynx to the esophagus. To prevent aspiration, swallowing requires reconfiguration of breathing patterns. The fundamental element of the pharyngeal phase swallow-breathing pattern is inhibition of inspiration until the bolus passes the laryngeal opening and enters the esophagus. Failure of swallow-respiratory coordination results in food material aspirating and penetrating the airway (dysphagia). The stereotypical motor pattern for the pharyngeal swallow phase is characterized by conversion of the respiratory motor pattern into a "swallow-breathing"pattern. Swallow and respiratory central pattern generators (sCPG, rCPG, respectively) share breathing-modulated elements. We reasoned that afferent stimulation that elicits pharyngeal swallow will recruit non-respiratory neurons and retask specific populations of respiratory neurons to reconfigure the respiratory neural network to produce a swallow breathing pattern. Non-respiratory neurons were recruited during induction of pharyngeal swallow. Specific groups of expiratory and inspiratory neurons were facilitated or inhibited during swallow suggesting retasking of these respiratory neuronal elements. The recruitment and retasking of brainstem neural elements during pharyngeal swallow suggests reconfiguration of the brainstem respiratory neural network to produce the swallow breathing pattern. We propose that the swallow-respiratory pattern is controlled by neuronal assemblies dynamically organized into regulatory elements required for the expression of airway defensive behaviors. These behavioral control assemblies for swallow are composed of recruited sCPG neurons that exhibit functional interactions with the rCPG and reconfigure the respiratory neural network to generate the swallow-breathing pattern.

Support: NIH HL109025.

Chemosensation

16:00h - 16:30h

I34 - 5-HT chemoreception and sudden death in epilepsy

George Richerson[1], Gordon Buchanan[2]
[1]*University of Iowa, IOWA CITY, United States of America*
[2]*Yale University, NEW HAVEN, United States of America*

Sudden unexplained death in epilepsy (SUDEP) is the most common cause of death in epilepsy patients. Cardiac and respiratory causes have been implicated.. Apnea occurs during 51% of seizures in humans. 5-HT dysfunction has been implicated in the pathophysiology of SUDEP. Here we studied seizures in *Lmx1bf/f/p* mice, in which nearly all 5-HT neurons have been genetically deleted. Seizures were induced via graded pilocarpine or electroshock. All mice experienced seizures following the first dose of pilocarpine. 75% of *Lmx1bf/f/p* mice experienced grade 3 seizures following the first dose, whereas no WT mice did. Grade 4 seizures progressed to status epilepticus in all animals. In the post-ictal period following prolonged seizures *Lmx1bf/f/p* mice displayed profound reduction of respiratory rate and irregular rhythm. All *Lmx1bf/f/p* mice and 75% of WT mice progressed to death. Seizures induced by electroshock were relatively short. *Lmx1bf/f/p* mice experienced seizures at lower stimulus intensity than WT mice. In both genotypes, the tonic phase was accompanied by respiratory arrest. Seizures were more severe across stimulus intensities in *Lmx1bf/f/p* mice compared to WT mice. Seizure-related mortality increased with increasing stimulus intensity and was greater in *Lmx1bf/f/p* mice compared to WT mice. Following seizures that ultimately resulted in death, there was immediate flattening of EEG activity, lack of recovery of breathing, and persistence of cardiac activity for up to 6 min following terminal respiration. These results indicate that respiratory mechanisms are more directly responsible for death than cardiac arrest and that 5-HT neuron dysfunction leads to post-ictal breathing abnormalities.

16:30h - 17:00h

I35 - KCNQ channels determine serotonergic modulation of ventral surface chemoreceptors and respiratory drive

Daniel Mulkey[1], Joanna Hawryluk[1], Ana Takakura[2], Thiago Moreira[2], Anastasios Tzingounis[1]
[1]*University of Connecticut, STORRS, United States of America*
[2]*University of SÃO Paulo, SÃO PAULO, Brazil*

Chemosensitive neurons in the retrotrapezoid nucleus (RTN) regulate breathing in response to CO_2/H^+ changes. Their activity is also sensitive to neuromodulatory inputs from multiple respiratory centers, and thus they serve as a key nexus of respiratory control. However, molecular mechanisms that control their activity and susceptibility to neuromodulation are completely unknown. Here, we show *in vitro* and *in vivo* that KCNQ channels are critical determinants of RTN neural activity. In the acute brain slices we show that KCNQ channels set basal firing rate of RTN chemoreceptors and mediate their responsiveness to serotonin. Further, we show that blocking KCNQ channels in the RTN *in vivo* blunts the ventilatory response to serotonin in awake and anesthetized animals. These results indicate that KCNQ channels are essential downstream effectors of serotonin in the RTN. Given that serotonergic dysfunction may contribute to respiratory failure, our findings suggest a new therapeutic avenue for respiratory complications associated with multiple neurological disorders.

17:00h - 17:30h

I36 - Astrocytes as functional brain pH sensors

Alexander Gourine[1], Sergey Kasparov[2]
[1]*University College London, LONDON, United Kingdom*
[2]*University of Bristol, BRISTOL, United Kingdom*

Astrocytes are the most abundant type of brain glial cells. By having contacts with cerebral vasculature as well as multiple neurons, astrocytes are in a position to 'taste' the chemical composition of the arterial blood entering the brain and integrate this information with that of brain parenchyma. Does it reflect their functional importance for the operation of brain interoceptors, which monitor key homeostatic parameters including pH, PCO_2 and possibly PO_2 levels? We found that astrocytes which reside within the 'classical' brainstem chemoreceptor areas located near the ventral surface of the medulla oblongata are highly chemosensitive. They respond to small physiological decreases in pH with vigorous elevations in intracellular Ca^{2+} and release of ATP. ATP spreads astroglial Ca^{2+} excitation within the neuropil, activates key chemoreceptor neurons and respiratory neurons of the medullary rhythm-generating circuits and induces adaptive increases in breathing. Mimicking astroglial pH-evoked Ca^{2+} responses by selective light stimulation of astrocytes expressing channelrhodopsin-2 activates chemoreceptor neurons via ATP-dependent mechanism and triggers robust respiratory responses *in vivo*. Thus, medullary astrocytes appear to be highly sensitive to physiological chemosensory challenges and have the ability to impart chemosensory information onto a modified pattern of cardiorespiratory activity.

17:30h - 18:00h

I37 - Carotid body chemoreceptors in integrative chemoreflexes: sometimes they are hot, sometimes they are not

Richard Wilson[1], Arijit Roy[1], Marie-Noëlle Fiamma[2]
[1]*University of Calgary, CALGARY, Canada*
[2]*Laboratoire ER 10 UPMC, PARIS, France*

Inputs from the principle peripheral respiratory and autonomic chemoreceptors, the carotid bodies, and the central respiratory chemoreceptors in the brainstem converge within the central cardiorespiratory control system to regulate breathing and autonomic function. Huge strides have been made in understanding the cellular identity of central chemoreceptors and the mechanism of O_2 sensing in the carotid body. Yet, a number of fundamental issues related to the integration of central and peripheral chemosensitivity remain contentious. These include:

1. The role played by peripheral chemoreceptors in exercise hyperpnoea and CO_2 chemosensitivity.
2. The mathematical nature of peripheral and central chemoreceptor integration.

Here we examine the carotid bodies in an integrative context, reviewing recent data that address these contentious issues. We suggest that the carotid bodies are sometimes hot: they can be demigod chemoreceptors capable of sustaining breathing in the absence of central chemoreceptors and under some conditions may be capable of detecting changes in metabolism in the absence of changes in blood gases. But other times they are not hot, rather they sub-serve central CO_2 chemoreceptors through sympathetic and parasympathetic reflex loops.

Plenary lecture 3 by Gert Holstege

18:30h - 19:30h

I38 - Respiration is one of the tools for survival of the individual and survival of the species

Gert Holstege
UMCG, GRONINGEN, Netherlands

A living organism has only two goals, survival of the individual and survival of the species. In order to achieve these goals the organism has the disposal of organs and muscles. They need to be activated in such a way that their actions fit this goal. The smooth muscles of the various organs are controlled by hormones released in the bloodstream, as well as by sympathetic and parasympathetic preganglionic motoneurons. The striated muscles are controlled by somatic motoneurons. In order to produce effective behavior, hormonal and motoneuronal stimulations are coordinated by cell groups in brainstem and higher brain regions.
Respiration, coughing, sneezing, micturition, defecation, ejaculation, parturition and vocalization need precise control of pressure in thorax, abdomen, bladder, uterus and rectum. This pressure is controlled by different motor systems in the brainstem. An overview will be given which brainstem nuclei control which pressure. Since most of the above mentioned behaviors consist of different combinations of pressure in thorax, abdomen and pelvic organs, these basic brainstem centers need to be properly coordinated. This coordination takes place at higher brainstem levels and di-encephalon. Finally, these coordinating regions, in turn, need to be informed about whether or not the environmental situation allows generating the specific motor activities. This information is supplied by prefrontal cortical regions, which, based on visual, auditory, olfactory, somatosensory, and vestibular information, decide whether or not the various motor systems can be activated, all in the context of survival of the individual and survival of the species.

MONDAY, AUGUST 20th

Sleep and Wakefulness

08:30h - 09:00h

I41 - Affective brain areas contributing to breathing

Ronald Harper, Rajesh Kumar, Paul Macey, Jennifer Ogren
Univ of California at Los Angeles, LOS ANGELES, United States of America

The neural damage resulting from hypoxia, perfusion, and other consequences of obstructive sleep apnea (OSA) or congenital central hypoventilation syndrome (CCHS) appears in areas that serve multiple roles, including emotional drives to breathe, or involve neurotransmitter systems that serve both affective functions and breathing roles. The damage, assessed with structural magnetic resonance imaging (MRI) procedures, shows tissue loss or water diffusion changes indicative of injury, and impaired axonal integrity between structures; damage is preferentially unilateral. Functional MRI responses in affected areas also are time- or amplitude- distorted to ventilatory or autonomic challenges. Among the structures injured in both OSA and CCHS are the insular, cingulate, and medial ventral prefrontal cortices, as well as cerebellar deep nuclei and cortex, anterior hypothalamus, caudal raphe, portions of the basal ganglia and, in CCHS alone, the locus coeruleus. Caudal raphe and locus coeruleus injury has the potential to modify serotonergic and adrenergic modulation of upper airway and arousal characteristics, as well as affective drive to breathing. Several of the rostral sites mediate aspects of dyspnea, especially affected in CCHS, while others participate in initiation of inspiration after central breathing pauses. The ancillary injury to central structures associated with sleep-disordered breathing can elicit multiple other distortions in function in addition to effects on breathing regulation.

09:00h - 09:30h

I40 - C/EBP Homologous Binding Protein (CHOP) Underlies Neural Injury in Sleep Apnea Model

Sigrid Veasey[1], Yu-Ting Chou[2], Guanxia Zhan[1], Yan Zhu[1], Polina Fenik[1], Lori Panossian[1], YanPeng Li[1], Jing Zhang[3]
[1]University of Pennsylvania, PHILADELPHIA, United States of America
[2]Chang Gung Memorial Hospital, CHIAYI, Taiwan
[3]Peking University First Hospital, BEJING, China

Obstructive sleep apnea (OSA) is associated with cognitive impairment and neuronal injury. Long-term exposure to intermittent hypoxia (LTIH) in rodents, modeling the oxygenation patterns in sleep apnea, results in oxidative injury to specific neuronal populations. OSA oxygenation results in NADPH oxidase (Nox2) injury to hypoglossal motoneurons and catecholaminergic wake neurons, associated with functional consequences of the oxidative injury. Mechanisms underlying Nox2 activation in LTIH are largely unknown. Brainstem motoneurons susceptible to LTIH injury show uncompensated endoplasmic reticulum stress responses with increased C/EBP homologous protein (CHOP). In this newest series of studies, we first determined whether CHOP is upregulated in other brain regions susceptible to LTIH oxidative Nox2 injury and then determined whether CHOP plays an adaptive or injurious role in the LTIH response. To integrate these findings with previous studies examining LTIH neural injury, we examined the role of CHOP in Nox2, HIF-1a responses, oxidative injury and apoptosis and neuron loss. Relative to wild type mice, CHOP-/- mice conferred resistance to oxidative stress (superoxide production/carbonyl proteins) in brain regions examined: cortex, hippocampus and motor nuclei. CHOP deletion prevented LTIH upregulation of Nox2 and HIF-1Î± in the hippocampus, cortex and brainstem motoneurons and protected mice from neuronal apoptosis and motoneuron loss. Endogenous CHOP is necessary for LTIH-induced HIF-1Î±, Nox2 upregulation and oxidative stress; CHOP influences LTIH-induced apoptosis in and loss of neurons. Findings support the concept that minimizing CHOP may provide neuroprotection in OSA and that CHOP is upstream of injuries identified to date.

09:30h - 10:00h

I39 - Sleep-wake control of the upper airway by noradrenergic neurons with and without intermittent hypoxia

Leszek Kubin
University of Pennsylvania, PHILADELPHIA, PA, United States of America

Enhanced activity in upper airway muscles, such as the genioglossus innervated by hypoglossal (XII) motoneurons, allows obstructive sleep apnea (OSA) patients to breathe satisfactorily when they are awake. Sleep causes a loss of this hyperactivity which leads to recurrent apneic episodes that are resolved with or without arousal by enhanced respiratory drive caused by hypoxia and hypercapnia. In rodents with non-collapsible upper airway, we identified norepinephrine and to a lesser extent serotonin as the main mediators of the wake-related drive to XII motoneurons, a drive that is progressively diminished during transitions into slow-wave sleep and then rapid eye movement (REM) sleep. A major portion of this drive originates in the lateral pontine A7 group, a relatively small group of noradrenergic neurons that has yet unknown pattern of sleep-wake activity but exhibits decrements of Fos expression when REM sleep-like state is elicited pharmacologically. Another portion of this drive originates in the caudal A1/C1 group, a group that may be unique in that it does not exhibit REM sleep-related decrements of activity, as indicated by Fos expression and extracellular recordings. In rats subjected for 35 days to chronic intermittent hypoxia (CIH) as a model of OSA, the density of noradrenergic terminals increases in the XII nucleus, XII motoneurons exhibit upregulation of alpha1-adrenergic receptor protein, and the endogenous noradrenergic excitatory drive to XII motoneurons is increased. This suggests that upper airway hyperactivity in OSA patients is mediated by CIH-induced upregulation of noradrenergic input to upper airway motoneurons.

(Supported by HL-47600.)

I42 - Genomic determinants of end-organ susceptibility to sleep apnea: human and murine correlates

David Gozal, Leila Kheirandish-Gozal, Hui-Leng Tan, Abdelnaby Khalyfa
The University of Chicago, CHICAGO, United States of America

Epigenetic processes such as DNA methylation enable the environment to modulate gene transcription during fetal and early post-natal development, ultimately leading to stable phenotypic changes throughout the lifespan. Sleep perturbations including sleep disruption and sleep apnea are frequent occurrences during the last trimester of pregnancy and during childhood. Furthermore, sleep apnea in children is associated with severity-dependent alterations in cognitive, cardiovascular and metabolic functions. Selective gene hypermethylation occurs in pediatric sleep apnea suggesting an increased propensity for activation of inflammatory processes. As a corollary to the latter changes, altered distribution and function of T cell lymphocytes occurs and is strongly associated with endothelial dysfunction. Similarly, the offspring of pregnant mice exposed to sleep fragmentation (SF) will develop altered metabolic phenotypes (i.e., increased weigh and visceral fat, serum lipids, higher leptin, and both systemic and adipose tissue insulin resistance) along with alterations in the methylome, particularly in metabolism-related genomic pathways. These studies may unravel the unique role of sleep during pregnancy and childhood as an important determinant of epigenetic changes that ultimately result in heightened risk for obesity and cardiovascular and metabolic dysfunction.

Supported by National Institutes of Health HL-65260

Vocalization and singing

11:00h - 11:30h

I43 - Muscular correlates of PAG induced sound modulation

Hari Subramanian[1], Gert Holstege[2]
[1]*Florey Neuroscience Institutes, MELBOURNE, Australia*
[2]*UMCG, University of Groningen, GRONINGEN, Netherlands*

Chemical stimulation in the midbrain periaqueductal gray (PAG) produces different types of vocalization, depending on where in the PAG the stimulation takes place. In the cat mews can be elicited in the lateral and ventrolateral PAG, while hisses only in the central parts of the ventrolateral PAG. The basis of the PAG generating vocalization is its projection to the nucleus retroambiguus (NRA), a group of premotor interneurons in the caudal ventrolateral medulla. The neurons in the rostral NRA project to the motoneurons of the pharynx and larynx, other neurons in the more rostral NRA project to the phrenic motoneurons innervating the diaphragm and the external and internal intercostal motoneurons. Further caudally in the NRA neurons project to abdominal muscle motoneurons and even further caudal to the pelvic floor and to motoneurons involved in sexual posture. The PAG projections from the lateral and ventrolateral PAG project to different NRA interneurons than the PAG neurons in the more central parts of the PAG. In this presentation we examine the muscular correlates of PAG induced sound modulation (mew, howl and hiss) via investigating the recruitment patterns of the laryngeal (posterior cricoarytenoid, cricothyroid and thyroarytenoid), internal and external abdominal obliques, internal intercostals, the crural and costal diaphragm, the genioglossus (tongue) and the digastric (mouth opening) muscles. We propose a functional neuroanatomical framework for sound production and modulation in mammals.

11:30h - 12:00h

I44 - 'Breathing for singing' -The breathing support; tradition and research

Viggo Pettersen
University of Stavanger, STAVANGER, Norway

The aim of this paper is to present an overview of findings in eleven studies exploring muscular patterns and muscle activation levels in selected muscles by classical singers. Muscle activity in upper trapezius, sternocleidomastoideus, the scalenes, posterior neck, pectoralis major and the diaphragm was investigated during inhalation and phonation. Muscle activity was recorded by surface electromyography. The movements of the anterior and dorsal sections of the diaphragm were monitored by ultrasound imaging.
A phasing of upper trapezius activity to intercostal and abdominal muscle activity was discovered, all muscles supporting the expiration phase. During phonation upper trapezius contributes in the compression of the upper thorax, thus serving as an accessory muscle of expiration.
The sternocleido and the scalenes show correlated activity patterns during inhalation and phonation. During demanding singing, expiratory phased sternocleido/scalene activity peaks produce a counterforce to the compression of upper thorax at high pitches.
Substantial muscle activity is observed in the posterior neck during inhalation and phonation. During phonation professional opera singers activate the expiratory phased upper trapezius, the intercostals and the abdominal muscles to higher levels than student singers do.
Ultrasound imaging is a promising tool for surveying the movement of diaphragm. Especially the anterior section of the diaphragm is easily assessed, however, also the dorsal part may indirectly be surveyed by ultrasound imaging of the movement of the left kidney. The caudal adjustments, observed in both anterior and dorsal diaphragm during phonation, are apparently influenced by the diaphragm’s own activity.

Plenary lecture 4 by William Milsom

17:00h - 18:00h

I45 - The evolution of respiratory processes

William Milsom
University of British Columbia, VANCOUVER, Canada

Through evolution, a muscular pump that evolved for suspension feeding became the respiratory pump. Gas bladders that evolved for buoyancy control and gas exchange became the lungs. The transition from gill (water) to lung (air) breathing primarily required a change in control of the valving mechanisms associated with the ventilatory pump. The evolution of the aspiration pump involved a shift from a buccal pump driven by branchiomeric muscles innervated by cranial nerves to an aspiration pump driven by axial muscles innervated by spinal nerves. Presumably, the neural circuitry involved in producing water flow for feeding became the neural circuitry involved in producing breathing. With the appearance of air breathing the dominant pair of oscillators producing the respiratory rhythm appears to have shifted caudally from a para-trigeminal to a para-facial site and ultimately to a para-vagal site (the preBotzinger Complex). There was an associated switch from a respiratory pattern initiated by active expiration to one beginning with active inspiration. There was also a switch in the primary respiratory drive from one associated with oxygen uptake to one associated with CO_2 excretion and, thus, in the chemoreceptors associated with ventilatory control. Critically, these evolutionary changes did not occur independently but co-evolved together.