Quelling the neuroinflammatory cytokine storm with Bioelectrics

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Turning Points: From Healthy Cells and Systems to Neurological Disease States
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Loma Linda

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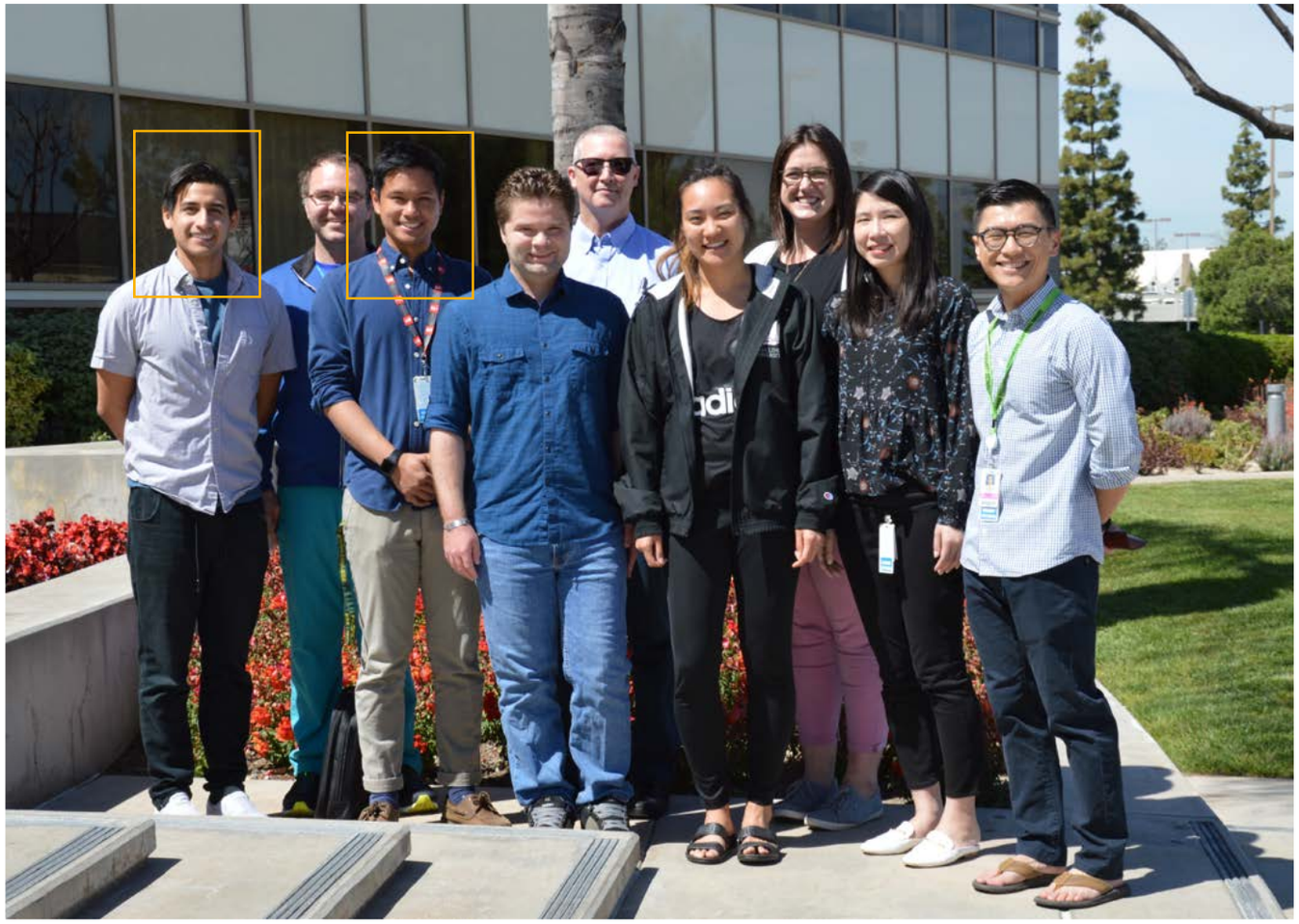
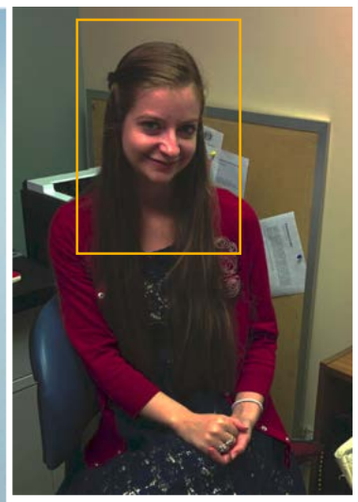
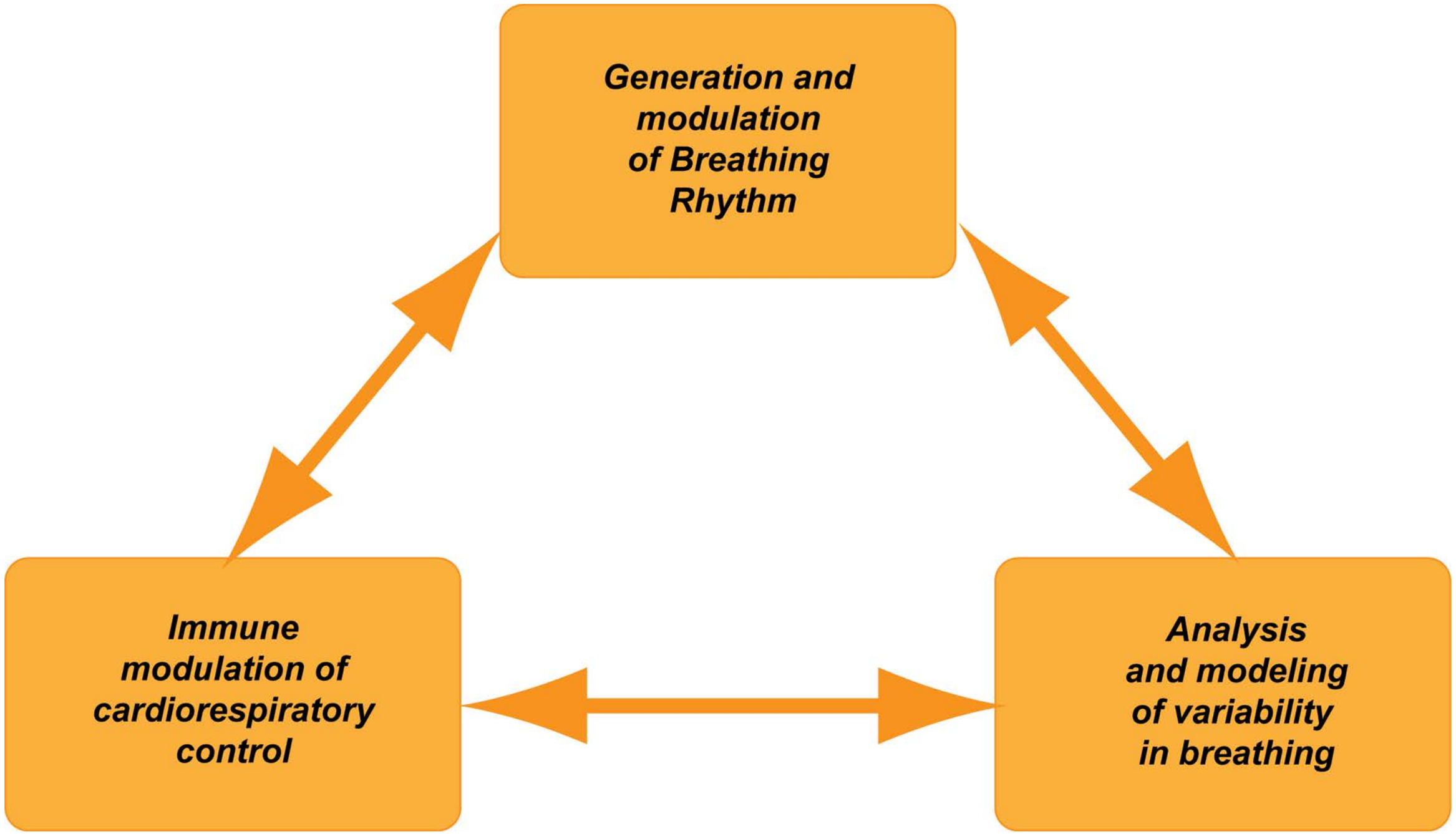




Outline

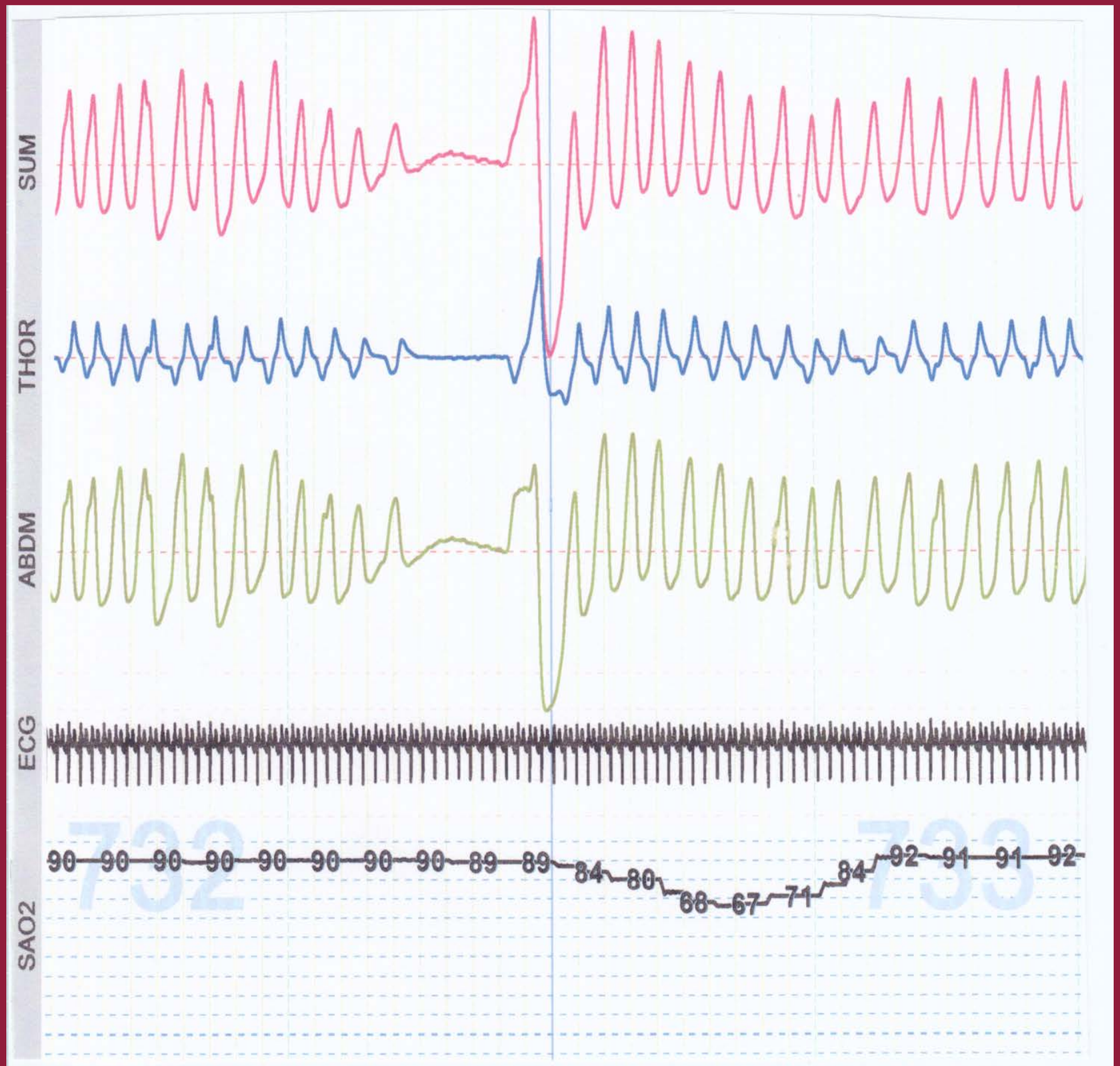
- Using *neonatal rodent models* to understand premature breathing patterns in humans
- Understanding how neuroinflammation alters brainstem neural networks and modulates autonomic control circuits
- Using *vagus nerve stimulation* (VNS) to prevent central neuroinflammation


Generation and modulation of Breathing Rhythm
Immune modulation of cardiorespiratory control
Analysis and modeling of variability in breathing

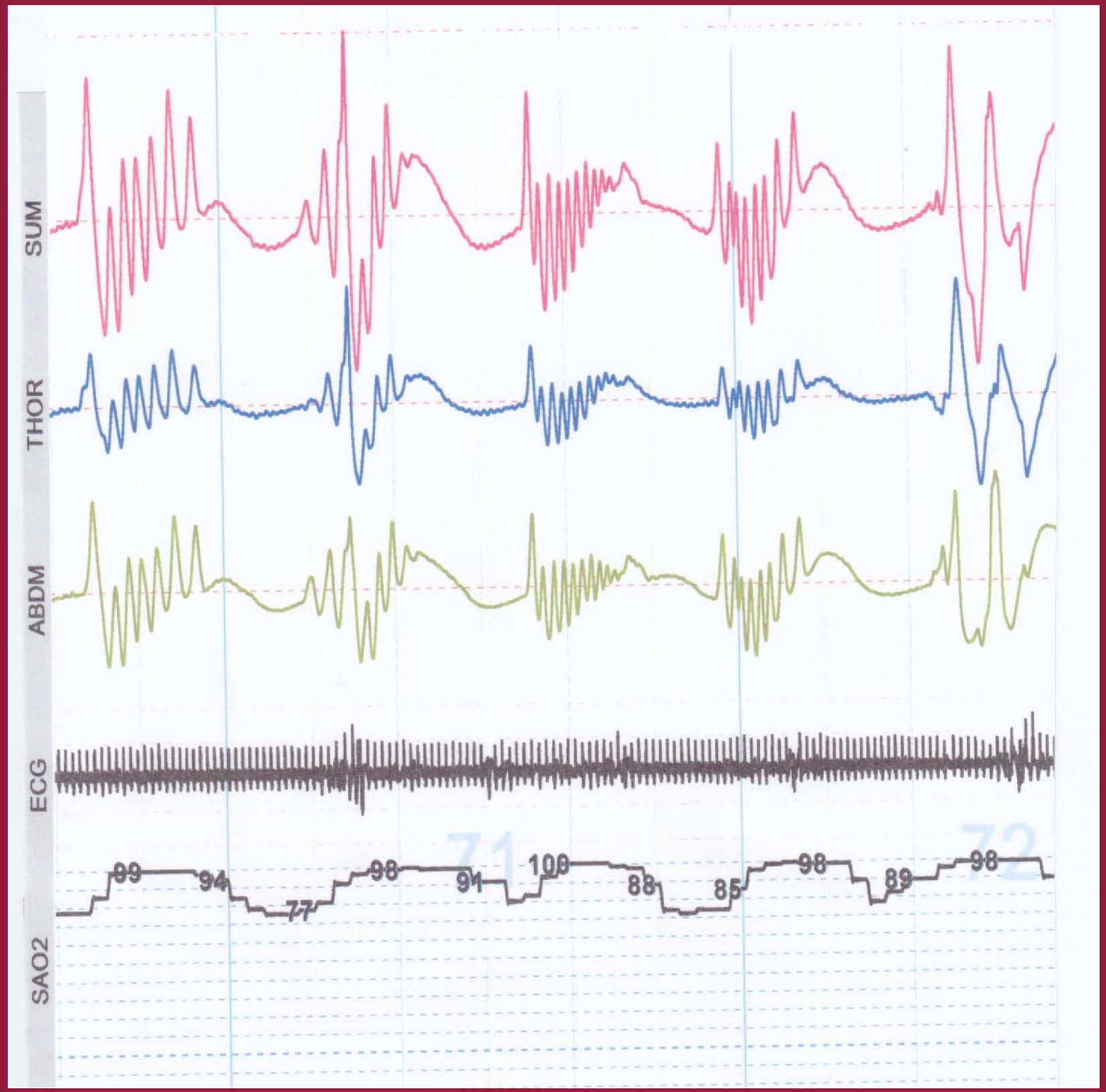
Premature babies and respiratory control

- 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***.

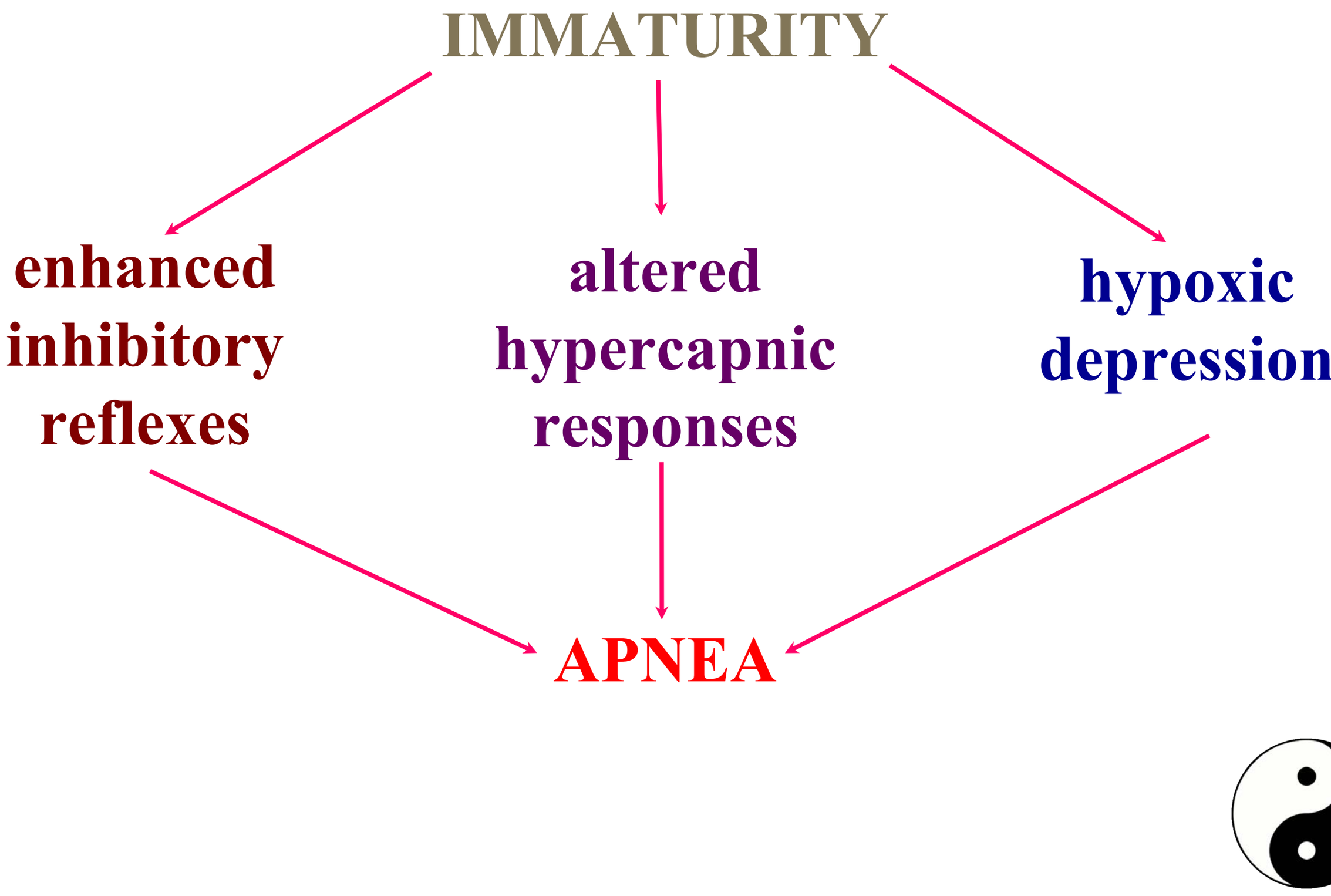
Inductance plethysmography—apnea of prematurity



Inductance plethysmography—periodic breathing

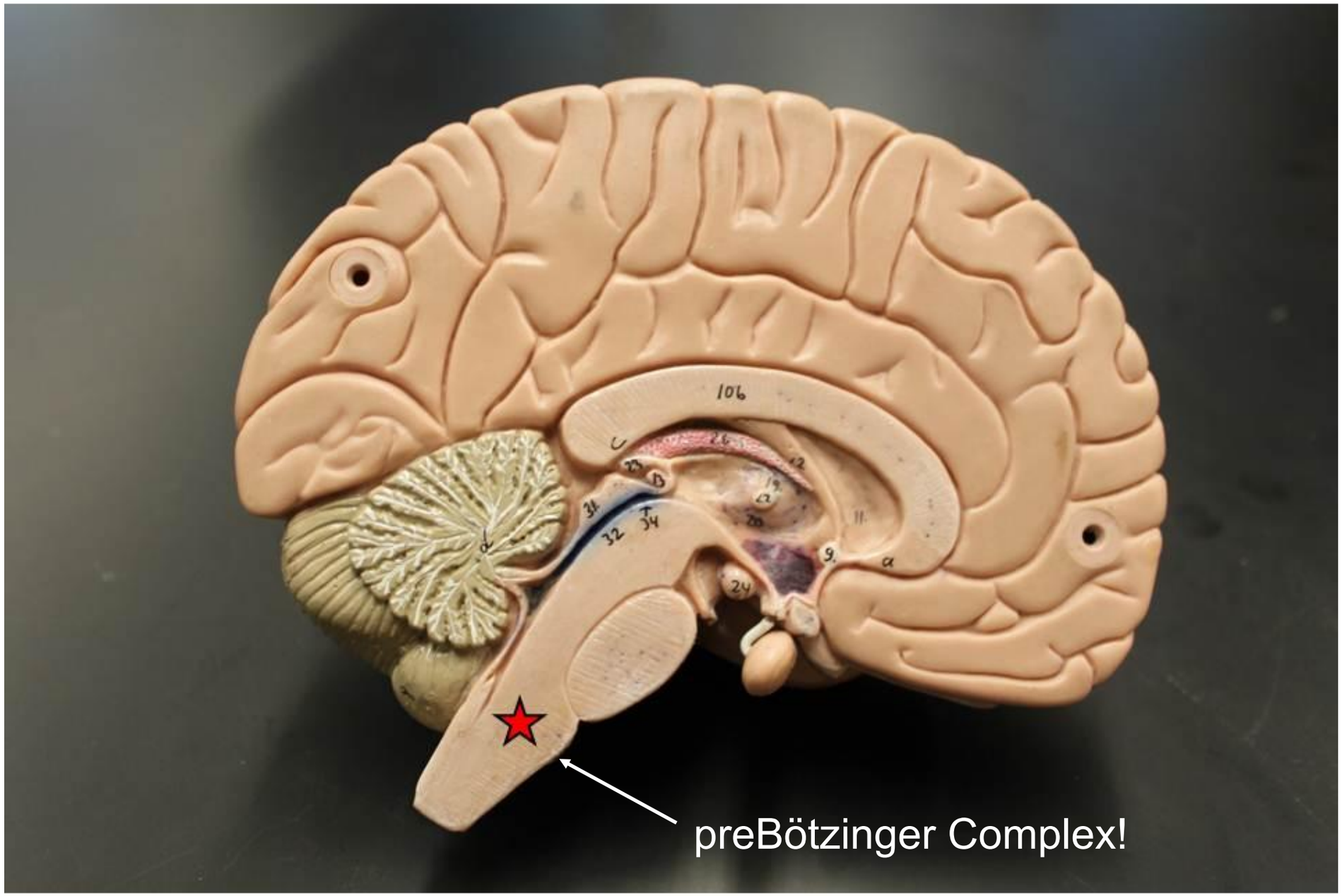


Respiratory Reflexes and Neonatal Apnea

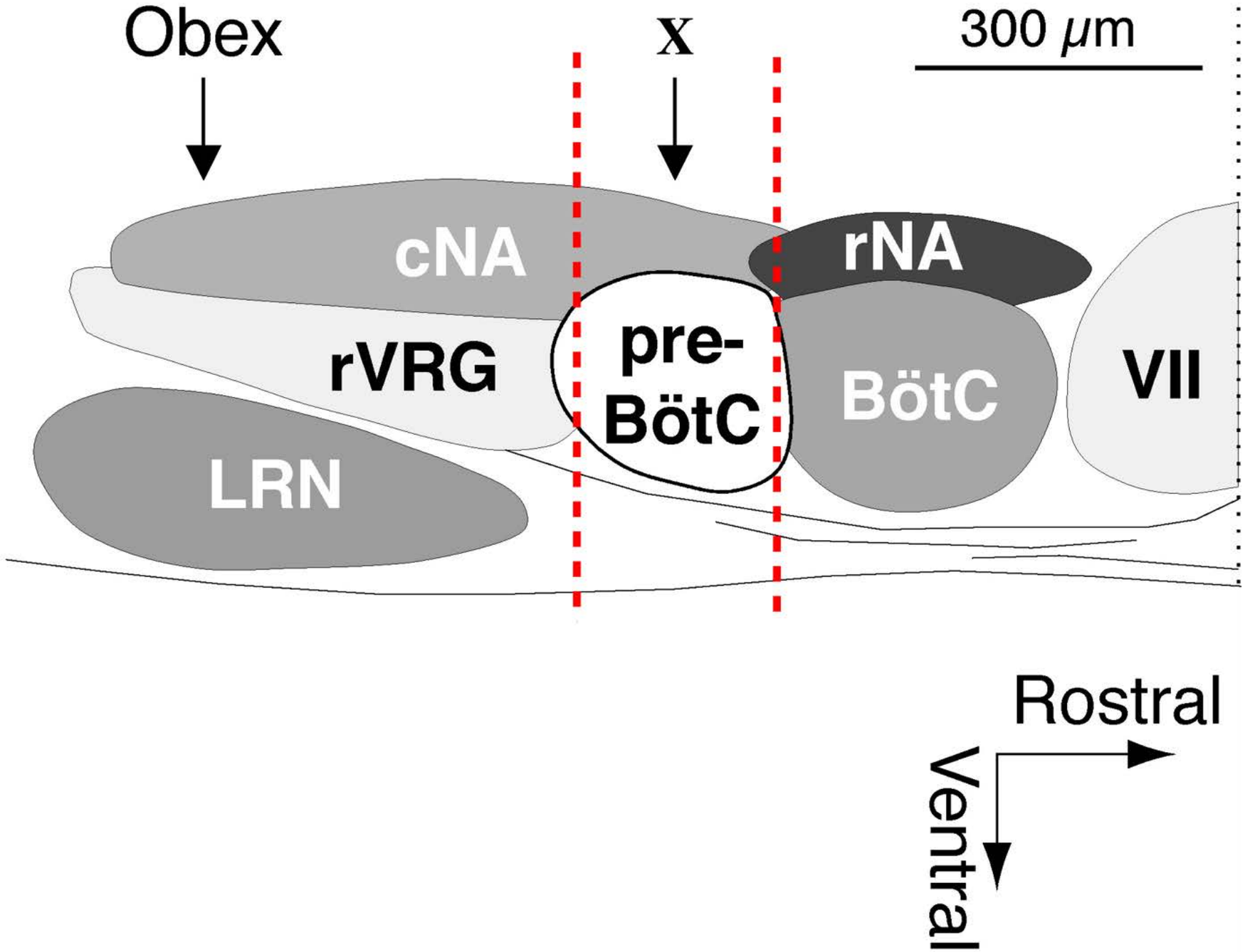


Generation and modulation of Breathing Rhythm

Breathing rhythm originates in the *medulla oblongata*

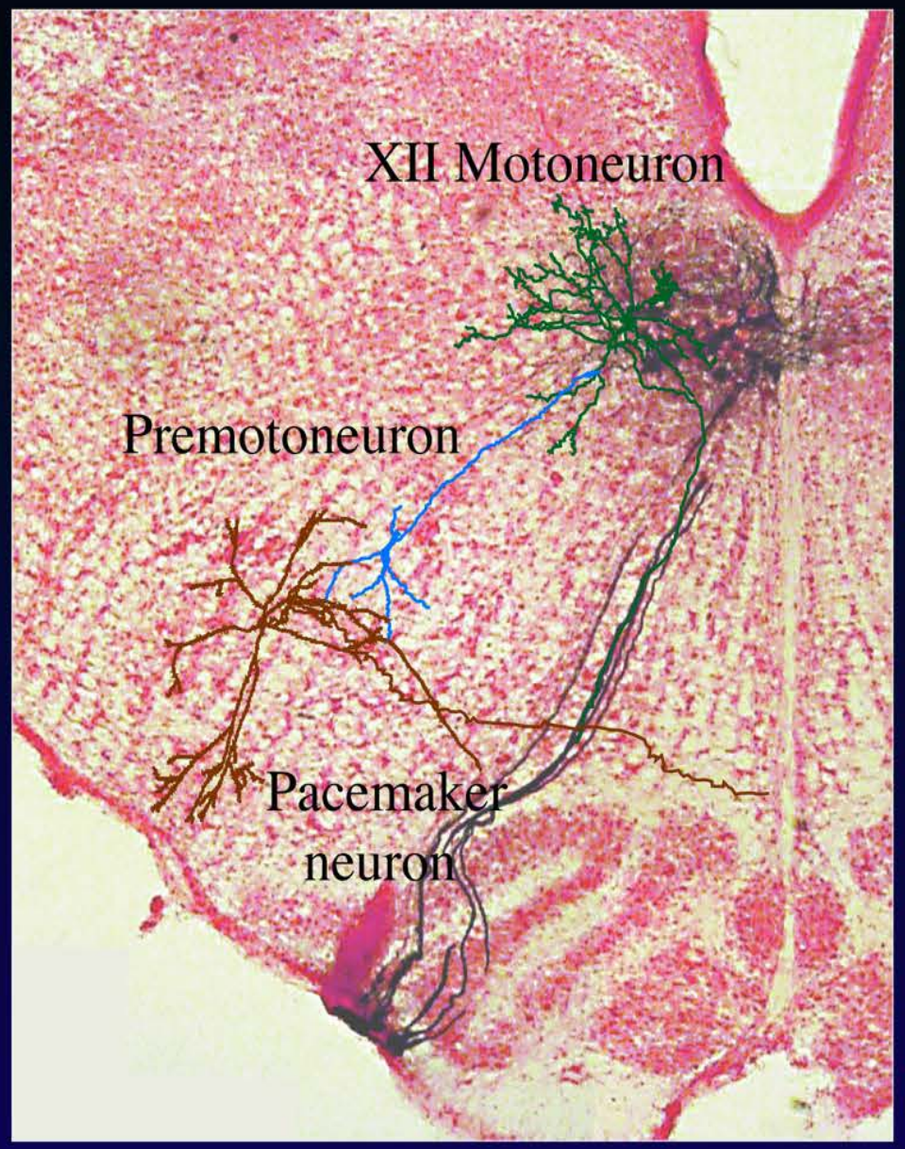


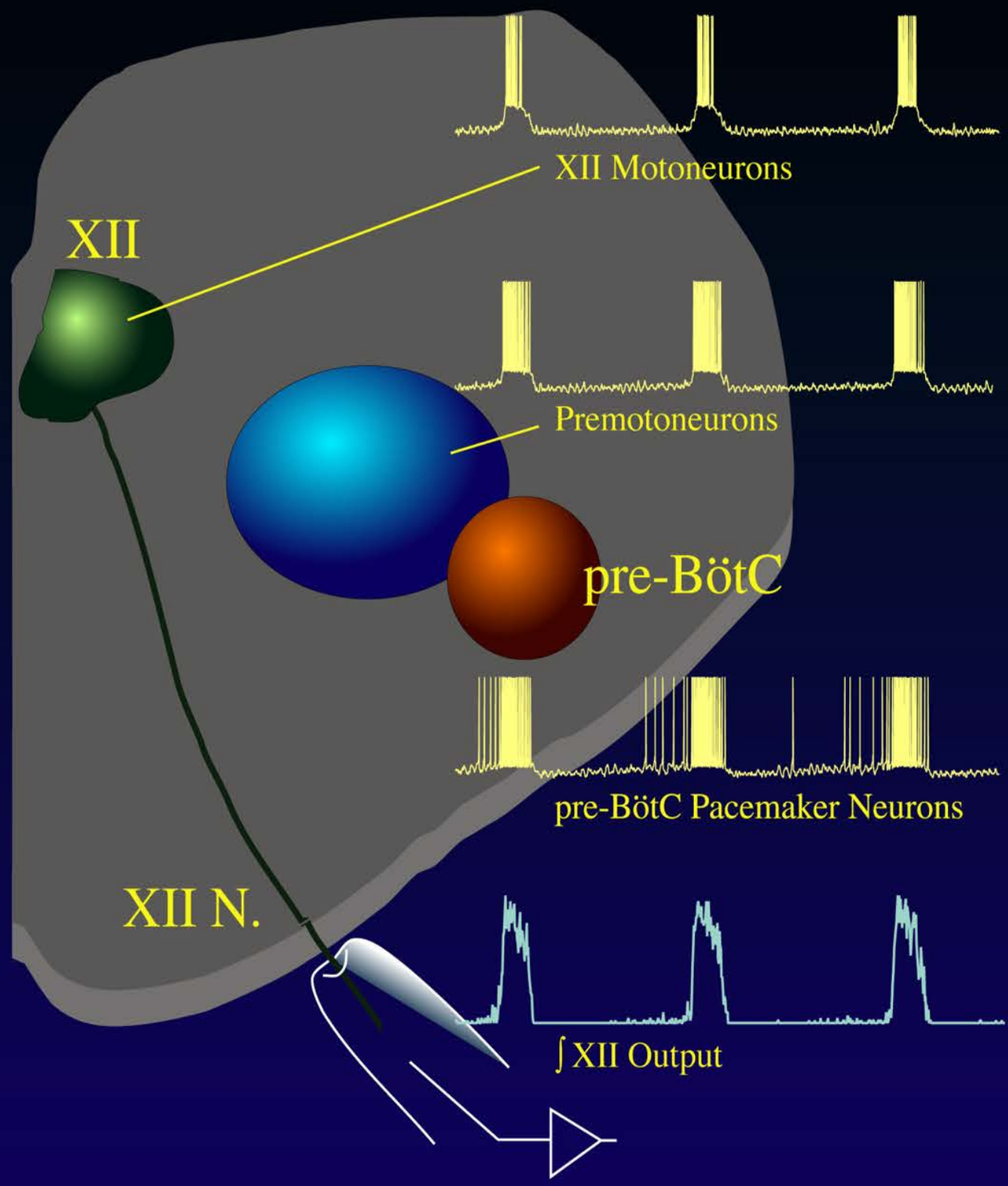
Sagittal section of brainstem



Adapted from Rekling and Feldman, *Annu Rev Physiol*. 1998;60:385-405

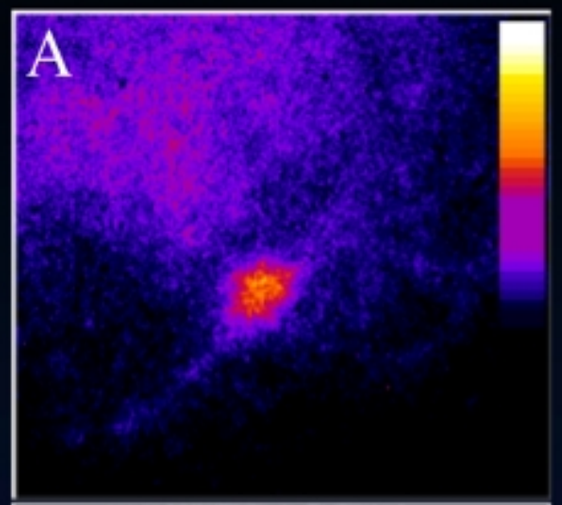
The Respiratory Neural Circuit *in vitro*

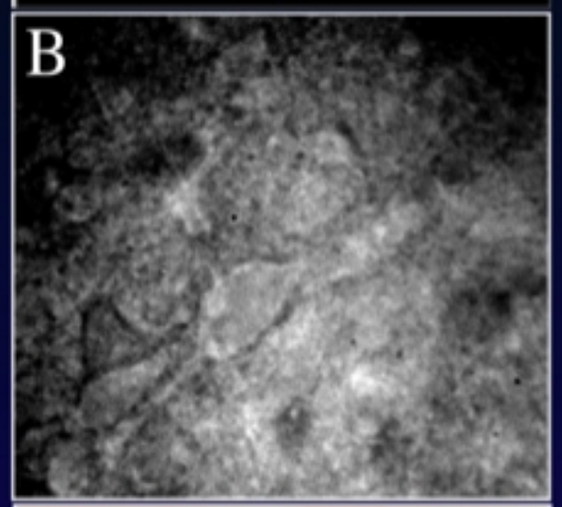


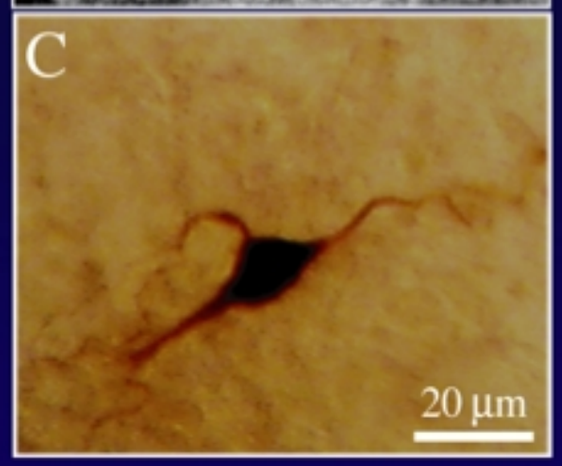


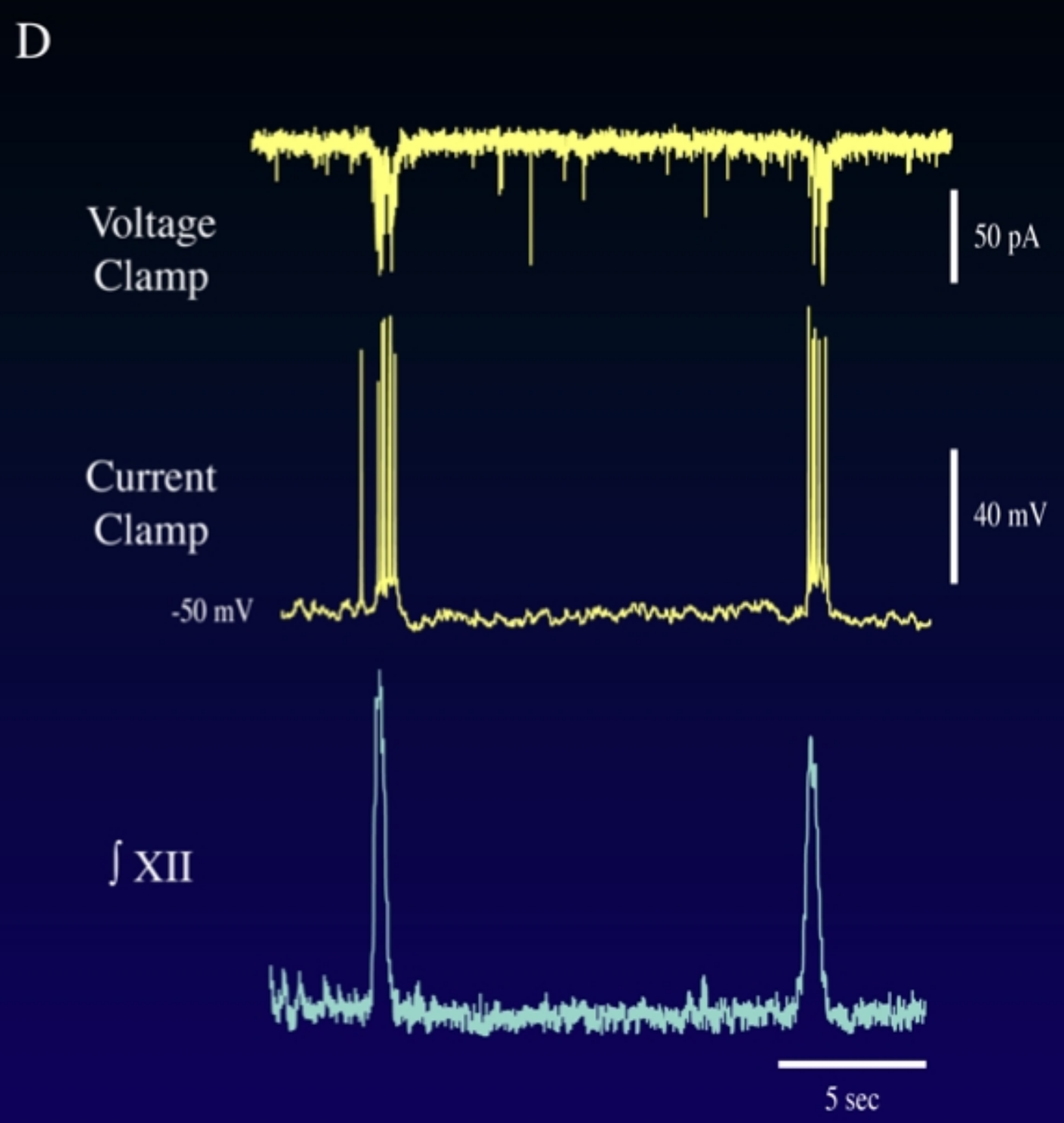
Koizumi et al. *J Neuroscience*, 2008

Patch-Clamp Recording from Optically-Identified Respiratory Premotoneurons



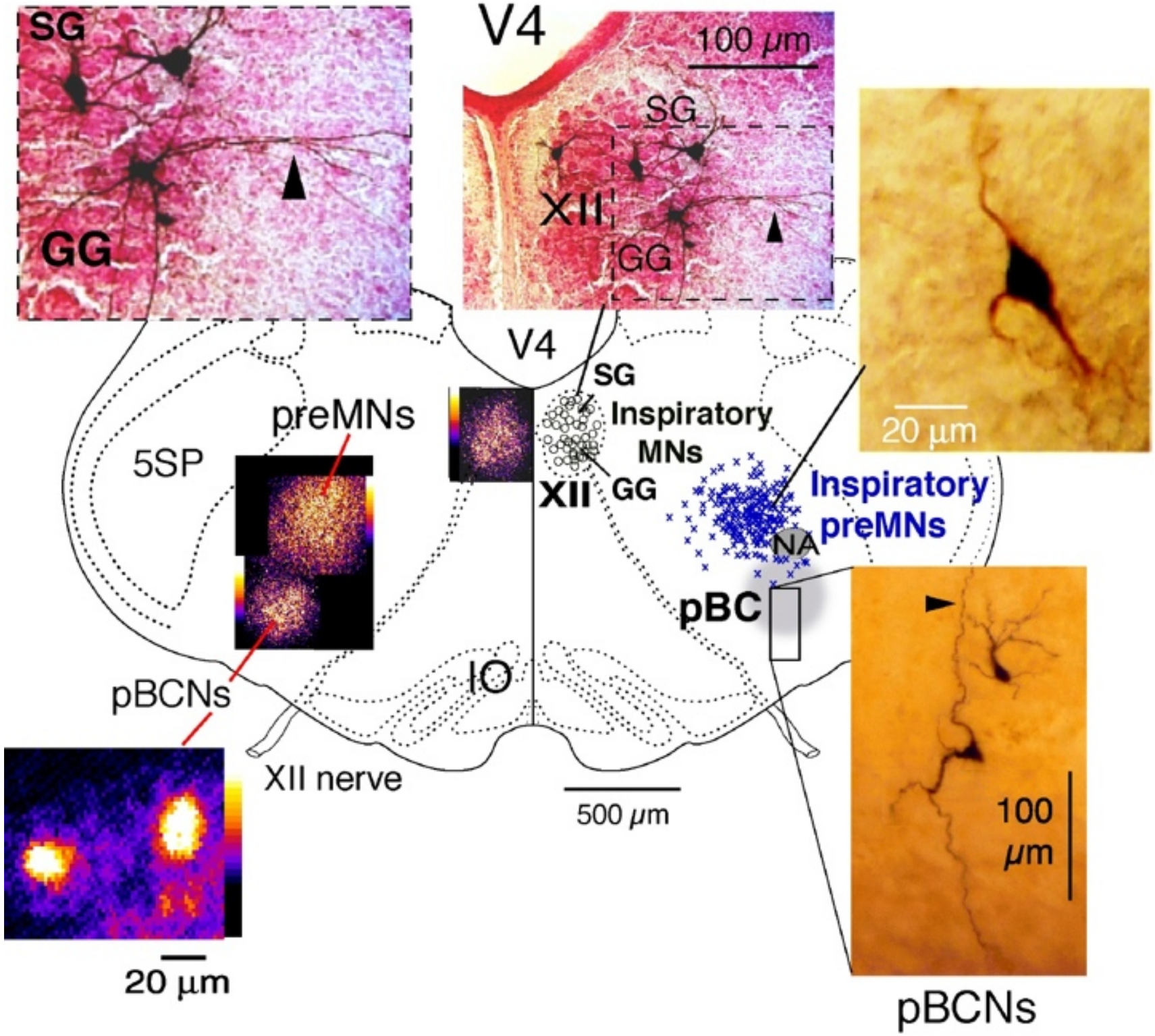






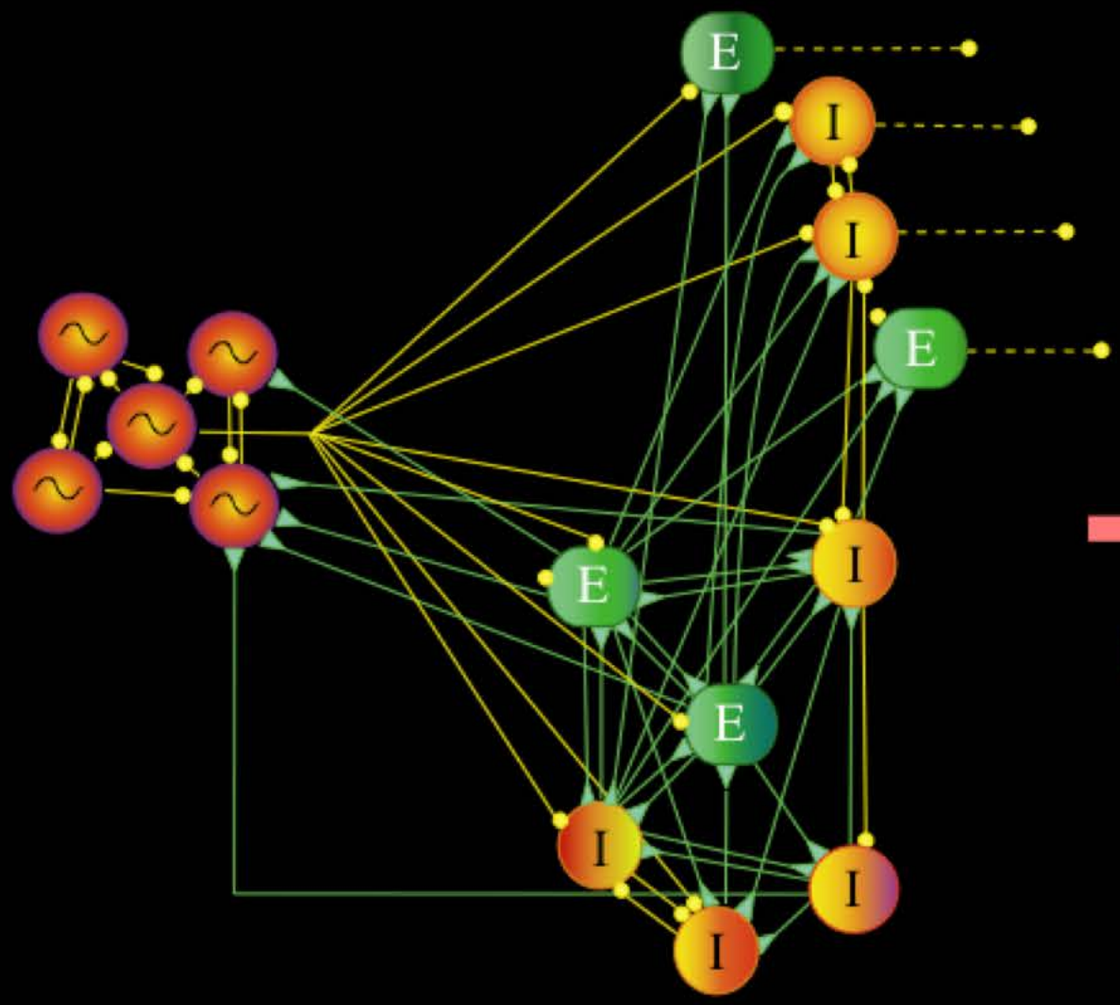
Koizumi et al. *J Neuroscience*, 2008

Morphology of inspiratory-related neurons in the brainstem

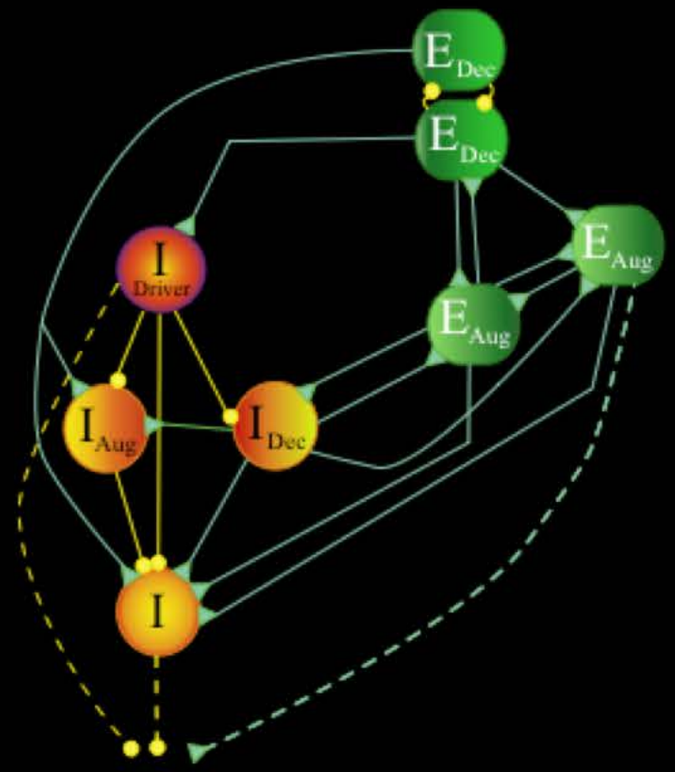


Koizumi, et al., 2008, *J Neurosci*

Maturation affects firing pattern and connectivity

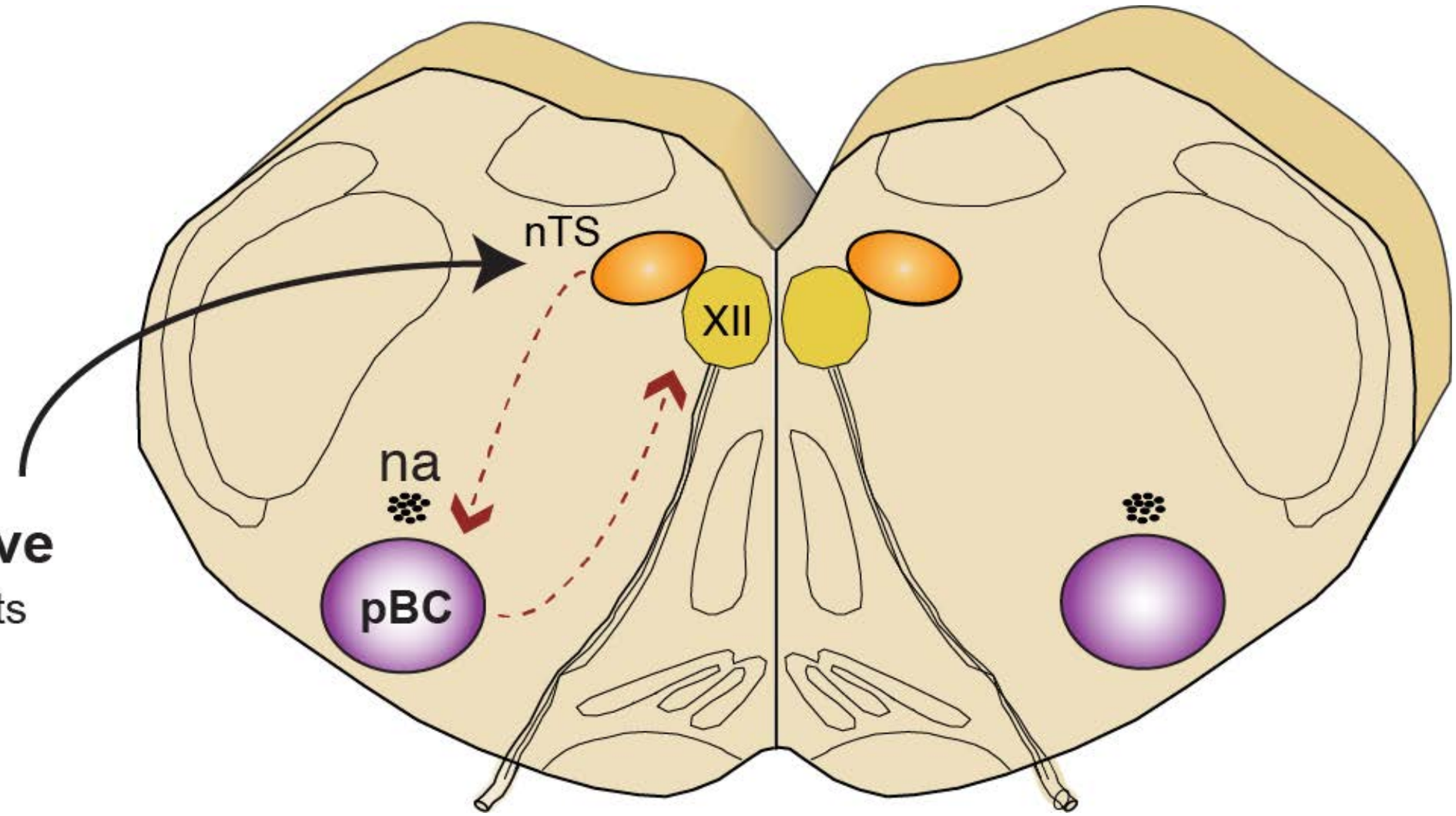


DEVELOPMENT
??



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….

http://urbanministryblog.org/wp-content/uploads/2011/01/starbucks-baby2.jpg

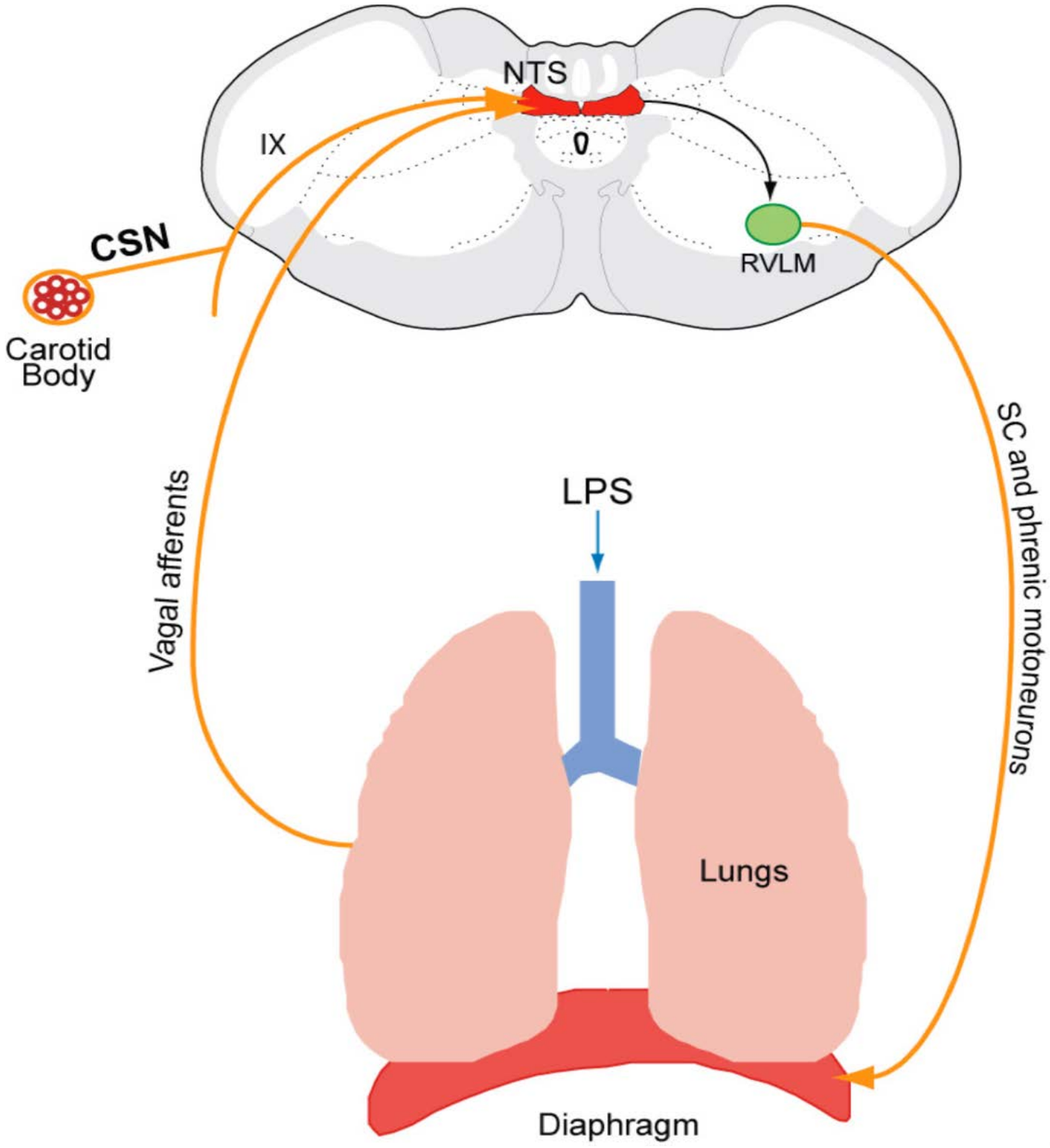
Immune modulation of cardiorespiratory control

Inflammation and respiratory control

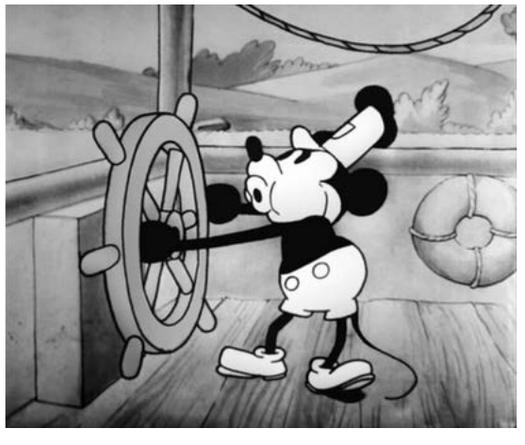
• Perinatal inflammation/infection is a major source of morbidity and mortality in the newborn population.

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

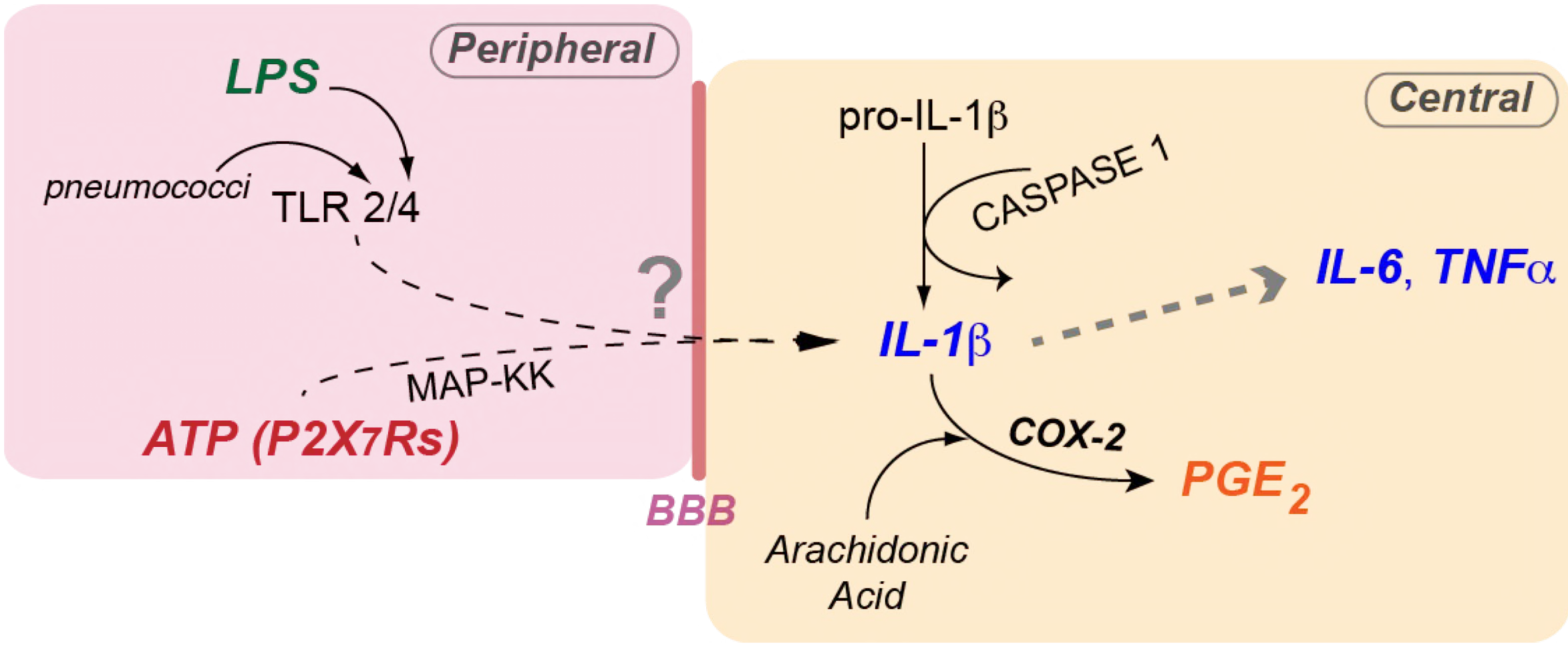
• Infection frequently manifests as respiratory perturbations—like *apnea, tachypnea*, or *periodic breathing*—that are challenging to treat.



P11 rats or mice (approximately full-term)



“Pro-inflammatory” Cytokine cascade



Why these cytokines?

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

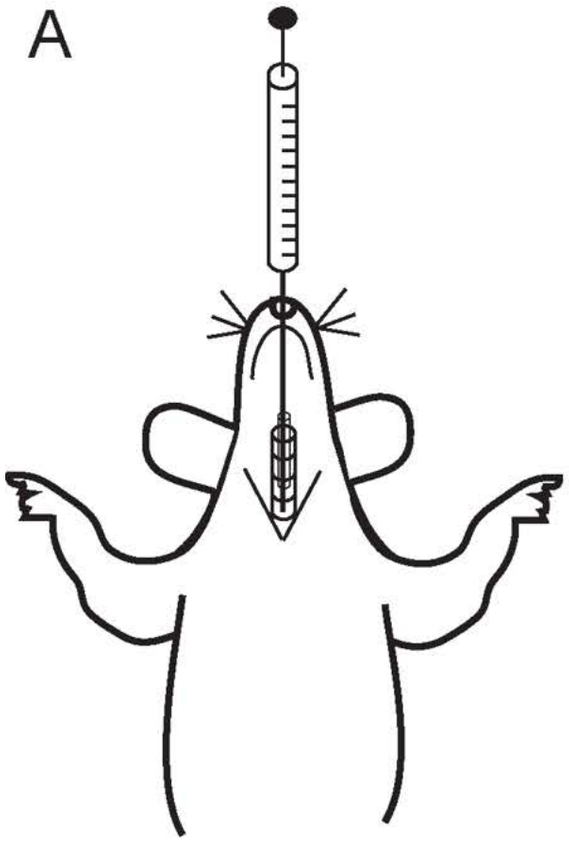
• **Interleukin-6** (IL-6): An interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory *myokine*.

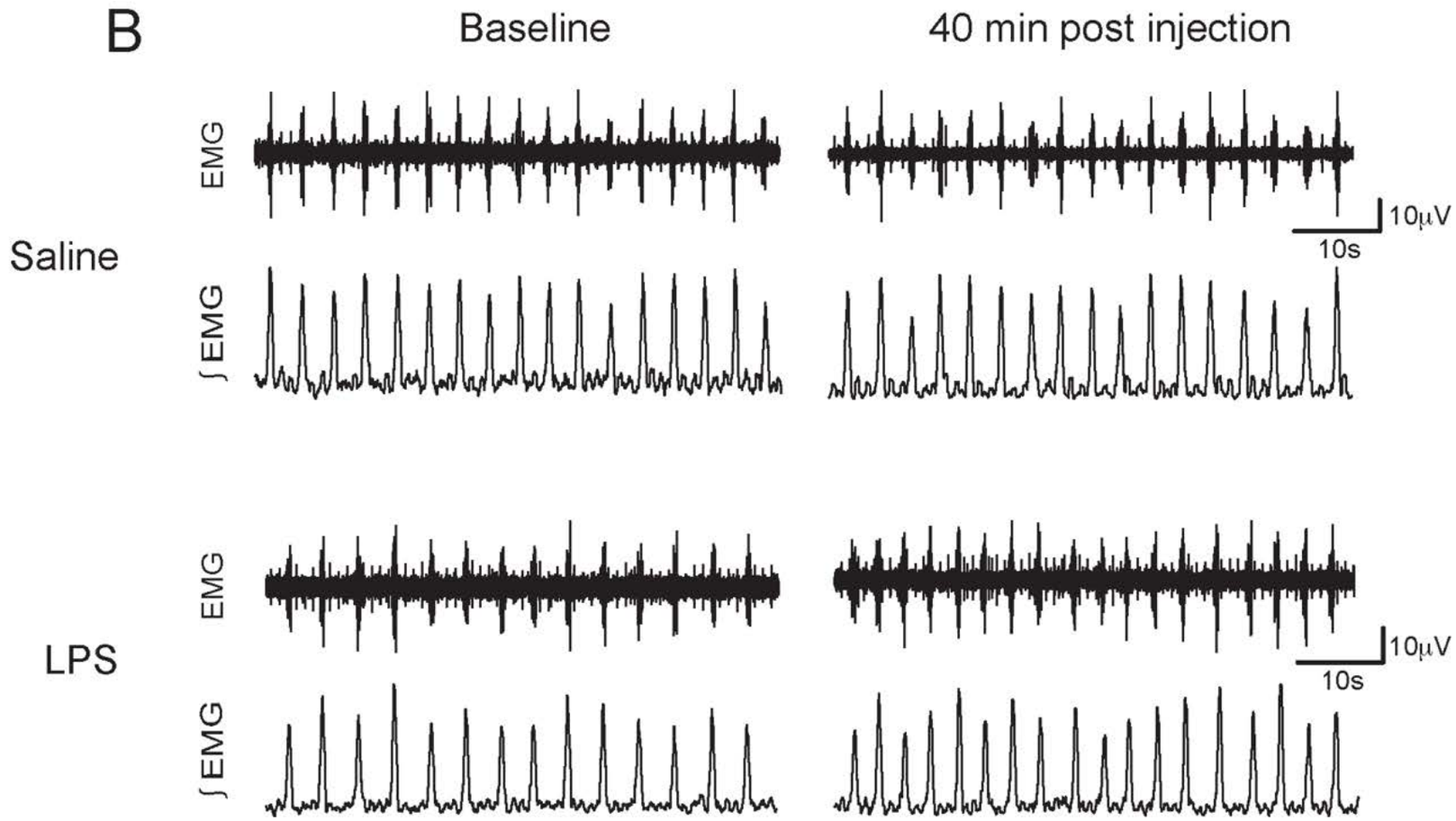
• **Tumor necrosis factor** α (TNFα): Discovered in the late 60s/early 70s. Another acute phase inflammatory cytokine. Also known to modulate synaptic activity in the CNS.

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)

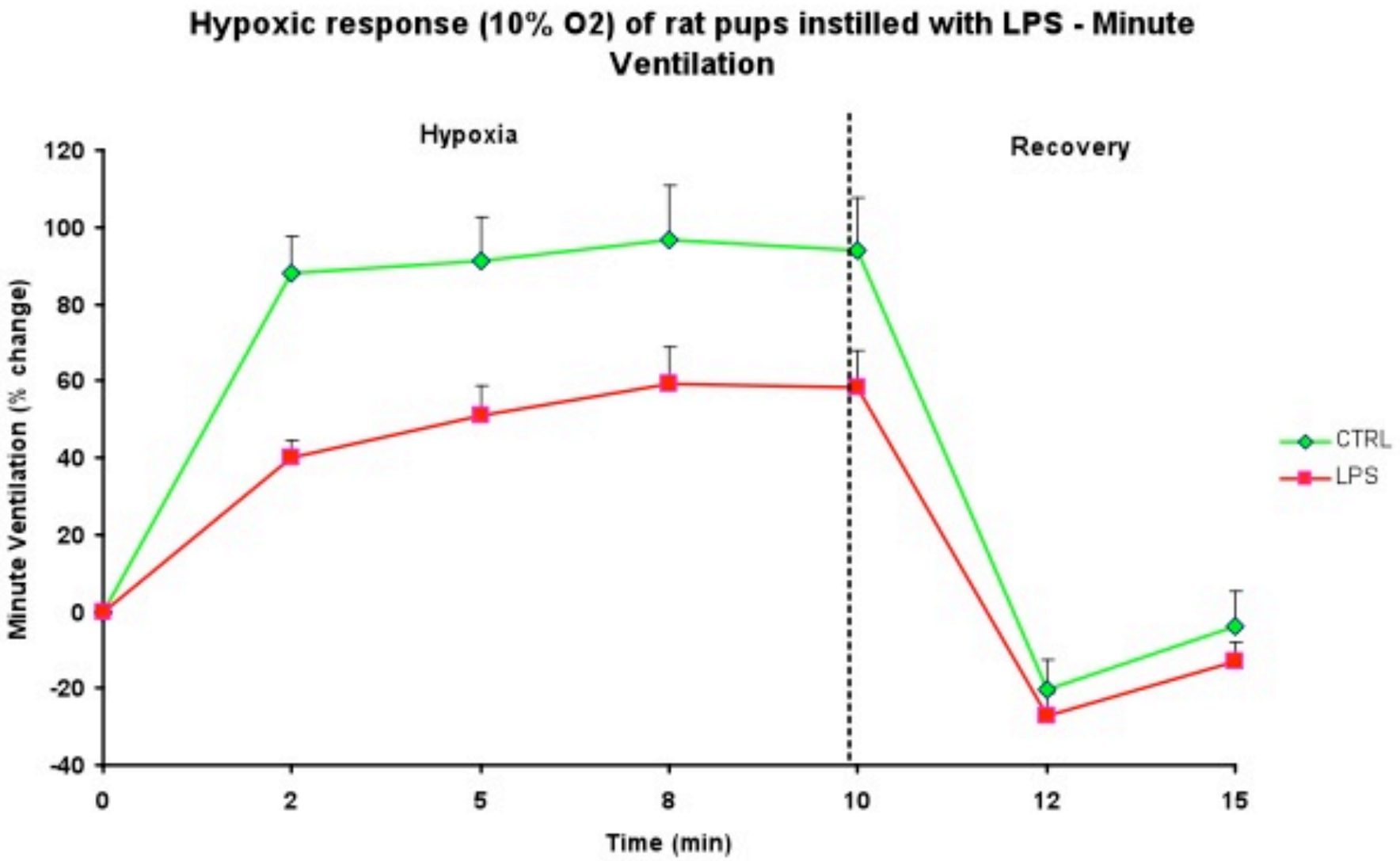




- Ketamine/xylazine or isoflurane
- LPS @ 0.5 – 1.0 μg/g *or* Saline
- *In vivo* (monitor for 2 to 4 hours
- *In vitro*/staining (harvest after 4 hours)

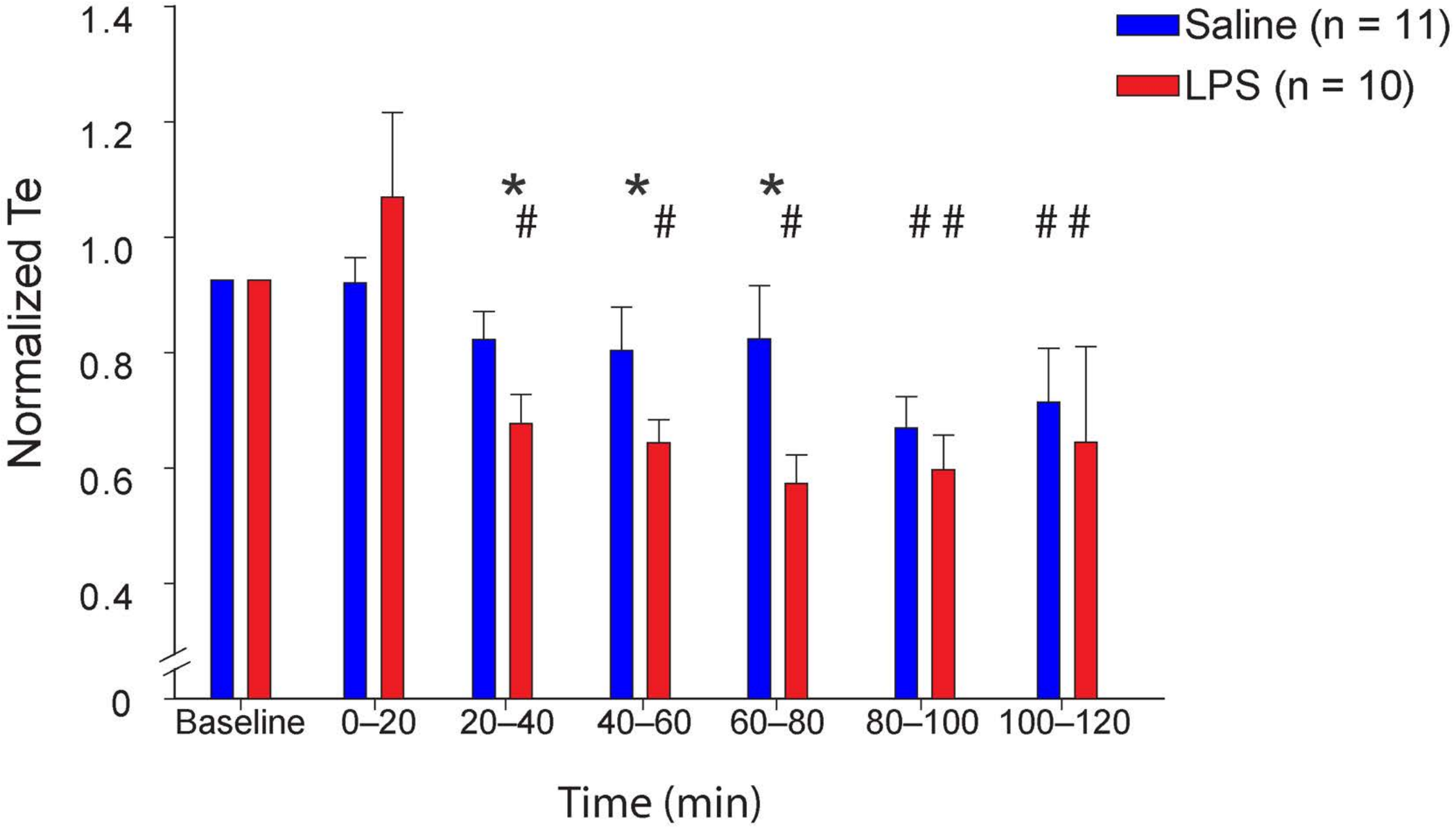
Cathy Mayer and Brooke Boyer

Inflammation alters chemoreflexes



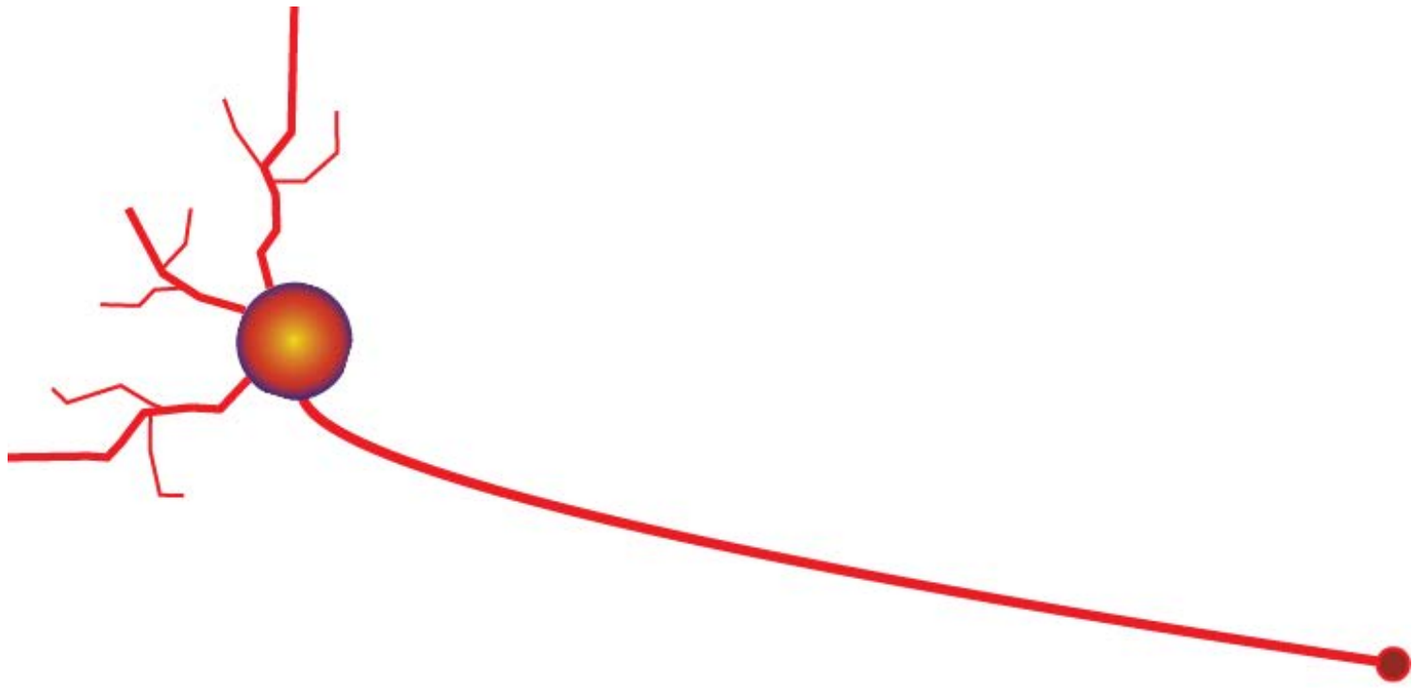
Balan et al., *Resp. Physiol. Neuriobiol*., 2011

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



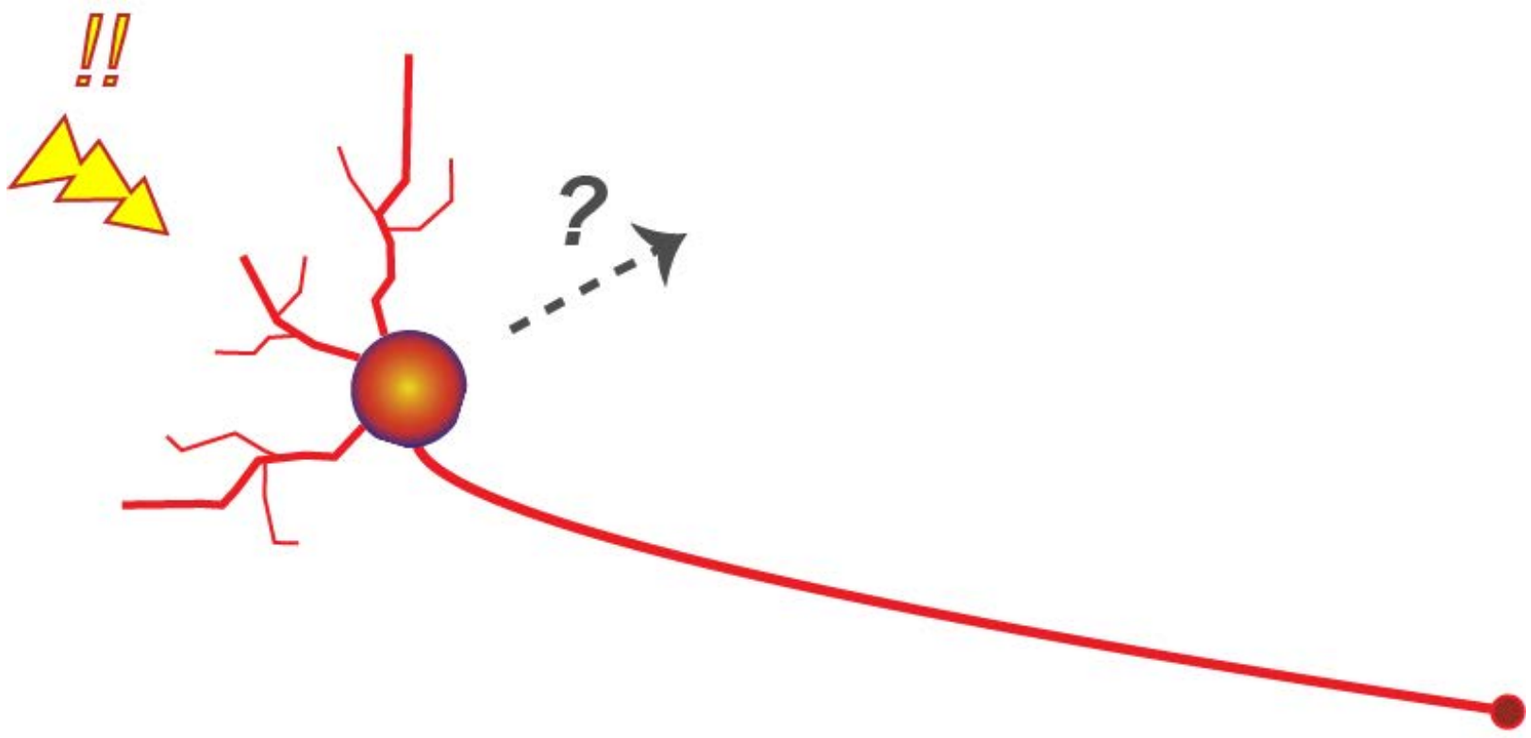
Gresham et al. *Resp Physiol & Neurobiol*, 2011

Acute inflammatory up-regulation: The canonical model



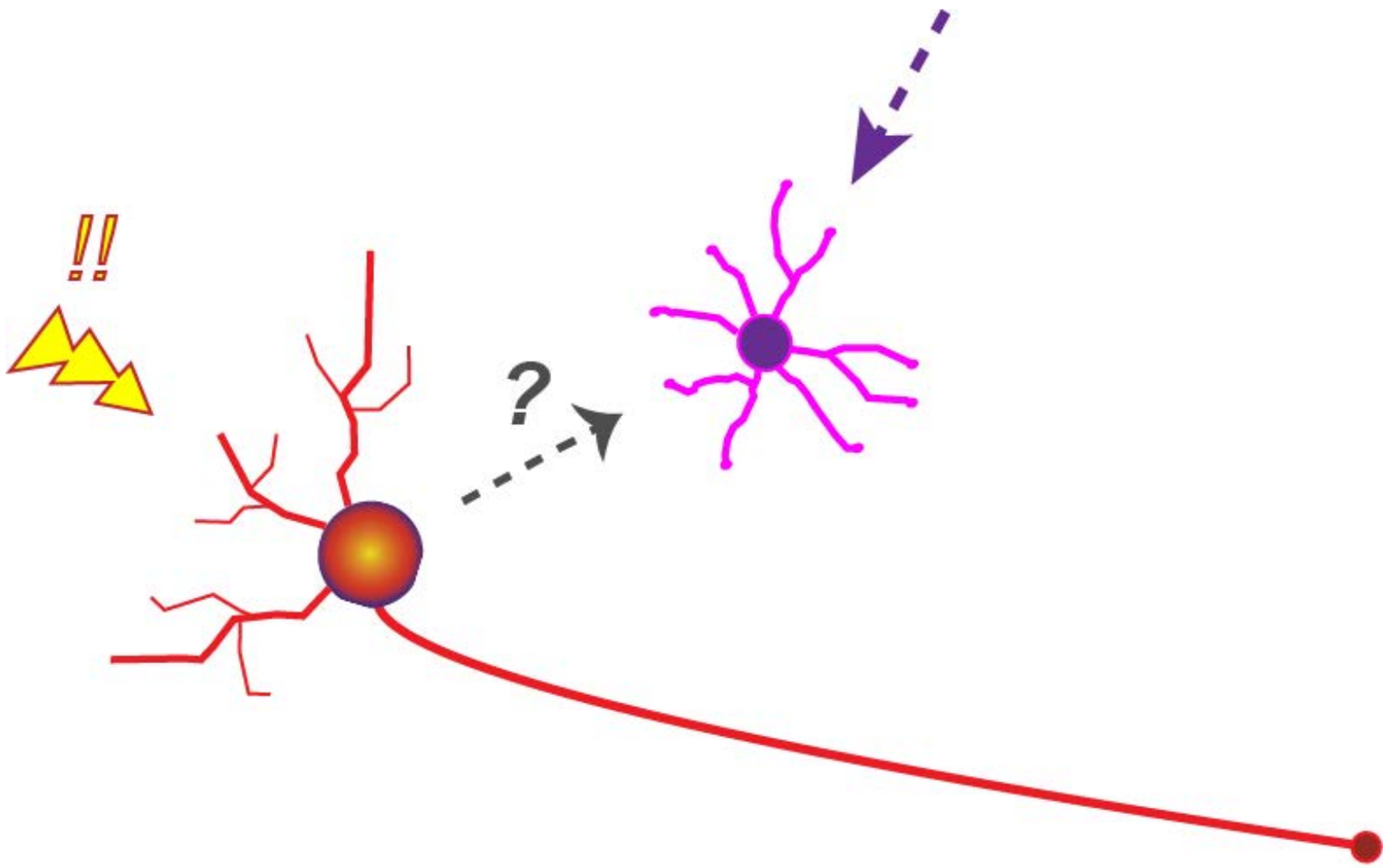
Neuron

Acute inflammatory up-regulation: The canonical model

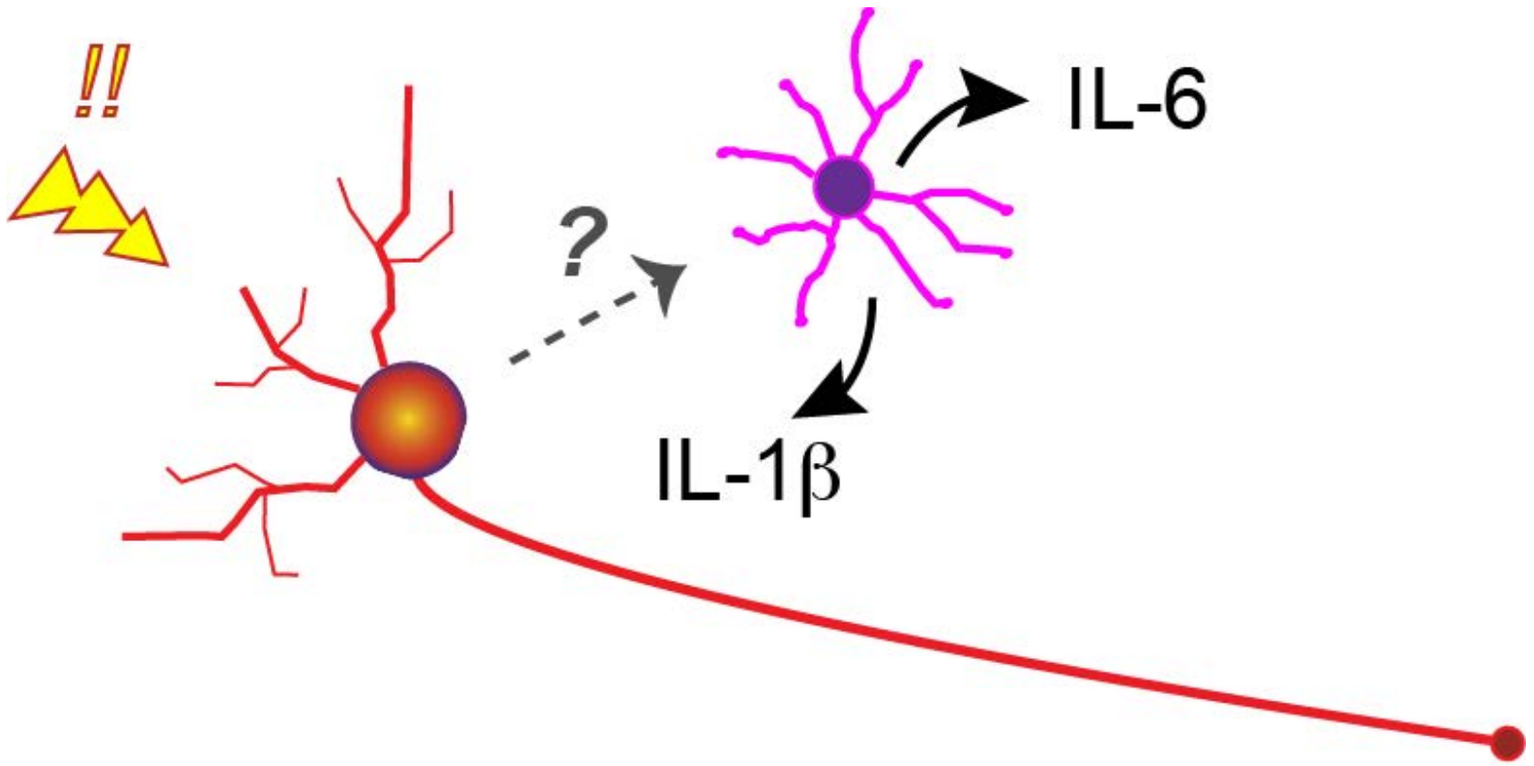


Neuron

Acute inflammatory up-regulation: The canonical model

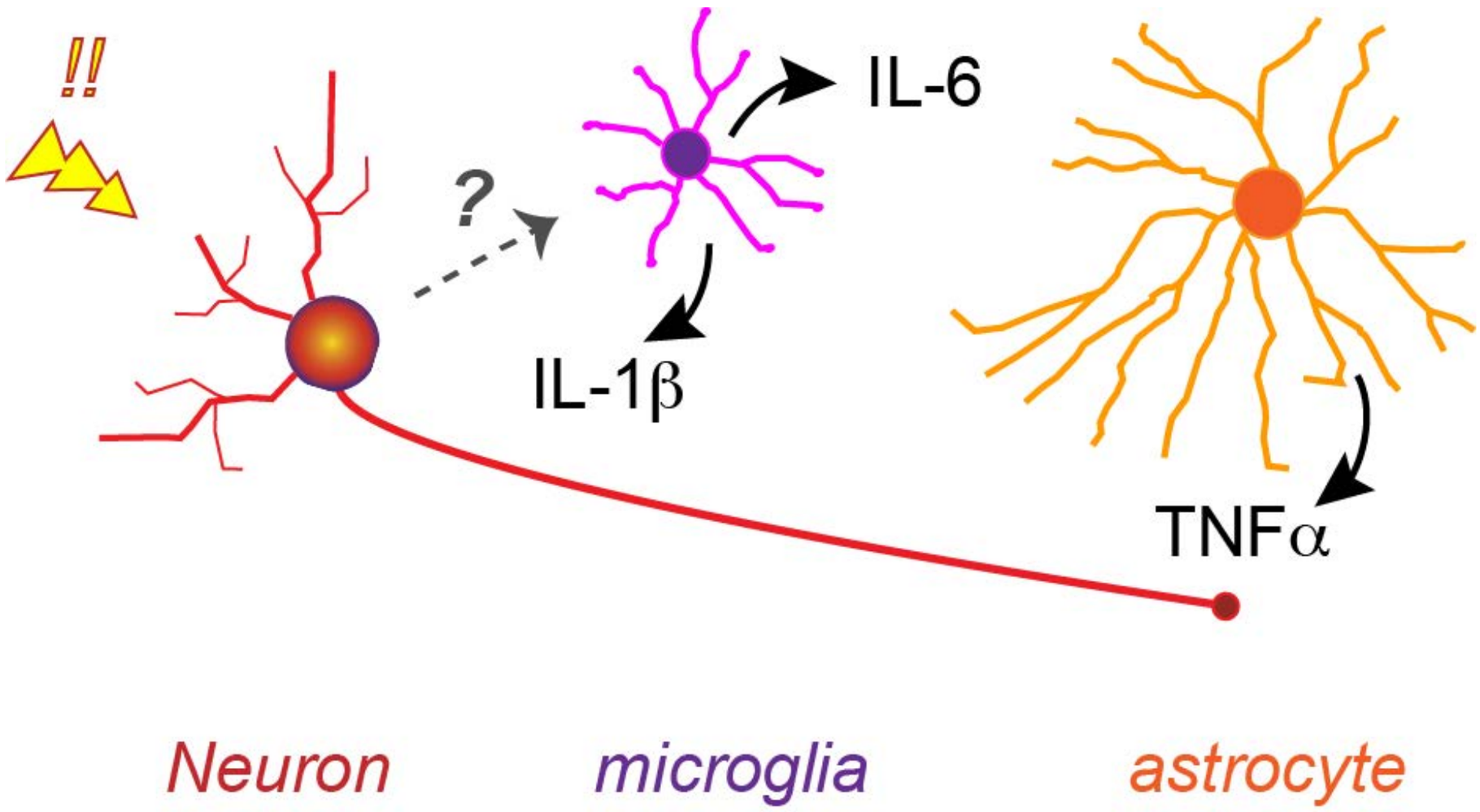


Acute inflammatory up-regulation: The canonical model

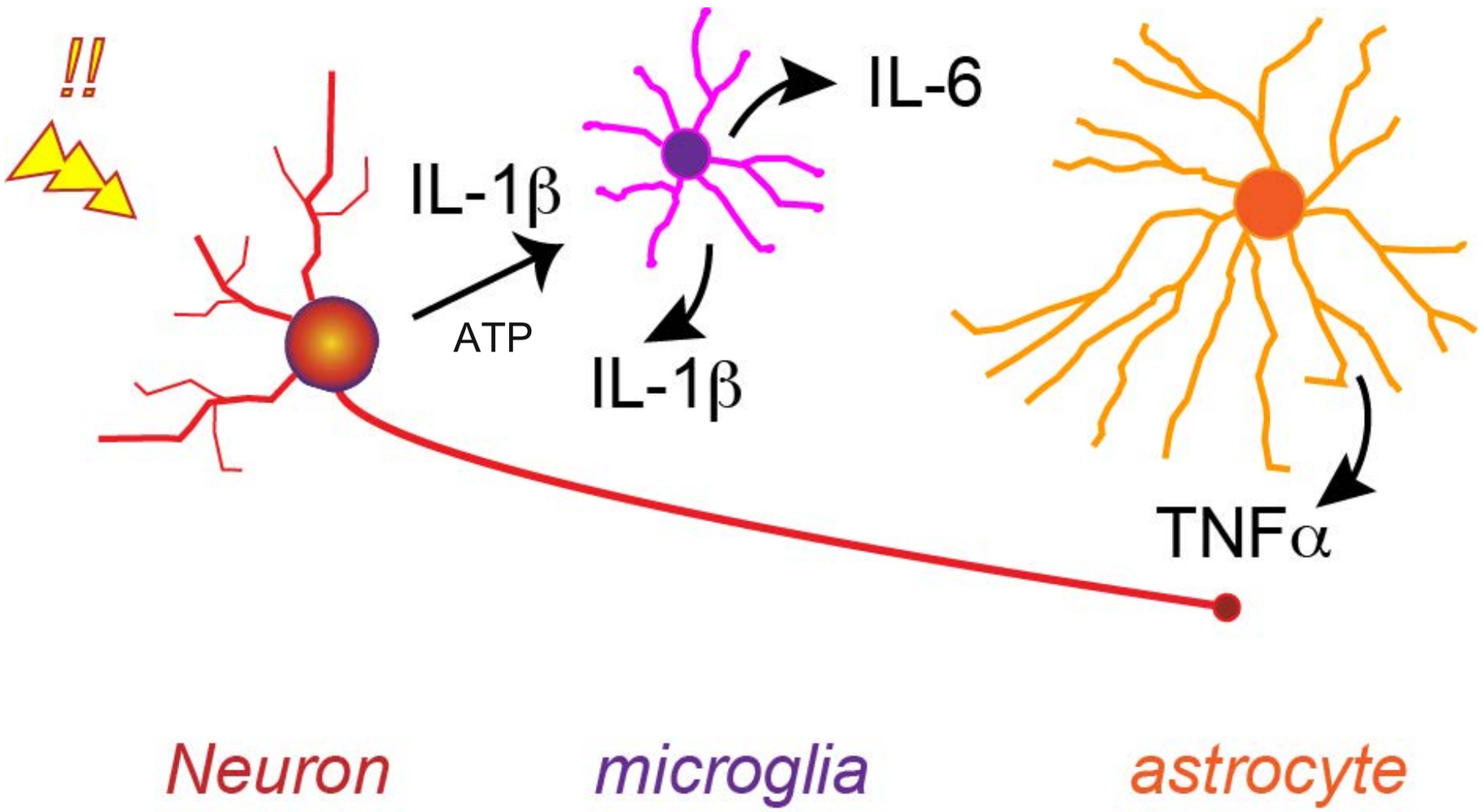


Neuron *microglia*

Acute inflammatory up-regulation: The canonical model



Acute inflammatory up-regulation: Our "new" model

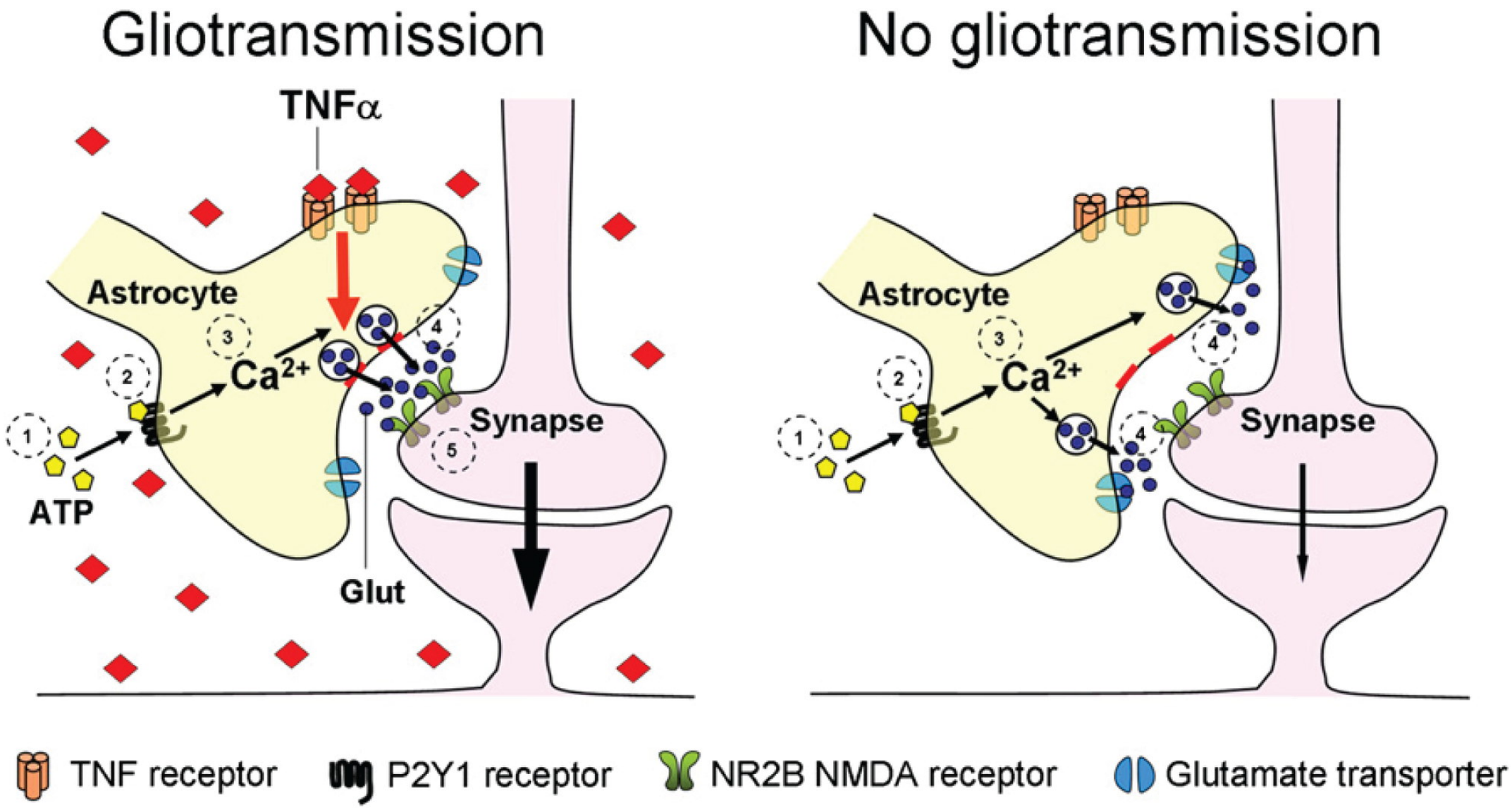


Jafri et al. *Resp Physiol Neurobiol*, 2013

Hypothesis

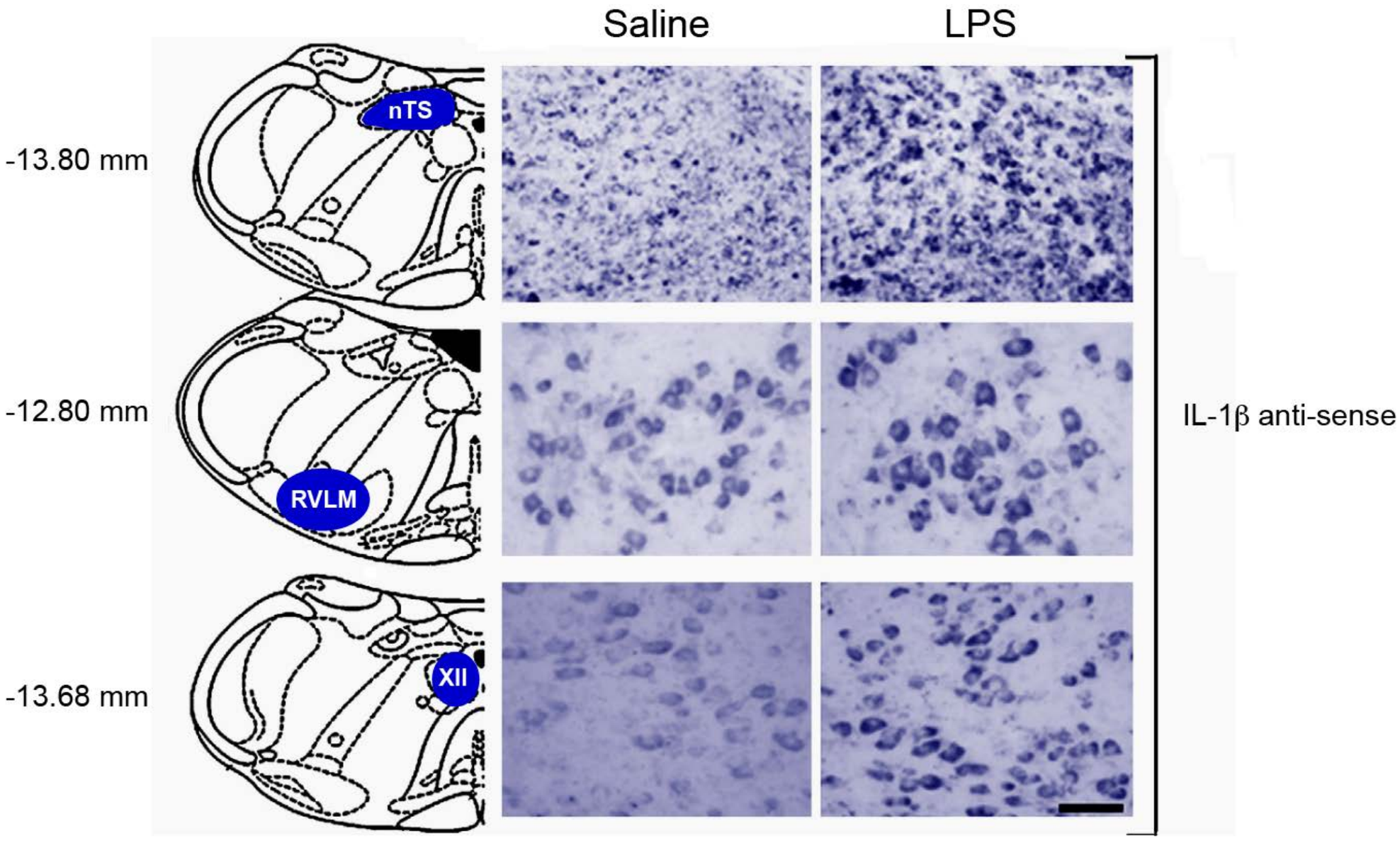
- Inflammation-induced cytokine release signals the production of proinflammatory cytokines in the brainstem and this alters signaling throughout the CNS.
  - LPS induces a cascade of cytokine (IL-1β, IL-6, TNFα and others) release *from neurons and microglia*.
  - These cytokines modulate processing of vagal afferent input at the *nTS*, rhythm-generation at the pBC, and motor output at the *XII* nucleus.
- Release of prostaglandins (e.g. PGE_2) then changes synaptic processing at this first-order input to the CNS.

Cytokines and purines modify synaptic transmission ***normally***



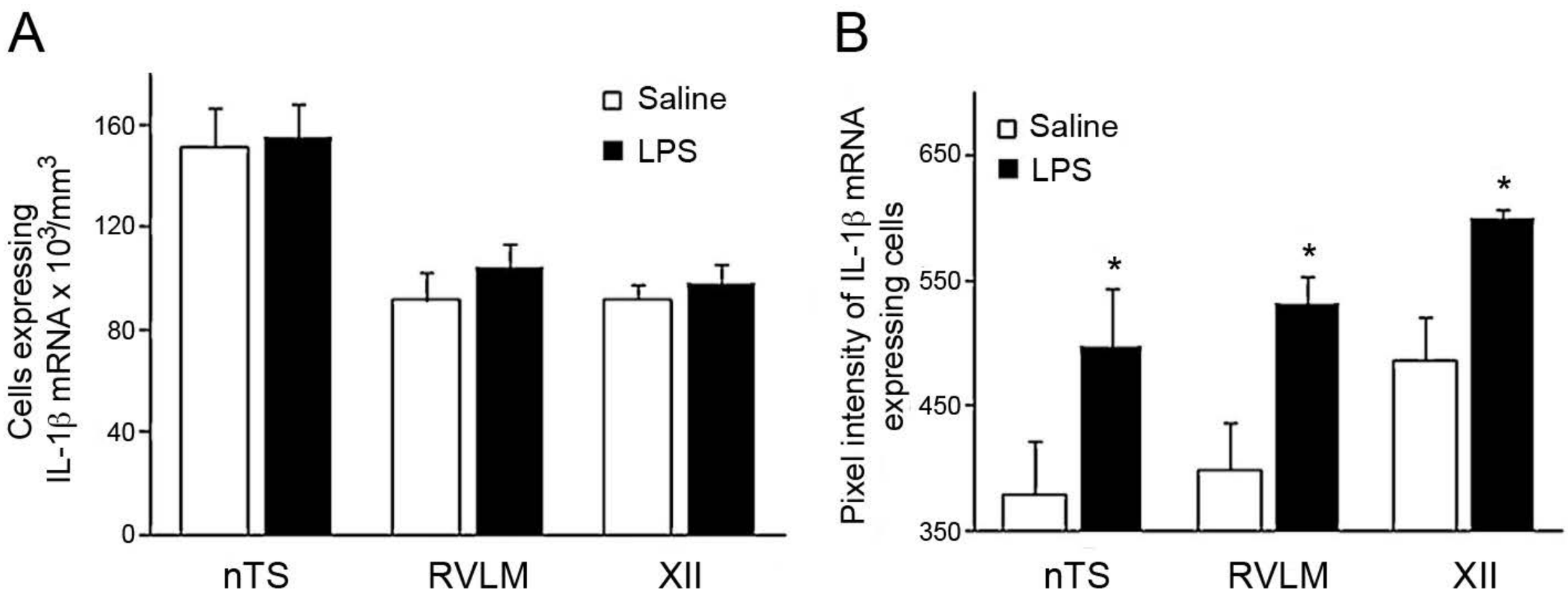
Santello et al., *Neuron* 2011

LPS-induced IL-1β message in respiratory regions of brainstem



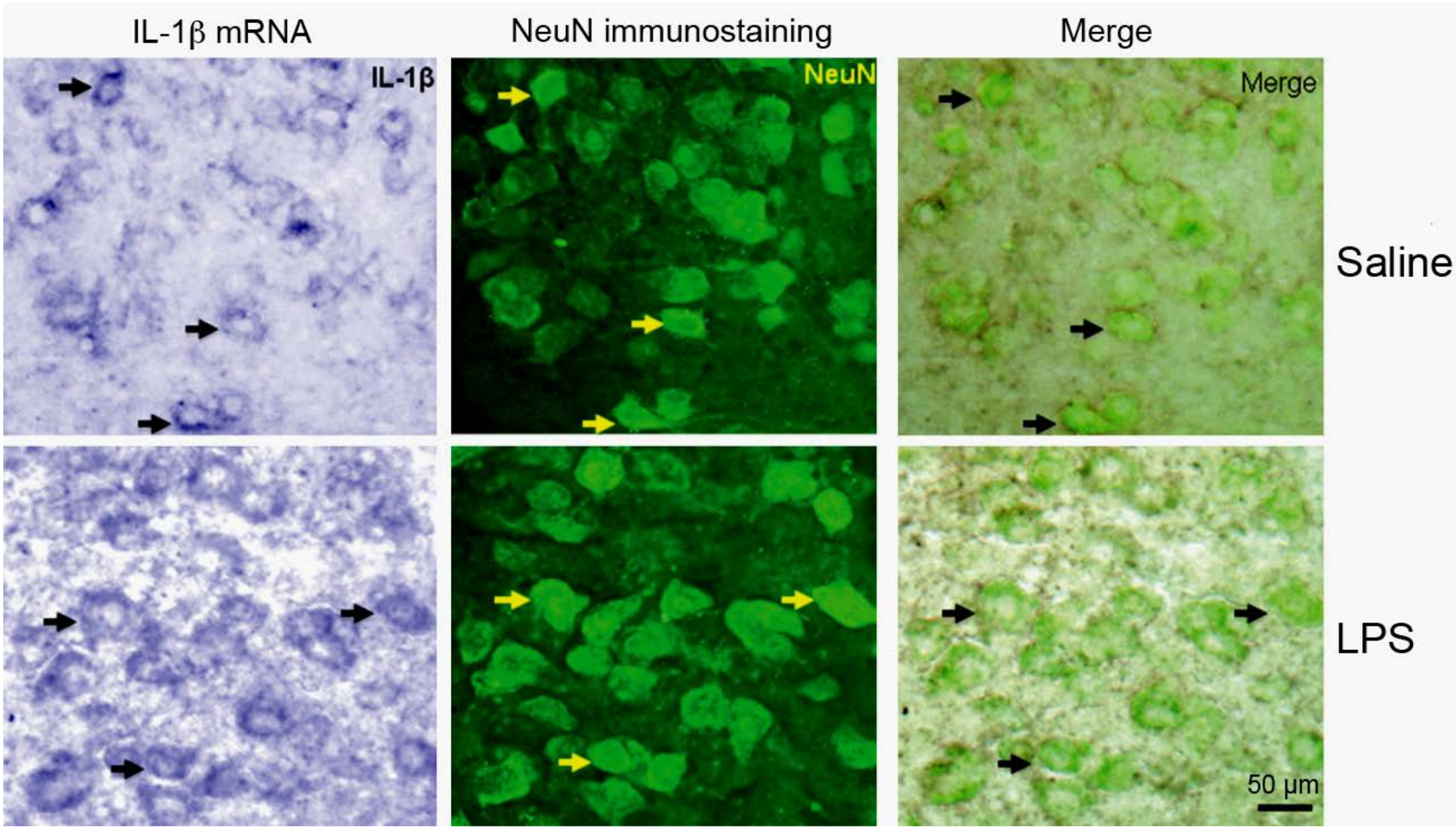
Jafri et al. *Resp Physiol Neurobio* (2013)

IL-1β mRNA expression increased in respiratory areas



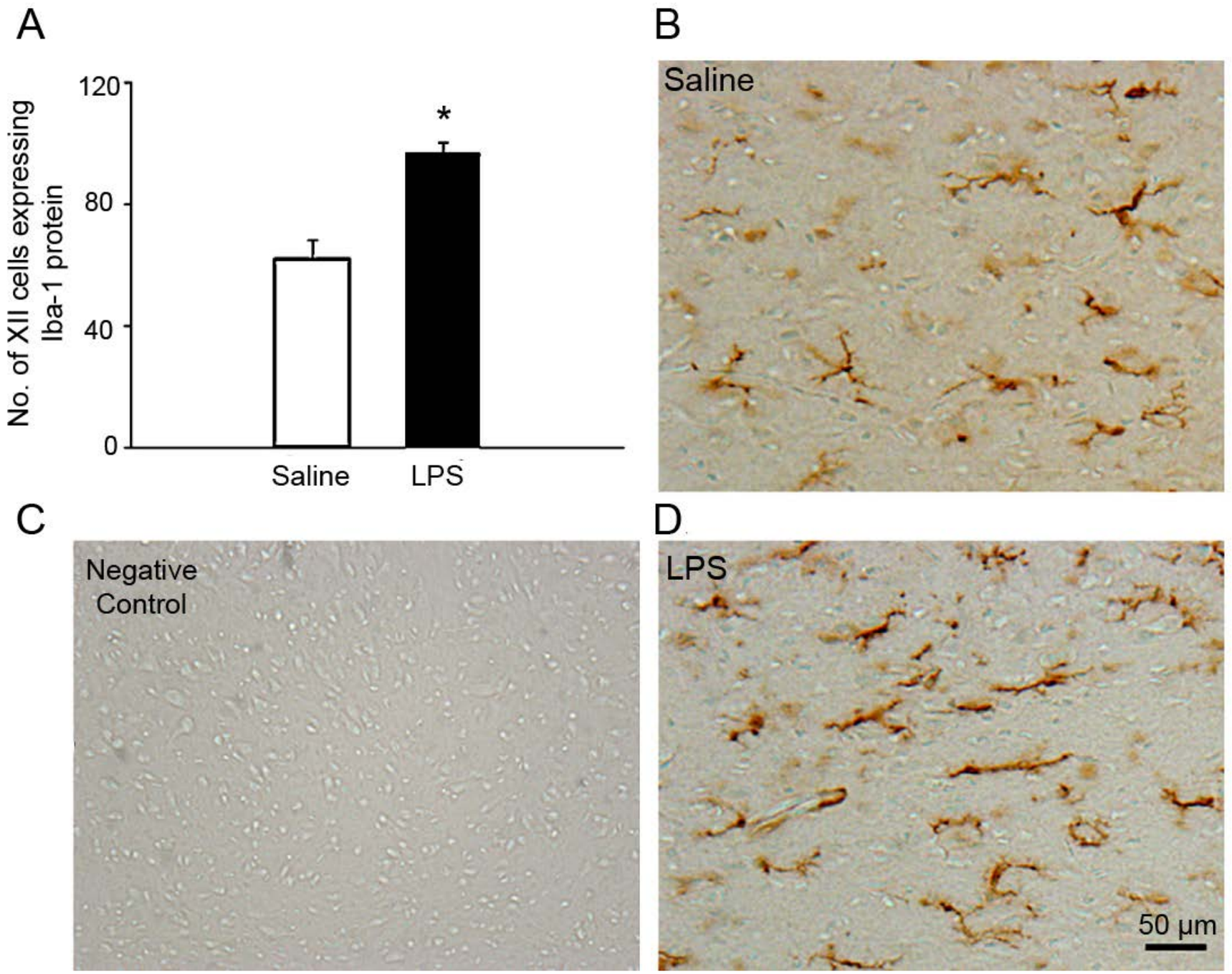
Jafri et al. *Resp Physiol Neurobio* (2013)

IL-1β mRNA is expressed in XII motoneurons



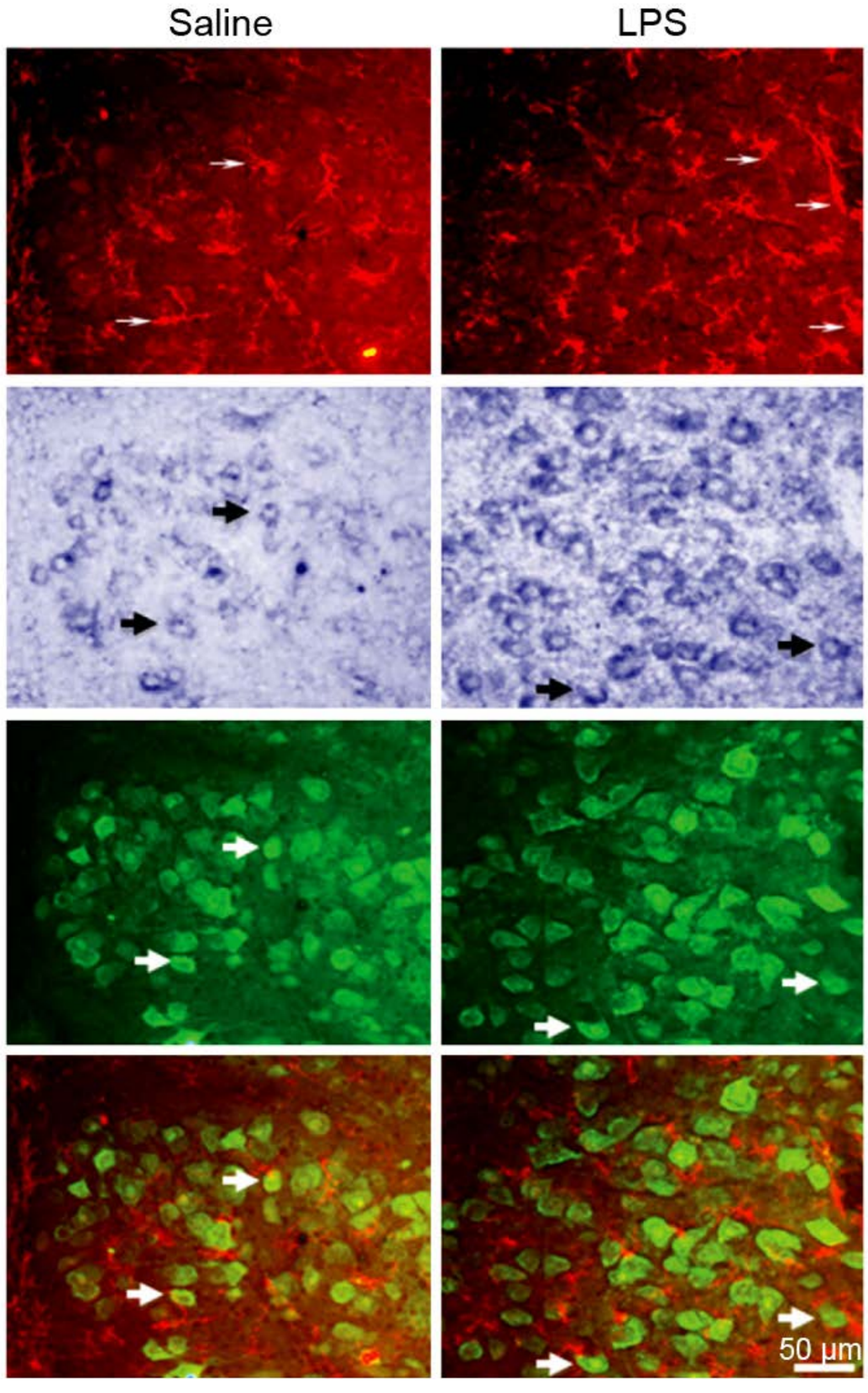
Jafri et al. *Resp Physiol Neurobio* (2013)

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



Jafri et al. *Resp Physiol Neurobio* (2013)

Microglia appear NOT to express IL-1β



Iba-1 Immunostaining

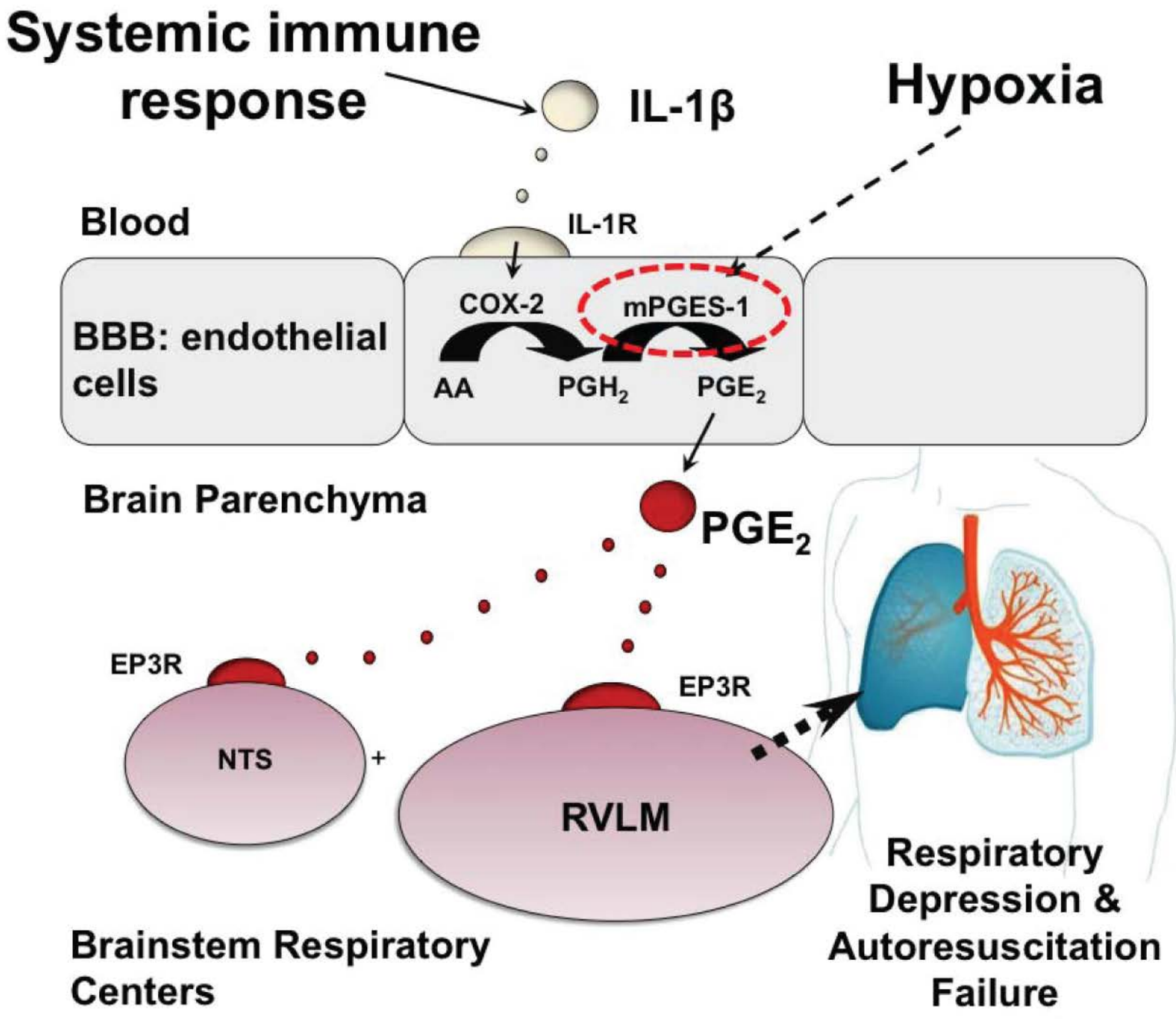
IL-1β mRNA (in situ hybridization)

NeuN Immunostaining

Merge (triple labeled)

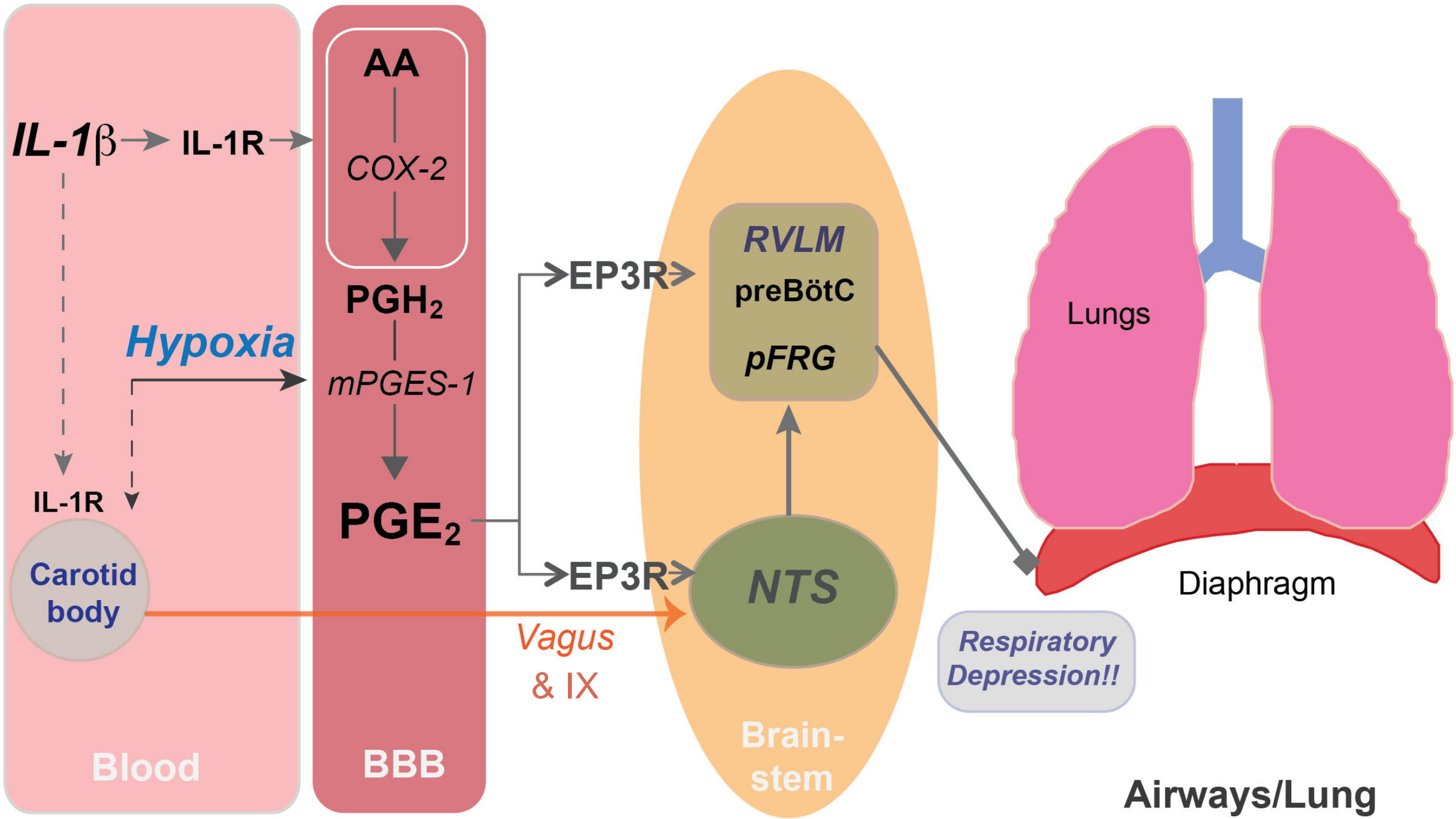
Jafri et al. *Resp Physiol Neurobiol,* 2013

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



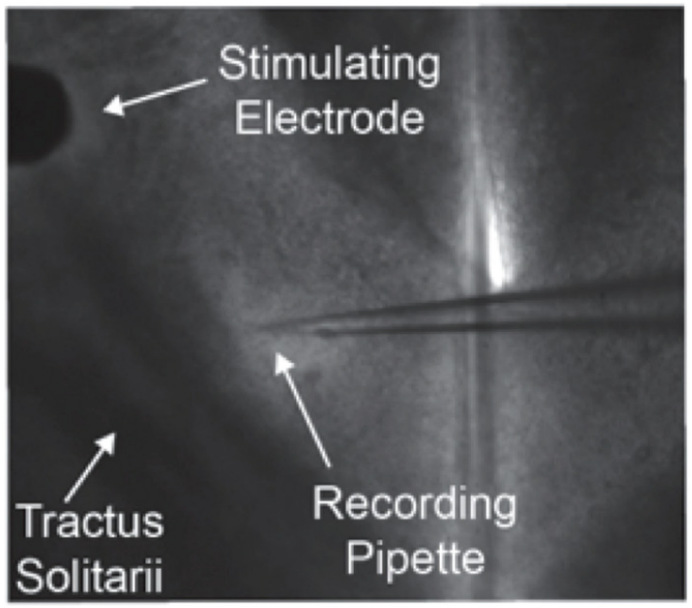
Herlenius, *Resp. Physiol. Neurobiol.*, Sept. 2011

Acute inflammation alters inflammatory drive in the CNS



Changes in nTS neural dynamics after inflammation/lung injury

Horizontal slice preparation



Getsy et al. *Resp Physiol Neurobiol.* 2019

Changes in nTS neural dynamics after inflammation/lung injury

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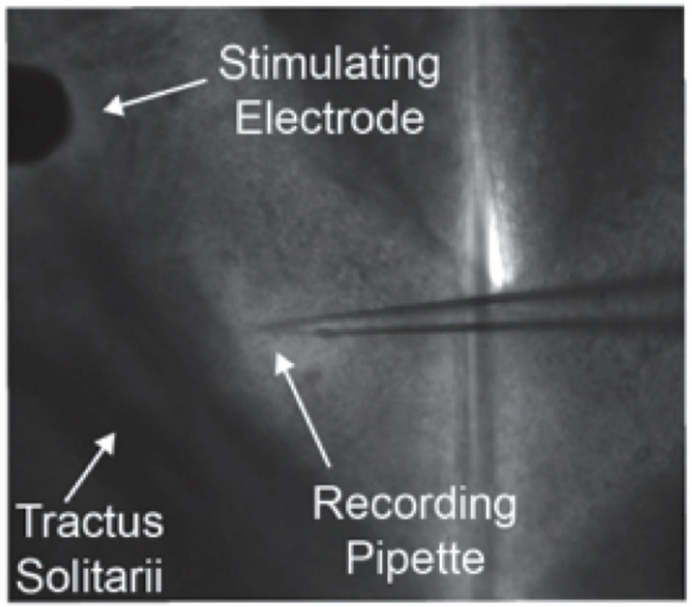
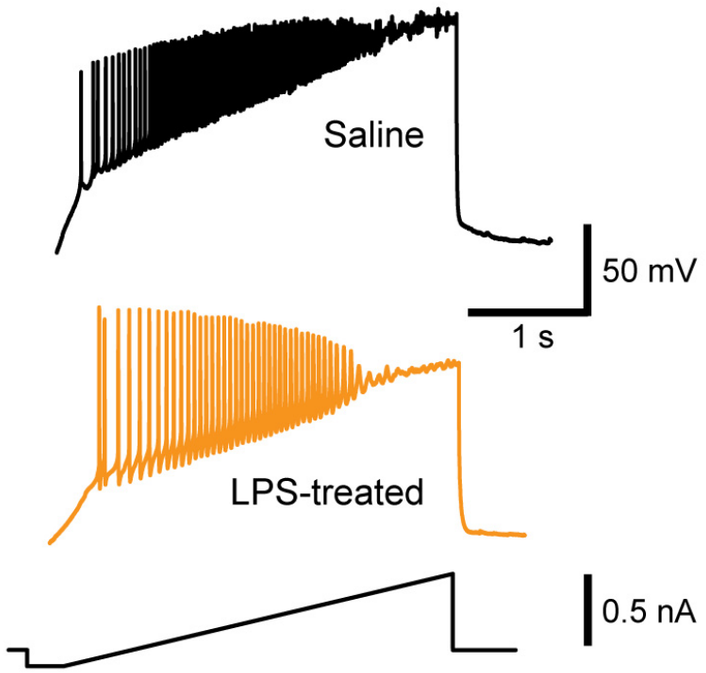
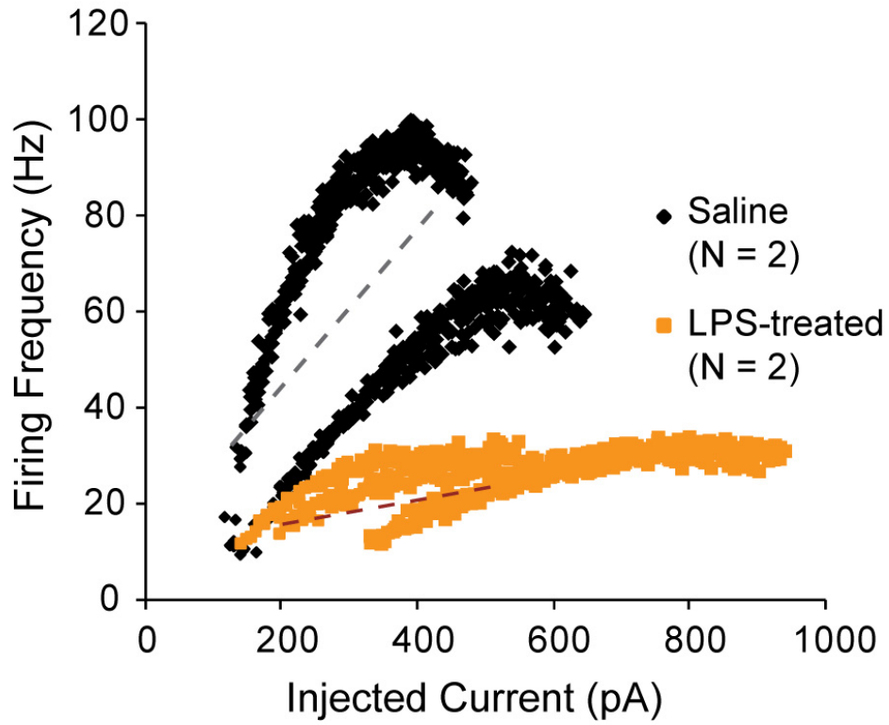


Current-Clamp ramps

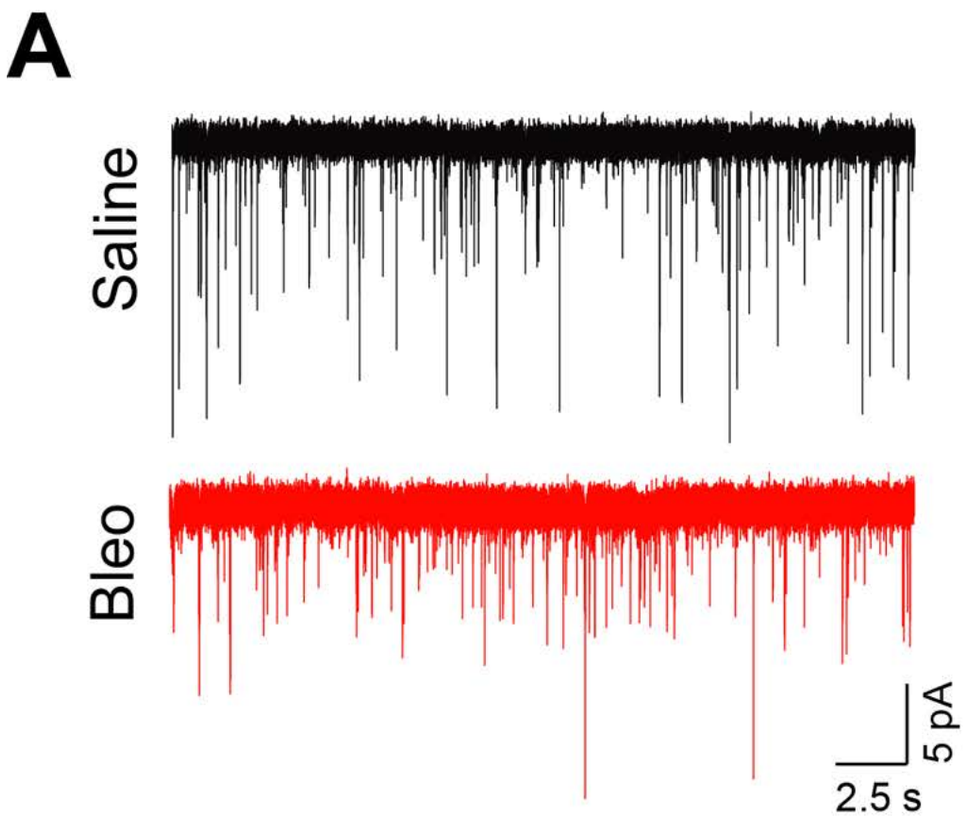


LPS blunts the f-I relationshp



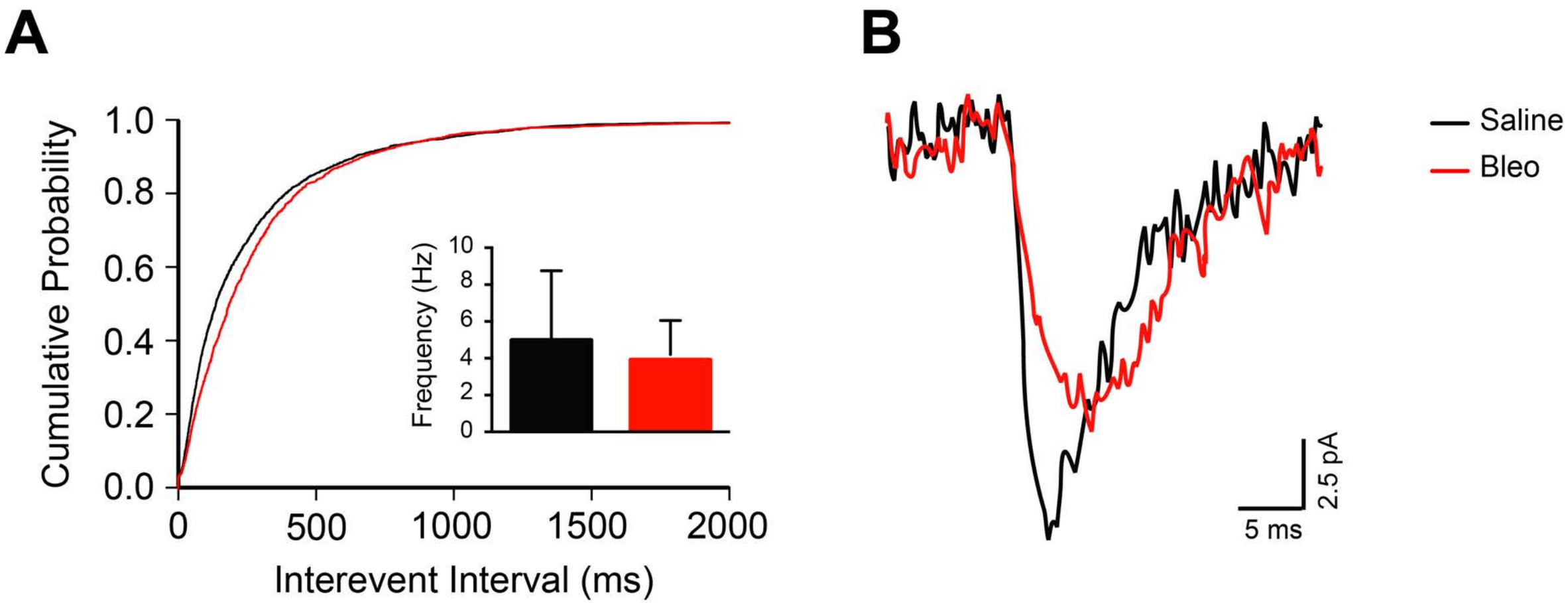
Getsy et al. *Resp Physiol Neurobiol,* 2019

nTS neurons have smaller sEPSCs after lung injury



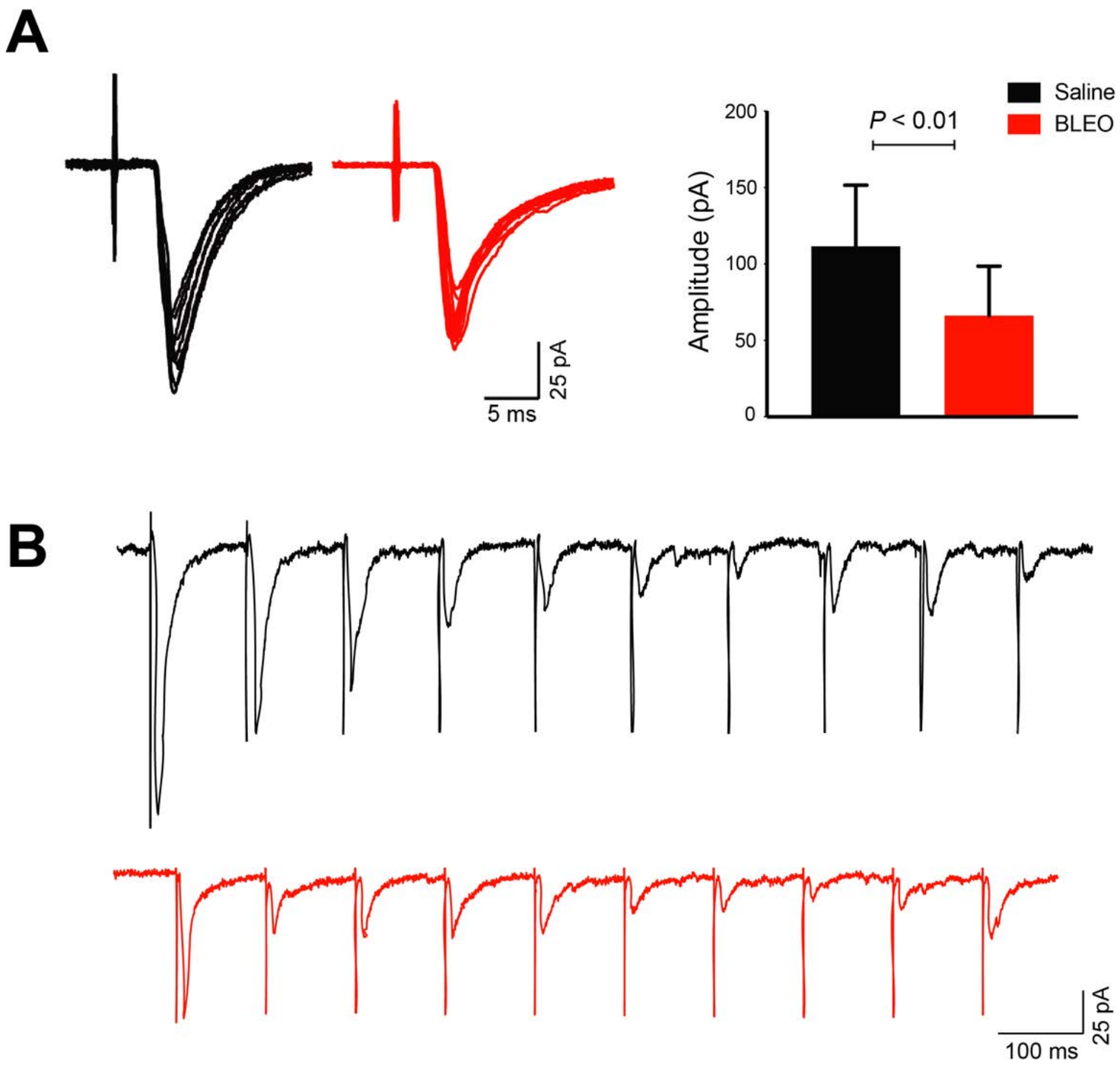
Getsy et al. *Resp Physiol Neurobiol,* 2019

Changes in nTS sEPSCs activity after lung injury



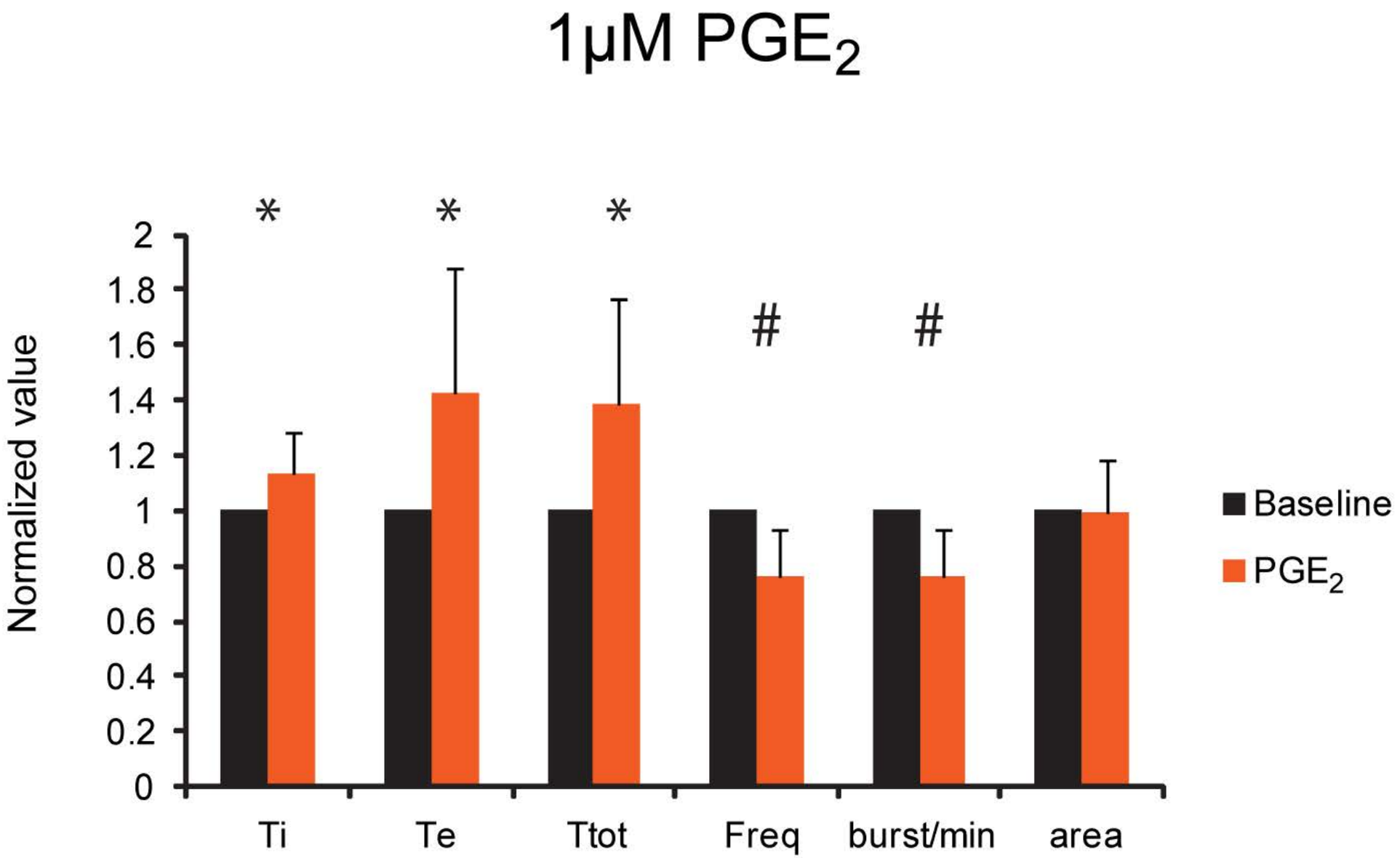
Getsy et al. *Resp Physiol Neurobiol.* 2019

nTS evoked EPSCs also show reduced amplitude



Paulina Getsy

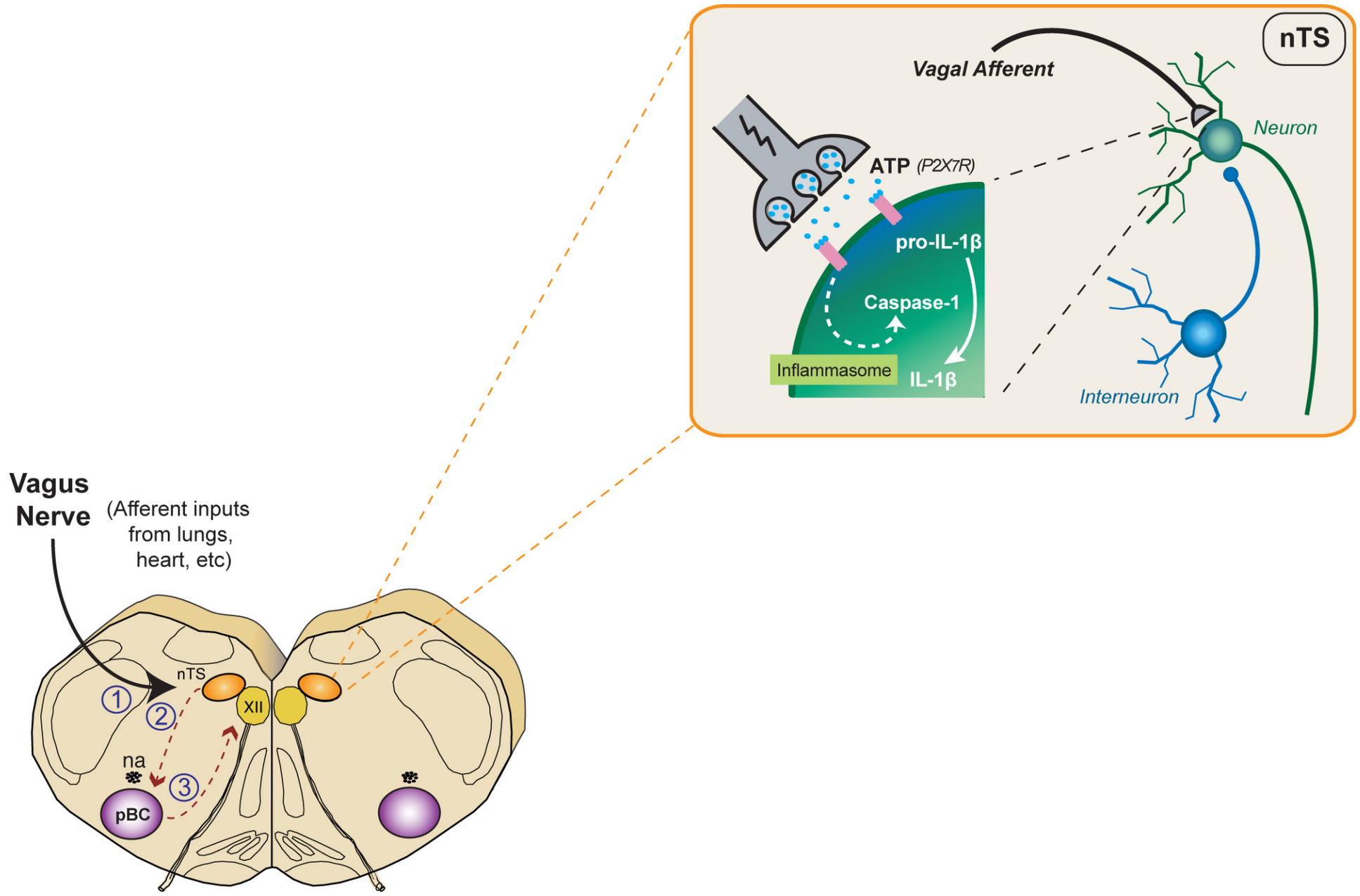
PGE_2 alters breathing pattern *in vitro*



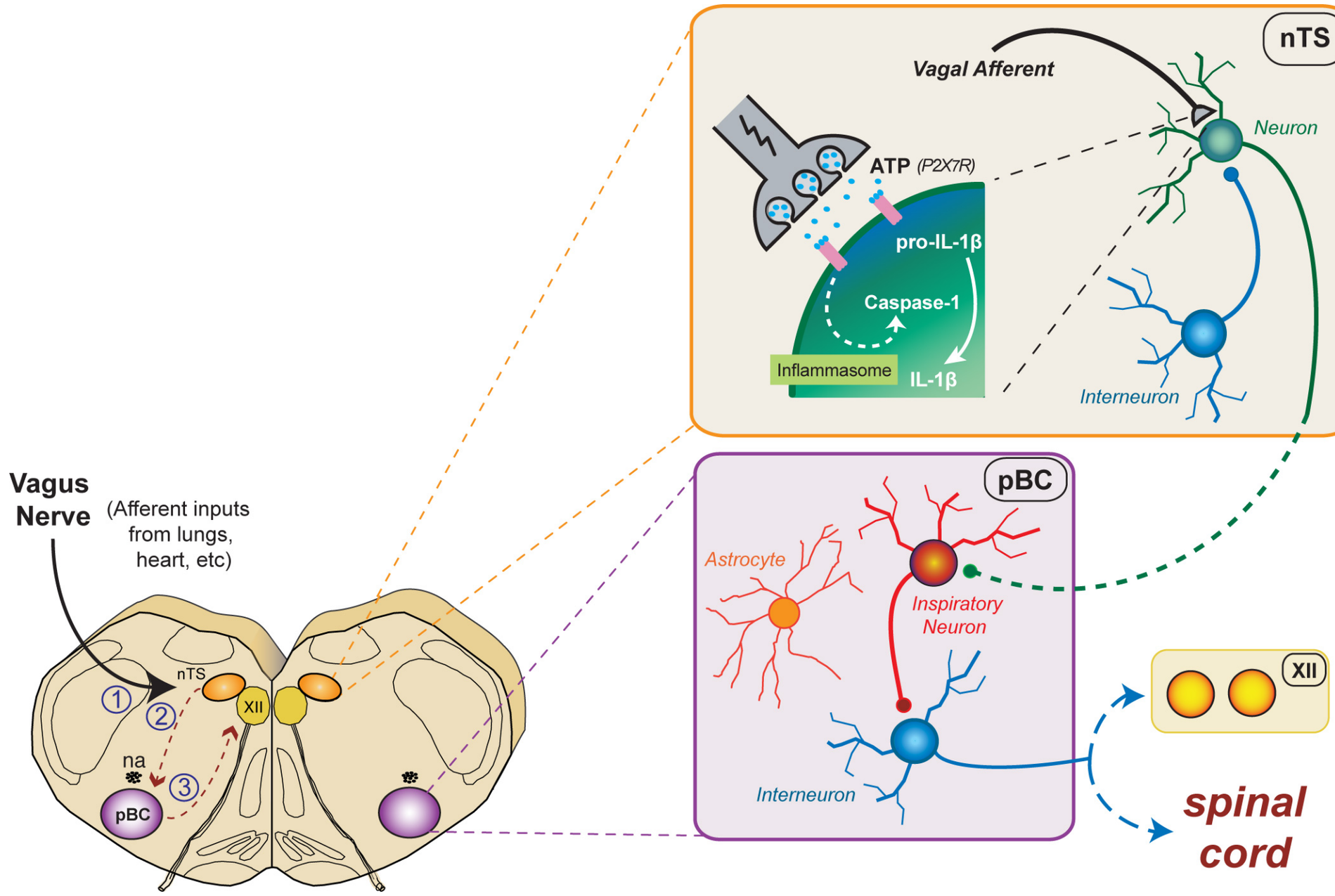
n = 8
* = p < 0.05
# = p <0.01

Ken Gresham

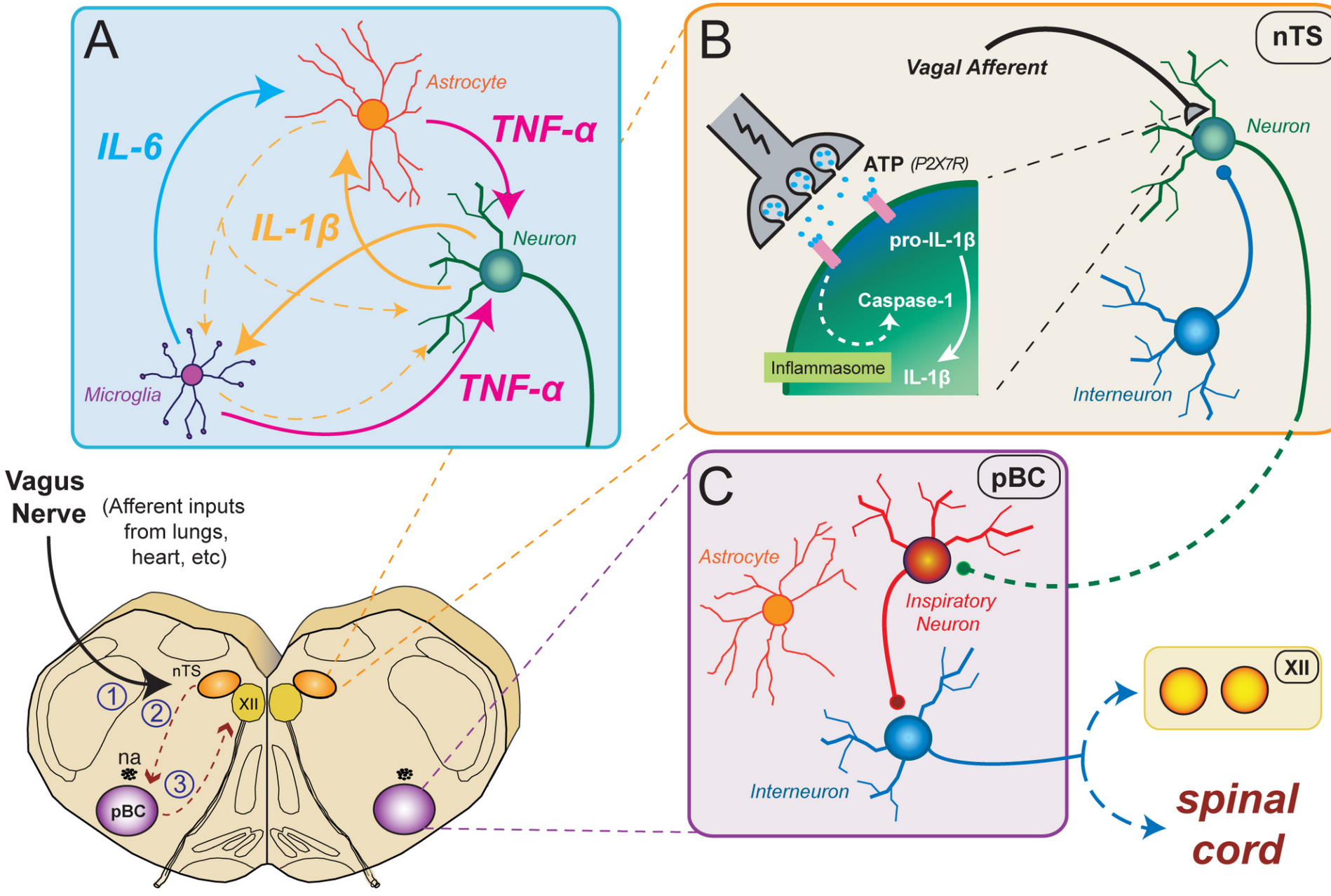
How do cytokines alter neural activity?



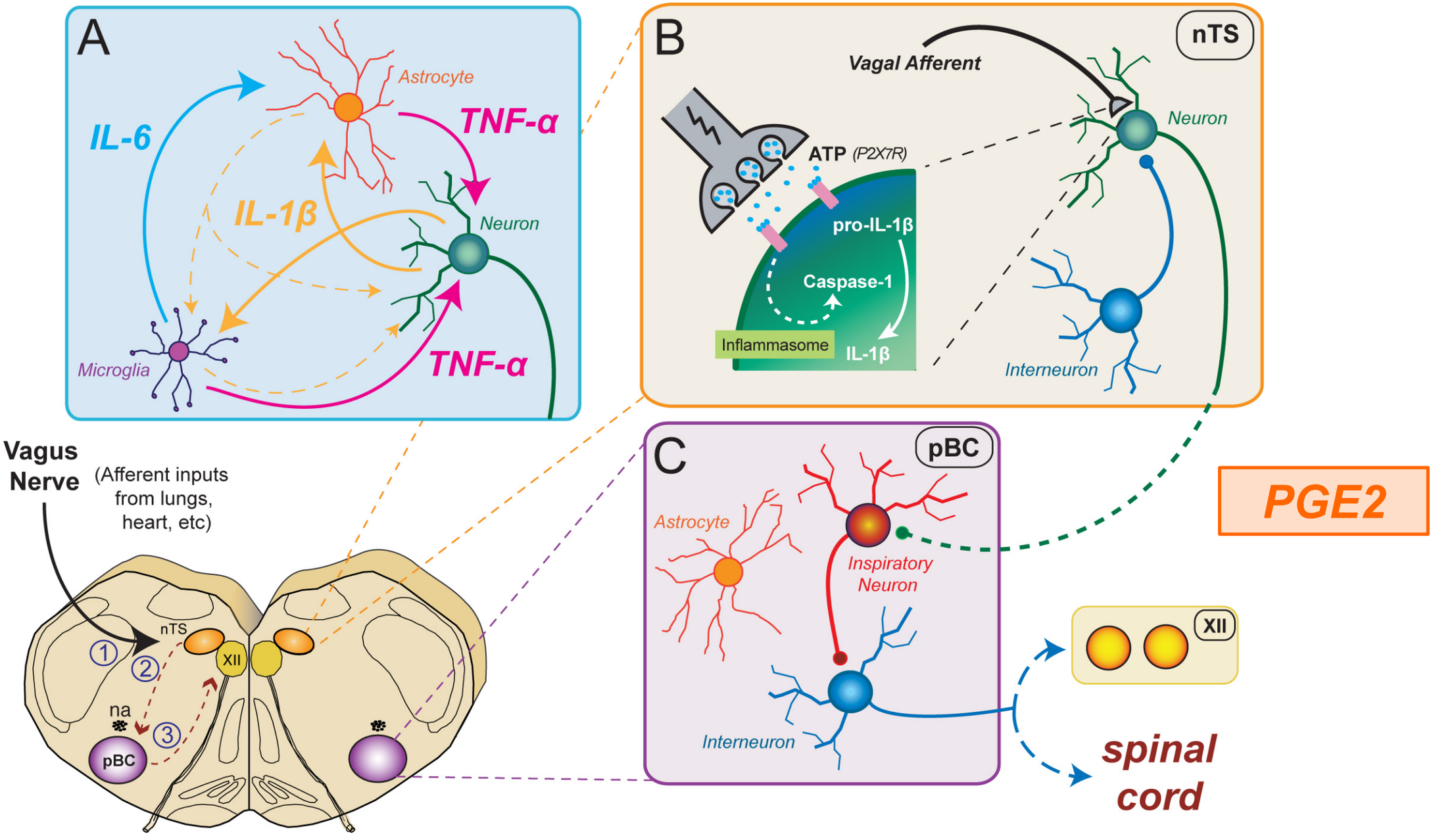
How do cytokines alter neural activity?



How do cytokines alter neural activity?



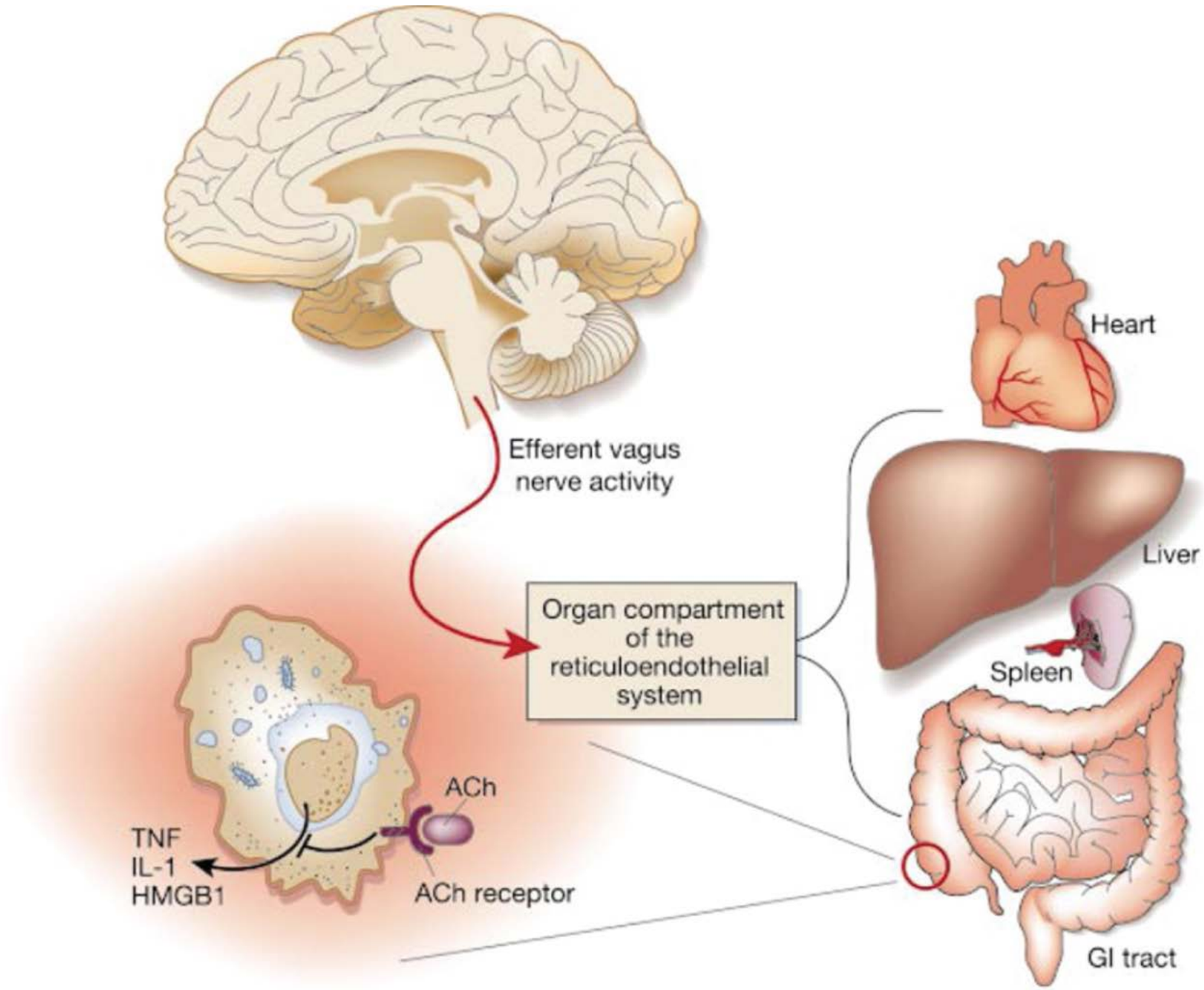
How do cytokines alter neural activity?



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

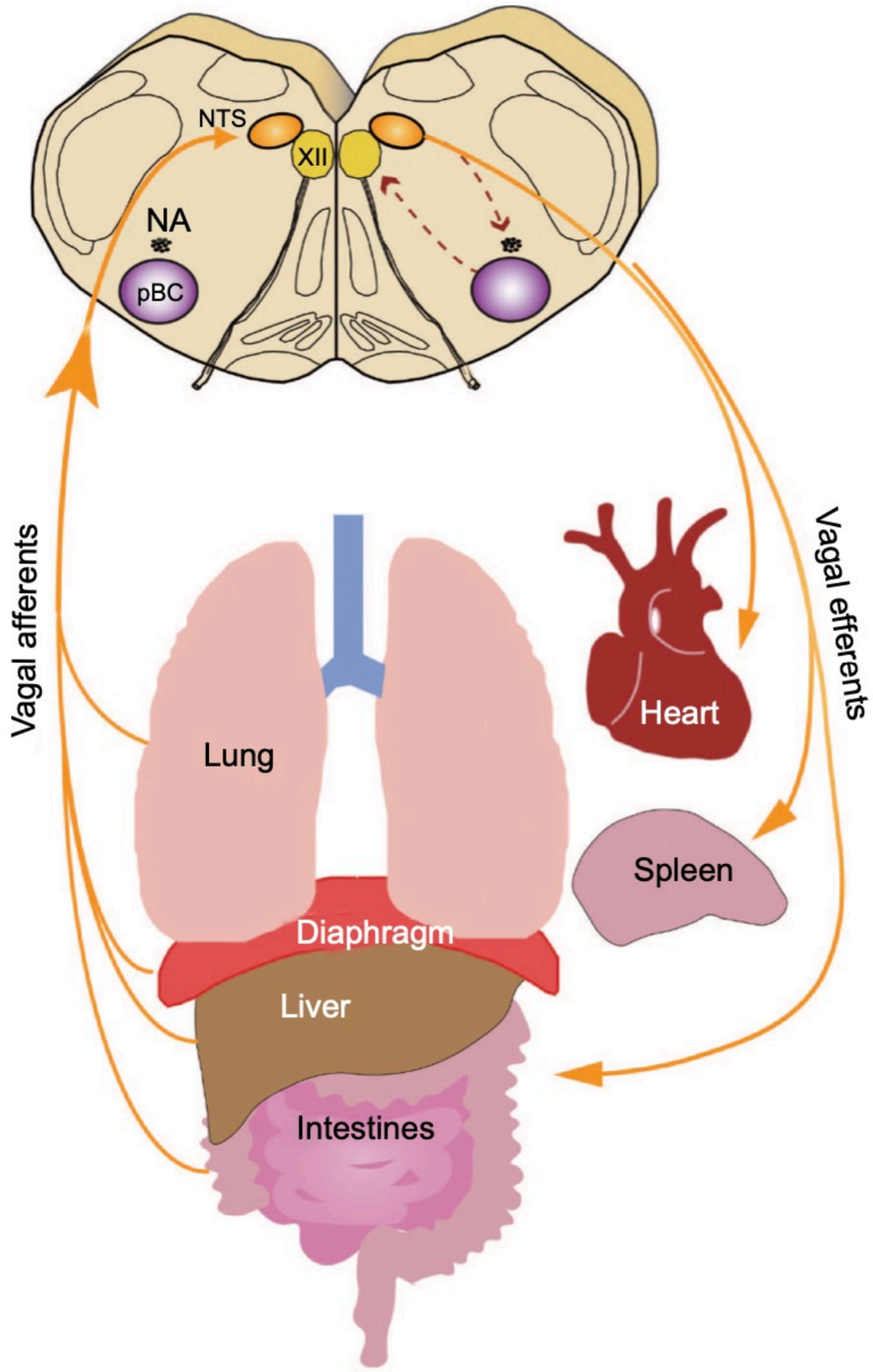


Tracey KJ, *Nature*, 2002

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

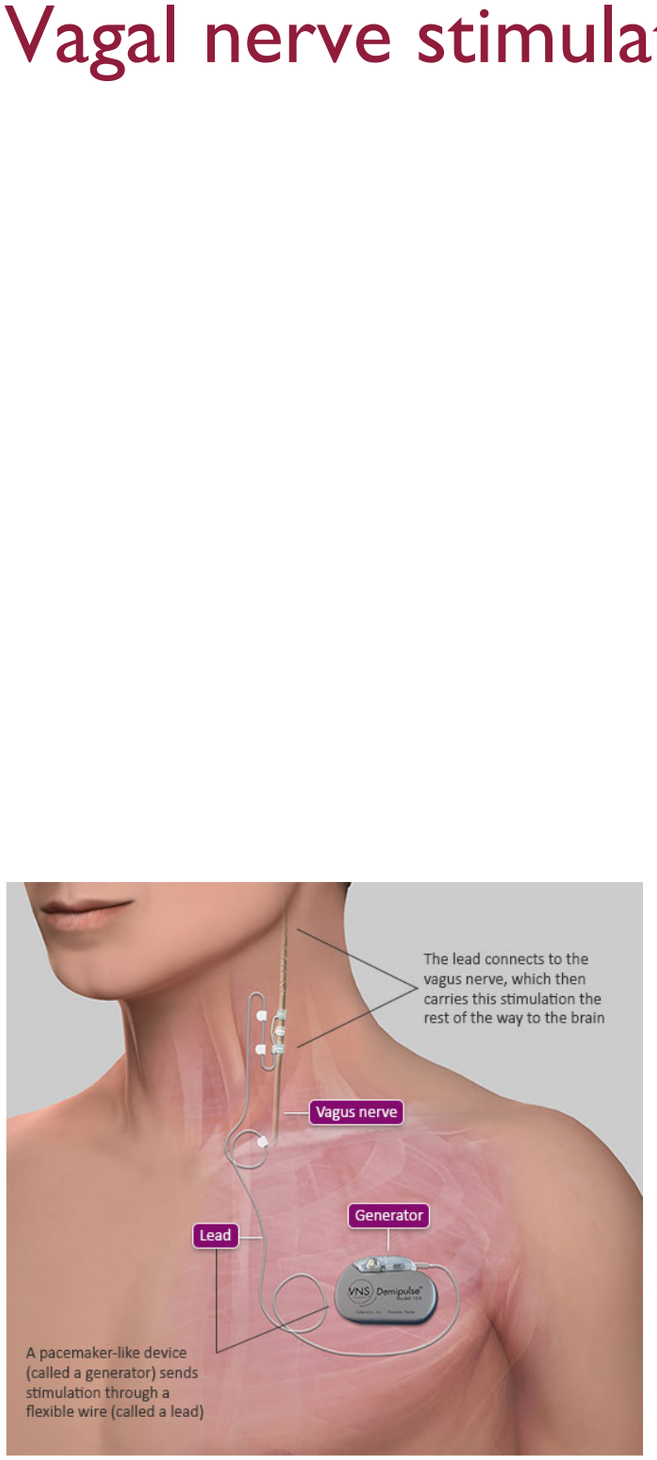


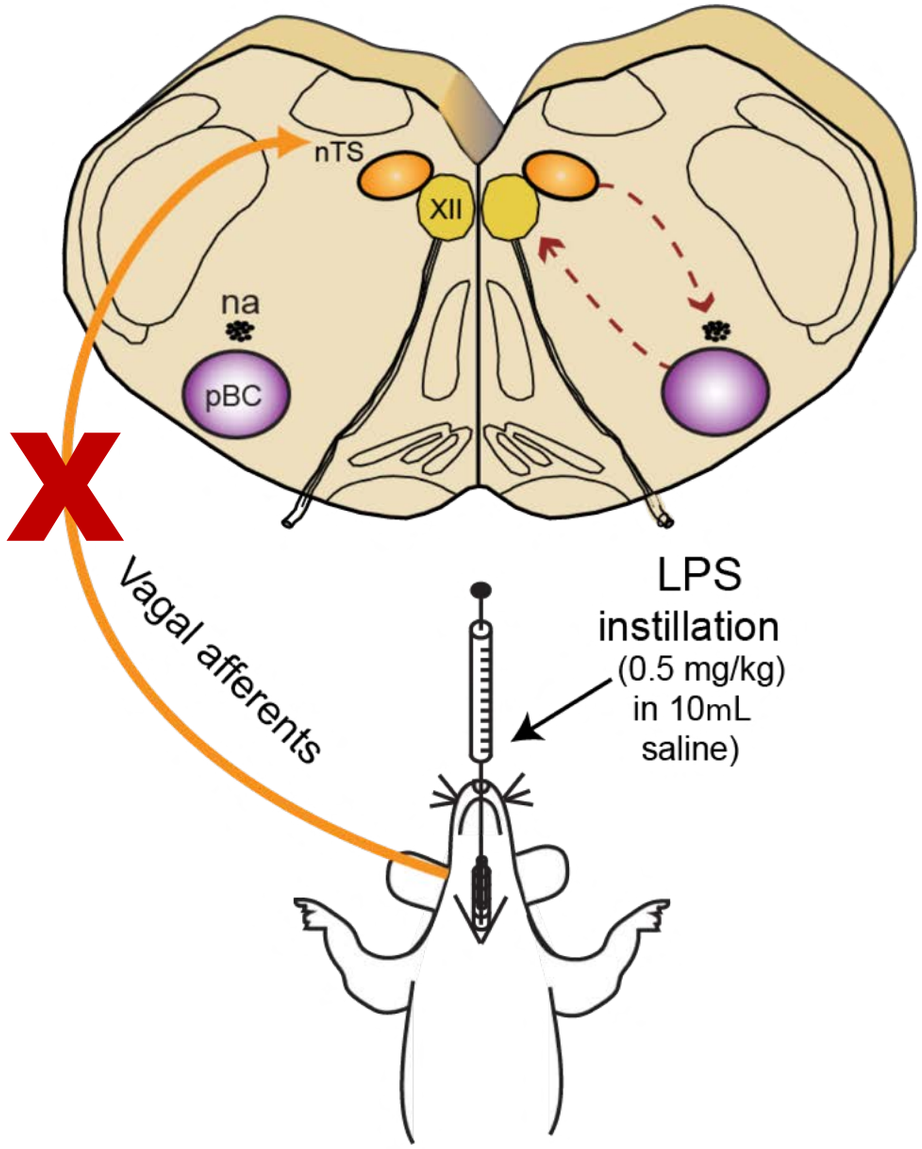
NTS = *nucleus tractus solitarius*
NA = *nucleus ambiguus*
pBC = preBötzinger Complex (rhythm generator)

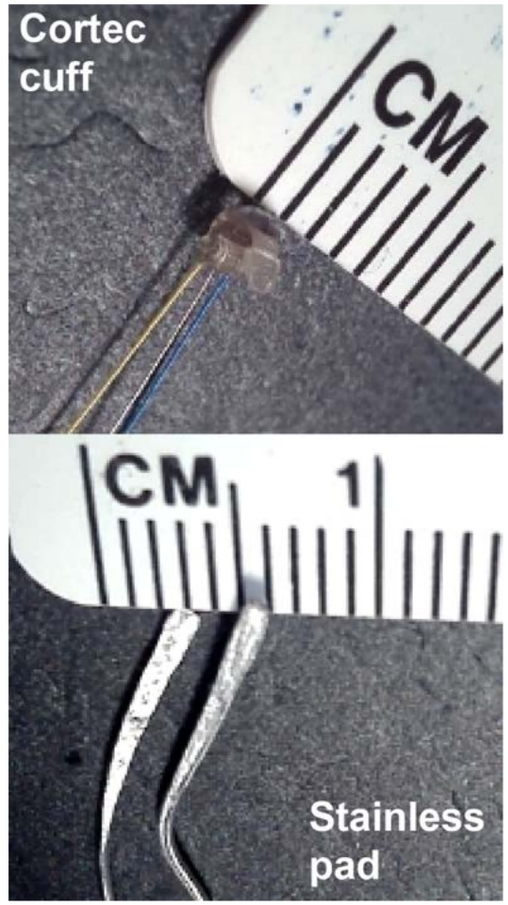
Vagus Nerve Stimulation

- 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







Johnson et al. *Resp Physiol Neurobiol*, 2016

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.

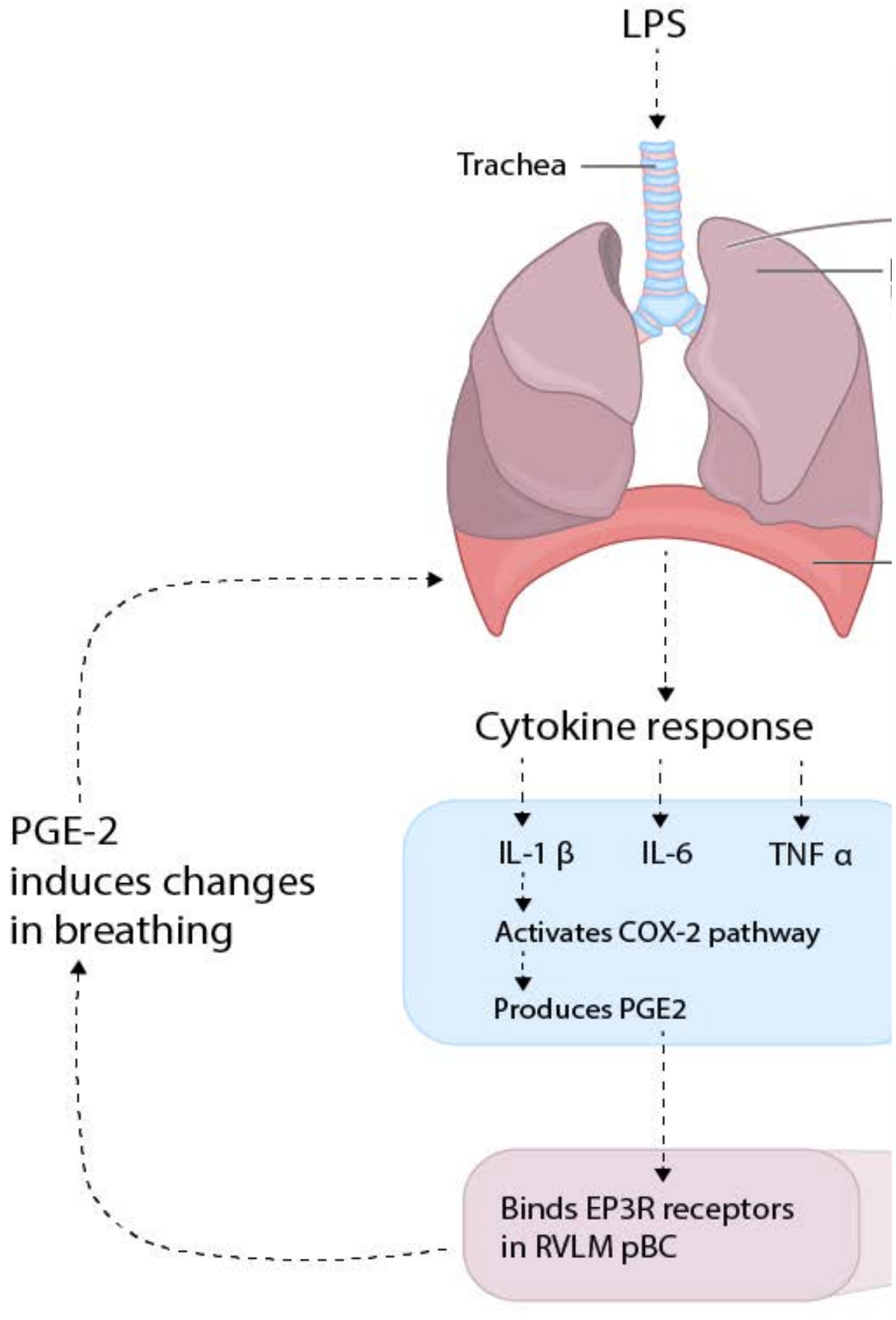
Research uses of VNS

- ***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

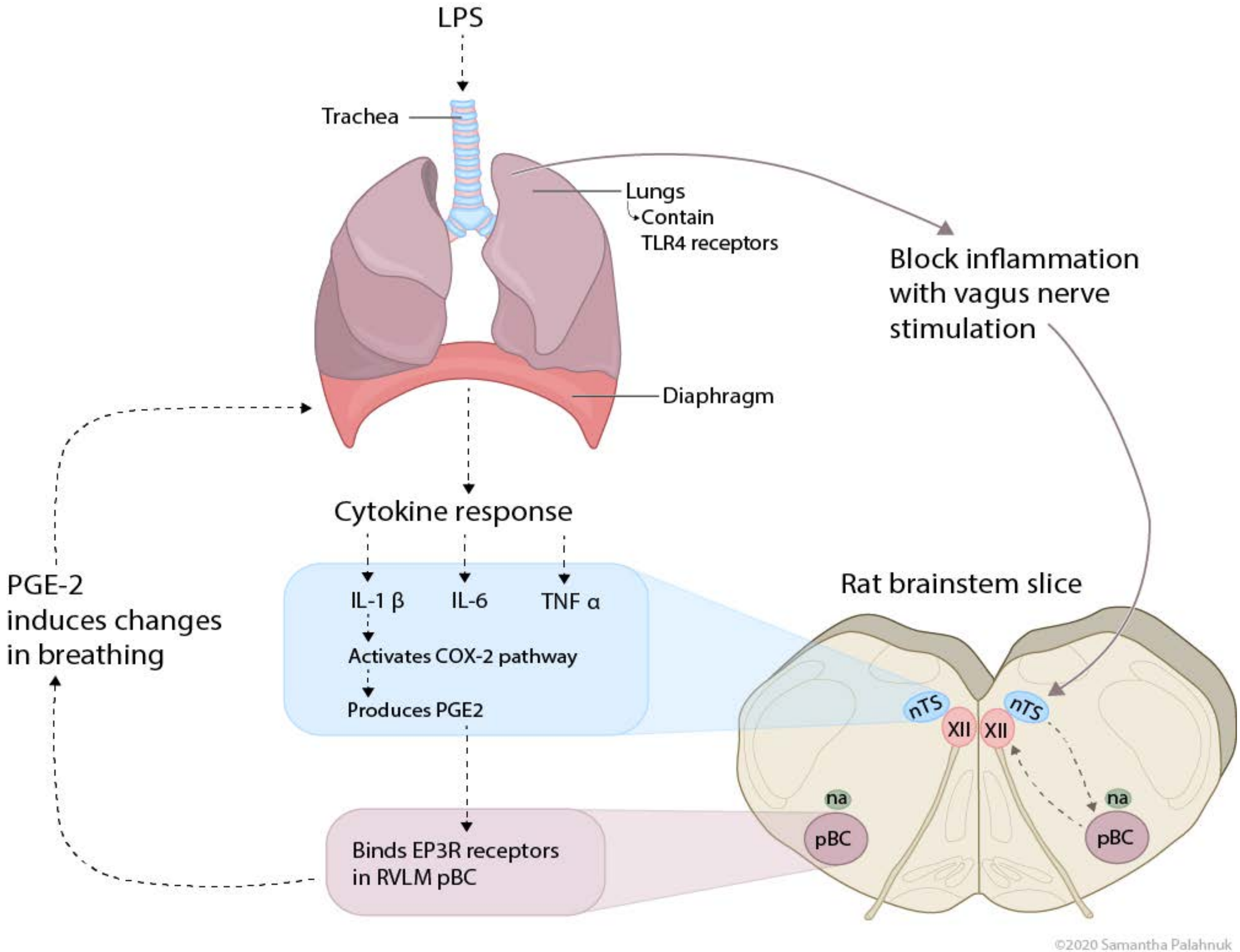
- ***Pain management***. The applications of VNS also extends to disorders associated with chronic or intermittent bouts of pain such as fibromyalgia and migraines.

- ***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.

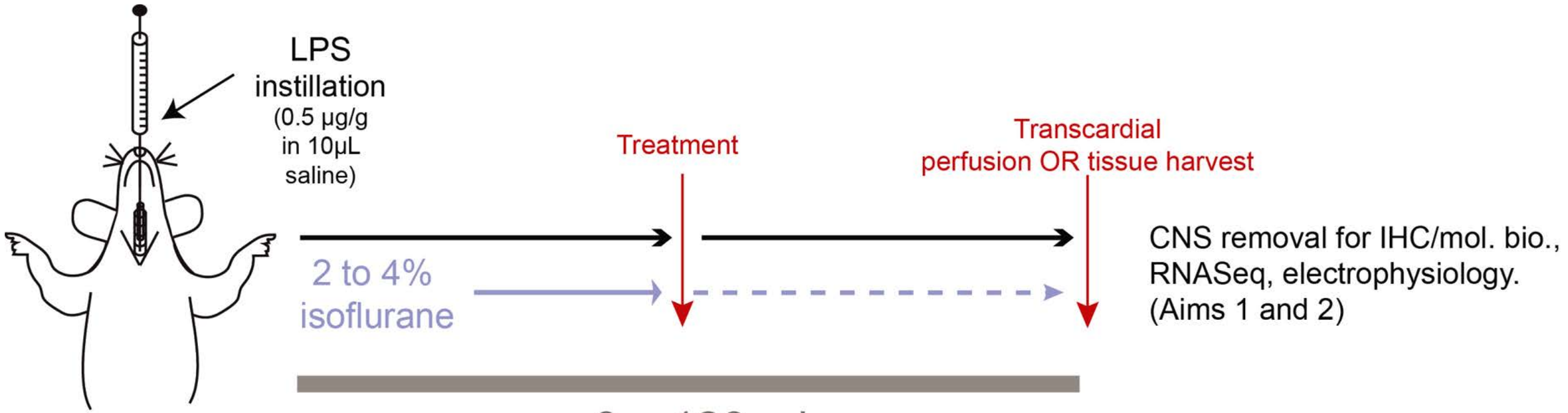
VNS and cytokines



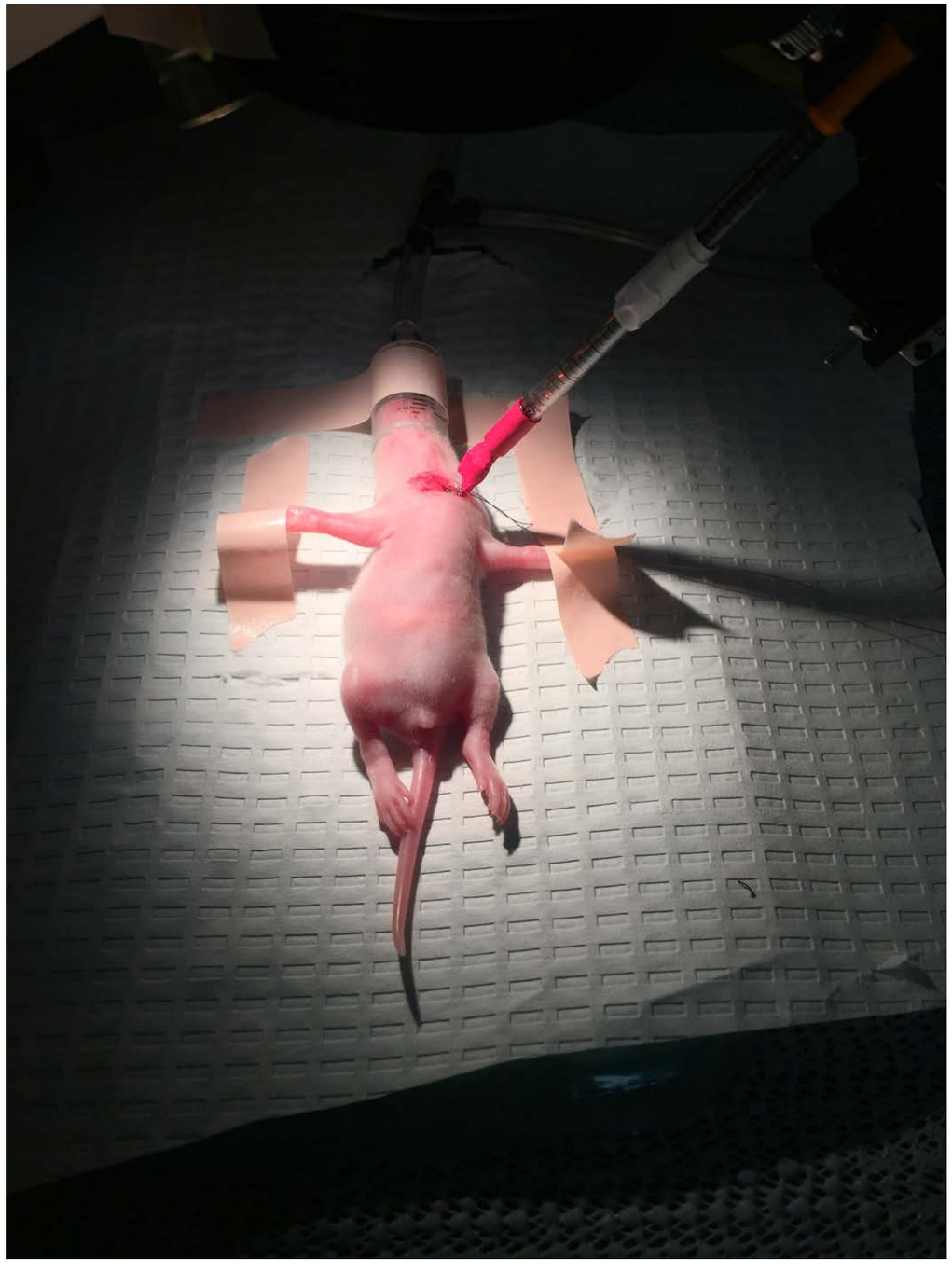
VNS and cytokines

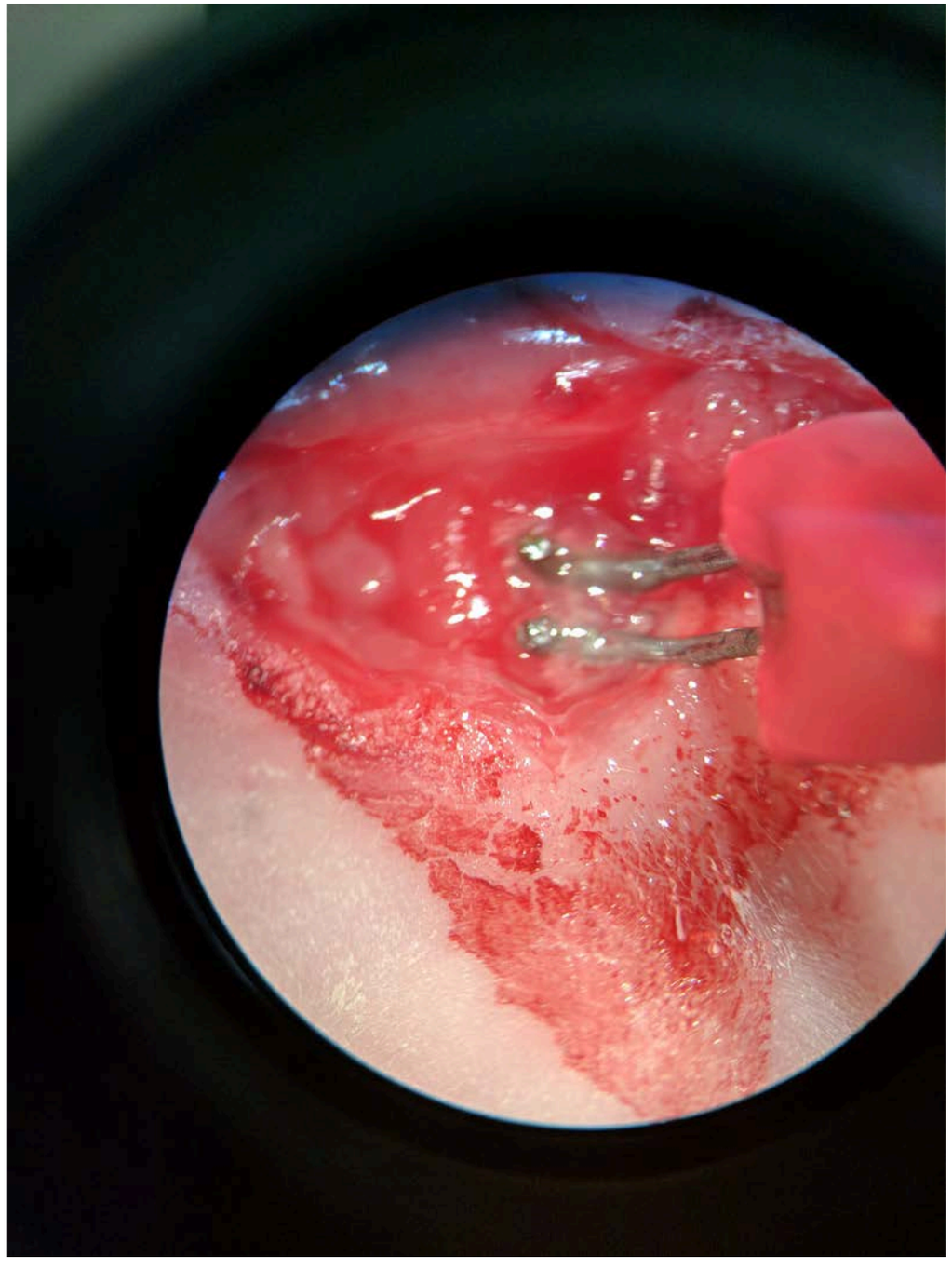


Methods

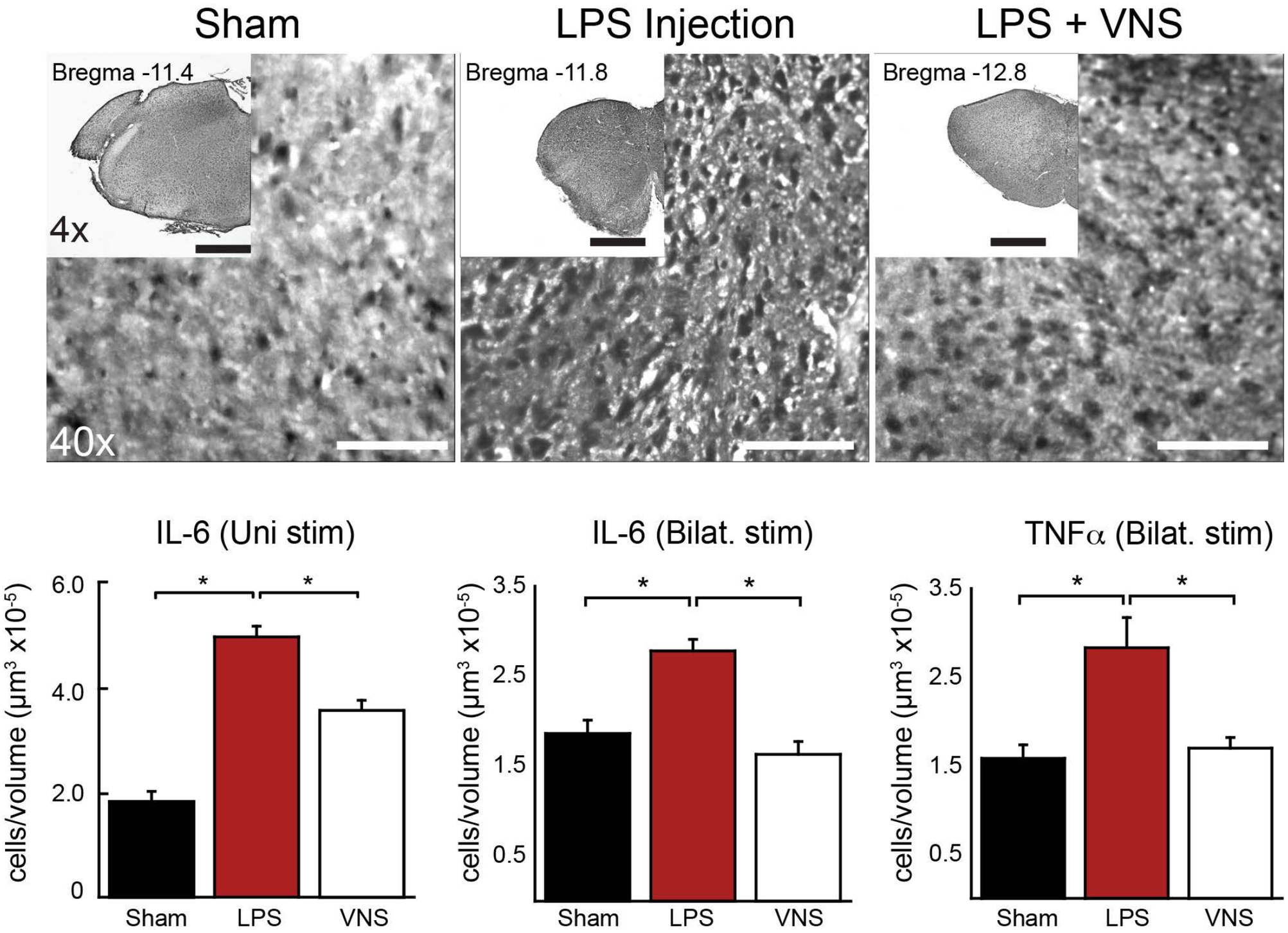

LPS
instillation
(0.5 µg/g
in 10µL
saline)
Treatment
Transcardial
perfusion OR tissue harvest
CNS removal for IHC/mol. bio.,
RNASeq, electrophysiology.
(Aims 1 and 2)
2 to 4%
isoflurane
0 – 120 min
Rat
(anesthetized at
time 0)
P10 to P12
day old
rat pups
(VNS Stim, 30 min)
For VNS+LPS
treatment
group
Treatments include:
PGE2, EP3 agonist/antagonist
+ 10% O2 (hypoxia) before and after
blockers.

Methods





IL-6 and TNFα are reduced after VNS

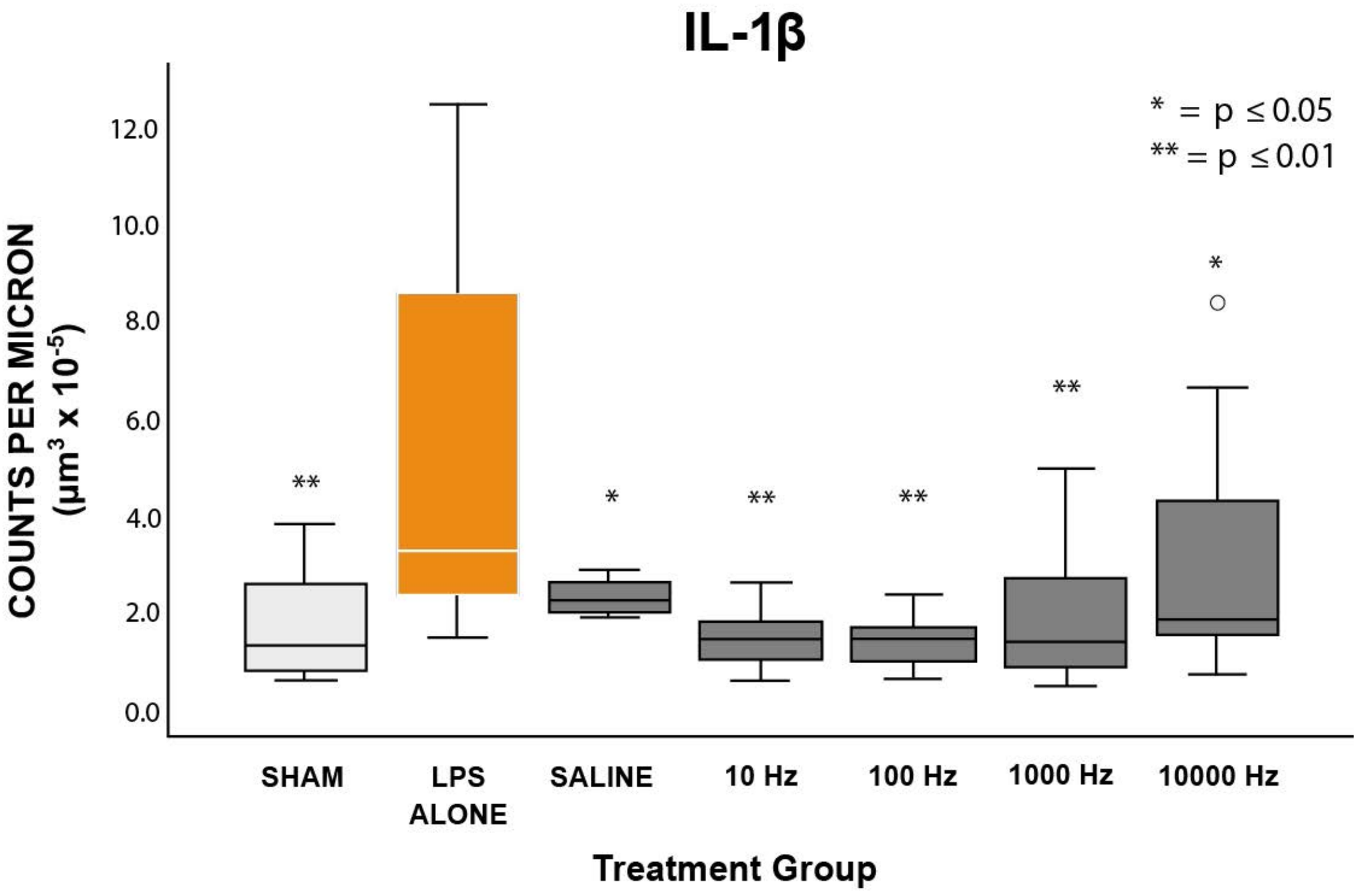


Johnson et al. *Resp Physiol Neurobiol*, 2016

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

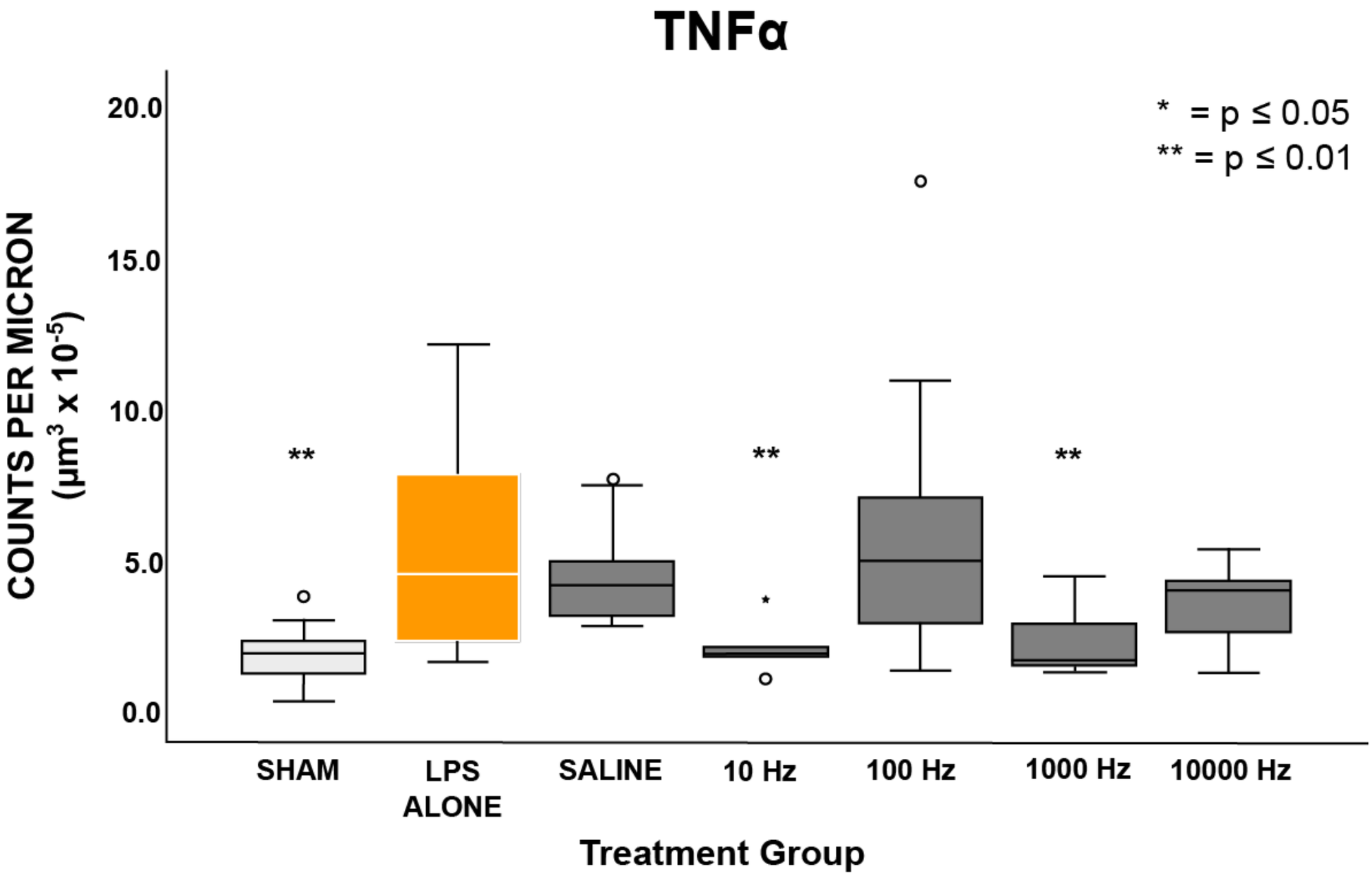
But, what are the OPTIMAL stimulation parameters to reduce inflammation?

VNS attenuates IL-1β across most frequencies



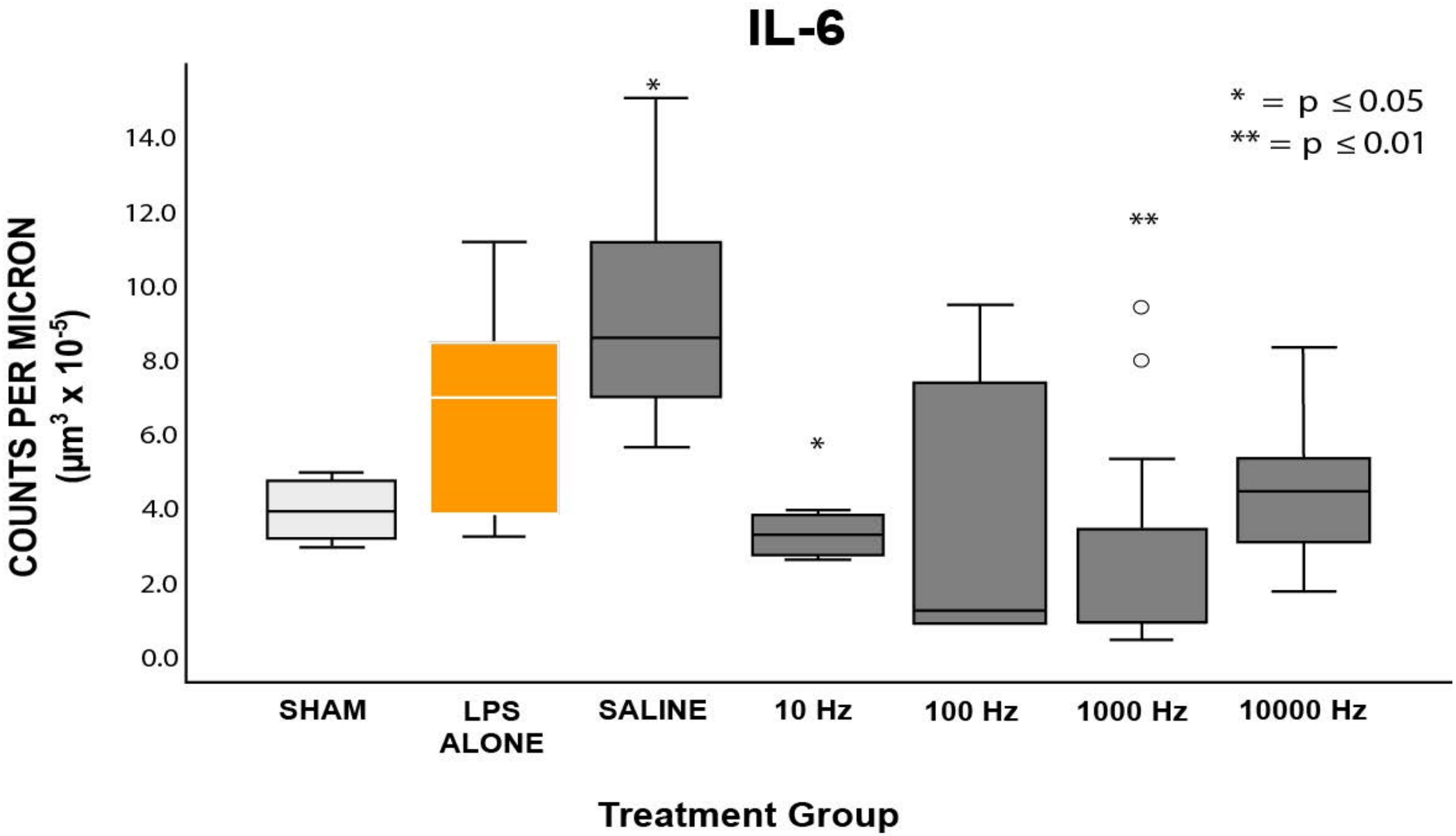
Cacho et al. submitted to *Peds Research*

VNS attenuates TNFα at higher stimulation frequencies



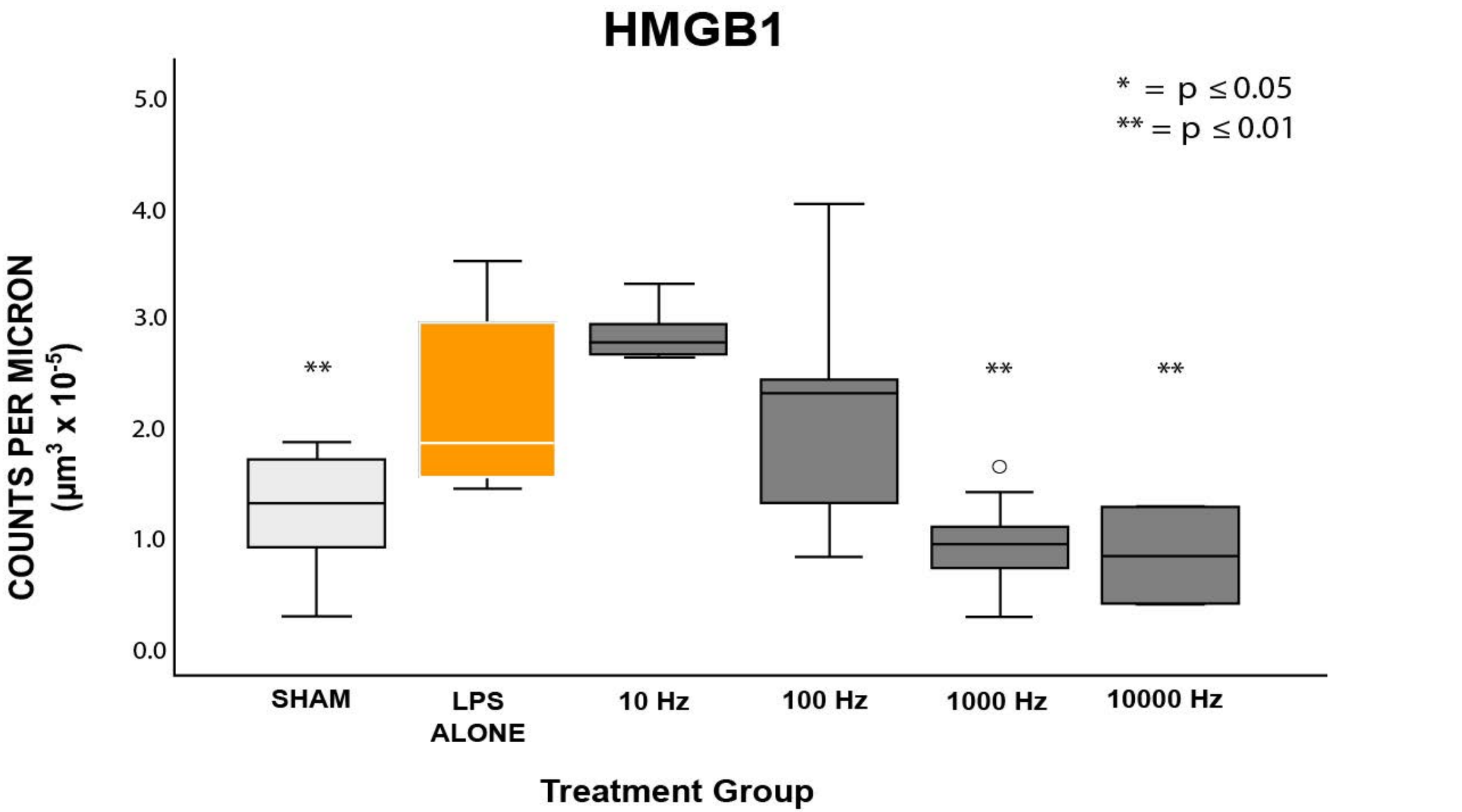
Cacho et al. submitted to *Peds Research*

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*

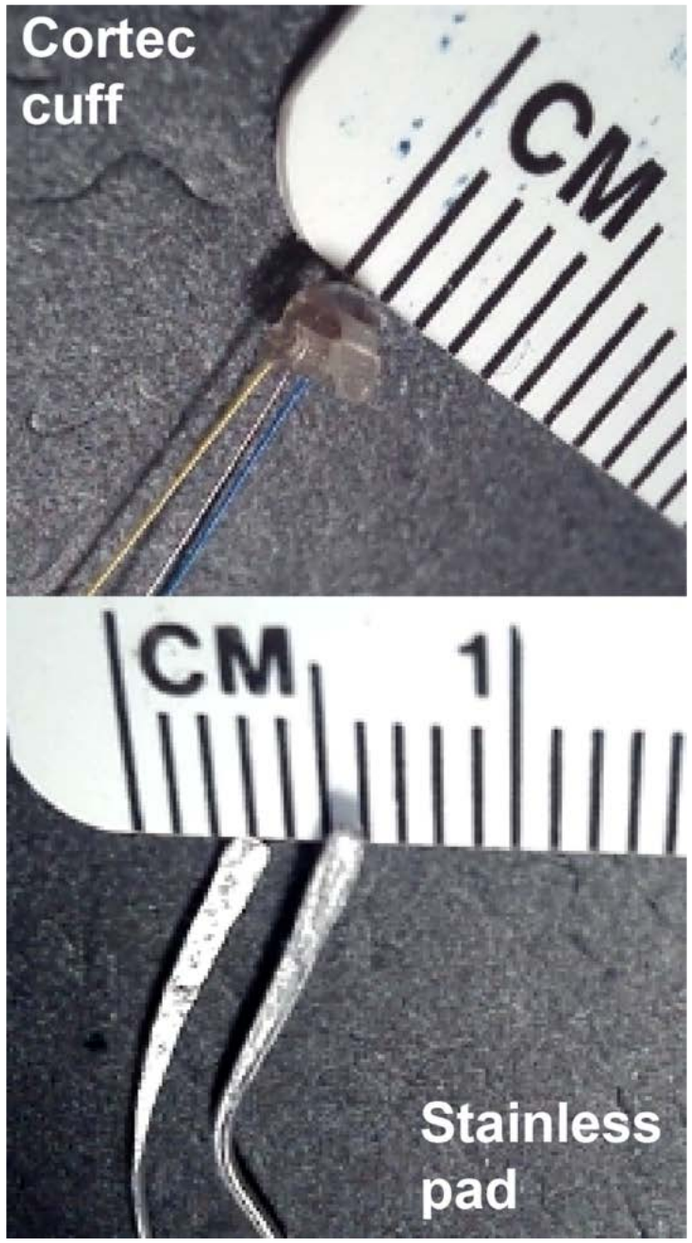
Future Directions

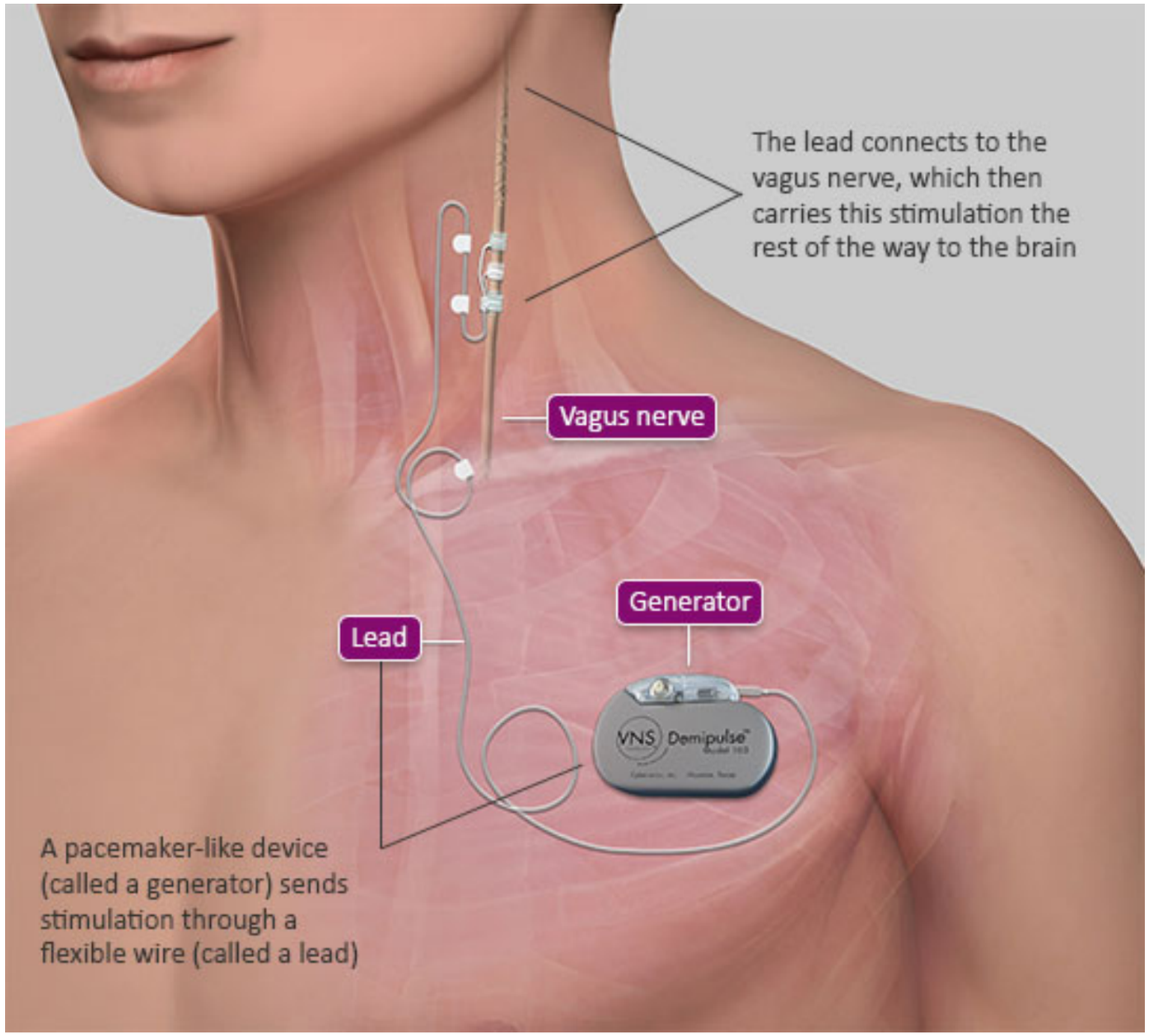
- The likelihood that we will get IRB approval to implant a vagus nerve stimulator in a preterm infant is *vanishingly small!*

- 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.

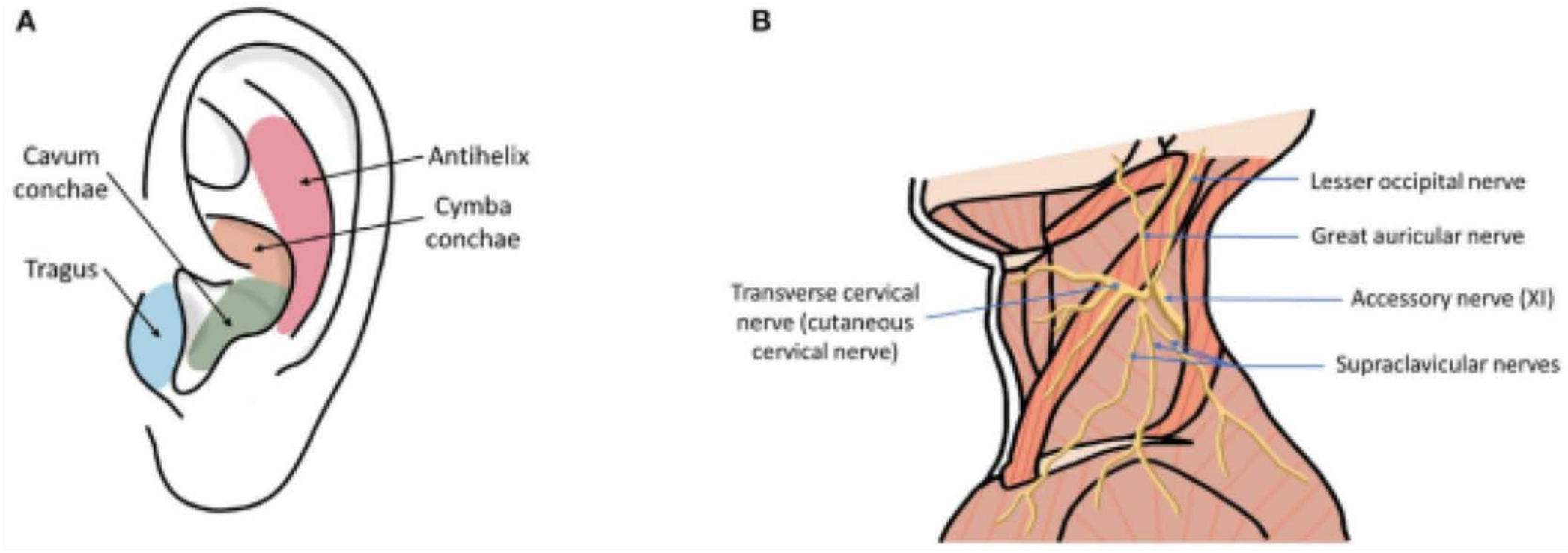
- An even more interesting option in the clinic would be the use of *transcutaneous auricular vagus nerve stimulation* (aVNS) which is non-invasive and easy to use in a clinical setting.

Can we modify the method of VNS to use non-invasive stimulators?





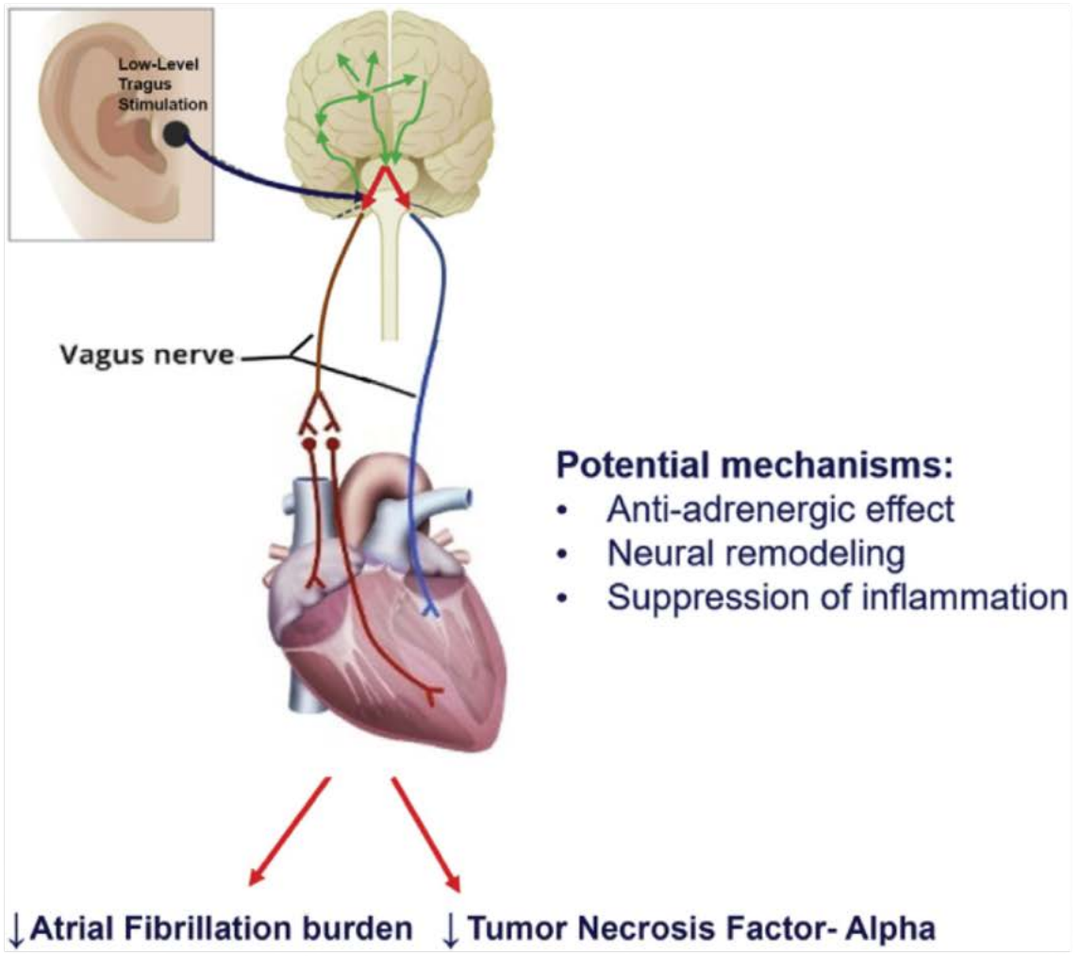
Transcutaneous Auricular Vagus Nerve Stimulation (aVNS)



Yap JYY et al. *Front Neuroscience*, 2020

Transcutaneous auricular vagus stimulation

CENTRAL ILLUSTRATION Neural Pathways and Potential Mechanisms Involved in Neuromodulation Using Low Level Tragus Stimulation for Atrial Fibrillation

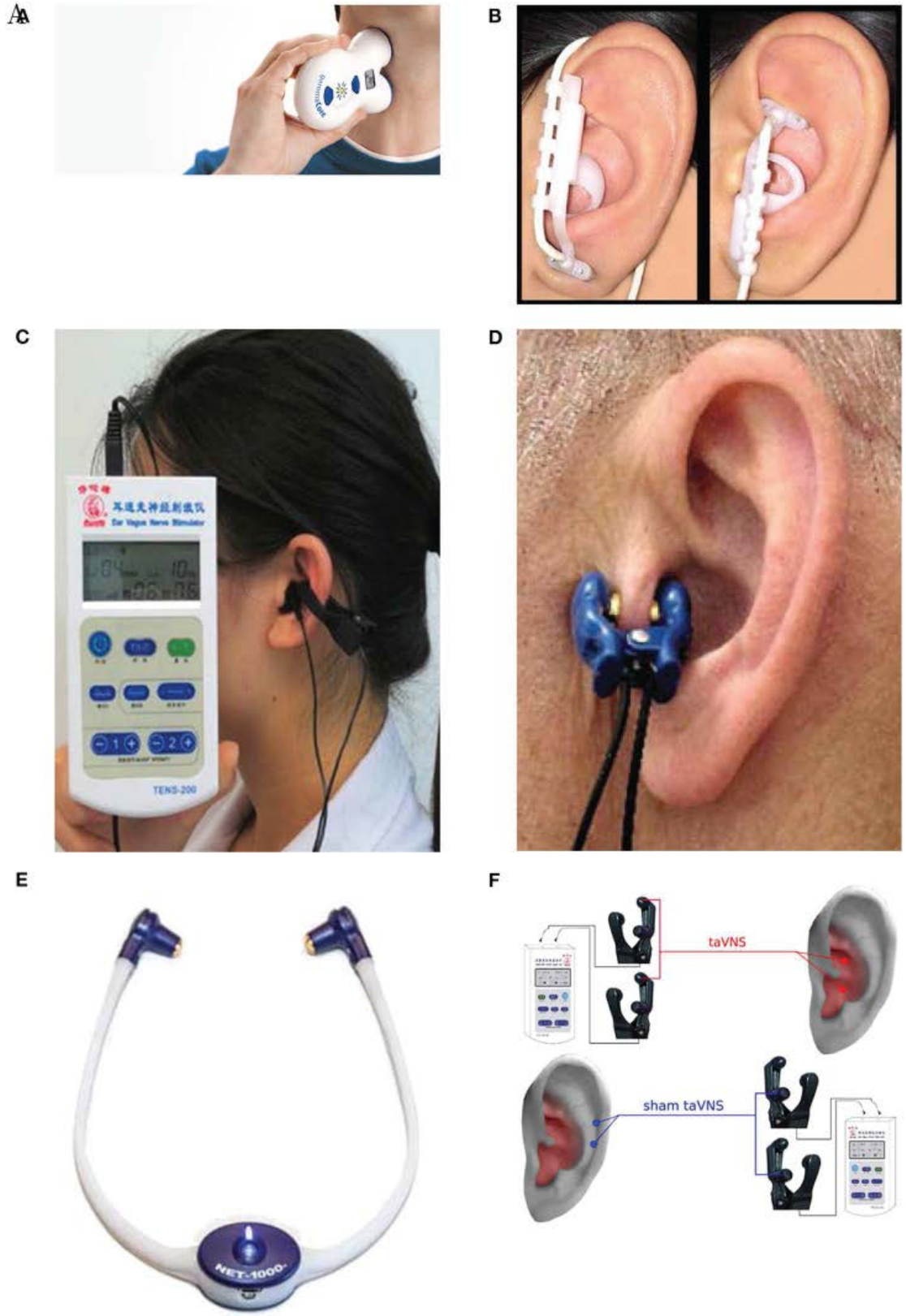


Stavrakis, S. et al. J Am Coll Cardiol EP. 2020;6(3):282–91.

Noninvasive neuromodulation using low-level tragus stimulation signifficantly 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.

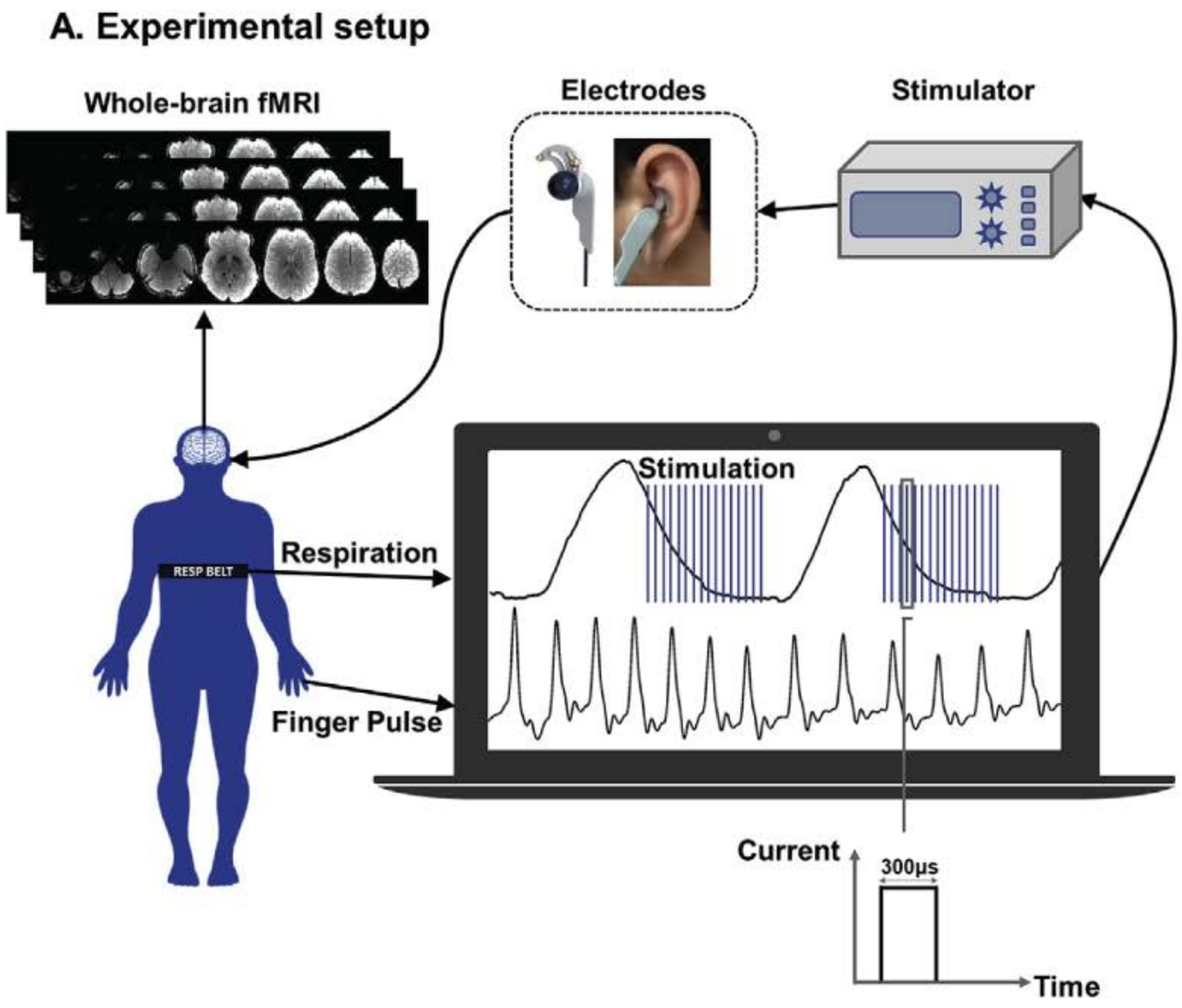
Stavrakos S et al., *JACC: Clinical Electrophysiology*, 2020

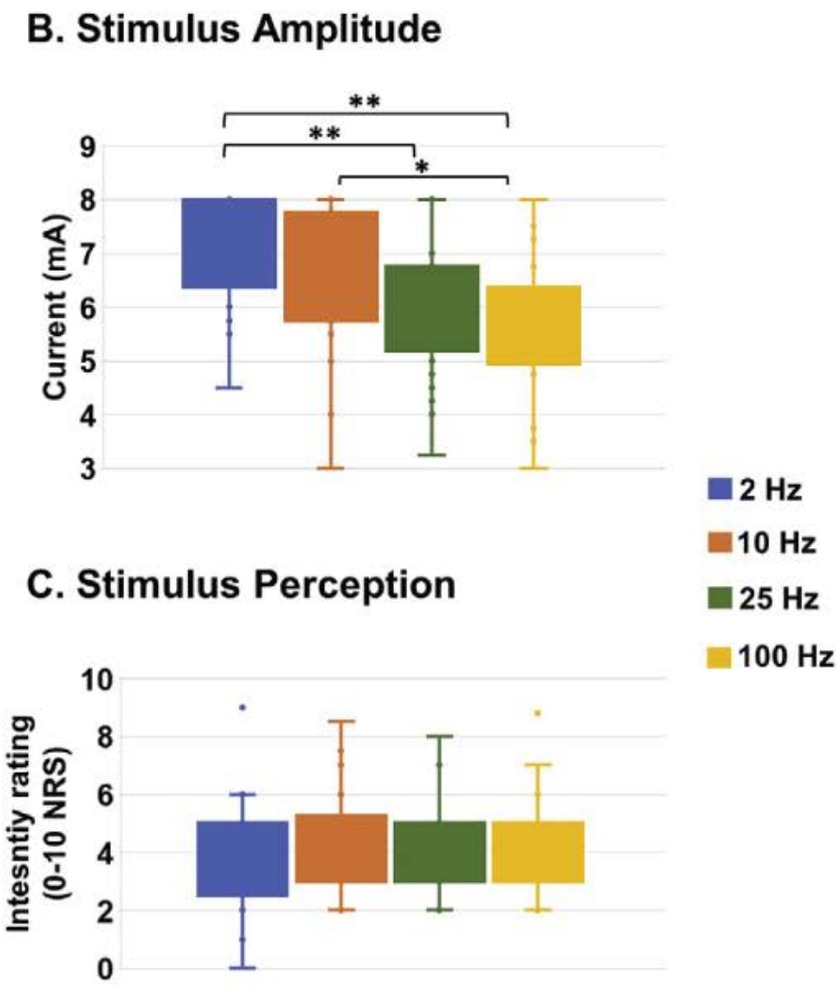
aVNS stimulators



Yap JYY et al. *Front Neuroscience*, 2020

aVNS protocols that replicate some of our work….





Sclocco R et al. *Brain Stimulation,* 2020

Summary

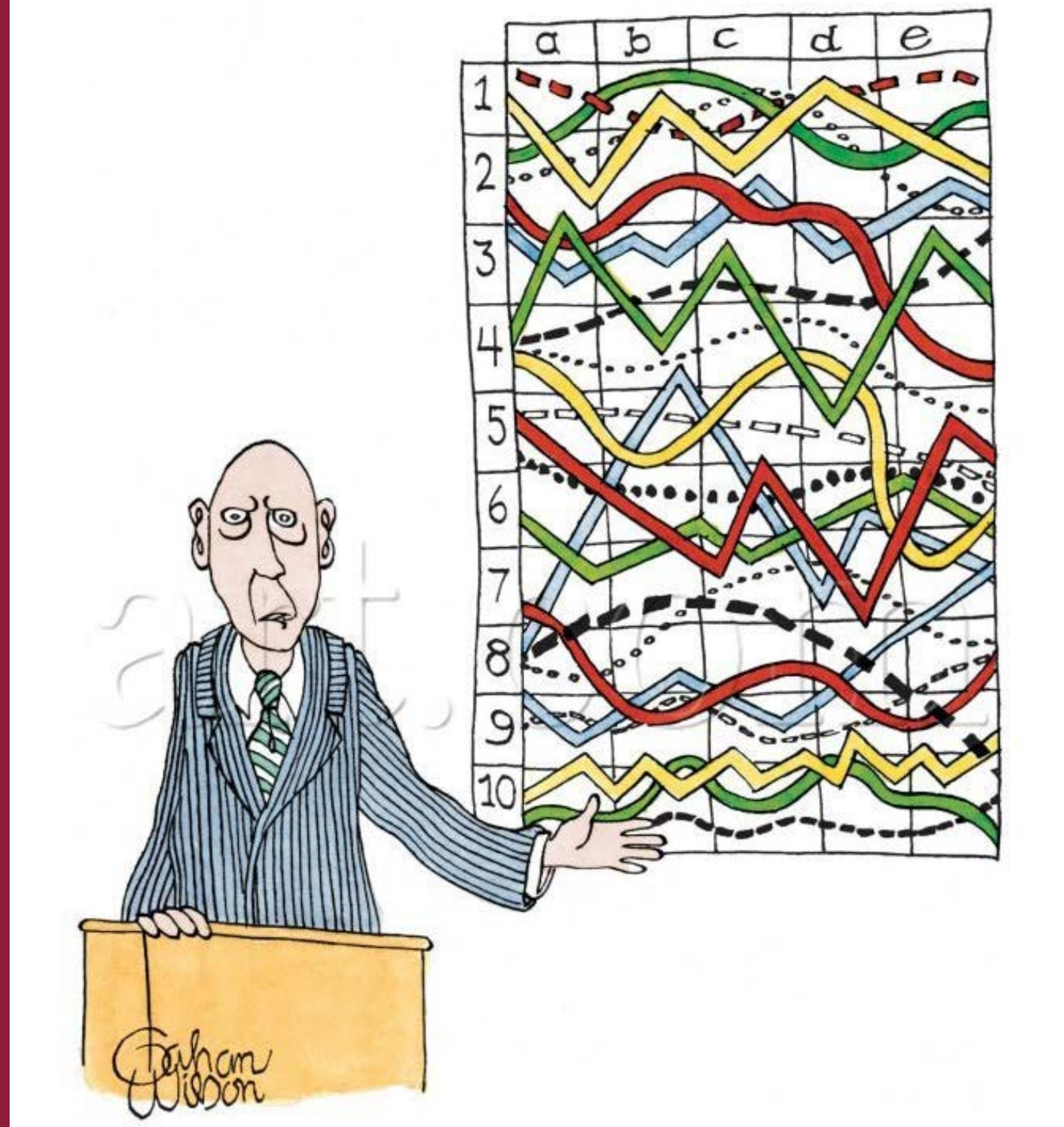
- Our laboratory has been focused on translational applications of developmental neurophysiology in neonates.

- 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??



"I'll pause for a moment so you can let this information sink in."

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