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Neuromuscular Network

CONTRACTILE MEASUREMENTS IN VIVO

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1. Update History

Version	Date of issue	Comments
01	5 December 2013	New document
02	4 June 2026	Updated SOP

2. Objective

The purpose of this SOP is to provide a simple yet detailed method to test skeletal muscle contractile function in rodent models of neuromuscular disorders in vivo. This non-invasive and non-terminal procedure can be performed longitudinally either in mice or rats – as well as in larger mammals, not discussed in this SOP – and involves stimulating a whole muscle group: anterior crural muscles (ankle flexors or dorsi flexors, i.e., primarily tibialis anterior and extensor digitorum longus), posterior crural muscles (ankle extensors or plantar flexors, i.e., primarily gastrocnemius, soleus, and plantaris) or anterior thigh muscles (knee extensors or quadriceps femoris) (1 – 5). For the sake of simplicity, this document focusses on isometric torque measurements, although this technique also allows us to perform lengthening (eccentric) contractions to assess susceptibility to injury and recovery (6 – 8).

3. Scope and Adaptability

Contractile force represents one of the most comprehensive functional indicators of overall muscle health in animal models of neuromuscular disorders. In vivo assessment of muscle force, particularly torque – defined as the application of a force at some distance from and perpendicular to an axis of rotation – is considered a reliable and physiologically relevant method for evaluating muscle function.

3.1 Advantages

The in vivo torque test is performed in anesthetized animals, without the need for any surgical intervention, allowing for rapid recovery at the end of the procedure. This means that the test can be repeated multiple times throughout a preclinical study protocol, for example to monitor disease progression and/or the effects of a treatment, as well as the recovery from injury. In parallel, normal muscle innervation and blood supply are preserved, thus allowing us to perform the measurement under physiological conditions. Finally, since the animal is asleep, there is no variability related to its willingness to perform the test, as is the case for in vivo behavioral assessments.

3.2 Disadvantages

The test requires expensive equipment, and only a single animal can be tested at the time. Experimenters need to be well-trained to allow correct placement of the needle electrodes.

4. Caution

Although this technique does not require complex surgical procedures or special handling of the animals, certain precautions must be taken to perform the procedure correctly and obtain reliable results.

- The operator should have at least a basic knowledge of rodent hind limb anatomy and muscle physiology to approach the technique and position the electrodes properly.

- The positioning of the animal, the stabilization of the hind limb (without interfering with the muscle being tested), and the positioning of the electrodes are essential for the correct execution of the technique.

- The animal's body temperature (~36 °C) should be kept constant, as this may affect force output.

- As with other functional assessments, this test should be performed at consistent times of day whenever possible. Mice are nocturnal animals, and circadian variations may influence muscle function, potentially affecting the comparability of results (9).

5. Materials

The equipment required to perform in vivo torque measurements includes:

- Anesthesia machine (isoflurane)
- Electric razor and/or depilatory cream, adhesive tape, betadine
- Stimulator equipped with needle electrodes
- Force transducer
- Heated platform for placing the animal with knee clamp (e.g., a trans osseous pin/27G needle and a tower to support the pin/needle) and pedal
- PC with software for data acquisition and analysis connected to an interface

Although it is possible to use custom-designed instruments, complete equipment for studying muscle physiology in vivo can be purchased from specialized companies; the most used device is the 3-in-1 1300A (mouse)/1305A (rat) Whole Animal System (Aurora Scientific Inc. – ASI, Aurora, ON, Canada; Figure 1).

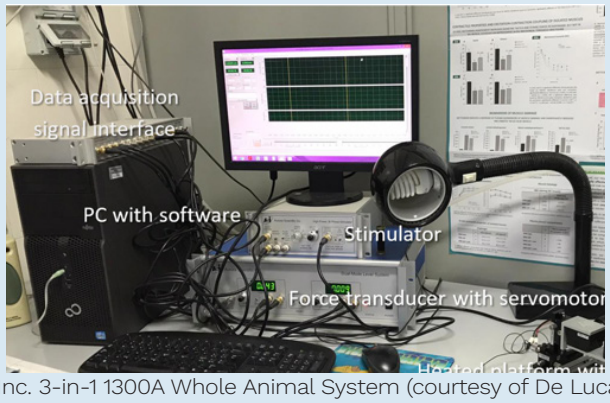

Figure 1. Aurora Scientific Inc. 3-in-1 1300A Whole Animal System (courtesy of De Luca's laboratory, Department of Pharmacy – Drug Sciences, University of Bari "Aldo Moro").

6. Methods

01. This procedure can be used for rats or mice. To begin, place the animal supine under inhalation anesthesia (~ 3-4 % isoflurane for induction in an induction chamber, then ~ 2% isoflurane via a nose cone for maintenance, both with 1.5 L/min 100% O2) using a precision anesthetic vaporizer (e.g. cat # 91103, Vet Equip, Inc., Pleasanton, CA; Harvard Apparatus Fluovac and Datex Ohmeda Isotec 4, Holliston, MA, USA). Be sure to keep the animal warm using a temperature-controlled platform on which the animal rests (e.g., mod. 809B ASI).

02. Prep the skin of the right hind limb by completely removing hair between ankle and pelvis, with an electric shaver and/or hair removal cream. Proper hair removal is essential to obtain a good clamp of the knee joint and electrodes placement. Optionally clean with betadine and 70% alcohol to prevent seeding skin bacteria into the soft tissue or bone. Confirm proper anesthesia by lack of a deep tendon reflex (no foot withdrawal in response to pinching).

03. With the animal in supine position on the platform, secure the right leg into the knee clamp (if using a 1300A or 1305A apparatus from ASI, this will be a U-shaped holder). The lateral tibia is secured in place with a brace that comes from the other side of the U-shaped holder which is also held with a set screw.

04. Place the foot of the right limb onto a footplate connected to a dual-mode servomotor (e.g., mod. 300C-LR, ASI), or onto a stepper motor (e.g., mod. T8904, NMB Technologies, Chatsworth, CA) and a torque sensor (e.g., mod. QWFK-8M, Sensotec, Columbus, OH), so that it is aligned orthogonally to the tibia at 90° (Figure 2). Adjust the distance between the platform and the pedal/servomotor by using micromanipulators. Secure the foot and the animal (i.e., nose cone, abdomen, and tail) in place with adhesive tape.

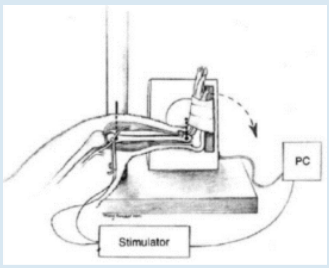

Figure 2. Mouse leg positioning for in vivo contraction (torque) measurements. Lengthening contractions are produced when the dorsiflexors are stimulated while the footplate forces the foot into plantar flexion (dotted arrow) (Lovering et al., 2011).

05. **Contractions will be elicited via a pair of transcutaneous needle electrodes (e.g., Chalgren Enterprises Inc., CA, USA; 723742, Harvard Apparatus, Cambridge, MA, USA), connected to a stimulator (e.g., high-power, bi-phase stimulator mod.**

701C, ASI), in turn controlled by a data acquisition signal interface (e.g., mod. 604A, ASI) and by dedicated software (e.g., Dynamic Muscle Control, ASI).

06. Before positioning the needle electrodes, the upper parts of the needles (hubs) should be taped together to make it easier to keep them in place simultaneously. Ensure that the needle tips are in proximity to one another but not touching when positioning them. Needles are placed subcutaneously near the peroneal nerve (also known as fibular nerve) to measure dorsiflexion or near the sciatic/tibial branch nerve for plantarflexion. Accurate electrode placement is the most technically demanding part of the procedure and requires practice to avoid repositioning which could damage the muscle before measurement. Do not place the needles too deep, and only puncture once. Electrode placement for plantar flexors is generally easier due to their larger muscle size and tendon attachment point, which gives approximately tenfold greater torque compared to dorsiflexors. In contrast, dorsiflexor measurements require greater precision to avoid co-contraction of the plantar flexors. For these reasons, plantar flexors are more commonly assessed in practice, and reference data for dorsiflexor measurements are currently very limited.

Figure 3 gives a clear view of how the mouse should be properly placed and secured on the heated platform during the experiment, and how the electrodes are correctly positioned in the leg.

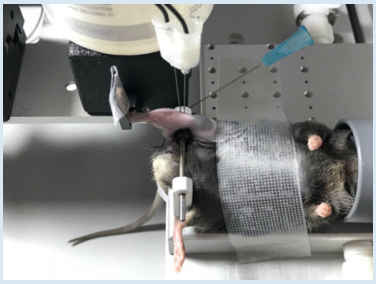

Figure 3. In vivo hind limb plantar flexor torque measurement in an anesthetized mouse. The right hind limb is secured in a knee clamp, with the foot attached to a servomotor footplate at 90°. Transcutaneous needle electrodes are positioned near the sciatic/tibial branch nerve to elicit contractions, while the mouse is kept under inhalation anesthesia and stabilized with adhesive tape (courtesy of De Luca's lab, Department of Pharmacy – Drug Sciences, University of Bari "Aldo Moro").

07. Via the live data monitor of the software, perform a series of initial twitches, evoked with 0.2 ms single square wave pulses, to adjust the current (from 25 to 40 mA) to maximize torque production. An increase in twitch amplitude in response to an increase in voltage confirms that opposing muscles are not being simultaneously stimulated. After this, a torque frequency curve is obtained by stimulation of the muscle group at increasing frequencies (usually 200 ms pulses at 1, 10, 30, 50, 80, 100, 120, 150, 180, 200 Hz every 30 s). Maximal isometric torque is usually reached between 80 and 100 Hz in healthy mice. If lengthening contractions are used to induce injury, torque measurements are performed before and after injury on the same rig that is used to induce injury. The magnitude of injury can be regulated by manipulation of variables such as angular velocity, timing of muscle activation, % maximal activation, range of motion, and the number of lengthening contractions. The specific protocol depends on the magnitude of injury desired by the investigator and is not discussed in this SOP.

08. At the end of the experiment, the electrodes are removed; then, the animal is freed from the tape and knee joint clamp, and a small amount of betadine is applied to the exposed skin and the knee area of the tested leg.

The animal is then placed in a warm recovery chamber with 1L/min 100% O2. When the animal can ambulate in the chamber normally, it can be returned to its cage.

09. Data collection and analysis is performed by means of dedicated software, e.g., the LabVIEW software, National Instruments, Austin, TX, or the 615A Dynamic Muscle Control and High-Throughput Analysis software suite, that combines three LabVIEW stand-alone executable programs into one complete package.

7. Evaluation and Interpretation of Results

Isometric peak twitch torque and tetanic torque – frequency curve values at increasing frequencies are normally recorded in N*cm. On an excel worksheet, or another program for data elaboration of operator's choice, these can be converted to N*mm and then normalized to the body weight of each mouse to obtain N*mm/kg. This data can then be used to produce additional high-quality figures.

7.1 Reference Values

Reference data have been collected by the De Luca lab at the University of Bari "Aldo Moro" **(Figure 4)**. Hind limb plantar flexor torque was determined in C57BL/10ScSn-Dmdmdx/J (BL10-mdx) and D2-mdx mouse models of Duchenne muscular dystrophy in a direct comparison with C57BL/10ScSnJ (BL10-WT) and DBA/2J (D2-WT) wildtype mice with matching genetic backgrounds. Both dystrophic mouse models showed a strong reduction in force at all frequencies of stimulation between the age of 8 to 52 weeks. Notably, dystrophic and wildtype mice on a DBA/2J genetic background were weaker than BL10 counterparts at frequencies ≥ 50 Hz.

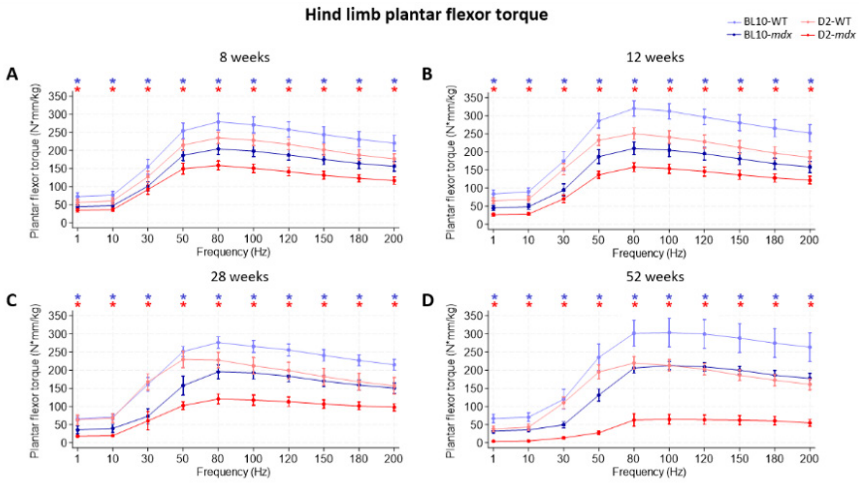

Figure 4. Hindlimb plantar flexor torque force frequency curves, normalized by body weight, acquired at the age of 8 (A), 12 (B), 28 (C) or 52 weeks (D). Mice were assessed in a longitudinal manner, with all animals being assessed at 8 weeks of age (n=34 males per strain), and smaller subsets at the other timepoints (n=26 at 12 weeks, n=18 at 28 weeks and n=10 at 52 weeks). The stars indicate a significant difference (P<0.05) between BL10-WT and BL10-mdx (blue stars) or D2-WT and D2-mdx (red stars) mice.

6. Statistical Analysis

The statistical analysis of contractile measurements should be based on the hypothesis of interest. Contractile measurements are collected at multiple frequencies per muscle; however, we are typically most interested in torque differences at specific frequencies rather than over the range of all frequencies.

Analysis at each frequency between groups is testing the null hypothesis that the central value of the assessment between two or more groups is equal and requires cross-sectional statistical methods. These include Student's t-test or analysis of variance if the assessment is normally distributed, and Wilcoxon rank sums (Mann-Whitney U tests) or Kruskal-Wallis tests if the assessment is not normally distributed. If we are comparing more than two groups at a given frequency using analysis of variance or a Kruskal-Wallis test, we must perform post-hoc comparisons between groups and account for multiple comparisons to maintain an overall type I error rate of 5%. The p-values from the post-hoc comparisons should be adjusted for the number of comparisons done using one of the several correction methods (i.e., Bonferroni, Sidak, Tukey, etc.).

Analysis of torque over contractions require more complex statistical models to adequately test the hypothesis of interest. The most common model used is a mixed effects linear regression model, sometimes referred to as a mixed model for repeated measures. However, it should be noted that the pattern of torque over frequencies is commonly not linear and often requires a non-linear regression model. If the pattern over contractions is not linear (a clear example is shown in the reference data in figure 4), it is highly recommended that a statistician be consulted as non-linear modelling can be quite challenging.

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