Video Article

Behavioral and Locomotor Measurements Using an Open Field Activity Monitoring System for Skeletal Muscle Diseases

Kathleen S. Tatem\(^1\), James L. Quinn\(^1\), Aditi Phadke\(^1\), Qing Yu\(^1\), Heather Gordish-Dressman\(^1,2\), Kanneboyina Nagaraju\(^1,2\)

\(^1\)Center for Genetic Medicine Research, Children's National Medical Center
\(^2\)Department of Integrative Systems Biology, George Washington University School of Medicine and Health Sciences

Correspondence to: Kathleen S. Tatem at KTatem@cnmc.org

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Abstract

The open field activity monitoring system comprehensively assesses locomotor and behavioral activity levels of mice. It is a useful tool for assessing locomotive impairment in animal models of neuromuscular disease and efficacy of therapeutic drugs that may improve locomotion and/or muscle function. The open field activity measurement provides a different measure than muscle strength, which is commonly assessed by grip strength measurements. It can also show how drugs may affect other body systems as well when used with additional outcome measures. In addition, measures such as total distance traveled mirror the 6 min walk test, a clinical trial outcome measure. However, open field activity monitoring is also associated with significant challenges: Open field activity measurements vary according to animal strain, age, sex, and circadian rhythm. In addition, room temperature, humidity, lighting, noise, and even odor can affect assessment outcomes. Overall, this manuscript provides a well-tested and standardized open field activity SOP for preclinical trials in animal models of neuromuscular diseases. We provide a discussion of important considerations, typical results, data analysis, and detail the strengths and weaknesses of open field testing. In addition, we provide recommendations for optimal study design when using open field activity in a preclinical trial.

Video Link

The video component of this article can be found at https://www.jove.com/video/51785/

Introduction

Animal models have been useful for learning about disease mechanisms, but their utility in predicting treatment efficacy in clinical trials has frequently been challenged\(^1,3\). Numerous “promising” preclinical studies are published each year; however, very few of the proposed interventions show positive results when moved to clinical trial. These discrepancies are often attributed to publication bias, overoptimistic conclusions, and poorly designed and executed pre-clinical studies that lead to irreproducible results\(^1,3\).

With the current advances in drug development for neuromuscular disorders, there is an increasing need for well-designed preclinical trials. In particular, there is a need for rigorous methodologies that can be carried out in a standardized and blinded fashion, with validated, reproducible, and translatable outcome measures. As a member of the Congenital Muscle Disease Consortium, with the desire to conduct more rigorous preclinical studies, we share here our Standard Operating Procedure (SOP) for Open Field Activity. This SOP was previously validated\(^6\) and published as a part of TREAT-NMD’s SOPs for Duchenne muscular dystrophy (DMD) animal models\(^5\). We have used this methodology to phenotype and test the therapeutic efficacy of numerous drugs in a variety of animal models of neuromuscular disease, including Lama2\(^9\,-2,J\) (Dy2J) mice, the animal model for congenital muscular dystrophy (CMD)\(^5,7\). In turn, this article is adapted from our previously published TREAT-NMD SOP\(^5\).

The open field activity monitoring system comprehensively assesses locomotor and behavioral activity levels of mice, which can be correlated with locomotive function. The test is also widely used to assess anxiety like and exploratory behaviors\(^8,10\). In particular, the open field is a useful tool for assessing locomotive impairment in animal models of neuromuscular disease\(^11,12\) and the efficacy of therapeutic drugs that may improve locomotion and/or motor function\(^5,7,13,14\). The open field activity assessment provides a different measure than muscle strength, which is commonly measured with grip strength, and it shows how drugs may affect other body systems (i.e. the central nervous system) as well\(^5\). In addition, the open field activity measure, total distance traveled, mirrors the 6 min walk test, a clinical trial outcome measure, which focuses on submaximal exercise performance and quality of life\(^15,16\). Overall this makes the open field activity test a beneficial secondary or auxiliary outcome measure to use in preclinical trials. However, the open field activity monitoring system also has significant challenges associated with it. The test is behavioral and can be quite variable as it is influenced by a multitude of external factors. For example, this behavior can be influenced by exploratory drive (i.e. cognition), anxiety, sickness, circadian rhythm, environmental factors, genetic background, in addition to motor output\(^8\). As a result it is imperative to conduct this measure in a standardized fashion with a controlled environment. The protocol presented here describes our open field activity SOP in detail. It provides step-by-step procedures and further discussion of important considerations to control...
environmental conditions and help reduce variability, typical results, data analysis, and the assessment’s strengths and weaknesses in more detail.

**Protocol**

**NOTE:** Open field activity monitoring system uses an open field Plexiglas chamber with photocell emitters and receptors equally spaced along the perimeter of the chamber (Figure 1). These photocell emitters and receptors create an x-y grid of invisible infrared beams. When an animal is placed in the chamber, it moves about, causing beam breaks. Vertical sensors are also present to assess vertical activity levels (i.e. rearing behavior) as well. The analyzer records the beam break information and rapidly analyzes it. The computer software then calculates multiple activity measures over the preset time period. These measures include: horizontal activity (units), vertical activity (units), total distance traveled (cm), movement time (sec), and rest time (sec).

**NOTE:** In general the testing room should be temperature- and humidity-controlled, with even illumination. Testing chambers should be evenly distributed about the room and should not be placed in direct light, dark corners, or shadowed areas. All instrument acclimation and testing should be performed at the same time each day (e.g., in the morning) and by the same individuals. These individuals should be blinded to the animal treatment group, and genotype when possible.

The following protocol has been performed under the guidance and approval of the Children's National Medical Center IACUC.

1. **Instrument Acclimation**

   1. Place the mice in the testing room in their home cages for approximately 10 min to acclimate. Leave the room during this acclimation period.
   2. Return to the testing room and turn on the activity chambers. Even though data are not being collected at this time, this will further mimic the testing environment.
   3. Gently remove each mouse from their home cage and immediately place them in the test chambers. If the activity chamber contains a center divider that divides the chamber into quadrants (Figure 1), place one mouse into each empty quadrant.
   4. Once all animals are loaded into the test chambers, place the lid on top of each test chamber. Leave the room during this acclimation time.
   5. Following the 60 min, return to the room. Remove the lid from each test chamber and gently return each mouse to his or her respective home cage.
   6. Clean each chamber with disinfectant and paper towels. Ensure no dirt particles are left in the chamber.
   7. Repeat Steps 1-6 for 4 consecutive days.

   **NOTE:** If multiple sessions are being run each day, thoroughly clean each test chamber in between each session.

   **NOTE:** Perform acclimation one week prior to initial data collection. If animals are tested multiple times throughout a study, only perform acclimation prior to the first round of testing to avoid habituation. In addition, randomly assign the animals to a new box each session. Track the box assignments throughout the duration of the study.

2. **Data Collection**

   1. Place mice in the testing room in their home cages for 10-30 min to acclimate. Leave the room during this time.
   2. Following the 10-30 min, return to the testing room. Turn on the activity chambers and open the accompanying computer software on the computer connected to the chambers. If the chamber contains a quadrant divider, insert the partition at this time.

      **NOTE:** If the test chamber contains a quadrant divider, two animals can be placed in the test chamber during data collection. One animal can be placed in the front-left quadrant and one in the back-right quadrant (Figure 1).

      **NOTE:** Do not place animals in all four quadrants during data collection or in the same row or column. Placing the animals in these orientations will interfere with the x-y grid of infrared beams and the animal movement will be inaccurately measured.

   3. Configure the computer software to perform a prebeam check. This configuration will allow one to run a pre-beam check following the experimental setup and prior to the insertion of animals into the test chambers (see below).

      **NOTE:** When the prebeam check is run, the computer software assesses the function of the x-y infrared beams. For example, it can determine if the photocell emitters and receptors are blocked and unable to appropriately detect movement within the chamber.

   4. Set the primary data collection parameters in the computer software to collect six 10 min blocks of data (i.e. collect data for a total of 60 min), and then enter the appropriate date, filename, and Mouse ID numbers.

   5. Once all parameters are set, run the prebeam check. If a chamber does not pass the prebeam check it is most likely due to poor alignment of the center quadrant divider or test chamber. If this happens, realign the center quadrant divider and test chamber until the sensors are no longer blocked and the system states that the test chamber is ready. If this does not correct the problem, reference the instrument manual.

   6. When all test chambers are ready, gently remove the mouse from his or her home cage and immediately place him or her into the test chamber. Note the ID of the mouse and make sure it matches the one entered into computer.

   7. Once all animals are appropriately loaded into the test chambers, place the lid on top of each chamber. Then select the appropriate command in the computer software to start data collection. At this time, the analyzer and computer software will start recording activity levels according to the data collection parameters.

   8. Leave the testing room for the remainder of the testing period.

   9. Upon completion of the testing period (i.e. 60 min later), immediately return to the testing room. Save the data, and then return each animal to their respective home cage.

10. Clean all units with a disinfectant and paper towels.

    **NOTE:** If multiple sessions are being run each day, thoroughly clean each test chamber in between each session.

11. Export data to a spreadsheet and then exit the software program.

12. Check the data to ensure they were recorded. If the data were not recorded, or the animals slept throughout the entirety of the data collection time period, perform an extra day of data collection.

    **NOTE:** An animal is considered to be “sleeping” if it does not move throughout the entire 60 min testing duration.
13. Repeat Steps 2.1-2.12 for 4 consecutive days.
   NOTE: If animals are tested at multiple time points throughout the duration of a study, do not perform open field activity measurements more than once a month to avoid habituation. In addition, randomly assign the animals to a new box each session. Track the box assignments throughout the duration of the study.

3. Data Analysis

1. Calculate the mean horizontal activity (units), vertical activity (units), total distance traveled (cm), movement time (sec), and rest time (sec) per mouse and group. The computer software calculates and reports the total horizontal activity (units), vertical activity (units), total distance traveled (cm), movement time (sec), and rest time (sec) over the data collection period (i.e., 60 min) for each mouse. Calculate the mean for each of the aforementioned parameters from the 4 days of data collection.
2. Prior to performing any statistical analyses, assess the normality of the data using the Shapiro-Wilk test, and check for outliers using the Grubb's test. Remove any significant outliers (p <0.05).
3. For normally distributed data, compare means between groups using either an independent sample t-test or a one way ANOVA and rank sum test with p-values adjusted for multiple comparisons depending on the total number of treatment groups.
4. For non-normally distributed data, compare median values between groups using either the Wilcoxon rank sum test, or a Kruskal-Wallis test with p-values adjusted for multiple comparisons depending on the total number of treatment groups.

Representative Results

When analyzing open field activity data, we focus on a few select measurements that provide an assessment of activity levels that generally reflect locomotive function. These parameters include: horizontal activity, vertical activity, movement time, rest time, and total distance traveled. In general, animals with reduced muscle function will be less active and have lower ambulatory activity. This is generally associated with decreased horizontal activity, vertical activity, total distance traveled and movement time, and increased rest time\(^\text{6,7,14,17}\). On the contrary, animals with unimpaired muscle function or those treated with therapeutics that decrease the progression of deteriorating muscle pathology are more likely to display higher activity levels\(^\text{6,7,14,17}\).

To show an example of typical results obtained using this protocol in animal models of neuromuscular disease, we provided data from a longitudinal study we previously conducted in the Dy2J congenital muscular dystrophy (CMD) animal model\(^\text{6}\). In short, the Dy2J model contains a truncated form of the LAMA2 gene that results in hindlimb paralysis, demyelination, and dystrophic skeletal muscle changes. The impact of this muscle pathology on activity levels is observed in these mice. For example, the Dy2J mice in the study tended to exhibit lower horizontal activity levels, and less distance traveled, compared to their age and gender-matched BL6 wild-type controls throughout the duration of the study (Figure 2); however, these differences were not always significant. The lack of significance is most likely due to the small sample size, and high intra-group variation in the BL6 data. Variation is typical of open field activity data; however, these data in particular lack sufficient power to determine if these groups are statistically different from one another. Typically an \(n = 10-12\) should be used to detect statistically significant differences\(^\text{6,14,17}\). For example, when a larger sample size is used, as was done in the SJL study (see the second BL6 bar in Figures 3B, 3D, and 3E) significant differences between groups can be observed. The Dy2J animals also showed a complete loss of vertical activity levels, which mirrored their hindlimb paralysis, and in turn, an inability to rear (Figure 2B)\(^\text{6}\). Lastly, it is important to note the sex differences in the activity levels. For example, the females tended to be more active than males, displaying higher levels of horizontal activity, vertical activity, and total distance traveled (Figure 2); however these differences were not statistically significant.

We have also provided data from several previous studies conducted in other animal models of neuromuscular diseases, as well to highlight several additional factors that affect activity levels (Figure 3). For example, activity levels vary by genetic background\(^\text{15}\). BL10 wild type mice exhibit greater horizontal activity, vertical activity, and total distance traveled compared to age- and gender matched BL6 wild type mice (Figure 3). This is an important observation to note, as utilizing the incorrect control strains in a study can render the data unusable. Second, activity levels vary by disease model and phenotype (Figure 3). For example, the SJL mouse, the animal model for limb girdle muscular dystrophy-2B (LGMD-2B), shows the lowest level of horizontal activity and total distance traveled, followed by the Dy2J mouse and the mdx mouse, the animal model for Duchenne muscular dystrophy (Figures 3A, 3C). However, Dy2J mice, because of their hindlimb paralysis, display the lowest level of vertical activity (Figure 3B). It is also important to note that the higher level of activity in the mdx phenotype is most likely attributed to the increased activity level of the BL10 background strain. Finally, this figure highlights the importance of animal age/disease pathology at time of testing. For example, at 30 weeks of age, no differences can be detected in activity levels between mdx mice and their age- and sex matched BL10 wild type controls (Figure 3). However, at 6 weeks of age, around the peak necrotic phase in the mdx mouse model, mdx mice display a significant decrease in vertical activity, and horizontal activity. A decrease is also observed in total distance traveled, but this difference is not significant (Figure 3).
Figure 1. Open field activity apparatuses. Two open field apparatuses with center quadrant dividers, and tops. If removable center quadrant dividers are present, animals should only be placed in the front left (1, 3) and back right chambers (2, 4) of each box during testing to obtain valid readings.

Figure 2. Open field activity data. Typical open field activity data for Dy2J (grey line, n = 3) and age- and gender matched BL6 control strain (black line, n = 3) mice at 14, 19, 23, 25, and 30 weeks of age (A - F). A) Horizontal activity (arbitrary units) males, B) Horizontal activity (arbitrary units) females, C) Vertical activity (arbitrary units) males, D) Vertical activity (arbitrary units) females, E) Total distance traveled (cm) males, F) Total distance traveled (cm) females. Data were collected over 4 consecutive days and averaged per mouse and group. The same mice were tested at each time point. Data represent mean ± SEM. Data previously published. Dy2J and BL6 mice were compared with an Independent sample T-test at each time-point. A p-value <0.05 was considered significant. * p <0.05, **p <0.01, ***p <0.001.
Figure 3. Open field activity data multiple strains. Typical behavioral activity data from male BL10 (6 weeks of age, n = 8; 25-30 weeks of age, n = 10), mdx (6 weeks of age, n = 9; 25-30 weeks of age, n = 15), BL6 (control group for Dy2J mice, n = 3; control group for SJL mice, n = 13), Dy2J (n = 3), and SJL (n = 13) mice at varying ages. A) Horizontal activity (arbitrary units) data from BL10 and mdx mice at 6 and 25-30 weeks of age, B) Horizontal activity (arbitrary units) data from BL6, Dy2J, and SJL mice at 25-30 weeks of age, C) Vertical activity (arbitrary units) data from BL10 and mdx mice at 6 and 25-30 weeks of age, D) Vertical activity (arbitrary units) data from BL6, Dy2J, and SJL mice at 25-30 weeks of age, E) Total distance traveled (cm) data from BL10 and mdx mice at 6 and 25-30 weeks of age, and F) Total distance traveled (cm) data from BL6, Dy2J, and SJL mice at 25-30 weeks of age. Dy2J, animal model for CMD with laminin α2 gene mutation on the BL6 background; SJL, animal model for limb girdle muscular dystrophy-2B (LGMD-2B); mdx, animal model for DMD on the BL10 background. There is no dysferlin-sufficient SJL control. Data are means ± SEM. B, C, and D contain data previously published6,17. Data were not normally distributed; therefore, data were compared using a Wilcoxon rank sums test. A p-value of p <0.05 was considered significant. The following comparisons were made for each parameter: a) BL10 and mdx mice at 6 weeks of age, p <0.05; b) BL10 and mdx mice at 25-30 weeks of age, not significant; c) Dy2J and BL6 matched control strain mice at 25-30 weeks of age, p <0.05; d) SJL and BL6 matched control strain at 25-30 weeks of age, p <0.001; e) BL10 mice at 25-30 weeks of age and BL6 (control group for Dy2J mice) mice at 25 weeks of age, p <0.01; f) BL10 at 25-30 weeks of age and BL6 (control group for SJL mice) at 25-30 weeks of age, p <0.001.

Discussion

The open field activity measurement is an in vivo assay that can be beneficial for assessing disease progression and drug efficacy in animal models of neuromuscular disease6,7,11-14. As shown in Figure 2, it provides an assessment of activity levels that generally reflect locomotive function. This is a different measure than muscle strength, making it an ideal secondary or auxiliary outcome measure to perform in a preclinical drug study. In addition, it is a clinically relevant15, noninvasive measure, which can be performed multiple times throughout the duration of a study. However, behavioral and locomotive activity is also influenced by additional factors as well (i.e. experimenter handling, environmental conditions, and cognition) creating variation in open field activity data. The goal of this paper is to provide a well-tested and standardized protocol that reduces variation and allows results to be compared across multiple labs, in hopes of enhancing translation within our field.

A major disadvantage of this measure is that it is highly variable and influenced by numerous external factors. However, we took this into consideration when developing the protocol. We assessed a variety of testing protocols ranging in duration from 1-5 days of data collection. In the end, we determined that performing instrument acclimation prior to data collection to familiarize the animals with the test chamber environment and performing 4 days of data collection significantly reduced the amount of variation in the outcome data5. This protocol was originally designed to assess behavioral and locomotive activity levels in the mdx mouse model; however, this protocol was recently validated...
in the Dy2J animal model as well. It is suggested that the protocol be standardized within your lab for each animal model prior to using it in a preclinical trial.

Open field activity varies by genetic background, sex, age, and circadian rhythm. This requires animals of the same age, sex, and genetic background to be assessed at the same time. During the planning stages, careful thought should be put into deciding at what age or ages open field activity levels will be assessed. Each animal model has its own distinct disease progression and locomotive and behavioral phenotype, which vary in severity and by age (Figure 2 and Figure 3). Therefore, it is important to determine clinically and pathologically relevant time points to assess open field activity measures. The total number of animals needed in each treatment group to detect statistically significant differences varies by animal model, age, and sex as well. Consequently, relevant sample size calculations should also be performed during the planning stages to determine the total number of animals needed in each treatment group to detect statistically significant differences. These calculations should also take into consideration additional outcome measures used in the study (e.g., grip strength measurements or histological analyses). Based upon our power calculations, we typically use 10-12 animals per treatment group. In addition, special attention should be paid to what control strain is used in the study. There is a tendency for improper control strains to be used in preclinical studies. For example, BL6 mice are often used as a control strain for mdx mice; however, the mdx mouse is on a BL10 background. As seen in Figure 3, BL10 mice are much more active than BL6 mice, which make it impossible to compare mdx and BL6 data. When conducting preclinical studies with mdx mice, BL10 mice should be used as the control strain. Furthermore, if a study is being conducted with the Dy2J mice, BL6 mice should be used as the control strain.

Small environmental changes can also significantly affect activity levels. These include lighting, temperature, humidity, odor, noise, and human activity. Therefore, it is very important that testing be performed in a temperature- and humidity controlled room with non-direct lighting at the same time each day. The test chambers should be evenly spaced throughout the room and not placed under direct lighting or in shadowed or dark corners. Animals should be randomly assigned to their test chambers each day to reduce the effects of varying environmental conditions throughout the room, and they should be allowed to acclimate to the testing room for 10-30 min prior to data collection. Make sure to track the box assignment of each animal throughout the duration of the study to ensure that any influence of box/environment is equally distributed among the different treatment groups. The individuals loading the animals into the test chambers and handling the animals throughout the duration of the study should be blinded to the treatment group, and animal strain when possible. In many cases affected genotypes are markedly different from related controls and blinding is not possible. However, individuals should always be blinded between treated and untreated groups. Furthermore, all individuals should leave the room during data collection to reduce noise and distractions within the room, and all chambers should be thoroughly cleaned following each session of data collection. These actions will significantly reduce variation in the data. It is important to note that animals are also highly susceptible to adaptation. Therefore, it is suggested that animals be removed from the test chamber directly following the 60 min of data collection each day and that open field activity levels be assessed no more than once a month.

The total distance traveled and total movement time measurements tend to be the most sensitive open field activity measurements. In the Dy2J model, the vertical activity measurement tends to be the most sensitive open field activity measure (Figure 3); however, it can be difficult to capture accurate vertical activity measurements in smaller animals. For example, it is possible that a smaller animal will exhibit rearing behavior and the sensor will not capture it due to the height of the vertical sensor. As a result, we recommend testing animals no earlier than 5 weeks of age. It is also possible that an animal will sleep throughout the duration of a data collection session. If this is the case, it is appropriate to add an additional day of data collection. Finally, poor alignment of the quadrant divider or blockage of sensors within the box can result in inaccurate data as well. Therefore, it is very important to perform a sensor precheck, prior to testing, and review all data following the end of each data collection session.

Caution should also be taken when analyzing open field activity data. Open field activity data has a tendency to be non-normally distributed and have outliers. Prior to performing any statistical analyses, our biostatisticians highly recommend checking the data for normality and outliers. If data are non-normally distributed, one should consider utilizing a nonparametric test when comparing means. In addition, all data should be analyzed by an individual blinded to what the treatment groups are.

Overall, the open field activity measure has major advantages: a) it is a comprehensive assessment of both locomotor and behavioral activity, which is strongly, but not always correlated with locomotive function; b) it is an easy measure to perform; c) it requires no animal handling during testing; d) it is a noninvasive measure that can be performed more than once throughout the duration of a study; e) no special training is needed to perform the test; f) multiple animals can be tested at one time; and g) it is a clinically relevant outcome measure. However, when testing therapeutics, keep in mind that other factors can affect an animal’s behavior, and in turn open field activity measurements. Drugs can have CNS and or other body wide effects, and behavior can also be influenced by a stressful environment. As a result, it can be difficult to distinguish if changes in locomotive or behavioral activity levels are related to changes in muscle function, muscle strength, or are a result of side effects from the drug. Therefore, additional functional, histological and or molecular assays should be conducted as well. This standardized protocol has also been successfully utilized in other muscle diseases; however, as seen in Figure 3, pilot studies should be performed initially to assess the sensitivity of the measure in the animal model.

Disclosures

The authors have nothing to disclose.

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This paper is one of several in a series of SOPs for routinely used methodologies in the congenital muscle disease field. It reflects the efforts discussed and established by over 20 experts in the field of congenital muscle disease at the recent Congenital Muscle Disease Consortium Workshop, held in April 2013 in Washington, D.C.

References