SMA_M.2.1.002

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Grip strength

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1. OBJECTIVE

The grip strength test is a widely-used non-invasive method designed to evaluate mouse limb strength that has been used to investigate the effects of neuromuscular disorders and drug. It is based on the natural tendency of the mouse to grasp a bar or grid when it is suspended by the tail. During this test the mouse grips with both forelimbs (or hind-limbs) a single bar or a mesh. There are three kinds of grip strength tests used: Mesh Grip Test, Wire Grip Test, and Automatic Grip Strength (GS Meter). The Mesh Grip Test measures the ability of the mouse to remain clinging to an inverted or tilted surface such as a wire grid or a cage lid for a period of time, usually up to 1 minute; the Wire Grip Test measures the ability of the mouse to hang on a wire with its forepaws for a preset length of time or until grip fails, while in the Automatic Grip Strength the mouse grasps a horizontal metal bar or grid while is pulled by the tail. The bar or grid is attached to a force transducer that peak pull-force achieved on its digital display. A thorough evaluation of automated force meter has been already performed by the Treat NMD consortium for mdx mice and is available online (M.2.2_001; http://www.treatnmd.eu/research/preclinical/SOPs/).

2. SCOPE AND APPLICABILITY

The purpose of this assay is to assess the animals fore and/or hind limb strength. This method can be used to measure disease progression and neurobehaviour as well as to test effect of specific therapeutic interventions in mouse models of neuromuscular disorders; increases in grip strength have been interpreted as evidence of increased muscle strength. The grip strength test is useful to assess strength in animal models of spinal muscular atrophy (SMA) and the effect of experimental therapies. This test can be performed reliably in SMA mice starting at 2-4 weeks of life (mesh grip ≥ postnatal day 12 (P12); bar test ≥ P10; automated ≥ P28.

Advantages

- It is a simple, non-invasive test.

- It can be performed longitudinally. It is particularly useful in SMA mice that live longer than 2 weeks.

Disadvantages

- Cannot be used in mice less than P10.

- Some mice have to be excluded from analysis due to poor cooperation with the task.

- Multiple trials (3-5) per animal are required to generate reliable data

- Several variables affect the test reproducibility: parametric factors (sampling frequency of the load cell, angle at which the animal is pulled, system type, and speed of each trial); state of arousal at the time the test is administered (morning-afternoon); body temperature,

fatigue due to previous physical activity; diet restriction-induced changes in body weight and muscle mass.

Specific Considerations

- Duration: Since the test has to be performed one mouse at a time with a period of rest time between each of the three-five trials per animal it requires several minutes (5-9 minutes). This results in a considerable time investment, particularly if several experimental groups are being concurrently evaluated.

- Variability: Since this is an in vivo behavioural test, by its nature, it has variability. In carefully controlled conditions, the variability within a group of animals of similar gender, age, and genotype should range between 10-25%. A similar variability should be observed among repeated measures (3 to 5) performed in the same animal during the trial. If greater variability is observed, it may indicate the presence of a bias in the assay (animal fatigue, variability in administering the test, or an ambient source of variation such as light, temperature, noise, etc). In order to reduce variability, it is important that the same examiner perform the test for all experimental groups under study and for the entire duration of the experimental session.

- Learning/habituation bias: To avoid the bias of habituation, it is advisable that the number of iterations in each session be limited (usually < 5). If the assay is repeated too frequently, the mouse may lose interest in performing the task.

Specific Limits

Mesh Grip test

The inverted-grid test may lack sensitivity in adult mice, and accurate assessment of grip strength is dependent on homogeneity of mouse weight across the cohorts. In addition, the mice tend to move around when the grid has been inverted, and consequently different muscle groups are being used or rested while the animal is moving. Butchbach et al. (2007) reported that the test can be performed with delta 7 SMA mice at P11 and P14 of life.

Wire Grip test

Only SMA mice older than P12 can perform this task. The results depend on animal weight and on the willingness of the mice to hang on to the grip bars. Variability of mouse weights across the cohorts affects accurate assessment of grip strength.

Automatic Grip Strength

The mechanical grip strength meters suffer from a single major problem: the unwillingness of the mice to hang on to the grip bars. Invariably several mice have to be excluded from the study on the grounds of non-participation and multiple trials per animal are usually required to generate reliable data. Sometimes mice refuse to hang on to the grip bars, this problem is reduced with the mesh. El- Khodor et al., (2008) reported that this test can be performed only on mice older than 28 days of age.

3. CAUTIONS

Because the grip strength measures may be affected negatively by several variables (e.g., volition, cognition, and fatigue) that are independent of muscle dysfunction, the experimental conditions must be well controlled**.** First, when considering test validity, it is essential that the tests selected are appropriate for the particular level of motor organization and function that is under experimental manipulation. When considering test reliability, it is important that the test design provides objective measurement, ideally by automated data collection. In order to detect real differences between experimental groups, the test must be sensitive. The most efficient approach to achieve these conditions is to include full repeated measures designs (the same measures are collected multiple times for each subject over time in a longitudinal study), to adopt the most powerful post hoc statistical analysis (like Bonferroni or Tukey tests), and to increase animal numbers.

- One attempt to increase the sensitivity of the grip strength test has been to add different masses to a grid that the mouse grips while suspended by its tail.
- In the mesh grip test, mouse movement can be overcome by agitating the grid before inverting it partially, as the animals increase their grip as the grid is shaken.
- In the strength grip meter, the unwillingness of the mice to hang on to the grip bars can be overcome to some extent by providing a metal grid for the mice to hang on to rather than a bar.

It is important that:

- For each kind of test, the same examiner should perform all measures to minimize human variability.
- The tests should preferably be performed in a blinded fashion, so that the examiner does not know the genetic status or the treatment state of the mice at the time of the test.
- The test should be performed at the same time of day, on the same day of the week, and in a protected environment.
- To assess the influence of the environment (the season of the year, food provided or variations in the animal housing) or of the experimental protocol, it is important to evaluate a group of wild-type mice as a control.
- Large group of animals (at least 6-8) that are of similar age and sex should be used. If possible, wild-type, heterozygous, and homozygous SMA mice should be littermates.

4. MATERIALS

The basic materials needed for the mesh and wire grip test are simple and cheap and consist of a single bar or a mesh and a timer.

- The mesh is made of a plastic or metallic material with 1 cm^2 grids (Butchbach et al., 2007).

- The bar is a simple metal rail small enough to be gripped (approximately 0.3-0.5 mm).

In contrast, for automatic grip strength (GS meter) measures, the materials are more elaborate and expensive and require a computer connection. The bar or grid, usually made of stainless steel, is attached to a precision force transducer to retain the peak force applied on a digital display. The data appear on the display can be collected either manually or on-line through a cable connection with a computer. A number of different force maters are commercially available (from Panlab, UgoBasile, Columbus Instruments, TSE Systems, etc.). Given that the automatic grip strength can only be measured after P28, this system has not been not widely used for severe SMA mice. Actually, new treatments are available for severe SMA mice which led to a remarkable increase of survival (Porensky et al., 2012; Passini et al., 2011) and for this treated animals this test can be performed. As milder SMA mouse models become available, this measure is likely to be more frequently used. A thorough evaluation of automated force meter has been performed by the Treat NMD consortium for mdx mice and is available online (M.2.2_001). To date, the application of automatic grip strength in SMA mice was reported by Passini et al. 2010: Grip Strength Meter, Chatillon; Columbia Instruments (Columbus, OH). Also Osborne et al., 2012 evaluated the grip strength in a in a novel allelic series of SMAmouse models using the Chatillon-Ametek DigitalForce Gauge, DFIS 2 (Columbus Instruments) to determine the strength exerted by the forelimbs of an animal in response to a constant downward force.

5. METHODS

At least 6 to 8 mice per group are generally needed if statistical significance is to be reached for all of the tests presented. All of the tests should be administered in a blinded fashion such that the group assignment is unknown to the examiners.

Wire Grip test

Fore limb strength measurement:

- Control mice as well as SMA mice are timed for how long they can support their weight holding onto a metal rail suspended in midair.
- The metal rail is suspended by the examiner or using a specific support
- Distance from the ground about 10 cm (soft surface beneath)
- The examiner take the mouse from the tail and allow the mice to grasp the metal rail
- Each mouse is subjected to five trials with at least a 10 min rest period between tests.

- The test can be performed measuring the time (without a pre-fixed limit) or defining a maximum cut-off (for example: 1 minute)

For the Delta 7 mice and "SMA2" mice (Grondard et al., 2005) the test is performed usually between P10-P15, however can be performed also a later times.

Mesh Grip test

Grip strength is assessed using a suspension test on inverted mesh grid.

Fore and hind-limb strength measurement:

- Each pup is placed on a wire mesh (1 cm^2 grids)
- Visually check that the grip is good
- The mesh with the mouse is inverted.
- The latency for the pup to release the mesh is recorded.
- The distance from the ground is about 10 cm (soft surface beneath)
- Repeat the test a set of number of times (3-5 times, not more than 5 times)

Grip strength is measured usually on PND11 and PND14 for the Delta 7 mice, however can be performed also a later times.

Automatic grip strength

A Grip Strength Meter is used to measure forelimb and forelimb/hind limb grip strength as an indicator of neuromuscular function. The Grip Strength Meter (GSM) consists of a base plate, a trapezoidal stainless steel grip, and a force sensor for recording data.

Grip Strength Meter, Chatillon; Columbia Instruments (Columbus, OH)

Allow the mice to acclimatize to the testing room for approximately 10 minutes before beginning the test.

- Place the Grip Strength Meter horizontally on a stable surface, away from drafts or vents that could disturb the measurement by the sensor.
- Gently clean the grid with a 50% EtOH solution and dry with a kimwipe
- Turn on the sensor. The unit of measurement of the sensor is delivered in grams mode
- Reset the display on the sensor to zero
- Prepare a record sheet to include the details of the mouse identification, trials, measurements etc.
- Remove a mouse from its home cage, gripping the base of the tail between the thumb and the forefinger.

Forelimb measurement:

- Gently lower the mouse over the top of the grid so that only its front paws are allowed to grip the smooth metal pull bar at the top of the apparatus.
- Keep the torso horizontal and pull the mouse back steadily and with a uniform force until the grip is released down the complete length of the grid.
- The force applied to the bar at the moment the grasp is released is recorded as the peak tension.
- Record the value.
- Tare the machine.
- Repeat twice for each animal.

Forelimb and hind limb measurement:

- Gently lower the mouse over the top of the grid so that both its front paws and hind paws are allowed to grip the smooth metal pull bar at the top of the apparatus.
- Keep the torso parallel to the grid and the mouse back steadily and with a uniform force until the grip is released down the complete length of the grid.
- Record the value.
- Tare the machine.
- Repeat twice for each animal.

Before testing different cages, clean the grid with 50% ethanol and dry with a kimwip. Grip strength is measured between PND12 and PND16 for the Delta 7 mice.

6. EVALUATION AND INTERPRETATION OF RESULTS

It is recommended that grip performance data be collected using consistent techniques, a thorough understanding of how the equipment of each test system functions, and rigorous methodology. During the first test, the mice might present an initial period of non-participation probably due to the learning process. Thus the tests have to be repeated 1-2 days later. However, if the test is repeated frequently, pay attention to any sign of habituation bias.

Interpretation of grip performance data must critically consider the factors that contribute to the overall grip performance measurement. Usually the SMA delta 7 and SMA2 mice show a significantly reduced strength measures compared to wild-type and heterozygous animals.

This is evident as early as the test can be administered (~P10). For the analysis, it is possible to compare the absolute values of grip time or force.

7. REFERENCES

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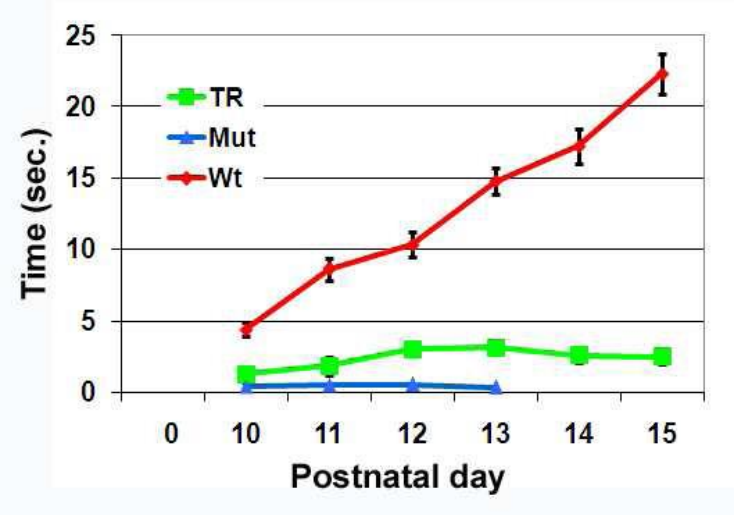
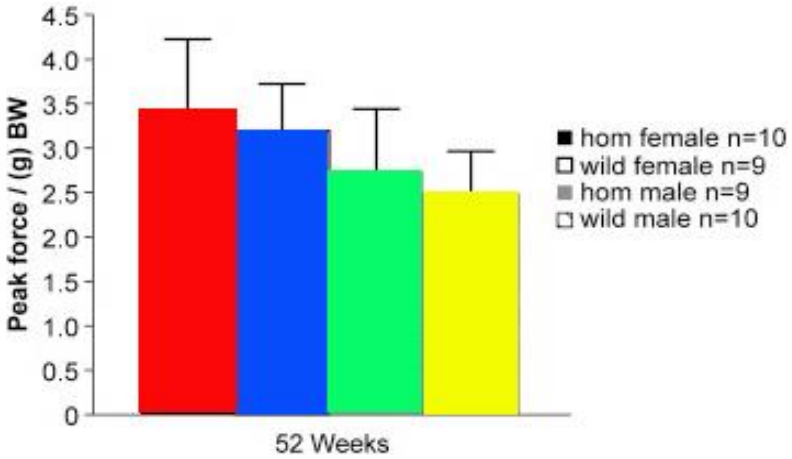
8. APPENDIX

A) Example of wire-grip test data (modified Corti et al., 2008).



Grip time in Neural Stem Cells (NSC)-SMA mice (blue color, n = 24) or untreated SMA (green, n = 24) and unaffected littermate WT controls (red color, n = 24). The grip time was statistically different in the untreated and treated SMA mice ($P < 0.00001$) at 12–13 days of age. Error bars represent SD.

B) Example of automatic grip strength test data (Osborne et al., 2012).



Modified from Osborne et al., 2012.

Neither homozygous Smn1C/C mutant males nor females exhibit diminished grip strength (as normalized to gram of BW) at either 24 weeks (C) or 12 months of age (D) at JAX. Data are represented as mean ± SD.