SMA_M.2.1.001

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Use of pen test (balance beam) to assess motor balance and coordination in mice

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1. OBJECTIVE

The objective of the present SOP is to describe how the pen test (also known as the balance beam)[1] can be used to evaluate the disease-related impairment of motor balance and coordination in mouse models of spinal muscular atrophy (SMA). The goal is to be able to use the test to better assess the benefits of a specific treatment on young mice. Ultimately, one needs to develop preclinical animal tests that should predict the efficacy of a new treatment in patients. Therefore, we propose that motor balance and coordination are properties that can be tested in SMA mice before and after administration of a therapeutic. The pen test is suitable for this purpose due to its simplicity and speed of performance, and because no training is required. In this test, a mouse suspended by its tail is slowly lowered from above to a pen (diameter of 1 cm) horizontally held approximately 20 cm above the surface of a bench (Figure 1). Usually, the mouse will grab onto the pen and start walking on it without difficulty. Any deviation from this normal behavior is recorded, and the time till fall is recorded (latency to fall). After 1 min, any mouse that has not fallen is returned to its cage, and the time is recorded as 1 min. The test is repeated three to five times each day. The results from such measurements can be compared between various treatment groups (for example, plus or minus therapeutic treatment).

2. SCOPE AND APPLICABILITY

The pen test can be used to assess the severity of the SMA phenotype in mouse models and/or to evaluate the efficacy of therapeutic interventions such as those afforded by gene/cell therapy, pharmacological compounds, or nutritional intervention. This test can be performed as early as the first week of life on neonates.

Advantages

The pen test is non-invasive and can be performed longitudinally throughout most of the lifespan of SMA mice. As such, it is a useful tool to assess the effect of both short and long-term treatments. Also advantageous is that the test can be completed in a short time and thus the animals do not fatigue and retain interest in the test. Finally, no training of the experimenter is required to perform the test.

Disadvantages

The test can only be performed on one mouse at a time, which means that testing will require several minutes for each individual animal. If several experimental groups are being

assessed, the entire procedure can take up a significant amount of time.

Other variables can also influence the experimental outcome (see section on cautions below).

3. CAUTIONS

As with any pre-clinical test, there are a number of variables that can impact on the results obtained. For example, environmental factors may contribute to the level of anxiety in mice. The temperature, humidity, ventilation, noise intensity and lighting intensity should be at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained. Other variables that need to be kept constant include the time of day that the test is performed and the number of trials per day. Furthermore, the dimensions and the material of the balance beam (in this case a pen) need to be standardized – diameter of 1 cm and horizontally held by hand approximately 20 cm above the surface of a bench.

An important consideration is that the same person assesses all the mice in a group to minimize investigator variability. For the efficacy of therapeutic experiments, the person who assesses all the mice should not know which group is treatment or control. As such, in the ideal case, the examiner should be blinded.

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. This might cause a consistent time investment, in particular if several experimental groups are being concurrently evaluated.

4. MATERIALS

The material necessary for the pen test is fairly simple. Any pen with a diameter of 1 cm is suitable. Variations to this theme are common – e.g. use of balance beams made of plexiglass with a diameter or width of 1 cm.

5. METHODS

Several mice for each treated or control group are needed to achieve statistical significance. The pen is held horizontally approximately 20 cm above the surface of a bench. The mouse is suspended by the tail to a height where the front paws are at the same height as the pen. The mouse is allowed to grab onto the pen and to begin to walk on it, with its

four limbs. The latency at which each mouse falls off the pen is recorded. If the mouse is able to maintain balance and stay on the pen for at least 1 minute, it is returned to the cage and the time recorded is 1 minute.

The test is repeated a set number of times (usually between 3 to 5 times) and the time of latency to fall in each test is recorded. The mean of all values, as well as the maximum/minimum time is used for the analysis.

6. EVALUATION AND INTERPRETATION OF RESULTS

When the pen test is used to evaluate motor balance and coordination in SMA mice, the objective data obtained are used to determine the duration of time an animal is able to stay on the pen up to a maximum of 1 minute. Severe SMA mice are generally unable to grab onto the pen and therefore fall off within the first second (Figure 2). Any improvement induced by treatment can then be readily measured. The mean of all values obtained in 3 to 5 repeats of the test is used for the analysis. Performance in the pen test can be monitored over days to determine if any particular treatment strategy is having a positive influence.

7. REFERENCES

1. Gomez, C.M. *et al.* Slow-channel transgenic mice: a model of postsynaptic organellar degeneration at the neuromuscular junction. *J Neurosci* 17, 4170-4179 (1997).

8. APPENDIX

Figure 1. Examples of mice being subjected to the pen test. The mice in the left and right panels were able to stay on the pen for an extended time whereas the mouse in the middle panel had difficulty in staying on the pen (photos courtesy of Dr. Hong Liu).

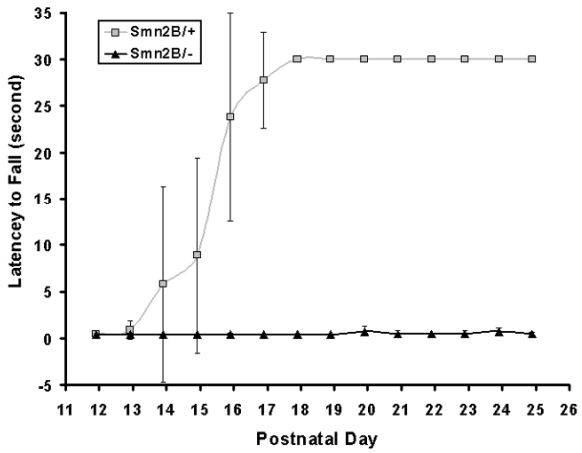

Figure 2. Pen test performed beginning at P12 on SMA mice or littermate controls. The latency to fall was recorded for each mouse. The normal mice were able to stay on the pen for an extended time (and the test was terminated at 30s in this instance) whereas SMA mice had difficulty in staying on the pen (data courtesy of Dr. Hong Liu).