Webinar Q&A Report:

Beyond Isometric Twitch: Utilizing lengthening, shortening and isotonic contraction tests for muscle function research

Q: What is your intra animal variability for torque measures on the same animal in vivo. Also you have normalized the data - is there an optimal normalization method in your view?

[R.Grange]: Intra animal variability ~5%; We typically normalize the torque data to body mass; ideally optimal normalization would be to the cross sectional area of the muscles activated – but of course this is difficult to accomplish with an in vivo preparation.

Q: what type of muscle is tibialis anterior and gastrocnemius in mice?

[R.Grange]: The mouse TA has predominantly fast fibers; the gastrocnemius has superficial white and deeper red muscle portions – therefore a mixed fiber type muscle.

Q: What is the sample rate of the system? Can you assess the rate of force development in these experimental conditions?

[R.Grange]: We typically sample at 1000 Hz, which is sufficient to determine rate of force development

[M.Borkowski]: Great Question. The output of the instruments themselves is an analog voltage so it can theoretically be sampled at nearly any rate. The software we typically package the instrument with (Dynamic Muscle Control), can sample up to 10kHz. Since contraction development is occurring over many milliseconds this is certainly fast enough to assess this.

Q: Can you use the transducer/motor on the stage of a microscope where you may follow other parameters (e.g. fluorescence)?

[M.Borkowski]: Yes absolutely. We have a chamber developed for the dual mode lever system which is designed to mount on an inverted microscope. We have many other chambers that work with our other instruments as well for researchers studying small fibers or cells as well.

Q: What is the physiological relationship for measuring torques versus frequency at values above 30 Hz? or, why to measure at so high frequencies if the body doesn't not do so?

[R.Grange]: The purpose of the torque frequency relationship is to establish the minimal (twitch) and maximal (tetanic) torque responses, but importantly the intermediate frequencies which provides some insight on the operating range of the muscle(s) (i.e., muscles provide the power to move a limb at these intermediate frequencies. Do they mimic directly the in vivo activation – no, because rate coding (i.e., activation frequencies) and recruitment of motor units operate in vivo; we can't at present duplicate that natural force control.

[M.Borkowski]: Electrical stimulation is recruiting motor units differently than in-vivo hence the higher stimulation rates. Nonetheless we need to apply these stimuli to find the maximal torque/force value of the muscle. We need to find the maximal strength of the muscle to have a proper reference for comparison between other muscles or between two different animal models.

Q: Is a physiological relaxation possible. i.e. for cardiac muscle? Physiological relaxation being combination of isometric and isotonic.

[R.Grange]: If the timing of the isometric and isotonic portions of the cardiac relaxation phase are known, then I believe a protocol could be developed to measure them

Q: Can the stretch protocols you demonstrated be used on smooth muscle and cardiac muscle samples? What about vascular smooth muscles?

[R.Grange]: Yes; protocols can be derived on the basis of known physiological characteristics.

[M.Borkowski]: Yes, dynamic contractions do occur in smooth and cardiac muscle, and standard experimental protocols exist. They are however different from the ones we describe in the webinar (which focuses on skeletal muscle).

Q: I am curious about using our system to measure connective tissue properties such as elastic modulus, or stiffness. Thank you for briefly describing one such experiment. What other potential measurements could be done with connective tissue?

[R.Grange]: We have measured stiffness of skeletal muscle and tendon using the stress-strain relations, including determination of the yield point. If there are specific characteristics you would like to investigate, then appropriate protocols can be developed.

[M.Borkowski]: Most of the experiments strictly done on connective tissue focus almost entirely on the stress-strain relationship. Since connective tissue has no active contractile component only passive tension can be measured by stretching the tissue in different ways. Breaking stress of the connective tissue is often measured as well.

Q: What is an effective way to minimize any compliance that might exist due to sutures used in the Aurora in-vitro water bath?

[R.Grange]: We use 4-O silk suture which is very stiff relative to the muscle. I have colleagues who use thin steel wire. However, the wire still needs to be connected to the tendons, and this could still introduce compliance.

[M.Borkowski]: I know of one very experienced lab who used Kevlar thread for this purpose and said that the compliance artifact was greatly minimized by doing so.

Q: Can we do power and force velocity relationship for whole muscle? Or can I measure shortening velocity in it (in-situ)

[R.Grange]: I assume by whole muscle you mean an isolated muscle prep such as the EDL or soleus. If so, then yes, the force-velocity assay can be performed, and from these data a power curve generated.

[M.Borkowski]: Yes absolutely, this is regularly done. Power is calculated from the force-velocity relationship which can certainly be done in whole muscle. Shortening velocity using the tetanic after load method can be done in-situ as well.

Q: Can this sort of physiological analysis be done on single isolated fibers (e.g. FDB fibers)?

[M.Borkowski]: Certainly. The presenter of our last webinar, showcased some of this data. It should be mentioned that with smaller, more delicate preparations, the tests are often simpler (isometric) so as not to risk damaging them accidentally during the experiment.

Q: How do you control length of the dual mode lever system?”

[M.Borkowski]: The instruments require an analog voltage input to control them, and are expecting a voltage waveform to have the same profile of the desired movement. The voltage input scales from -10 volts to +10 volts for full scale motion in each direction.

One of the best ways to accomplish this is by using some sort of DAQ card though a PC which can send voltages that are synchronized with a stimulator trigger. The output of the instrument can then be recorded as well. This functionality is provided with Dynamic Muscle Control software. The original version was in fact written by Dr Grange himself.

Q: How long should a test last for any one particular muscle or mouse? Or, at what point is the viability of the muscle compromised from being too long in a bath chamber or undergoing too many contractions?

[R.Grange]: There are a number of factors that determine the length of time for an experiment for a given muscle. But assuming dissection and hanging the muscle were not compromised, then the types of contractions that the muscle undergoes and the time between the contractions would influence the viability.

I suggest that for longer experiments, the muscle be tested in some standard way (e.g., a tetanus at some set interval to see if there is a loss of force (e.g., greater than 5-10% of the initial values), after which no further experimentation is done. To simply test the duration of the muscle in the bath under the experimental conditions (temperature, duration), test this muscle as described above but do no additional experiments on it.

If you have additional questions for Aurora Scientific or Dr. Grange regarding content from their webinar or wish to receive additional information about *in-vivo*, *in-situ*, and *in-vitro* muscle experimentation please contact them by email:

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