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Non-invasive measurement of ankle flexion/extension torque for longitudinal follow-up of strength in small rodents

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1. OBJECTIVE

The present SOP aims to describe the methods that have been developed for the *in vivo* assessment of ankle flexion/extension isometric torque using the "DynaMice", a specific dynamometer designed at the Institut de Myologie for small rodents (mice, hamsters, rats or guinea pigs).

2. SCOPE AND APPLICABILITY

All conditions described are specific to the DynaMice and some aspects might not be applied to other systems or conditions.

The DynaMice has been validated for mice and hamsters but may be useful for rats and guinea pigs as well, keeping in mind that possible dimensional re-arrangements of the system may be necessary. The experiments are performed while the animals are anesthetized. The muscles are activated using direct electrical stimulation delivered via subcutaneously inserted needles. This approach is preferred as direct nerve stimulation has been observed to be less repeatable for this particular evaluation.

3. CAUTIONS

Consistent training and experience with the DynaMice equipment and protocols must be acquired before the results should be considered reliable.

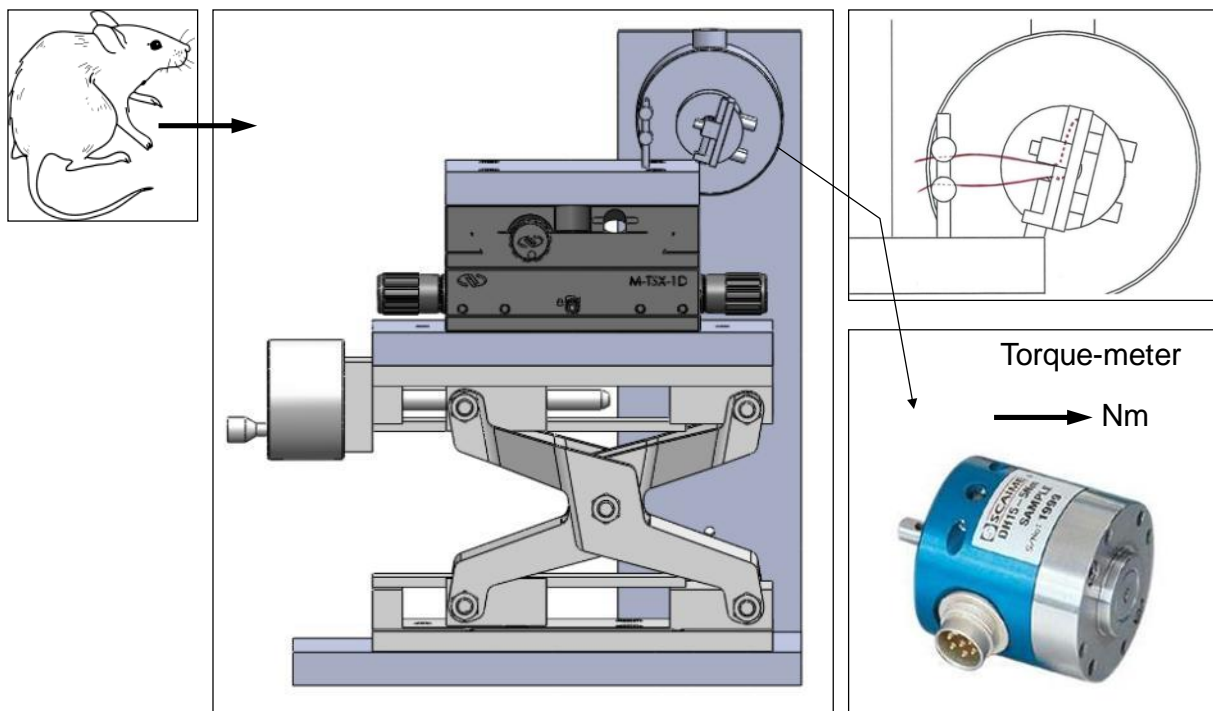
4. MATERIALS

The different elements that are currently used with the system at the Institut de Myologie are listed below in brackets. Rigorous validation should be carried out for any alternative elements used:

- method of anesthetizing animals (intra-peritoneal injection of a combination of ketamine, xylazine and midazolam)
- stimulator (Isolated Pulse Stimulator Model 2100, A-M Systems, Sequim, WA, USA)
- two monopolar (28G) electrodes (Teca disposable monopolar needles 902-DMF37-TP, CareFusion, Middleton, WI, USA)
- DynaMice (equipped with a torquemeter SCAIME DH 15 - 0.05 Nm, Annemasse, France)
- the conditioning electronic box for strength visual feedback and BNC output

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- data acquisition system (DAQ board National Instruments model USB 6229)
- laptop
- data acquisition and processing software (Protags developed in Labview)



Scheme of the DynaMice system

5. METHODS

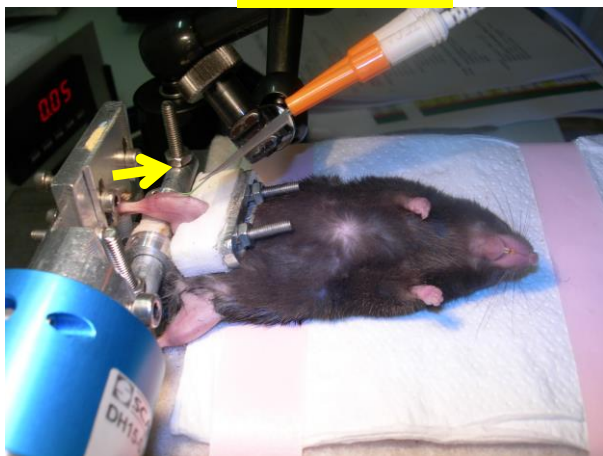
The mouse is anaesthetized by intra-peritoneal injection of a mix of ketamine, xylazine and midazolam (80, 15 and 0.8 mg/kg body weight, respectively), which allows for ~1 hour experiment. If a slightly longer experiment time is needed, the animals can be re-injected with a 5 times smaller dose. The lower limbs are shaved and the animal is installed in supine position with hip, knee and ankle at 90° in a homemade dynamometer set up (namely the "DynaMice") similar to devices previously described (Huguet et al., 2013; Ridgley et al., 2009). The ankle joint axis must be perfectly aligned with the torque-meter axis. This is achieved by making three-dimensional adjustments to the micrometric platforms.

Subcutaneous electrodes are inserted at the level of the bulk of the tibialis anterior or of the gastrocnemius muscles to trigger ankle dorsi- or plantarflexion, respectively. The maximal isometric torque tetanus is obtained by direct application over the muscle of a 300

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milliseconds train of 200 microseconds biphasic square pulses at 100 Hz. Stimulation intensity is progressively increased by step of 0.5 mA until the maximal torque is reached. When the torque begins to decrease after a plateau has been reached, the intensity must be decreased and adjusted so the antagonist muscles are not recruited. Stimulation intensity eliciting the maximal tetanus torque ranges between 3 and 10 mA. The optimal intensity is then used for the fatigue protocol, which consists of a continuous 100 Hz electrical stimulation imposed for 20 seconds.

Dorsiflexion

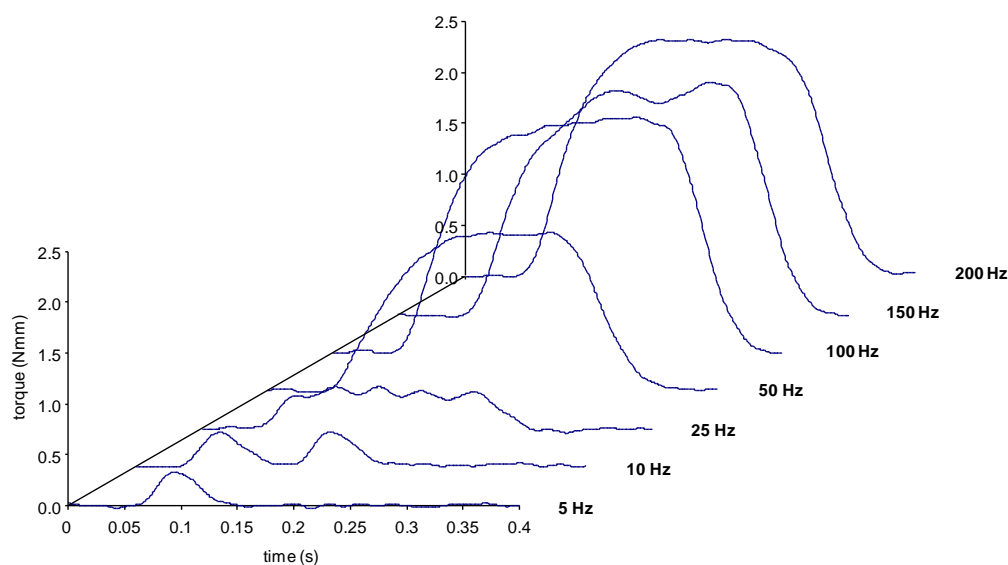


Plantarflexion



Positioning of the mouse during experiments

Ankle torque is measured with a torque transducer. Stimulation and torque signals are acquired using a data acquisition system. A custom-made LabView-mediated software is used for signal recording (10 kHz sampling rate) and analysis.

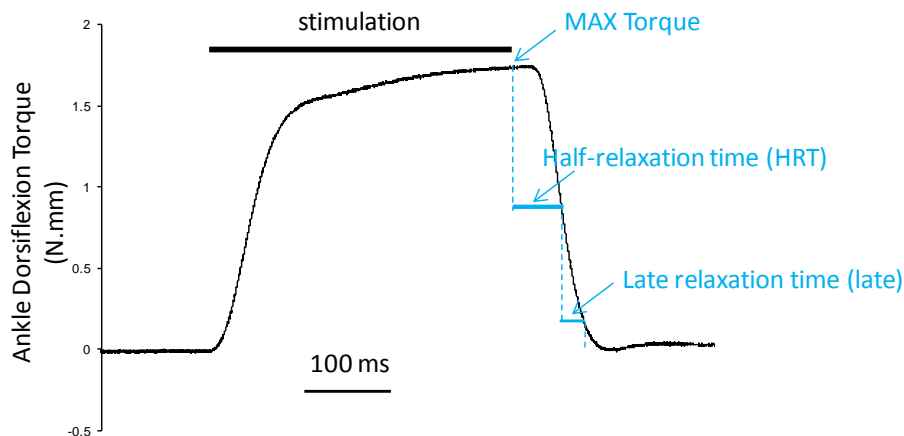


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Progressive fusion of twitches with increasing stimulation frequency (ankle flexion)

6. EVALUATION AND INTERPRETATION OF RESULTS

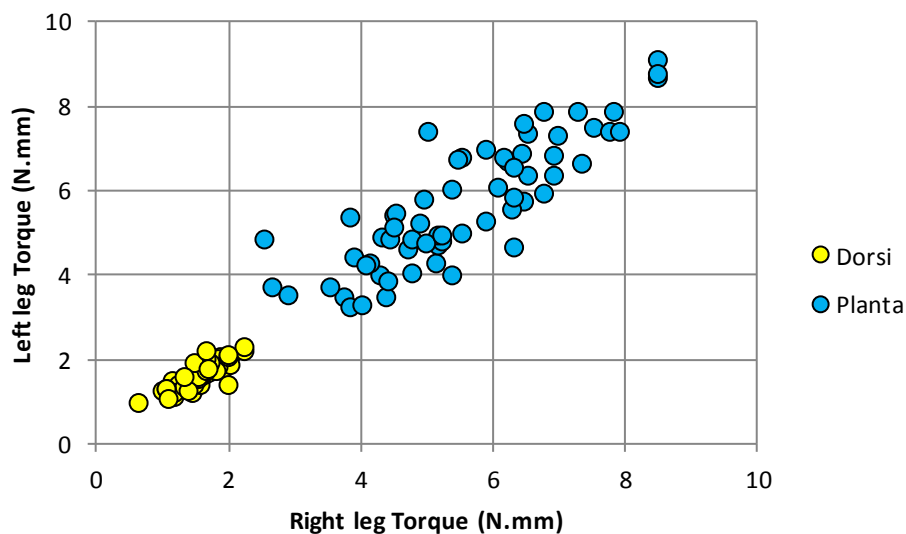
Tetanus peak torque, half-relaxation time (time for tetanic torque after the end of the stimulation to decline to 50%) and late relaxation time (time for torque to decline from 50% to 10% of the tetanic torque at the end of the stimulation) are determined. The torque elicited by the fatigue protocol is measured at different times during the continuous stimulation by averaging over 200 milliseconds the contractile response centered on 4, 10 and 19 seconds after the start of the stimulation. It is expressed as percentage of the maximal torque plateau achieved at the beginning of the stimulation (maximal torque averaged over a period of 200 milliseconds).



Computation of relaxation times

The repeatability of the method has been assessed by comparing the tetanic peak torques of the right and left hindlimbs of the same mice. Good reliability has been observed in wild type and DMSXL mice (ICC=0.97). However, reliability should be assessed for each model because pathophysiological conditions could have possible deleterious effects on muscle contraction reproducibility.

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Repeatability between left and right sides (ICC=0.97)

7. REFERENCES

Decostre V, Vignaud A, Matot B, Huguet A, Ledoux I, Bertil E, Gjata B, Carlier PG, Gourdon G, Hogrel JY. Longitudinal in vivo muscle function analysis of the DMSXL mouse model of myotonic dystrophy type 1. *Neuromusc Dis* 2013, in press. doi: <http://dx.doi.org/10.1016/j.nmd.2013.07.014>.

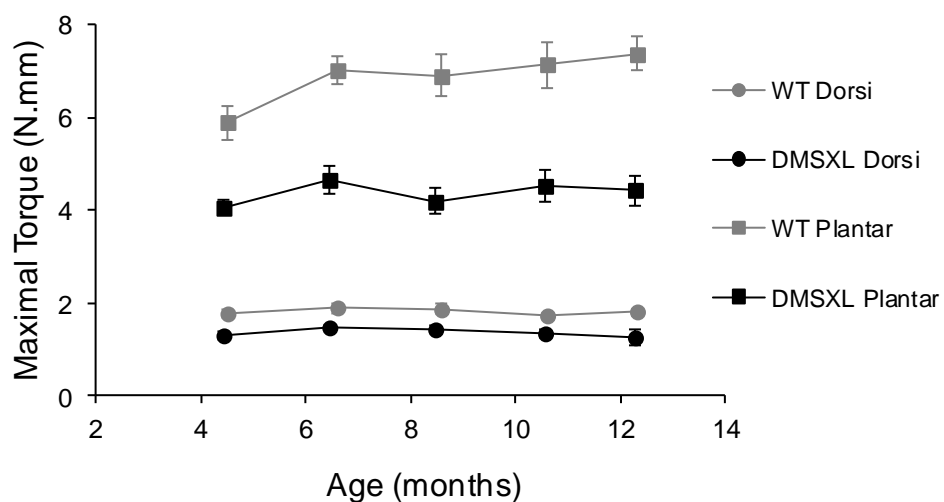
Huguet A, Medja F, Nicole A, et al. Molecular, physiological, and motor performance defects in DMSXL mice carrying >1,000 CTG repeats from the human DM1 locus. *PLoS Genet* 2012; 8: e1003043.

Ridgley JA, Pinniger GJ, Hamer PW, Grounds MD. The physiological effects of IGF1 (class 1:Ea transgene) overexpression on exercise-induced damage and adaptation in dystrophic muscles of mdx mice. *Pflugers Arch* 2009; 457: 1121-1132.

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8. APPENDIX

An example of follow-up is given in the following figure. An evaluation of DMSXL mice (model of myotonic dystrophy type 1) has been performed every two months between the ages of 4 to 12 months.



Dorsi- and plantar-flexion torque follow-up over 8 months in DMSXL and WT mice.

The protocol has also been successfully applied to a hamster model of delta-sarcoglycanopathy.