Quelling the neuroinflammatory cytokine storm with Bioelectrics

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Acknowledgements

Loma Linda

- Jonathan Abdala, Rhaya Johnson, Vadim Gospodarev, Brad Cacho, Tyler Hillman, Lianne Pak, Lorraine Siebold, Billy Wang
- Jane Huang, Jovicarole Raya, Beau Young, Earl Lee, Abby Dobbins, Melisa Custer, Noah Osman, Kathleen Conner (CSUSB, UCR)
- Michael Morikone (CSUSB, U Nebraska)
- Arlin Blood, Sean Wilson (CPB)
- Stephen Ashwal (LLU Peds Neurology)

CWRU

- Peter MacFarlane, Cathy Mayer, Abdelmadjid Belkadi, Julie Di Fiore, Kannan Balan, Prabha Kc
- Richard Martin
- Ken Loparo, TED Dick, Frank Jacono,
- Michael DeGeorgia
- Peter Thomas, Casey Diekman (NJIT)

Funding: R01-HL081622 (NHLBI),
R03-HD064380 (NICHD), R21-HD092941-01 (NICHD),
NNH16ZTT001N-FG (NASA)
Outline

‣ Using *neonatal rodent models* to understand premature breathing patterns in humans

‣ Understanding how neuroinflammation alters brainstem neural networks and modulates autonomic control circuits

‣ Using *vagus nerve stimulation* (VNS) to prevent central neuroinflammation
Generation and modulation of Breathing Rhythm

- Immune modulation of cardiorespiratory control
- Analysis and modeling of variability in breathing
Premature babies and respiratory control

- In the U.S. and U.K., 8–18% of all births (>500,000 babies/year!) are premature (< 37 weeks gestational age).

- Respiratory problems are common, particularly infant respiratory distress syndrome (IRDS) and chronic lung disease (bronchopulmonary dysplasia).

- Neurological problems include apnea of prematurity, hypoxic-ischemic encephalopathy (HIE), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH).

- Premature babies are susceptible to infection, including sepsis, pneumonia, and urinary tract infection.

- Infection frequently manifests as respiratory perturbations—like apnea, tachypnea, and/or periodic breathing.
Inductance plethysmography—apnea of prematurity
Inductance plethysmography—periodic breathing
Respiratory Reflexes and Neonatal Apnea

IMMATURITY

- enhanced inhibitory reflexes
- altered hypercapnic responses
- hypoxic depression

APNEA
Generation and modulation of Breathing Rhythm
Breathing rhythm originates in the medulla oblongata

preBötzinger Complex!
The Respiratory Neural Circuit in vitro

Patch-Clamp Recording from Optically-Identified Respiratory Premotoneurons

Morphology of inspiratory-related neurons in the brainstem

Koizumi, et al., 2008, J Neurosci
Maturation affects firing pattern and connectivity

Smith et al. Resp Physiol, 2000
Regions involved in breathing control

Vagus Nerve
(Afferent inputs from lungs, heart, etc)
This is (sort of!) how apnea of prematurity is treated....
Immune modulation of cardiorespiratory control
Inflammation and respiratory control

• Perinatal inflammation/infection is a major source of morbidity and mortality in the newborn population.

• Neonatal infection can be acquired by aspiration of infected amniotic fluid either *intra-uterine* or during vaginal delivery, resulting in systemic infection in 1 – 4% of neonates born to mothers with chorioamnionitis.

• Infection frequently manifests as respiratory perturbations—like *apnea, tachypnea, or periodic breathing*—that are challenging to treat.
P11 rats or mice (approximately full-term)
“Pro-inflammatory” Cytokine cascade

**Peripheral**
- **LPS**
- **pneumococci**
- **ATP (P2X7Rs)**
- **TLR 2/4**
- **MAP-KK**

**Central**
- **pro-IL-1β**
- **CASPASE 1**
- **IL-1β**
- **COX-2**
- **PGE₂**
- **Arachidonic Acid**
- **IL-6, TNFα**

**BBB**
Why these cytokines?

• **Interleukin-1β (IL-1β):** First described in 1972, this cytokine is an important *early* mediator of the inflammatory response and invokes cell proliferation, differentiation, and apoptosis.

• **Interleukin-6 (IL-6):** An interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory *myokine*.

• **Tumor necrosis factor α (TNFα):** Discovered in the late 60s/early 70s. Another acute phase inflammatory cytokine. Also known to modulate synaptic activity in the CNS.

All three of these are early, acute phase pro-inflammatory cytokines that initiate the immune response. They are considered “classic” pro-inflammatory cytokines—which is why we have focused on them.

*They are also trophic factors during development!*
Methods – *in vivo* rats (postnatal day 10–11)

- Ketamine/xylazine or isoflurane
- LPS @ 0.5 – 1.0 µg/g or Saline
- *In vivo* (monitor for 2 to 4 hours)
- *In vitro*/staining (harvest after 4 hours)

Cathy Mayer and Brooke Boyer
Inflammation alters chemoreflexes

Expiratory time (Te), is reduced in Control vs. LPS-exposed rats

Acute inflammatory up-regulation: The canonical model

Neuron
Acute inflammatory up-regulation: The canonical model
Acute inflammatory up-regulation: The canonical model
Acute inflammatory up-regulation: The canonical model
Acute inflammatory up-regulation: The canonical model

Neuron

microglia

astrocyte
Acute inflammatory up-regulation: Our “new” model

Hypothesis

- Inflammation-induced cytokine release signals the production of proinflammatory cytokines in the brainstem and this alters signaling throughout the CNS.
  - LPS induces a cascade of cytokine (IL-1β, IL-6, TNFα and others) release from neurons and microglia.
  - These cytokines modulate processing of vagal afferent input at the nTS, rhythm-generation at the pBC, and motor output at the XII nucleus.
  - Release of prostaglandins (e.g. PGE₂) then changes synaptic processing at this first-order input to the CNS.
Cytokines and purines modify synaptic transmission *normally*
LPS-induced IL-1β message in respiratory regions of brainstem

IL-1β mRNA expression increased in respiratory areas

IL-1β mRNA is expressed in XII motoneurons

Iba-1 (activated microglia) is greater in XII after LPS

A

![Bar graph showing the number of XII cells expressing Iba-1 protein](image)

B

Saline

C

Negative Control

D

LPS

50 μm

Microglia appear NOT to express IL-1β

Hypoxia alters IL-1β signaling in the brainstem breathing circuitry

Acute inflammation alters inflammatory drive in the CNS

IL-1β → IL-1R → AA → COX-2 → PGH₂ → mPGES-1 → PGE₂ → IL-1R

Hypoxia → Carotid body

Blood → BBB

RVLM → preBötC → pFRG → NTS → Brainstem

Lungs → Diaphragm

Respiratory Depression!!

Vagus & IX → Airways/Lung
Changes in nTS neural dynamics after inflammation/lung injury

Horizontal slice preparation

Stimulating Electrode

Tractus Solitarii

Recording Pipette

Getsy et al. Resp Physiol Neurobiol. 2019
Changes in nTS neural dynamics after inflammation/lung injury

Getsy et al. Resp Physiol Neurobiol, 2019
nTS neurons have smaller sEPSCs after lung injury

A

Saline

Bleo

B

Cumulative Probability

Amplitude (pA)

0.0 0.2 0.4 0.6 0.8 1.0

0 20 40 60 80 100

Saline

Bleo

P < 0.05

Getsy et al. Resp Physiol Neurobiol, 2019
Changes in nTS sEPSCs activity after lung injury

A

Cumulative Probability

0.0 0.2 0.4 0.6 0.8 1.0

0 500 1000 1500 2000

Interevent Interval (ms)

B

Saline

Bleo

2.5 pA

5 ms

Getsy et al. Resp Physiol Neurobiol. 2019
nTS evoked EPSCs also show reduced amplitude
PGE$_2$ alters breathing pattern *in vitro*

1μM PGE$_2$

- **Ti**
- **Te**
- **Ttot**
- **Freq**
- **burst/min**
- **area**

* = p < 0.05
# = p < 0.01

n = 8

Ken Gresham
How do cytokines alter neural activity?
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How do cytokines alter neural activity?
When CNS injury occurs, what treatment options are available and how do we assess and promote “good,” anti-inflammatory process while attenuating “bad,” pro-inflammatory responses?
Can we use something besides antibiotics, corticosteroids, or pharmacological blockade to reduce/prevent neuro-inflammation in the CNS?
The anti-inflammatory reflex

The Vagus nerve

- The vagus nerve provides extensive afferent & efferent innervation of the viscera and is a key interface between CNS circuits and the autonomic control circuitry of the brainstem.

- The vagus is a mixed autonomic nerve originating in the medulla oblongata and projects bilaterally along the neck (bundled with the carotid artery) to the esophagus before branching to innervate the viscera.

- The anatomy of the vagus and its projections have been discovered through tract tracing or gross dissection.

- The physiology of the vagus is still an area of active investigation.
The Vagus nerve

NTS = nucleus tractus solitarius
NA = nucleus ambiguus
pBC = preBötzinger Complex (rhythm generator)
Vagus Nerve Stimulation

- Inflammation stimulates the release of pro-inflammatory cytokines which activate vagal afferents and induce central neuroinflammation.

- Vagal c-fibers are implicated in this inflammatory upregulation and their first-order synapse is in the *nucleus tractus solitarius* (NTS).

- Vagal efferents are implicated in anti-inflammatory responses via the cholinergic anti-inflammatory pathway.

- We have previously shown that vagus nerve stimulation (VNS) modulates pro-inflammatory cytokine expression in the central nervous system (CNS) using high frequency stimulation.

- *However, the optimal VNS parameters to reduce inflammation are not yet known.*
Vagal nerve stimulation to “knock down” cytokine upregulation

FDA-approved clinical uses of VNS

- **Treatment of epilepsy.** In 1988, the first chronic implantable stimulator was used to treat drug-resistant epilepsy.

- VNS has been approved by the FDA since 1997 to treat partial onset seizures that are drug-resistant.

- **Treatment of depression.** Chronic or severe depression affects up to 1.5% of the general population, and many of these patients obtain little relief from pharmaceutical treatment.

- Although VNS was not originally developed to treat depression, the FDA approved VNS for the treatment of chronic or recurring depression in 2005.
Research uses of VNS

• **Sepsis.** Sepsis is a multibillion dollar health care burden typically due to systemic bacterial infection and chronic activation of the pro-inflammatory cytokine cascade. VNS is being used experimentally to quash runaway inflammation.

• **Pain management.** The applications of VNS also extends to disorders associated with chronic or intermittent bouts of pain such as fibromyalgia and migraines.

• **Cardiovascular disease.** VNS must alter cardiovascular control due to the convergence of inputs in the autonomic control centers of the brain stem, but for how long and to what extent is unknown. The descending cardiac branch of the vagus is key for normal cardiac function.
VNS and cytokines

LPS

Trachea

Cytokine response

- IL-1 β
- IL-6
- TNF α

Activates COX-2 pathway

Produces PGE2

PGE-2 induces changes in breathing

Binds EP3R receptors in RVLM pBC
VNS and cytokines

LPS

Trachea → Lungs → Diaphragm

Lungs Contain TLR4 receptors

Cytokine response:
- IL-1 β
- IL-6
- TNF α

Activates COX-2 pathway

Produces PGE2

Binds EP3R receptors in RVLM pBC

PGE-2 induces changes in breathing

Block inflammation with vagus nerve stimulation

Rat brainstem slice

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Methods

Rat (anesthetized at time 0)

LPS instillation (0.5 μg/g in 10μL saline)

2 to 4% isoflurane

(VNS Stim, 30 min)

For VNS+LPS treatment group

Treatment

Transcardial perfusion OR tissue harvest

CNS removal for IHC/mol. bio., RNaseq, electrophysiology. (Aims 1 and 2)

0 – 120 min

P10 to P12 day old rat pups

Treatments include:
PGE2, EP3 agonist/antagonist + 10% O2 (hypoxia) before and after blockers.
Methods
IL-6 and TNF$\alpha$ are reduced after VNS

So if we use “typical” clinical VNS parameters (current/frequency) we can reduce cytokine expression.

But, what are the OPTIMAL stimulation parameters to reduce inflammation?
VNS attenuates IL-1β across most frequencies

Cacho et al. submitted to Peds Research
VNS attenuates TNFα at higher stimulation frequencies

The chart shows the counts per micron (μm³ x 10⁵) for different treatment groups. The groups include SHAM, LPS ALONE, SALINE, 10 Hz, 100 Hz, 1000 Hz, and 10000 Hz. The data points are marked with asterisks indicating statistical significance: * = p ≤ 0.05, ** = p ≤ 0.01.
IL-6 is a confusing bugger in response to VNS!

Cacho et al. submitted to Peds Research
The alarmin, HMGB1, exhibits a dose-dependent decrease with VNS.

Cacho et al. submitted to *Peds Research*
Future Directions

‣ The likelihood that we will get IRB approval to implant a vagus nerve stimulator in a preterm infant is vanishingly small!

‣ Transcutaneous stimulation would allow us to stimulate non-invasively and attempt to get sufficient current to the vagus nerve and have an impact on inflammation.

‣ An even more interesting option in the clinic would be the use of transcutaneous auricular vagus nerve stimulation (aVNS) which is non-invasive and easy to use in a clinical setting.
Can we modify the method of VNS to use non-invasive stimulators?
Transcutaneous Auricular Vagus Nerve Stimulation (aVNS)

Yap JYY et al. Front Neuroscience, 2020
Transcutaneous auricular vagus stimulation

Noninvasive neuromodulation using low-level tragus stimulation significantly decreased atrial fibrillation burden and decreased tumor necrosis factor alpha levels. The potential mechanisms of this effect are shown. Also shown are the neural pathways involved in this effect. Low-level tragus stimulation targets the auricular branch of the vagus nerve, an afferent nerve (blue arrows) that relays information to central vagal projections in the brain stem. The signal undergoes processing in the brain stem and in higher centers (green arrows), which in turn provide the efferent neural signal to the heart (red arrows), which reaches the target organ through the vagus nerve.

Stavrakos S et al., JACC: Clinical Electrophysiology, 2020
aVNS stimulators

Yap JYY et al. Front Neuroscience, 2020
aVNS protocols that replicate some of our work....
Our laboratory has been focused on translational applications of developmental neurophysiology in neonates.

Intratracheal LPS stimulates IL-1β production in the brainstem (nTS, RVLM, and XII) of rodents, activating the COX2 pathway and, ultimately, releasing prostaglandins and other chemokines/cytokines that alter neural network activity.

Bioelectric stimulation may be valuable in controlling acute or chronic inflammation and, using aVNS, may be easily incorporated into current clinical practice.
Thank you for your attention!

Questions??

“T’ll pause for a moment so you can let this information sink in.”
References


References


