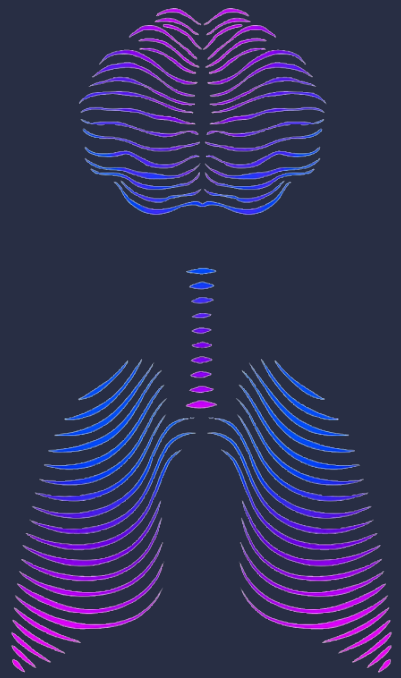

# Breathing for Life

## 16th Oxford Conference on Breathing, Emotion, and Beyond

*Conference Program*

September 30 – October 4, 2024
Williamsburg, VA

# *Thank you to our Sponsors*

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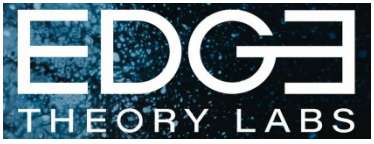
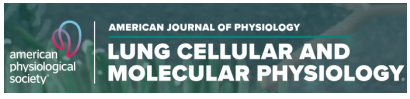




WILLIAM & MARY





# Contents

# Mission Statement

*Breathing for Life* is a preeminent international conference now in its 16th iteration since 1978. We, the meeting organizers, aim to assemble a diverse range of respiratory neurobiologists from different nations and cultures, at various career stages, of all genders and backgrounds. We aim to unite a diverse cadre of delegates to synthesize new knowledge and understanding while building a strong foundation among trainees. Our programming and plan for the meeting are designed to disseminate the latest research on the neural control of breathing as well as the influence of breathing neural activity on numerous brain functions including – but not limited to – cognition, emotion, and contemplative disciplines (e.g., meditation). *Breathing for Life* embraces all forms of neuroscience research including molecular genetics, developmental biology, cellular neurophysiology, systems and behavioral neuroscience, mathematical modeling, data science, as well as the interdisciplinary intersection of such disciplines. While disseminating new knowledge regarding respiratory neurobiology, we are reaching out to the larger community of neuroscience, physiology, and beyond to convey a deeper understanding of – and practices that benefit – breathing, brain health, and wellness in general.

# Executive Committee

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# Brief History of the Oxford Conference

The Oxford Conference grew from a fortuitous meeting between a physiologist and mathematician at the Polish National Academy of Sciences in 1971. Physiologist Dan Cunningham was seated at dinner with mathematician Ryszard Herczynski. Cunningham recounted his struggles describing the pattern of breathing. The two began to work out a solution on dinner napkins, and then wondered how much more could be achieved by bringing together physiologists and modelers. Thus, was born the idea of a conference on mathematical modeling and the neural control of breathing. Their idea was realized in 1978, at the meeting hosted by The Queen's College at Oxford University. This inaugural meeting featured 90 delegates from 10 countries. Subsequently, the traditionally triennial conference has become an established forum at the interface of neuroscience and respiratory physiology. It has been hosted in seven countries on four continents. In September 2017, it returned home to Merton College of Oxford University for the first time in 40 years. It has since resumed its peripatetic journey to Japan in 2022, and now back to the colonial capital of the United States in 2024.

| Year | Venue | Organizers |
|---|---|---|
| 1978 | Oxford, United Kingdom | E.R. Carson, D.J.C. Cunningham, R. Herczynski, D.J. Murray-Smith, E.S. Peterson |
| 1982 | Lake Arrowhead, California, USA | B.J. Whipp, S.A. Ward, J.W. Bellville, D.M. Wiberg |
| 1985 | Solignac, France | G. Benchetrit, P. Baconnier, J. Demongeot |
| 1988 | Grand Lake, Colorado, USA | G.D. Swanson, F.S. Grodins, R.L. Hughson |
| 1991 | Fuji, Japan | Y. Honda, Y. Miyamoto, K. Konno, J.G. Widdicombe |
| 1994 | Egham, Surrey, United Kingdom | S.J.G. Semple, L. Adams, B.J. Whipp |
| 1997 | Huntsville, Ontario, Canada | R.L. Hughson, D.A. Cunningham, J. Duffin |
| 2000 | North Falmouth, Massachusetts, USA | C-S. Poon, H. Kazemi |
| 2003 | Paris, France | J. Champagnat, M. Denavit-Saubie, G. Fortin |
| 2006 | Lake Louise, Alberta, Canada | M.J. Poulin, R.J.A. Wilson |
| 2009 | Nara, Japan | I. Homma, Y. Fukuchi |
| 2012 | Almelo, The Netherlands | G. Holstege, M. Dutschmann, H.H. Subramanian |

| Year | Venue | Organizers |
|---|---|---|
| 2014 | Sydney, New South Wales, Australia | P.M. Pilowsky, M. Farnham, S. Mohammed, P. Nedoboy |
| 2017 | Oxford, United Kingdom | A.V. Gourine, D. Paterson, R.T.R. Huckstepp, P. Hosford |
| 2022 | Odawara, Japan | Y. Okada, H. Onimaru, Y. Oku, T. Kuwaki, F. Miwakeichi |
| 2024 | Williamsburg, Virginia, USA | D.A. Bayliss, C.A. Del Negro, L.H. Gargaglioni, D.K. Mulkey, T. Pitts |

# Area Dining

Please also see the online guide published by the Williamsburg Lodge to explore attractions and restaurants in the area. Scan the QR code to access!

**Amber Ox**
A seasonally inspired brewpub with a modern approach.
Location: 525 Prince George St.
Phone Number: (757) 790-2299

**Aromas Café**
Fast-casual coffeehouse serving breakfast, lunch, and dinner with a strong emphasis on fresh, healthy items, fine teas and freshly roasted coffees.
Location: 431 Prince George St.
Phone Number: (757) 221-6676

**Alter Ego**
A modern pop-up style eatery, where the menu changes as often as their mission! Located inside the Precarious Beer Hall.
Location: 101 S. Henry St.
Phone Number: (757) 808-5104

**Bake Shop**
Inspired by passion! Hand crafted baked goods and pastries and delicious coffee.
Location: 416 Prince George St.
Phone Number: (757) 229-6385

**Baskin-Robbins**
A huge variety of ice creams, milk shakes, malts, smoothies, sundaes, and frozen yogurt.
Location: 416 Prince George St.
Phone Number: (757) 229-6385

**Berret's Seafood**
Voted by locals “Best Seafood Restaurant” and "Best Crabcake" year after year. Excellent wines and microbrews. Seasonal outdoor dining and live music nightly.
Location: 199 S. Boundary St.
Phone Number: (757) 253-1847

**Blue Talon Bistro**
Celebrated Chef David Everett presents his French-inspired “serious comfort food” offering a break from the day’s demands with great food, wine, and service in a relaxed atmosphere. Serving breakfast, lunch, and dinner.
Location: 424 Prince George St.
Phone Number: (757) 476-2583

**Cheese Shop**
Offers 200+ imported and domestic cheeses, charcuterie, freshly baked breads, specialty foods and over 4,000 bottles of wine in the wine cellar. Enjoy a cheese plate and glass of wine on our patio. Owned and operated by the Power family since 1971.
Location: 410 Duke of Gloucester St.
Phone Number: (757) 220-0298

**Christiana Campbell's Tavern**
Colonial Williamsburg-operated tavern for a traditional colonial meal in the context of colonial garb and tradition.
Location: 101 South Waller St.
Phone Number: (855) 263-1746

**Chowning's Tavern**
Colonial Williamsburg-operated tavern. Chowning's Tavern features hearty, colonial-inspired pub fare in an alehouse atmosphere.
Location: 109 East Duke of Gloucester St.
Phone Number: (855) 270-5114

**Culture Café**
Strives to unite cultures and food by serving small plates. A uniquely communal dining experience where the only thing more stimulating than the culinary collision of flavors from different cultures is the company of those around you.
Location: 747 Scotland St.
Phone Number: (757) 378-2556

**DoG Street Pub**
An American Gastropub. Duke of Gloucester (DoG) Street Pub's focus is simply presented with an understanding that a superb craft beer is the perfect complement to a perfect meal.
Location: 401 W. Duke of Gloucester St.
Phone Number: (757) 293-6478

**Downstairs Fat Canary**
A complement and contrast to the namesake restaurant above, it offers a more casual setting to enjoy distinctive food and beverages. Seating is offered on a first-come, first-served basis.
Location: 401 W. Duke of Gloucester St.
Phone Number: (757) 220-0298

**Fat Canary**
Located in the heart of Colonial Williamsburg, Fat Canary has received the AAA Four Diamond award every year since opening, in 2003. Fancy but worth it!
Location: 410 W. Duke of Gloucester St.
Phone Number: (757) 229-3333

**Golden Horseshoe Gold Course Clubhouse Grille**
Cap off a round on the Gold Course with a cold drink at the Horseshoe Bar, dine in the restaurant, or soak in views of the 18th hole from the outdoor terrace. Menu showcases local oak smoked BBQ brisket, pork, chicken and weekly specials. Also featuring vegetarian options, burgers, salads and cobbler.
Location: 401 South England St.
Phone Number: (855) 318-5868

**Green Leaf Café**
One of Williamsburg's most enduring Pubs, featuring 40 unique taps. Conveniently located across from the WIlliam & Mary football stadium (Zable), a few blocks from Colonial Williamsburg.
Location: 765 Scotland St.
Phone Number: (757) 903-2697 or (757) 903-2713

**Hound's Tale**
A casual, cozy bistro-type restaurant. It features affordable fresh, tasty bites that range from comfort foods with a twist, to dishes that evoke memories of travels abroad. Great spirits include a variety of regional beers and wines as well as an array of delightful, hand-crafted cocktails.
Location: 515 Prince George St.
Phone Number: (757) 221-6678

**Hound's Tale - Corner Barkery**
Dog-friendly bar for lunch & dinner. Local brews available on draft and in can. Unique cocktails. Dine inside or on either the front or back patios.
Location: 501 Prince George St.
Phone Number: (757) 221-6678

**Illy caffe**
A family owned premium coffee company based in Trieste, Italy. Their mission is to sustainably support the production of the best coffee in the world.
Location: 435 W. Duke of Gloucester St.
Phone Number: (757) 208-0006

**Kilwins**
Chocolate, fudge, and ice cream in fresh hand-crafted waffle cones.
Location: 421 Prince George St.
Phone Number: (757) 378-2727

**King's Arms Tavern**
Originally opened in 1772, this authentic reproduction public house serves up the Colonial Williamsburg experience. Everything is true to the time period in colonial elegance. Inspired by 18th-century recipes, but with updates to suit 21st-century tastes.
Location: 416 East Duke of Gloucester St.
Phone Number: (855) 240-3278

**Mellow Mushroom**
Stone-baked pizzas made-to-order. 30 beers on tap, including local breweries and handcrafted cocktails. Al Fresco patio dining and next door to Precarious Beer Project.
Location: 110 South Henry St.
Phone Number: (757) 903-4762

**Oishii**
Family-owned casual kitchen serving Japanese-style food.
Location: 515 Prince George St.
Phone Number: (757) 220-6880

**Ol' Dominion Burger**
All-natural, hormone & antibiotic free beef raised in Virginia. All burgers are cooked well-done and are juicy! If you're not into beef, no worries! They serve a killer veggie patty option thatLocated inside the Precarious Beer Hall.
Location:  110 S. Henry St.
Phone Number: (757) 808-5104

**Paul's Deli**
This place is a lovable dive. Paul's Deli has been a family tradition since 1977.
Location: 761 Scotland St.
Phone Number: (757) 229-8976

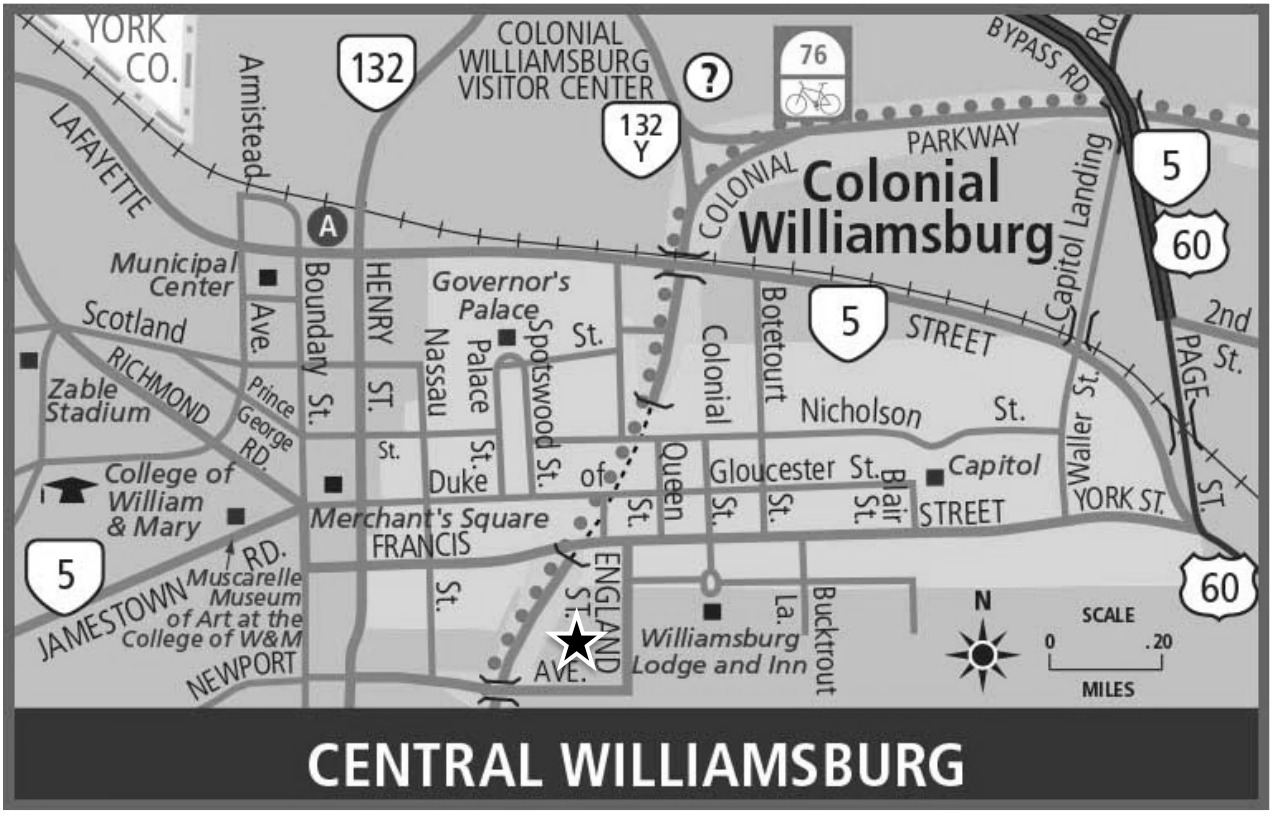
**Precarious Beer Project**
Precarious Beer Hall is home of Precarious Beer Project, a modern brewing company focused on creating cutting edge, progressive beers! Featuring food from Electric Circus Taco Bar and Ol' Dominion Smashburger Bar.
Location: 110 South Henry St.
Phone Number: (757) 808-5104

**Retros Good Eats**
Self-service cafe for hot dogs, hamburgers, draft root beer, fries, and frozen custard (ice cream). Inexpensive and very good.
Location: 435 Prince George St.
Phone Number: (757) 253-8816

**Rick's Cheese Steak Shop**
Cheesesteaks! Rick's gives the customers a large selection of options for their orders.
Location: 603 Prince George St.
Phone Number: (757) 221-9566

**Saladworks**
A quick, casual-style restaurant offering signature salads as well as create-your-own options.
Location: 110 North Henry St.
Phone Number: (757) 345-6099

# Central Williamsburg Map



# Schedule

| Monday September 30, 2024 | | |
|---|---|---|
| **Time** | **Event** | **Location** |
| 2:00 – 4:00 PM | On-site Registration | Colony Foyer |
| 4:00 – 6:00 PM | Welcome Reception | Colony Room |
| 6:00 – 7:00 PM | Brief Remarks & Orientation | Colony Room |
| **Tuesday October 1, 2024** | | |
| **Time** | **Event** | **Location** |
| 6:00 – 7:00 AM | Cold Plunge with EDGE Therapy Labs | Garden Patio |
| 7:00 – 8:30 AM | Buffet Breakfast | Tidewater Room |
| 7:45 – 8:10 AM | *Nano-talks I*: Oscillators | Tidewater Room |
| 8:30 – 10:00 AM | *Scientific Session I* – Respiratory Oscillators I: Inspiration and Active Expiration | Colony Room |
| 10:00 – 10:30 AM | **Break** | |
| 10:30 – 12:00 PM | *Scientific Session II* – Respiratory Oscillators II: PiCo, Lateral Parafacial, & Pontine Microcircuits | Colony Room |
| 12:00 – 1:30 PM | On-site Lunch | Tidewater Room |
| 1:30 – 3:00 PM | *Scientific Session III* – Sensory Processing I: Peripheral Chemoreception | Colony Room |
| 3:00 – 3:30 PM | **Break** | |
| 3:30 – 4:00 PM | *Nano-talks II:* Chemoreception | Colony Room |
| 4:00 – 5:00 PM | **Meet the Experts (PIs)** | |
| 5:00 – 5:30 PM | **Free Time** | |
| 5:30 – 7:00 PM | Poster Session with beer and wine | Colony Room |

| | | |
|---|---|---|
| 7:00 – 9:00 PM | On-site Dinner | Tidewater Room |
| **Wednesday October 2, 2024** | | |
| **Time** | **Event** | **Location** |
| 6:00 – 7:00 AM | Body By Breath: Diaphragm Dynamics Workshop with Jill Miller | Colony Room |
| 7:00 – 8:30 AM | Buffet Breakfast | Tidewater Room |
| 7:45 – 8:10 AM | *Nano-talks III:* Neuroplasticity | Tidewater Room |
| 8:30 – 10:00 AM | *Scientific Session IV* – Sensory Processing II: Central Chemoreception | Colony Room |
| 10:00 – 10:30 AM | **Break** | |
| 10:30 – 12:00 PM | *Scientific Session V* – Respiratory Neuroplasticity | Colony Room |
| 12:00 – 1:30 PM | On-site Lunch | Tidewater Room |
| 1:30 – 3:00 PM | *Scientific Session VI* – Sex Differences in Breathing | Colony Room |
| 3:00 – 3:30 PM | **Break** | |
| 3:30 – 5:30 PM | *Scientific Session VII* – Breathing Disorders I | Colony Room |
| 5:30 – 6:00 PM | *Nano-talks IV:* Disorders | Colony Room |
| 6:00 – 7:30 PM | Poster Session with beer and wine | Colony Room |
| 7:30 PM | **Off-site Dinner *(on your own)*** | |
| **Thursday October 3, 2024** | | |
| **Time** | **Event** | **Location** |
| 6:00 – 7:00 AM | Yoga for All Levels with Full Circle Yoga | Colony Room |
| 7:00 – 8:30 AM | Buffet Breakfast | Tidewater Room |
| 7:45 – 8:10 AM | *Nano-talks V:* Emotion & Lung-Brain | Tidewater Room |
| 8:30 – 10:00 AM | *Scientific Session VIII* – Breathing & Emotion I | Colony Room |
| 10:00 – 10:30 AM | **Break** | |
| 10:30 – 12:00 PM | *Scientific Session IX* – Breathing & Emotion II | Colony Room |

| | | |
|---|---|---|
| 12:00 – 1:30 PM | On-site Lunch | Tidewater Room |
| 1:30 – 3:00 PM | *Scientific Session X* – Upper airway regulation | Colony Room |
| 3:00 – 5:30 PM | **Free Time** | |
| 5:30 – 7:00 PM | *Scientific Session XI* – Breathing Disorders II | Colony Room |
| 7:00 – 9:00 PM | On-site Dinner | Tidewater Room |
| **Friday October 4, 2024** | | |
| **Time** | **Event** | **Location** |
| 8:30 – 10:00 AM | *Scientific Session XII* – Genomics & Data Science | Colony Room |
| 10:00 – 10:30 AM | **Break** | |
| 10:30 – 12:00 PM | *Scientific Session XIII* – Congenital Central Hypoventilation Syndrome (CCHS) | Colony Room |
| 12:00 – 1:30 PM | **Meet the Experts (PIs) & Lunch** | |

# Program- Tuesday October 1, 2024

| **Nano-talks I: Oscillators \| Moderator: Luciane Gargaglioni (Brazil)** | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 7:45 AM | Carlos da Silva Junior | USA | Evaluating the role of KCNQ ion channels in breathing rhythmogenesis and opioid-induced respiratory depression in adult mice |
| 7:50 AM | Juliana Reis Souza | Brazil | Effects of activation of glutamatergic interneurons of the lateral parafacial region on the ventilatory parameters of mice during normocapnia and hypercapnia |
| 7:55 AM | Nathalia Salim | Brazil | Excitation of somatostatinergic interneurons of the lateral parafacial region of mice during normocapnia and hypercapnia |
| 8:00 AM | Grigory Loginov | USA | The role of enkephalinergic system in respiratory control |
| 8:05 AM | Mariana Del Rosso Mello | Australia | Photoinhibition of pre-Bötzinger neurons that project to the facial nucleus affect the nasofacial activity with minimal effect on breathing in rats |
| **Scientific Session I: Oscillators \| Moderator: Daniel Zoccal (Brazil)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 8:30 AM | Jack Feldman | USA | Hypothesis: Burstlets and synchronization are essential to generation of breathing rhythm |
| 8:50 AM | Nathan Baertsch | USA | Homeostatic and non-homeostatic breathing control - from cellular properties to brain-wide circuits |
| 9:10 AM | Donatella Mutolo | Italy | Respiratory muscle control and preBötzinger complex neural circuitry alterations in mice lacking CDKL5 |

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|---|---|---|---|
| 9:20 AM | Jeehaeh Do | USA | The role of a molecularly defined inhibitory inspiratory neuron in breathing demonstrates that inspiration must suppress expiration |
| 9:30 AM | Yamusada Okada | Japan | Proposal of a respiratory rhythm-generator model consisting of longitudinally distributed multiple oscillators along the neuraxis |
| 9:40 AM | Jonathan Rubin | USA | Proposal of a respiratory rhythm-generator model consisting of longitudinally distributed multiple oscillators along the neuraxis |
| 9:50 AM | Discussion | | |
| **Scientific Session II : Respiratory Oscillators II \| Moderator: Jens Rekling (Denmark)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 10:30 AM | Silvia Pagliardini | Canada | The lateral parafacial region and active expiration |
| 10:50 AM | Alyssa Huff | USA | Disruption to swallow and its coordination with breathing when silencing the caudal Nucleus of the Solitary Tract and the Postinspiratory Complex |
| 11:10 AM | Annaliese Eymael | Australia | Premotor organisation of laryngeal control in mice |
| 11:20 AM | Sufyan Ashhad | India | PreBötzinger Complex subthreshold oscillations underlie rapid switches in breathing dynamics |
| 11:30 AM | Babita Thadari | USA | The role of phenytoin in generating multi-rhythmic inspiratory patterns in the preBötzinger complex |
| 11:40 AM | Kayla Baker | Canada | Inhibitory GABAergic Cells in The PreBötzinger Complex Mediate Rhythmic Breathing In-Vivo |
| 11:50 AM | Discussion | | |

| Scientific Session III: Sensory Processing I \| Moderator: Benedito Machado (Brazil) | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 1:30 PM | Nanduri Prabhakar | USA | O2 sensing by the carotid body |
| 1:50 PM | Silvia Conde | Portugal | Novel Interoceptive Properties of the Carotid Body in Health and Disease |
| 2:10 PM | Brigitte Browe | USA | Carotid body activity coordinates changes in breathing and hippocampal physiology |
| 2:20 PM | Pedro Favoretto Spiller | Brazil | Acute oxygen sensing by carotid body chemoreceptor cells of wild-type and mitochondrial complex III-deficient mice expressing an alternative oxidase |
| 2:30 PM | Marina Rincon Sartori | Canada | A role for mitochondria in Spinal Oxygen Sensing (SOS) and the autonomic dysfunction associated with neurodegenerative diseases |
| 2:40 PM | Karla Rodrigues | Brazil | Respiratory profile of adenosine A2A receptors knockout mice exposed to short term sustained hypoxia |
| 2:50 PM | Discussion | | |
| **Nano-talks II: Chemoreception I \| Moderator: Dan Mulkey (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 3:30 PM | Pamela Medeiros | Brazil | Physical Exercise Prevents Neurodegeneration in Cardiorespiratory Nuclei and Breathing Deficits in The 6-OHDA Model of Parkinson's Disease |
| 3:35 PM | Nicolleta Memos | USA | Butyrylcholinesterase is dispensable for the neonatal autoresuscitation reflex |
| 3:40 PM | Ryan Budde | USA | Rapid terminal apnea following diving reflex with carotid body resection or seizure |
| 3:45 PM | Monica Strain | USA | Enhanced peripheral chemoreception contributes to disordered breathing in Rett syndrome |

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|---|---|---|---|
| 3:50 PM | Eliandra da Silva | Brazil | Orexinergic signaling in the caudal nucleus of the solitary tract contributes to the CO2 ventilatory response during light/inactive state |
| 3:55 PM | Renato Martins Sá | Brazil | Astrocytes of the lateral parafacial region are sensitive to high CO2 but play no major role in controlling pulmonary ventilation of mice |

# Program- Wednesday October 2, 2024

| **Nano-talks III: Neuroplasticity I \| Moderator: Kimberly Iceman (USA)** | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 7:45 AM | Kayla Burrowes | USA | Long lasting suppression of phrenic motor plasticity after mild acute inflammation is mediated by persistent p38 MAPK activity |
| 7:50 AM | Aaron Jones | USA | Phrenic motor neuron Bmal1 expression regulates phrenic long-term facilitation in rats |
| 7:55 AM | Michael Frazure | USA | Calcium-sensitive potassium currents underly functional diversity of hypoglossal motoneurons |
| 8:00 AM | Alec Butenas | USA | Acute Intermittent Hypercapnia Elicits Phrenic Long-Term Facilitation During the Daily Rest, but not Active Phase in Rats |
| 8:05 AM | Jessica Whitaker-Fornek | USA | Endogenous Opioids and Breathing Development |
| **Scientific Session IV: Sensory Processing II \| Moderator: Davi Moraes (Brazil)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 8:30 AM | Stephen Abbott | USA | Interdependent functions of central chemoreceptors and peripheral chemoreceptors in respiratory homeostasis |
| 8:50 AM | Muriel Thoby Brisson | France | Search for therapeutic candidates to treat respiratory deficits associated with Congenital Central Hypoventilation Syndrome |
| 9:10 AM | Satvinder Kaur | USA | Fork head Box protein 2 (FoxP2) expressing neurons in the lateral parabrachial area regulate respiratory responses to hypercapnia |

| | | | |
|---|---|---|---|
| 9:20 AM | Natasha Kumar | Australia | The effect of long-term recurrent intermittent hypercapnia on central respiratory chemoreflex function |
| 9:30 AM | Luis Gustavo Patrone | Brazil | The role of BK channels in mouse ventilatory response to CO2 and the chemosensitity of locus coeruleus neurons |
| 9:40 AM | Deanna Arble | USA | The Molecular Circadian Clock of Phox2b-expressing Cells Drives Daily Variation of the Hypoxic but Not Hypercapnic Ventilatory Response in Mice |
| 9:50 AM | Discussion | | |
| **Scientific Session V: Respiratory Neuroplasticity \| Moderator: Erica Levitt (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 10:30 AM | Alicia Vose | USA | Acute Intermittent Hypoxia Improves Airway Protection During Swallowing in Chronic Cervical Spinal Cord Injury |
| 10:50 AM | Erica Dale | USA | Epidural stimulation for recovery of respiration after spinal cord injury |
| 11:10 AM | Allison Brezinski | USA | Neuromodulation of cervical excitatory interneurons enhances the hypercapnic response in chronic cervical spinal cord injury |
| 11:20 AM | Alexandria Marciante | USA | Diurnal regulation of APOE genotype on phrenic motor plasticity and associated genes |
| 11:30 AM | Gordon Mitchell | USA | Spinal microglia regulate phrenic long-term facilitation via hypoxia-evoked phrenic motor neuron fractalkine release |
| 11:40 AM | May Smith-Hublou | USA | Impacts of deep brain stimulation on breathing in Parkinson disease |
| 11:50 AM | Discussion | | |

| Scientific Session VI: Sex Differences \| Moderator: Kristi Streeter (USA) | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 1:30 PM | Luciane Gargaglioni | Brazil | Sex differences in breathing control |
| 1:50 PM | Adrianne Huxtable | USA | Neonatal inflammation induces lasting sex-, region-, and stimulus-dependent changes in adult microglia from respiratory control regions |
| 2:10 PM | Taylor Holmes | USA | Daily diaphragm pacing attenuates breathing deficits after spinal cord injury in male, but not female, rats |
| 2:20 PM | Anthony Marullo | Ireland | Microbiota-metabolite-muscle axis: Gut feelings about breathing |
| 2:30 PM | Mariana Bernardes Ribeiro | Brazil | Elimination of noradrenaline synthesis in the Locus coeruleus neurons disrupts CO2-ventilatory and metabolic responses during development in both male and female mice |
| 2:40 PM | Ted Dick | USA | During Sepsis, Breathing and the Brainstem Concentrations of Proinflammatory Cytokines Differ between Male and Female Rats |
| 2:50 PM | Discussion | | |
| **Scientific Session VII: Breathing Disorders I \| Moderator: Marie-Noëlle Fiamma (FRAN)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 3:30 PM | Gary Sieck | USA | Don't Take My Breath Away: Aging and Diaphragm Neuromotor Control |
| 3:45 PM | Ana Takakura | Brazil | The respiratory-sleep disruptions and laterodorsal tegmental nucleus in a mouse model of Parkinson's disease |
| 4:00 PM | Dan Mulkey | USA | Developmental progression of respiratory dysfunction in a mouse model of Dravet syndrome |
| 4:15 PM | Brian Dlouhy | USA | Focal ablation of the amygdala may prevent seizure-induced hypoventilation and sudden unexpected death in epilepsy (SUDEP) |

| | | | |
|---|---|---|---|
| 4:30 PM | Steve Crone | USA | Respiratory deficits in a mouse model of epilepsy caused by altered PI3K/mTOR signaling in the forebrain |
| 4:45 PM | Matthew Fogarty | USA | Untangling compensation from pathology in MNs innervating upper airway, diaphragm and limb muscles in a mouse model of Amyotrophic Lateral Sclerosis |
| 5:00 PM | Discussion | | |
| **Nano-talks IV: Disorders \| Moderator: Teresa Pitts (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 5:30 PM | Dipak Patel | USA | Htr1B's role in the autoresuscitation reflex and its Implications to SIDS |
| 5:35 PM | Andersen Cheng | USA | Machine Learning for Identification of Phenotypes in Cardiorespiratory Activity During Autoresuscitation Reflex in Neonate Mice |
| 5:40 PM | Yasmin Aquino | Brazil | Gender-specific respiratory and neuroanatomical impairments in a mice model of Parkinson's disease |
| 5:45 PM | Alyssa Mickle | USA | Detangling the spinal respiratory network's response to external electrical stimulus in a model of spinal cord injury |
| 5:50 PM | Michael Maxwell | Ireland | PREDNAC DMD: Respiratory effects of combined chronic glucocorticoid and antioxidant intervention in the mdx mouse model of Duchenne muscular dystrophy |
| 5:55 PM | Luiz Fernando Pedrão | Brazil | NADPH Oxidase, Apocynin, And Its Effects On Apoptotic And Survival Pathways In The 6-OHDA Rat Model Of Parkinson's Disease |

# Program- Thursday October 3, 2024

| Nano-talks V: Emotion & Lung-Brain \| Moderator: Doug Bayliss (USA) | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 7:45 AM | Josh Goheen | Canada | Breathing is More Related to Anxiety Than Depression |
| 7:50 AM | Jeffrey Gu | USA | Emotional control of breathing: Inhibitory monosynaptic connections between the amygdala and preBötzinger complex |
| 7:55 AM | Nicholas Burgraff | USA | Mechanisms of Fentanyl-Induced Respiratory Muscle Rigidity |
| 8:00 AM | Ryan Phillips | USA | Disentangling pain modulation and respiratory depression at the level of the rostral ventromedial medulla |
| 8:05 AM | Steven Pratscher | USA | Group Breathwork Intervention for Adults with Chronic Pain: A proof-of-concept study of Guided Respiration Mindfulness Therapy |
| **Scientific Session VIII: Breathing and Emotion I \| Moderator: Greg Funk (Canada)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 8:30 AM | Anton Sirota | Germany | Role of breathing in studying the mechanisms of learning and memory consolidation |
| 8:50 AM | Karen Hegland | USA | The urge-to-cough: a respiratory sensation related to upper airway (dys)function |
| 9:10 AM | Paul Dallaghan | USA | Breath Practice Clinical Trial Outcomes on Telomere Length, Stress and Anxiety |
| 9:25 AM | Sung Han | USA | A top-down slow breathing circuit that alleviates negative affect |

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| 9:40 AM | Elora Reilly | USA | Mapping whole-brain excitatory and inhibitory networks for the modulation of respiratory rhythm |
| 9:50 AM | Discussion | | |
| **Scientific Session IX: Breathing & Emotion II \| Moderator: Christopher del Negro (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 10:30 AM | Justin Feinstein | USA | Amygdala-driven apnea and the chemoreceptive origin of anxiety |
| 10:50 AM | Peng Li | USA | Neural circuitry for sighing |
| 11:10 AM | Yae Sugimura | Japan | Amygdala-to-preBötzinger complex neurotransmission constitutes a direct pathway for emotional and pathological modulation of breathing |
| 11:20 AM | Rosana Burgos Pujols | USA | Episodic slow breathing in mice markedly reduces fear responses |
| 11:30 AM | Richard Kinkead | Canada | Acute stress augments obstructive apneas and related O2-desaturations in supine "sleeping" rats via potentiation of cholinergic inhibition and reduction of hypoglossal motoneuron excitability |
| 11:40 AM | Anna Hudson | Australia | The detection, perception and neural processing of respiratory loads in healthy ageing and chronic obstructive pulmonary disease (COPD) |
| 11:50 AM | Discussion | | |
| **Scientific Session X: Upper airway regulation \| Moderator: Bowen Dempsey (AU)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 1:30 PM | Teresa Pitts | USA | Pavlov's dog is still hungry |
| 1:50 PM | Nozomu Nakamura | Japan | The respiratory brain: inspiratory onset as a temporal prediction for cognitive processes and mental health |

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| 2:10 PM | Chloe Edmonds | USA | Aerodigestive coordination through development: what animal models can teach us |
| 2:20 PM | Kevin Yackle | USA | The breath shape controls intonation of mouse vocalizations |
| 2:30 PM | Jane Butler | Australia | Motor unit recruitment and rate coding strategies in human genioglossus during flow limited breathing in obstructive sleep apnoea |
| 2:40 PM | Ann Revill | USA | Muscarinic modulation at hypoglossal motoneurons across postnatal maturation |
| 2:50 PM | Discussion | | |
| **Scientific Session XI: Breathing Disorders II \| Moderator: Ralph Fregosi (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 5:30 PM | Mai ElMallah | USA | Respiratory pathology in Duchenne Muscular Dystrophy |
| 5:50 PM | Lauren Tabor Gray | USA | Combined Respiratory Training to Improve Pulmonary and Cough Function in pALS |
| 6:10 PM | Ken O'Halloran | Ireland | JANUS HYPOTHESIS: Compensation and de-compensation of peak inspiratory performance in mouse models of Duchene muscular dystrophy |
| 6:25 PM | Gaspard Montandon | Canada | Comparative biology perspective of opioid-induced respiratory depression: from zebrafish to rodents |
| 6:40 PM | Debolina Biswas | USA | Respiratory pathology in the TDP-43A315T mouse model of Amyotrophic Lateral Sclerosis |
| 6:50 PM | Discussion | | |

# Program- Friday October 4, 2024

| **Scientific Session XII: Genomic & Data Science \| Moderator: Tina Picardo (USA)** | | | |
|---|---|---|---|
| **Time** | **Presenter** | **Country** | **Title** |
| 8:30 AM | Russell Ray | USA | CardioRespiratory Physiomics: novel approaches for big data in physiology |
| 8:50 AM | Donald Bolser | USA | Brainstem circuit motifs and network strategies regulating the respiratory network: Lessons from a 32 year database |
| 9:10 AM | Savannah Lusk | USA | Precision in Motion: Breathe Easy and the Future of Respiratory Data Analysis |
| 9:20 AM | Kingman Strohl | USA | Apolipoprotein A2 Brainstem Expression and Proteomic Profiling |
| 9:30 AM | Eric Herlenius | Sweden | GENES(IS) of Inspiration – Single cell transcriptomic Atlas of the Brainstem Breathing Rhythm Generator |
| 9:40 AM | Md Rakibal Mowla | USA | Human forebrain responses to breathing modulated by arousal states |
| 9:50 AM | Discussion | | |
| **Scientific Session XIII: CCHS \| Moderator: Patrice Guyenet (USA)** | | | |
| **Time** | **Presenter** | **Country** | **Title** |
| 10:30 AM | Doug Bayliss | USA | Molecular physiology of Phox2b-expressing brainstem neurons |
| 10:50 AM | Luiz Hernandez-Miranda | Germany | Impairment of dB2 neurons causes congenital hypoventilation |
| 11:10 AM | Yingtang Shi | USA | Neuron-specific effects of distinct poly-alanine expansions of Phox2b in mice |
| 11:20 AM | Matthew Ricetti | USA | Formation of breathing circuits in the mouse: the role of the homeobox gene Gsx2 |

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| 11:30 AM | George Souza | USA | Genetically-targeted ablation of central chemoreceptors in the retrotrapezoid nucleus combined with carotid body denervation in mice causes respiratory failure |
| 11:40 AM | Kathryn Lehigh | USA | The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression |
| 11:50 AM | Discussion | | |

# Schedule of Posters

| **Tuesday October 1st \| 5:30-7:00pm** | |
|---|---|
| **Presenter** | **Title** |
| Marlusa Amarante | Characterization of swallow-related sympathetic nerve activity |
| Emmanuel Araujo | Oxytocinergic signaling contributes to breathing regulation in the retrotrapezoid nucleus |
| Joe Arthurs | Parabrachial Tac1 neurons are conditionally activated during non-homeostatic breathing patterns |
| Tanya Bentley | Breathing Practices for Stress Reduction: Conceptual Framework of Implementation Guidelines Based on a Systematic Review of the Published Literature |
| Evgeny Bondarenko | The roles of glutamatergic and glycinergic preBötzinger Complex neurons in inspiratory rhythm generation in vivo |
| Eduardo Bravo | Amygdala stimulation during loss of wakefulness drive of breathing increases the risk of fatal apnea. |
| Rachel Clements | Mechanism and Function of PACAP Expression in the Neonatal Retrotrapezoid Nucleus |
| Greg Conradi Smith | Connectivity and synaptic weight distribution effect the spiking network dynamics of a minimal model of the brainstem preBötzinger Complex |
| Bowen Dempsey | A Phox2b+ pontine nucleus essential for ingestion |
| Rishi Dhingra | Asymmetric neuromodulation in the respiratory network contributes to rhythm generation |

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| Mathias Dutschmann | Significance and state-dependent modulation of mammalian glossopharyngeal nerve motor system. |
| Romain Guinamard | Pharmacological targeting of TRPM4 to modulate spontaneous rhythmic activities. Presentation of three models: breathing activity, cardiac rhythm and uterine contractions. |
| Eriko Hamada | Persistent Glossopharyngeal Nerve Respiratory Discharge Patterns after Ponto-Medullary Transection |
| John Hayes | Kynurenic acid injected into the nucleus tractus solitarius stunts swallow production |
| Jose Luis Herrero Rubio | Intracranial Responses to Respiratory Challenges in Sensorimotor and Insular Cortices are modulated by the Strenght and the Perception of the Challenge. |
| Makito Iizuka | Hiccup center is localized in the subpostremal region of the nucleus tractus solitarius |
| Zainab Khalid | Compensatory cellular plasticity in cervical excitatory following spinal cord injury |
| Gjinovefa Kola | Effects of xylazine on opioid-induced respiratory depression. |
| Peter MacFarlane | Critical windows of brainstem neural development characterized by a lethal vulnerability to endotoxin |
| Simon McMullan | Inhibitory control of motor and respiratory components of orienting by the substantia nigra pars reticulata is state-dependent |
| Jill Miller | Body by Breath: The Science and Practice of Physical and Emotional Resilience *GRAPHICAL ABSTRACT POSTER* |
| Maria Nikodemova | Persistent suppression of phrenic long-term facilitation following acute inflammation requires microglial TGFbeta signaling to phrenic motor neurons |

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| Ryan Pauly | Serotonin Neuron Influence on Pontine Breathing Circuitry Impaired by Opioids |
| Mateus Ramos Amorim | Melanocortin receptor 4 agonist, setmelanotide, treats opioid-induced respiratory depression |
| Jens C. Rekling | Genetically encoded sensors and actuators in the study of preBötzinger neurons in organotypic slice cultures. |
| Ariane Rhone | Radiofrequency ablation of a focal amygdala site affects apnea susceptibility: Implications for Sudden Unexpected Death in Epilepsy (SUDEP) |
| Jordan Skach | Inhibitory subpopulations in preBötzinger Complex play distinct roles in modulating inspiratory rhythm and pattern |
| Andrew Tryba | Neuromodulation of the preBötzinger Complex produces a unique network state driving activity |
| Alysha Michaelson | Repetitive acute intermittent hypoxia consisting of 10-min hypoxic episodes during the mid-active phase increases tidal volume in intact rats |
| Vivian Biancardi | The hyperpolarization-activated inward current, Ih, in the preBötzinger Complex contributes to the hypoxic ventilatory response in anaesthetized adult rats |
| Luke Ehlert | The Influence of Music on Respiratory Rhythm: Anatomical and Functional Insights into Musical Entrainment |
| Marie-Nöelle Fiamma | Preclinical model of obstructive sleep apnea and TRPV1 R antagonist as drug candidate |
| Tara Janes | Hypothalamic progestin infusion enhances the hypercapnic ventilatory response in female rats |

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| Neeharika Reddy Mattayavalahally Nagesh | Exploring the Impact of Perinatal Exposure to the cannabinoid THC on Sleep and Respiratory Development Across Early Life Stages |
| Caroline Szujewski | Locus Coeruleus mediated tolerance to opioid-induced respiratory depression in repeat opioid use |
| Jean-Charles Viemari | Role of $Na_v$ 1.1 and $Na_v$ 1.6 Sodium Channels in Mediating Inspiratory Rhythm Generation and Gasping: Implications for Respiratory Dysfunction in Epilepsy |
| Ryan S. Phillips | A biophysical model of burstlets and bursts in the respiratory preBötzinger complex |
| Subhkinder Kumar | Investigating the thalamus in breathing control using electrical stimulation and intracranial recordings in humans |

| **Wednesday October 2nd \| 6:00-7:30pm** | |
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| **Presenter** | **Title** |
| Davi Moraes | Ventral medullary parvalbumin expiratory neurons control respiratory pattern formation and active expiration |
| Raheja Nishika | The Effects of Slow-Paced Breathing Techniques on Cognitive Performance: Current Findings and Future Directions |
| Yasin Seven | Neurotropism of the Carbon Nanoparticles |
| Barbara Smith | Sigh frequency and magnitude following acute intermittent hypoxia |
| Mihaela Teodorescu | Altered Control Of Breathing In A Rat Model Of Allergic Lower Airway Inflammation |
| Daniel Zoccal | Breathing irregularities and post-natal hypoxemia in a genetic model of essential hypertension |
| Yasmin Aquino | Gender-specific respiratory and neuroanatomical impairments in a mice model of Parkinson's disease |
| Ryan Budde | Rapid terminal apnea following diving reflex with carotid body resection or seizure |
| Nicholas Burgraff | Mechanisms of Fentanyl-Induced Respiratory Muscle Rigidity |
| Kayla Burrowes | Long lasting suppression of phrenic motor plasticity after mild acute inflammation is mediated by persistent p38 MAPK activity |
| Alec Butenas | Acute Intermittent Hypercapnia Elicits Phrenic Long-Term Facilitation During the Daily Rest, but not Active Phase in Rats |

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| Andersen Chang | Machine Learning for Identification of Phenotypes in Cardiorespiratory Activity During Autoresuscitation Reflex in Neonate Mice |
| Carlos Aparecido da Silva Junior | Evaluating the role of KCNQ ion channels in breathing rhythmogenesis and opioid-induced respiratory depression in adult mice |
| Mariana Del Rosso de Melo | Photoinhibition of pre-bötzinger neurons that project to the facial nucleus affect the nasofacial activity with minimal effect on breathing in rats |
| Michael Frazure | Calcium-sensitive potassium currents underly functional diversity of hypoglossal motoneurons |
| Josh Goheen | Breathing is More Related to Anxiety Than Depression |
| Jeffrey Gu | Emotional control of breathing: Inhibitory monosynaptic connections between the amygdala and preBötzinger complex. |
| Aaron Jones | Phrenic motor neuron Bmal1 expression regulates phrenic long-term facilitation in rats. |
| Grigory Loginov | The role of enkephalinergic system in respiratory control |
| Renato Willians Martins Sá | Astrocytes of the lateral parafacial region are sensitive to high CO2 but play no major role in controlling pulmonary ventilation of mice |
| Michael Maxwell | PREDNAC DMD: Respiratory effects of combined chronic glucocorticoid and antioxidant intervention in the mdx mouse model of Duchenne muscular dystrophy |
| Pamela Medeiros | Physical Exercise Prevents Neurodegeneration In Cardiorespiratory Nuclei And Breathing Deficits In The 6-OHDA Model Of Parkinson's Disease |

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| Nicoletta Memos | Butyrylcholinesterase is dispensable for the neonatal autoresuscitation reflex |
| Alyssa Mickle | Detangling the spinal respiratory network's response to external electrical stimulus in a model of spinal cord injury |
| Dipak Patel | Htr1B's role in the autoresuscitation reflex and its Implications to SIDS |
| Luiz Fernando Pedrão | NADPH Oxidase, Apocynin, And Its Effects On Apoptotic And Survival Pathways In The 6-OHDA Rat Model Of Parkinson's Disease |
| Ryan Phillip | Disentangling pain modulation and respiratory depression at the level of the rostral ventromedial medulla |
| Steven Pratscher | Group Breathwork Intervention for Adults with Chronic Pain: A proof-of-concept study of Guided Respiration Mindfulness Therapy |
| Juliana Reis Souza | Effects of activation of glutamatergic interneurons of the lateral parafacial region on the ventilatory parameters of mice during normocapnia and hypercapnia |
| Nathalia Salim | Excitation of somatostatinergic interneurons of the lateral parafacial region of mice during normocapnia and hypercapnia |
| Eliandra Silva | Orexinergic signaling in the caudal nucleus of the solitary tract contributes to the CO2 ventilatory response during light/inactive state |
| Monica Strain | Enhanced peripheral chemoreception contributes to disordered breathing in Rett syndrome. |
| Jessica Whitaker-Fornek | Endogenous Opioids and Breathing Development |

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| Thiago Moreira | Selective stimulation of chemosensitive retrotrapeoid nucleus rescue fentanyl-induced respiratory depression |
| Philippe Haouzi | Effects of metformin-induced hyperlactatemia on breathing control and gas exchange at rest and in exercise |
| Mateus Ramos Amorim | Targeting Melanocortin 4 Receptor to Treat Sleep-Disordered Breathing |

# Meet the Experts (PIs)

- Tuesday October 1st, 4:00 – 5:00 pm
- Friday October 4th, 12:00 – 1:00 pm

**Panelists**

- Jack Feldman: When is a paper a paper?
- Don Bolser: Industry
- Gordon Mitchell: Path to tenure
- Ana Takakura: Women in science
- Silvia Pagliardini: Women in science
- Greg Funk: Dealing with rejection
- Natasha Kumar: Grantsmanship

# Abstracts

Interdependent functions of central chemoreceptors and peripheral chemoreceptors in respiratory homeostasis.

Stephen Abbott

University of Virginia

The control of ventilation to maintain blood gas homeostasis relies on a distributed network of central chemoreceptors combined with peripheral chemoreceptors. This network generates a homeostatic ventilatory drive that matches alveolar ventilation with arterial PCO2, PO2, and pH, defending against asphyxia. This presentation will highlight recent studies characterizing the functional interactions between central and peripheral chemoreceptors that maintain respiratory homeostasis by leveraging genetically targeted approaches to stimulate, inhibit, and measure the activity of central CO2 chemoreceptors in the retrotrapezoid nucleus (RTN) of mice. This work provides evidence that RTN neurons are essential for the effects of arterial PCO2 on respiratory drive and that interactions between RTN neurons and carotid body peripheral chemoreceptors are essential for respiratory homeostasis.

HL148004.

Characterization of swallow-related sympathetic nerve activity

Marlusa Karlen-Amarante[1], Alyssa Huff[2], Kimberly E. Iceman[1], Clinton L. Greene[3], Teresa Pitts[1]

[1] Department of Speech Language Hearing Sciences, Dalton Cardiovascular Center, University of Missouri, Columbia, MO, USA; [2] Center for Integrative Brain Research, Seattle Children's Research Institute. Seattle, WA, USA; [3] Veterinary Medicine Surgery, University of Missouri, Columbia, MO, USA

Sympathetic tone controls vascular tone, and thus the homeostatic maintenance of arterial blood pressure. While the cervical sympathetic nerve (cSN) is known to innervate the upper airways, the activity of the cSN during swallow behavior and any role it might play in swallow dysfunction have not been the subject of many studies. In this study, we characterized cSN activity in rodents to assess its coordination with swallow and breathing. To achieve this aim, we recorded electromyography (EMG) of diaphragm, submental, and laryngeal complex muscles, and neurograms of cervical sympathetic, hypoglossal, and vagus nerves in baseline conditions and during swallow stimulation by injecting 0.1 mL of water into the mouth. Bursts of cSN activity were coincident with both inspiration and swallow. Water swallows triggered higher amplitudes of cSN activity compared to the respective baseline respiratory cycle. Our data suggest that swallow triggers an excitatory vasomotor sympathetic pathway which is involved in the control of upper airway motor activity.

Funded by NIH Grants: NS110169, HD110951.

Targeting Melanocortin 4 Receptor to Treat Sleep-Disordered Breathing

Mateus R. Amorim[1], O Aung[2], Noah Williams[1], Frederick Anokye-Danso[3], Junia L de Deus[1], Jiali Xiong[4], Olga Dergacheva[5], Shannon Bevans-Fonti[1,3], Jeffrey S. Berger[2], Mark N Wu[4], Rexford Ahima[1], David Mendelowitz[5], and Vsevolod Y. Polotsky[1,2,5,6*]

[1]Department of Anesthesiology and Critical Care Medicine, George Washington University, Washington, DC; [2]Medical College of Wisconsin, Milwaukee, WI; [3]Department of Medicine, Johns Hopkins University, Baltimore, Maryland; [4]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; [5]Department of Pharmacology and Physiology, George Washington University, Washington, DC; [6]Department of Medicine, George Washington University, Washington, DC

There is no effective pharmacotherapy for sleep disordered breathing (SDB). A melanocortin receptor 4 (MCR4) agonist, setmelanotide (SET), is used to treat genetic obesity caused by abnormal melanocortin and leptin signaling. We hypothesized that SET can treat SDB in diet induced obese mice. We performed a proof-of-concept randomized crossover trial of a single dose of SET vs vehicle and a two-week daily SET vs vehicle trial in obese mice. We also examined co-localization of Mcr4 mRNAs with a marker of CO2 sensing neurons PHOX2b in the brainstem and performed chemogenetic studies expressing Cre-dependent designer receptors exclusively activated by designer drugs (DREADD) in Mcr4-Cre mice. SET increased minute ventilation across sleep/wake states, greatly enhanced the hypercapnic ventilatory response (HCVR) and abolished apneas during sleep. PHOX2b+ neurons in the nucleus of the solitary tract (NTS) and the parafacial region expressed Mc4r. Chemogenetic stimulation of the MC4R+ neurons in the parafacial region, but not in the NTS, augmented the HCVR without any changes in metabolism. Parafacial MC4R neurons projected to the respiratory pre-motor neurons expressing cholera toxin B after C3-C4 spinal cord injections. In conclusion, MC4R agonists enhance the HCVR and treat SDB by acting on the parafacial MC4R+ neurons.

Support: NIH (R01 HL128970, R01 HL133100, and R01 HL138932), AHA (24CDA1270910).

Gender-specific respiratory and neuroanatomical impairments in a mice model of Parkinson's disease

Yasmin C. Aquino[1], Giovanna M. Rodrigues[1], Thiago S. Moreira[2], Ana C. Takakura[1]

[1]Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, 05508-000, Sao Paulo, SP, Brazil; [2]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, 05508-000, Sao Paulo, SP, Brazil

The neuropathology of Parkinson's disease (PD) involves progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in classical and non-classical symptoms, including respiratory deficits. PD is observed at a two-fold higher rate in men compared to women. Thus, our aim is to examine the influence of sex hormones on respiratory disorders within the PD model. C57BL/6 mice (CEUA: 3325170822) underwent ovariectomy (OVX), orchiectomy (ORX), or sham surgery. Bilateral injection of vehicle or 6- hidroxydopamine (6-OHDA) into the striatum was performed to induce the PD model. Respiratory parameters were evaluated using whole-body plethysmography. In male mice, ORX did not induce changes in the SNpc and respiratory regions, nor did it affect the respiratory rate in animals that received 6-OHDA injection. However, in female PD animals, OVX intensified the reduction of:1) dopaminergic neurons in SNpc from 77 to 90%, 2) neurokinin 1 receptor (NK1r) density in pre-Bötzinger complex from 25 to 37%, 3) phox2b+ neurons in the retrotrapezoid nucleus from 35 to 43% and 4) respiratory rate from 11 to 17%. Therefore, OVX exacerbated SNpc and respiratory region neurodegeneration, along with a reduction in respiratory frequency in 6-OHDA-treated animals, suggesting potential modulatory role of female sex hormones in neuroprotection.

FAPESP, CNPq, CAPES.

Oxytocinergic signaling contributes to breathing regulation in the retrotrapezoid nucleus

Emmanuel V. Araújo[1], Phelipe E. Silva[1], Ana C. Takakura[2], Daniel K. Mulkey[3], Thiago S. Moreira[1]

[1]Department of Physiology and Biophysics; Institute of Biomedical Sciences, University of Sao Paulo, São Paulo, Brazil; [2]Department of Pharmacology; Institute of Biomedical Sciences, University of Sao Paulo, São Paulo, Brazil; [3]Department of Physiology and Neurobiology; University of Connecticut, Connecticut, USA

Neurons in the retrotrapezoid nucleus (RTN) are activated by high CO2/H+ and regulate several aspects of the breathing cycle. RTN neurons are also subject to modulation by various stress- and arousal-dependent inputs including oxytocinergic signaling from the paraventricular nucleus (PVN) of the hypothalamus. However, the extent to which oxytocinergic signaling contributes to chemoreception and breathing regulation at the RTN level is not clear. To test this, we characterized effects of an oxytocin agonist (TGOT) on activity of RTN neurons and breathing in vitro and in anesthetized mice maintained on C57B6/J background. In brain slices from Phox2bcre/+::Ai14 (7-11 days postnatal), cell-attached voltage clamp (Vhold -60 mV) shows that exposure to TGOT (2 nM) increased RTN neural activity from 1.8 ± 0.32 Hz to 3.9 ± 0.3 Hz. This response was dose- dependent but prone to desensitization at concentrations > 32 nM. In anesthetized adult oxytocincre/+::Ai32 mice, unilateral TGOT (1 μM - 30 nL) injection or optogenetic stimulation of oxytocinergic terminals in the RTN stimulated diaphragm electromyography (DiaEMG) amplitude (TGOT: 16.3 ± 10.9 vs. saline: 2.7 ± 4.2% and optogenetic stimulation: 18.7 ± 8.3% of baseline) without changing DiaEMG frequency. These results suggest that oxytocinergic signaling can activate RTN neurons.

FAPESP, CNPq, CAPES-PROEX, NIH/NHLBI.

The Molecular Circadian Clock of Phox2b-expressing Cells Drives Daily Variation of the Hypoxic but Not Hypercapnic Ventilatory Response in Mice

Aaron A. Jones, Gabriella M. Marino, Allison R. Spears, Deanna M. Arble

Department of Biological Sciences, Marquette University, WI 53233, USA

Breathing exhibits 24-hr rhythms, including daily changes in minute ventilation (VE) and ventilatory chemoreflexes. However, the mechanisms that drive these daily changes are not well understood. We hypothesized that the daily breathing and chemoreflex rhythms were dictated by the molecular circadian clock. We used whole-body plethysmography to assess ventilatory function in transgenic BMAL1 knockout (KO) mice to determine the role of the molecular clock in regulating daily rhythms in ventilation and chemoreflex. Unlike their wild-type littermates, BMAL1 KO mice exhibited a blunted daily rhythm in VE and failed to demonstrate daily variation in the hypoxic ventilatory response (HVR) or hypercapnic ventilatory response (HCVR). We then assessed ventilatory rhythms in BMAL1fl/fl; Phox2bCre/+ mice, which lack BMAL1 exclusively in Phox2b- expressing chemoreceptor cells (BKOP). BKOP mice lacked daily variation in HVR, similar to BMAL1 KO mice, but continued to exhibit circadian variations in VE and HCVR comparable to controls. These data indicate that the molecular clock regulates daily rhythms in VE, HVR, and HCVR. Moreover, the molecular clock of Phox2b- expressing cells is specifically necessary for daily variation in the hypoxic chemoreflex. These findings suggest that disruption of circadian biology may undermine respiratory homeostasis, which, in turn, may have clinical implications for respiratory disease.

Supported by Marquette University funds.

Parabrachial Tac1 neurons are conditionally activated during non-homeostatic breathing patterns

Joe Arthurs[1], Nicholas Bush[1], Elora Reilly[1], Steven Guan[1], Nathan Baertsch[1,2]

[1]Seattle Children's Research Institute; [2]University of Washington

Homeostatic breathing is continuously regulated by reflexive neural circuits that provide physiological feedback. However, in the awake state, breathing is also conditionally altered by behaviors and emotions. Relatively little is known about the circuits that mediate this “non-homeostatic'' breathing control. We previously identified a subpopulation of Tac1, but not Calca, expressing parabrachial (PBN) neurons that drives breathing frequencies that far exceed those relevant for the homeostatic breathing, but only in the awake state. Here, we combine cell- type-specific silencing and multi-unit recording approaches to further test the hypothesized role of this circuit in non-homeostatic breathing control. Silencing these neurons with either tetanus toxin, optogenetic, or chemogenetic strategies had minimal effects on breathing in either room air or during homeostatic challenges. “Optotagging” during Neuropixels multi-unit recordings in awake mice suggested that this PBN subpopulation is mostly quiescent during resting homeostatic breathing, but switches to tonic spiking during periods of non- homeostatic breathing including rapid sniffing bouts induced by odorants. Isoflurane anesthesia abolished light- evoked activity of optotagged neurons, underlying their state-dependent function. We conclude that this PBN subpopulation functions as a switch-like mechanism that is inactive at rest and conditionally activated during non- homeostatic breathing patters that are unique to the awake state.

R01HL166317.

PreBötzinger Complex subthreshold oscillations underlie rapid switches in breathing dynamics

Sufyan Ashhad[1,2], Evgeny Bondarenko[1], Omar Ali[1], Jack L. Feldman[1]

[1]Department of Neurobiology, DGSOM, UCLA, Los Angeles, California 90095-1763, USA;
[2]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India

Our study explores the mechanisms that allow mammals to switch breathing dynamics instantaneously for vocalization, active sensing, such as sniffing, and essential reflexes. This robustness and flexibility of breathing, which has remained enigmatic, is the focus of our investigation. In slow-breathing anesthetized adult mice, preBötC local field potentials and population activity oscillate at a higher harmonic(s) of breathing frequency. These oscillations represent preBötC burstlets, which either fail to propagate or trigger inspiratory bursts that propagate to inspiratory motoneurons. The propagation of preBötC burstlet rhythm exhibited attractor dynamics, i.e., pre-inspiratory synchronization generates a burstlet that must cross a tipping point(s) to drive the next inspiration. The preBöC excitation-inhibition (E-I) balance regulated this tipping point. For instance, chemogenetic inhibition of preBötC glycinergic neurons lowered the tipping point(s) for burstlet propagation, resulting in burstlet-driven low-amplitude breaths. Crucially, with decreased inhibition, breathing transitioned rapidly between two dynamical modes: i) high amplitude lower frequency and ii) low amplitude breaths centered at the higher harmonics of the high amplitude breaths. Motivated by these findings we tested the hypothesis that subthreshold preBötC rhythms underlie instantaneous switch in breathing dynamics crucial for coordinating orofacial behaviors. In auditory-stimulated awake-behaving mice, stimulus-induced sniffs were at higher harmonics of the preceding baseline breathing frequency. These experiments reveal that preBötC rhythm is not centered at a single breathing frequency, and its dynamics can be modulated by inputs affecting the E-I balance of the network. These observations are explainable by the burstlet theory of preBötC rhythmogenesis.

Homeostatic and non-homeostatic breathing control - from cellular properties to brain-wide circuits

Nathan A Baertsch

Seattle Children's Research Institute, Norcliffe Foundation Center for Integrative Brain Research
University of Washington, Departments of Pediatrics, Physiology and Biophysics, and Center of Excellence in Neurobiology of Addiction, Pain, and Emotion (NAPE)

Breathing originates from the preBötzinger complex (preBötC) and neighboring regions of the brainstem collectively referred to as the ventral respiratory column. The biophysical properties of this network produce regular rhythmic activity that is continuously regulated by reflexive feedback mechanisms to ensure homeostasis. Yet, despite its vital physiological function, breathing must occasionally override this homeostatic regulation, allowing conditional integration with behavioral or emotional contexts such as olfactory exploration, fear, and pain. Opioids can differentially affect circuits mediating this homeostatic and non-homeostatic breathing control, leading to life-threatening respiratory depression that can be difficult to predict due to its state- and context-dependence. Our lab utilizes computational modelling, viral mediated circuit mapping and manipulation, and electrophysiological recording techniques to deepen our understanding of these aspects of breathing control.
Specifically, recent and ongoing work: 1) illustrates how changes in spike shape can dynamically tune the interdependent cellular and network properties of the preBötC that produce rhythm, 2) defines parabrachial neurons that are conditionally activated to drive rapid breathing in the awake state, 3) characterizes the neurotransmitter phenotype and opioid-sensitivity of brain-wide circuits that project to the preBötC, and 4) explores the cells and circuits that mediate endogenous opioid signaling and their role in breathing control.

R01HL166317, R00HL145004.

Inhibitory GABAergic Cells in The PreBötzinger Complex Mediate Rhythmic Breathing In-Vivo

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The preBötzinger Complex (preBötC) is a key medullary structure critical to generating inspiration. Approximately half the preBötC neurons are inhibitory expressing γ-aminobutyric acid (GABA) or glycine but how these cells control respiratory rhythm is unclear. We identified the role of preBötC-GABA cells in modulating respiratory rhythm in-vivo using optogenetics. We injected Cre-dependent adeno-associated viruses in the preBötC to express either excitatory channelrhodopsins or inhibitory archaerhodopsins in vesicular GABA transporter (encoded by Vgat) cells. We measured diaphragm activity in anesthetized mice or respiratory activity using whole-body plethysmography in awake mice while unilaterally stimulating Vgat cells. In anesthetized mice, photostimulation (470 nm) of preBötC-Vgat cells depressed breathing by delaying the subsequent inspiration. Diaphragm activity did not change when stimulated during inspiration but was abolished with long photostimulations (> 5 sec). Photoinhibition (554 nm) of preBötC-Vgat cells triggered an inspiration during expiration. In awake mice, unilateral photostimulation inhibited diaphragm activity when mice were calm, but not when behaviorally active. Our results show that preBötC-GABA neurons can stop or start diaphragm activity, suggesting these cells are necessary and sufficient to generate rhythmic breathing. Our study is the first to demonstrate the function of preBötC-GABA neurons in-vivo, furthering our understanding of inhibitory circuits regulating breathing.

KB was awarded an Ontario Graduate Scholarship and a Canadian Institutes of Health Research (CIHR) Doctoral Award for her research and GM was supported by CIHR project grants.

Molecular physiology of Phox2b-expressing brainstem neurons

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Congenital central hypoventilation syndrome (CCHS) is caused by mutations in PHOX2B, a transcription factor required for the development and function of brainstem autonomic and respiratory systems. The mechanisms that account for the dysautonomia and reduced respiratory and arousal reflexes associated with CCHS have not been determined, and the broad gene regulatory function of Phox2b in relevant brainstem nuclei has not been explored. We developed a Cre-dependent, viral shRNA-mediated approach to deplete Phox2b expression in respiratory chemoreceptor neurons of the retrotrapezoid nucleus (RTN) and examine effects on: a) single cell transcriptome; b) target neuron connections; and c) regulation of breathing and arousal. Initial candidate analysis from transcriptomic studies suggests a Phox2b requirement for expression of key genes that define RTN neurons, including those that mediate RTN-intrinsic CO2 chemosensation. In addition, Phox2b depletion disrupts projections to multiple brainstem targets. Accordingly, we find that CO2-stimulated breathing and arousal reflexes are blunted after Phox2b knockdown in RTN neurons. Together, these data provide new insights into the gene regulatory functions of Phox2b in a brainstem cell group relevant to CCHS and support the concept that transcription factors that direct early cell differentiation may also be required maintaining cell identity and function throughout life.

Supported in part by NIH HL108609 and Pilot Grant Awards from CCHS Family Foundation.

Breathing Practices for Stress Reduction: Conceptual Framework of Implementation Guidelines Based on a Systematic Review of the Published Literature

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Stress and anxiety plague populations worldwide, and voluntary regulated breathing practices offer an accessible, affordable solution. We examined peer-reviewed published literature to assess the stress-reduction benefits of such practices as a function of population, practice, and approach, and to identify criteria for optimizing intervention effectiveness. PubMed and ScienceDirect were searched for clinical trials evaluating isolated breathing-based interventions with psychometric stress/anxiety outcomes. Two independent reviewers conducted all screening and data extraction. Components of effective and ineffective interventions were evaluated to develop a conceptual framework of factors associated with stress-reduction effectiveness. Of 2904 unique articles, 58 met the inclusion criteria. Fifty-four of 72 interventions were effective. Effective breath practices avoided fast-only breathing and sessions <5 min, and included human-guided training, multiple sessions, and long-term practice. Not associated with effectiveness were variations in population, other breath paces, duration >5 min, and group versus individual/at-home practices. Additional factors potentially rendering interventions ineffective were extensive standing, interruptions, involuntary diaphragmatic obstruction, and inadequate training for complex practices. We identified simple yet important breath practice implementation criteria for effective stress reduction. Our evidence-based framework highlights the importance of slow breathing, adequate training, and continued practice for optimizing outcomes associated with this accessible, whole-health strategy.

This was an unfunded study.

Elimination of noradrenaline synthesis in the Locus coeruleus neurons disrupts CO2-ventilatory and metabolic responses during development in both male and female mice

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Recent data demonstrate that noradrenergic neurons of the locus coeruleus (LC-NA) are required for ventilatory and panic-like escape responses elicited by CO2. The chemosensitivity index of LC neurons depends on age, being larger in young (P10) neonate rats. Nevertheless, the effects of these alterations on breathing control, as well as whether this effect is sex-dependent, remain unresolved. We evaluated the respiratory and metabolic responses to normocapnia and hypercapnia (7% CO2) at postnatal period (P0-1, P6-7 and P12-13) of male and female wild type C56BL/6 mice (Dbhwt/wt), mice that lack Dbh expression required for NA synthesis in the LC En1Cre/wt Dbhflx/flx (DbhLC-null) and mice that have reduced Dbh expression in LC and other noradrenergic neurons En1wt/wt Dbhflx/flx (Dbhhypo). Our data show that DbhLC-null and Dbhhypo females have a reduced respiratory and metabolic response at all ages during CO2 exposure whereas in DbhLC-null and Dbhhypo males a similar response was observed only in P6-7 animals. In contrast, P12-13 DbhLC-null and Dbhhypo males showed an increased CO2-hyperventilatory response. Our findings suggest that the release of NA from the LC is important for hypercapnic ventilatory response in both sexes during development showing a sex-dependent effect on P12-13.

FAPESP, CNPq and NIH.

The hyperpolarization-activated inward current, Ih, in the preBötzinger Complex contributes to the hypoxic ventilatory response in anaesthetized adult rats

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Hypoxia triggers a biphasic ventilatory response (HVR), comprising an initial increase followed by a secondary depression that can be life-threatening in premature babies (Apnea of Prematurity/AOP). Dogma holds that there is no contribution of the CNS to the increase in ventilation evoked by hypoxia. More recent data, however, indicate that astrocytes in the preBötzinger Complex (preBötC) respond to hypoxia by releasing ATP, which acts via P2Y1 receptors to increase ventilation and attenuate the secondary depression. In vitro, the P2Y1R excitation of the preBötC network, and a subpopulation of inspiratory neurons, is partly mediated by the hyperpolarization- activated inward current, Ih (HCN channels). To test whether this ionic mechanism contributes to the HVR, we compared in anaesthetized rats the HVR evoked by 10% O2 in four groups: two control groups; bilateral preBötC injection of ZD7288; bilateral preBötC injection of shRNA to knockdown HCN1/2 mRNA. Bilateral ZD7288 microinfusion reduced the HVR compared to aCSF controls. ~40% bilateral knockdown of HCN1/2 reduced the hypoxia-induced frequency increase in paralyzed, ventilated rats, but did not affect the HVR in freely-behaving rats. Data suggest that Ih in the preBötC contributes to the HVR, but its contribution is obscured in freely- behaving animals with intact redundant chemosensory mechanisms.

CIHR, NSERC, WCHRI (Postdoctoral Fellowship to VB), FAPESP, CAPES.

Respiratory pathology in the TDP-43A315T mouse model of Amyotrophic Lateral Sclerosis

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Duke University

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. Mutations in the transactive response DNA binding protein 43 (TDP43) is associated with ALS. However, its impact on breathing is unclear. Since respiratory failure is a major cause of death in ALS, we sought to determine the role of TDP-43 mutations on the respiratory motor unit in the Prp-hTDP-43A315T mouse model that expresses human TDP43 with A315T mutation. We examined breathing using whole-body plethysmography and neuropathology in hypoglossal and phrenic respiratory motor units of these mice by examining the hypoglossal and putative phrenic motor neuron pools and nerves of the TDP43A315T mice. These mice had progressive breathing deficits at baseline and during hypoxia and hypercapnia. The male TDP43A315T mice had more severe disease than the female mice, including an early onset and rapid progression of disease and earlier death. Hypoglossal and putative phrenic motor neurons of TDP43A315T mice are decreased with significant activation of microglia and astrocyte, indicating pronounced neurodegeneration and neuroinflammation, along with axonopathy and demyelination in the hypoglossal and phrenic nerve of TDP43A315T mice. Thus, the TDP-43A315T mice have significant respiratory pathology and are a potentially useful model to assess the impact of novel therapies on breathing in ALS.

1R21NS098131-01 (MKE); and Children’s Miracle Network Hospital Research grant (DDB).

Brainstem circuit motifs and network strategies regulating the respiratory network: Lessons from a 32 year database

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Investigations of the respiratory network have revealed a system driven by inspiratory neurons in the preBotzinger complex and a motor pattern shaped by a variety of circuit strategies involving inspiratory and expiratory neurons. We have consolidated a database of brainstem neuron spike train recordings from experiments in cats in vivo.

These spike trains were obtained by simultaneous recordings with multiple tungsten electrode arrays (n=40-100 electrodes) that were placed in brainstem respiratory areas. This database includes spike trains of over 7,200 neurons. Functional interactions between neurons were identified by cross correlation analysis. Over 160,000 cross correlations were performed of which over 9,300 functional correlations were detected. Interrogation of the database supports: 1) approximately 40% of these cross correlations were solely central features indicative of parallel processes; 2) reciprocal inhibitory relationships can occur via complex subcircuits involving at least 3 or 4 neurons; 3) excitation was observed between inspiratory and expiratory neurons sometimes as often as inhibitory phenomena; 4) some functional relationships were biphasic consistent with excitatory phenomena that was “braked” by temporally-linked inhibition. These observations support a network that is composed of both serial and parallel process and is regulated in part by biphasic interactions between individual neurons.

NIH HL 163008; NIH HL 155721.

The roles of glutamatergic and glycinergic preBötzinger Complex neurons in inspiratory rhythm generation in vivo

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The preBötzinger Complex (preBötC) is the mammalian kernel for inspiratory rhythm generation. Our hypothesis of preBötC rhythm generation is that low amplitude (preinspiratory) burstlets are the rhythmogenic component driving preBötC inspiratory bursts. Our recent in vitro and modeling studies propose critical role of neuronal synchronization in generation of preBötC burstlets and bursts. Here, we further extend tests of Burstlet Theory and determine genetic specificity of subtypes of preBötC neurons that underlie production of burstlets and bursts in vivo. Using fiber optical recording of excitatory or inhibitory preBötC neuronal populations in slow breathing anesthetized mice, we observed burstlets, i.e., small amplitude rhythmic discharges that do not propagate to motor output, in both preBötC VGluT2+ and GlyT2+ neurons. Next, using ultrapotent inhibitory DREADD PSAM4 we determined that these two neuronal populations produce both burstlets and bursts. Inhibition of VGluT2+ preBötC neurons evoked an immediate and irreversible terminal apnea in anesthetized mice. Inhibition of GlyT2+ preBötC neurons evoked a breathing pattern, in which burstlets, normally observed only in preBötC, propagate to motor output. We conclude that burstlets are produced by excitatory VGluT2+ preBötC neurons in vivo, while GlyT2+ preBötC neurons provide feedback inhibition.

Supported by NIH Grants R35 HL171451 and R35 HL135779.

Neuromodulation of cervical excitatory interneurons enhances the hypercapnic response in chronic cervical spinal cord injury

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Respiratory dysfunction is the leading cause of death in individuals with cervical spinal cord injury (cSCI), and current treatments are invasive and have significant systemic effect; as such, developing new treatments to improve breathing post-injury is crucial. Cervical excitatory interneurons (eINs) play a key role in breathing and diaphragm motor recovery after injury; however, the fate and function of these neurons in adaptive breathing in chronic cSCI has not been investigated. We examined the role of cervical eINs in the hypercapnic response and investigated their integration into respiratory networks in both health and chronic cSCI. Using cellular-specific one-photon in vivo calcium imaging, we demonstrate a subpopulation of cervical eINs exhibit increased activation during hypercapnia. Further, we find that cervical eINs remain intact and viable 12 weeks after cSCI and, selectively stimulating these neurons using chemogenetics effectively enhances breathing during hypercapnia in health and chronic cSCI. Monosynaptic retrograde viral tracing from these cervical eINs demonstrates direct neural connectivity to raphe serotonergic neurons, providing anatomical evidence for their role in the hypercapnic ventilatory response. These findings underscore the importance of cervical eINs in adaptive breathing and present a novel target for developing therapeutic interventions to improve ventilatory function after cSCI.

Carotid body activity coordinates changes in breathing and hippocampal physiology

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A growing body of evidence suggests that breathing can influence cognitive performance, yet the interplay between these domains remains poorly understood. In rodents, exposure to intermittent hypoxia (IH) enhances carotid body (CB) activity, increasing sympathetic tone and causing dysregulated breathing. IH also leads to deficits in spatial memory through a HIF1a-dependent mechanism, which drives hippocampal remodeling and suppresses synaptic plasticity. This study examines the role of CB activity in IH-dependent alterations in hippocampal properties. We found that carotid body denervation (CBD) effectively prevents IH-dependent molecular remodeling, and impairment to long-term potentiation (LTP). Using the virally expressed biosensor, GRABNE, we measured intrahippocampal adrenergic tone and discovered that IH enhances hypoxia-driven increases in respiratory-coupled adrenergic tone. Additionally, in β2-adrenergic receptor null mice, IH does not induce HIF1a-dependent molecular remodeling nor suppress LTP. In vitro brain slice experiments further demonstrate that β2-adrenergic receptor signaling alone is sufficient to increase hippocampal HIF1a activity.
These findings indicate that CB activity plays a central interoceptive role necessary for coordinating changes between breathing and cognition in conditions such as sleep apnea.

R01HL163965.

Rapid terminal apnea following diving reflex with carotid body resection or seizure

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Seattle Children's Research Institute

Evidence suggests that sudden unexpected deaths in epilepsy (SUDEP), childhood (SUDC), and infants (SUID) share some overlapping mechanisms. In urethane anesthetized rats we established that animals with either continuous seizure or carotid body (CB) resection display high mortality to the diving reflex. We sprayed 0.2–2mL of ice water into the nasal cavity over 2–20 seconds. Animals with seizure or CB resection experience fatal apnea at 14-58% per-exposure mortality risk, while for healthy controls the risk is 0%. The fatal response to the reflex was rapid – with 90% silencing of medullary spiking 46.7 ± 8.4 seconds after stimulus onset. The laryngeal chemoreflex, initiated by placing a 0.1mL drop of acid on the laryngeal epithelium, yields similar data to the diving reflex. We hypothesize that these two reflexes (one trigeminal and one vagal) likely share some network architecture governing the fatal apnea. Using Neuropixels in urethane-anesthetized mice, we are currently exploring why certain medullary regions shut down even faster – down to 33.1 ± 9.8 seconds. Using transgenic mice, targeted viral expression, optogenetics, and typical peripheral respiratory measures will allow us to identify the neurons and their medullary locations critical for the sudden death.

This work is supported by R01HL126523 and a private donation to support SIDS research.

Episodic slow breathing in mice markedly reduces fear responses

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For millennia, controlled breathing practices have been used to rebalance emotions and reduce stress. We sought to delineate neural mechanisms underlying the effects of controlled breathing in humans, such as in meditation, which can reduce depression, anxiety, stress, and pain. Here, we developed a murine model where breathing frequency is substantially slowed. The protocol in awake mice involves periodic optogenetic stimulation of AAV (ChR2)-transfected preBötC GlyT2+ neurons. When breathing was slowed for 20-30 min/day for 4 weeks, subsequent evaluation of these mice showed significant reductions in stress-related changes in behavior compared to controls. Thus, the positive effects of slow breathing on emotional state are present in mice and cannot be attributed to top-down influences, e.g., volitional control, or placebo effects. The fact that we see effects in mice and humans suggest an evolutionary conservation of this effect, likely across many if not all mammalian species. Our study paves the way for investigations of the neural mechanisms underlying body-brain interactions related to the effects of controlled breathing.

Supported by NIH Grant RO1 AT012412 and R21 MH134158.

Mechanisms of Fentanyl-Induced Respiratory Muscle Rigidity

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Opioid-induced respiratory depression (OIRD) is the leading cause of mortality in opioid overdose cases, with fentanyl being a major driver. Research has shown that opioids like fentanyl act on mu-opioid receptors (MORs), impairing brainstem respiratory centers and reducing respiratory rhythm generation. Fentanyl is particularly fatal due to its ability to cause "wooden-chest syndrome" (WCS), which is refractory to naloxone. This potent synthetic opioid induces increased muscle tone, predominantly affecting the chest wall and abdominal muscles, compromising respiratory function. In urethane-anesthetized mice, 500 μg/kg fentanyl reduced respiratory rate by nearly 50% and elevated tonic diaphragmatic activity, indicating muscular contraction. Fentanyl acts on MORs and directly blocks subthreshold K+ channels, leading to membrane depolarization. Using HEK-293 cells expressing Kv10.2, fentanyl blocked EAG-like K+ currents, reducing outward currents by over 70%. In spinal cord sections from C3-C5, bath-applied fentanyl enhanced excitability in 44.44% of phrenic motor neurons, with 44.44% showing inhibition and 11.11% no change. This depolarizing effect, unaffected by synaptic isolation and not reversed by naloxone, suggests a direct effect on motoneuronal excitability independent of MORs. Our results reveal fentanyl's novel non-MOR mechanism, enhancing spinal phrenic motor neuron excitability and contributing to diaphragm rigidity through off-target blockade of K+ channels.

K99HL168211, NJB R01HL126523, JMR.

Long lasting suppression of phrenic motor plasticity after mild acute inflammation is mediated by persistent p38 MAPK activity

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Mild systemic inflammation elicited by a low dose of the TLR4 receptor agonist lipopolysaccharide (LPS; 0.1mg/kg), undermines a form of serotonin-dependent phrenic motor plasticity elicited by moderate acute intermittent hypoxia (mAIH), known as phrenic long term facilitation (pLTF). To date, pLTF suppression has been studied only during the period of active inflammation (3-24hrs). Thus, the duration of pLTF suppression is unknown. Here, we report that even mild inflammation persistently suppresses phrenic motor plasticity for more than 3 weeks after spinal pro-inflammatory markers have returned to normal (Nikodemova et al., ibid).
Mechanisms whereby acute inflammation undermines mAIH-induced pLTF during the acute versus post-inflammatory recovery phase are not well understood. Here, we demonstrate that, with acute inflammation (24 hours post-LPS), spinal adenosine-dependent p38 MAP kinase activation is necessary to suppress mAIH-induced pLTF. In contrast, whereas pLTF remains suppressed during the post inflammatory recovery phase (1-week post- LPS) via persistent p38 activity, it is no longer via an adenosine-dependent mechanism. Thus, mechanisms initiating pLTF suppression shifts between the acute vs post-inflammatory, recovery phases, yet they share common downstream signaling (i.e., p38 MAP kinase).

NIH R01HL149800 and R01HL148030.

Acute Intermittent Hypercapnia Elicits Phrenic Long-Term Facilitation During the Daily Rest, but not Active Phase in Rats

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Acute intermittent hypercapnia (AIHc) consisting of severe hypercapnic episodes triggers a form of respiratory motor plasticity known as phrenic long-term depression (pLTD), characterized by a sustained reduction in phrenic nerve burst amplitude. To date, studies of AIHc-induced pLTD were conducted exclusively using severe AIHc presented during the rodent rest phase (vs human active phase). Since we recently discovered that daily fluctuations in spinal adenosine levels significantly influence the magnitude and mechanisms of acute intermittent hypoxia-induced phrenic long-term facilitation (pLTF), we measured changes in phrenic nerve burst amplitude in anesthetized male Sprague-Dawley rats exposed to moderate AIHc (arterial $PCO_2$ ~48mmHg) consisting of 15, 1-min episodes delivered in both the mid-rest and mid-active phases (n=7 each). With such modest levels of hypercapnia, we observed pLTF versus pLTD; phrenic burst amplitude was elevated 90 min post-AIHc versus baseline when presented in mid-rest (62±13%; $P<0.001$), but not mid-active phase (23±22%; $P=0.218$). The unexpected finding of AIHc elicits pLTF and not pLTD is likely due to shorter and milder hypercapnic episodes (1 min vs. historical 5 min; $FiCO_2=3.5\%$ vs. historical 10%). Much remains to be understood about AIHc-induced respiratory motor plasticity, such as its regulation across the daily rest/active cycle.

Supported by: NIH R01 HL147554 & R01 HL148030 (PI: G. Mitchell).

Motor unit recruitment and rate coding strategies in human genioglossus during flow limited breathing in obstructive sleep apnoea

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The neural control of genioglossus is complex, with distinct patterns of single motor unit (SMU) activity observed during quiet breathing in wakefulness. This study examined SMU activity from genioglossus during sleep in 11 participants with OSA (apnoea hypopnoea index AHI>10 events/hour/sleep). Forty-four SMUs were recorded with fine-wire electrodes in the genioglossus and epiglottic pressure was measured with a catheter. Participants slept supine with continuous positive airway pressure (CPAP), where 2 tonic and 16 inspiratory units were active.

During transient CPAP drops, which induced increasing epiglottic pressure reductions to induce airflow limitation, a further 26 phasic inspiratory SMUs were recruited; 10/26 became tonic inspiratory with increasing respiratory stimuli. All inspiratory units increased their activity with increasing negative pharyngeal pressure (original 16 SMUs by1.0±0.9 Hz/-1 cmH2O, mean±SD, range 0.1-3.5 Hz; recruited 26 SMUs by 1.3±1.4 Hz/-1 cmH2O, range 0.2-5.8 Hz). By contrast, tonic unit firing became phasically inhibited during inspiration, and deactivated by airflow limitation. No new tonic units were recruited. These data indicate that during sleep, inspiratory genioglossus SMUs increase their activity via recruitment and rate coding and that a limited subset of inspiratory hypoglossal motor neurons are recruited reflexly to compensate for airflow limitation and maintain airway patency.

National Health and Medical Research Council of Australia.

Machine Learning for Identification of Phenotypes in Cardiorespiratory Activity During Autoresuscitation Reflex in Neonate Mice

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Sudden Infant Death Syndrome (SIDS) remains one of the leading causes of infant mortality in the United States. Failure of the neonate autoresuscitation reflex is hypothesized to be a common endpoint for SIDS. However, not much is known about the biological causal underpinnings that affect the likelihood of autoresuscitation failure. In this project, we implement machine learning to explore autoresuscitation reflex failure through the study of neonate autoresuscitation ECG and plethysmography waveforms from mouse pups exposed to repeated anoxic challenges to induce apnea and a subsequent autoresuscitation reflex. We first implement UMAP for dimensionality reduction and fuzzy c-means clustering in order to identify clusters across different mouse models which exhibit similar patterns of cardiorespiratory behavior in response to anoxia. We then derive cluster feature importance scores by applying a random forest classifier predicting the estimated labels from the raw features, which allow us to find combinations of genotypes and physiological factors that typify each cluster. We use the results to characterize common patterns in cardiorespiratory responses to anoxia and to establish genetic and biological phenotypes associated with each pattern. These outcomes help illustrate the preeminent features that define specific potential cardiorespiratory phenotypes and provide novel mechanistic insights into autoresuscitation failure.

NIH 5R01HL161142.

Mechanism and Function of PACAP Expression in the Neonatal Retrotrapezoid Nucleus

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At birth, an infant faces numerous physiological challenges in the transition to extrauterine life, with the first breath signifying the beginning of a life-long process required for homeostatic regulation of blood gases. The retrotrapezoid nucleus (RTN) comprises a group of brainstem neurons that detect and respond to elevated levels of arterial CO2 (PCO2) and provide critical drive to the respiratory circuits that maintain blood gas homeostasis.

We recently found that neonatal mice (P2-P12) lacking the neuropeptide PACAP in RTN neurons have a blunted CO2-regulated respiratory response and increased respiratory instability under thermal stress. We additionally reported an upregulation of PACAP expression by the RTN following birth. The current work aims to uncover the physiological mechanisms that underlie this birth-associated activation of PACAP expression in the RTN, and to determine whether PACAP expression contributes to stabilizing respiratory output in the immediate postnatal period. Preliminary results suggest that hypoxic exposure, intended to drive hyperventilation and decrease PCO2, disrupts early PACAP expression in the neonatal RTN. Ongoing experiments are examining effects of PACAP deletion in RTN on respiratory control in the early neonate (<P1). We expect to reveal mechanisms underlying PACAP expression in the RTN, and its possible contributions to early respiratory efforts.

T32GM148379, UVA Wagner Fellowship.

Novel Interoceptive Properties of the Carotid Body in Health and Disease

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Interoception involves recognizing and being aware of the body's internal state, crucial for regulating functions like heartbeat, digestion, glucose metabolism, and respiration. The carotid body (CB) acts as an interoceptive organ, sending information to the brain through its sensitive nerve, the carotid sinus nerve, aiming to maintain homeostasis. While traditionally known for sensing oxygen, carbon dioxide, and pH levels, the CB is now recognized to possess additional interoceptive properties, detecting various mediators involved in blood pressure regulation, inflammation, and glucose homeostasis, among other physiological functions. Furthermore, in the last decades CB dysfunction has been linked to diseases like sleep apnea, essential hypertension, and diabetes

In this talk, I will explore the novel interoceptive properties of the CB related to glucose and energy regulation and its relation with the control of breathing. Additionally, I will delve into the neural pathways and circuitries implicated in CB-mediated energy homeostasis, while discussing strategies to modulate CB activity and its reflex responses.

Connectivity and synaptic weight distribution effect the spiking network dynamics of a minimal model of the brainstem preBötzinger Complex

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The brainstem preBötzinger Complex (preBötC) generates two breathing-related rhythms: one for inspiration on a timescale of seconds and another for sighs on the order of minutes. The preBötC exhibits: 1) adjustable frequency with inspiration about 20-fold more labile than sigh; 2) network excitability focal excitation of 4-9 constituent neurons; 3) rhythm breakdown following laser ablation of ~15% of its constituent neurons; and 4) miniature 'burstlets' that trigger full-sized bursts and motor output. Here we present a minimal spiking model - based on the activity model of Borrus et al., J. Physiol. 602(5) 809-834, 2024 - that reproduces these essential aspects of preBötC rhythms. A 3-variable dynamical system representing network activity (a), synaptic depression (s), and cellular adaptation (θ) give rise to the inspiratory rhythm. The intracellular calcium oscillation subserving the sigh rhythm is modeled via a 2-variable slow subsystem representing cytosolic and endoplasmic reticulum Ca2+ concentrations. Parameter studies focusing on properties 1-4 (above) are performed using 500 spiking neurons to assess the importance of network connectivity (e.g., Erdős–Rényi or Barabási–Albert) and synaptic weight distribution (e.g., constant, normal, or log-normal) on these crucial benchmarks for a realistic preBötC model.

This work was supported by the National Institutes of Health grants R01-HL104127 (PI: C.A.D.N.) and R01- AT010816 (PIs: G.D.C.S. and C.A.D.N.), and National Science Foundation grant DMS 1951646 (PI: G.D.C.S.).

Respiratory deficits in a mouse model of epilepsy caused by altered PI3K/mTOR signaling in the forebrain

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Respiratory deficits are a major contributor to Sudden Unexpected Death in Epilepsy (SUDEP). However, it is not clear how epilepsy, primarily associated with altered activity in the forebrain, influences breathing, primarily driven by respiratory centers in the brainstem. To investigate this problem, we employed a genetic mouse model lacking phosphatase and tensin homolog (PTEN) in excitatory forebrain neurons. These animals experience tonic-clonic seizures, cortical dysplasia, and premature mortality. We used continuous electromyography (EMG) and electroencephalography (EEG) recordings to measure breathing and seizure activity in these animals over the course of epilepsy progression. Our analysis revealed frequent apneas and decreased diaphragm activity shortly after seizure-onset, progressing to slow breathing late in disease, ultimately resulting in respiratory failure and premature death. Furthermore, we identified connections between forebrain neurons lacking PTEN and neurons in the nucleus of the solitary tract, a region in the brainstem important for regulation of breathing. Future experiments will test whether forebrain neurons that project to the nucleus of the solitary tract drive breathing abnormalities and premature death in this epilepsy model.

CURE Epilepsy Foundation NIH R21 NS121644.

Evaluating the role of KCNQ ion channels in breathing rhythmogenesis and opioid-induced respiratory depression in adult mice

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Rhythm and pattern information for inspiratory breathing movements emanates from the preBötzinger Complex (preBötC). Although the ionic and synaptic mechanisms that initiate and sustain inspiratory bursts are well understood, those involved in burst termination remain unclear. Furthermore, the mechanisms by which opioids depress breathing are not well understood either. KCNQ2/KCNQ3 heteromeric ion channels underlying M-type potassium currents have been associated with both burst termination and opioid-induced respiratory depression (OIRD) due to their subthreshold voltage dependence and non-inactivating kinetics. We tested those roles using short-hairpin RNA (shRNA) to knock down Kcnq2 and Kcnq3, thus M-currents, in preBötC glutamatergic neurons of 10-14 weeks-old mice. We predicted that this attenuation would slow down breathing by increasing inspiratory time and blunt the effects of opioids. Surprisingly, we found that KCNQ2/KCNQ3 currents neither influence inspiratory breath duration nor mediate the depressant effects of opioid drugs because knockdown of Kcnq2/Kcnq3 subunits by <65% in the preBötC of adult mice did not affect any measures of breathing including the depressant effects of fentanyl. Our results do not refute existing explanations for burst termination involving synaptic depression and non-M-type intrinsic currents, nor do they contradict the role of non-gated potassium channels and presynaptic inhibition in OIRD.

NIH R01 NS107296.

Epidural stimulation for recovery of respiration after spinal cord injury

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Over half of traumatic spinal cord injuries (SCI) occur at the cervical level, leading to respiratory compromise or failure. Approximately 20% of cSCI patients will require ventilator support and there are few therapeutic options for recovery. Epidural stimulation has emerged as a strategy to restore a variety of motor, sensory, and autonomic functions in experimental and clinical conditions after SCI. To date little is known how eStim elicits motor function at the neuronal level. Even less is known about the capacity for epidural stimulation to promote long-lasting spinal plasticity and to date no previous studies have explored the potential for eliciting respiratory plasticity. We have shown that long-term, closed-loop epidural stimulation elicits facilitation in evoked diaphragm activity that outlasts the stimulation. Further we see immediate activation of spontaneous diaphragm bursting with eStim in acute preparations. We are now beginning to parse the spinal neuronal populations involved in acute closed- and open- loop stimulation paradigms, further elucidating what becomes activated and what can lead to longer-term plasticity once stimulation has ended. Ultimately, these data will serve to inform development of future investigations of the mechanistic basis of epidural stimulation's efficacy essential for advancing therapeutic applications to the neural system controlling breathing.

Breath Practice Clinical Trial Outcomes on Telomere Length, Stress and Anxiety

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Based on a randomized study in a diverse human population we report a significant lengthening of telomeres, and significant improvements in perceived stress and anxiety scores, from pre- to post-intervention. A study of 96 participants was conducted in residence at the Kaivalyadhama Yoga Institute, India. Three groups were studied: a passive control who did not receive any intervention; an active control who continued their training in āsana body- based practice; and an active intervention group who received a comprehensive yoga approach with emphasis on a combined practice of breath control-lower abdomen activation-inner focus. Along with significant improvements in mental health markers, the intervention group reported a significant increase in telomere length, not found in either of the control groups. A higher 'Engaged Participation' as a measure of sincerity was found to be the most significant and strongest predictor of this outcome. This presentation will unpack the yoga protocol, the study design made to represent an ecologically valid real world practice scenario, and the positive longevity results on the first known study to report on breath practice and telomere length.

No funding to declare.

Photoinhibition Of Pre-Bötzinger Neurons That Project To The Facial Nucleus Affect The Nasofacial Activity With Minimal Effect On Breathing In Rats

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The pre-Bötzinger Complex (preBötC) is a major generator of breathing that also modulates cardiovascular function, emotional state and orofacial behaviours. The identity of the neurons responsible for these different functions is not determined. Here, we identified the preBötC neurons that projects to facial nucleus (7n) motoneurons to coordinate nasofacial activity. We injected a cre-dependent virus that expresses a somatic- targeted inhibitory opsin - AAV-DIO-GtACR2-muGFP-Kv2.1 into the preBötC of Sprague-Dawley rats (60-80 g, n=19). In eleven of these rats, a retrograde virus expressing cre-recombinase (AAVrg-Cre) was injected into the 7n. Three weeks later, blood pressure, heart rate, and diaphragm, abdominal and mystacial pad muscle activity were recorded in either anesthetised or conscious rats, with optic fibres located in the preBötC. Our results show that selective photoinhibition of preBotC□7n neurons affects mystacial pad activity, with minimal effects on breathing. These effects are altered between anesthetised and conscious states. Postmortem analysis showed that excitatory and inhibitory neurons were transduced and send collaterals to multiple brainstem nuclei involved in regulating autonomic activity. The results suggest that distinct preBötC populations predominantly regulate specific functions, but that collateral projections to other nuclei contribute to the coordinate these functions with breathing.

Australian Research Council DP231003058, National Health and Medical Research Council 1156727.

A PHOX2B+ Pontine Nucleus Essential For Ingestion

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The first phase of feeding consists in acquiring solid foods from the environment by biting, and their preparation for swallowing by chewing. These actions require the precise coordination of tens of orofacial muscles for the jaw and tongue. The siege for this motor patterning is known to be in the reticular formation, a complex and poorly mapped region of the hindbrain, but the neuron groups involved are still elusive. Here, we characterize a group of reticular interneurons located in the supratrigeminal area that express the homeodomain transcription factor Phox2b. This nucleus — Sup5Phox2b— is premotor to both jaw-closing and jaw-opener motoneurons and receives direct input from cranial sensory afferents, motor cortex and satiation related nuclei. Its activity differentially tracks lapping, biting and chewing movements, suggesting its involvement in the elaboration of distinct orofacial motor patterns in vivo. Acute global activation or inhibition of Sup5Phox2b by optogenetics both interrupt volitional feeding sequences. Thus, Sup5Phox2b is an obligatory subcortical node, topologically and genetically defined, in the neural circuits that control the oral phase of feeding.

ANR-19-CE16-0029-177 01 ANR-17-CE16-0006-01 FRM EQU202003010297.

Asymmetric neuromodulation in the respiratory network contributes to rhythm generation

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Like other brain circuits, the mammalian respiratory network is modulated by neurotransmitters that activate slow metabotropic receptors. In many cases, exogenous neuromodulation only subtly alters the respiratory motor pattern. However, some neuromodulators, like opioids, can evoke respiratory arrest. We hypothesized that neuromodulatory transmission is organized asymmetrically across the network to contribute to rhythm generation, and developed a computational model that encapsulates this hypothesis. Then, we compared model predictions of increasing the strength of subsets of neuromodulatory connections based on the net effect on their post- synaptic targets with pre-Bötzinger complex ensemble activities before and after systemic administration of neuromodulatory receptor agonists in situ. Increasing slow inhibition in the model predicted a mild increase in respiratory frequency without changing the distribution of neuronal activities generated by the network, which we confirmed experimentally following administration of the 5HT1aR agonist 8-OH-DPAT. Increasing slow excitation in the model predicted a collapse of network activity associated with a reduction in the number of active neurons sparing a subset with tonic or bursting activities. These predictions were confirmed following administration of the μ-opioid receptor agonist fentanyl. We conclude that asymmetric neuromodulation contributes to respiratory pattern generation, which may explain the varied effects of neuromodulation on breathing.

NIH R01 HL-161582.

During Sepsis, Breathing and the Brainstem Concentrations of Proinflammatory Cytokines Differ between Male and Female Rats.

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In male rats, we have associated ventilatory changes during sepsis with increased expression of IL-1β, a proinflammatory cytokine, in the dorsomedial medulla. Here, we hypothesize that during sepsis the ventilatory behavior and brainstem cytokine concentrations differ in female compared to male rats. CWRU-IACUC approved our protocol, which we applied to Sprague-Dawley males (n=24) and females rats (n=42) (23 proestrus &19 nonproestrus) and formed groups of naïve, sham, and inoculated (Escherichia coli). We measured the baseline ventilatory pattern (VP) using plethysmography. At 12h, we measured VP and harvested serum and brainstem, lung, and liver tissues to measure (Luminex) cytokines (IL-1β, IL-6, IL-17, KC, and TNFα) of each group. At baseline, fR was greater in male than female rats in proestrus but not nonproestrus. In the sham group, fR did not differ between the sexes but in the inoculated group, fR was much greater in males than female rats. Luminex revealed a differential distribution of cytokines in the brainstem. The caudal medulla had greater concentrations of IL-1β and IL-6 in males than females, whereas, IL-1β and IL-6 were greater in the rostral medulla of females than males. These results support the hypothesis but require confirmation.

National Institutes of Health (NIH) U01EB021960, R01 HL-161582 and the VA Research Office I01BX000873.

Focal ablation of the amygdala may prevent seizure-induced hypoventilation and sudden unexpected death in epilepsy (SUDEP)

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Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with intractable epilepsy, often due to seizure-induced apnea. Seizure spread to the amygdala and electrical stimulation of a focal amygdala site (AIR site) inhibit breathing, causing apnea that can persist for minutes. Patients are unaware of this respiratory suppression and do not experience air hunger. To investigate mechanisms underlying amygdala seizure-induced apnea and SUDEP, we studied epilepsy patients with intracranial electrodes. In an infant who experienced near-SUDEP and required resuscitation, seizures originating in the amygdala and hippocampus coincided with near-fatal apnea. Stimulation of the amygdala evoked apnea whereas the hippocampus did not, suggesting the apneic effects were specific to the amygdala. Surgical resection of these structures prevented post-surgery diffuse seizure activity from evoking apnea, suggesting that resection of the amygdala may prevent seizures outside the amygdala from evoking apnea and SUDEP. Supporting these findings, AIR site ablation in another patient prevented stimulation and seizure-induced apnea. Providing a mechanism for these inhibitory effects on breathing, amygdala stimulation in other patients reduced hypercapnic ventilatory and dyspneic responses. Together, these findings suggest that focal ablation of the AIR site may be a strategy to prevent seizure-induced hypoventilation and SUDEP.

K08 NS112573 01 A1.

The role of a molecularly defined inhibitory inspiratory neuron in breathing demonstrates that inspiration must suppress expiration.

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The speed of our breathing can be modulated in two ways, a general or tonic tuning of the rhythm and its moment-by-moment control. Each inspiration emerges as a ramping activity of excitatory neurons in the preBötzinger Complex (preBötC) in the presence of both tonic and phasic inhibition. It's conceivable that tonic inhibition sets a threshold for the preBötC ramp to overcome, while brief phasic inhibition can rapidly suppress or terminate it. Physiologically, the latter can manifest as a failure to inspire and a prolonged expiration. The contribution of each of these modalities to breathing control remains incompletely characterized due to an inability to selectively manipulate either. Optogenetic silencing all inhibitory mouse preBötC neurons in vivo revealed an abnormal hyperexcited inspiratory state, consistent with a role of inhibition to control the general level of preBötC activity. In contrast, we found that silencing a specific sub-population of preBötC inhibitory cells stopped breathing, locking it in expiration. These cells were active during the inspiration breath phase and acutely silencing them suppressed preBötC inspiratory units while converting surrounding expiratory patterned units into tonically active. This data demonstrates that some preBötC inhibitory neurons must "turn-off" expiration to breathe, and consistently, ablation of these specific neurons is lethal. Together, these results reveal that a necessary mechanism for respiratory rhythm generation is the reciprocal interaction between inhibitory inspiratory and expiratory cells.

NIDA, Simons Foundation, Klingenstein-Simons Foundation, Program of Breakthrough of Biomedical Research Sandler Foundation.

Significance and state-dependent modulation of mammalian glossopharyngeal nerve motor system.

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The glosspharngeal nerve selectively innervates the stylopharyngeus muscle (SPm) is known to dilate the upper airway and to stabilize the lateral pharyngeal wall during inspiration. The collapse of the lateral pharyngeal wall has recently become a key determinant for the assessment of obstructive sleep apnea severity. Here we use plethysmography recordings and the in situ perfused brainstem preparation of rat to investigate the significance of the glossopharyngeal-SPm motor system in maintaining inspiratory airway patency and its state-dependent modulation using an in situ perfused brainstem preparation. We show that bilateral sectioning of the SPm triggers inspiratory flow limitations, implicating a major role of SPm in maintaining inspiratory upper airway patency in intact animals. Pharmacologically evoked REM sleep like states (Bicuculline injections targeting the sublaterodorsal tegmental nucleus and caudal laterodorsal tegmental nucleus) in situ correlated with a profound suppression of hypoglossal nerve activity while inspiratory and pre-inspiratory activity of the glossopharyngeal nerve activity persists. Our data suggest that the glossopharyngeal-SPm motor system may act as a save guard for inspiratory upper airway patency during the naturally occurring REM sleep upper airway hypotonia and thus may have major implication for current and future OSA research.

The Influence of Music on Respiratory Rhythm: Anatomical and Functional Insights into Musical Entrainment

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Music can synchronize brain dynamics, influencing emotional, cognitive, and physical states. The therapeutic benefits of music, known for centuries, remain elusive due to the complexity of the underlying mechanisms. Comparable to music, volitional breathing share many of the same medicinal properties. We recently demonstrated that the primary sensory cortex (SI), a brain region responsible for higher-order sensory processing, also exerts an efferent motor control (locomotion). Given that musical rhythms can synchronize neurons, we hypothesize that auditory sensations could modulate respiratory rhythms via neural connectivity between the auditory, and primary somatosensory cortex and brainstem respiratory centers. In our study, mice were exposed to four different stimuli (ambient noise, white noise, Mozart's K.448, and Fur Elise) while undergoing ventilatory assessments over multiple trials. Our data reveal that breathing frequency increases with the complexity of musical pieces, following an inverted U pattern. The breathing frequency decreased once the peak was reached, suggesting a complex interaction between music and respiratory rhythm. We also delineated the anatomical underpinnings of musical entrainment of breathing using anterograde and retrograde transsynaptic viral tracings. The results demonstrate that the auditory and primary sensory cortical regions of the brain are connected to respiratory rhythm-generating centers and spinal motor networks.

NIH R01NS129794.

Respiratory pathology in Duchenne Muscular Dystrophy

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Duke University

Duchenne muscular dystrophy (DMD) is a devastating X-linked recessive disease with no current cure. Respiratory insufficiency is a prominent feature of DMD and many patients eventually die from respiratory failure. Although DMD is classically known as a muscular disease, recent work from our group and others show that dystrophin deficiency also causes significant central nervous system (CNS) pathology. However, the impact of the CNS pathology in the respiratory-related morbidity and mortality in DMD patients is unknown. Using humanized mouse models of DMD, we sought to examine the impact of dystrophin deficiency on respiratory pathology and neuropathology. We then assessed the ability of adeno-associated virus (AAV) gene therapy carrying a microdystrophin (μDys) transgene to correct this pathology. Since AAV-μDys gene therapy is already FDA approved for boys with DMD, this work has strong clinical implications. We performed behavioral and ex vivo diaphragm contraction studies. We also examined the histological, transcriptional and molecular impact of dystrophin loss on the respiratory centers, nerves and motoneurons of the medulla and cervical spinal cord.
Finally, we used a novel AAVcc47 to deliver μDys and target respiratory pathophysiology and neuropathology in DMD. Our results show that different mouse models of DMD display significant respiratory pathology, neuropathology and transcriptional changes. We also found that AAV gene therapy partially restored the respiratory pathology and ongoing studies will examine the impact of AAV gene therapy on neuropathology in DMD.

NHLBI R01HL171282.

Premotor organisation of laryngeal control in mice

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Laryngeal muscles critically support upper airway patency, speech production, and protect the airways during feeding. When contracted, the thyroarytenoid (TA) muscles valve the airway, serving as an important point of control for both volitional and autonomic aerodigestive behaviours. In this study, we mapped the premotor neural inputs to the larynx to determine the cell types and connections that underpin its versatile action. Rabies based monosynaptic retrograde tracing from the TA revealed an extensive and genetically diverse premotor network, spanning from the brainstem to the spinal cord. Key premotor pools included neurons expressing: paired-like homeobox 2b (Phox2b) in the nucleus tractus solitarius (nTS), forkhead box protein P2 (FOXP2) within the Kölliker-Fuse (KF), tryptophan-hydroxylase 2 (TPH2) in the caudal raphe, and choline acetyltransferase (ChAT) in the spinal cord. We identified axon collaterals originating within this network that terminated on phrenic, infrahyoid, suprahyoid, and lingual motor pools, indicating the existence of highly collateralised premotor neurons that 'hardwire' the larynx with key respiratory and oral effectors.

IPAP Hilcrest Foundation: 2022/0722, 2023/0745.

Acute oxygen sensing by carotid body chemoreceptor cells of wild-type and mitochondrial complex III-deficient mice expressing an alternative oxidase

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In carotid body glomus cells, hypoxia favors the generation of mitochondrial signaling molecules, NADH and reactive oxygen species (ROS), which inhibit membrane K+ channels to induce cell depolarization and transmitter release, triggering reflex hyperventilation and sympathetic activation. Previous experiments on conditional knockout mice lacking RISP (TH-RISP), a component of the mitochondrial complex (MC) III, in catecholaminergic tissues have shown complete abolition of systemic hyperventilation and the glomus cell response to hypoxia, indicating that the functional connection between MCI and MCIV is necessary for O2 sensing by glomus cells.

Here, we tested whether expression of alternative oxidase (AOX) from Ciona intestinalis, in wild-type mice (TH- WT-AOX mice) and MCIII-deficient mice (TH-RISP-AOX mice) can bypass MCIII and MCIV and impact sensitivity to hypoxia or rescue responsiveness to hypoxia in RISP-null cells. To date, we have observed that: i) AOX expression does not affect the hypoxic ventilatory response in TH-WT-AOX mice; ii) AOX does not affect sensitivity of WT glomus cells to hypoxia or cyanide; iii) TH-RISP-AOX mice do not exhibit hypoxic ventilatory response. To date, our data indicates that AOX is not able to bypass complexes III and IV in WT mice or rescue acute O2-sensing in MCIII-deficient glomus cells.

São Paulo Research Foundation (FAPESP), Brazil.

Amygdala-driven apnea and the chemoreceptive origin of anxiety

Justin Feinstein

University of Iowa Float Research Collective

The neurophysiological processes driving the chronic and unrelenting nature of anxiety remain elusive. This presentation will examine the close link between the amygdala and the brain's chemoreceptive system, with a focus on how this may be an important missing link for what is driving the chronicity of anxiety and the pronounced hypersensitivity toward carbon dioxide commonly found across the spectrum of anxiety disorders. Recent evidence from lesion and intracranial stimulation studies will be reviewed, highlighting a novel role for the amygdala in both the induction of apnea and the inhibition of fear due to rising levels of carbon dioxide. Such a role is plausible given the strong inhibitory connections linking the central nucleus of the amygdala with respiratory and chemoreceptive centers in the brainstem. This dual brain-behavior relationship is not well-known in the fields of psychiatry and psychology, but it can be formally tested in future studies in both humans and non- human animals, and it may have substantial implications with regard to both the etiology and treatment of anxiety.

Hypothesis: Burstlets and synchronization are essential to generation of breathing rhythm.

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Breathing in postnatal mammals is essentially continuous, robust, yet labile with breathing rate that can change almost instantaneously. What are the mechanisms at the core of the circuits generating breathing pattern?

Hypotheses based on groups of pacemaker neurons have failed critical experimental tests that that show that pacemaker neurons are not essential for generation of breathing rhythm. Moreover, these hypotheses assume fixed oscillators that can only be modulated slowly and do not have the contrasting traits of robustness, flexibility and lability implemented by the same network elements. Their continued prevalence in the discussion is propped up by the unproven notion that since breathing is vital for life, there must be multiple mechanisms and redundancy. Here we present data in vitro and in vivo experiments, with supportive modeling, that rhythm generation is a emergent network property, where burstlets, a phenomena resulting from synchronization of putatively rhythmogenic excitatory neurons, can account for the essential properties of breathing in intact mammals. In contrast, inhibitory neurons provide feedback and maintain excitation-inhibition (E-I) balance to regulate network synchrony and its propagation to pre(motor) neurons (but are not essential for generation of breathing rhythm). This two-step network assembly allows a failproof breathing rhythm, that can also be instantaneously regulated by changing the network's E-I balance via afferent inputs.

NHLBI, NIH R35 HL135779, GR35HL171451.

Preclinical model of obstructive sleep apnea and TRPV1 R antagonist as drug candidate

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BACKGROUND: Obstructive sleep apnea syndrome (OSA) is characterized by recurrent collapses of the upper airways during inspiration. A key factor is instability in respiratory control caused by overzealous respiratory chemoreflexes that compound mechanical effects of obesity. Treatment of severe OSA is mainly based on mechanical treatments. Importantly, no drugs are approved to treat OSA. Our previous work demonstrates that apnea-like stimuli increased carotid body sensitivity by modulating TRPV1 receptors. AIM: This study evaluates a potential treatment for OSA targeting peripheral chemoreceptors with an antagonist of TRPV1 receptor (AMG-9810) using a preclinical mouse model of OSA (New Zealand Obese mice) and whole- body plethysmography. METHODS: The ventilation of eight 4-month-old male mice was recorded after receiving an intraperitoneal injection of AMG-9810 or vehicle. Injections were randomized and spaced by one week. Automated breath-by- breath analysis of ventilation was performed and prolonged inspiratory events (“Ti apneas”) were quantified. RESULTS: The number of Ti apneas was significantly reduced with AMG-9810 compared to vehicle ($p<0.0005$). The mass of the mice remained constant after treatment. OUTCOME: Our preclinical mouse model of OSA was pharmaco-respondent to AMG-9810, suggest a complementary pharmacological approach to current therapeutic measures. Further studies are required to test efficacy of chronic treatment.

Sorbonne Université and INSERM Mitacs Globalink Research Award.

Untangling compensation from pathology in MNs innervating upper airway, diaphragm and limb muscles in a mouse model of Amyotrophic Lateral Sclerosis

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Mayo Clinic

ALS is a neurodegenerative disease exhibiting progressive muscle weakness and death of corticospinal and motor neurons (MNs). Airway defense manoeuvres (eg. cough) and aerodigestive behaviours (eg. speech, swallow) are impaired, resulting in death by respiratory failure within ~3 years of diagnosis. In ALS, fast fatigueable motor units are more vulnerable than slow and fast fatigue resistant units, consistent with the selective death of larger MNs from the brainstem (including hypoglossal) and spinal cord MNs.

In this presentation, we show that the tongue is surprisingly resilient to denervation, despite marked hypoglossal MN loss. Intriguingly, larger hypoglossal MNs exhibit compensatory dendritic changes at ages prior to MN death, whilst the larger MNs of early-denervated muscles exhibit degenerative changes from the earliest examined ages. The specific mechanisms underpinning the compensation of otherwise vulnerable larger hypoglossal MNs is likely to be of translatable interest.

R56- HL166204.

Calcium-sensitive potassium currents underly functional diversity of hypoglossal motoneurons

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Precise control of the tongue muscles is vital for functional breathing and swallowing. We previously demonstrated that hypoglossal motoneurons innervating specific tongue muscles have different excitability profiles. Here, we compare calcium-sensitive potassium currents in motoneurons innervating the superior longitudinalis (SL; intrinsic) and genioglossus (GG; extrinsic) muscles. Back labeled SL or GG motoneurons from neonatal rat pups were visualized under a fluorescence microscope. Recordings were obtained in whole-cell patch clamp. Potassium currents were measured following bath application of tetrodotoxin (TTX) and TTX plus cadmium chloride (Cd2+). Calcium-sensitive potassium currents were calculated by subtracting recordings made in TTX plus Cd2+ from those made in TTX alone. Resting membrane potential was more depolarized in SL (-56 mV ± 6) than GG motoneurons (-63 mV ± 3, $p=0.001$). Frequency-current curves were steeper in SL than GG motoneurons ($p<0.01$). At depolarized voltage steps, calcium-sensitive potassium currents constituted a greater percentage of sustained outward current in SL (20%) than GG (7%; $p=0.02$) motoneurons. We suggest that the increased excitability in SL compared to GG motoneurons is due in part to increases in the density or kinetics of calcium-activated potassium channels. This may reflect intrinsic mechanisms, or activity-dependent plasticity secondary to differences in muscle use.

This research was supported by NIH grants 5R01DC020889-02 and T32HL007249.

Sex hormones and control of breathing

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Sex is a factor that can influence behaviors, including breathing and stress-related responses. Sex-dependent effects on the respiratory control system are observed in offspring following prenatal chronic exposure to drugs like cannabinoids, antidepressants, and anxiolytics, with males being more affected than females. Further, there is a sex dependence on the prevalence of some respiratory-related diseases, including the panic disorder respiratory subtype, where women have two to three times higher probability of developing panic disorder (PD) than men during their lifetime. One of the brain areas that may be involved in these sex-dependent responses is the locus coeruleus (LC). LC is a sexually dimorphic noradrenergic (NA) nucleus that integrates signals associated with anxiety, stress, arousal, and breathing. Our findings reveal that male LC-NA neurons exhibit activation after severe CO2 stimulation, while female counterparts do not, highlighting a noteworthy difference in CO2 response between the sexes. Additionally, LC-NA release is important for the tonic excitatory control of ventilation in males, but not in females. In addition, embryonic disruption of LC-NA has selective, sex-specific effects on ventilatory and behavioral responses to CO2. Together, these findings show that studies involving both males and females are important to understand the impact of these differences, as well as their therapeutic implications in respiratory and psychiatric diseases.

FAPESP, CNPq and NIH.

Aerodigestive coordination through development: what animal models can teach us

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Feeding and respiration are fundamental and critical life functions that rely on the same anatomical spaces, structures, and muscles. The performance and the physiology of both behaviors are ultimately loops, taking sensory input from soft tissues and bones and converting that information into muscular activation in the central nervous system (CNS), including the brain stem and the cerebral cortex. Focusing on individual parts or specific levels of this elaborate system has provided an essential detail of how the system works. Integration among these individual functional components in time and space is critical for successful performance, that is, keeping the pathway of food separate from that of air. We use our term and preterm infant pig model of swallow-breathe coordination as an example to explicate the hierarchical nature of physiology and its impact on performance.

Clarifying these terms, and the roles they play in the biology of dysphagia will help both the researchers studying these problems as well as the clinicians applying the results of those studies to our patients.

Breathing is More Related to Anxiety Than Depression

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Background: Understanding the physiological correlates of emotions like anxiety and depression are crucial for developing effective interventions. This study explored the relationship between spontaneous breathing dynamics (i.e., speed, variability, and timescales) and symptoms of anxiety and depression, as assessed by the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI), respectively. Methods: A diverse sample of 122 subjects participated in this exploratory study. Participants breathed spontaneously for five minutes while staring at a cross. Breathing signals were decomposed into preprocessed raw, breathing rate, and amplitude time series. A set of metrics were computed from these signals in order to quantify the temporal characteristics and complexities embedded in the data. These measures included their mean, variability, power spectral density, autocorrelation function, Lempel-Ziv complexity, and entropy. These measures were used as a set of predictor variables for two separate Partial Least Squares Correlation (PLSC) models designed to identify the relationship between spontaneous breathing dynamics and anxiety and depression symptoms. Results: We found one significant latent variable across both analyses, $p = 0.02$, from the anxiety PLSC which explained 55% of the cross-block covariance. Degrees of compression (i.e., Lempel-Ziv complexity; lzc) were strongly associated with anxiety symptoms, particularly the feelings of lightheadedness and indigestion. Conclusions: This study underscores the potential of using non-invasive physiological markers such as spontaneous breathing measures, to predict symptoms of anxiety and depression. A key finding from our data is that breathing is more related to anxiety than depression.

This project was supported by an NSERC Discovery Grant (DGECR-2022-00309) and a Canada Research Chair (Tier II, CRC-2020-00174) to JAEA. This project was supported by the European Union‘s Horizon 2020 Framework Program for Research and Innovation No. 785907; Human Brain Project SGA2GN () awarded to GN. GN is also grateful to CIHR, NSERC, and SSHRC for supporting his tri-council grant from

the Canada–UK Artificial Intelligence (AI) Initiative The self as agent–environment nexus: crossing disciplinary boundaries to help human selves and anticipate artificial selves‘ (ES/T01279X/1).

Emotional control of breathing: Inhibitory monosynaptic connections between the amygdala and preBötzinger complex.

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We demonstrate a direct relationship between the central amygdala (CeA), a major output hub of the limbic system associated with emotional brain function, and the brainstem preBötzinger complex (preBötC), which generates the fundamental rhythm and pattern for breathing. This connection is monosynaptic and inhibitory, involving GABAergic CeA neurons whose axonal projections act predominantly via ionotropic GABAA receptors to produce inhibitory postsynaptic currents in preBötC neurons. Connections between these two key brain sites may serve as a mechanism for the emotional control of breathing. Moreover, this pathway provides a mechanism to arrest breathing in the context of freezing to assess threats and plan defensive action. The existence of this pathway may further explain how epileptic seizures invading the amygdala cause long-lasting apnea, which can be fatal. These results elucidate a link between emotional brain function and breathing, which underlies survival- related behavior in mammals and pertains to human anxiety disorders.

This study was supported by the U.S. National Institutes of Health (NIH) grant R21 NS134005 (PI: CADN) and the Japan Society for the Promotion of Science (JSPS) KAKENHI 18H02722 and 21H02816 (PI: FK), as well as JSPS KAKENHI 20K09207, Nakatomi Foundation, and Takeda Science Foundation grants (PI: YT).

Pharmacological targeting of TRPM4 to modulate spontaneous rhythmic activities. Presentation of three models: breathing activity, cardiac rhythm and uterine contractions.

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TRPM4 is a Ca2+-activated non-selective cation channel which was shown to participate in numerous physiological processes. Among these, it participates in the modulation of vital spontaneous rhythmic activities. Indeed, TRPM4 enhances spontaneous electrical activity of sinoatrial pacemaker cardiac cells. It modulates the activity of specific interneurons in the preBötzinger complex responsible from the onset of breathing activity. It also participates in smooth muscle activity, for example in the spontaneous uterine contractions. Mechanisms linking TRPM4 activities and rhythm generation often remain not sufficiently understood. Despite this, pharmacological modulation of TRPM4 appears to be an interesting opportunity to correct defects in these rhythmic activities. Here we present three examples in which TRPM4 pharmacological targeting was able to modulate rhythmic activities. TRPM4 is inhibited by unspecific molecules (flufenamate and glibenclamide) but also by more specific molecules including 9-phenanthrol. This latter modulates spontaneous activity of preBotzinger inspiratory neurons from mouse isolated brainstem slices. It reduces spontaneous beating in isolated mouse atria without effect on tissue from Trpm4 knock-out mice. The same was also observed for spontaneous contractions isolated uterus rings from mice. Even if many steps remain to be taken before considering the pharmacological targeting of TRPM4 in medicine, this option is worth exploring.

Fédération Française de Cardiologie; FHU CARNAVAL project (GSC G4).

Persistent Glossopharyngeal Nerve Respiratory Discharge Patterns after Ponto-Medullary Transection

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Shape and size of the nasopharyngeal airway is controlled by muscles innervated facial, glossopharyngeal, vagal, and hypoglossal cranial nerves. Contrary to brainstem networks that drive facial, vagal and hypoglossal nerve activities (FNA, VNA, HNA) the discharge patterns and origins of glossopharyngeal nerve activity (GPNA) remain poorly investigated. Here, an in situ perfused brainstem preparation (n=19) was used for recordings of GPNA in relation to phrenic (PNA), FNA, VNA and HNA. Brainstem transections were performed (n=10/19) to explore the role of pontomedullary synaptic interactions in generating GPNA. GPNA generally mirrors FNA and HNA discharge patterns and displays pre-inspiratory activity relative to the PNA, followed by robust inspiratory discharge in coincidence with PNA. Postinspiratory (early expiratory) discharge was, contrary to VNA, generally absent in FNA, GPNA or HNA. As described previously FNA and HNA discharge was virtually eliminated after pontomedullary transection while an apneustic inspiratory motor discharge was maintained in PNA, VNA and GPNA. After brainstem transection GPNA displayed an increased tonic activity starting during mid-expiration and thus developed prolonged pre-inspiratory activity compared to control. In conclusion respiratory GPNA

reflects FNA and HNA which implies similar function in controlling upper airway patency during breathing. That GPNA preserved its pre-inspiratory/inspiratory discharge pattern in relation PNA after pontomedullary transection suggest that GPNA premotor circuits may have a different anatomical distribution compared HNA and FNA and thus may therefore hold a unique role in in preserving airway patency.

This work was supported by the Clinical and Translational Science Collaborative of Northern Ohio. funded by the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Science Award grant, UM1TR004528 (KS); EH is supported by The Japanese Respiratory Society Fellowship Grant.

EFFECTS OF METFORMIN-INDUCED HYPERLACTATEMIA ON BREATHING CONTROL AND GAS EXCHANGE AT REST AND IN EXERCISE

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What is the contribution of an increase in blood lactate, associated with a metabolic acidosis, to the overall ventilatory (relative hyperventilation) and gas exchange (increased $\dot{V}CO_2/\dot{V}O_2$ ratio) response to exercise? In a population of 163 diabetic patients treated by metformin, we were able to retrospectively identify a group of asymptomatic diabetic patients with a high resting blood lactate (from 2 to 5.3 mM, n= 61), a well described side effect of biguanides. Patients with high resting lactate were further classified in 4 groups: La < 2 mM (1.30 ± 0.36 mM, n=102, group 0), 2<La<3 mM (2.38 ± 0.28 mM, group 1, n= 37); 3<la<3.6 mM (3.18 ± 0.23 mM, group 2, n=12); and 3.5<La<5.3 mM (4.04 ± 0.57 mM, group 3, n=12). Resting hyperlactatemia (in group 3) was associated with a very modest increase in minute ventilation (~ 2 l/min) with no increase in RQ (0.85± 0.12, as group 0) and did not alter the ventilatory and gas exchange response to exercise (no difference between groups), despite the exercise being performed with high lactate. Hyperlactatemia cannot explain, per se, the characteristics of the ventilatory and pulmonary gas exchange responses during heavy exercise.

A top-down slow breathing circuit that alleviates negative affect

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Breathing is profoundly influenced by both behavior and emotion and is the only physiological parameter that can be volitionally controlled. This indicates the presence of cortical-to-brainstem pathways that directly control breathing centers in the brainstem, but the neural circuit mechanisms of top-down breathing control remain poorly understood. Here, we identify a top-down breathing pathway from dorsal anterior cingulate cortex (dACC) neurons to pontine reticular nucleus GABAergic inhibitory neurons (PnC(GABA)), which then project to the ventrolateral medulla (VLM). dACC→PnC(GABA) activity correlates with slow breathing cycles, volitional orofacial behaviors, and is influenced by anxiogenic conditions. Optogenetic stimulation of the dACC→PnC(GABA)→VLM circuit simultaneously slows breathing and suppresses anxiety-like behaviors, whereas optogenetic inhibition increases both breathing rate and anxiety-like behaviors. These findings suggest that the dACC→PnC(GABA)→VLM circuit plays a crucial role in coordinating slow breathing and reducing negative affect. Our study elucidates a circuit basis for top-down control of breathing which can influence emotional states.

Kynurenic acid injected into the nucleus tractus solitarius stunts swallow production

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Swallowing is a behavior that requires the well-formed coordination of a series of muscles to convey a bolus of food/liquid down the esophagus. These muscles are controlled by motoneurons in different areas of the hindbrain and spinal cord and are also the target of other brainstem circuits such as respiratory networks. We conducted experiments in anesthetized, spontaneously breathing cats to determine the effects of drugs microinjected into the nucleus tractus solitarius (NTS) on swallow, while recording electromyograms from pharyngeal, laryngeal, and respiratory-related muscles. Kynurenic acid (KYNA) is an endogenously produced metabolite of the essential amino acid tryptophan, and is a non-specific ionotropic glutamate receptor antagonist that may also have effects through other receptor pathways that are not fully understood. We found that when KYNA was injected into the NTS bilaterally it inhibited the coherent production of swallow from pharyngeal water stimuli. After microinjection of KYNA, swallows from water stimuli were impeded for up to an hour or more but gradually recovered. These data suggest a relatively long-term action by KYNA beyond simple acute antagonism of ionotropic glutamate receptors that creates an “open circuit” between the afferent sensory feedback from pharyngeal water stimulation among elements of the swallow pattern generator.

NIH HL155721, NIH HL163008, NLHB HL103415, VEGA Project 1/0072/16, and VEGA Project 1/0253/15.

The urge-to-cough: a respiratory sensation related to upper airway (dys)function

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The urge-to-cough (UtC) is a respiratory sensation that precedes cough and can be elicited by multiple sensory modalities including central neural, chemical, and mechanical. Davenport (2007) described the UtC as "a component of the brain motivation system that mediates cognitive responses to cough stimuli." Thus, although the cough motor response can be produced reflexively via brainstem control pathways, in physiologically "normal" instances of cough, the UtC plays a critical role the generation of cough. Specifically, the UtC sensation is positively correlated with the magnitude of cough motor response to increasing stimulus intensity. The UtC has been studied extensively across multiple patient populations, including those with heightened UtC, and those with blunted UtC. Heightened (sensitized) UtC is associated with the presence of chronic refractory cough, and blunted (desensitized) UtC is associated with impaired airway protective functions including swallowing and cough. The goal of this presentation is to review the role of UtC as a contributor to dysfunction of behaviors of the upper airway and explore the sensorimotor control pathways that drive this phenomenon. We will also explore the ways in which the UtC may be used therapeutically with patients to either down-regulate or up-regulate the cough response.

NIH – NIDCD; American Parkinson Association.

GENES(IS) of Inspiration – Single cell transcriptomic Atlas of the Brainstem Breathing Rhythm Generator

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In the Genesis of rhythmic breathing, the preBötzinger complex (PreBötC) is essential. However, characterization and in-depth identification of cell types using molecular signatures are lacking. Here, we used a combination of single cell RNA-sequencing and spatially-resolved transcriptomics to characterize the molecular landscape of the PreBötC and its surrounding. Punchouts of the PreBötC from 2-day old CD-1 mice were obtained for further SmartSeq3 cDNA library preparation and analysis. Using uniform manifold approximation and projection (UMAP) and based on identified cell type markers genes, 35 different cell clusters were identified. The non-neural cells were clearly distinguishable and typically 8270 genes in each non-neuronal cell were found. The neurons expressed more than 9500 genes/cell. The excitatory (SlC17a6) and the inhibitory neurons (SLC32a) constituted about half of cells. They were heterogeneous with 14 GABA/glycinergic and 16 glutamatergic clusters respectively, identifiable using 3-6 molecular markers/cluster. Validation of the results using spatial transcriptomics methods further characterized and revealed 3D interaction between specific cell types and several hundreds of novel markers for distinct cell populations. By reveling the in depth transcriptomic and spatial profile of respiration-related neurons in the neonatal brainstem we help to unravel the underlying mechanism and specific targets for the genesis of inspiration and therapeutics.

This study was supported by grants to EH from the Swedish Research Council (2016-01111 & 2019-01157 and 2023-02613), the Stockholm County Council (2019-0400, 2019-0974), the Swedish Brain (FO2017-0203, FO2019-0087, FO2021-0313 and FO2023-0231), the Swedish National Heart and Lung (2015-0558, 20180505 and 20210579), the Axel Tielmans, and the Freemasons Children's House foundations. In addition, WSP, NR and

AS were partly supported by the Freemasons Children's House foundation scholarship and WSP was supported 1 year by the StratNeuro Karolinska Institutet Postdoc grant 2020.

Furthermore, the authors acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council, and SNIC/Uppsala Multidisciplinary Center for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure.

Impairment of dB2 neurons causes congenital hypoventilation

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Breathing homeostasis originates from complex networks of brainstem neurons that generate, modulate and adjust the respiratory rhythm in response to physical demands. Genetic alterations contribute to the onset of several respiratory disorders, but the dysfunctional neural circuits associated with them are largely unknown. Congenital hypoventilation disorders are life-threatening and characterized by pronounced alveolar hypoventilation, central apnea and impaired chemoreflexes. Mutations in the genes encoding the transcription factors PHOX2B and LBX1 correlate with congenital hypoventilation disorders. Here, I will provide data illustrating that the developmental impairment of distinct, and previously undescribed, groups of medullary neurons co- expressing both factors (that is, dB2 neurons), account for specific respiratory functions and phenotypes seen in congenital hypoventilation diseases. These neurons locate to the caudal medulla and are key for the generation of appropriate respiratory tidal volumes, the neonatal hypercarbic reflex, and for the maintenance of neonatal respiratory stability. Our data provide functional evidence for the critical role of specific groups of dB2 neurons in neonatal respiratory physiology and establish dB2 neurons dysfunction as causative in congenital hypoventilation.

Deutsche Forschungsgemeinschaft (grant # 514060831).

Intracranial Responses to Respiratory Challenges in Sensorimotor and Insular Cortices are modulated by the Strength and the Perception of the Challenge.

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We adapted a novel Respiratory Resistance Sensitivity Task (RRST) to precisely quantify how the brain processes ascending respiratory signals. In this task, the airways are partially obstructed during the inspiratory cycle by a resistance. Ten epilepsy patients implanted with intracranial electrodes (iEEG) completed this task, detecting loads of different magnitudes (subthreshold vs. suprathreshold) while breathing through a mouthpiece. Compared to non-loaded, loaded inhalations showed a decrease in airflow and an increase in mouth-pressure, as expected. At the neural level, loaded inhalations showed increased high frequency activity (HFA, 70-150hz) in the sensorimotor, insular, and frontal (orbitofrontal, rostro-medial, anterior cingulate) cortices. This effect was proportional to the magnitude of the load, with larger loads eliciting higher HFA compared to smaller loads. At the behavioral level, loads that were consciously perceived by the subjects showed higher HFA than those that were not. In addition, when the subjects breathed harder in reaction to a given load, the coherence between the sensory and motor cortices was enhanced (15-30hz). These results reveal 1) the key cortical areas contributing to the detection of respiratory sensations, 2) the neuronal “gain” mechanisms underlying the perceptual detection of loads of different magnitudes, and 3) the respiratory-motor compensatory strategies necessary at the cortical level to not only detect a load but to react to meet ventilatory demands.

NHLBI (R01HL163578).

Daily diaphragm pacing attenuates breathing deficits after spinal cord injury in male, but not female, rats.

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Diaphragm pacing (DP) is used to manage respiratory insufficiency following cervical spinal cord injury. Anecdotal evidence indicates DP may offer rehabilitative benefit. We considered sex as a biological variable and tested the hypothesis that DP improves breathing in a rodent model of injury. Male and female Sprague Dawley rats were implanted with custom diaphragm electromyography electrodes and received a left C2 hemisection (C2Hx). We delivered rhythmic stimulation to the paralyzed hemidiaphragm for 4 consecutive days (1 hr/day) starting 1-week after C2Hx. We recorded ventilatory output via whole body plethysmography before, 4 days after injury, and 24 hours after DP to determine the rehabilitative impact of pacing. Our data shows that 24 hours after DP, males (n=6) have elevated tidal volume (mL/breath/100g) compared to injured controls not receiving DP (n=7; p=0.0222). In females, we observed no difference between DP (n=7) and injured controls (n=7; p=0.7892). Our data also shows that the DP-induced improvement in males is driven by phrenic afferents, since animals pre-treated with dorsal rhizotomy to remove afferent input to the spinal cord prior to receiving DP (n=6) are not different than controls (p=0.9927). Collectively, our data suggests a differential sex-based response to DP following C2Hx.

This work was supported by funding from the National Institute of Health, grant numbers: R00 HL143207-01 (KAS), UL1TR001436 (TCH) and the APTA Acute Care Physical Therapy Seed Grant (TCH).

The detection, perception and neural processing of respiratory loads in healthy ageing and chronic obstructive pulmonary disease (COPD)

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Impairments in the detection, perception and neural processing of inspiratory loads in healthy ageing and COPD have implications for airway protection and breathlessness. This study investigated load detection and perception in 18 participants with COPD, 17 age-matched controls (AMC) and 23 young controls (YC) and respiratory-related evoked potentials (RREPs) in 11, 10 and 14 per group, respectively. Load detection was assessed as the minimal change in airway resistance detectable, load perception as the perceived level of effort to breathe through supra- threshold loads, and RREPs with electroencephalography during airway occlusions. YC could detect a smaller proportion of maximal inspiratory pressure compared to AMC and COPD groups ($p<0.001$). For load perception, the relationship between Borg ratings and inspiratory pressure was steeper in AMC than YC ($p<0.01$), with no difference between AMC and COPD groups. All RREP peaks were similar across groups, except N1 amplitude was greater in YC compared to AMC and COPD groups ($p = 0.011$). There was no correlation between either load detection threshold or RREP amplitudes and load perception in AMC or participants with COPD. In older adults, heightened perception of loaded breaths may be due to an increased effort to breathe and impaired processing of respiratory sensory inputs.

National Health and Medical Research Council (Australia) Rebecca L. Cooper Medical Research Foundation.

Disruption to swallow and its coordination with breathing when silencing the caudal Nucleus of the Solitary Tract and the Postinspiratory Complex

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Postinspiratory Complex's (PiCo) role in swallow generation, thought to be controlled by the caudal Nucleus of the Solitary Tract (cNTS), remains unknown. We hypothesize that silencing PiCo specific neurons dysregulates swallow-breathing coordination and PiCo is not necessary for swallow production, rather the cNTS is necessary to trigger a swallow. Inhibitory DREADDs were injected into PiCo and/or cNTS. Swallows were stimulated by 1) injection of water into the oral cavity and/or 2) optogenetic stimulation of PiCo neurons. We found breathing was slowed, postinspiration abolished, and swallowing, along with its interaction with breathing, were altered after activation of the inhibitory DREADD, located in PiCo. Silencing the cNTS resulted in an inability to evoke swallows either by water stimulation or optogenetic stimulation. PiCo is important for postinspiration, normal swallow production, swallow-breathing coordination, and relies on direct feedback from the cNTS to trigger swallow.

HL144801 (JMR), HL151389 (JMR), HL144454 (JMR), HL126523 (JMR), HL160102 (ADH).

Neonatal inflammation induces lasting sex-, region-, and stimulus-dependent changes in adult microglia from respiratory control regions

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Early life inflammation is common and leads to consequences on respiratory neural control by abolishing adult phrenic respiratory motor plasticity in both sexes later in life. However, phrenic motor plasticity can be restored by acute administration of an anti-inflammatory in adulthood, suggesting that the loss of neuroplasticity is due to persistent adult inflammation. To begin dissecting underlying mechanisms for this, we evaluated the effects of neonatal inflammation induced by an acute systemic lipopolysaccharide challenge (on postnatal day 4) in both adult male and female microglia from the medulla and cervical spinal cord, which are key sites of respiratory control and neuroplasticity. We found that adult microglia demonstrated sex- and region-specific increases in microglia number (assessed by flow cytometry) and inflammatory gene expression (isolated microglia gene expression and transcriptomics). Further, microglia from adults previously exposed to neonatal inflammation also exhibited sex-, region-, and stimulus-dependent responses to an acute, inflammatory challenge in adulthood, suggesting that the microglia were reprogrammed by the initial neonatal insult. Collectively, our data support the idea that neonatal inflammation increases lasting, microglial-specific inflammation, which abolishes adult respiratory plasticity through sex-specific mechanisms, leaving adults more vulnerable to subsequent inflammatory stimuli.

NIH HL141249 (AGH).

Hiccup center is localized in the subpostremal region of the nucleus tractus solitarius

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When blue light stimulation was applied to the dorsal head of neonatal transgenic rats expressing the mutant channelrhodopsin ChRFR in Phox2b-positive neurons, sucking, swallowing, and hiccups were frequently evoked. Therefore, in this study, we investigated whether these movements could be induced in the brainstem-spinal cord preparations from these neonatal transgenic rats. Motor activity was recorded from the trigeminal nerve (V), hypoglossal nerve (XII), 4th cervical ventral root (C4VR), and 1st lumbar ventral root (L1VR). Photostimulation evoked burst activity of approximately 1.5 Hz in C4VR, with synchronous bursts in V and XII, but not L1VR. These results suggest that the evoked rhythmic activity was not swallowing or sucking, but hiccups. Using voltage- sensitive dyes, it was found that depolarization signals in phase with the hiccup motor burst were localized in the AP and subpostremal NTS. Electrical coagulation of the AP did not abolish the photo-evoked hiccup motor rhythm, but bilateral coagulation of the subpostremal NTS did. In the subpostremal NTS, both Phox2B-positive and -negative neurons fired synchronously with the hiccup motor rhythm. This study is the first to provide evidence that the hiccup center is localized in the subpostremal NTS and that Phox2B-positive neurons are involved in the induction of hiccups.

Japan Society for the Promotion of Science KAKENHI (no. 20K07266 to M.I., no. 16K07003 to H.O.).

Hypothalamic progestin infusion enhances the hypercapnic ventilatory response in female rats

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The central CO2-chemoreflex drives breathing during rest and homeostatic challenges. While the retrotrapezoid nucleus (RTN) is crucial, other brain structures, such as orexinergic neurons in the dorsomedial hypothalamus, likely contribute to ventilatory responses to CO2. These neurons project to respiratory-related brainstem neurons and potentially co-express receptors for progesterone, a respiratory stimulant. Previous studies, including ours, have demonstrated that systemic administration of the progestinic drug etonogestrel (ETO) can 'rescue' CO2- chemoreflexes following RTN impairment and in pre-clinical models of Congenital Central Hypoventilation Syndrome. In this study, we explored whether ETO's stimulatory effects are mediated through the hypothalamus. Respiration was measured in adult female Sprague-Dawley rats using plethysmography before and during a four- week ETO infusion into the dorsomedial hypothalamus. Rats were instrumented with cannulae connected to Alzet pumps containing ETO (0.056 ng/μL) or vehicle. In a separate cohort, chemoreflexes were impaired by lesioning the RTN two weeks prior to cannula installation. In healthy rats, unilateral ETO infusion modestly increased tidal volume during 5% and 7% CO2 exposure without affecting breathing frequency. Preliminary data from RTN- lesioned rats suggest that bilateral ETO infusion modestly recovered chemoreflexes by increasing tidal volume.

These data suggest a hypothalamic role in CO2-chemoreflex regulation in healthy and RTN impaired rats.

TJ: Canadian Institutes of Health Research (CIHR) Fellowship, Canadian Lung Association Fellowship.
ASPS: Women and Children's Health Research Institute (WCHRI) summer scholarship, Natural Sciences and Engineering Research Council of Canada (NSERC) summer scholarship.
SC: Canadian Lung Association Fellowship.

SP: Canadian Institutes of Health Research (CIHR) operating grant, Women and Children's Health Research Institute (WCHRI) operating grant.

Phrenic motor neuron Bmal1 expression regulates phrenic long-term facilitation in rats.

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Acute intermittent hypoxia (AIH) elicits phrenic long-term facilitation (pLTF), a well-studied form of respiratory motor plasticity. With an AIH protocol consisting of 15, 1-min hypoxic episodes ($PaO_2$ ~40-55 mm Hg), AIH- induced pLTF exhibits profound diurnal regulation where pLTF magnitude is 4-fold greater in the rest versus active phase. Since many daily physiological and behavioral rhythms are driven by the endogenous circadian clock, and clock genes are rhythmically expressed in the phrenic motor system, we hypothesized that the molecular clock within phrenic motor neurons differentially regulates pLTF across the daily rest/active cycle in young male Sprague-Dawley rats (3-6 months old). Using intrapleural injections of small-interfering RNAs (siRNAs) to selectively knock down the core clock gene Bmal1 within phrenic motor neurons, we discovered that AIH-induced pLTF is greatly reduced in the mid-rest (68 ± 10% vs 122 ± 6%) but marginally increased in the mid- active phase (43 ± 8% vs 33 ± 4%), when comparing rats given siBmal1 versus non-targeting siRNA injections. Collectively, these data demonstrate that the molecular circadian clock of phrenic motor neurons is a powerful regulator of AIH-induced pLTF.

NIH HL148030 & 147554 (GSM) and NIH T32HL134621-5 (ABM).

Fork head Box protein 2 (FoxP2) expressing neurons in the lateral parabrachial area regulate respiratory responses to hypercapnia

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We have identified a population of neurons in the central lateral (PBcl), lateral crescent and Kölliker-Fuse (KF) parabrachial subnuclei that are activated by CO2 and project to the respiratory sites in the medulla and spinal cord and many of them express the transcription factor, Fork head Box protein 2 (FoxP2-PBcl/KFFoxP2). We hypothesized that these PBcl/KFFoxP2 neurons may be important in the respiratory response to CO2, as they express cFos and have increased intracellular calcium during exposure to CO2. We also found that optogenetically photo-activating PBFoxP2 neurons increased respiration, whereas either photo-inhibition of PBFoxP2 or genetic deletion of PB/KFFoxP2 neurons reduced the respiratory response to CO2 stimulation without preventing awakening. Our results indicate that PBcl/KFFoxP2 neurons play an important role in the respiratory response to CO2 exposure during NREM sleep, and that other pathways that also contribute to the response cannot compensate for the loss of the PBcl/KFFoxP2 neurons. Our findings suggest that augmentation of the PBcl/KFFoxP2 response to CO2 in patients with sleep apnea in combination with inhibition of the PBelCGRP neurons may avoid hypoventilation and minimize EEG arousals. We are now investigating their role in reversal of opioid induced respiratory depression.

This research work was supported by funding from USPHS grants 1P01 HL149630-01 and NS112175.

Compensatory cellular plasticity in cervical excitatory following spinal cord injury

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Breathing disfunction is the leading cause of death following cervical spinal cord injury (cSCI), highlighting the need for strategies to improve respiratory function after cSCI. Spinal interneurons (INs) facilitate and modulate motor and sensory functions within the central nervous system. Notably, cervical excitatory INs (eINs) can generate rhythmic phrenic motor output without supraspinal descending inputs. While cervical eINs are not essential for normal breathing in health, they become critical for maintaining breathing after cSCI. The underlying mechanism for this functional shift remains unknown. Serotonin, a vital neuromodulator, is linked to recovery after SCI. Our research indicates that raphe serotonin system directly connects to to cervical eINs. We hypothesize that cSCI causes hyperexcitability of cervical eINs through serotonergic receptor plasticity, potentially altering their intrinsic properties. Our findings show significant downregulation of 5-HT1A, 5-HT2A and upregulation of 5-HT2B and 5-HT7 receptors. These receptor changes are validated through whole-cell patch-clamp recordings in acute spinal cord preparations and pharmacological manipulation to identify the specific receptors responsible for this hyperexcitability. Overall, our results suggest that the increased excitability of cervical eINs post-SCI underlies critical compensatory respiratory mechanisms, offering potential targets to enhance breathing function following cSCI.

Acute stress augments obstructive apneas and related O2-desaturations in supine “sleeping” rats via potentiation of cholinergic inhibition and reduction of hypoglossal motoneuron excitability

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Anomalies in airway motor control during sleep contribute to obstructive sleep apnea (OSA). While OSA affects ~1 billon of adults worldwide, the origins of the problem are uncertain. Because stress disrupts brain function, we hypothesised that exposure to a single stress session induces cardiorespiratory disturbances associated with OSA in adult male rats.

Stress was induced by immobilisation 24h prior to the experiments. Controls were handled briefly. In vivo experiments were performed in urethane-anesthetised rats (1.6 g/kg) instrumented to monitor: diaphragmatic (DIA) and genioglossus (GG) EMG, respiratory flow, O2 saturation. Obstructive apneas: no airflow with DIA activity with a SpO2 drop (> 3%). Rats were place in supine position to favors airway obstruction. Whole cell recordings of hypoglossal motoneurons were performed on tissue slices (250µM) from control and stress rats. By comparison with controls, stress augmented obstructive apnea frequency (4X) and the severity of apnea-induced O2 desaturations (2X). Atropine (0.5 mg/kg) reduced obstructive apneas and prevented O2 desaturations in stressed but not controls. Stress augmented the atropine-induced rise in GG activity (~2X higher). Current- clamp recordings showed that stress increased rheobase and attenuated the current-dependent firing rate. Acute stress is sufficient to disrupt airway control in ways that favor significant obstructive apneas.

Supported by the Canadian Institutes of Health Research and the Foundation of the Québec Heart & Lung Institute.

Effects of xylazine on opioid-induced respiratory depression.

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We use the perfused brainstem preparation of rats to investigate the interplay of the α-2 receptor agonist xylazine and the μ-opioid receptor agonist fentanyl on drug-induced respiratory arrest. We performed a variety of experiments using fentanyl, xylazine, or a fentanyl-xylazine mix applied systemically or locally in the pre-Bötzinger complex (pre-BötC) and Kölliker-Fuse nuclei (KFn). We show that respiratory arrest after systemic fentanyl- xylazine application cannot be recovered by naloxone and co-application of naloxone and the α-2 antagonist yohimbine only recovered a highly variable and unstable respiratory motor pattern. Microinjection of a xylazine- fentanyl mix into the pre-BötC triggered rapid shallow breathing patterns with strongly reduced motor nerve discharge amplitudes, contrary microinjection of this mix into the pons evoked pronounced apneusis and reduced respiratory frequency. Both local manipulations rendered the respiratory network highly vulnerable to opioid- induced respiratory arrest at low doses of fentanyl. Despite xylazine acting only locally subsequent application of naloxone failed to recover stable respiratory activity. Thus, the treatment of opioid respiratory depression in the presence of adulterant sedatives may require additional antidots to counteract opioid respiratory depression but simply adding α-2 receptor antagonist may not suffice.

The effect of long-term recurrent intermittent hypercapnia on central respiratory chemoreflex function

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The central respiratory chemoreflex maintains arterial CO2 stability via regulation by brainstem neural circuits. Central chemoreceptors including retrotrapezoid nucleus (RTN) neurons control respiratory adjustments on a breath-by-breath basis and are highly stimulated during hypercapnia. Concurrent intermittent hypoxia (IHx) and hypercapnia (IHc) are common in respiratory disorders, yet previous research has focused mainly on IHx. Both stimuli converge on the same brain regions, making their study crucial for understanding the brain response to IHc. Our recent findings on chemoreflex function using a mouse model of long-term recurrent IHc (18 x 5 min 8% CO2/21%O2/balance N2, for 7 days) will be discussed. In adult mice, we found that previous IHc exposure does not change chemoreflex responses (cFos protein expression after 1 hour of 10% CO2) in RTN neurons, or brainstem autonomic neurons (C1 and A1 catecholaminergic neurons) compared to mice exposed to room air for 7 days (n=7/group). In another study, previous IHc exposure significantly increased neuroplasticity (delta FosB protein expression) in RTN neurons, but not C1 or A1 neurons, when compared to room air exposed mice (n=7/group; $p<0.01$ 2-way ANOVA). There were no differences in plasma corticosterone levels between groups, measured by ELISA, indicating IHc does not affect stress levels.

Australian Research Council of Australia (DP180101890).

Investigating the thalamus in breathing control using electrical stimulation and intracranial recordings in humans

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We examined the role of the human thalamus in breathing control by recording local field potentials (LFPs) from the thalamus and electrically stimulating focal sites within the thalamus in five epilepsy patients with intracranial electrodes.
To determine how the thalamus responded to breathing, coherence between breathing and LFPs were determined. Several sites located in the anterior and posterior thalamus showed strong coherence with automatic breathing in awake and anesthetized states. When breathing was dependent on a ventilator while under anesthesia, only posterior sites located in the ventro-posterior lateral nucleus showed strong coupling with breathing.
Electrical stimulation of the thalamus inhibited breathing and caused apnea. Anatomical parcellation of the thalamus localized apneic sites to thalamic anterior nuclei. Stimulation of posterior thalamic sites evoked apnea but not to the degree as the anterior nuclei. Examining mechanisms for this inhibition, electrical stimulation of the thalamus inhibited the hypercapnic ventilatory response.
These findings suggest that the thalamus can inhibit breathing and cause apnea. Moreover, the rhythm of automatic breathing, both under awake and unresponsive anesthetized states is represented in the thalamus. Posterior thalamic sites may be more engaged in representing the bottom-up sensory effects (e.g., of lung contractions) of respiration.

The medullary 5-HTergic neuron subtype called Tac1-Pet1 augments breathing during quiet wake and counters morphine-induced respiratory depression.

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Brainstem serotonin (5-HT)-producing neurons critically modulate breathing, with distinct 5-HTergic neuron subtypes contributing to specific respiratory functions (1,2). The Tac1-Pet1 neuron subtype, named by gene expression, distributes soma across midline raphe obscurus (ROb), raphe magnus (RMg), and lateral paragigantocellularus. Tac1-Pet1 neurons innervate brain regions involved in respiratory motor output (i.e., hypoglossal, phrenic, nucleus ambiguus) and nuclei essential for respiratory rhythm generation and modulation (i.e., preBötzinger, parabrachial). Functionally, Tac1-Pet1 neurons are required for mounting a full respiratory response to hypercapnia (3). Until now, their sufficiency for driving breathing had not been assessed, though our axonal projection data and other prior work on 5-HTergic ROb neurons suggest that activating these neurons may augment breathing (4,5). We combined our mouse intersectional genetic platform with DREADD chemogenetics to activate Tac1-Pet1 neurons and measure respiration via awake whole-body plethysmography. We found that acute activation of Tac1-Pet1 neurons increases respiratory rate and minute ventilation – comparable to activating Pet1+ neurons en masse. Further, Tac1-Pet1 neuron activation mitigates morphine-induced depressed respiratory rate and waveform abnormalities. We conclude that Tac1-Pet1 neuron activity is sufficient to augment breathing and can counter opiate-induced disordered breathing. We plan to assess Tac1-Pet1 neuron modulation of motor output (genioglossus and diaphragm EMG) and respiration across sleep-wake states.

HBI Young Scientist Transitions Award FY 2021; F32 NS106762 04/01/18-3/31/2021; P01HL49630.

Neural circuitry for sighing

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A sigh is a critical breathing function that spontaneously occurs every several minutes to reopen collapsed alveoli and maintain normal pulmonary function. Sighing is strongly triggered by physiological inputs, including hypoxia, and is also induced by various emotional states, such as stress, relief, and sadness. However, the mechanisms by which sighs are generated and regulated by different inputs remain largely unknown. Using genetic and neurogenetic tools, we have undertaken a series of projects to illuminate the neural circuitry for sighing and its physiological and emotional controls. Initially, we identified the peptidergic circuit in the medulla as the core molecularly identified circuit underlying a breathing pattern variant. We then discovered a two-step peptide neural circuit from lateral hypothalamic area to the medulla that controls sighing in claustrophobia-like behavioral states. More recently, we discovered a neural circuit from the carotid body to the medulla that specifically mediates sighing in response to hypoxia. Together, these studies highlight a neural circuitry where diverse inputs, associated with different emotional and physiological states, converge on a common core circuit to regulate sighing. Our work not only enhances our comprehension of respiratory function but also illuminate the brain's wiring for breathing control.

National Institutes of Health.

The role of enkephalinergic system in respiratory control

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While numerous studies examine the interactions between exogenous opioids and the respiratory network, little is known about the role of endogenous opioids in breathing control. This work investigates the role of enkephalins, endogenous opioid peptides encoded by the Penk gene, in the preBötzinger Complex (preBötC) - the brainstem inspiratory rhythm-generator. To test the necessity of enkephalinergic neurons in respiratory rhythmogenesis, we permanently silenced the preBötC Penk+ population. Mice in the experimental group developed severe tachypnea (rapid and shallow breathing), and about 40% of animals died within two weeks post manipulations.
These functional observations are supported by spatial transcriptomics revealing extensive enkephalin co- expression with inhibitory neurotransmitters. To assess the direct contribution of enkephalin signaling to respiratory control, we selectively disrupted opioid signaling. Acute blockade of opioid receptors did not produce respiratory changes under normoxic, hypercapnic, or hypoxic conditions. Likewise, permanent knock-out of the Penk gene with the CRISPR technology did not affect breathing characteristics in awake animals. Utilizing optogenetic techniques in urethane-anesthetized mice, we recorded a difference between respiratory frequencies in Naloxone-treated and control groups during optogenetic stimulations implicating the involvement of opioid signaling. Translating our findings into awake and behaving animals will further illuminate enkephalin’s involvement in respiratory modulation.

R00HL145004 (Baertsch).

Precision in Motion: Breathe Easy and the Future of Respiratory Data Analysis

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Whole-body barometric flow-through plethysmography is vital for studying respiratory output in rodent models of genetic and disease conditions. Despite its importance, data analysis is time-consuming and prone to observer bias, with researchers often using costly commercial software or manual annotation. This creates a barrier for more precise custom respiratory measurement systems due to the lack of consistent and high-throughput data analysis tools. We introduce Breathe Easy, an open-source pipeline that processes raw recordings and metadata into respiratory outcomes, publication-ready graphs, and robust statistical analyses. Featuring a user-friendly GUI, it allows users to upload data files, set waveform thresholds, and define experimental variables. The Python-based Signal Analysis Selection and Segmentation Integration (SASSI) module selects quality breaths, while the STatistics and Graph Generator (STAGG) creates graphs and performs linear mixed-effects modeling. Validation against expert manual selection showed high-quality breath selection. Breathe Easy's capability was demonstrated on a near terabyte-sized data set from a two-year study of an Alzheimer's Disease mouse model, finding no association between forebrain pathology and respiratory outcomes despite notable respiratory features associated with Alzheimer's Breathe Easy provides a customizable, automated platform for analyzing respiratory and metabolic data, enabling high-throughput studies and machine-learning applications on large datasets.

This work was supported by the following NIH grants: F32-HL160073 (SL), R01-HL130249 (RR), R01-HL161142 (RR), and R01-NS076708 (RR).

Critical windows of brainstem neural development characterized by a lethal vulnerability to endotoxin

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During the second postnatal week in the rat, the CNS (particularly the brainstem) exhibits abrupt, and in some cases transient, shifts in constitutive neurochemical expression. These developmental events represent critical and important transitional stages of CNS neurochemical and immuno-development. Further, exposure to pro- inflammatory insults such as hypoxia and the gram-negative endotoxin lipopolysaccharide (LPS) during this period results in an unexpected rate of mortality not observed in younger, or older animals. Here we show that the lethality to LPS is associated with respiratory control dysfunction (impaired ventilatory responses to acute hypoxia), exaggerated bradycardic responses, and thermoregulatory collapse. Mortality from LPS is also associated with aberrant systemic and brainstem pro-inflammatory responses, all of which can be prevented by phosphodiesterase and microglia inhibitors. These data have important implications for our understanding of the vulnerability of the respiratory and autonomic control systems, particularly during necessary stages of brainstem neurodevelopment. Microglia- and/or phospho-diesterase inhibitors may be novel prophylactic options aimed at preventing respiratory, autonomic, and thermoregulatory dysfunction in neonatal sepsis, a problem that kills thousands of infants annually in the US.

William & Louis Briggs Research Chair.

Diurnal regulation of APOE genotype on phrenic motor plasticity and associated genes

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Apolipoproteins (ApoE) function as CNS lipid carriers. Certain APOE alleles are associated with neuroprotective (ApoE2) or neuro-neutral (ApoE3) phenotypes; ApoE4 is associated with neurodegeration, and impairs neuroplasticity. We recently discovered that specific APOE alleles predict human expression of acute intermittent hypoxia (AIH)-induced plasticity in cortico-spinal pathways to the phrenic/diaphragm motor system in the daily active phase. However, although AIH-induced phrenic motor plasticity exhibits a profound diurnal rhythm, the impact of APOE genotype across the daily rest/active cycle is unknown. Thus, anesthetized, vagotomized, paralyzed and ventilated knock-in humanized (h)APOE3 (control) or hAPOE4 rats were presented with 15,1 min moderate hypoxic episodes to determine if APOE4 undermines respiratory plasticity (phrenic long-term facilitation; pLTF) in a time-of-day-dependent manner. Whereas rest phase pLTF was similar in both hAPOE3 (121±12%) and hAPOE4 rats (122±5%), active phase pLTF was significantly greater in hAPOE3 (33±11%) vs hAPOE4 rats (-26±7%; $p<0.001$). Similarly, genes regulating the circadian clock, intercellular signaling, and phrenic motor plasticity exhibited comparable rest phase expression in hAPOE3 or hAPOE4 rats, but were differentially expressed in the active phase. Understanding genetics and time-of-day interactions in regulating respiratory plasticity will help identify individuals most/least likely to respond to neurotherapeutics, revealing new targets for precision interventions of AIH.

NIH R01HL149800 (GSM) and T32HL134621-5 (ABM).

Astrocytes of the lateral parafacial region are sensitive to high CO2 but play no major role in controlling pulmonary ventilation of mice

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Studies indicate that astrocytes in the medullary respiratory chemoreceptive areas are responsive to CO2, pH, and O2 levels, and can regulate ventilation. Nonetheless, the influence of astrocytes in the parafacial lateral region (pFL) on respiratory chemoreception remains uncharted. We hypothesised that astrocytes of the pFL region, which contain expiratory neurons active during respiratory challenges, control the ventilatory response to hypercapnia of mice. Using Aldh1l1Cre/+ERT2 mice, the experiment involved bilateral injections of AAV-DIO- hM4D(Gi)-mCherry or AAV-DIO-hM3D(Gi)-mCherry into the pFL region. The contribution of pFL astrocytes to ventilatory control was assessed in vivo through the activation of DREADDs by intraperitoneal injection of JHU37160 (0.1 mg/Kg) under normocapnic or hypercapnic conditions (7% CO2). Additionally, the impact of hypercapnia on intracellular [Ca2+] of pFL astrocytes was measured in medullary slices using mice expressing genetically encoded calcium indicator (Aldhcre/+/Gcampflox/+). The findings revealed that JHU37160 did not change ventilation in Aldhcre/+hM4D(Gi) and Aldhcre/+hM3D(Gq) mice under either normocapnia or hypercapnia. On the other hand, hypercapnia induced a significant increase in intracellular [Ca2+] of pFL astrocytes in vitro (p=0.001; 35 astrocytes from 5 separate experiments). These results show that pFL astrocytes are sensitive to hypercapnia in vitro but play no major role in controlling ventilation during hypercapnia in vivo.

FAPESP 2022/02138-9, 2018/15957-2 and 2020/00201-0.

Microbiota-metabolite-muscle axis: Gut feelings about breathing

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Gut bacteria produce metabolites (i.e., short-chain fatty acids (SCFA), secondary bile acids and neurotransmitter substrates) that act as fuel sources and modulators of cell signalling influencing host-muscle development, growth and maintenance. Skeletal muscle is the largest metabolic organ and has endocrine functions eliciting systemic effects that can alter microbial populations in the gut. The bidirectionality of signaling creates a gut-muscle axis.
We characterised the axis in a model of no microbial signaling (germ-free mice) and altered signaling (mdx mice). In the absence of microbial signaling, we observed diaphragm weakness and increased fatigue in males only, with increased SDH (oxidative) and GPDH (glycolytic) enzyme activities, but no change in fibre cross-sectional areas. Pink1 transcript expression levels are decreased in germ-free male diaphragm suggesting impaired mitophagy.

Muscular dystrophy mice (mdx) harbour unique microbial populations. Diaphragm force is halved in mdx mice associated with profound remodelling. Our metabolomic analysis revealed a decrease in acetate in mdx faecal and plasma samples, indicating an impairment in microbial derived SCFA signaling, which may exacerbate mdx diaphragm dysfunction. Our studies suggest that disrupted microbial signaling has downstream consequences for diaphragm muscle function. The gut-muscle axis may be a therapeutic target in a range of muscle wasting disorders.

Funded by Science Foundation Ireland SFI/19/6628 INSPIRE DMD.

Exploring the Impact of Perinatal Exposure to the cannabinoid THC on Sleep and Respiratory Development Across Early Life Stages

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Background-Perinatal cannabis exposure is associated with significant risks such as sudden infant death syndrome(SIDS), preterm birth, and developmental challenges. Despite increasing perceptions of cannabis as safe during pregnancy, especially with its legalization, frequent use during pregnancy is linked to adverse outcomes in offspring. This study examines the impact of perinatal Δ-9-tetrahydrocannabinol(THC) exposure on sleep and breathing patterns in rats to understand the long-term effects on these critical functions.

Methods-Pregnant rats were exposed to THC or a vehicle solution via osmotic pumps from gestational day 5 to postnatal day (P) 14. Sleep patterns were assessed by implanting electromyography (EMG) electrodes in nuchal muscles from P0 to P14. From P20 onwards, rats were further equipped with electroencephalography (EEG) electrodes for detailed sleep assessments. Respiratory function was measured using head-out plethysmography from P0 to P11 under normoxic, hypoxic, and hypercapnic conditions. Results-THC-exposed rats showed a reduced percentage of active sleep at P7, which is more typical of more mature P10 behaviour in control rats. Additionally, chemoreflex responses to both O2 and CO2 were attenuated in THC-exposed groups across developmental stages, indicating compromised respiratory adaptability. Conclusion-Early THC exposure disrupts sleep-wake patterns and respiratory function in postnatal rats, highlighting the need for further research on the long-term neurodevelopmental effects of perinatal cannabinoid exposure.

Canadian Institutes of Health Research (CIHR).

Exploring the role of the Kolliker-Fuse nucleus in respiratory rhythmicity, control and disorders using mathematical modeling

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The respiratory central pattern generator includes rhythmically interacting populations of neurons in the ventral respiratory column in the medulla. It has become clear, however, that neuronal populations within the pons also can play a major role in the generation and control of respiratory rhythmicity. For example, the Kölliker–Fuse nucleus (KF), which is part of the parabrachial complex, participates in the generation of eupnoea under resting conditions and the control of active abdominal expiration when increased ventilation is required. Moreover, dysfunctions in KF neuronal activity are believed to play a role in the emergence of respiratory abnormalities seen in Rett syndrome (RTT), a progressive neurodevelopmental disorder associated with an irregular breathing pattern and frequent apnoeas. Because relatively little is known about the intrinsic dynamics of neurons in the KF, their synaptic connectivity, and how these features affect breathing pattern and irregularities, computational modeling represents an ideal tool for testing hypotheses and formulating predictions about these factors. In this work, we compare several potential models that simulate eupnoeic as well as RTT-like breathing phenotypes. Using dynamical systems analysis, we establish which KF characteristics and circuitry are compatible with experimental observations and propose experiments that can distinguish between these competing frameworks.

US NSF awards DMS 1612913 and 1951095, Brazilian National Council for Scientific and Technological Development (CNPq) grant no. 303481/2021-8, São Paulo State Research Foundation (FAPESP) grant no. 2022/05717-0, US National Institutes of Health NCCIH grant no. R01AT008632.

PREDNAC DMD: Respiratory effects of combined chronic glucocorticoid and antioxidant intervention in the mdx mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is characterised by respiratory muscle injury and weakness, ultimately leading to respiratory failure. The dystrophin-deficient mdx mouse model of DMD shows impaired respiratory muscle performance, fibrosis and inflammation in early disease.
Previously we showed that 1% chronic N-acetyl cysteine (NAC) supplementation (3 months daily) alone has no overt beneficial effects on respiratory system performance in the mdx mouse model of DMD, suggesting limited utility alone. Glucocorticoids are the current standard treatment for DMD and work by anti-inflammatory action. Here we investigated the effect of α-methyl-prednisolone (PRED) and PRED+NAC (PREDNAC).

One-month-old male mdx mice received PRED (0.8mg/kg methyl-prednisolone i.p. weekly) or PREDNAC (0.8mg/kg methyl-prednisolone i.p. weekly, 1% NAC in drinking water daily) for 3-months. At 4 months of age, we assessed breathing by whole-body-plethysmography followed by assessment of respiratory electromyogram (EMG) activities and inspiratory pressure during baseline, airway-occlusion, post-vagotomy, and asphyxia in anaesthetised mice and ex vivo assessment of diaphragm force-generating capacity.

Neither treatment influenced breathing or diaphragm force-generating capacity in mdx mice. Treatment significantly increased diaphragm and parasternal EMG activity, but inspiratory pressure was unchanged. We conclude that neither treatment has a major beneficial effect on respiratory system performance in the mdx mouse model of DMD.

SFI 19/FFP/6628 INSPIRE DMD.

Inhibitory control of motor and respiratory components of orienting by the substantia nigra pars reticulata is state- dependent

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The ability to generate coordinated behavioural (e.g. orienting) and physiological (e.g. respiratory) responses to external cues is highly conserved across diverse species. Here we investigate a potential role for GABAergic neurons in the substantia nigra pars reticulata (SNr) in controlling orienting behaviours and supportive respiratory responses in the rat. Anterograde tracing of SNrGAD1 neurons revealed dense terminal fields within the deep superior colliculus (SC), confirming innervation of regions known to mediate orienting and respiratory responses, and the ventromedial and paracentral/centrolateral thalamic nuclei. No labelling was observed in the pons, medulla, cortex or striatum.

Optogenetic inhibition was then used to examine whether tonic drive from SNrGAD1 neurons gates orienting-like behaviours. In awake rats, unilateral SNr inhibition evoked contralateral orienting accompanied by rapid and variable breathing, consistent with previously reported responses to SC stimulation. However, responses to SNrGAD1 inhibition were state-dependent. In quiet wakefulness, SNrGAD1 inhibition evoked motor and respiratory responses in >70% trials, falling to 3% during sleep. No effects were observed under anaesthesia.

We conclude that responses to SNrGAD1 inhibition likely result from disinhibition of SC outputs. The state- dependency of responses may reflect variable baseline activity of SNr neurons in these conditions.

Perpetual Foundation.

Physical Exercise Prevents Neurodegeneration In Cardiorespiratory Nuclei And Breathing Deficits In The 6-OHDA Model Of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disease with death of dopaminergic neurons in the Substantia Nigra (SN). It presents classic symptoms, and respiratory problems. There is neurodegeneration in cardiorespiratory regions such as nucleus of solitary tract (NTS), retrotrapezoid nucleus (RTN), preBötzinger complex (preBötC), rostral ventral respiratory group (rVRG) and nucleus ambiguus (NA), noted in 6- hydroxydopamine (6-OHDA) PD animal model, causing a high loss in cardiorespiratory function.

Results: 6-OHDA reduced TH+ neurons in SN and EX did not reverse it as expected, confirming the PD model. At normoxia, 6-OHDA animals showed reduced respiratory frequency (fR), prevented with EX as well during hypercapnia. We observed a reduction in phox2b neurons in cNTS, iNTS and RTN in sedentary groups and this was prevented with EX. The same was observed in NK1R-expressing neurons in rVRG and preBötC. Neurodegeneration was also seen in ChAT neurons in NA, prevented with EX. All the respiratory impairments and neurodegeneration was prevented after EX in the 6-OHDA PD model.

CEUA: 1342290421; CAPES - 88887.684788/2022-00, FAPESP (2021/08562-4).

Butyrylcholinesterase is dispensable for the neonatal autoresuscitation reflex

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Sudden Infant Death Syndrome (SIDS) is the leading cause of non-natural infant death under the age of one year of age and has a vastly unknown etiology but is best explained via a triple risk model that proposes SIDS occurs in a vulnerable infant during a critical developmental period when triggered by an external stressor. It is hypothesized that failure of arousal followed by failure of the autoresuscitation reflex is a common endpoint in most SIDS cases. Many SIDS cases are associated with genetic and molecular perturbations. A recent study identified decreased blood BChE activity associated with SIDS, however it is unclear how BChE perturbation leads to SIDS. We functionally tested the relationship between BChE perturbation and the neonatal autoresuscitation reflex. Male and female BChE loss of function mice were exposed to repeated bouts of anoxia utilizing our novel testbed, Looper, and allowed to autoresuscitate until death occurred. Despite earlier published findings, our results show that partial and total loss of BChE function does not affect neonatal autoresuscitation and survival, nor any cardiorespiratory parameters during recovery following challenges. Our data demonstrate the first functional assessment of BChE in a SIDS like setting; highlighting that BChE is dispensable for successful autoresuscitation.

Precision Core funding U54 OD030165. NIH/NHLBI R01HLN161142-01 and R01HL130249-07.

Repetitive acute intermittent hypoxia consisting of 10-min hypoxic episodes during the mid-active phase increases tidal volume in intact rats

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Repetitive acute intermittent hypoxia (AIH) elicits spinal, respiratory motor plasticity. AIH-induced plasticity can be initiated by serotonin or adenosine receptor activation on phrenic motor neurons. However, when both pathways are activated, powerful crosstalk inhibition suppresses plasticity. Traditionally, our group studied AIH protocols consisting of 3, 5-min hypoxic episodes during the rodent rest phase (i.e., human active phase), which elicits serotonin-dominant (adenosine-constrained) plasticity. However, when the same protocol is delivered in the mid- active phase, diurnal variations in basal spinal adenosine levels shift plasticity in favor of an adenosine-driven (serotonin-constrained) mechanism. Spinal adenosine levels increase in the mid-active phase, and are raised further by hypoxia-evoked adenosine formation, particularly during longer hypoxic episodes. Thus, time-of-day and hypoxic episode duration can be manipulated to alter the dominant mechanism driving respiratory motor plasticity. Here, we tested the hypothesis that repetitive AIH consisting of 10-min moderate hypoxic episodes delivered in the mid-active phase evokes respiratory motor plasticity, thereby increasing breathing ability. We report that repetitive AIH consisting of 3, 10-min hypoxic episodes (10.5% O2; 5-min intervals) during the mid- active phase for 5-7 consecutive nights significantly increases tidal volume during eupneic breathing one day post-exposure ($p < 0.01$), likely by an adenosine-driven mechanism.

NIH R01HL147554.

Detangling the spinal respiratory network's response to external electrical stimulus in a model of spinal cord injury

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After spinal injury, closed-loop epidural stimulation (sub-threshold stimulus at C4 during breathing efforts) restores some spontaneous ipsilesional diaphragm EMG which can last a short period post-stimulation. It is unknown what role in-phase (vs. out of phase of respiratory effort) stimulation plays. C2-hemisected (n=8) and intact (n=6) anesthetized rats were stimulated in- and out-of-phase with the drive to breathe. For in-phase stimulated hemisected rats, both the ipsilesional and contralesional hemidiaphragm EMG greatly increased in peak amplitude from baseline during stimulation and remained somewhat elevated from baseline for the 30 breaths post-stimulation. During out-of-phase stimulation, the ipsilesional hemidiaphragm both responded between breaths to the electrical stimulus and contracted in phase with the contralesional hemidiaphragm. The contralesional hemidiaphragm did not respond between breaths to this 'inappropriately' timed stimulus. Both ipsilesional and contralesional hemidiaphragm EMG peak activity remained slightly elevated from baseline after out-of-phase stimulation, with no continuation of between breath activity. In non-injured rats, peak amplitude was increased during stimulation, but this did not last post stimulation. The ipsilesional spinal cord thereby seems receptive to all inputs, while the contralesional side responds only to those in phase with respiratory drive. Finally, the injured cord is more primed for respiratory plasticity than intact.

R01HL153102 (ED), SPARC OT2OD023854 (ED), Craig H. Neilsen Pilot Grant (ED), T32HL134621 (AM), Bryan Robinson Endowment Grant in the Neurosciences (AM).

Body by Breath: The Science and Practice of Physical and Emotional Resilience *GRAPHICAL ABSTRACT POSTER*

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Co-founder Tune Up Fitness Worldwide, inc.

Body By Breath explores the body wide benefits of breathing as a deliberate exercise. Body By Breath details the benefits of soft self-massage tools to support improved, even optimal, breath mechanics, which can have profound impact on body function. In part 1, Body by Breath presents an embodied journey through the physiology of breathing, with an emphasis on the diaphragm, its central muscular node. Special chapters explore such topics as: i) the critical role of fascia and sensing; ii) neural control of breathing, and; iii) how breathing supports and interacts with performance. Part 2 proposes four primary application tools for self-improvement. In particular, by describing a combination of self-massage, movement, breathing and Yoga Nidra, Body by Breath delineates the keys to access whole body mobility, pain reduction, self-regulation and insight.

Spinal microglia regulate phrenic long-term facilitation via hypoxia-evoked phrenic motor neuron fractalkine release

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Although microglia regulate important CNS functions, including neuroinflammation and neuroplasticity, little is known concerning microglial regulation of respiratory motor plasticity. Acute intermittent hypoxia (AIH) elicits a model of spinal respiratory motor plasticity, phrenic long-term facilitation (pLTF). Whereas AIH consisting of moderate hypoxic episodes (mAIH) elicits pLTF by a serotonin-dominant, adenosine-constrained mechanism, adenosine drives severe AIH (sAIH)-induced pLTF. Neurons communicate with nearby microglia via fractalkine signaling, triggering extracellular adenosine accumulation. During hypoxia, neuronal fractalkine triggers sufficient adenosine accumulation to constrain or drive pLTF with mAIH and sAIH, respectively. To test the hypothesis that the relevant fractalkine comes from phrenic motor neurons, we injected siRNAs targeting fractalkine mRNA (or non-targeting controls) into the intrapleural space of adult male rats (3 consecutive days) to selectively knock down fractalkine mRNA within phrenic motor neurons. Phrenic motor neuron fractalkine knock down: 1) enhanced mAIH-induced pLTF (104±7%; n=7; vs controls: 56±5%; n=4; p=0.002), but 2) attenuated sAIH-induced pLTF (22±9%; n=7; vs controls: 102±16%; n=4; p<0.001), consistent with the idea that phrenic motor neurons are the source of relevant hypoxia-evoked fractalkine release, triggering the microglia-dependent adenosine formation that regulates AIH-induced pLTF. These findings increase understanding of inter-cellular mechanisms regulating AIH-induced phrenic motor plasticity.

Supported by NIH R01HL148030 (GSM), R01HL149800 (GSM), NIH T32HL134621-5 (ABM).

Comparative biology perspective of opioid-induced respiratory depression: from zebrafish to rodents

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Opioid drugs are widely used as pain therapies but can be abused and lead to overdose. In fact, opioid drugs present side-effects including respiratory depression that can be lethal with overdose. Using a comparative approach spanning zebrafish and rodents, our research identifies the neural circuits that are inhibited by opioid drugs and the molecular mechanisms regulating this inhibition. Our goal is to develop better therapeutic strategies to prevent respiratory depression while preserving the analgesic properties of opioid drugs. Using knockout mice, we found that tachykinin-1 expressing neurons (or neurons expressing substance P) mediate respiratory depression by the opioid fentanyl. Importantly, respiratory depression by fentanyl was reversed when substance P medullary neurons were stimulated by optogenetics in mice, suggesting that these neurons can be targeted to prevent respiratory depression. By leveraging data found in rodents, we identified in larval zebrafish new molecular targets, such as voltage-gated calcium channels, and drug compounds targeting these channels that could potentially reverse or prevent respiratory depression by fentanyl. By combining basic research in rodents and drug discovery in larval zebrafish, we are identifying the neural circuits mediating opioid-induced respiratory depression and are developing novel therapies to alleviate the respiratory side-effects of opioid drugs.

Canadian Institutes of Health Research.

Ventral medullary parvalbumin expiratory neurons control respiratory pattern formation and active expiration.

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Parvalbumin (PV) is a marker of inhibitory neurons. Previous studies described the presence of PV neurons in the ventral medullary region of rats, closely associated with the location of Bötzinger Complex (BötC) expiratory neurons. Herein, we addressed whether PV is expressed by BötC inhibitory expiratory neurons. We also evaluated the role of BötC PV neurons in the control of ventilation (VE) and respiratory pattern formation. Mice expressing Cre-recombinase in PV neurons were used to analyze the expression of PV in physiologically identified BötC expiratory neurons and their projection, as well as to evaluate the effects of inhibition and activation of BötC PV neurons on respiratory pattern formation and pulmonary ventilation (VE) during resting and high chemical drive. BötC post-inspiratory (PI) glycinergic neurons express PV. By projecting to preBötzinger Complex, PV-PI+ are involved in the respiratory pattern formation, by controlling Hering-Breuer reflex expiratory response, PI glottal adduction and VE. On the other hand, PV-PI+ neurons inhibit active expiration during resting, as well as control its incidence and ventilatory responses to hypercapnia/acidosis by projecting to lateral parafacial region. The target-specific of glycinergic inhibition from BötC PV+ neurons to different respiratory cells is another mechanism controlling inspiration and expiration.

FAPESP and CNPQ.

Human forebrain responses to breathing modulated by arousal states

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Forebrain control of breathing remains understudied. While the brainstem generates respiratory rhythm, the forebrain modulates respiration for various tasks by sending signals to the brainstem. To understand the mechanisms of this control, we examined how different brain regions represent breathing during responsive (awake) and unresponsive states (sleep, anesthesia, and ventilator support). We collected respiration signals and local field potentials (LFPs) from eight patients with intractable epilepsy. Recordings were taken during (i) automatic breathing while awake, (ii) automatic breathing during sleep, (iii) automatic breathing under anesthesia, (iv) breathing with ventilator support, (v) ventilator support with high tidal volume, and (vi) ventilator-induced apnea. After preprocessing the data, we calculated the coherence between respiration signals and LFPs at the breathing frequency. State-dependent changes in respiratory entrainment were analyzed using a linear mixed-effect model. Our findings revealed that multiple brain regions entrain respiration across all states, with distinct patterns observed in different conditions. Temporal regions are entrained during the awake state, the amygdala and hippocampus during sleep, and parietal regions during anesthesia. Significant changes in respiratory entrainment were noted in the postcentral gyrus, fusiform gyrus, insula, and parietal regions. These results demonstrate the forebrain's diverse responses to breathing, influenced by the arousal state.

NINDS K08 NS112573 04.

Developmental progression of respiratory dysfunction in a mouse model of Dravet syndrome

Milla BM and Mulkey DK

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Dravet syndrome (DS) is an early-onset epilepsy caused by mutations in the SCN1A gene, which encodes Nav1.1 channels that preferentially regulate activity of inhibitory neurons early in development. DS is associated with a high incidence of sudden unexpected death in epilepsy (SUDEP) by a mechanism that may involve respiratory failure. Consistent with this, Scn1a is expressed by neurons in brainstem respiratory centers, thus suggesting loss of Scn1a directly impair respiratory function. In support of this possibility, we show that Scn1a+/- mice exhibit a blunted ventilatory response to CO2/H+ prior to overt seizure activity that worsens with disease progression. Scn1a+/- mice also show a blunted ventilatory response to hypoxia, the severity of which correlates with mortality. We also found that pharmacological activation of Nav1.1 increased inhibitory control and rescues activity deficits of RTN neurons in slices from Scn1a+/- mice. We conclude that disordered breathing may be an early biomarker of SUDEP in DS, and at the cellular level loss of Scn1a disrupts RTN neurons by mechanisms involving disinhibition and pharmacological activation of Nav1.1 re-establish inhibitory control of RTN neurons and rescue activity deficits.

This work was supported by the following National Institutes of Health Grants: R01HL104101 (D.K.M.), R01HL137094 (D.K.M), R21NS134132 (D.K.M.) and F31 NS120467 (B.M.M).

Respiratory muscle control and preBötzinger complex neural circuitry alterations in mice lacking CDKL5

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CDKL5 deficiency disorder (CDD) is a rare genetic disease resulting from mutations in the CDKL5 gene. Sleep apneas have been reported for CDKL5-knockout (CDKL5-KO) mice, a CDD model. This research aimed to elucidate the role of CDKL5 kinase in the control of breathing by discriminating central (CSA) and obstructive (OSA) sleep apneas in CDKL5-KO mice and by exploring changes in the somatostatin neurons expressing high levels of neurokinin-1 receptors (NK1R) within the preBötzinger complex (preBötC), a medullary area essential for breathing. Sleep stage discrimination and diaphragmatic activity recording were performed in 10 wild-type (WT) and 12 CDKL5-KO mice. They were studied using whole-body plethysmography for 7 hours during the light (resting) period. Sleep apneas were categorized as CSA or OSA. Immunohistochemistry was performed in a sub- group of animals to evaluate the number of preBötC somatostatin neurons and NK1R expression. CDKL5-KO mice exhibited higher apnea rates and greater OSA prevalence during rapid-eye-movement sleep than WT mice. Furthermore, the number of preBötC somatostatin neurons and NK1R expression decreased in KO mice. These findings indicate the pivotal role of CDLK5 in regulating normal breathing, suggesting its potential involvement in shaping preBötC neural circuitry and controlling respiratory muscles during sleep.

Grants from the University of Florence and the University of Bologna supported this work.

The respiratory brain: inspiratory onset as a temporal prediction for cognitive processes and mental health

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The timing of the respiratory cycle modulates memories, which can be applied to anticipate what will happen in the future. We found that inspiratory onset derived from the PreBötzinger complex (PreBötC) in the ventrolateral medulla alters hippocampal ensemble dynamics and subsequently improves and deteriorates memory performance, suggesting that inspiratory onset may play a key role in the temporal prediction in the brain. Importantly, PreBötC activity can be phasically generated in divergent manner. Since divergence can contain a feedforward signal that is equivalent to a prediction, PreBötC activity might have a certain role in corollary discharge and efferent copies sent to the hippocampus and prefrontal cortex. Recent studies propose that the hippocampal-entorhinal circuit fits the active-perception loop, where corollary discharge from the PreBötC might form memory encoding in the hippocampus. Conversely, an inaccurate coincidence with the respiratory copies may trigger abnormal and overactive hippocampal and cortical formations associated with stress and cognitive impairment. These considerations may support a better understanding of brain-lung interactions and could be applied to even a potential strategy for treating neuropsychiatric disorders.

Japan Society for the Promotion of Science, Takeda Science Foundation, Hyogo Innovative Challenge.

Persistent suppression of phrenic long-term facilitation following acute inflammation requires microglial TGFbeta signaling to phrenic motor neurons

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Even mild inflammation impairs phrenic long-term facilitation (pLTF) elicited by moderate acute intermittent hypoxia (mAIH). Until now, it was assumed that pLTF is impaired only during active inflammation and would rapidly recover once inflammation subsides. However, we now report that mild inflammation induced by low dose lipopolysaccharide (LPS; 0.1 mg/kg) impairs pLTF for up to a month after pro-inflammatory markers have returned to normal. This finding has profound implication for clinical trials using therapeutic AIH to improve respiratory and limb function in patients with SCI or ALS since inflammation from bladder, skin and lung infections is common in these conditions. Thus, a history of inflammation may be a major factor contributing to the incidence of “low responders” reported in human therapeutic AIH trials to date. We now report that the mechanism of pLTF impairment switches from adenosine- during active inflammation (Burrowes et al., ibid) to TGF□-dependence in the recovery phase. Nevertheless, pLTF is suppressed by microglia- phrenic motor neuron interactions in both cases. Additional study of mechanisms whereby inflammation suppresses respiratory motor plasticity is warranted.

Supported by NIH R01HL148030 (GSM), R01HL149800 (GSM), NIH T32HL134621-5 (ABM).

JANUS HYPOTHESIS: Compensation and de-compensation of peak inspiratory performance in mouse models of Duchene muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a debilitating neuromuscular disease with end-stage respiratory failure. We have characterised respiratory control over the natural history of disease in mouse models of DMD. In the most widely studied animal model of DMD, the dystrophin deficient mdx mouse, we established that diaphragm weakness and impaired EMG activity appear as early as 1-month of age, with decreased peak inspiratory pressure. However, a remarkable compensation is subsequently afforded by accessory muscles of breathing that serve to recover peak inspiratory generating capacity by 4 months of age with a subsequent slow decline in performance by 16-months of age. Loss of compensation precipitates respiratory compromise in late- stage muscular dystrophy. We proposed the Janus hypothesis, describing the two faces of fibrosis, wherein fibrotic remodelling of the diaphragm paradoxically confers an early advantage in peak pressure-generating capacity, but subsequent fibrosis and impairment of accessory muscles contributes to the temporal decline in respiratory performance and ultimately respiratory failure. We recently characterised the D2.mdx mouse, which elaborates a more progressive disease profile than mdx mice. Our studies further support the Janus hypothesis demonstrating early compensation and subsequent de- compensation of peak inspiratory performance between 4 and 8 months of age in D2.mdx mice.

Science Foundation Ireland 19/FFP/6628 INSPIRE DMD.

Proposal of a respiratory rhythm-generator model consisting of longitudinally distributed multiple oscillators along the neuraxis

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The maintenance of respiratory rhythm is vital for life and must be robust in various situations. The fundamental respiratory rhythm is generated by the pre-Bötzinger complex (preBötC). When the preBötC is acutely destroyed, respiration ceases. However, if preBötC function is gradually disturbed, breathing can persist, suggesting the presence of auxiliary respiratory rhythm generators that enhance robustness and adaptability. Our and other laboratories' research has identified multiple respiration-synchronized microcircuits with intrinsic oscillatory properties. Onimaru's group reported that the amygdala exhibits respiration-synchronized oscillation. Certain microregions in the hypothalamus might generate auxiliary respiratory rhythms. The parafacial respiratory group (pFRG) can independently generate respiratory rhythm, demonstrated by persistent cranial nerve rhythms after removal of the brainstem caudal to the pFRG in neonatal rats. A rhythm-generating oscillator in the high (C1-C2) spinal cord also drives the brainstem oscillator and independently generates rhythmic phrenic activity. We propose a model where respiratory rhythm is primarily generated by the preBötC, supported by oscillators in the amygdala, hypothalamus, pFRG, high spinal cord, and lumbar spinal cord. Under psychological stress, the rhythmically activated amygdala facilitates respiration, and during locomotion, the lumbar spinal cord's central pattern generator (CPG) rhythmically drives both legs and respiratory muscles. This model's validity requires experimental investigation.

JSPS KAKENHI Grant Number 24K14597.

The lateral parafacial region and active expiration

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Breathing is an essential behavior for life. In humans and other mammals the part of the brain that controls respiration is located in the brainstem, where neurons control the rhythmic contraction of respiratory muscles from the early stages of gestation until the last breath. For a number of years respiration was thought to be under the control of a sole system pacing rhythmic inspiration. Recent evidence, however, has shown that a second region in the brainstem may also be essential for generating rhythmic expiration during high metabolic demand and across sleep states. Our laboratory is interested in delineating the function of this expiratory oscillator in both physiological and pathological conditions. The talk will highlight some recent work that illustrates the role of the expiratory oscillator in respiration, its location and its neuromodulation.

Canadian Institutes of Health Research; Natural Sciences and Engineering Research Council of Canada.

Htr1B's role in the autoresuscitation reflex and its Implications to SIDS

Dipak Patel, Benjamin Frankfort, and Russell Ray

American Heart Association and Baylor College of Medicine

Sudden Infant Death Syndrome (SIDS) is a leading cause of death under the age of 1 with an unknown etiology. Limited data indicates that a failure in the autoresuscitation reflex (ARR) is a common endpoint in many SIDS cases after the infant has fallen into a terminal apnea and bradycardia, often while rebreathing and sleeping face down. The ARR is a series of gasps an infant takes to break the apnea and revitalize their cardio-respiratory system. Multiple postmortem studies have revealed a variety of brainstem serotonin abnormalities in subsets of SIDS cases while in-vivo modeling has shown that that serotonin plays a key role in the ARR. However, the cellular and molecular pathways involving serotonin signaling in ARR remain largely unknown. Here we show that loss of serotonin receptor 1B (Htr1B) disrupts the AAR. Using our automated neonatal cardio-respiratory assessment platform, Looper, Neonate mice lacking Htr1B show a decrease in survival to anoxic gas challenges mimicking face down infant sleeping, a heterozygous male specific vulnerability, a delay in heart rate and respiratory rate recovery, and a delay in gasping and gasping frequency. Our results identify a critical component of the serotonin signaling pathway in neonate autoresuscitation.

American Heart Association Predoctoral Fellowship.

The role of BK channels in mouse ventilatory response to CO2 and the chemosensitity of locus coeruleus neurons

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Big conductance potassium (BK) channels contribute to K+ flow and electrical activity in various cell types, including neurons. The firing rate of CO2/pH-sensitive neurons in the locus coeruleus (LC) is influenced by Ca2+- activated K+ (BK) channels. In addition, the chemosensitivity index of LC neurons decreases over the first weeks of life, concomitant with an increase in the expression of BK channels on the neural membrane. Dysfunctions in BK channel activity lead to exacerbated hypercapnic responses, and several pathological conditions have been correlated with chemosensitivity disturbances. The present study evaluated the hypercapnic ventilatory response (HcVR – 7% CO2) of BK KO male and female mice during the first weeks of life (P0-1, P6-7, and P12-13), as well as the CO2 chemosensitivity of LC neurons in adult male and female mice (baseline and 10% CO2). Preliminary data show increased ventilation in female KO mice of postnatal ages P6-7, and increased VE and VO2 at P12-13 age compared to control group during hypercapnia. Electrophysiological data indicate an over 3-fold increase in CO2 response in LC neurons of adult male BK KO mice. These findings suggest that BK channels play an important role in HcVR during development in females, but act as a brake on neural CO2 chemosensitivity of adult males.

FAPESP and CNPq.

Serotonin Neuron Influence on Pontine Breathing Circuitry Impaired by Opioids

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Opioid-induced respiratory depression (OIRD) is due to mu opioid receptor (MOR) activation and can cause fatality in overdose. Stimulation of serotonergic raphe neurons and 5HT2A/C receptors stimulates breathing and may act as a MOR-independent target for treating OIRD. Despite this, opioids' actions on serotonin neurons and their inputs to OIRD-implicated lateral parabrachial (LPB) neurons are poorly understood. Herein, we hypothesized that medullary serotonin neurons innervate the LPB and enhance respiratory activity, even under fentanyl. To test this hypothesis, we used mice expressing channelrhodopsin-2 (ChR2) in serotonin neurons. We employed an in situ working heart-brainstem preparation to record respiratory output during serotonin neuron activation and fentanyl application. Optical stimulation of medullary raphe neurons enhanced respiratory output, which was attenuated but not blocked by fentanyl. To characterize serotonin inputs to the LPB, we used brain slice electrophysiology with slices containing the LPB from mice expressing ChR2 in serotonin neurons. We observed optically-evoked slow outward currents that were blocked by the 5HT1A antagonist, WAY100635, and fast post-synaptic currents that were attenuated by met-enkephalin and blocked by the GABA-A antagonist gabazine. These data indicate that serotonin neurons may modulate breathing through complex inhibitory actions via GABA and serotonin release in the LPB.

NIDA R01 DA 047978 to ESL

NADPH Oxidase, Apocynin, And Its Effects On Apoptotic And Survival Pathways In The 6-OHDA Rat Model Of Parkinson’s Disease

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Parkinson's disease is a neurodegenerative disease characterized by the death of dopaminergic neurons in Substantia Nigra. It presents motor symptoms, such as dyskinesia, postural instability and respiratory problems. It's known that the 6-OHDA animal rat model presents neurodegeneration in respiratory control regions such as nucleus of solitary tract (NTS), retrotrapezoid nucleus (RTN), preBötzinger complex (preBötC) and rostral ventral respiratory group (rVRG), which causes loss in ventilatory function. Oxidative stress seems to be one cause of this impairment in breathing and previous work have shown that apocynin prevented respiratory nuclei degeneration and breathing dysfunction in PD model. We evaluated the effects of treatment with apocynin on NOX2 expression and in the signaling of respiratory nuclei and respiratory deficits in 6-OHDA animals. Our results show an abnormal signaling for survival pathways in the preBötC and RTN, which led to neuronal death, and treatment with apocynin prevented it. Also, NOX2 expression was altered in the RTN and preBötzinger, and apocynin prevented these alterations. Thus, survival signaling is important in the respiratory neurodegeneration in 6-OHDA model, and treatment with APO prevents it, revealing that NOX2 is valuable in the neurodegeneration of respiratory nuclei in the 6-OHDA model.

CAPES - 88887.940799/2024-00; FAPESP - 2019/00065-1; 2021/12538-1.

Disentangling pain modulation and respiratory depression at the level of the rostral ventromedial medulla

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Opioid medications are pivotal in pain management but are often accompanied by severe side effects, such as opioid-induced respiratory depression (OIRD), the primary cause of death in opioid overdoses. The respiratory side effects and pain-relieving properties of opioids are primarily attributed to the activation of μ-opioid receptors (μOR) that are expressed in respiratory and pain modulatory circuits, respectively. However, pharmacological manipulations of the rostral ventromedial medulla (RVM), the primary output node of the descending pain modulatory system and a critical mediator of opioid analgesia, have been shown to significantly contribute to OIRD, making it somewhat unclear if the pain-relieving benefits of opioids are completely separable from respiratory depression. Here, using intersectional genetic techniques in mice, we explore the roles of RVM subpopulations in descending pain modulation and the control of breathing based on their excitatory/inhibitory phenotypes and the expression of Oprm1 (the gene encoding μOR). We show that RVM subpopulations can strongly influence pain thresholds and exert bidirectional and state-dependent control of breathing, in some cases causing prolonged apneas. These findings clearly illustrate the intersection of pain and respiration at the level of the RVM and point toward potential targets for reversing OIRD or generating pain relief without respiratory depression.

K01 1K01DA058543-01.

Pavlov's Dog is Still Hungry

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As Pavlov demonstrated, feeding is highly dependent on the autonomic nervous system. Feeding is a process of sensing, orally ingesting, chewing, swallowing, and ultimately digesting food. Swallow and laryngeal adduction are essential for the vital suite of behaviors comprising ingestion and airway protection. Swallow, and even just glottal adduction or imagined swallow can produce transient but powerful autonomic effects. These effects include both sympathetic and parasympathetic outputs to affect heart rate, blood pressure, arousal, and respiration. However, relatively little is known about specific mechanisms of sympathetic and parasympathetic control during swallowing. We will present swallow recordings from humans, cats, rats, and mice which demonstrate changes in blood pressure and/or heart rate consistent with multi-modal contributions. For example, following vagotomy, there is a significant increase in blood pressure during swallow, consistent with a hypothesis of sympathetic contribution. Following spinal cord transection at the T1 level, there is a small decrease in blood pressure during swallow, consistent with parasympathetic contribution. The role of the autonomic nervous system during swallow—including parasympathetic and sympathetic outflows as well as visceral sensation—has broad implications for potential mechanisms of dysphagia in patients such as those with lung transplant and spinal cord injury.

Supported by NIH grants NS110169, HL155721, HL163008, HD110951, OT20D001983, the Craig H. Neilsen Foundation Pilot Research Grant 546714.

# $O_2$ Sensing by the Carotid Body

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Carotid bodies (CB) are sensory organs for monitoring arterial blood O2 levels. CB chemoreflex is vital for maintaining cardiorespiratory homeostasis under hypoxia. Glomus cells, primary O2 sensing cells in the CB, express cystathionine gamma-lyase (CSE), an H2S-generating enzyme, and hypoxia increases H2S generation in a stimulus-dependent manner. CSE null mice exhibit impaired CB neural activation and ventilatory stimulation by hypoxia, and loss of H2S generation by low O2. H2S generation by hypoxia require interaction of CSE with hemeoxygenase-2, which generates carbon monoxide (CO). Persulfidation of olfactory receptor 78 (Olfr78) by H2S is an integral component of carotid body activation by hypoxia. Hypoxia and H2S increased persulfidation in CB glomus cells and persulfidated cysteine240 in Olfr78 protein. Olfr78 mutants manifest impaired CB sensory nerve, glomus cell, and breathing responses to H2S and hypoxia. Glomus cells are positive for GOlf,, adenylate cyclase 3 (Adcy3), and cyclic nucleotide-gated channel alpha 2 (Cnga2), key molecules of odorant receptor signaling. Adcy3 or Cnga2 mutants exhibit impaired CB and glomus cell responses to H2S and breathing responses to hypoxia. These findings suggest that H2S through redox modification of Olfr78 participates in carotid body activation by hypoxia to regulate breathing.

Supported by NIH-HLBI, PO1 HL-144454.

Group Breathwork Intervention for Adults with Chronic Pain: A proof-of-concept study of Guided Respiration Mindfulness Therapy

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Chronic pain is a major public health problem. Due to the persistent, costly, and complex nature of chronic pain, there is an urgent need for safe and effective treatments. Respiration is a vital physiological function that is also bidirectionally related to pain, stress, and emotions. Breathwork, or the conscious control of breathing for therapeutic purposes, has promise as a novel treatment for chronic pain. The primary objective of this proof-of- concept study was to examine the acceptability and clinical significance of a single breathwork session for adults with chronic pain. The breathwork intervention is called Guided Respiration Mindfulness Therapy and involves a sustained (1-hour) conscious connected breathing pattern where there is no pause between inhale and exhale. Participants included 10 adults with various types of chronic musculoskeletal pain. We found that the group breathwork intervention was highly acceptable and satisfying. Almost all participants (90%) reported a clinically significant improvement in pain intensity immediately following the session with 60% maintaining a clinically meaningful response at the 2-week follow-up. It is plausible that this type of breathwork intervention could produce clinically significant effects for those with chronic pain. Future research will examine the efficacy and biopsychosocial mechanisms of this intervention.

Funding: K01AT012066 from the National Center for Complementary and Integrative Health.

The Effects of Slow-Paced Breathing Techniques on Cognitive Performance: Current Findings and Future Directions

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Slow-paced breathing has emerged as a prominent strategy to alleviate anxiety symptoms by altering physiological processes like respiration rate, $PETCO_2$, and neurotransmitter levels, which modify interoceptive and cognitive states. We hypothesized that changes in $PETCO_2$ and respiration rate influence cognition through anxiety modulation. Participants completed a psychomotor vigilance task while $PETCO_2$ and respiration rate were altered using slow-paced breathing. Reaction times (RT) were measured during spontaneous respiration, extended-inhalation, and extended-exhalation breathing, corresponding to baseline, elevated, and reduced $PETCO_2$. Our findings reveal a significant relationship between $PETCO_2$ and cognitive performance. While respiration rate remained stable for paced conditions, $PETCO_2$ varied based on condition. In altered $PETCO_2$ conditions, RT were significantly higher for extended-exhale (mean difference= 0.0752, $p< 0.05$) and extended- inhale (mean difference= 0.1032, $p< 0.05$), compared to spontaneous respiration. However, there was no significant difference in RT between extended-exhale and extended-inhale (mean difference= 0.028, $p> 0.05$). This suggests that $PETCO_2$ is crucial for attention modulation. Future research will use intracranial electrochemistry in epilepsy patients for sub-second monoamine and pH recordings during slow-paced breathing exercises, correlating these with $PETCO_2$ and cognitive states. This approach will offer unprecedented insights into the relationship between respiration, $PETCO_2$, cognition, and real-time neuromodulatory dynamics in human subjects.

Ray Gaskins Health Sciences Graduate Fellowship.

Melanocortin receptor 4 agonist, setmelanotide, treats opioid-induced respiratory depression

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The primary cause of death associated with opioids is opioid-induced respiratory depression (OIRD) and obesity is a major risk factor that increases mortality. Naloxone is used to reverse OIRD, but this drug is a competitive antagonist of μ-opioid receptor (MOR) and reverses analgesia, which limits its therapeutic use. Alternative non- opioid receptor antagonist-based approaches to OIRD treatment and prevention are needed. Previous studies suggest that melanocortin 4 receptor pathway (MC4R), which is implicated in body weight regulation, may impact control of breathing. An MC4R agonist, setmelanotide (SET), is approved by FDA to treat genetic obesity caused by abnormal melanocortin and leptin signaling. We hypothesized that SET can treat OIRD in mice. C57BL/6J male mice with diet-induced obesity were treated with IP morphine (10 mg/kg) and then 15 min later with either SET (1 mg/kg IP) or vehicle VEH (IP) in a random order. Breathing was recorded by barometric plethysmography, and pain sensitivity was measured by the tail-flick test. In mice with OIRD, SET induced a 3-fold reduction of the apnea index, from 67 ± 7 to 22 ± 4 events per hour ($p < 0.001$), and decreased apnea duration as compared to the VEH treatment. SET increased respiratory rate from 102 ± 4.5 to 130 ± 5.6 breaths per minute ($p < 0.05$). SET did not affect opioid analgesia. Photostimulation of MC4R+ ChR2-expressing fibers in the parafacial region elicited short-latency postsynaptic current in pre-motoneurons projecting to the phrenic motoneurons in the C3-C4 ventral horns of the spinal cord. In conclusion, SET effectively treated OIRD by increasing respiratory rate and inducing a significant decrease in the number of apneas without decreasing analgesia. Parafacial MC4R (+) neurons are a likely site of respiratory effects of SET.
NIH (R01 HL128970, R01 HL133100, and R01 HL138932), AHA (24CDA1270910).

Targeting Melanocortin 4 Receptor to Treat Sleep-Disordered Breathing

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There is no effective pharmacotherapy for sleep disordered breathing (SDB). A melanocortin receptor 4 (MCR4) agonist, setmelanotide (SET), is used to treat genetic obesity caused by abnormal melanocortin and leptin signaling. We hypothesized that SET can treat SDB in diet induced obese mice. We performed a proof-of-concept randomized crossover trial of a single dose of SET *vs* vehicle and a two-week daily SET vs vehicle trial in obese mice. We also examined co-localization of *Mcr4* mRNAs with a marker of CO2 sensing neurons PHOX2b in the brainstem and performed chemogenetic studies expressing *Cre*-dependent designer receptors exclusively activated by designer drugs (DREADD) in *Mcr4-Cre* mice. SET increased minute ventilation across sleep/wake states, greatly enhanced the hypercapnic ventilatory response (HCVR) and abolished apneas during sleep. PHOX2b+ neurons in the nucleus of the solitary tract (NTS) and the parafacial region expressed *Mc4r*. Chemogenetic stimulation of the MC4R+ neurons in the parafacial region, but not in the NTS, augmented the HCVR without any changes in metabolism. Parafacial MC4R neurons projected to the respiratory pre-motor neurons expressing cholera toxin B after C3-C4 spinal cord injections. In conclusion, MC4R agonists enhance the HCVR and treat SDB by acting on the parafacial MC4R+ neurons.
NIH (R01 HL128970, R01 HL133100, and R01 HL138932), AHA (24CDA1270910).

Mapping whole-brain excitatory and inhibitory networks for the modulation of respiratory rhythm

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Breathing is altered by behavior and emotion, yet little is known about the cell-types that transmit these non- homeostatic breathing commands to preBötzinger complex (preBötC). Interestingly, higher-order brain regions cannot be distinguished based on whether they project to excitatory or inhibitory preBötC neurons. We hypothesized that preBötC inputs would map to distinct brain regions based on the inhibitory or excitatory phenotype of the projecting neurons. To test this, mice that express tdTomato only when both Cre and FlpO are present (Ai65) were bred with mice that express Cre in either inhibitory (VgatCre) or excitatory (Vglut2Cre) neurons. Adult offspring received a unilateral preBötC injection of a retrograde AAV that expresses FlpO (AAVrg- FlpO), thereby specifically and efficiently activating tdTomato expression in either inhibitory or excitatory neurons that project directly to the preBötC. Whole-brain imaging revealed distinct populations of neurons in many mid- and forebrain regions with little overlap between inhibitory and excitatory projections. Example inhibitory projections include CeA, BNST, Zona Incerta, and RVM, whereas projections from LC, PBN, Hypothalamus, and CTX are excitatory. Defining the transcriptional phenotypes of these preBötC inputs establishes an essential foundation for future functional studies to determine the role of these circuits in the control of breathing.

R01 HL1660317 (Baertsch) R00 HL145004 (Baertsch).

Effects of activation of glutamatergic interneurons of the lateral parafacial region on the ventilatory parameters of mice during normocapnia and hypercapnia

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The lateral parafacial region (pFL), responsible for generating expiratory motor activity during hypercapnia, contains excitatory neurons and interneurons (with local projections). The role of pFL excitatory interneurons in regulating ventilation during normocapnia and hypercapnia is unclear. We used mice expressing the Cre recombinase in VGLUT2-positive neurons [VGLUT2(cre/cre)] that were microinjected with cre-dependent adeno- associated virus to express hM3D-Gq only in pFL glutamatergic interneurons and evaluated the effects of its chemogenetic activation, using JHU37160 (0.1mg/kg; i.p.), on ventilatory parameters of non-anesthetized mice during resting and hypercapnia (7% CO2). At resting, JHU injection reduced respiratory frequency (fR; 147±56 vs 213±60 cpm; p=0.002) and increased tidal volume (VT; 9.9±3 vs 7.9±1 μL.g-1; p=0.02) of VGLUT2(cre/cre+hM3D-Gq) (n=10), while did not change the ventilatory parameters of VGLUT2(cre/cre) (n=15) mice. JHU injection did not change the hypercapnia-induced ventilatory responses of VGLUT2(cre/cre+hM3D-Gq) (n=10) mice when compared to VGLUT2(cre/cre) (n=12) [(fR: 212±75 vs 251±50 cpm) (VT: 12±4 vs 12±4 μL.g-1) (pulmonary ventilation: 2757±1802 vs 3251±1702 μL.g-1.min-1) (Cycle duration: 207±47 vs 207±32 ms) (Duration of inspiration: 72±33 vs 78±11 ms) (Duration of expiration: 134±27 vs 131±25 ms)]. Our data demonstrate that the pFL glutamatergic interneurons are important for controlling ventilation of mice during rest.

FAPESP (2022/15704-2 and 2018/15957-2), CNPq and CAPES.

Genetically-encoded sensors and actuators in the study of preBötzinger neurons in organotypic slice cultures.

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The preBötzinger complex (preBötC) remains rhythmically active in organotypic slice cultures, and neurons can be efficiently transduced with AAVs containing genes for genetically-encoded sensors and actuators.

Here, we used the fast opsin Chronos, genetically-encoded calcium-, glutamate- and GABA-sensors, to study dendritic properties, dynamic changes in input resistance, presynaptic modulation of calcium transients, and opioid modulation of glutamate release in preBötC neurons. Using a calcium sensor confined to the nucleus, we visualized calcium transients in the nucleus of preBötC neurons during inspiratory bursts.

These experiments demonstrate that preBötC neurons have a dendritic length constant of ~200 μm, an input resistance that decreases in a v-shape during inspiratory bursts, show GABAergic inhibition of presynaptic calcium transients, and that the opioid mediated slowing of respiratory rhythm involves presynaptic reduction of glutamate release. The nuclear calcium sensor spatially demarcates a distinct preBötC core and periphery in the cultures and the calcium transients in the core are greater than in the periphery. Application of DAMGO decreases burst frequency but is not associated with cells dropping out of the rhythm completely.

In conclusion, AAV transduced organotypic slice cultures are useful in studying somadendritic properties, and modulation of transmitter release in preBötC neurons.

Independent Research Fund Denmark, Lundbeck Foudation.

Muscarinic modulation at hypoglossal motoneurons across postnatal maturation

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Muscarinic modulation of inspiratory bursting at hypoglossal motoneurons potentiates inspiratory bursting in neonatal rodents and inhibits inspiratory bursting in adult rodents. We speculate that changes in muscarinic acetylcholine receptor expression contribute. Thus, we examined changes in muscarinic acetylcholine receptor expression at hypoglossal motoneurons across postnatal maturation using immunofluorescence methods. We hypothesized there is an increase in inhibitory (M2) and decrease in excitatory (M1, M3, M5) muscarinic acetylcholine receptors at hypoglossal motoneurons with postnatal maturation. Our results indicate that M1 and M5 receptor expression levels decreased with postnatal maturation, whereas M3 receptor expression levels increased transiently around postnatal days 17-19. By comparison, surprisingly, M2 receptor expression levels were unchanged across maturation. Using the rhythmic medullary slice preparation and pharmacological agents, we also tested the contribution of M1, M2, and M3 receptors to the muscarinic modulation of inspiratory bursting at hypoglossal motoneurons. Preliminary data indicate that M1 activation contributes significantly to muscarinic potentiation of inspiratory bursting in the first postnatal week and that M2 activation may contribute significantly to the net effect of muscarinic modulation in the second postnatal week. Overall, these results begin to elucidate the mechanisms underlying the changes in cholinergic modulation at hypoglossal motoneurons with postnatal maturation.

NIH/NHLBI R15 (R15HL148870) (Revill); NIH/NHLBI R25 HL126140 (Moreno, Garcia, Parthasarathy, subaward to Revill).

Radiofrequency ablation of a focal amygdala site affects apnea susceptibility: Implications for Sudden Unexpected Death in Epilepsy (SUDEP)

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We have previously identified an amygdala site in human that inhibits respiration when electrical stimulation is delivered or when seizures spread to that area [Rhone et al. 2020, Harmata et al. 2023], which we have called the "amygdala inhibition of respiration" (AIR) site. Crucially, patients had no awareness of apnea, no panic, and no alarm. We hypothesized that this site may be critical in Sudden Unexpected Death in Epilepsy (SUDEP). Radio frequency thermocoagulation (RFTC) of brain tissue is emerging as a useful tool for management of intractable epilepsy. In RFTC, a lesion is created at the seizure focus using radiofrequency electrical current. RFTC is delivered in patient's hospital room, takes minutes, is well tolerated, and is safe [Catenoix et al. 2018]. We evaluated neural responses, respiratory physiology, and behavior before and after RFTC of the mesial temporal lobe in patients undergoing intracranial electroencephalographic monitoring for seizure localization. We found that focal RFTC lesion of the AIR site prevented stimulation-induced apnea. Before RFTC of the AIR site, spontaneous and electrical-stimulation-evoked seizures resulted in apnea requiring clinical intervention. After RFTC of the AIR site, no apnea was observed. Focal AIR site lesions may provide a future therapeutic target for SUDEP prevention.

NIH K08 NS112573-01.

Formation of breathing circuits in the mouse: the role of the homeobox gene Gsx2

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In sudden infant death syndrome (SIDS), neuronal breathing circuitry can function apparently normally at birth but fail at some point in the first year. Gsx2 is a transcription factor expressed in hindbrain neural progenitors of the dA3 domain which give rise to glutamatergic neurons within the nucleus tractus solitarius (nTS) and catecholaminergic neurons of the A1/C1 and A2/C2 groups. These neurons are essential for integrating and regulating respiratory responses to hypoxia and hypercarbia and have been implicated in SIDS. Gsx2 null mice die within hours of birth after becoming cyanotic. Here, we show that loss of Gsx2 results in a loss of bHLH factor Ascl1 in dA3 progenitors at E11.5. As a result, embryonic brainstem neurons marked by Phox2b and Tlx3 are severely reduced in number. By E18.5, the Gsx2-null mutants (Gsx2-KO) display reduced glutamatergic (vGlut2+/Phox2b+) nTS neurons and catecholaminergic neurons (TH+/Phox2b+ A1/C1 and A2/C2). Surprisingly, our preliminary studies demonstrate that Gsx2-KO mice breathe rhythmically at birth with fewer apneas than control littermates before becoming cyanotic. Furthermore, Gsx2 null mutants aspirate milk when fed, suggesting a failure to gate between swallowing and breathing. Our data indicate that Gsx2 is a critical regulator of glutamatergic nTS and catecholaminergic neuron fate.

This study was supported by NIH grant R01 NS124660.

A role for mitochondria in Spinal Oxygen Sensing (SOS) and the autonomic dysfunction associated with neurodegenerative diseases

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BACKGROUND: Low oxygen (hypoxia) damages CNS neurons and is a risk factor in most neurodegenerative diseases. We discovered that spinal thoracic sympathetic preganglionic neurons (SSPNs) are spinal oxygen sensors. The oxygen sensing mechanism involves reactive oxygen species (ROS) and superoxide dismutase (SOD), both of which are implicated in ALS. We propose that overactivity of this system results in SSPN death, and ALS-associated autonomic dysfunction. HYPOTHESIS: As mitochondria are a main source of ROS, we hypothesize that mitochondria play an important role in spinal oxygen sensing. METHODS AND RESULTS: Using a rat in situ, artificially-perfused spinal cord preparation we show that blocking mitochondrial complexes I, III or IV with rotenone, antimycin or cyanide, respectively, increases SSPN activity by 1.5-4.5 fold, but importantly, does not abolish sympathetic hypoxic responses. Similarly, sympathetic hypoxic responses persist with mitochondrial antioxidant SkQ1, although our data suggest that hypoxic responses are reduced substantially.CONCLUSIONS: Mitochondria-derived ROS is not essential for oxygen sensing, as blockade of mitochondrial respiratory complexes or mitochondrial-derived ROS failed to abolish the hypoxic responses. Nonetheless, mitochondria-derived ROS augments hypoxic responses and may therefore contribute directly to overexcitation of the sympathetic activity and eventual cell death associated with ALS autonomic dysfunction .

This work was supported by CIHR and NSERC BRAIN CREATE Fellowships (MS) and CIHR operating grant (RJAW).

Respiratory profile of adenosine A2A receptors knockout mice exposed to short term sustained hypoxia

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Adenosine and A2A receptors are involved with autonomic and respiratory responses to hypoxia. Herein knockout mice for A2A receptors (A2AKO) and wild-type (WT) were exposed to sustained hypoxia (SH, 24h - FiO2 0.1) and then we evaluated: a) the cardiorespiratory changes in response to SH in awake mice, b) the activities of phrenic (PN), abdominal (AbN), sympathetic (tSNA) nerves in the in situ preparation, and c) the excitatory transmission in NTS neurons using patch clamp in brainstem slices. A2AKO mice exposed to normoxia presented higher respiratory frequency in vivo (241±25 vs 184±11 breaths.min-1, P<0.0001) than WT. The frequency of PN in situ was higher in the A2AKO than in WT mice (2.05±0.45 vs 0.28±0.04 Hz, P<0.001) under normocapnia (5% CO2), while the incidence of Late-E events in AbN (active expiration) of A2AKO exposed to SH was similar to that observed in the A2AKO exposed to normoxia. SH increased the amplitude of evoked glutamatergic currents of NTS neurons from WT mice (-429±87 vs -210±32 pA, P=0,0203) but not from A2AKO (-268±61 vs -307±37pA, P>0,9999). These findings support the concept that adenosine and A2A receptors play a key role in modulating the baseline respiratory frequency and in generating active expiration in mice.

FAPESP (2018/15957-2, 2021/01767-0, 2021/08833-8 and 2022/05237-8) and CNPq (309338/2020-4).

A biophysical model of burstlets and bursts in the respiratory preBötzinger complex

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Burstlet theory posits that inspiratory oscillations arise from an emergent network process in a subpopulation of the preBötzinger complex (preBötC) dedicated to rhythm (burstlet) generation. For inspiration to occur on a particular cycle, a secondary pattern-generating subpopulation must be recruited to generate a full network oscillation (burst) required for motor output. This view is supported by experimental observations that, in low excitability states, the preBötC generates a regular rhythm featuring a mixture of large (burst) and small (burstlet) amplitude network oscillations (Kam et al., 2013). Experiments have shown that the burstlet-to-burst transition is Ca2+-dependent and suggest that the recruitment of pattern-forming neurons depends on intracellular Ca2+ transients and activation of a Ca2+-activated nonspecific cationic current (ICAN) in non-rhythmogenic, pattern- forming preBötC neurons. In this computational study, we build upon these previous findings to show that periodic amplification of synaptically triggered Ca2+ transients by calcium-induced calcium release (CICR) and subsequent ICAN activation provides a plausible mechanism that can produce the observed conversion of burstlets into bursts and can explain diverse experimental findings associated with this process. Altogether, our modeling work provides the first mechanistic basis for the conceptual framework of burstlet theory and the experimental observations that this theory seeks to address.

National Science Foundation DMS1951095.

Role of $Na_v$ 1.1 and $Na_v$ 1.6 Sodium Channels in Mediating Inspiratory Rhythm Generation and Gasping: Implications for Respiratory Dysfunction in Epilepsy

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Epilepsy affects more than 65 million people worldwide, making it one of the most common chronic neurological disorders. Patients with epilepsy have a higher risk of sudden death (SUDEP). A growing body of evidence now strongly indicates that a substantial subset of SUDEP is due to seizure-induced respiratory arrest. The inspiratory rhythm relies on the interplay between synaptic and intrinsic properties. Among the intrinsic properties, the persistent sodium current plays an important role in sustaining the bursting pacemaker activity in preBötC neurons. The persistent sodium current is, in part, mediated by the voltage-gated sodium channel Nav 1.6. However, the preBötC expresses different subunits. Here, we investigated the role of Nav 1.1 and Nav 1.6 using different mice models, including a Dravet Syndrome mouse model. In vivo, Scn1aR1407X mice have a slower respiratory frequency compared to wild-type and Scn8a null mice (Scn8amed/+). *In vitro*, the Scn1aR1407X mice have a slower rhythm compare to wild-type and Scn8amed/+ mice. In anoxia, gasping is also affected after the blockade of Nav 1.1 and Nav 1.6 sodium channels. Taken together, Nav 1.1 and Nav 1.6 may mediate the persistent sodium current in preBötC neurons and contribute to the inspiratory rhythm generation in normoxia and anoxia.

Locus Coeruleus mediated tolerance to opioid-induced respiratory depression in repeat opioid use

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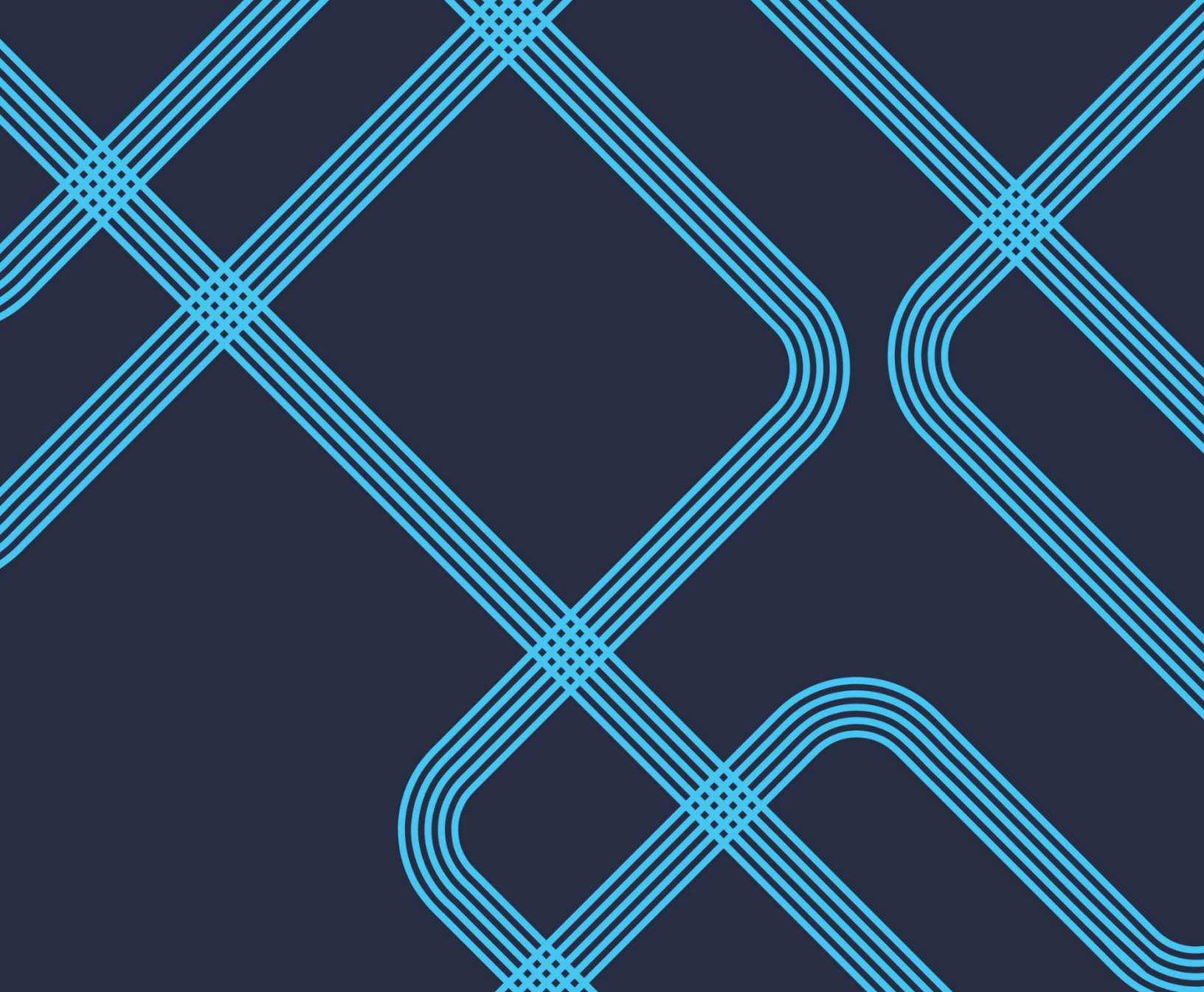
Despite the insights gained in understanding the mechanisms of opioid-induced respiratory depression (OIRD), limited progress has been made toward resolving how repeat opioid use (ROU) impacts the control of breathing. The locus coeruleus (LC) is a principal source of noradrenergic neuromodulation that can influence breathing. It is also involved with reward anticipation that is associated with opioid use. Using fiber photometry with whole-body plethysmography in a mouse model of repeat fentanyl administration, we tested the hypothesis that ROU leads to context-based OIRD tolerance supported by LC activity. ROU led to the development of adaptive ventilatory response characterized by increased inspiratory drive and an improved VO2-to-breathing relationship during OIRD. While the adaptive response coincided with enhanced LC activity coupled to breathing, changing the context of fentanyl use altered LC activity and reduced the tolerance developed with ROU. Furthermore, optogenetic LC activation minimized OIRD magnitude in fentanyl-naïve subjects. These findings illustrate the importance of the LC in facilitating OIRD tolerance from learned associations to ROU. Failure to establish context associations and LC activity may contribute to the unpredictable nature of overdose and death among chronic opioid users.

Support: R01HL163965, R01DA057767, R01HL169679



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