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Behavioral Phenotyping for neonates: Righting reflex

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1. OBJECTIVE

The righting reflex test is a simple, rapid test to assess locomotor abilities in mice. It evaluates general body strength and can be affected by weakness in limb muscles, trunk muscles and/or general poor health, by scoring or measuring the ability of mice to return to their four paws after having been placed in a supine position or on their side. Because of its simplicity, and like other, non-terminal tests, it allows the longitudinal study of the progression of a locomotor impairment, and/or its improvement by therapeutic compounds.

2. SCOPE AND APPLICABILITY

This test is particularly adapted to mouse mutants that are too young or too affected to be tested on other tests like the rotarod, the gait analyses systems or the openfield. It does not require any apparatus and can therefore be performed behind any health status barrier without the burden of having to move devices from one animal room to the other.
Righting reflex can be conducted starting at PND2 and carried out throughout the entire life of the mouse, however it is most applicable prior to weaning when the neuromuscular system is still developing. The nature of most neuromuscular disorders renders some muscles more affected than others, over time this can result in compensatory increases in strength within unaffected muscles that can influence righting reflex time in older mutant mice.

3. CAUTIONS

The righting reflex test lacks the precision of more quantitative tests like the rotarod or gait analysis and should therefore not be used to document the natural history or phenotypic changes of mutants with mild impairment that can undergo these tests.
A number of factors must be taken into account when conducting righting reflex. Some are listed below:

A. It is imperative to conduct this, and all phenotypic experiments in a blinded fashion.
B. Righting reflex can be affected by general health. This is important to consider when interpreting results as a poor score may be indicative of muscle weakness, but it may also be due to problems of feeding, maternal neglect, physical injury, cardiac dysfunction, ect. It is recommended that a daily weight be taken in conjunction with righting reflex, as this can be used as a measure of general health.
C. Neonatal mice (<P10) are unable to thermoregulate their body temperature, and should not be removed from their dam for more than 3-4 minutes at a time. As pups approach hypothermia their phenotypic results will differ compared to non-hypothermic controls.
D. If righting reflex is performed with other phenotypic assays an appropriate rest period must be incorporated (~5min) to remove fatigue related artifacts from the

results. It is not recommended that righting reflex be performed on consecutive days to avoid fatigue and artificial increases in muscle strength due to exercise.

E. Pups can be labeled a number of different ways to ensure proper identification longitudinally. It is important to ensure that your method does not affect phenotyping results.

F. If the righting reflex is being used in conjunction with other phenotypic tests, a test order should be standardized. Righting reflex should be performed prior to any exhaustive tests, such as hind limb suspension, or on alternate days.

4. MATERIALS

A flat, smooth surface required for the test, for instance a bench top or air table. It is not recommended to perform the test on textured surfaces (e.g. cage lids) because the texture can make the righting either more difficult, or easier. Consistency of the surface used across the whole experiment is required. Additionally, a hand–held timer can be used.

5. METHODS

5.1. Procedure

- Once a litter is born have someone else mark and genotype the mice. **Do not** reveal the typing of the mice until the final righting reflex date. Blinding is essential for accurate, unbiased phenotyping.
  - Litters over 10 should be culled to an N of 10 to avoid competition for nutrition.
  - Litters under 5 pups should not be phenotype as the increased maternal care will result in a milder phenotype.
- Remove the mouse from its cage and record body weight. Be sure to note if any of the mice have sustained injury or are in poor health.
- Invert the mouse on its back, stabilizing it with your finger so that all 4 paws are facing up in the air.
- Remove your finger and start the timer
- Stop the timer once the mouse has flipped over onto its stomach AND has all 4 paws touching the bench top.
  - If the mouse has not inverted after 60 seconds score the test a fail.
  - The test is not over if the mouse has one paw underneath its body. Wait until all 4 paws are flush with the surface.
  - Score for the ability to right itself can be:
    0 = Remain in dorsal position
    1 = Struggles to right itself
    2 = Slow righting reflex
    3 = Rights itself up. Time taken to turn over can be measured.
- Return the mouse to the cage with its mother.

Average the scores or times for three repetitions of the test per animal. As mutant mice can fatigue on this test, better reproducibility between repetitions is achieved if at least 5 minutes of rest is allowed between successive tests. For instance, the first test can be performed through the entire cohort once, then again back to first mouse for a second, and third times.

5.2 Notes

There are a number of variations to righting reflex (1, 2). The above description is the protocol from the DiDonato lab and Jaxon Labs optimized for neo and peri-natal mouse models from P0 to P12 (3, 4).

6. EVALUATION AND INTERPRETATION OF RESULTS

After the final day of righting reflex, unblind the groups and perform the appropriate statistical analysis (statistical analysis is dependent on the parameters of the experiment). It is important to consider the root cause for changes in righting reflex time. The majority of the time changes in score result from muscle weakness, but this is not always the case. Culling of litters and avoiding hypothermia are examples of measures that can be taken to maintain the general health of your pups and reduce non-neuromuscular changes in righting reflex time.

7. REFERENCES

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8. APPENDIX

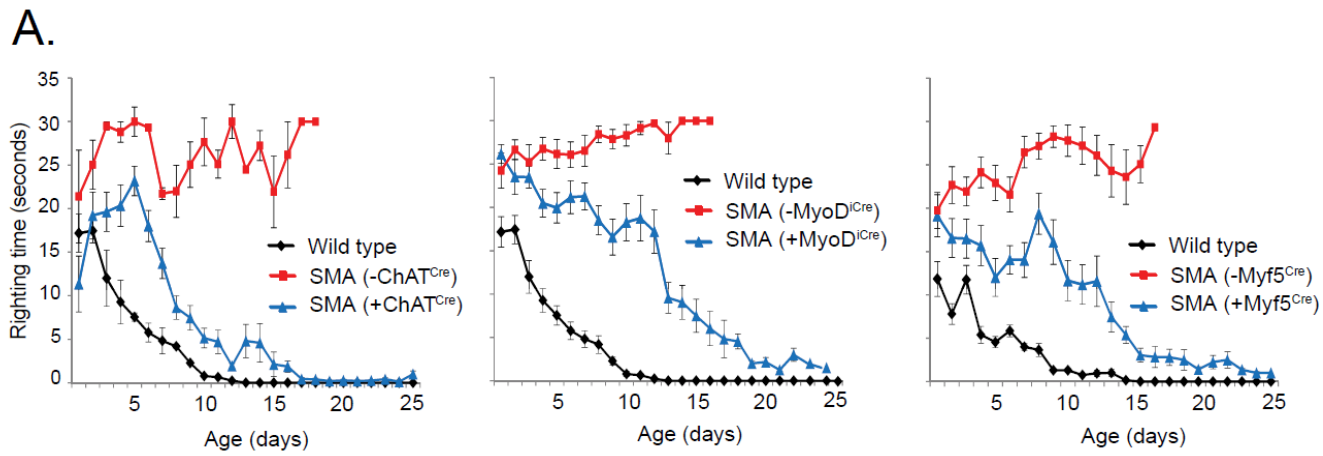

Fig. 1 Righting latency is reduced in Cre + SMA mice compared to Cre− SMA mice in all 3 lines of mice, p<0.0001 (ChatCre: n=11 WT, 12 Cre− SMA, and 27 Cre+ SMA, MyoDiCre: n=11 WT, 12 Cre− SMA, and 18 Cre+ SMA, Myf5Cre: n= 10 WT, 14 Cre− SMA, and 15 Cre+ SMA mice).

From: Tara L. Martinez, Lingling Kong, Xueyong Wang, Melissa A. Osborne, Melissa E. Crowder, James P. Van Meerbeke, Xixi Xu, Crystal Davis, Joe Wooley, David J. Goldhamer, Cathleen M. Lutz, Mark. M. Rich, and Charlotte J. Sumner: SMN in motor neurons determines synaptic integrity in spinal muscular atrophy. J. Neurosci. 2012 June 20; 32(25): 8703–8715.