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Use of Grip Strength Meter to Assess the Limb Strength of dyw Mice

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1. OBJECTIVE

Grip strength of mice can be measured non-invasively in vivo by taking advantage of the rodent’s instinctive tendency to grab as they are gently pulled backward. This method can be used to test forelimb grip in which case a horizontal bar is attached to a force transducer or using a grid, in which case strength of both forelimb and hindlimb are measured together. The advantage is that the methods are reproducible and inexpensive and can be measured repeatedly in rodents to discern the natural history and effect of therapy or genetic modifications (Connolly, 2001; Connolly, 2002).

2. SCOPE AND APPLICABILITY

The purpose of this assay is to measure strength and can be tested easily at the time of weaning (3-4weeks). Mice are conscious and this method can be used longitudinally at fixed intervals to assess effect of intervention. The phenotype of the severely affected dyw and Dy 2J mice limits the outcomes one can assess. Both the dyw and dy2J mice are small at birth and remain small with the dyw mice being the most severely affected. They develop hind limb contractures making assessment with walking very difficult.

Advantages:

Measurement of forelimb grip strength is non-invasive and does not damage muscle and can be tested for most of the lifespan. While mdx mice clearly fatigue when grip strength is performed without rest in between, dy/dy mice did show only about 10% fatigue (Connolly, 2001). Limited information regarding dy^{w} mice fatigue is available (see below).

Disadvantages:

a. Variability: Variability exists in all in vivo testing and, in other rodent models there is an expected sex, weight, and age effect. In longitudinal testing, most were able to grip the bar at ages 3, 4, 6, and 8 weeks but by 10 weeks most have died or are too weak to grip bar (unpublished observation AC). Strength was very impaired with range of 4-11 gms/gram body weight. There was however, significant fatigue from the first pulls to last pulls which ranged from 10 to 25%. Therefore, in order to use this assay, the issue of fatigue must be addressed.

b. Dy^{w} mice, untreated progress (Kuang, 1999) and become too weak to grip the bar (unpublished observation AC). While this is a disadvantage it is closely linked temporally to death (unpublished observations AC). Therefore, this does suggest that effective treatment should lead to prolongation of ability to perform this test.

c. Hindlimbs may not be readily measured: As dy^{w} mice have disproportionate and earlier involvement of hindlimb weakness grid testing will likely be ineffective.

d. Learning/habituation bias: The tendency to grip is innate in healthy mice and this test has been readily performed in mdx, mdx:$utrn^{-/-}$, dy/dy, dy^{2J} and dy^{w} mice. Testing can usually begin safely at 3 weeks. However, if mice habituate or loose interest, testing at 1-4week intervals may prevent this. In healthy animals and mdx mice studied over two years, no such effect was seen. To avoid this potential problem, it is recommended that animals be tested no more frequently than weekly.

3. CAUTIONS

Strength determination is affected by general time of day and fatigue and by the operator. Therefore, it is important that these be standardized.

a. The same operation should measure all mice in a group and should be blinded to treatment effect to avoid personal bias.
b. The test should be performed at the same time of the day and the same day of the week by the same person.

4. MATERIALS

The grip meter must be equipped with a precision force gauge which will allow digital display. In the case of dy^{w} mice, it is likely that a trapeze bar will work best.

The following chapter corresponds to (or: is adapted from) the SOP DMD_M.2.2.001, Materials, page 5, by Annamaria De Luca and is pasted here with her consent on behalf of the working group members:

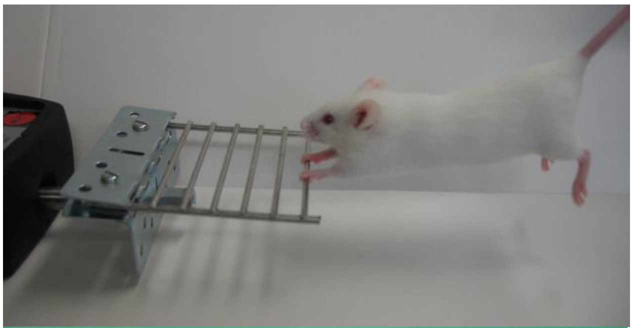
The basic material needed is a digital force meter equipped with precision force gauges to retain the peak force applied on a digital display and with a grid or wire system that allows mouse grip by either or both paws. Data can be collected either manually (reading the values on the display) or on-line through a RS232 connection with a computer. A number of different force meters for the determination of grip and limb strength are commercially available (PanLab, Ugo Basile, Columbus Instruments, TSE Systems, etc). However, they are not considered to be technically equivalent as there are major differences in design and electronics. A grip strength meter with an axial force transducer is to be preferred to one with a lever-type force transducer (the results of the later is easily inferred by the physical law of the lever).

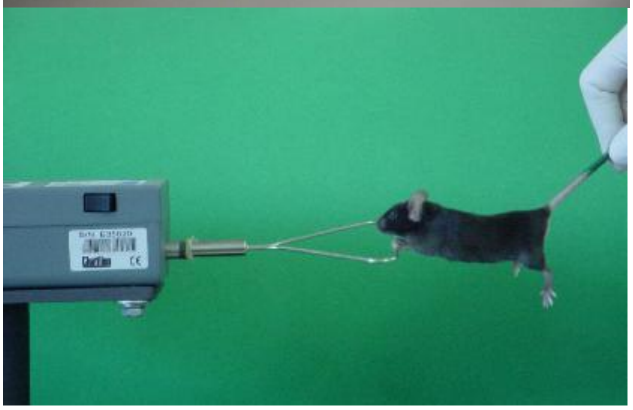
- All commercially available force meters are digital dynamometers produced by different brands and re-sold for the specific animal use by biological research companies. These may re-sell it either directly or via local country specific representatives, with prices in the order of 1200-3000€ (for meter and grid/wire, based on direct or mediated sell, money exchange rates, taxes etc). Few commonly used models are listed below:

a) Chatillon® DFE Series available with capacities from 2 lbf (1 kgf, 10 N) accuracy of better than 0.25% full scale; AMETEK TCI Division • Chatillon Force Measurement Systems; Largo, Florida (USA); re-sold by Columbus Instrument, USA

b) CENTOR Easy force meter, 25N maximal force (see figure below); CatNo: CNR EA 25; produced by Andilog Technologies SA, Chaville, France; re-sold by PanLab and others

Grid or wire for the grip strength measurements (specifically designed grids (grids only!) are sold by PanLab for 350€, however can easily be assembled from materials which can be purchased from a local do-it-yourself store)



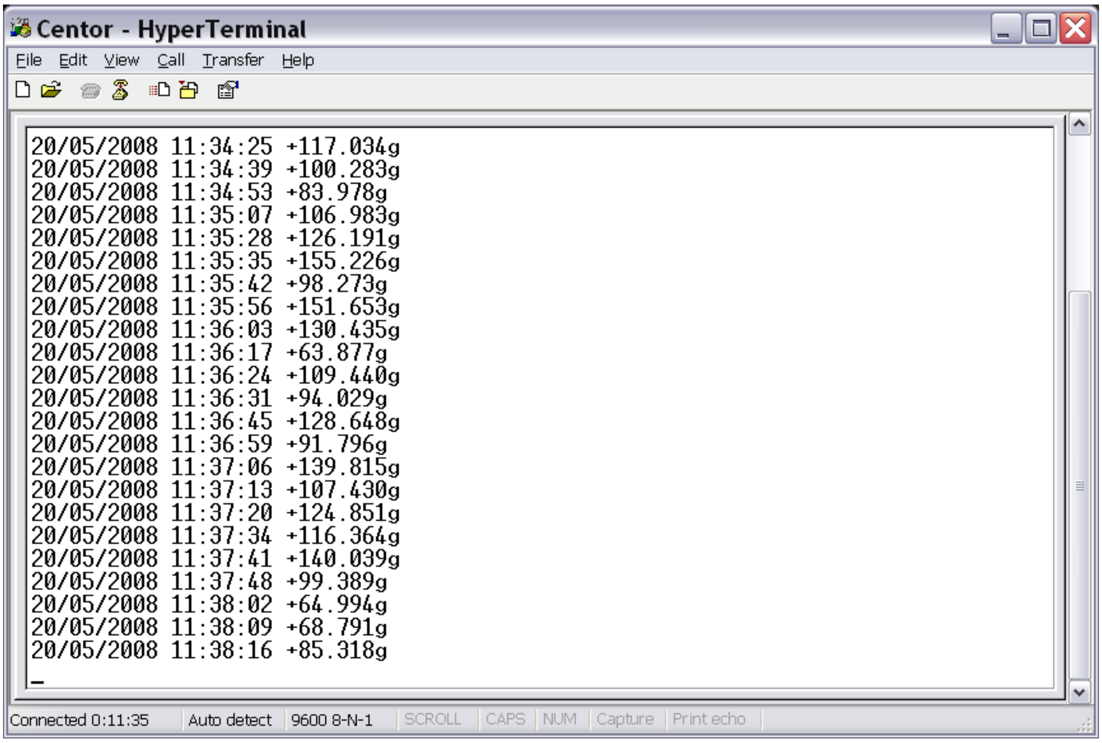


1) Custom made computer cable for RS232 connection (optional, for the direct transfer of data to a computer). The cable terminal diagram is the following:

Centor	Cable	Computer
SubD 15 pin, m	**Function**	**RS232 (9 pin SubD, f)**
3	RS232 RxD	2
4	RS232 TxD	3
13	Shield	5
shield	Shield	shield

2) A software (optional for the direct transfer of data to the computer; only in conjunction with the cable) such as HyperTerminal software for the PanLab meter (data transfer settings; 9600 Baud, 8 data bits, 1 stop bit, no parity, no flow control; as part of the Windows operating system the HyperTerminal program is free of charge). Generally, after acquisition and for further processing, the results can be

saved as a text file or can be copy-pasted into any other application, i.e. Excel thus allowing easy management and statistical analysis of the original data. Using the optional cable and software, the data are transferred directly to the computer, along with a date and time stamp. This ensures the best possible traceability and avoids possible writing or typing errors.



Screenshot of the data as can be seen, if the data are transferred from the Centor Easy force meter to the HyperTerminal program. Similar data sheet can be obtained with other software for data acquisition.

Special comments:

The proper functioning of the force meter can be verified by exerting a defined maximal force on it as follows:

a) Affix a paper clip to the grip bar (aim: paper clips are magnetic, the grip bar is usually made of stainless steel and is thus not magnetic).

b) Attach a magnet to the paper clip in a defined manner (ideally the contact to the magnet should be just on one edge of the paper clip (not on the side) and the place of contact on the magnet should be also defined: this is easier if you use a small magnet).

c) With a slow, constantly increasing force, pull the magnet until the paper clip is released. If the contact between the paper clip and the magnet is sufficiently defined, the force necessary to break the contact will be relatively constant.

First, perform the check with the magnet in the middle area of the bar: constant values with a data spread of <2% should result. A larger spread of the data will indicate that the electronics are not functioning appropriately (the electronics of some grip strength meters are inappropriate, i.e. Ugo Basile). If the first test was successful, then repeat the same test first on the left and then again on the right side of the bar: the results should be the same, independently of which side of the bar the measurement was made. A difference in the measurement values from the left and right side indicate that there might be a lever-effect: some of the marketed grip strength meters are error prone due to the selection of a force transducer where the results are dependent on the animal's holding position on the bar. Therefore, such force transducers (e.g. the one from Ugo Basile) are not reliable. It is sufficient to perform these tests just once, on purchase and installation of the device.

The diameter of the pulling bar is also of particular importance. A very thin pull bar (as that of Ugo Basile) may allow a very tight grasp that requires a very strong pull to break it. In addition, the use of a very thin bar or wire could result in an animal wounding its paws. A very thick bar could result in weak grip strength values. A suitable bar should be 1-2 mm in diameter and composed of non-flexible metal, allowing an efficient grasp that can easily be broken by the operator.

The force meter has to be fixed to a firm base (so to be stable during the pull and not move as a result of mouse grasp), at a defined height above the table or surface. Positioning the force meter on the edge of a desk or table-top is a good idea, as upon breaking the grasp, the mice are then not likely to hit the top of the table.

The force meter has to be configured for the recording of the maximal force according to the manual and in the units (lb, kg, N) preferred by the experimenter.

5. METHODS

Fore limb grip strength measurement.

a. Set the meter to 0 and use grams as a unit for values
b. Gently lift the mouse by the tail to the height where front paws are just in front of and above the grip bar.
c. Move the mouse gently backward until the mouse grabs the bar with both forepaws. This must be clearly seen.

d. The mice are analyzed on a horizontal platform or with a grip bar. Gently pull the mouse away at a constant speed until its grip is broken. Peak tension (grams of force) is recorded on a digital force gauge as mice release their grip. The transducer will save the value at the time of the release. The transduced must be again set to 0 before each new measurement. Repeat the test five or six times and record all.

e. If the plateform is used, the measurement is discounted if the hindlimbs touch the horizontal plated. The measurement should also be discounted if the animal uses only one paw, uses hind limbs or releases with no resistance.

f. Weigh the mouse so that force can be calculated as strength per gram body weight.

Strength Calculation.

The single best recorded value (Max Force) or the average of the three best are recommended to give the strength score. While the mean value of each session can be calculated for analysis this method is less favored as it is likely that fatigue (see below) will go unanalyzed in this paradigm. In addition, the results can be expressed as a multiple of the body weight by dividing the grip strength value by the body weight of each mouse. For longitudinal studies, time-dependent variation (increment/decrement) with respect to initial values can also be used to evaluate effect of pathology/therapeutic strategies.

Fatigue Calculation.

Calculates the degree of fatigue by comparing the first two pulls to the last two pulls. The decrement between pulls 1 + 2 and pulls 4 +5 (or 5-6 if six pulls are recorded) gives a measure of fatigue. In the formula (4+5)/(1+2) (or (5+6)/(1+2)), animals with no fatigue have a value of 1. If this number is subtracted from one so that an animal without fatigue has a calculated fatigue value of 0, and an animal that can only complete 3 pulls, and thus fatigues completely, has a value of 1. These values were then expressed as percentages. An animal without fatigue would have 0% fatigue; an animal with complete fatigue would have a value of 100%.

6. EVALUATION AND INTERPRETATION OF RESULTS

While the exact size of test groups needed depends on the effect size of a given intervention, a minimum of 6-10 mice per group are generally required if statistical significance is determined. This assay ideally allows one to look at absolute forelimb grip strength across or forelimb grip strength a function of body weight across time (by dividing the grip strength value by the body weight of each mouse). While the dydy mice do not fatigue as much as mdx mice in the natural history study as they aged (weeks 16, 20, and24) this fatigue averaged 10% (Connolly, 2001) and therefore careful attention to the this ratio (first pulls versus last pulls) across time is important. For longitudinal studies, time-dependent variation (increment/decrement) with respect to initial values can also be used to evaluate effect of therapeutic strategies.

7. REFERENCES

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8. EXPERIMENTAL DATA

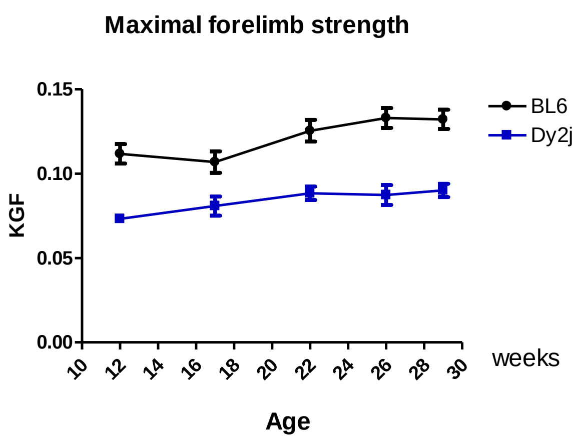

Fig.1. Forelimb grip strength in Dy2J mice. Five successful forelimb strength measurements within 2 minutes were recorded. The maximum values of each day over 5 day period were used. Dy2J homozygous mice had significant lower maximal forelimb strength when compare to the BL6 control group.