CLASSIFICATION AND MECHANISM OF FATIGUE RECEPTORS

Funding: Wyoming EPSCoR

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What is muscle fatigue?

- Ever heard the saying, “Its all in your head you can push through.”
  - And at the same time wonder, “What could be causing this?”

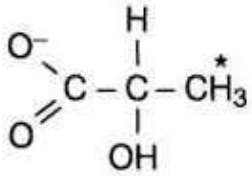
- Fatigue can be described as a sensation of muscle tiredness and pain

  - Fatigue is caused by use of muscles
    - Specifically metabolite build up in the interstitial space of muscle

  - Too much fatigue can cause pain

So, what's the "Big Idea"?

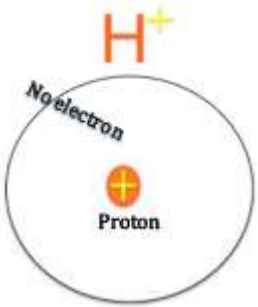
- Pain "Nociceptive" neurons have been shown to be involved in fatigue.
- Its been suggested that a separate population of neurons are involved in fatigue. Suggested to cause sensation of muscle tiredness thought as "Fatigue Receptors"

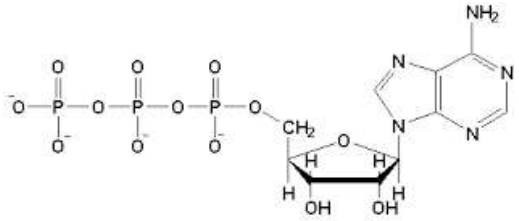


Lactate (Lac)

  - Previous human and animal studies show that neurons involved in fatigue respond to a cocktail of metabolites consisting of (Lactate, ATP, & protons)

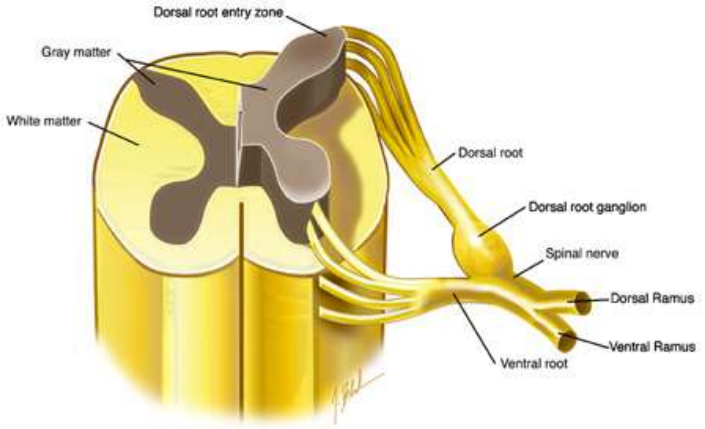
  - Research in human subjects hints that "Fatigue receptors" are activated at low to moderate concentrations of metabolites (Light, *et al.* 2013). No physical evidence of "Fatigue Receptor".





Adenosine triphosphate (ATP)

Our work



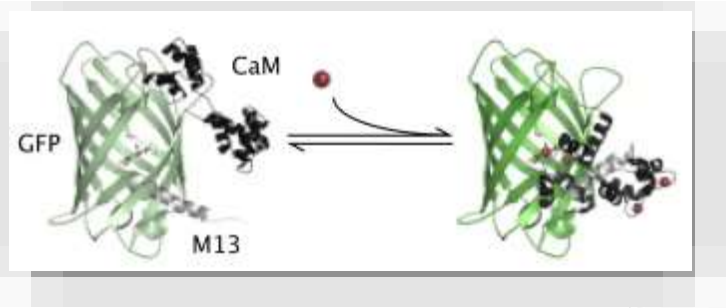
- We set out to show that fatigue neurons are a separate population and then to activate them at different metabolite concentrations.

- Hypothesis – To show that different levels of metabolites activate two separate populations of sensory neurons involved in the fatigue response. Fatigue receptors at moderate exercise (low) metabolic concentrations and nociceptive receptors at ischemic (high) metabolic concentrations.

- Used a novel mouse model to image the Dorsal Root Ganglion (DRG) a cluster of sensory neuron cell bodies located outside of the spinal cord. The DRG's were imaged at L3 & L4 that are innervate the target muscle used "the Gastrocnemius muscle".

Approach

- Use GCAMP3 mice that have a calcium indicator which flashes as a neuron is having an action potential. Allowing us to visualize a population of neurons.
  - Previous research was limited because they were only able to visualize a single neuron at a time.
- Expanded and developed on previous skin physiological *ex vivo* preparations to allow us to use GCAMP mice to preform these experiments.
- Introduce known metabolite concentrations found in muscles at different exercise levels



(Ex vivo)

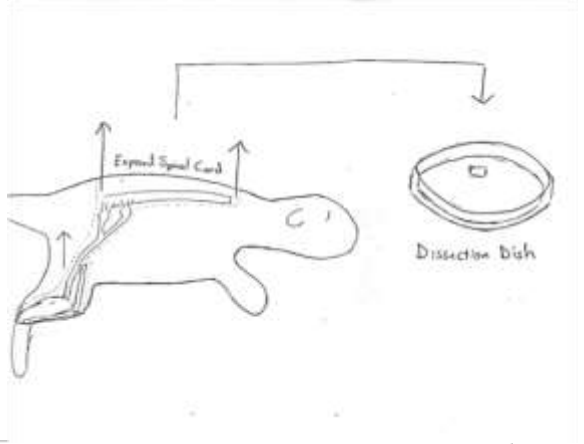
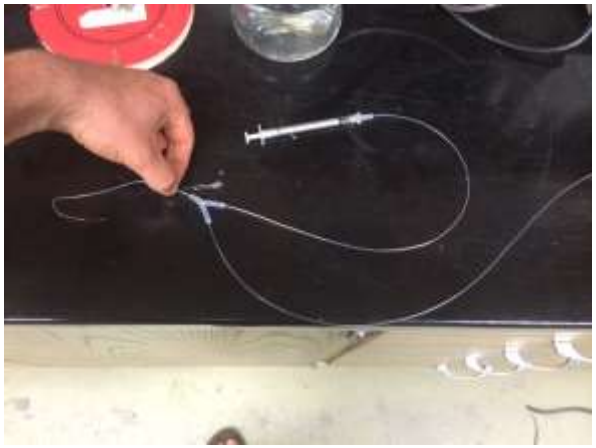


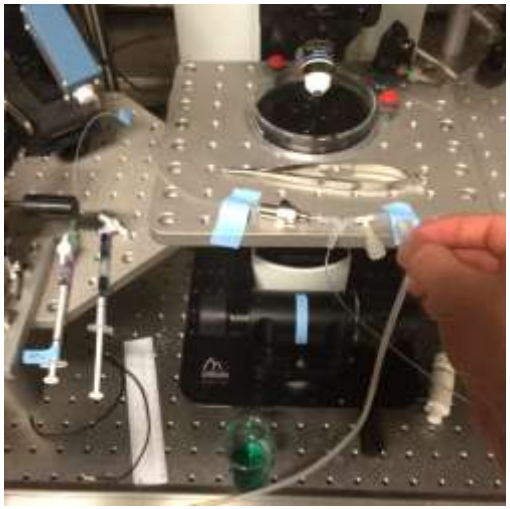
Table 1: Muscle Metabolite Solutions

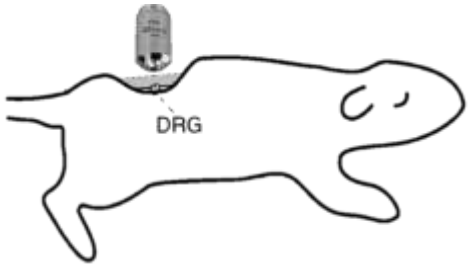
pH	ATP	Lactate	Exercise Level	Order of Presentation
7.4	300nM	1mM	Resting	1
7.0	1000nM	15mM	Moderate	2
6.6	5000nM	50mM	Ischemic	3

Troubleshooting and Optimization

- Ex vivo
  - Nourishing the tissue during experiments proved difficult
  - Next, how do we deliver the proper metabolite concentrations at the proper pH?
    - While supplying proper tissue nourishment
  - Developed a new delivery system that we are still optimizing
- While we are optimizing the ex vivo delivery system we used in vivo system for the following experiments

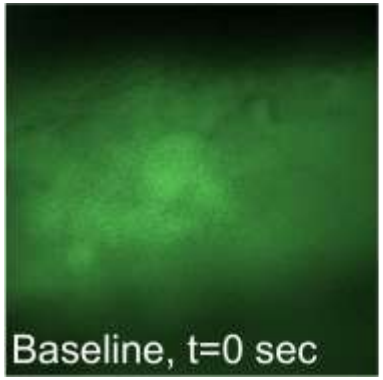


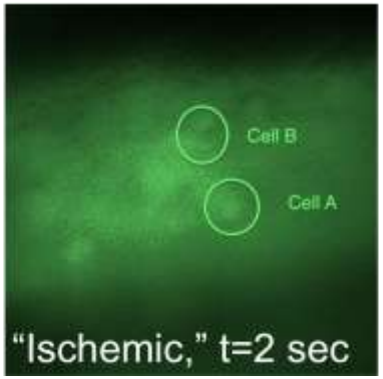


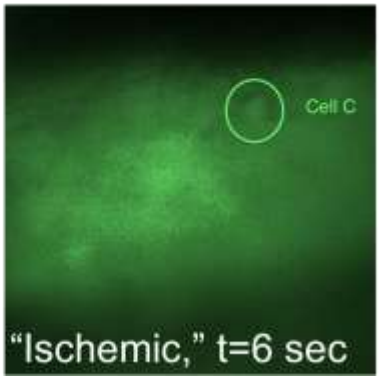


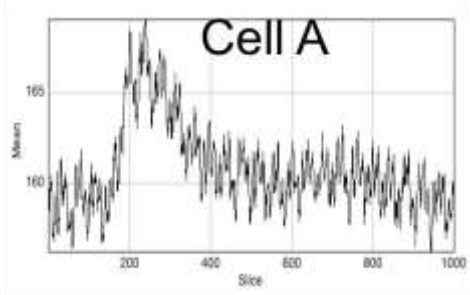
(In vivo)

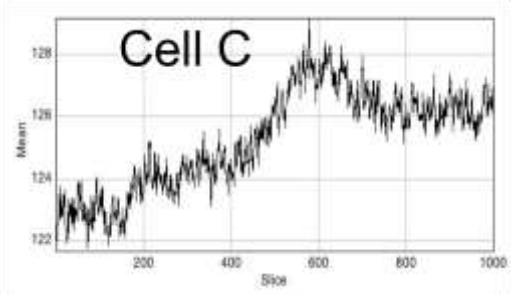
Proof of principle in mouse model

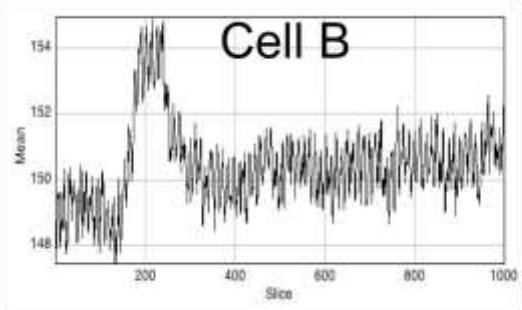








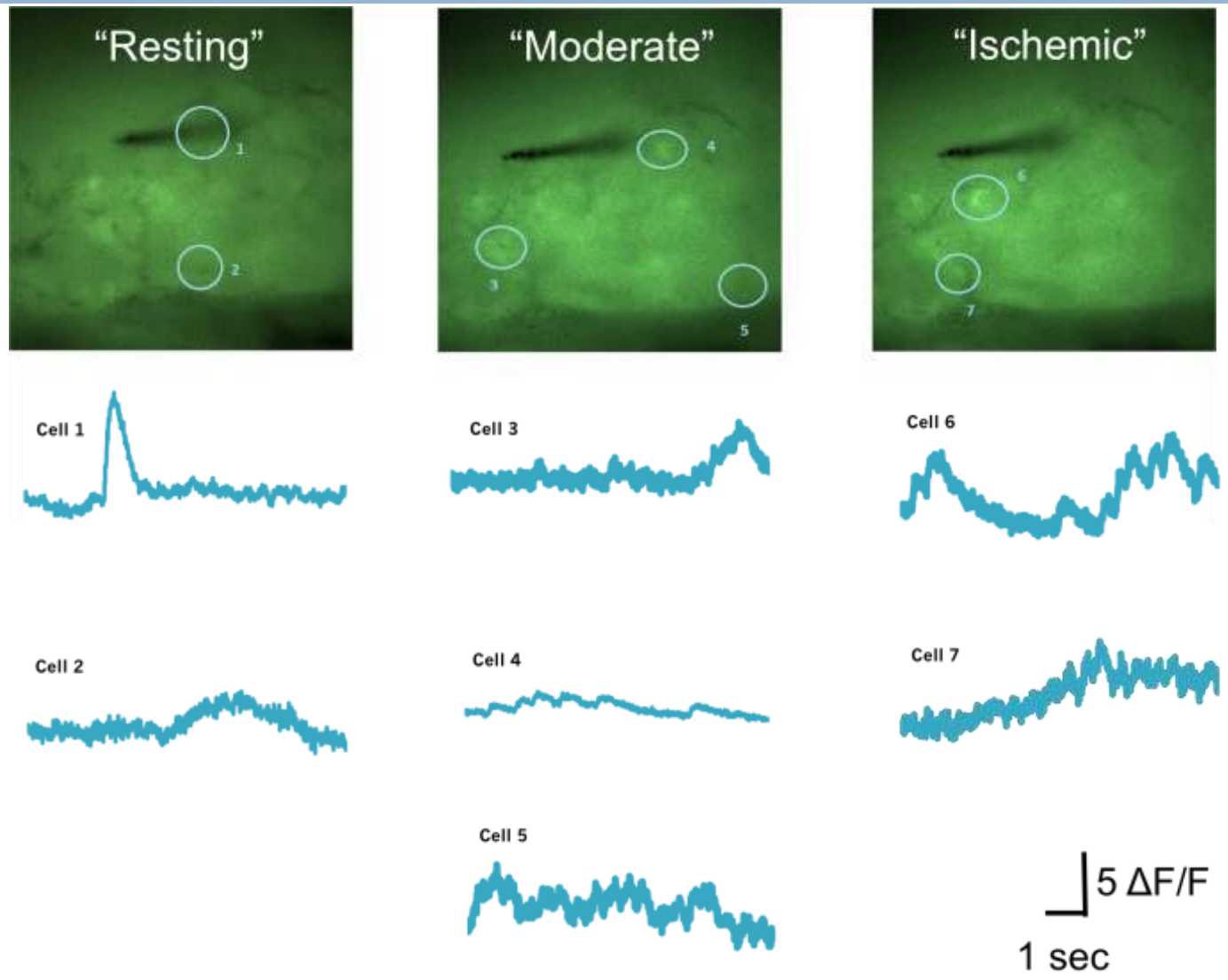




- GCAMP3 allows us to visualize neuron populations
- Ischemic metabolite concentrations activate what we believe to be nociceptive receptors
- Cell A & B activate first followed by cell C after diffusion

Fatigue receptors respond to moderate exercise physiological conditions (*in vivo*)

- Under control conditions mechanoreceptors respond to fluid build up
- Fatigue receptors activate at low to moderate metabolite concentrations
- Nociceptive receptors respond to high metabolite concentrations



Conclusions

- There are two populations of muscle sensory neurons involved in response to muscle fatigue
- One population responds to moderate levels of exercise
  - Fatigue receptors most likely signal non-painful muscle fatigue
- The other population of sensory neurons in muscle tissue respond to high levels of exercise (ischemic conditions)
  - These nociceptive receptors signal painful muscle fatigue to promote rest, clear acidic build up, rebuild muscle, and prevent damage
- Why are two populations of muscle sensory neurons needed?
  - Homeostatic hypothesis

Future Directions

- Replicate *in vivo* experiments
  - Also test different parameters
- Define the range of activation for fatigue & nociceptive neuron response
  - Once a population is found, find a cell and do a intracellular recording to support activation
- Test different muscles to find if there are different ratio's of populations innervating various muscles
- Further optimize *ex vivo* preparation

Acknowledgements

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  - For funding
- Dr. Charles J. Woodbury
- Kristen Smith

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