

# Risk Factors That Predispose Patients to Orofacial Pain



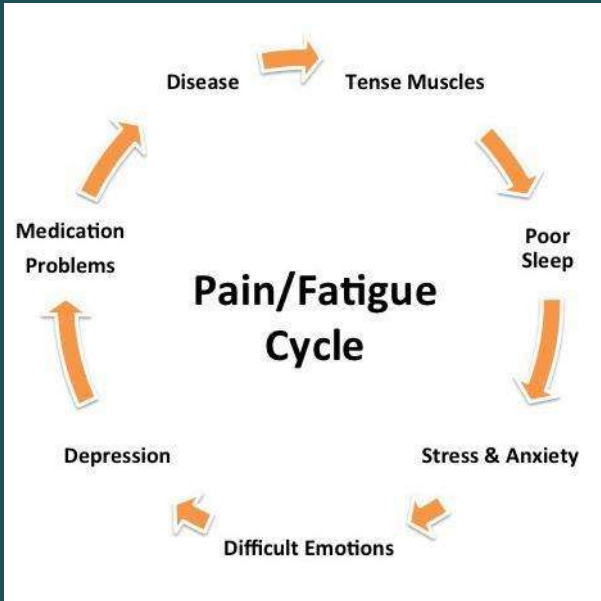
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Director

Center for Biomedical  
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Cluster Headache



Migraine



TMJ Disorders



Allergic Rhinitis



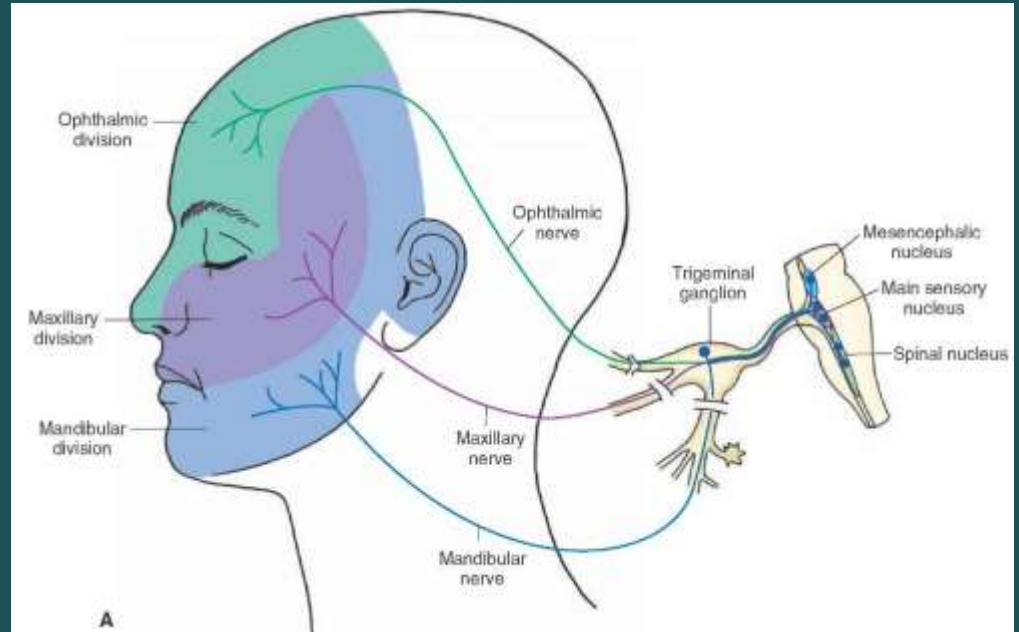
Tooth Ache



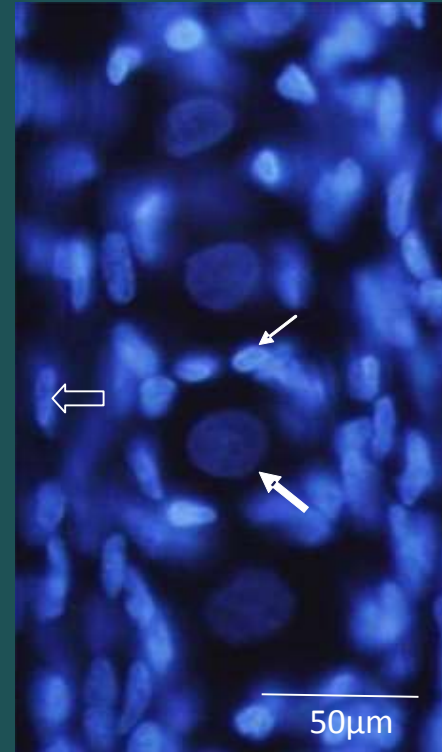
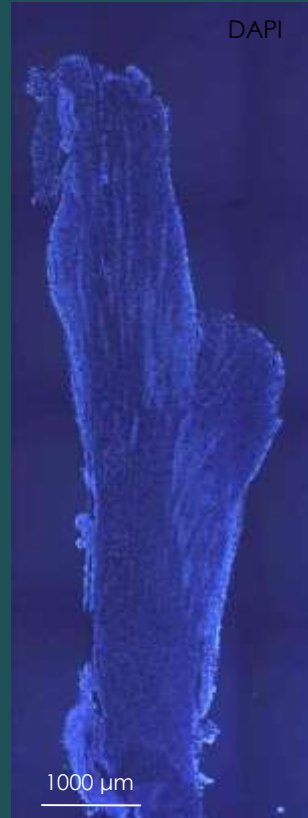
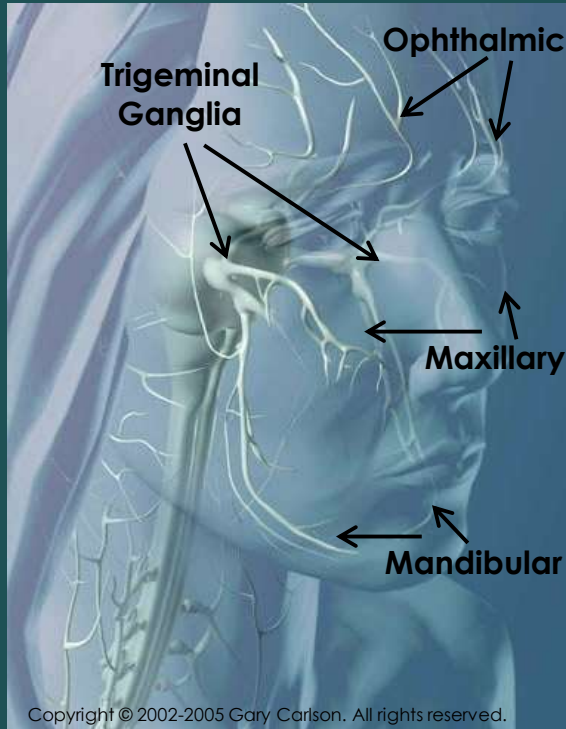
Trigeminal Neuralgia

# Trigeminal Nerve and Ganglion

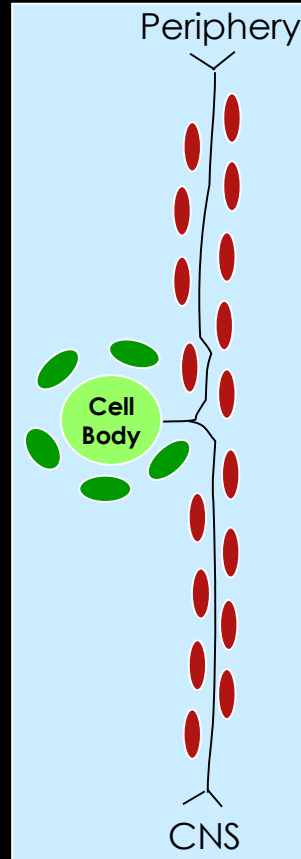
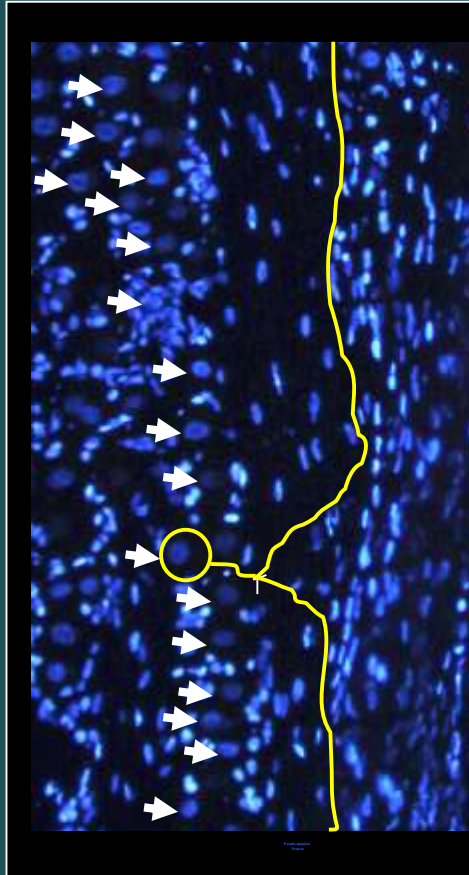
- Fifth cranial nerve- innervates head and face
- Comprised of three main divisions:
  - ophthalmic (V1)
  - maxillary (V2)
  - mandibular (V3)
- Cell bodies located in ganglion
- Cell types: Neurons, Schwann cells, satellite glia, macrophages
- Neurons may be A $\delta$  (myelinated, fast) or C (unmyelinated, slow) fibers
- Activated in response to thermal, mechanical, or chemical stimuli



# Trigeminal Ganglion Nerves and Cellular Organization – Functional Units Comprised of Neurons and Satellite Glial Cells



# Trigeminal Ganglion - Comprised of Neurons and Glia



> 90% of cells are glia  
- “glue” or “slime”

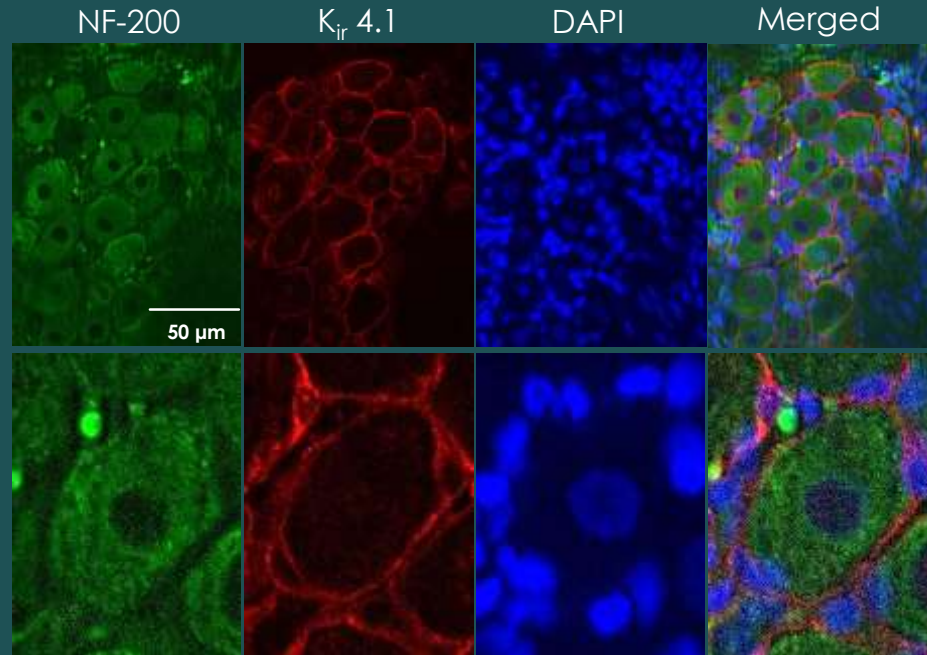
2 types of glia in ganglion:

1. Schwann cells  
- produce myelin
2. Satellite cells  
- release cytokines  
- modulate neuronal excitability and activation threshold

CNS diseases:

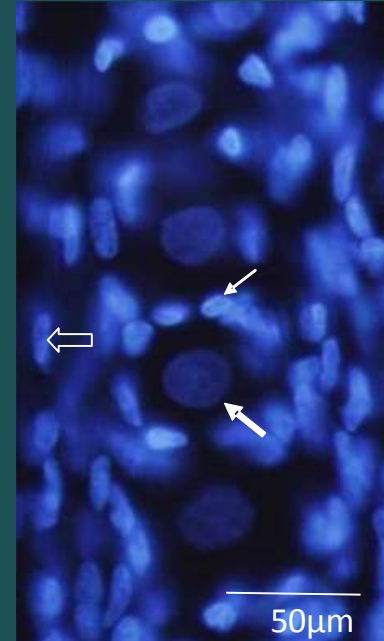
- involved in all stages of inflammation & pain
- involved in allodynia hyperalgesia

# Functional Units: Neuronal Cell Bodies and Associated Satellite Glia Cells



# Glial Cells Overview

- ▶ Function of Glial Cells
  - ▶ Glial cells “monitor” environment around neurons
    - ▶ Decrease excitatory molecules
    - ▶ Modulate state of neuronal excitability
- ▶ When glial cells are “abnormally” activated:  
release many inflammatory molecules
  - ▶ Lower activation threshold – cause sensitization
  - ▶ Contribute to hyperalgesia and potentially chronic pain
  - ▶ **CGRP** involved in initiating this nociceptive cascade



# Neuronal-Glial Cell Interactions in Pain

- ▶ Glial Cells of Peripheral and Central Nervous Systems

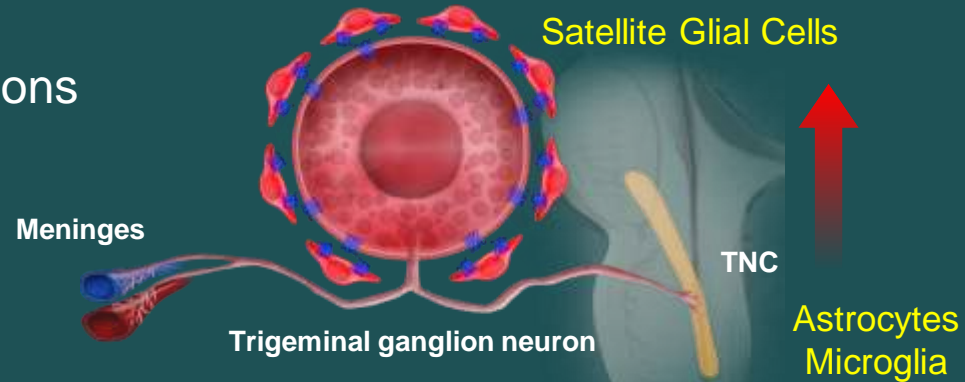
  - ▶ Role in sensitization/nociception

- ▶ Peripheral Glial Cells (Trigeminal Ganglion)

  - ▶ Satellite Glial Cells

- ▶ Central Glial Cells (TNC and other regions of spinal cord and brain)

  - ▶ Astrocytes and Microglia





  - ▶ Peripheral sensitization – activation of satellite glial cells

  - ▶ Persistent central sensitization and chronic pain are associated with activation of astrocytes and microglia



# Increased S100B Expression in Trigeminal Ganglion Neurons and Glia in Response to Capsaicin

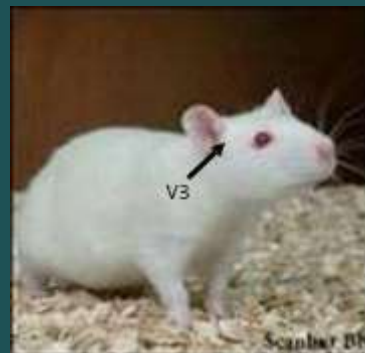
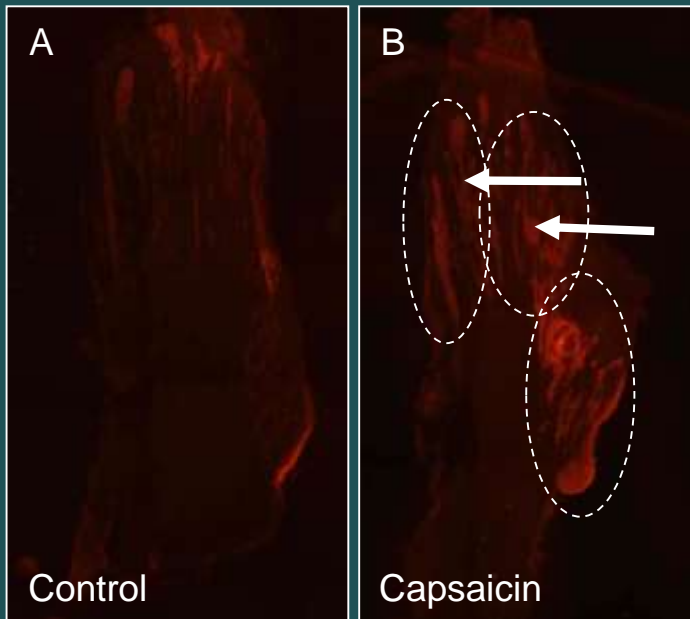
## S100B:

Calcium binding protein

Expressed in both neurons and glia

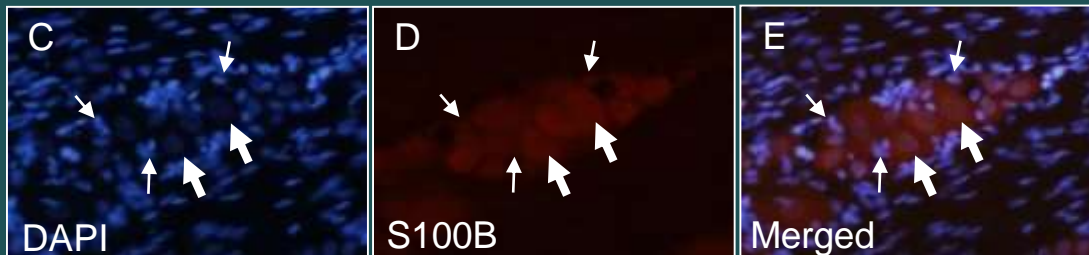
Secreted – role in inflammation

Marker of disease



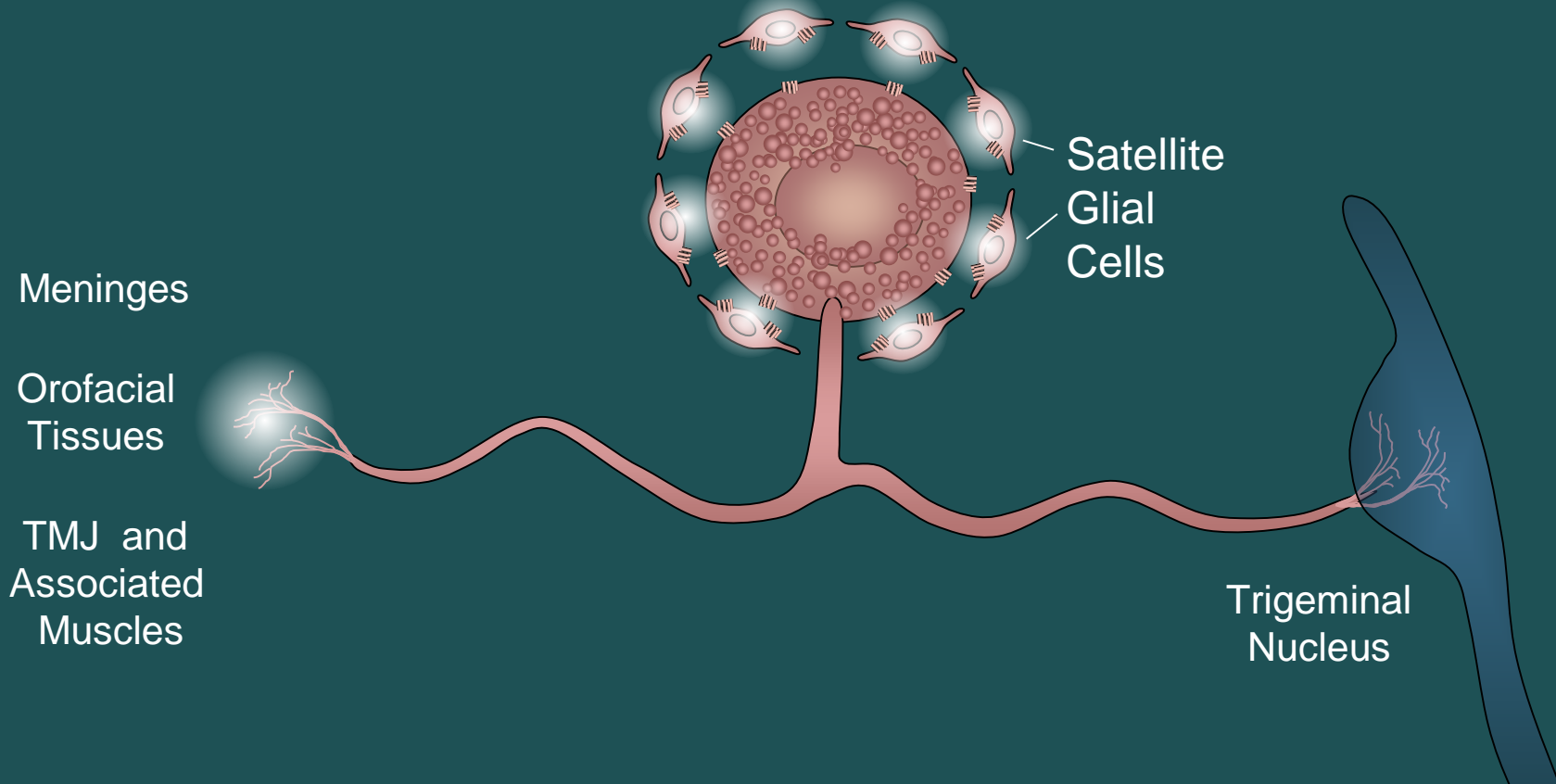
Capsaicin in TMJ capsule - 2 hrs

Thalakoti et al., Headache, 2007

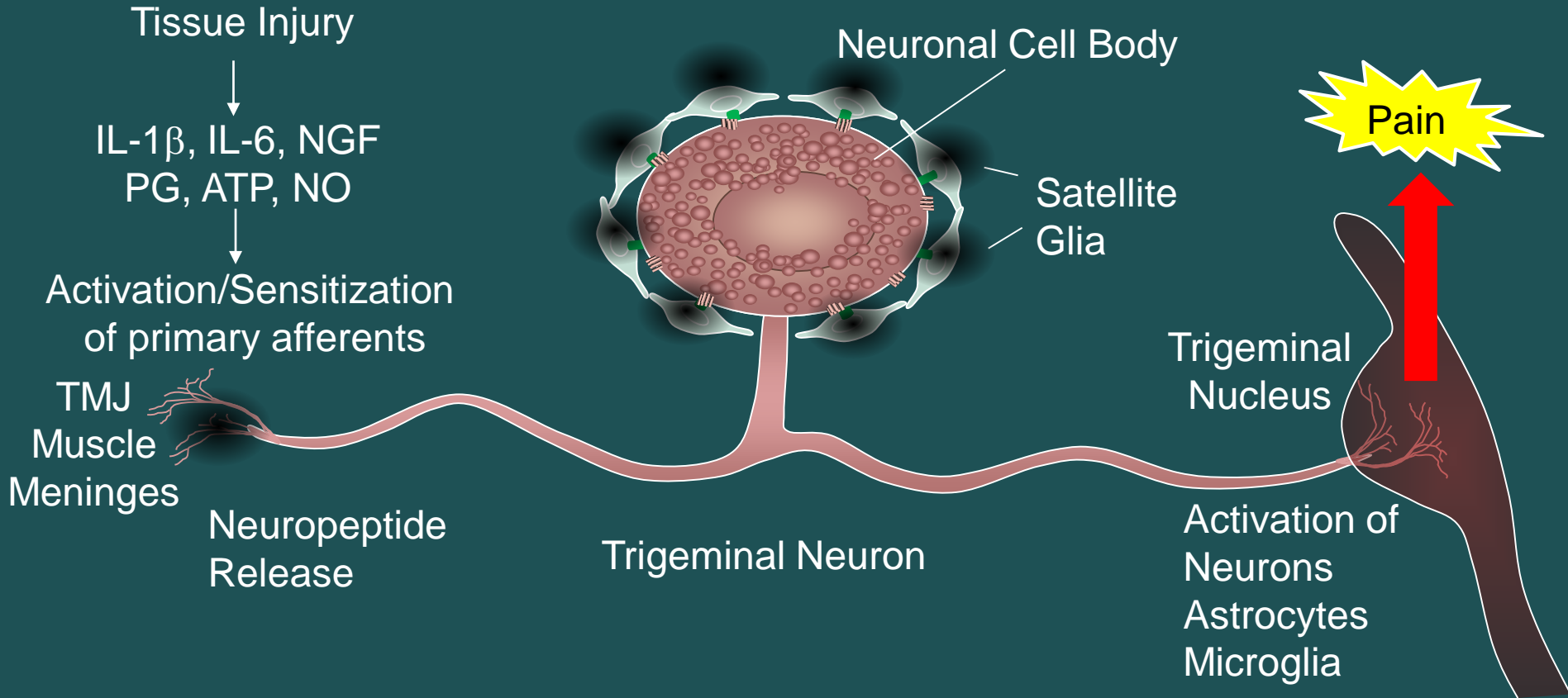


# Trigeminal Neurons and Satellite Glial Cells: Pathway for Pain Transmission

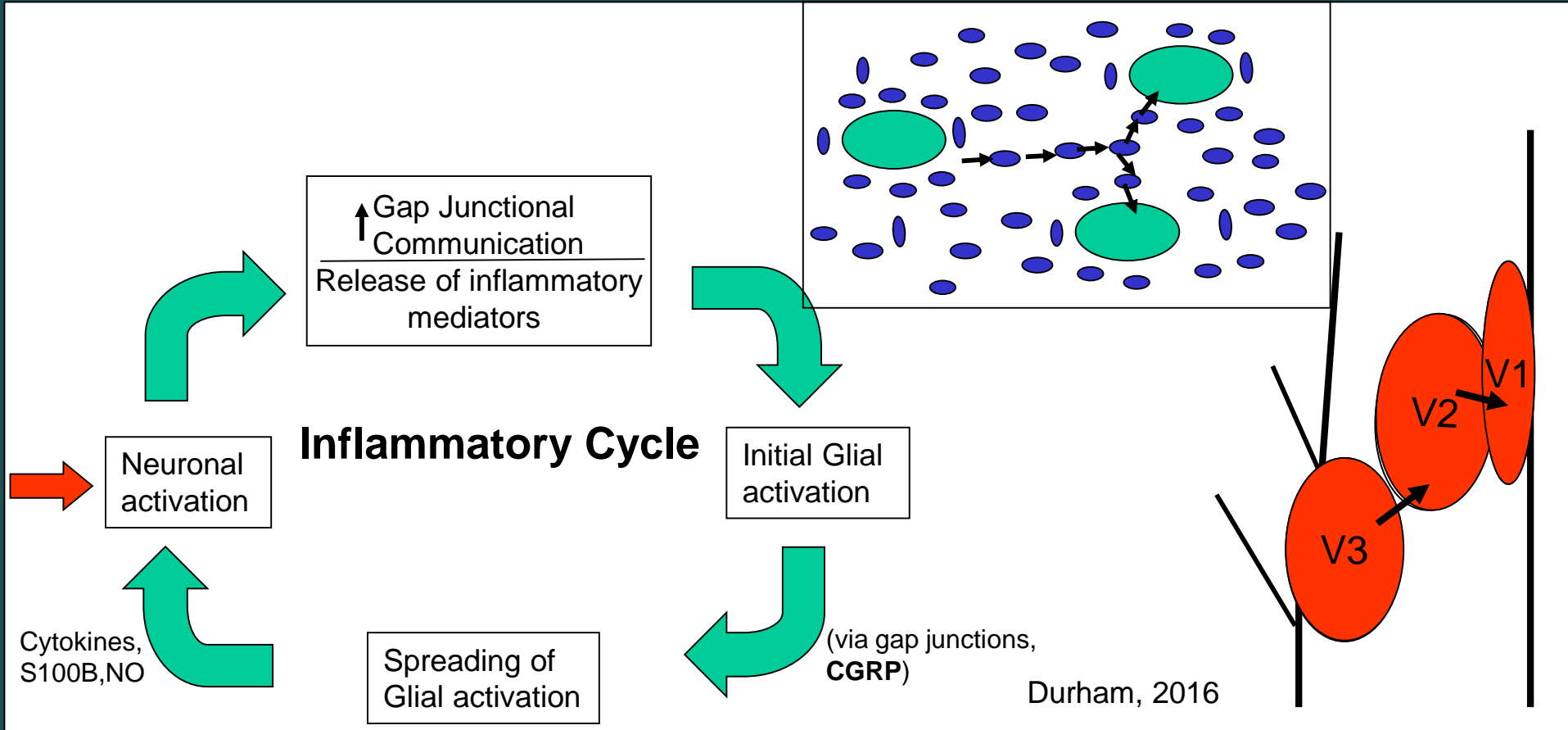
Trigeminal Ganglion Neuron



# Migraine-TMD-TN Pathology - Increased Expression of Pro-Inflammatory/Nociceptive Signaling Molecules and Increased Neuron-Satellite Glial Cell Communication

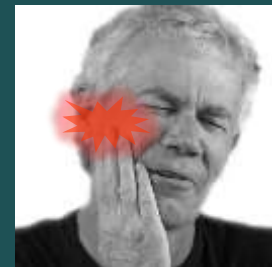
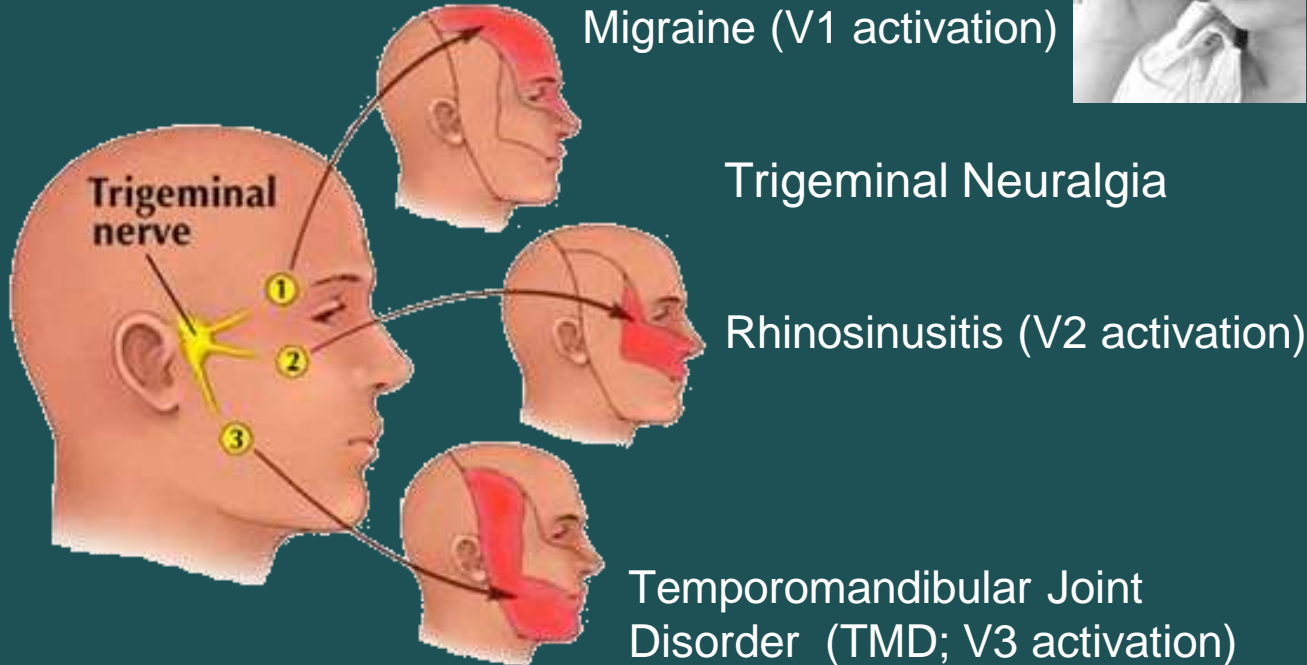


# Neuronal-Glial Cell Interactions: Possible Role in Initiation and Maintenance of Peripheral Sensitization and Priming

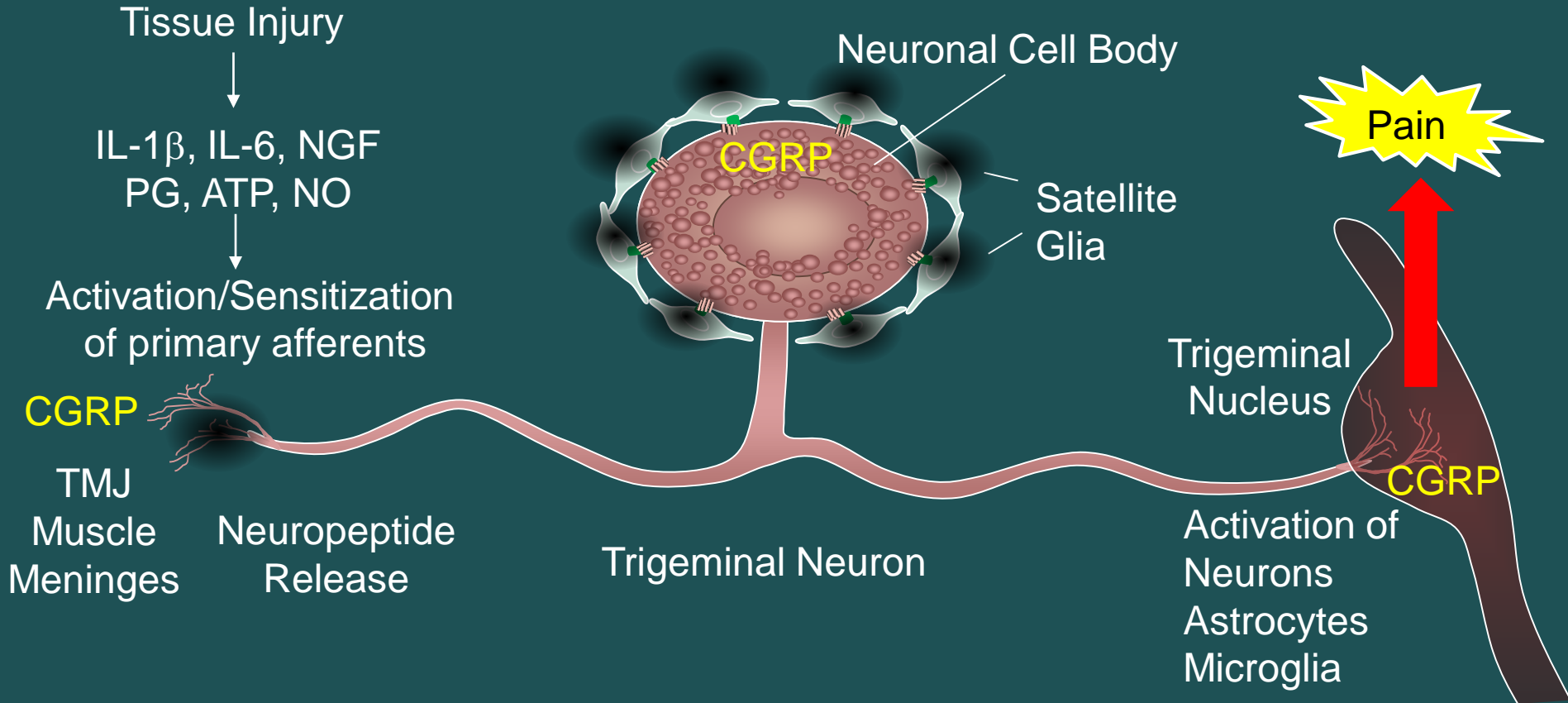


# Trigeminal Nerve Pathologies – High Comorbidity

Increased neuron-glia signaling in ganglion (convergence) may provide explanation for this clinical observation



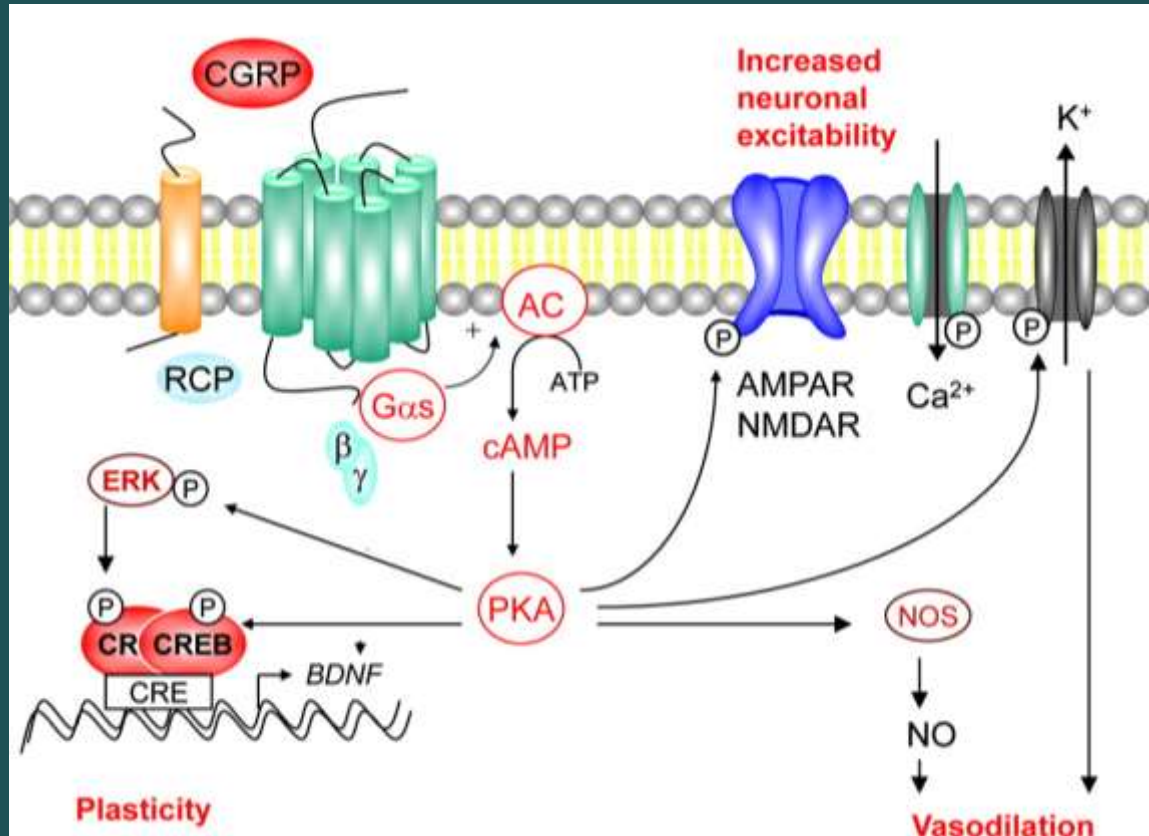
# Migraine-TMD-TN Pathology – Key Role of Calcitonin Gene-Related Peptide (CGRP)



# Calcitonin Gene-Related Peptide – Mechanism for Promoting Neuronal Sensitization

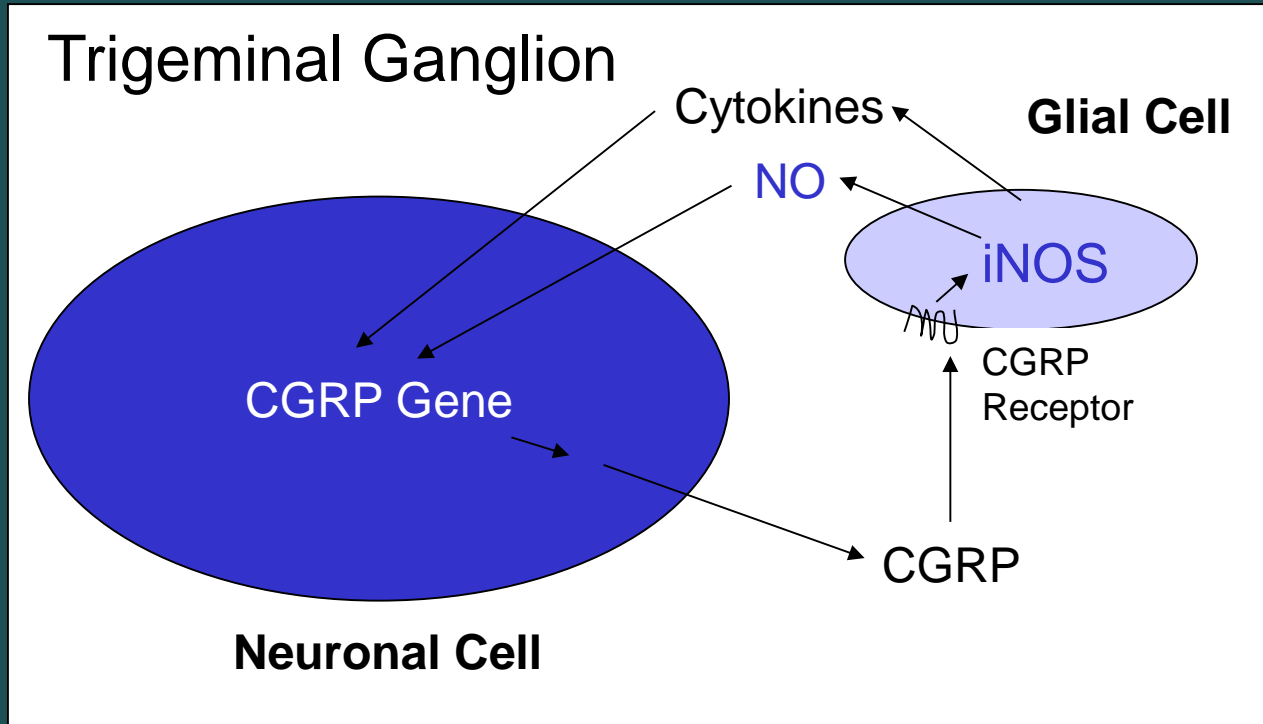
PKA =  
Protein  
Kinase A

Key role  
In lowering  
activation  
threshold  
of neurons



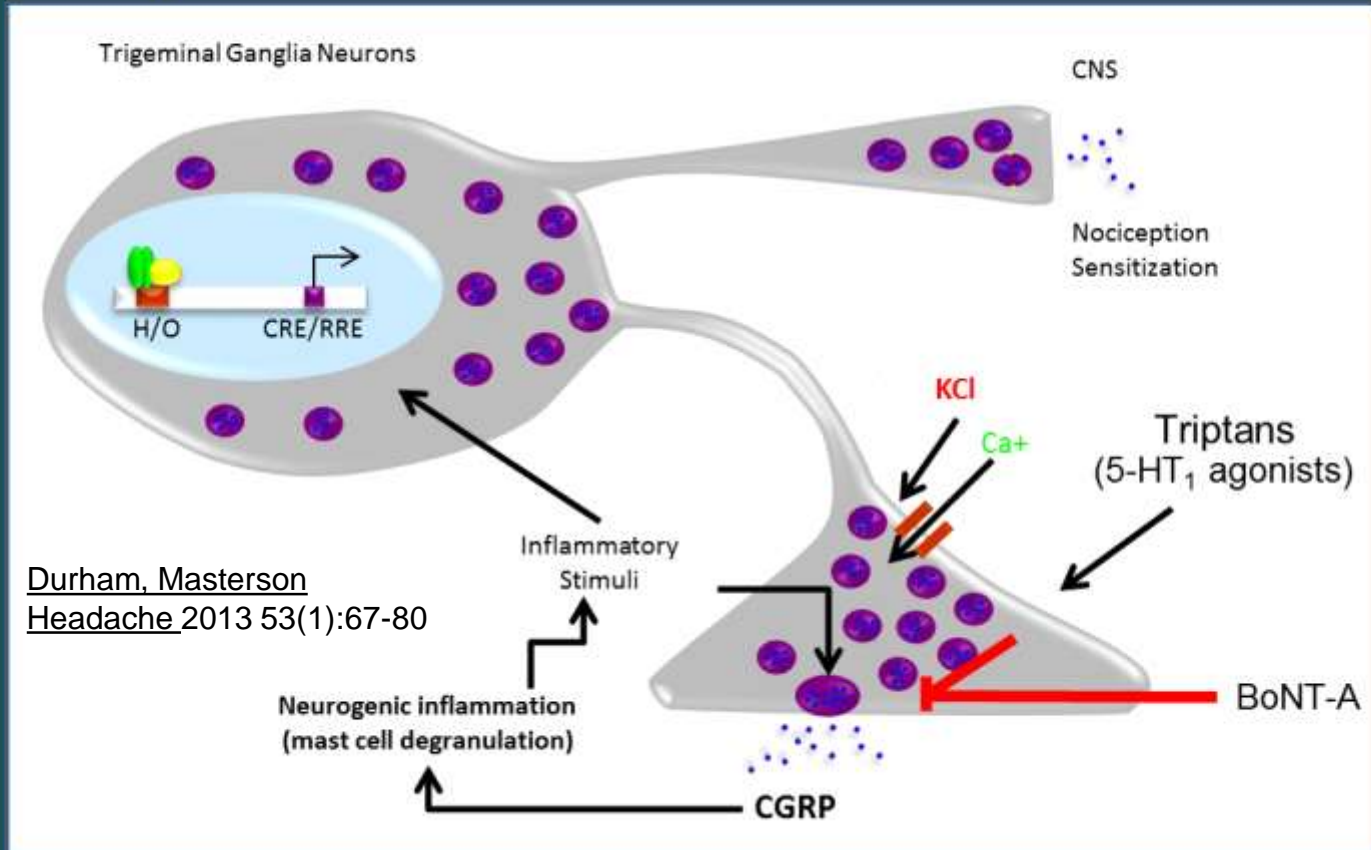
Blocking  
CGRP  
receptor  
activity inhibits  
trigeminal  
activation and  
sensitization

# CGRP Release from Neuronal Cell Body Promotes Peripheral Sensitization by Initiating an Inflammatory Loop



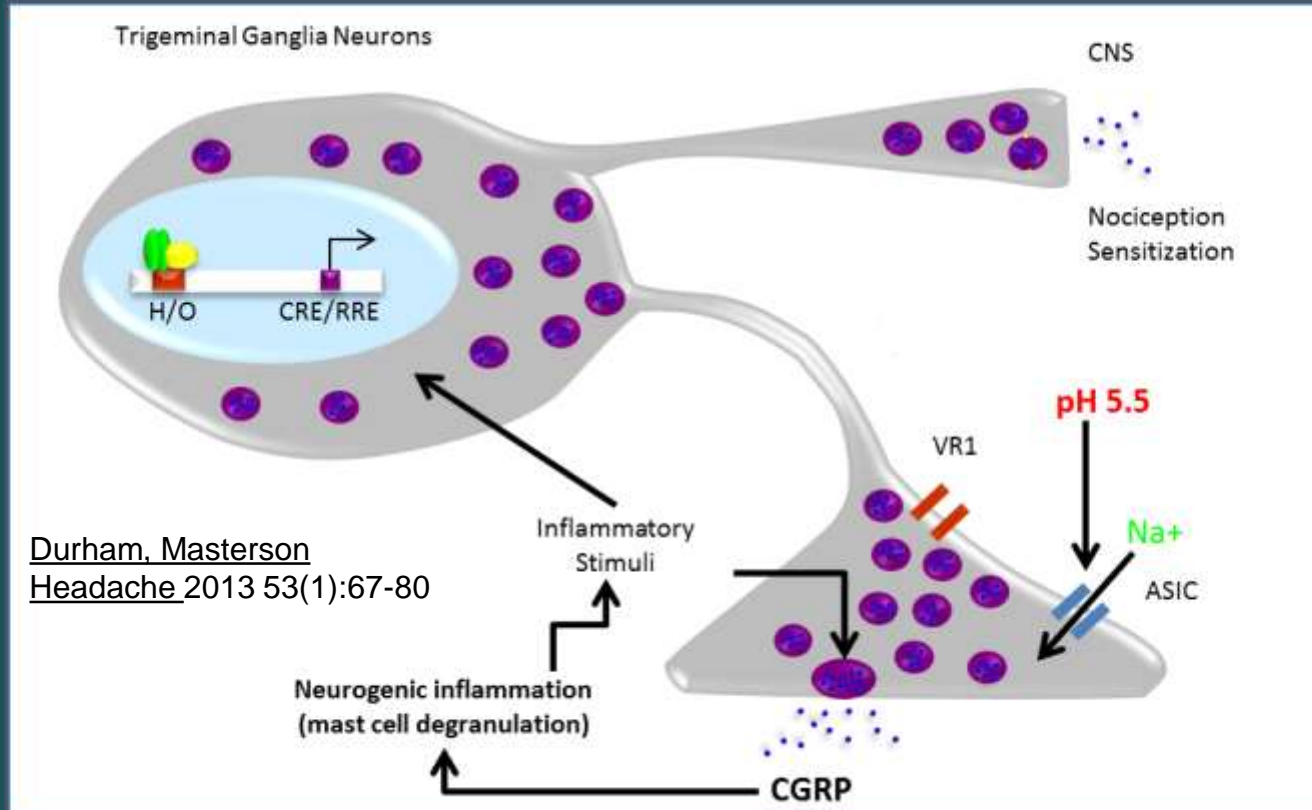


# Rizatriptan and Onabotulinumtoxin A Repress Calcium Dependent KCl-Stimulated CGRP Secretion



Durham, Masterson  
Headache 2013 53(1):67-80

# Rizatriptan and Onabotulinumtoxin A Do Not Repress Calcium Independent Proton Stimulated CGRP Secretion

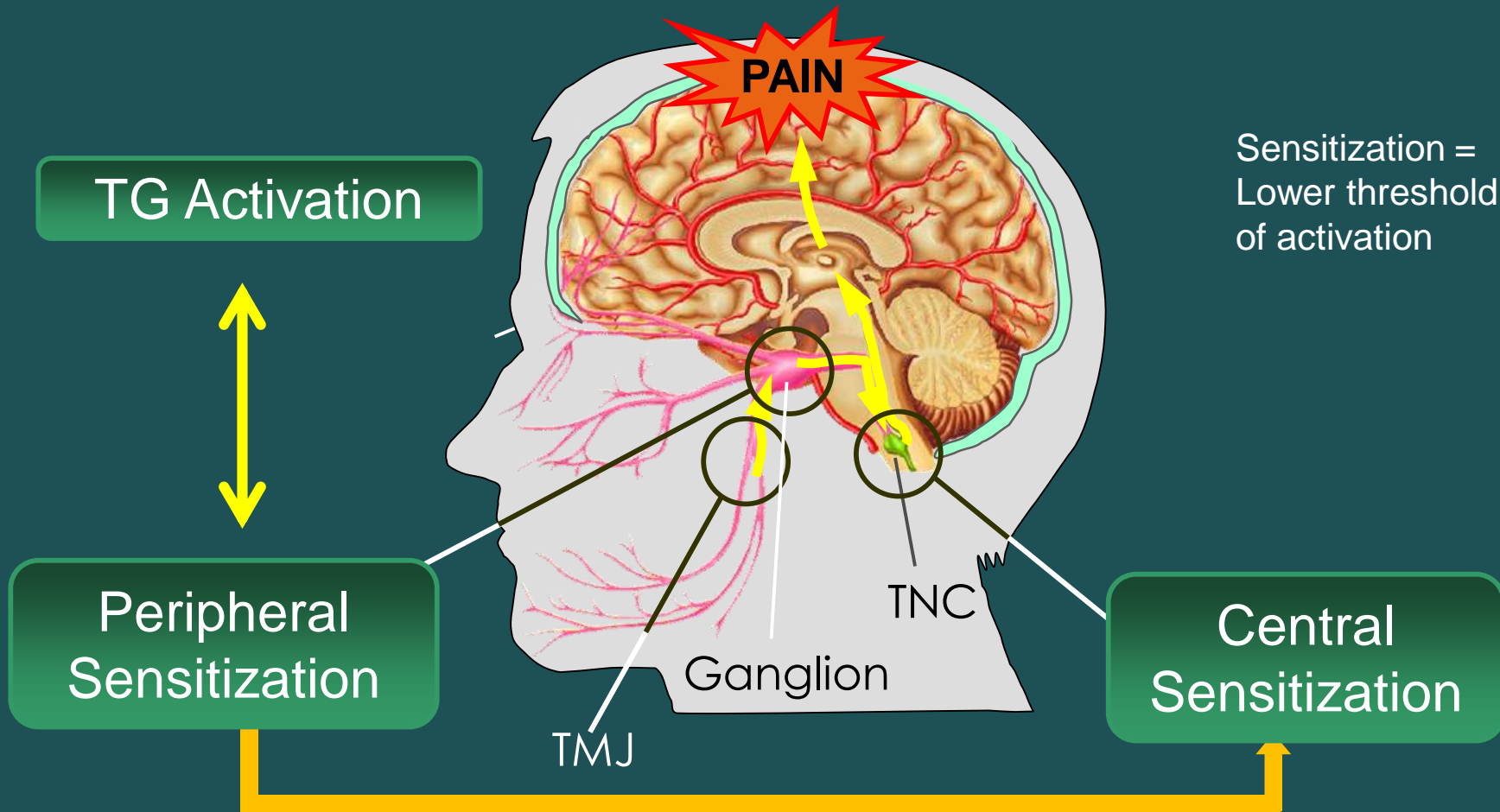


Durham, Masterson  
Headache 2013 53(1):67-80

# Summary and Conclusion

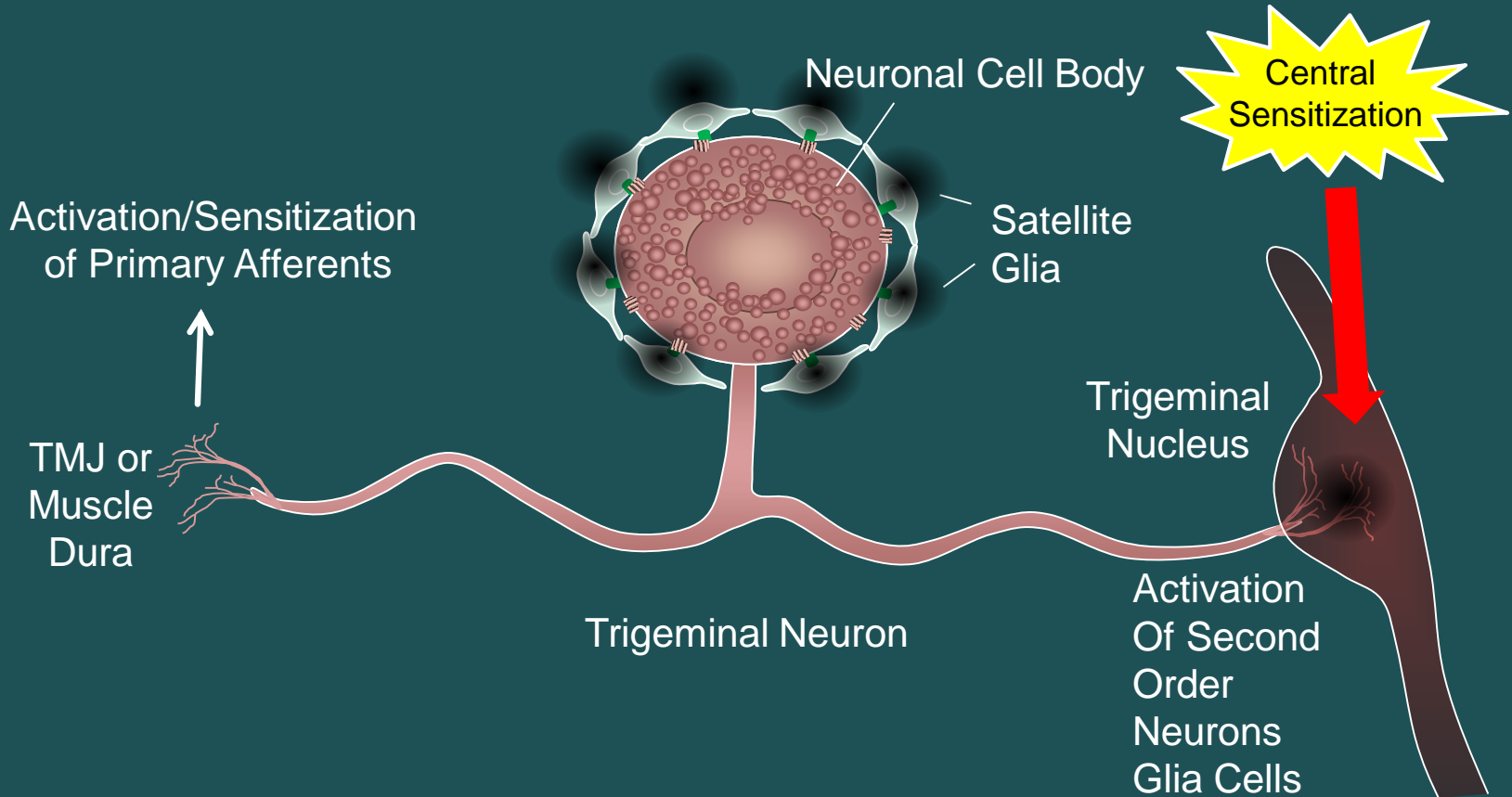
- ❖ Our results provide evidence of two distinct mechanisms involved in mediating the stimulated release of CGRP from trigeminal neurons
- ❖ One mechanism occurs via a calcium dependent mechanism that is blocked by the anti-migraine drugs rizatriptan and BoNT-A, while the other mechanism likely involves proton activation of ASICs and increases in intracellular sodium that was not repressed by Rizatriptan or BoNT-A
- ❖ Taken together, our findings provide evidence for a novel mechanism of calcium-independent CGRP release in response to protons that is not blocked by commonly used anti-migraine medications. (Amiloride)

# Neuronal Sensitization and Orofacial Pain

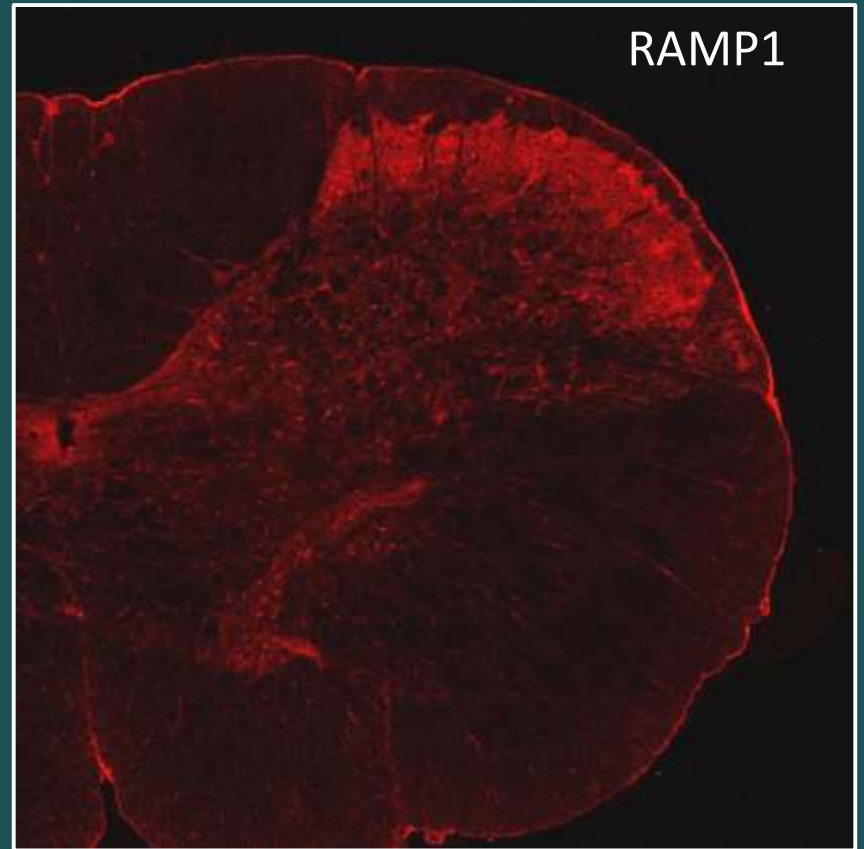


# Migraine and TMD Pathology

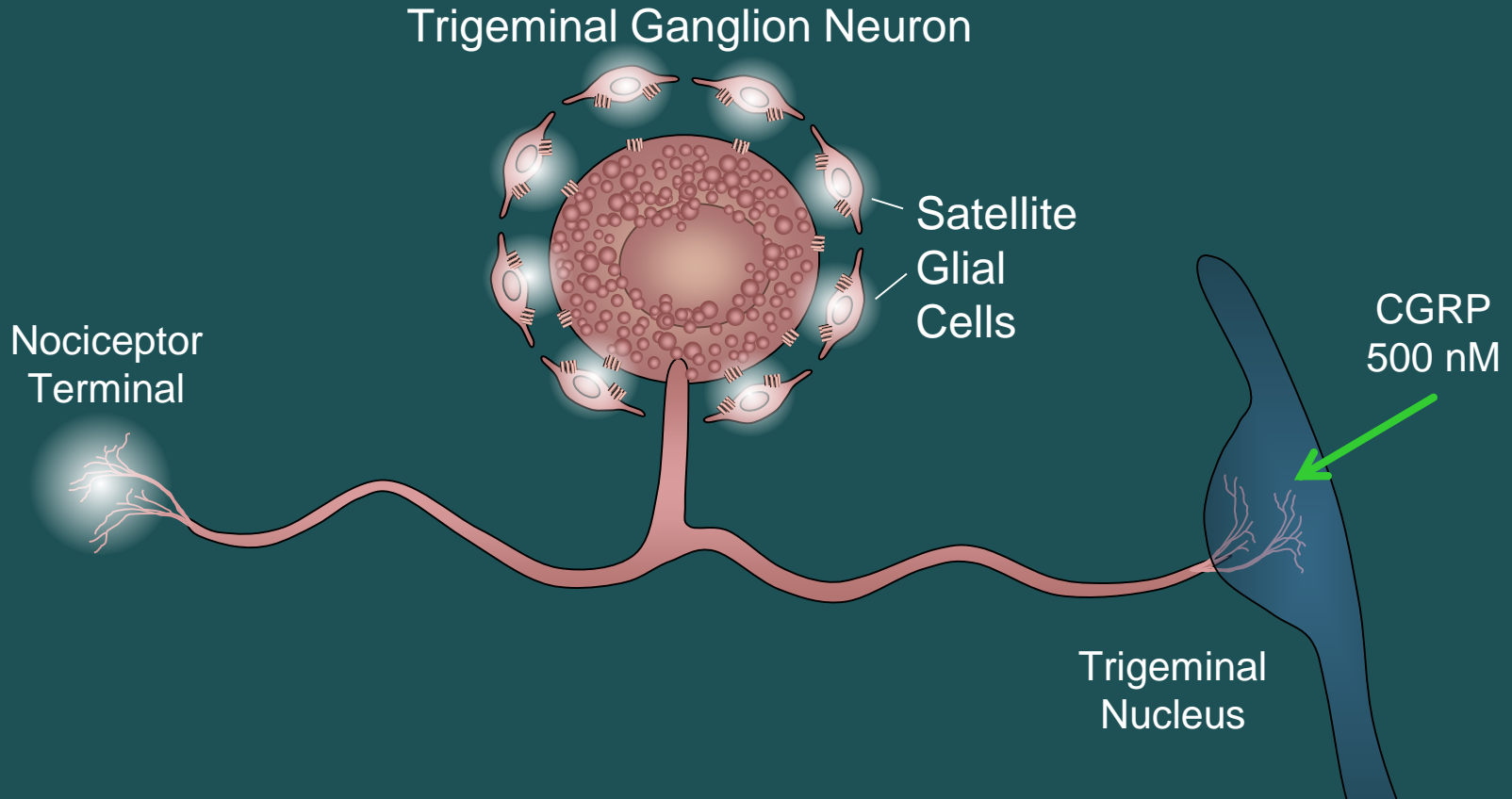
Can Central Sensitization Promote Peripheral Sensitization?



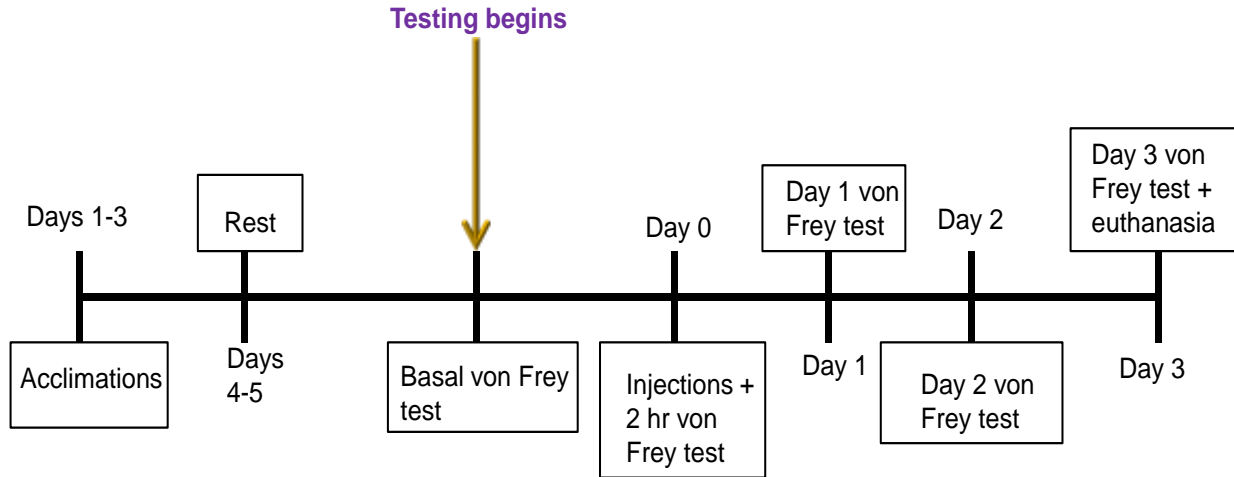
# CGRP and Receptor Protein RAMP1 are Abundantly Expressed in Medullary Dorsal Horn



# Can Elevated Levels of CGRP That Mediate Central Sensitization Also Promote Peripheral Sensitization?



# Sensitization to Mechanical Stimuli Behavior Experimental Timeline

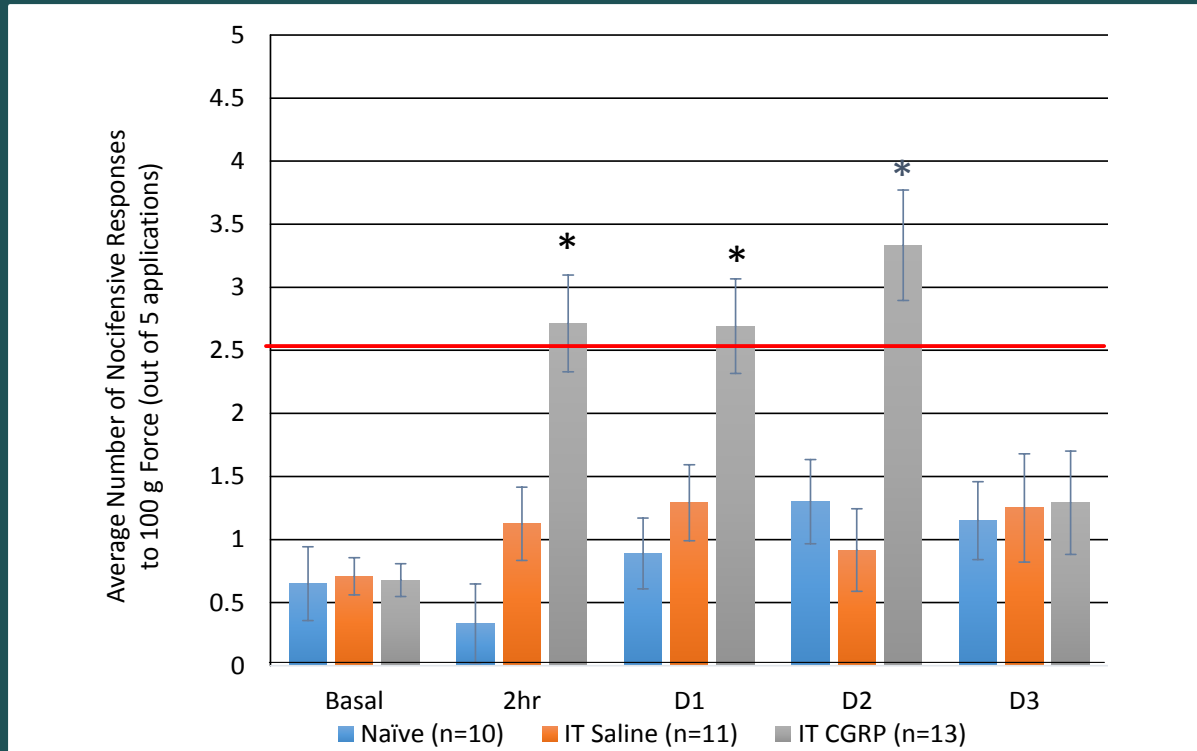


UGO Basile Durham Rat Holder



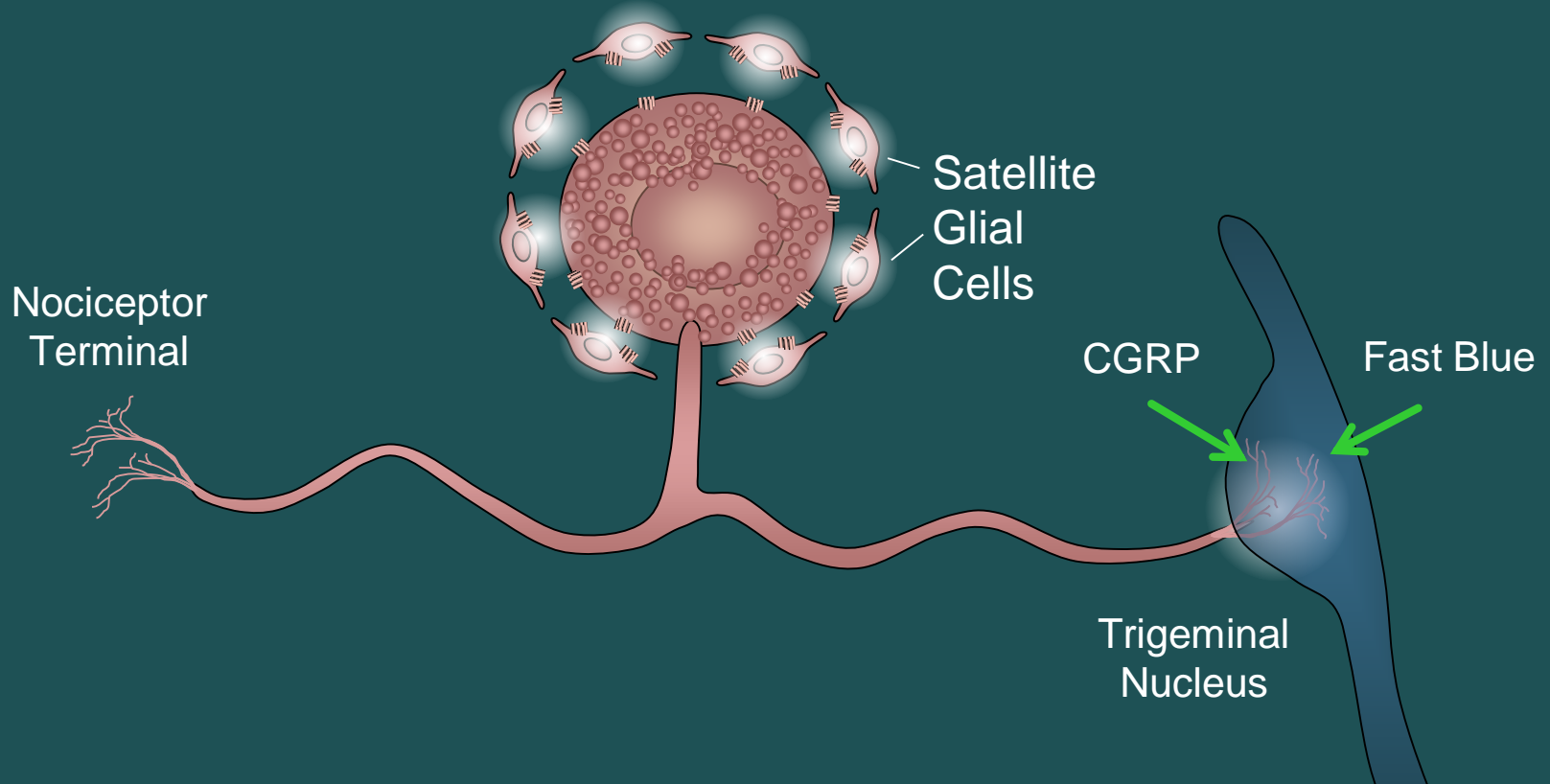


# CGRP Increases Number of Nocifensive Withdrawal Responses at 2, 24, and 48 Hours Post Injection - Resolution at 72 Hours



# Does Bidirectional Signaling Occur in the Trigeminal System?

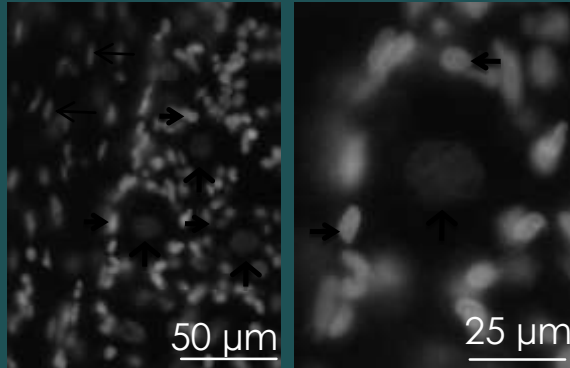
Does CGRP Promote Increased Neuron-Glia  
Communication in Trigeminal Ganglion



# Fast Blue is Retrogradely Transported to Cell Body of Trigeminal Neurons and Associated Satellite Glia



Longitudinal section from rat trigeminal ganglion showing neuronal bands



Enlarged image showing neurons surrounded by satellite glia cells

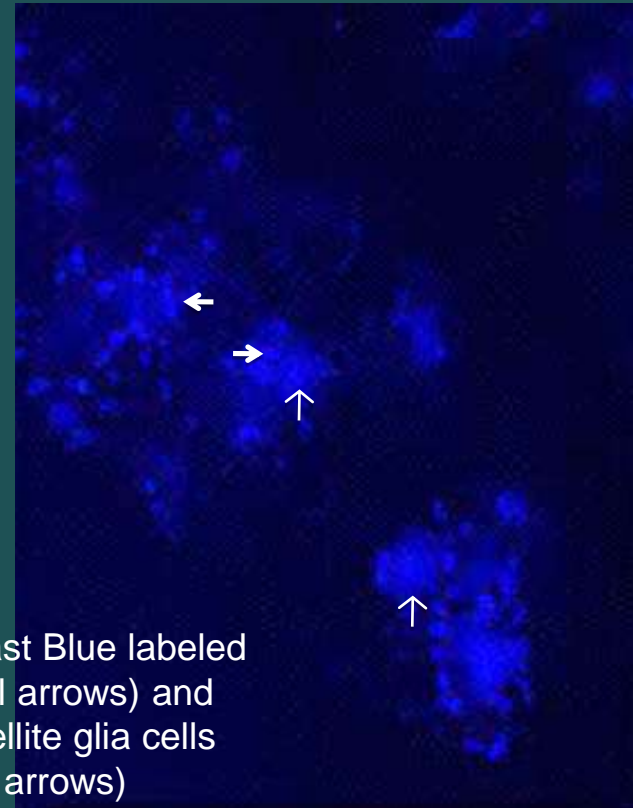
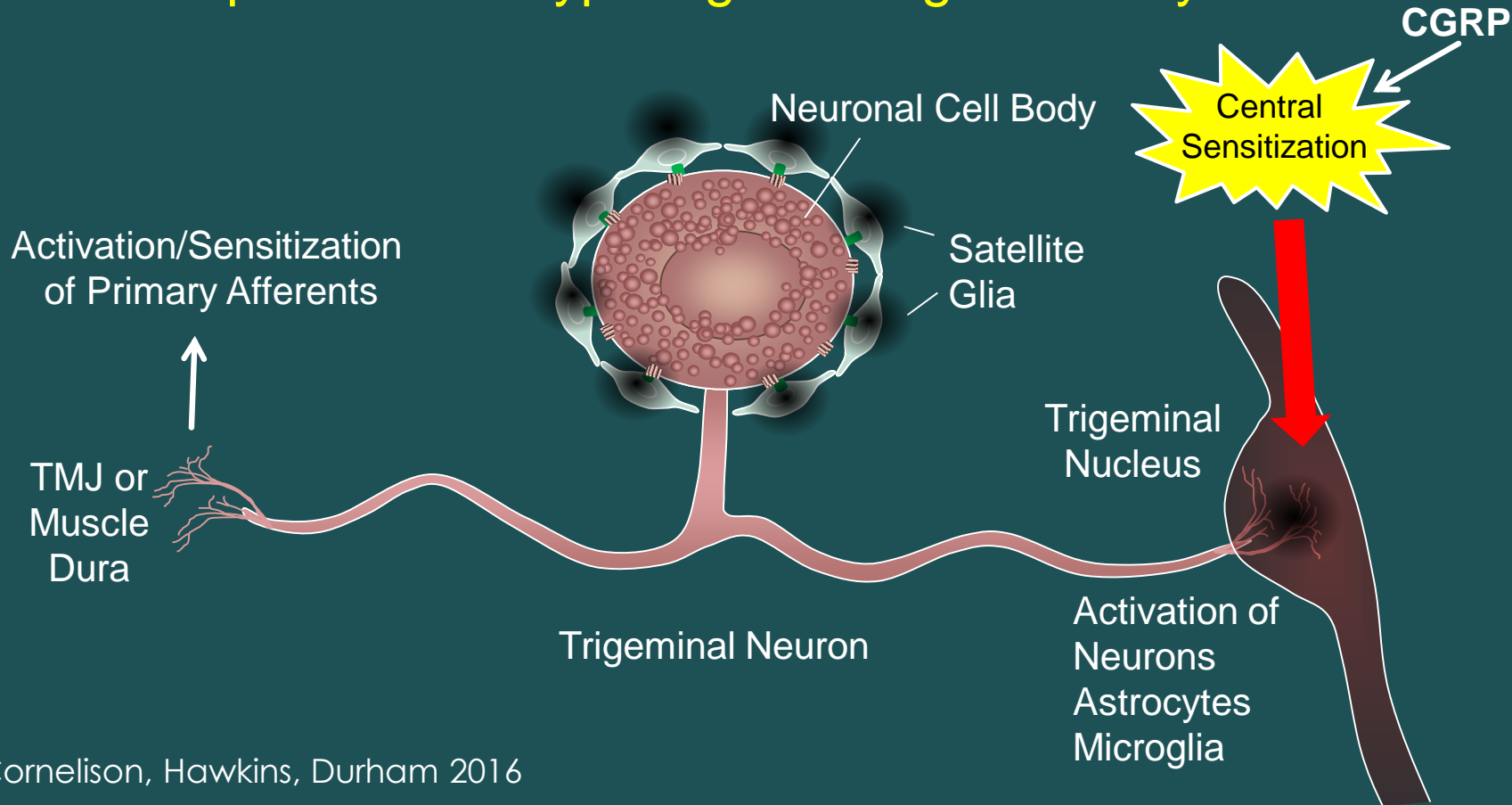


Image showing Fast Blue labeled neurons (vertical arrows) and surrounding satellite glia cells (horizontal arrows)

# Evidence CGRP Can Promote Peripheral Sensitization – Development of a Hypervigilant Trigeminal System

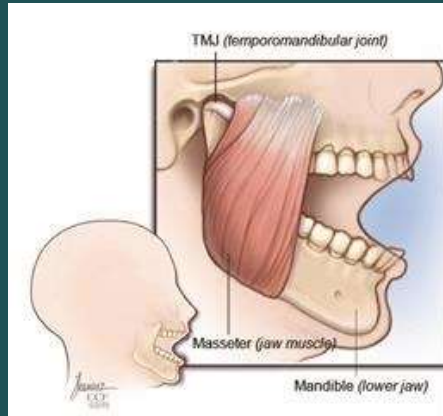


# Hypervigilant Nervous System – Genetic Predisposition and Environmental (Epigenetic) Factors

- ❖ Genetic hyperexcitability
  - ❖ Between episodes of migraine
  - ❖ During episodes of migraine
- Genetic hyperexcitability also associated with other chronic pain conditions
  - Between episodes of TMD and orofacial pain
  - During episodes of TMD and orofacial pain

# Risk Factors for Developing TMD

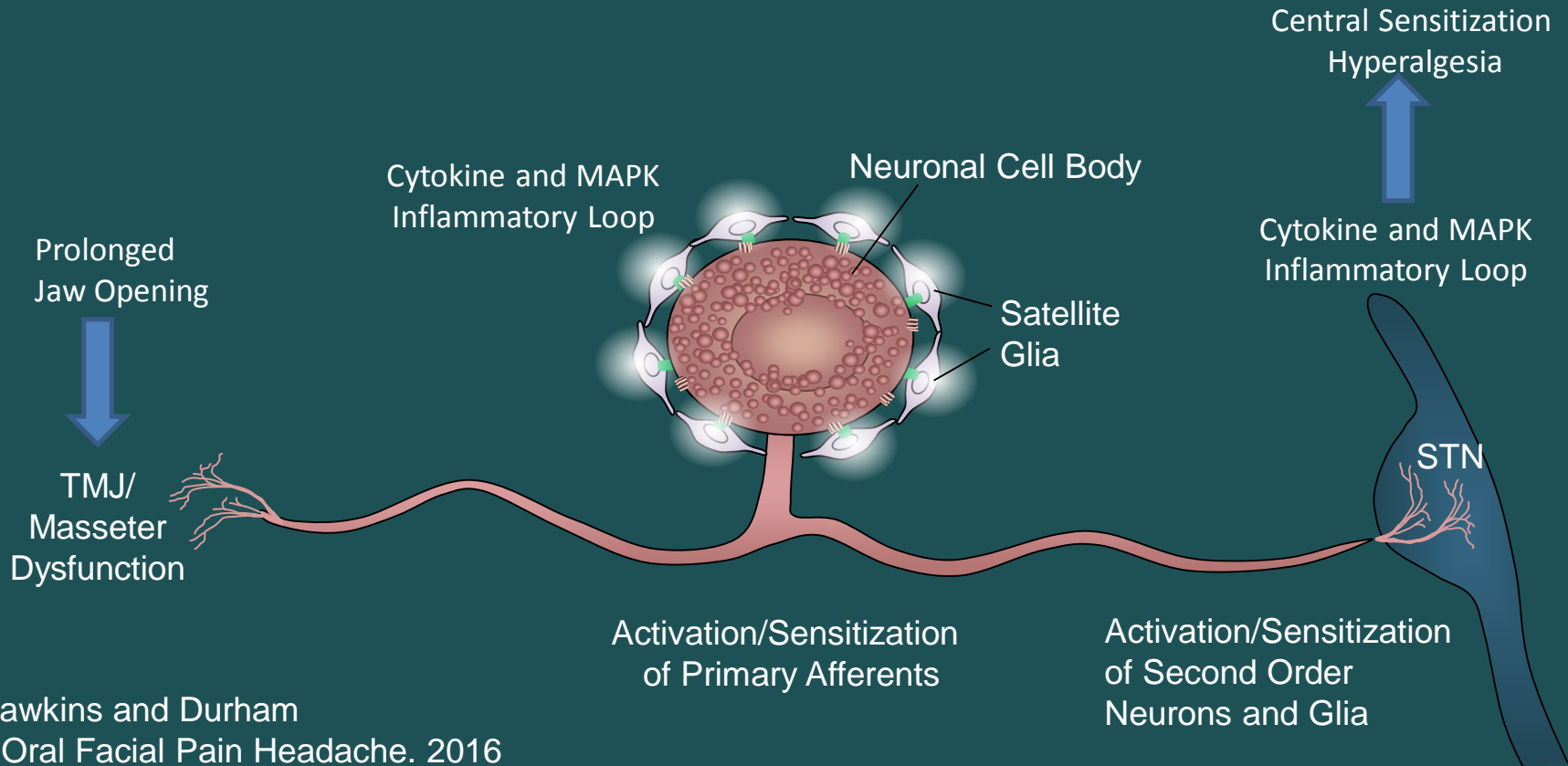
- ▶ Prolonged Jaw Opening
- ▶ Neck Muscle Pathology
- ▶ Female Gender



NIH funded OPFERA Study

# Prolonged Jaw Opening Promotes Sensitization of Trigeminal Nociceptive Neurons

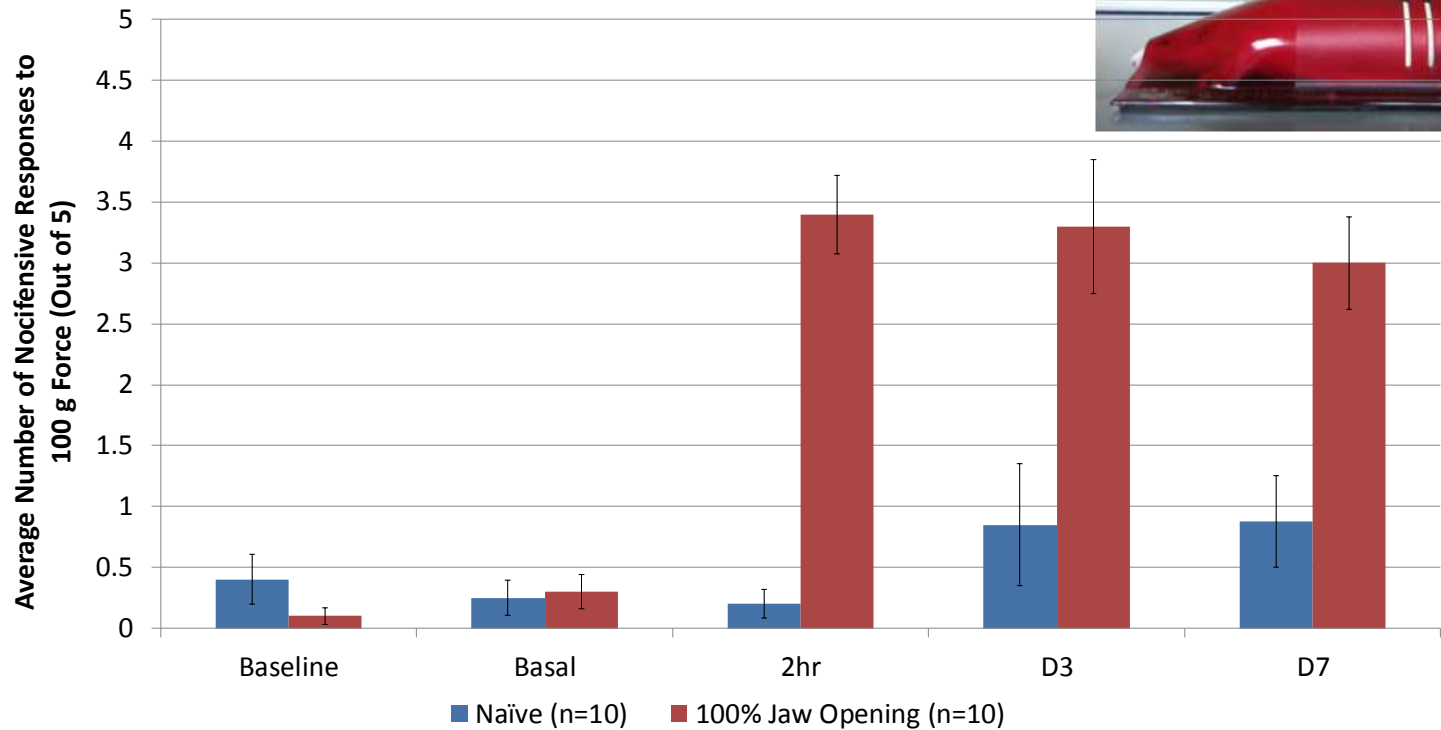
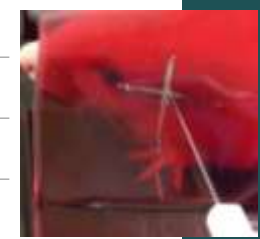
# TMD Pathology Involves Activation of Trigeminal System







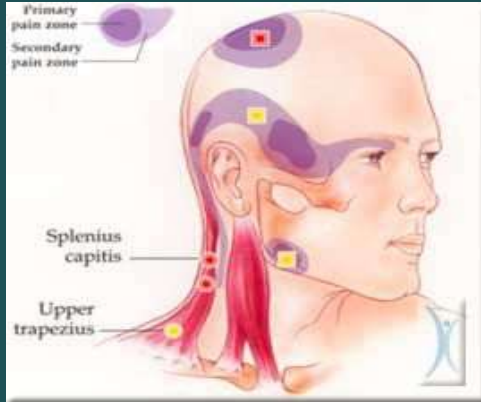
# Protracted Jaw Opening Results in a Prolonged Increase in Nocifensive Head Withdrawal Responses to Mechanical Stimulation of V3 Region



# Muscle Pathology Leads to Cellular Changes in Spinal Trigeminal Nucleus and Trigeminal Ganglia

- Sensitization of Trigeminal Nociceptors

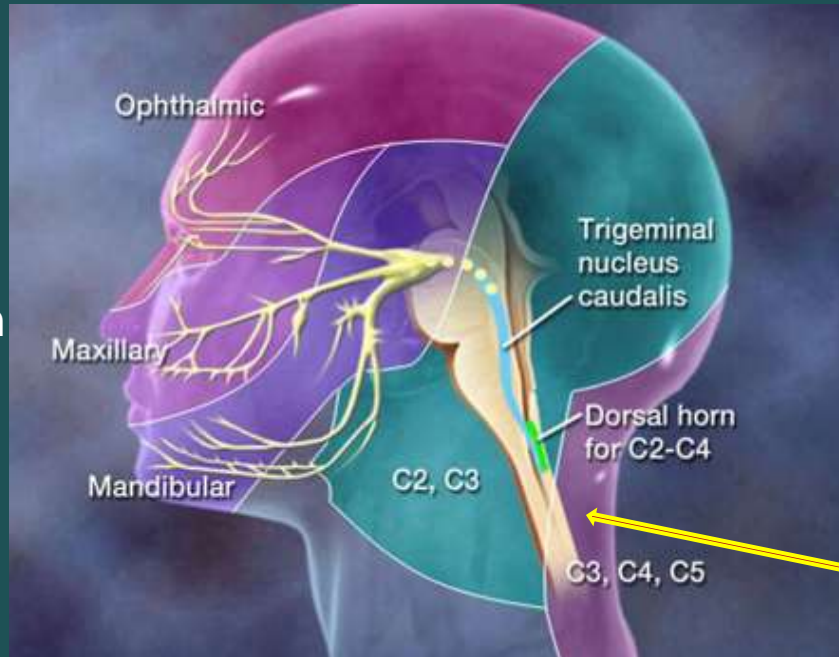
# Neck and Shoulder Muscles – Role in TMD Pathology



- ▶ Stress is often cited as trigger of migraine and TMD
- ▶ Stress can manifest as tension in the muscles in the head, face, neck, and shoulders
- ▶ Many TMD sufferers report muscle pain or sensitivity in their head and neck muscles during an attack
- ▶ Trapezius Muscle - common site of neck muscle tension
- ▶ Innervated by C2/C4 DRG that project to laminae I-IV
- ▶ Convergence of sensory stimuli in upper spinal cord

# Model of Neck Muscle Inflammation and Trigeminal Sensitization

Peripheral Sensitization



Central Sensitization

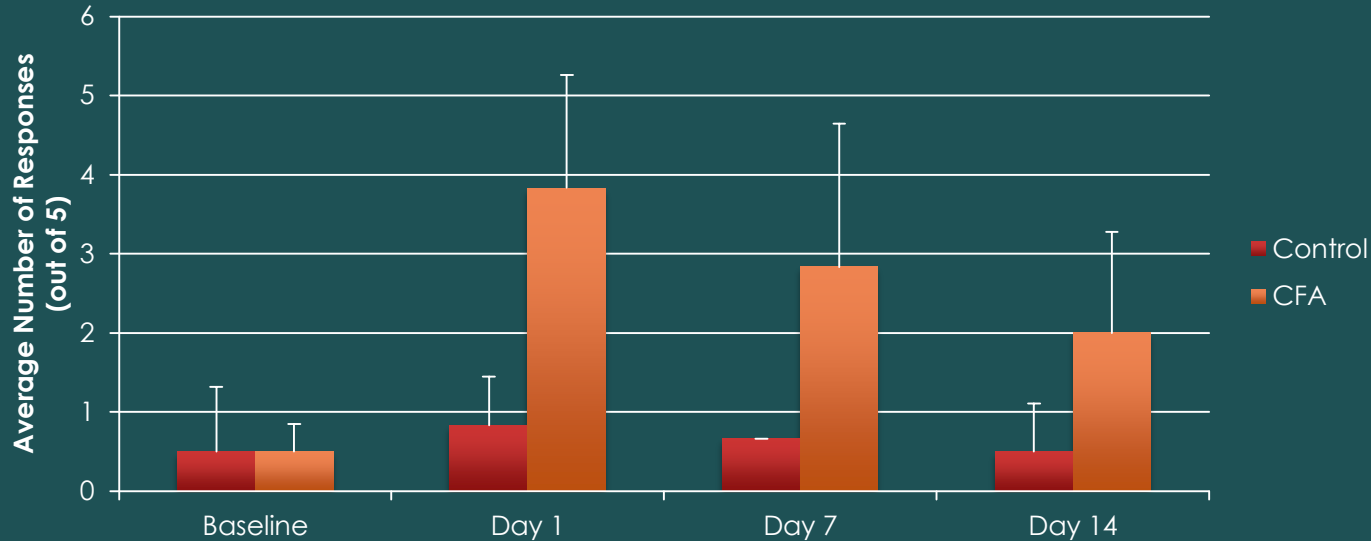
Muscle inflammation

- To test the hypothesis that activation of neurons that provide sensory innervation of the trapezius muscle promote cellular changes in the spinal trigeminal nucleus and trigeminal ganglia implicated in the development of central and peripheral sensitization of trigeminal neurons.

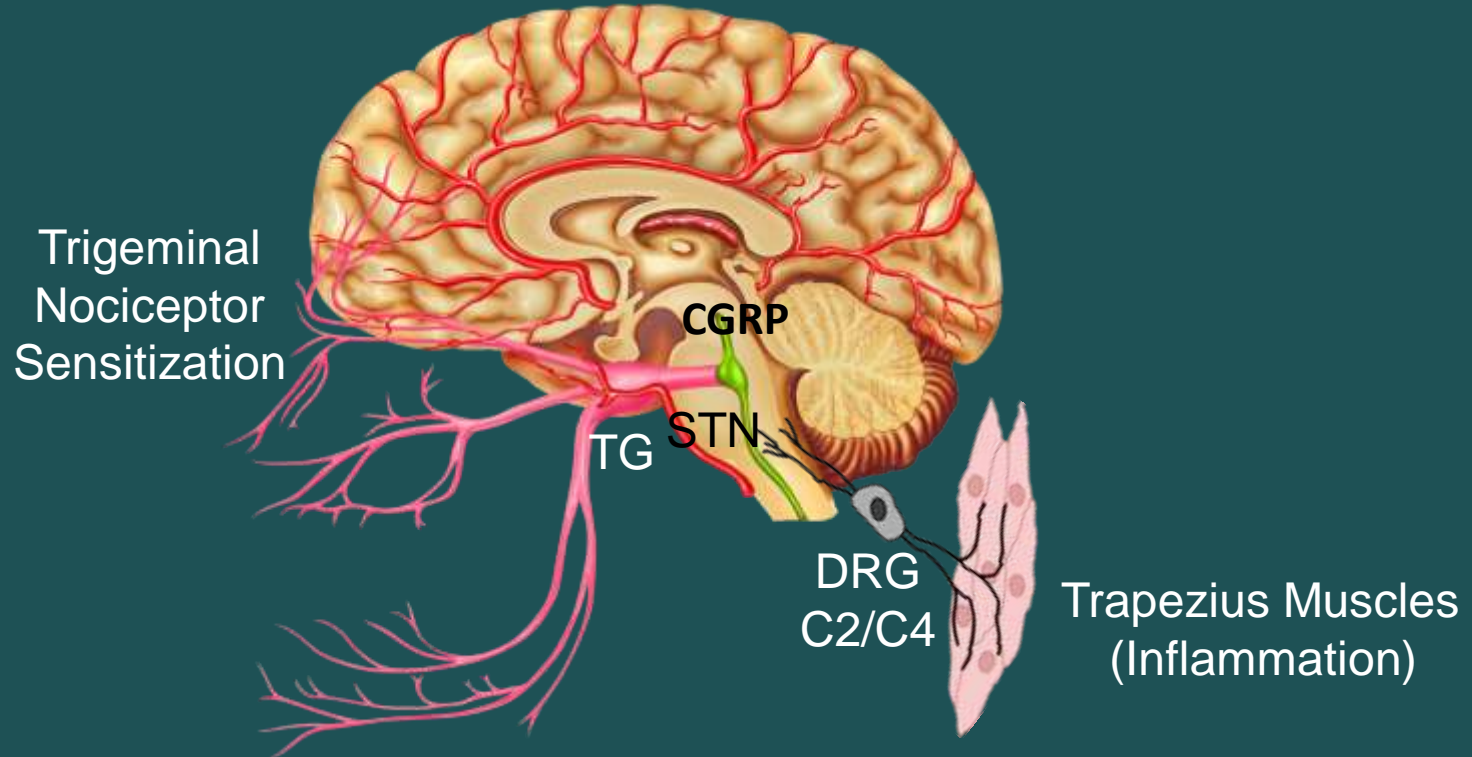
# Inflammation in Trapezius Muscle Promotes Prolonged Sensitization of Trigeminal Nociceptive Neurons



Hawkins et al. J of  
Orofacial Pain,  
2012



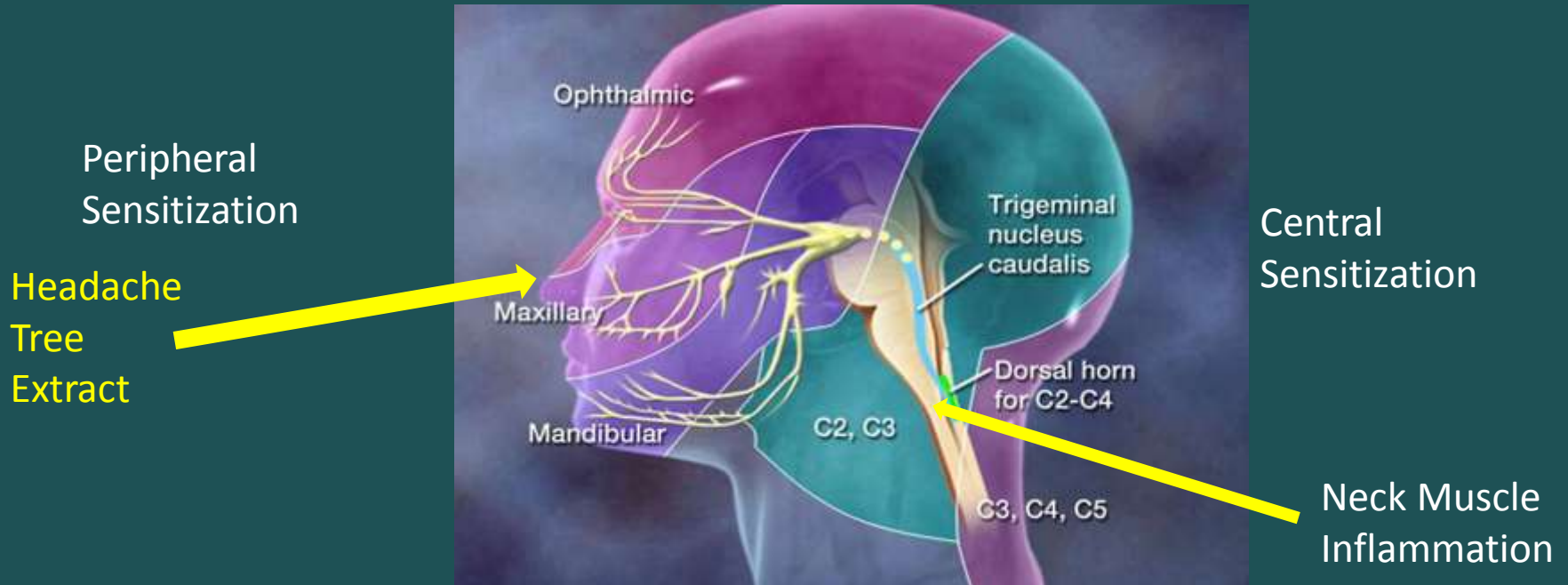
# Pathology in the Neck Muscles Promotes Cellular Changes Associated with Sensitization of the Trigeminal System: A Potential Risk Factor for Migraine and TMD



Sensitization of Trigeminal Nociceptive Neurons  
in Response to Prolonged Neck Muscle Inflammation:  
Risk for TMD and Migraine Pathology

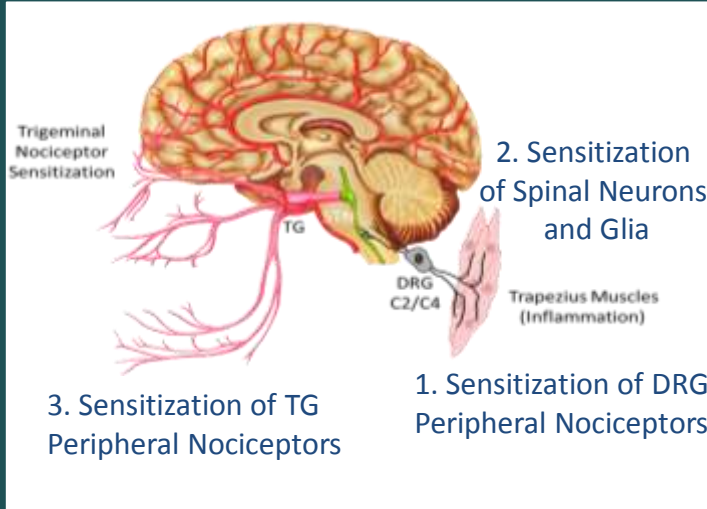


# Risk Factors and Trigeminal Sensitization

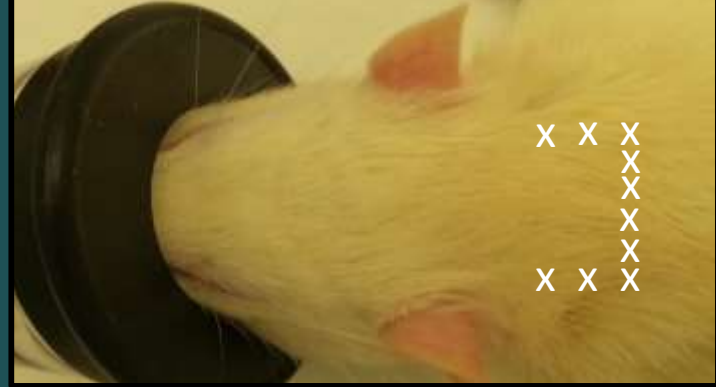


- Test the hypothesis that sensitization of the trigeminal system prior to activation of TRPA1 receptors by a pungent odor is sufficient to cause nociception and allodynia.

# Neck Muscle Inflammation Promotes Sensitization of Trigeminal Nociceptors



Injection Sites of CFA



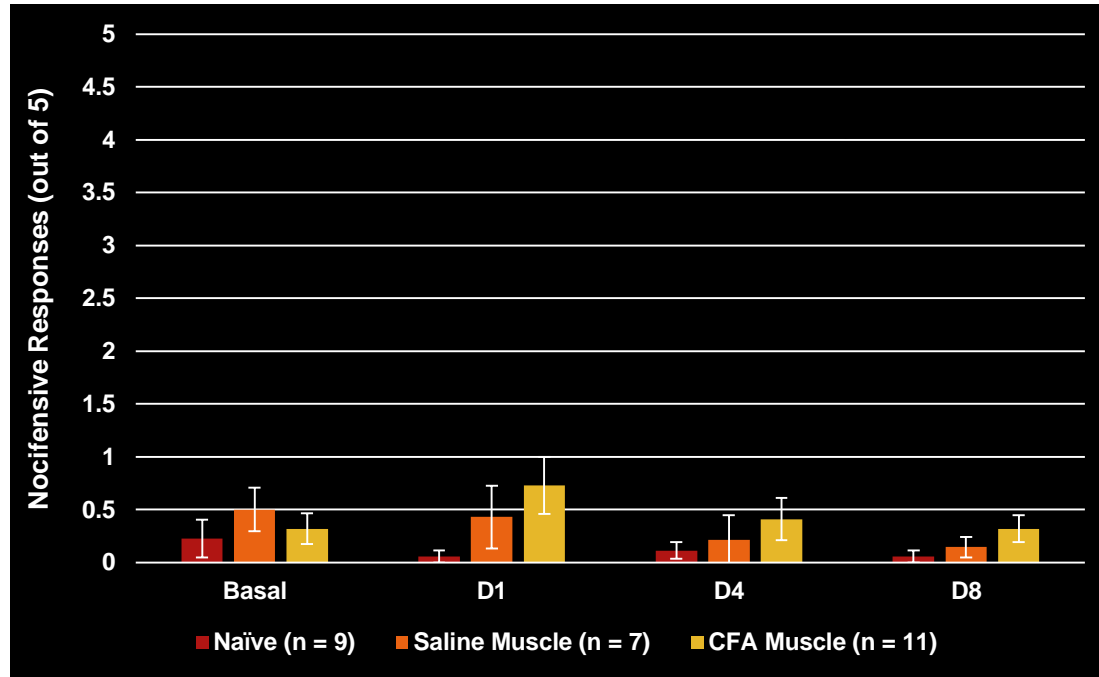
UGO Basile Durham Rat Holder



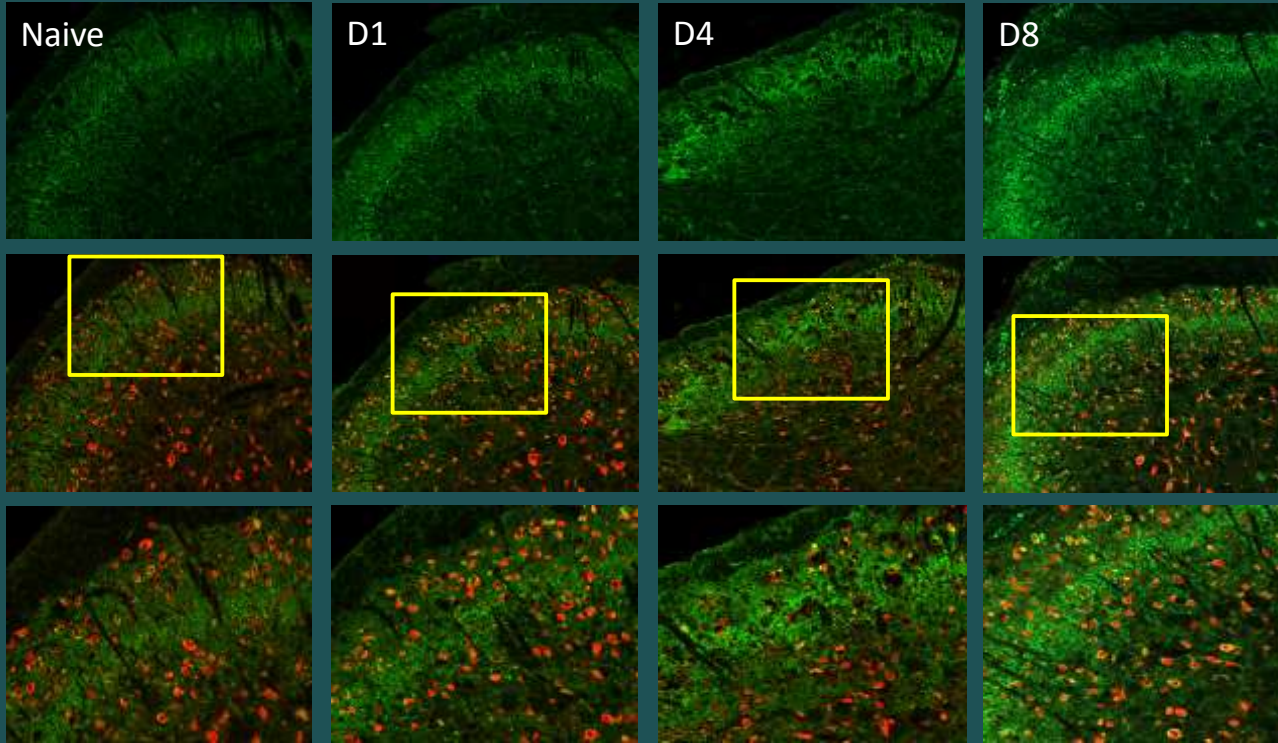
- Injection of 10  $\mu$ l of complete Freund's adjuvant (CFA) in 10 different sites -- heat killed bacteria -- promotes inflammation

# Minor Neck Muscle Inflammation Does Not Cause Nociception

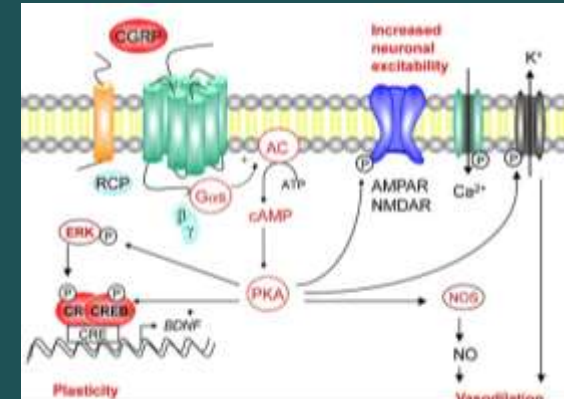
V1



# Neck Muscle Inflammation Promotes Increased PKA Expression



- Evidence of sensitized neurons in spinal cord
- Lower activation threshold to other stimuli
- Risk factor for orofacial pain

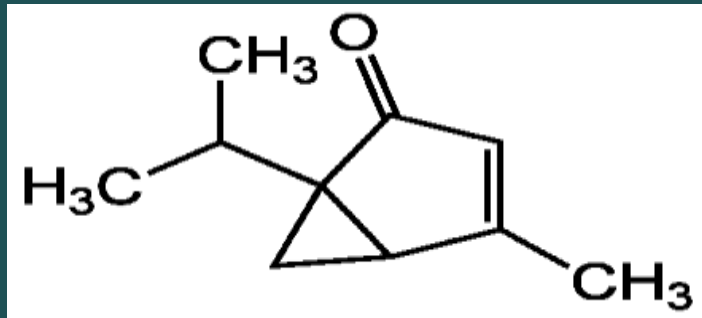


Naïve (n = 3)	D1 Post CFA (n = 3)	D4 Post CFA (n = 3)	D8 Post CFA (n = 3)
1.00 ± 0.02	1.15 ± 0.15	1.40 ± 0.30	1.79 ± 0.10
	P = 0.293	P = 0.182	P = 0.001

# Activation of Trigeminal Nerves Via TRPA1: Headache Tree Extract

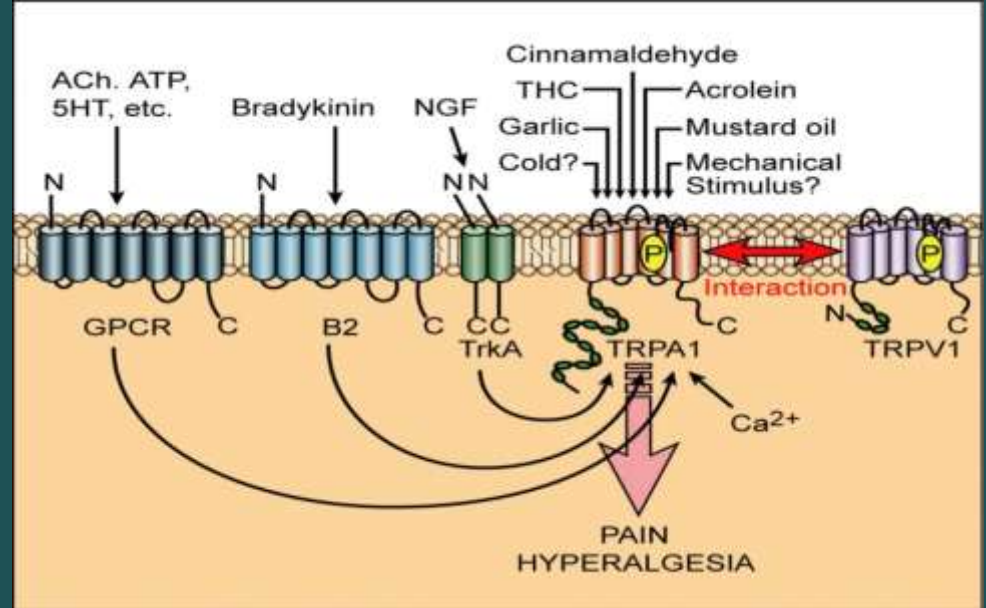


*Umbellularia californica*



Umbellulone

Nassini (Geppetti), Brain 2012



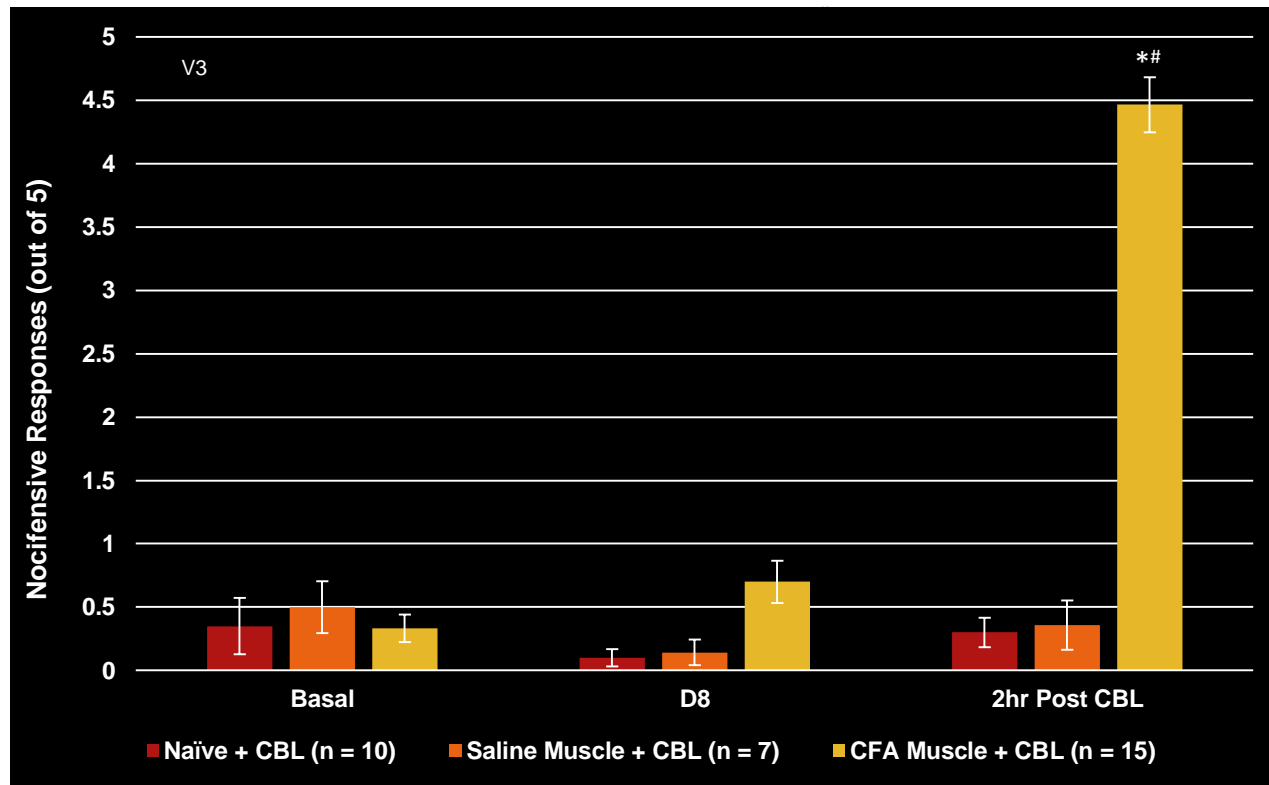
Pungent Odors: Trigger for migraine

# Experimental Design

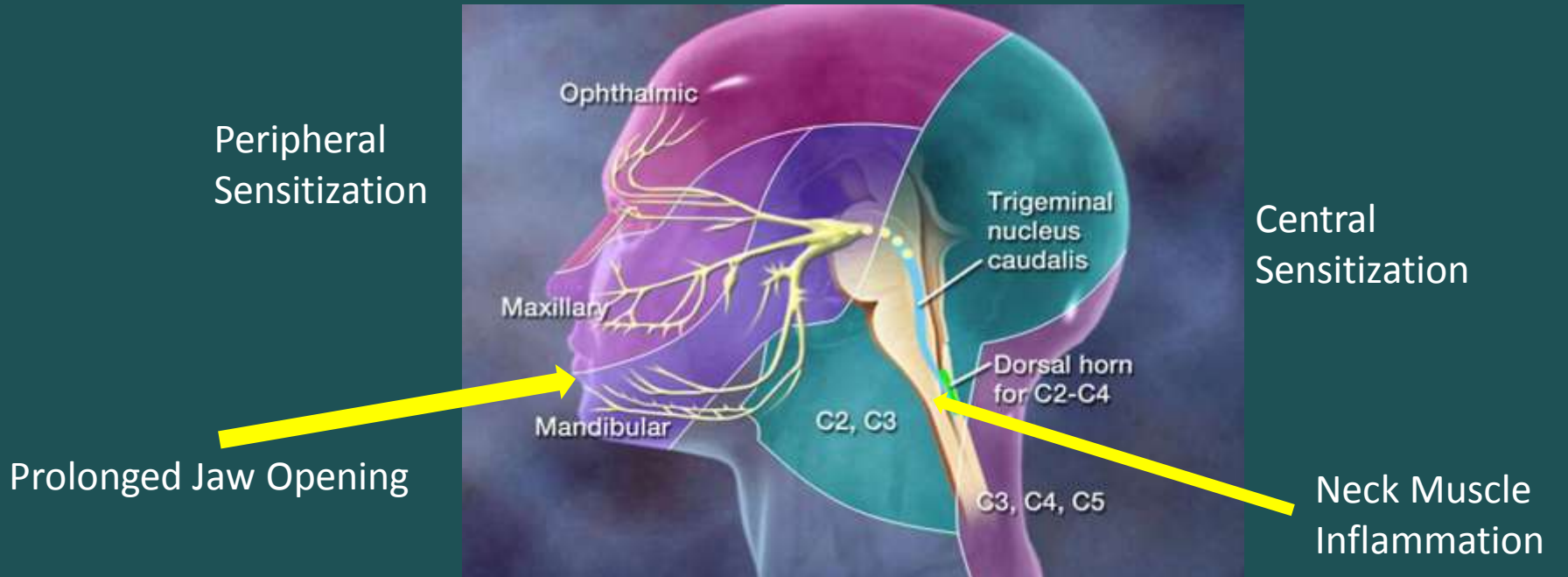
- ▶ Induction of Muscle Inflammation (**Risk Factor**)
  - Complete Freund's adjuvant (CFA)
- ▶ California Bay Leaf (CBL) Oil Exposure (**Trigger**)
  - Oil extract from leaves of the *Umbellularia californica* tree that acts on TRPA1 receptors in the trigeminovascular system upon inhalation
- ▶ Mechanical Nociception
  - UGO Basile Durham Rat Holder and von Frey filaments



# Minor Neck Muscle Inflammation Promotes Sensitization of Trigeminal Neurons: Activation with Pungent Odor



# Risk Factors and Trigeminal Sensitization



- Test the hypothesis that sensitization of the trigeminal system prior to prolonged jaw opening is sufficient to cause chronic nociception (pain) and allodynia.



# TMD Pathology Involves Activation of Trigeminal System



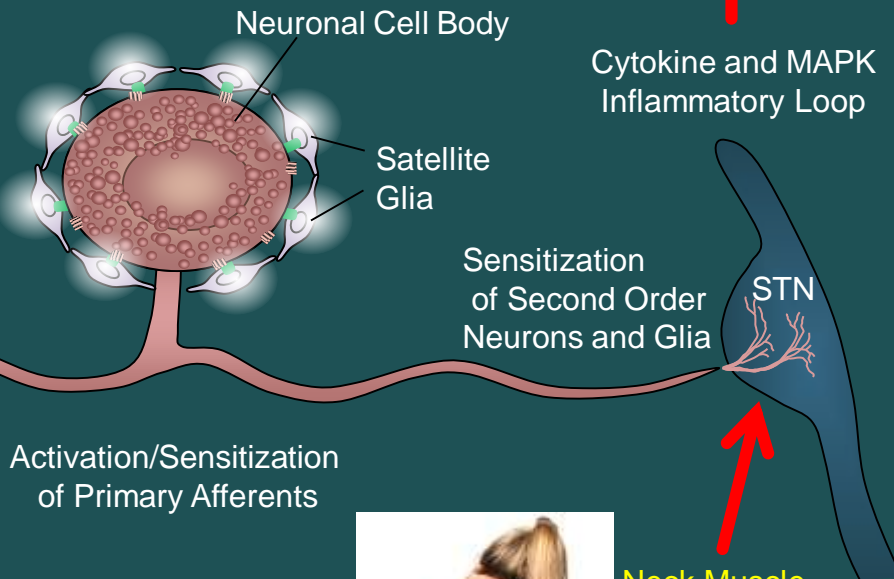
Prolonged  
Jaw Opening



TMJ/  
Masseter  
Dysfunction



Cytokine and  
MAPK  
Inflammatory  
Loop



Central Sensitization  
Hyperalgesia/Allodynia



Cytokine and MAPK  
Inflammatory Loop



Neck Muscle  
Inflammation



# Novel Model of TMD Pathology to Study Role of Risk Factors - Female Gender and Neck Muscle Inflammation



## CFA model:

All injections were performed under 5% isoflurane

Muscle inflammation: 100 microliter total of complete Freund's adjuvant (CFA) was injected into the neck muscle bilaterally at ten different areas

CFA is a composed of inactivated and dried mycobacteria (usually *M. tuberculosis*)

Naïve animals did not receive any treatment

# Novel Model of TMD Pathology to Study Role of Risk Factors - Prolonged Near Maximal Jaw Opening



## ▶ Jaw opening:

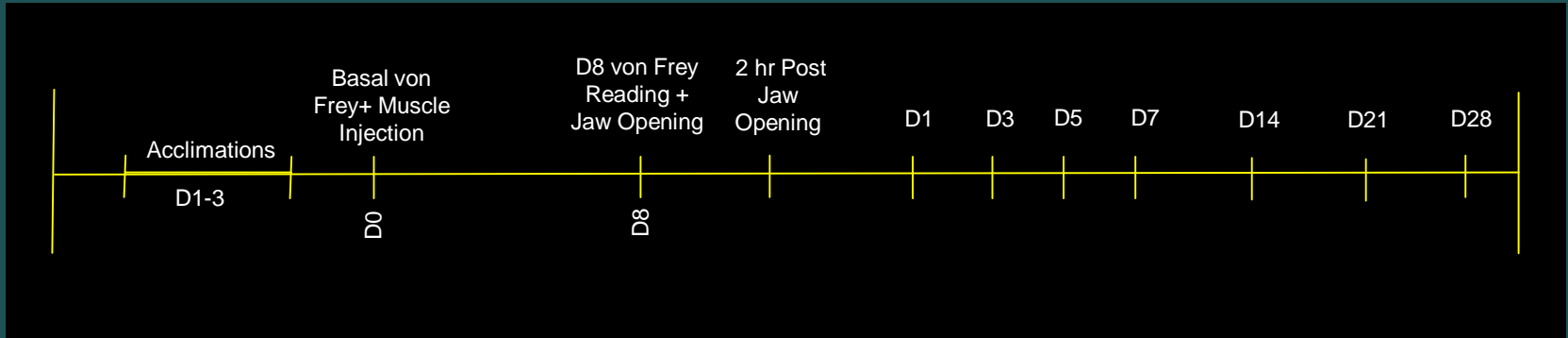
A mouth retractor is placed in between the upper and lower incisors and the retractor arms were then separated till the desired amount is reached. It is measured with the help of Vernier calipers

Animals were under 3% isoflurane for 20 mins

Maximum opening in males is 22 mm

Maximum opening in females is 20 mm

# Timeline



# Scoring of Nocifensive Response

- ▶ Normal/Mild: 0 - 2
- ▶ Moderate: 2 – 4
- ▶ Severe: 4 – 6

Unresponsive to mechanical stimulation (most severe pain state):  
Some animals went into a unresponsive state where no nocifensive withdrawal was observed even to the 180 g filaments

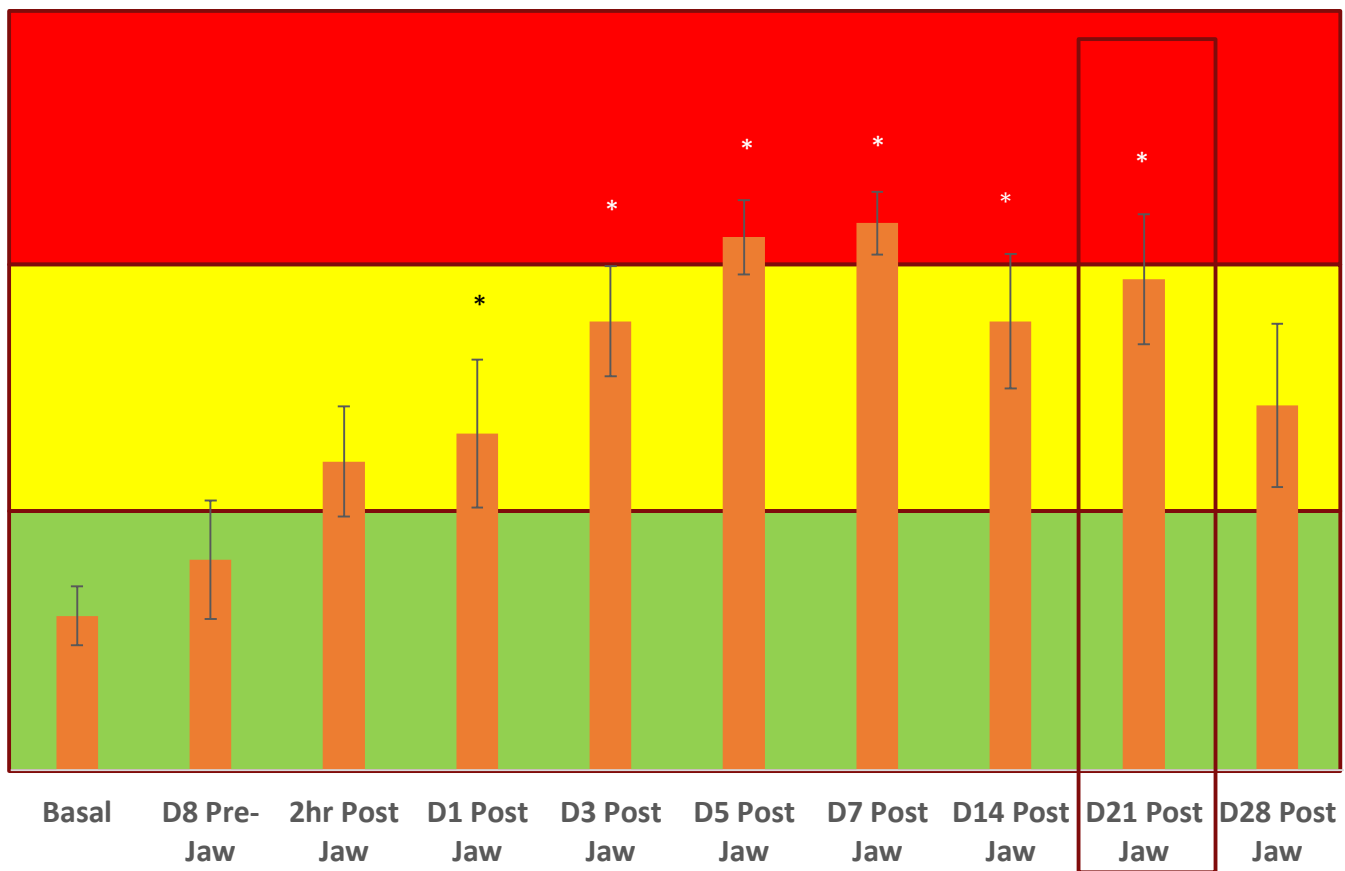
These animals were given a score of 6

# Prolonged Jaw Opening In Sensitized Animals Leads to Sustained Allodynia Over Masseter Region -- Males

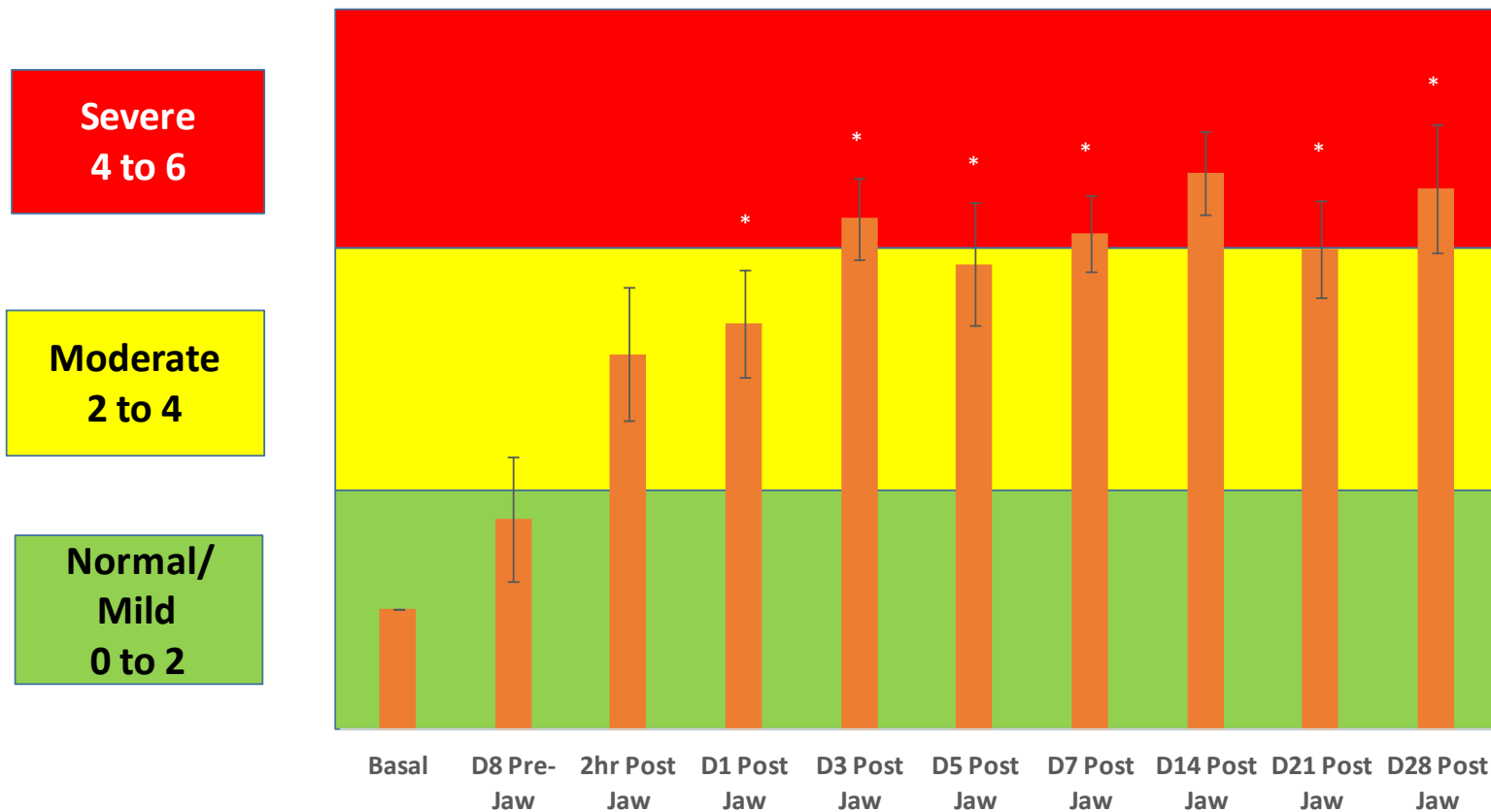
**Severe**  
4 to 6

**Moderate**  
2 to 4

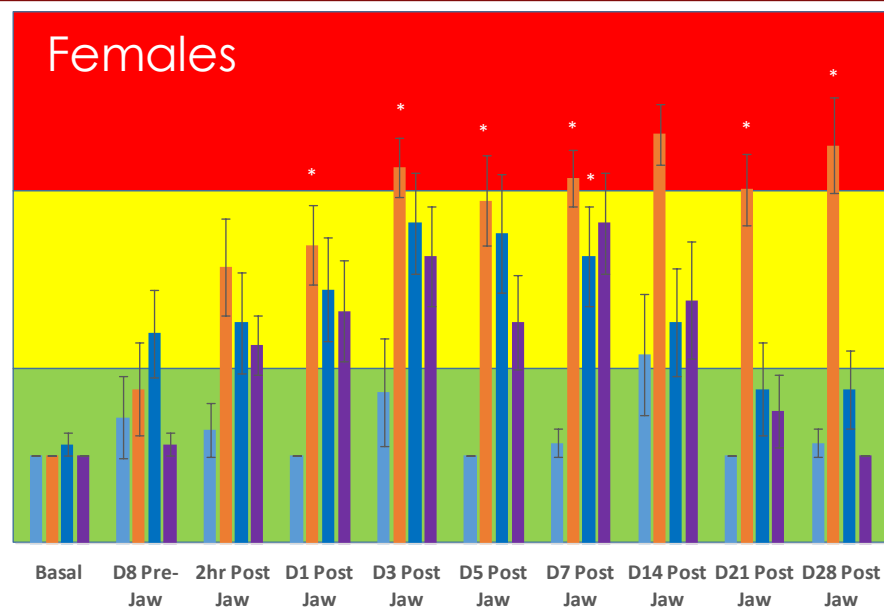
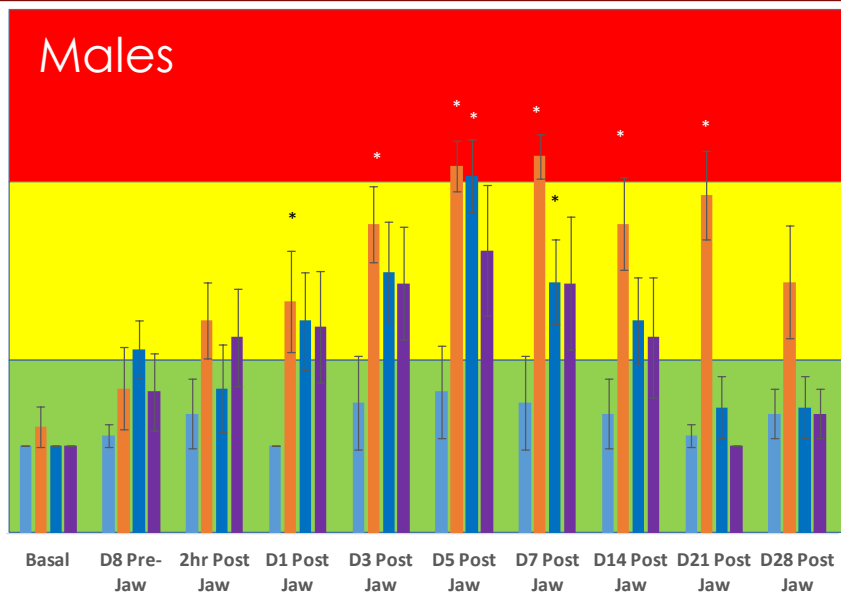
**Normal/  
Mild**  
0 to 2



# Prolonged Jaw Opening In Sensitized Animals Leads to Sustained Allodynia Over Masseter Region -- Females



# Summary - Neck Muscle Pathology and Prolonged Jaw Opening



**Severe**  
4 to 6

**Moderate**  
2 to 4

**Normal/  
Mild**  
0 to 2



# Summary of Risk Factors Involved in Promoting Transition from Acute to Chronic Pain States

1. An existing orofacial pain condition – high comorbidity
2. Increased levels of CGRP in STN (nicotine, early life stress)
3. Neck muscle inflammation
4. Prolonged jaw opening
5. Female gender
6. Sleep deprivation

❖ Multiple risk factors increase likelihood of developing a maladaptive chronic pain state

# CBLS LAB

