'Clamping in' novel physiology to insect muscle with a closed-loop virtual reality system

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Summary

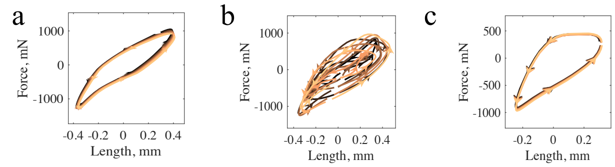
Fast-flapping insects have evolved muscles that are activated by stretch as opposed to neural signals [1]. The first flapping insects lacked stretch-activation, which was then layered on top of neural activation. However, it remains unclear how neural- and stretch-activation interact as a muscle evolves the capacity to be activated by stretch. Building on prior closed-loop muscle setups [2], we develop a 'virtual reality' system that couples neurally-activated hawkmoth (*Manduca sexta*) flight muscle to simulated stretch-activation. This allows us to manipulate the degree of stretch-activation *in-silico* while retaining all other muscle properties. We demonstrate that neurally-activated muscle can produce positive, stretch-activated work under tetanus, or at frequencies where stretch-activated forces entrain to the nervous system. The ability to 'clamp in' novel physiological properties to muscle in closed loop is a promising technique for studying state- and history-dependent properties of muscle.

Introduction

In most muscles, contraction is initiated by neural signals, which cause a cytosolic influx of calcium that drives force production in myofibrils. Many clades of insects break this rule, flapping at frequencies far exceeding the neural drive to their flight muscles [1]. These insects' muscles produce force in response to stretch as opposed to neural activation, decoupling the resulting wingbeats from calcium dynamics and enabling near-kHz wingbeat frequencies. However, the first flapping insects seemingly lacked stretch-activated physiology, which was then layered on top of neural activation dynamics before being reduced again in certain lineages such as moths. **How then does can neurally-activated muscle can evolve stretch-activation without interfering with its function?**

Methods

Inspired from prior real-time feedback control of isolated muscle, we modify a dual-mode muscle ergometer commonly used for open-loop muscle preparations to function in closed-loop, feeding real-time state information (force and length) from a live muscle to a computer model of stretch-activation with various time constants and magnitudes [2]. **Doing so enables us to impose stretch-activated physiology on a neurally-activated muscle**. We do this while stimulating the muscle tetanically, and at its wingbeat frequency. These two stimulation conditions allow us to manipulate the strength of intracellular calcium fluctuations, which are reduced in tetanus. We hypothesize that relatively constant neural activation will prevent interference between stretch- and neural activation, enabling stretch-activation to overcome nonlinear interactions between the two activation modes. When stimulated at wingbeat frequency, we predict stable behavior will emerge only when the timescales of neural and stretch-activation are close, causing stretch-activated forces to entrain to the neural driving frequency.



a). Under tetanic stimulation, hawkmoth muscle with virtual stretch-activation produces stable, positive work. b). Under in-vivo stimulation, work-loop amplitude and thus work produced is highly variable. c). Under in-vivo stimulation, with a properly tuned stretch-activation timescale, stable work-loops are recovered.

Results and Discussion

We find that neurally-activated muscle can produce stretch-activated work in tetanus, at relatively constant calcium-dependent activation. This is evidenced by positive constant-amplitude work-loops across multiple cycles (Fig. 1a). When stimulated at wingbeat frequency, interference between stretch- and neural activation results in variable amplitude work-loops (Fig. 1b). However, this interference disappears when we match the two activation timescales, resulting in stable work production generated by stretch-activated forces that have entrained to the nervous system (Fig. 1c).

Our results reveal the evolutionary conditions most conducive to transitions between stretch-activation and neural activation in muscle. Relatively constant activation present under tetanus, results in stable work production and is reflective of the physiology of stretch-activated muscle function in fast-flapping insects like bees and flies [1]. In addition, matching the stretch-activated and neural timescales results in stable behavior via entrainment. Such matching may be a smooth evolutionary pathway for the repeated switching between actuation modes observed across insects.

Conclusion

Using a novel virtual reality system for 'clamping in' novel physiological properties to an isolated muscle, we determine the conditions under which neurally-activated muscle can generate stretch-activated work. This study highlights the utility of closed-loop muscle preparations for investigating the interactions between muscle physiological properties in the context of dynamic loading.

References

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