Please quote the use of this SOP in your Methods.

Behavioural and Locomotor Measurements Using Open Field Animal Activity Monitoring System

SOP (ID) Number	DMD_M.2.1.002
Version	3.0
Issued	October 18th, 2008
Last reviewed	July 12th, 2017

Author	Kanneboyina Nagaraju Research Center for Genetic Medicine Children's National Medical Center 111 Michigan Avenue, N.W. Washington, DC 20010

Working group members	George Carlson (Dept. Physiology Kirksville College of Osteopathic Medicine Kirksville, MO. 63501-1497, USA) Annamaria De Luca (Sezione di Farmacologia, Dipartimento Farmacobiologico, Facoltà di Farmacia, Università di Bari, Italy)

SOP responsible	Kanneboyina Nagaraju
Official reviewer	George Carlson

TABLE OF CONTENTS

1. OBJECTIVE

Behavioural and locomotor measurements are important parameters that help to define the phenotypes of mice with neuromuscular disorders such as muscular dystrophies. These assessments are done in an open field Plexiglas chamber equipped with multiple photocell receptors and emitters. Animal activity is measured via a grid of invisible infrared light beams. A number of equally spaced beams traverse the animal cage from front to back, and an equal number of beams traverse the same cage from left to right. The body of the mouse, when placed within the monitor, will cause some of these beams to be broken, thus revealing its position in the (X-Y) plane. Vertical sensors connected to the mainframe monitor rearing or jumping activity. The analyzer collects the beam status information from the activity monitor and subjects it to rapid analysis. Each time it receives the beam status, it is able to determine the position as well as distinguish horizontal and vertical activities. The computer software then calculates multiple variables such as total distance, horizontal activity, vertical activity, and movement time over a preset time period.

2. SCOPE AND APPLICABILITY

Open field activity monitoring provides a comprehensive assessment of the motor as well as behavioural activities of mice. Therefore, it is an ideal method for assessing the degree of locomotor impairment in myopathic models as well as to assess the efficacy of drugs that improve muscle function and locomotion [1] [2]. The open-field exposure is also commonly used as a measurement of anxiety-related behaviour [3] [4] and is different from other measures of muscle strength such as grip strength, whole body tension and hang time. It is possible that a particular treatment could improve locomotor activity without improving any measure of muscle strength and vice versa. Behaviour in the open-field test is influenced by genetic variation [5] [6], sex [7], age [8] and exposure to illumination [9] in the chambers. For example, bright light can be used as an aversive stimulus in the open-field paradigm leading to an increase in anxiety-related behaviours [10] [11]. The major advantages of the system include a) comprehensive assessment of the motor as well as behavioural activities of mice, b) easy method and no handling of animals during testing; c) non-invasive method of motor assessment, d) no special training is required to perform these experiments, e) multiple animals can be measured at one onetime, f) animals are relatively stress free and g) accurate indicator of animals locomotor activity.

3. CAUTIONS

Several factors, such as environmental conditions and the time of testing, can affect the behavioural activity, and the data need to be interpreted carefully. Behavioural activity is generally strain, sex and age dependent and therefore appropriate strain, sex and age matched control animals need to be evaluated at the same time. External factors within the testing room (room temperature, humidity, ventilation and light intensity) also need to be controlled. For example, it is better to use even illumination at an optimal intensity of 150-

200 Lux, and avoid direct light beams, darker corners and shadow areas, uneven positioning of chambers, excess noise and human activity during testing period, excess odours in the room, and distracting visual signals. Other limitations include a need for a specialized costly equipment and software to monitor activity. The system also cannot differentiate between various forms of stereotypy, such as grooming or head bobbing.

4. MATERIALS

- C57/BL10 and *mdx* mice. Sample size varies with the age of the mice for this behavioural measure. Younger mice (10-12 weeks age: N=11) and higher numbers at later age groups probably due the adaptation and learning effect. (See the appendix). (adapted from Spurney et al., 2009).
- Open field scanner (Digiscan)
- Animal activity monitoring system (e.g., VersaMax System from AccuScan Instruments, Inc. Columbus, Ohio, USA)
  PLEASE NOTE that this instrument is no longer commercially available, but similar devices from other companies can be purchased.
- A PC loaded with the software suite; Timer
- A mild spray cleaner and paper towels

5. METHODS

5.1 Instrument acclimatization

It is necessary to acclimatize mice to the test chamber environment before collecting digiscan data. This is accomplished by placing a mouse in any empty quadrant of the acrylic chamber assembly and leaving the mouse undisturbed for 60 min every day for 4 consecutive days. Acclimatizing mice for 30 min before data acquisition during data collection days will also help to reduce the variation in the data.

5.2 Data collection

1. Perform all data collection in an undisturbed environment, preferably in the mornings at the same time of the day, for an hour a day for 4 consecutive days to reduce variability in the behavioural measurements.
2. Turn on the analyzer using the switch on the front panel and open the software program in the PC connected to the analyzer. Make sure that the partitions that divide each chamber into four quadrants are inserted into the acrylic cage. Each cage can now house two test animals, with one placed in the front-left quadrant and one in the rear-right quadrant. Configure the software to perform both a pre- and post-beam check.

3. Enter the experimental parameters that are identical for each test chamber by first selecting ""Setup All Test Chambers" under the "Experiment" menu. The user should enter the experiment/drug name next to "User Identification."

4. Set the primary data collection duration (min), for example, 6 times/hr (every 10 min), and enter the "Data Filename" and Mouse ID of the test animal.

5. The buttons that designate the individual cages should turn green, and the caption should change from "Ready" to "Insert," indicating that there is no block between the sensors. If the buttons turn yellow and the caption reads "Pre-check," this indicates that there is an obstruction in front of one of the sensors. If this occurs, it is most likely the result of incorrect placement of the acrylic cage inside the activity monitor. Reposition the acrylic cage until the cage button turns green and the caption displays "Insert."

6. When all cages are ready, note the ID of the mouse and insert it into the test chamber, making sure that the mouse ID matches the one entered into the software. Set an external timer for 5 min and allow the mice to acclimate to the test chamber for that period of time before starting data collection. The buttons for each test chamber will now change to blue and will begin to count down the time remaining for the experiment.

7. While the animals are in the test chamber, try to minimize the amount of noise in the room to reduce the variability of the results. When the experiment time elapses, the test chamber buttons will change to red, and the caption will read "Remove," at which point each mouse should be removed from its test chamber. Click on "Remove Subjects" under the "Experiment" menu. This will perform a beam block post-check that is designed to validate the results by determining whether any beams are not being passed through the activity monitor. If this test fails, the test chamber buttons will change to yellow, and the caption will read "POST-CHECK." Failure of the beam block post-check indicates a malfunction of the activity monitor. In this situation, it is advisable that the data obtained be disregarded.

8. Following completion the experiment, exit the software program and save your data.

9. Clean the chambers with mild cleaning spray and ensure that no dirt particles are left in the chambers.

10. Process the data retrieved from the data points using Excel to organize and group the data. Group subjects by treatment group and ID number.

6. EVALUATION AND INTERPRETATION OF RESULTS

The data obtained consist of parameters that describe the behaviour and activity of the mice, for example, total distance, horizontal activity, vertical activity, movement time, rest time, and movement number. Animals with decreased muscle function will be less active (decreased horizontal and vertical activity, decreased movement time, and increased rest time), and the total distance travelled will be decreased, giving a sense of the level of ambulatory activity; animals with unimpaired muscle function or treated with agents that

decrease the progression of muscle pathology are usually more active, and the total distance travelled will be significantly higher than for the untreated mice.

7.   REFERENCES

Raben N, Nagaraju K, Lee E, Kessler P, Byrne B, et al. (1998) Targeted disruption of the acid alpha-glucosidase gene in mice causes an illness with critical features of both infantile and adult human glycogen storage disease type II. J Biol Chem 273: 19086-19092.

Nagaraju K, Raben N, Loeffler L, Parker T, Rochon PJ, et al. (2000) Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. Proc Natl Acad Sci U S A 97: 9209-9214.

Belzung C, Griebel G (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav Brain Res 125: 141-149.

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463: 3-33.

Sakai K, Crochet S (2001) Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. Neuroscience 104: 1141-1155.

Van der Staay FJ, Kerbusch S, Raaijmakers W (1990) Genetic correlations in validating emotionality. Behav Genet 20: 51-62.

Ramos A, Kangerski AL, Basso PF, Da Silva Santos JE, Assreuy J, et al. (2002) Evaluation of Lewis and SHR rat strains as a genetic model for the study of anxiety and pain. Behav Brain Res 129: 113-123.

Colorado RA, Shumake J, Conejo NM, Gonzalez-Pardo H, Gonzalez-Lima F (2006) Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats. Behav Processes 71: 51-58.

Bowman RE, Maclusky NJ, Diaz SE, Zrull MC, Luine VN (2006) Aged rats: sex differences and responses to chronic stress. Brain Res 1126: 156-166.

Cunha JM, Masur J (1978) Evaluation of psychotropic drugs with a modified open field test. Pharmacology 16: 259-267.

Valle FP (1970) Effects of strain, sex, and illumination on open-field behavior of rats. Am J Psychol 83: 103-111.

Raben N, Nagaraju K, Lee E, Plotz P (2000) Modulation of disease severity in mice with targeted disruption of the acid alpha-glucosidase gene. Neuromuscul Disord 10: 283-291.

Spurney C, Gordish-Dressman H, Guerron, A et al. (2009) Preclinical drug trials in the mdx mouse: Assessment of reliable and sensitive outcome measures. Muscle Nerve 39: 591-602.

8.   APPENDIX

The data collected over 4 days per mouse and group are calculated, and the means are compared using a one-way ANOVA test to compare each individual group to the other groups; the focus is usually on the interaction between the untreated group and the treated groups. The open field approach has been used extensively to assess the activity of mice in several mouse models of human muscle disease [1] [2] [12]. Representative data from bl10 and *mdx* mice are presented in appendix.
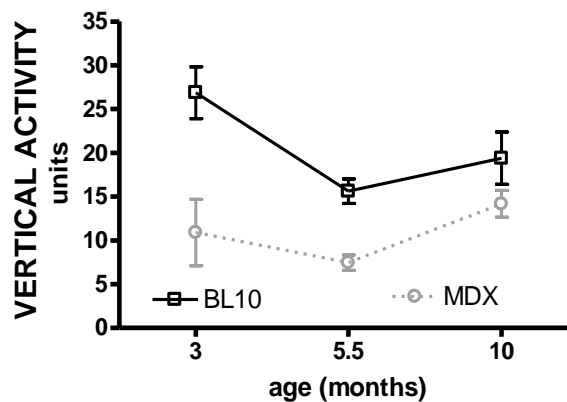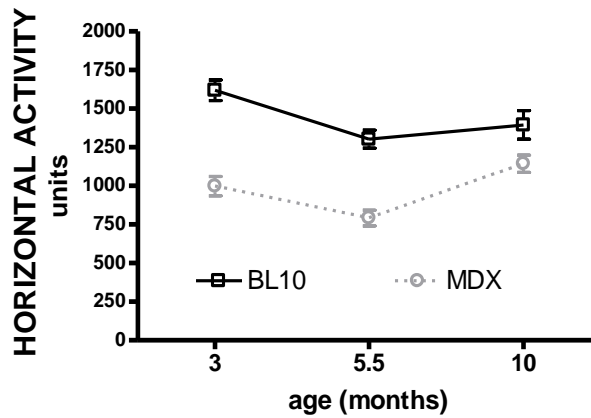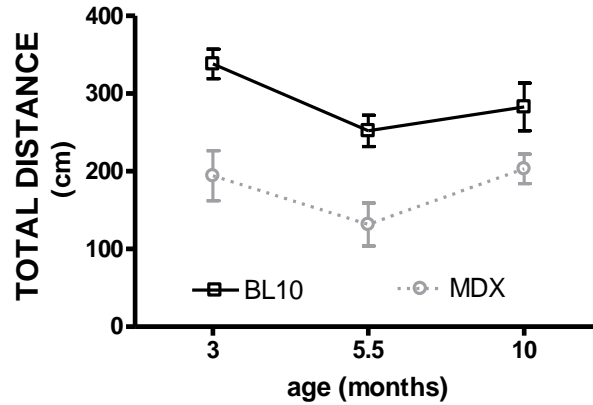
Figure 1. Open field activity (Horizontal activity (arbitrary units), Vertical activity (arbitrary units), and Total distance (cm) of BL10 control (n=14) and *mdx* mice (n=10) at different ages (mean±SE). Same animals were tested at different age (3, 5.5 and 10 months age). The sample size (n) for behavioural activity varies with age. For example a sample size of 11 mice is required at 3 months age but this significantly increases to n=17 at 5.5 months. There is high variation and no significant difference between control and mdx mice at 10 months age therefore sample size calculation for this age group is irrelevant (Spurney et al., 2009).