Video Article

Getting to Compliance in Forced Exercise in Rodents: A Critical Standard to Evaluate Exercise Impact in Aging-related Disorders and Disease

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URL: https://www.jove.com/video/51827
DOI: doi:10.3791/51827

Keywords: Behavior, Issue 90, Exercise, locomotor, Parkinson's disease, aging, treadmill, bradykinesia, Parkinsonism

Date Published: 8/22/2014

Citation: Arnold, J.C., Salvatore, M.F. Getting to Compliance in Forced Exercise in Rodents: A Critical Standard to Evaluate Exercise Impact in Aging-related Disorders and Disease. *J. Vis. Exp.* (90), e51827, doi:10.3791/51827 (2014).

Abstract

There is a major increase in the awareness of the positive impact of exercise on improving several disease states with neurobiological basis; these include improving cognitive function and physical performance. As a result, there is an increase in the number of animal studies employing exercise. It is argued that one intrinsic value of forced exercise is that the investigator has control over the factors that can influence the impact of exercise on behavioral outcomes, notably exercise frequency, duration, and intensity of the exercise regimen. However, compliance in forced exercise regimens may be an issue, particularly if potential confounds of employing foot-shock are to be avoided. It is also important to consider that since most cognitive and locomotor impairments strike in the aged individual, determining impact of exercise on these impairments should consider using aged rodents with a highest possible level of compliance to ensure minimal need for test subjects. Here, the pertinent steps and considerations necessary to achieve nearly 100% compliance to treadmill exercise in an aged rodent model will be presented and discussed. Notwithstanding the particular exercise regimen being employed by the investigator, our protocol should be of use to investigators that are particularly interested in the potential impact of forced exercise on aging-related impairments, including aging-related Parkinsonism and Parkinson's disease.

Video Link

The video component of this article can be found at https://www.jove.com/video/51827/

Introduction

Noninvasive lifestyle strategies that can either prevent or mitigate impairments to cognition and locomotion, both often associated with aging, are gaining traction as viable practices for maintaining health and well-being[1,2]. For example, moderate to rigorous exercise on a weekly and consistent basis in middle-aged men can significantly increase locomotor capabilities compared to similarly-aged peers with advancing age[3]. Furthermore, increasing evidence suggests that these lifestyle strategies, such as exercise, can mitigate or even reverse impairments associated with neurodegenerative diseases such as Parkinson's disease (PD)[4].

Efforts to understand the molecular mechanisms of aging-related impairments have also been directed toward identifying how noninvasive strategies, like exercise, reverse or attenuate the mechanisms that contribute to such impairments. Treadmill exercise is one such strategy being increasingly employed in models of PD[5-7] and cognitive impairment[1], wherein the mechanisms behind improvements in locomotor or cognitive function are still being determined. However, it is important to point out that aging is the neurobiological background of PD. Thus, for any potential translation of any exercise benefit in an animal model to be realized in the human condition it must take into consideration the neurobiological background of aging. For example, compensatory mechanisms that stave off locomotor impairment during PD progression could be impaired by the process of aging[8]. Thus, it stands to reason that exercise paradigms must be developed that not only consider the impact of aging in either the presence or absence of disease pathology, but that also could be initiated and maintained in aged rodents.

Therefore, in consideration of aging on the neurobiological background, the selection of the rat strain should be thoughtfully considered by the investigator. Several rat strains are available for aging studies, notably the Fischer 344 and the Brown-Norway/Fischer 344 F_1 (BNF) hybrid. The commonly used Sprague Dawley rat (an outbred strain) is also amenable for such use, as it is commonly used in neurodegenerative disease models, such as the 6-hydroxydopamine PD model. Our laboratory uses both the Sprague Dawley and the BNF strains in aging and neurodegenerative disease work. In this report, we will present results highlighting our exercise protocols using both strains. For those investigators strictly focusing on age-related studies, the BNF strain offers some important advantages. First, it is comparatively less vulnerable to aging-related disorders (such as tumors) and has exceptional longevity (typical life span exceeds 30 months) in comparison to other strains. They also have less variability in a variety of physiological and behavioral outcomes[9], and are also suitable to investigate approaches that intervene with the process of aging. Furthermore, experiments such as forced exercise demand considerable handling by the investigator, and the gentle disposition of the BNF strain is advantageous. Aging-related changes in striatal and midbrain dopamine tissue content, as well as locomotor activity changes are similar in BNF rats and primates[10-11]. Our laboratory also has extensive experience with characterizing and manipulating striatal and midbrain dopamine signaling as well as locomotor activity in the BNF strain[12-16]. Therefore, because the risk of other

neurobiologically-based diseases increases with aging, animal models of neurodegenerative disease should consider the use of rodent strains with an extensive track record of use in aging studies.

Our protocol herein also addresses some critical issues that the investigator must consider in the interpretation of their results obtained from an exercise protocol. The rodent treadmill apparatus (the use of which we will highlight in this report), is typically equipped with electric shock coils at the back of each lane of the treadmill that can be deactivated. However when these electric shock coils are activated, a small footshock is delivered to the subject as it comes in contact with the coils. This strategy is often employed in exercise studies to facilitate compliance to treadmill exercise. This is a critical point for consideration, particularly for those investigators involved in behavioral studies that are influenced by dopamine- or norepinephrine-signaling. Electrical footshock is a physiological stressor, and its impact on both neurotransmitter systems is well-documented, with increased activation of tyrosine hydroxylase[17-18]. Thus, increased biosynthesis of either neurotransmitter could confound the interpretation of any exercise effect, making the investigator liable for interpreting whether any observed change in behavior after exercise is strictly due to the exercise regimen or the footshock stress. Importantly, the proposed forced exercise regimen does not employ the use of footshock at any point in the treadmill acclimation or exercise training periods.

Successful exercise regimens also require maximum compliance to exercise from test subjects. The employment of footshock in order to achieve compliance could confound interpretation of experimental outcomes when the dependent measure is related to neurotransmitter signaling that is influenced by footshock (as previously discussed). Thus, the challenge is to get rodents, and in particular aged rodents, to comply with an exercise regimen. A desirable goal of any exercise regimen is to achieve nearly 100% compliance, as this will reduce the number of animals necessary to complete the exercise regimen. Specifically, maximum compliance to exercise and interpretation of exercise regimen outcomes can be obtained in our exercise regimen as a result of several pre-exercise procedures including, 1) a reverse light-dark cycle that enables animals to exercise during their active (wake) cycle, 2) ensuring locomotor performance baselines are equal prior to segregation of control and exercise groups, and 3) introducing the test subjects to an acclimation period that gradually introduces them to the requirements of the exercise regimen. Here, the aforementioned experimental considerations and steps necessary to achieve nearly 100% compliance to treadmill exercise in an aged rodent (>18 months of age) will be evaluated and presented. Finally, the environment associated with exercise may be stressful, and as such, present potential confounds to determine physiological effects specific to exercise. Our protocol also controls for the exposure to the potential stress-inducing environment of the treadmill by including the nonexercise group in every aspect of the acclimation phase (including modest treadmill running) and placing them on the stationary treadmill during the treadmill exercise training. Thus, this protocol aims to describe the measures necessary to determine the physiological impact of exercise alone.

Protocol

All of the following procedures are conducted in accordance with the Institutional Animal Care and Use Committee at LSU Health Sciences Center-Shreveport, and comply with the National Institute of Health guidelines.

1. Pre-exercise Procedures

Note: A timeline of all of the relevant procedures in this forced exercise regimen is diagrammed in **Figure 1**.

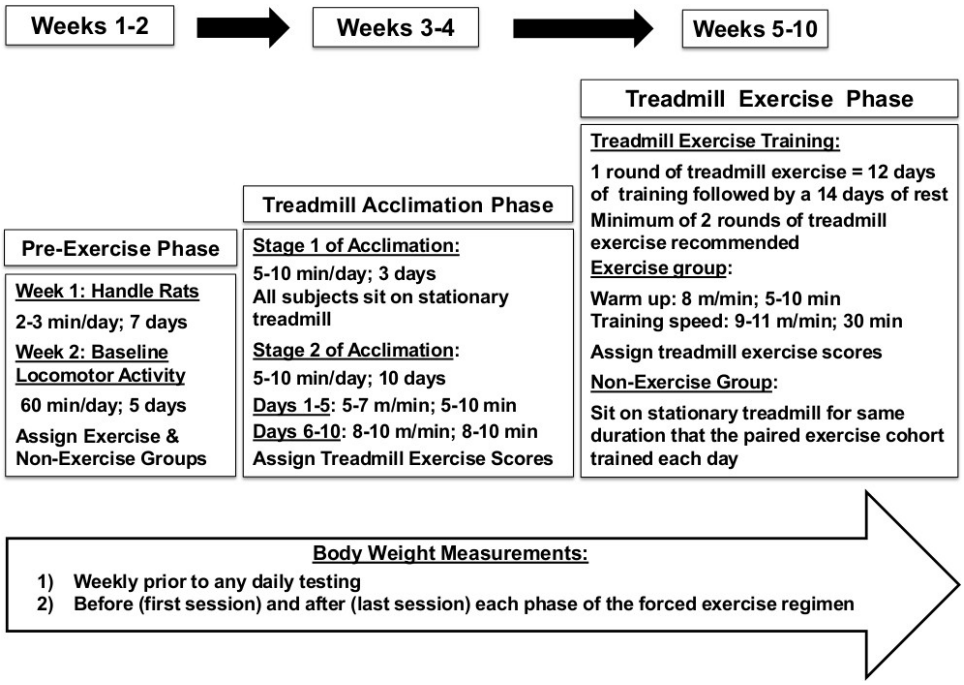

Figure 1. Forced exercise regimen timeline. This timeline details the important events of each phase of this exercise regimen during each week of the study. The pre-acclimation phase (weeks 1-2) involves experimenter handling and baseline locomotor activity. During the acclimation phase (weeks 3-4) all rats undergo 3 days of acclimation training where they sit on the stationary treadmill and then 10 days of acclimation training to the treadmill. A minimum of two rounds of exercise (1 round = 12 consecutive days) followed by a 2-week rest period is required during the exercise training phase (weeks 5-10). Behavioral and/or neurochemical measurements can be made during these rest periods. Furthermore, this regimen can be modified to include multiple rounds of exercise in this phase. Body weight is measured throughout all phases of the study on a weekly basis, and before (first session) and after (last session) each phase of this exercise regimen. Treadmill exercise training scores are assigned after all acclimation and exercise training sessions, and range from 1-4, with 4 being the highest possible score. Briefly, a training score of 4 is assigned when rats exercise the entire training session without assistance. Similarly, assign a training score of 3 when rats require minimal assistance (defined as: assistance for less than 25% of the time of the training session) from the experimenter. Assign a training score of 2 when rats require much assistance (defined as: assistance for greater than 25% of the time of the training session) from the experimenter during the exercise session. Finally, allocate a training score of 1 to rats that are noncompliant and fail to complete an exercise session.

1. Upon arrival to the animal colony, separate and singly house all subjects into standard shoebox cages. Provide food and water *ad libitum* to subjects throughout the entire study.
2. Maintain a reverse 12-hr light-dark cycle (lights on from 1800 to 0600 hr) in the animal colony, and allow subjects to acclimate to this cycle for a minimum of one week. Perform all behavioral experiments during the subject's active (dark) cycle, and 1-2 hr prior to the light-dark cycle change.
3. Beginning two weeks prior to any behavioral experiments, handle all test subjects for several minutes daily to familiarize the subject with the experimenter, and to decrease novelty-induced stress to unfamiliar environments, such as the locomotor chamber and the treadmill.
4. Measure each subject's body weight once a week, and immediately before (first session) and after (last session) each major interval of the exercise paradigm.

2. Baseline Locomotor Activity Assessments: Determining Forced Exercise Group Assignments

1. Assess each subject's spontaneous locomotor activity for 60 min/day for 5 consecutive days to establish baseline locomotor capabilities. Conduct locomotor activity sessions in automated open-field locomotor activity chambers consisting of a plexiglass box (45 x 45 x 20 cm^3) with a grid of infrared beams mounted horizontally and vertically as suggested in the product manual.
2. At the beginning of each day of the locomotor activity assessments, load, and set up the computer and software program connected to the open-field locomotor activity chambers in order to record the number of beam breaks (locomotor activity) during each session.
3. Add approximately 2 cups of pine-chip bedding to the floor of the locomotor chamber prior to each session.
4. Transport the subjects from the animal colony to the locomotor activity chambers in their home cages immediately prior to their locomotor activity session. Load subjects into their assigned locomotor chamber under a red light, and start the 60 min session on the computer once they have been placed into the chamber.
5. Upon completion of the locomotor activity session, remove subjects from the locomotor chamber and return them to their home cage under red light. Immediately return the subjects back to the animal colony.
6. Remove bedding from the chamber and clean the chamber walls and floor with 50% ethanol after each session.
7. Repeat steps 2.3-2.6 for additional locomotor sessions each day as needed until all subjects have been tested. Conduct all locomotor activity sessions in the dark, during the animal's active cycle, and 1-2 hr prior to the light-dark cycle change.

8. To account for the possibility of habituation to the activity chambers, rotate each subject among the available chambers such that locomotor activity is measured in a different chamber from the previous day.
    1. If multiple sessions need to be conducted in one day, change the order of testing such that subjects are tested in a different order than the previous session.

9. From the data generated by the software used to measure locomotor activity, determine each animal's baseline locomotor activity with the following 5 locomotor parameters: total distance traveled (cm), horizontal activity (number of beam breaks), movement number (number of initiated movements), time spent moving (sec), and movement speed (cm/sec).
    1. In order to assign exercise and nonexercise groups, calculate the mean value across all 5 sessions for each of the 5 locomotor parameters listed in step 2.9 above.

10. From these 5 parameters, determine exercise and nonexercise (control) groups such that an equal range of locomotor activity is represented in both groups. In this way, pair rats together based on similar movement capabilities, and assign one rat to the exercise group and one rat to the nonexercise group. Thus, for example, pair the two rats that exhibit the highest average of all 5 locomotor parameters together, and assign one rat to the exercise group and the other to the nonexercise group (group assignments are chosen at random). Repeat this process for the remaining subjects.
    1. Maintain pairing throughout the entire forced exercise regimen and for all data analyses.

11. If the experimenter is interested in the effects of exercise on locomotor activity, measure post-exercise locomotor activity using the same procedures described in steps 2.1-2.8.1 beginning the day after the last treadmill exercise session. NOTE: If the investigator is interested and/or the automated locomotor activity software permits, examine anxiety-related measures (*i.e.,* time spent in periphery vs. the center of activity chamber) to assess the relative anxiety of subjects during locomotor activity assessments.

3. Acclimation to the Treadmill

1. Conduct all treadmill exercise-related procedures on a motorized rodent treadmill with lanes that are separated by clear plexiglass walls during the animal's active cycle, and 1-2 hr prior to the light-dark cycle change.
2. In place of the electrical shock coils located at the back of each lane, use a plexiglass backstop designed specifically to fit the treadmill so that subjects can continuously exercise without coming into contact with the electrical shock coils. To prevent the backstop from sliding during the training sessions, hold the backstop in place with a 1.5 in c-clamp.
3. Due to the experimenter handling required for treadmill exercise training, conduct all treadmill exercise sessions with the lights on.
4. Transport subjects in their home cage to the treadmill immediately prior to their treadmill exercise training session. When the treadmill is stationary, load rats onto the treadmill in their assigned lane by sliding up the backstop just enough that subjects can maneuver under the backstop. Slide the backstop back down to the resting position once the subject is situated on the treadmill belt. Do not start the session until all subjects have been placed in their assigned lane for that particular session.
    1. During the first phase of acclimation, place each rat on one of the four lanes of the stationary treadmill (as described in step 3.4) for 5-10 min/day for 3 consecutive days. Increase the time of each session with each successive day (*e.g.,* 5 min on day 1, 7 min on day 2, and 10 min on day 3) as the rats become familiar with the treadmill apparatus and to reduce the potential for novelty stress to the treadmill apparatus. Important: Do not place rats anywhere on the treadmill (*e.g.,* deactivated electrical shock coils) except the stationary belt at this stage.

5. During the second phase of acclimation training, place each rat on one lane of the stationary treadmill in front of the backstop. When all subjects are in place, turn the treadmill on to a slow walking speed (*e.g.,* 6 m/min). Regardless of experimental grouping, train all rats to walk on the treadmill during this phase to ensure that any potential effects observed from the forced exercise regimen result from the exercise training and not simply from the ability to exercise.
6. As the belt of the treadmill begins to roll, ensure that all rats are walking in the forward direction. Assist rats as necessary by orienting or gently prodding them until they are moving forward. During the acclimation training period, train rats to walk on the treadmill such that they continuously walk on the treadmill without assistance from the experimenter.
    1. Acclimate all rats to the treadmill by gradually increasing the speed over a period of 5-10 min/day for 10 consecutive days. To further facilitate compliance to treadmill exercise, train the rats at low speeds (6-8 m/min) for 5-10 min during the first 5 acclimation sessions, and increase to more moderate speeds (9-10 m/min) for 5-10 min during the last 5 acclimation sessions.
    2. Ensure that all rats can maintain a speed of 9-10 m/min for 10 min by the last session of the acclimation training period.

7. Upon completion of the treadmill acclimation session, remove subjects from the treadmill in the same manner used to load subjects as described in step 3.4. Immediately return subjects to their home cage and transport them back to the animal colony.
8. Wipe down each individual lane including the walls and treadmill belt with 50% ethanol after each individual treadmill exercise training session.
9. Ascertain the exercise capabilities of each rat across the exercise sessions by assigning a treadmill exercise score at the conclusion of each daily session. Assign a training score of 4 when rats exercise the entire training session without assistance. Similarly, assign a training score of 3 when rats require minimal assistance (defined as: assistance for less than 25% of the time of the training session) from the experimenter. Assign a training score of 2 when rats require much assistance (defined as: assistance for greater than 25% of the time of the training session) from the experimenter during the exercise session. Finally, allocate a training score of 1 to rats that are noncompliant and fail to complete an exercise session.
   NOTE: The exercise scoring system is adapted, in part, from other studies[19].
    1. Exclude rats that cannot complete the acclimation phase due to noncompliance and/or an injury that prevents subjects from exercising (*e.g.,* this includes rats that earn a treadmill exercise training score of 1 for more than one consecutive day of acclimation training).

10. Average the treadmill scores across each training period (*e.g.,* acclimation training and exercise training) to determine the exercise capabilities of each subject. Ensure that all rats are able earn a treadmill exercise score of 4 during a training session by the end of the acclimation period.

1. If some rats are not compliant to the top exercise speeds maintained by others throughout a training session, adjust the speed for these rats so that they are able to continue exercising for the same amount of time but at a slower speed throughout the training sessions. In this instance, train slower rats in separate sessions and increase the speed as the subjects adjust to the slower speeds.

11. To avoid the potential for lane bias on the treadmill during treadmill exercise training, rotate each rat among the available lanes of the treadmill so that they are training in a different lane with each subsequent exercise session.

4. Treadmill Exercise Training

1. Begin the exercise training sessions the day following the last acclimation training session, and continue exercising rats for 12 consecutive days. Conduct these sessions during the animal's active cycle, and 1-2 hr prior to the light-dark cycle change.
2. Follow the same set-up procedures for each treadmill exercise training session used during acclimation training, as described in steps 3.2 and 3.4.
3. At the start of each session, turn on the treadmill to a lower, warm-up speed (8 m/min) than the training speed (9-11 m/min) that will be maintained throughout the remainder of the exercise training session. Continue with this warm-up speed for 5-10 min at the beginning of the session. Adjust the speed to 9-11 m/min for the remainder of the training session. Train subjects an average of 30 min each session for the entire 12 days of exercise training.
4. After each exercise training session, assign a treadmill exercise score to all rats in the exercise group using the same exercise scoring system described in step 3.9. Do not assign a training score during the treadmill exercise training period to nonexercise rats.
5. Conduct the exercise and nonexercise sessions separately. Place the nonexercise rats on the stationary treadmill for the same duration that their exercise cohort trained during each session.
6. As described in step 3.11, rotate each rat among the available lanes of the treadmill so that they are training in a different lane with each subsequent exercise session.
7. Upon completion of one round of exercise, rest subjects for 14 days. NOTE: This rest period was chosen based on the results from the pilot study described in **Figures 2A** and **2B**.
8. If desired, conduct various behavioral and/or neurochemical assessments at the end of the 12-day training period. Conversely, continue with subsequent rounds of exercise training following the 14 day rest period.

Representative Results

A diagram of the timeline of events in all three phases of the forced exercise regimen is shown in **Figure 1**. In addition to reversing the light-dark cycle, daily handling prior to any experimental testing, and stage 1 of acclimation training, where subjects sit on the stationary treadmill for 5-10 min/day for 3 consecutive days, were implemented to increase compliance to the forced exercise regimen. These procedures were added to the protocol in order to minimize stress associated with novel environments, and so that subjects would be more inclined to walk on the moving treadmill if they were already familiar with the stationary apparatus. These procedures outlined in **Figure 1** were employed following a pilot study conducted in BNF rats in which 50% of subjects were noncompliant to a forced exercise regimen previously conducted in Sprague Dawley rats. In this forced exercise regimen, 18-month-old Sprague Dawley rats were the test subjects that underwent 30 min of exercise for 12 consecutive days at speeds up to 15 m/min (**Figure 2**). These preliminary results indicate that one round of exercise (12 consecutive days) may increase locomotor activity (**Figure 2A**), with the duration of this effect of exercise on locomotor activity lasting out to one week post-exercise. Furthermore, a rebound effect of increased locomotor activity, relative to the original baseline, was observed following the same 12 day exercise regimen after one month of inactivity (**Figure 2B**). Thus, in the modified forced exercise regimen detailed in this protocol, one round of forced exercise consists of 12 consecutive days of exercise followed by a two week rest period. The results in **Figure 2** suggest that the proposed forced exercise regimen may increase locomotor activity in rats that exercise relative to their respective baseline activity.

As discussed previously, baseline locomotor activity assessments serve two critical purposes in this forced exercise regimen. First, baseline locomotor activity is used to assign test subjects to ensure equal representation of locomotor capabilities between the exercise and nonexercise (control) groups prior to exercise intervention, so that no bias is inadvertently introduced into the study. For example, an exercise group containing subjects that exhibit a higher percentage of greater locomotor activity compared to the nonexercise group introduces experimental bias. Second, for studies with a specific interest in locomotion, it must be noted that baseline activity does vary across individuals, and therefore determining the impact of forced exercise must be done by comparing activity (or other dependent measures) post-exercise to the baseline on an individual-by-individual basis. For example, an arbitrary value of 100 on a post-exercise assessment when compared to a baseline of 50 indicates a two-fold increase in activity. However, by the same token, a post-exercise assessment of 150, while greater than 100, represents only a 1.5-fold increase against a baseline of 100. Therefore, the optimal pre-exercise baseline is illustrated in **Figure 3**. When paired with their respective cohort, there is no significant difference in baseline locomotor activity between the assigned groups designated for the exercise and nonexercise groups for any of the five locomotor parameters including (A) total distance traveled (cm), (n=8 per group, p=0.7142, paired two-tailed t-test), (B) horizontal activity (# of beam breaks), (n=8 per group, p=0.7767, paired two-tailed t-test), (C) movement number, (# of initiated movements), (n=8 per group, p=0.6186, paired two-tailed t-test), (D) time spent moving (sec), (n=8 per group, p=0.9307, paired two-tailed t-test), and (E) movement speed (cm/sec) (n=8 per group, p=0.655, paired two-tailed t-test). Thus, these results show that an equal range of locomotor capabilities is represented in both groups, and that both the capacity and response to exercise will not be based on any differences in locomotor activity between the groups prior to acclimation training.

As previously discussed, ~100% compliance to this forced exercise regimen can be obtained with the addition of several pre-exercise and treadmill acclimation procedures. Importantly, the steps taken to achieve compliance in this regimen increase the sensitivity of detecting an exercise effect with employment of this forced exercise regimen. One way to measure an exercise effect is through body weight measurements. In this paradigm, body weights are measured on a weekly basis, and before (first session) and after (last session) each phase of the exercise regimen. As demonstrated in **Table 1**, exercise rats had a mean weight loss of 4.43±0.32% compared to their pre-exercise body weight. Comparatively, nonexercise rats had a mean weight loss of 0.05±0.55% compared to their pre-exercise body weight. Thus, as expected, there is a significant difference in total weight loss and percent change in body weight throughout the forced exercise regimen between exercise and nonexercise rats (n=7 per group, p<0.01, paired two-tailed t-test). Similarly, significant differences in weight loss and percent decrease in body

weight were observed during round 1 (n=7 per group, p<0.01, paired two-tailed t-test) and round 2 of forced exercise (n=7 per group, p<0.05). Notably, there were no significant differences in either parameter during the baseline or acclimation phases, indicating that only the forced exercise training periods affected body weight. This result indicates that the exercise regimen has a verifiable physiological effect on rats in the exercise group, which may be independent of other variables that the investigator is measuring. Given that body weights of these subjects ranged from 378-572 g throughout the study, a 4% decrease in body weight is not considered detrimental to the overall well-being of subjects, and also is within range of our previous pilot study forced exercise regimen with Sprague Dawley rats where a 6% decrease in body weight was observed over the same time period. Finally, we note that no significant correlation of body weight to locomotor activity has been observed in baseline assessments of individual body weights against individual locomotor activity parameters listed in **Figure 3**.

Treadmill exercise scores are assigned after each acclimation and treadmill exercise session, and enable the experimenter to ascertain the exercising capabilities of every subject throughout this regimen. For example, a treadmill exercise score of 4 is indicative of maximum compliance to the regimen for a particular session, whereas a treadmill exercise score of 1 is indicative of noncompliance to the regimen for a particular session. Thus, when the compliance criteria are employed in this regimen, treadmill exercise scores should reflect exercise compliance which is demonstrated by assigning scores of 3 or 4. During acclimation training, both exercise and nonexercise rats are trained to exercise on the treadmill and treadmill exercise scores are assigned for both groups during this phase only. Conversely, treadmill exercise scores are only assigned to exercise rats during each round of forced exercise. Results of treadmill exercise scores from both acclimation and treadmill exercise training are presented in **Figure 4**. During acclimation training, there is no significant difference in the mean treadmill exercise score between the exercise and nonexercise groups (n=7 per group, p=0.656, paired two-tailed t-test) as shown in **Figure 4A**. This indicates that both exercise and nonexercise rats were both compliant and able to acclimate to treadmill exercise similarly. **Figure 4B** demonstrates that exercise rats were compliant to treadmill exercise through two rounds of treadmill exercise training, as the mean treadmill exercise scores were not significantly different between these two rounds (n=7, p=0.2336, paired two-tailed t-test). Thus, once they were acclimated to exercise training, exercise rats remained compliant through two rounds of forced exercise. Finally, it is important to note that given the high average of treadmill exercise scores in both acclimation training (combined average exercise score =3.09) and exercise training (combined average exercise score=3.80), investigator involvement, in terms of assisting test subjects, during individual training sessions is minimal after the first few sessions of acclimation training.

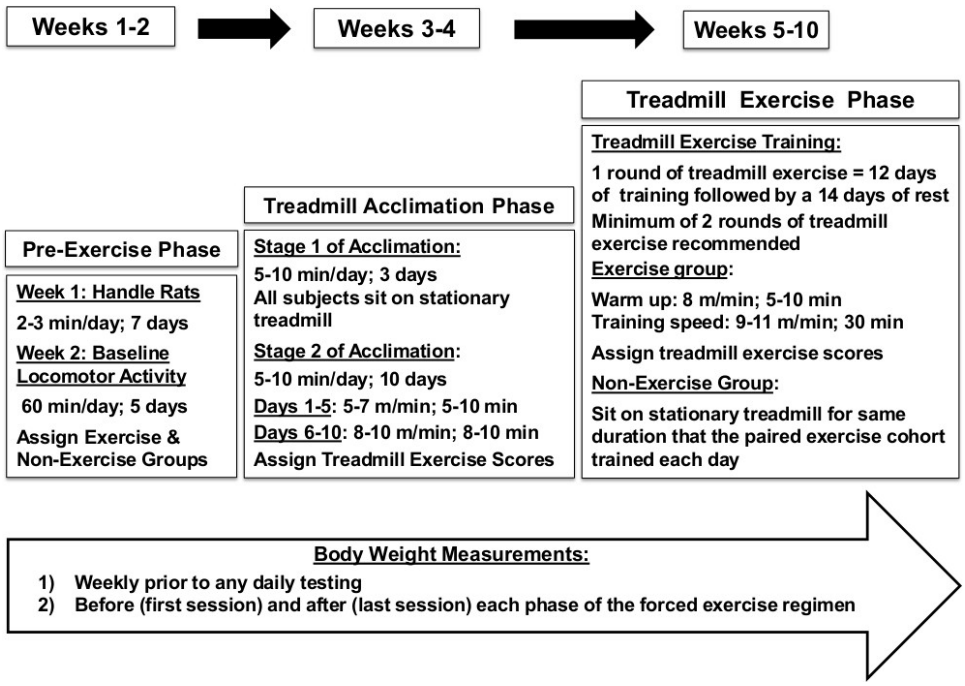

Figure 1. Forced exercise regimen timeline. This timeline details the important events of each phase of this exercise regimen during each week of the study. The pre-acclimation phase (weeks 1-2) involves experimenter handling and baseline locomotor activity. During the acclimation phase (weeks 3-4) all rats undergo 3 days of acclimation training where they sit on the stationary treadmill and then 10 days of acclimation training to the treadmill. A minimum of two rounds of exercise (1 round = 12 consecutive days) followed by a two week rest period is required during the exercise training phase (weeks 5-10). Behavioral and/or neurochemical measurements can be made during these rest periods. Furthermore, this regimen can be modified to include multiple rounds of exercise in this phase. Body weight is measured throughout all phases of the study on a weekly basis, and before (first session) and after (last session) each phase of this exercise regimen. Treadmill exercise training scores are assigned after all acclimation and exercise training sessions, and range from 1-4, with 4 being the highest possible score. Briefly, a training score of 4 is assigned when rats exercise the entire training session without assistance. Similarly, assign a training score of 3 when rats require minimal assistance (defined as: assistance for less than 25% of the time of the training session) from the experimenter. Assign a training score of 2 when rats require much assistance (defined as: assistance for greater than 25% of the time of the training session) from the experimenter during the exercise session. Finally, allocate a training score of 1 to rats that are noncompliant and fail to complete an exercise session.

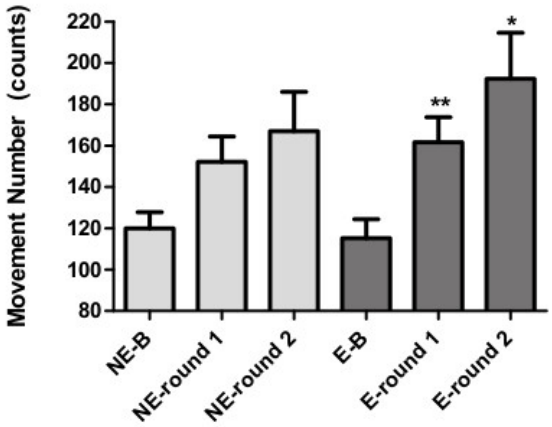

Figure 2. Pilot study of the impact of 12 days of forced exercise on locomotor activity for one week and the impact of the same regimen when initiated after one month of inactivity. Eighteen-month-old male Sprague Dawley rats underwent baseline locomotor assessment during their inactive cycle (nonwake period) under full nominal environmental lighting in an open-field locomotor chamber. The average of 5 daily, 60 min sessions was attained for each rat, and rats were then segregated to the nonexercise group (NE) or the exercise group (E). Baseline movement number (B) in the rats of the nonexercise group (NE-B) or exercise group (E-B) was not significantly different. Following 12 consecutive days of forced exercise (30 min/day, 12-15 m/min), locomotor activity was assessed during their active (wake) cycle (with dimmed lights). Testing subjects during their active cycle provided a natural stimulus that induces movement enabling comparisons against baseline measures wherein locomotor activity is predictably less when assessed during the sleep cycle and with ambient lighting. Rats that exercised (E-round 1) for 12 consecutive days exhibited 41% greater locomotor activity relative to their original individual baseline (n=5 per group, $p<0.01$, t=8.25, paired two-tailed t-test), whereas rats in the nonexercise group (NE-round 1) exhibited locomotor activity trending toward an increase, but not significantly different from their baseline (n=5 per group, $p=0.11$, t=2.05, paired two-tailed t-test). After one month of inactivity, the same rats underwent an identical second exercise regimen and rats in the exercise group (E-round 2) exhibited a 61% increase in locomotor activity compared to their respective original individual baseline activity levels (n=4 per group, $p<0.05$, t=5.34, paired two-tailed t-test), compared with a nonsignificant trend toward an increase in the nonexercise group (NE-round 2) (n=4 per group, p=0.13, t=2.08, paired two-tailed t-test).

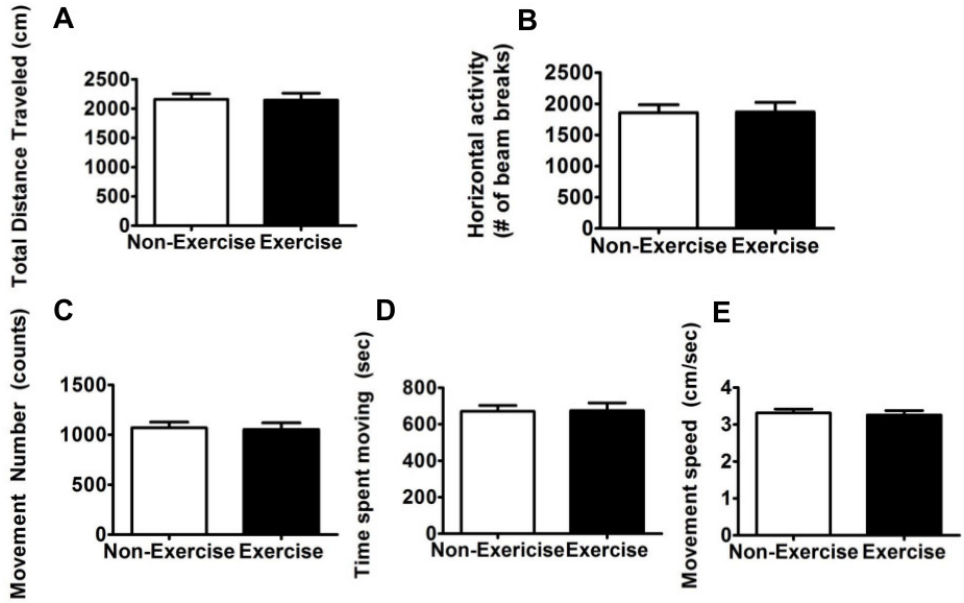

Figure 3. Comparison of baseline locomotor activity parameters in exercise and nonexercise groups. To establish baseline locomotor activity, 5 locomotor trials (60 min/day; 5 days) were conducted for in 18-month-old male BNF rats prior to treadmill acclimation training. The mean values of the five locomotor trials were determined for all five locomotor parameters measured. The means of the locomotor parameters were used to assign exercise and nonexercise (control) groups such that there was an equal range of locomotor activity represented in both groups. Subjects were paired based on similar mean values for all locomotor parameters. Once the pairs and groups were assigned a paired two-tailed t-test was performed for each of the 5 parameters to ensure that there were no significant differences in locomotor capabilities between the two groups. In sum, a paired two-tailed t-test showed no significant differences in (**A**) total distance traveled (cm), (n=8 per group, p=0.7142, t=0.3815), (**B**) horizontal activity (# of beam breaks), (n=8 per group, p=0.7767, t=0.2948), (**C**) movement number, (# of initiated movements), (n=8 per group, p=0.6186, t=0.5207), (**D**) time spent moving (sec), (n=8 per group, p=0.9307, t=0.9011), and (**E**) movement speed (cm/sec) (n=8 per group, p=0.655, t=0.4569). These results ensured that an equal range of locomotor capabilities was represented in both groups, and that both the capacity and response to exercise was not based on any differences in locomotor activity between the groups prior to acclimation training.

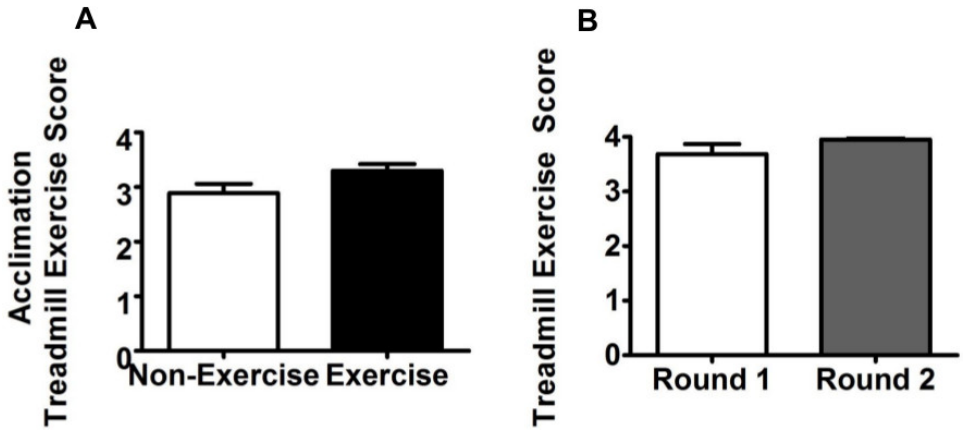

Figure 4. Treadmill exercise scores during acclimation (A) and treadmill exercise training (B). Treadmill exercise scores are assigned after every acclimation and treadmill exercise training period, and serve as an indicator of compliance during each session. Scores range from 1-4, with 4 being the highest. A score of 4 is assigned when subjects exercise the entire session without assistance. Scores of 3 and 2 are assigned when subjects exercise with minimal or maximal assistance from the experimenter, respectively. Finally, a score of 1 is assigned to subjects that do not finish the exercise session. Both exercise and nonexercise rats undergo acclimation training, and thus, both groups of rats are assigned treadmill exercise scores during acclimation. As shown in (**A**), there is no significant difference in the mean treadmill exercise scores between exercise and nonexercise rats. This suggests that both groups of rats were able to acclimate to treadmill exercise similarly, and furthermore, that both groups were compliant to acclimation training (n=7 per group, p=0.656, t=2.270, paired two-tailed t test). During treadmill exercise training, only the exercise rats are assigned treadmill exercise scores since nonexercise rats do not exercise in this phase. **(B**) demonstrates that exercise rats were able to maintain treadmill exercise scores of 3-4 through two rounds of exercise training. Similarly, mean treadmill exercise scores were not significantly different between the two rounds of exercise (n=7, p=0.2336, t=1.234, paired two-tailed t-test) and were compliant through two rounds of forced exercise.

		Nonexercise (n=8)		Exercise (n=7)			

Body Weight Measures						p-value	
Start Body Weight (g)		457.10±20.16		476.00±26.85			
End Body Weight (g)		450.80±21.63		454.90±25.55			
Baseline							
Weight Loss (g)		5.69±1.46		5.21±2.35		p=0.982	
Percent change in body weight (%)		1.35±0.36		1.10±0.51		p=0.811	
Acclimation-Phase 1							
Weight Loss (g)		3.88±0.56		3.93±0.74		p=0.850	
Percent change in body weight (%)		0.87±0.16		0.83±0.16		p=0.680	
Acclimation-Phase 2							
Weight Loss (g)		5.81±1.27		8.07±2.39		p=0.486	
Percent change in body weight (%)		1.29±0.26		1.61±0.47		p=0.744	
Exercise Round 1							
Weight Loss (g)		6.31±1.00**		13.50±1.78**		p<0.01	
Percent change in body weight (%)		1.45±0.24**		3.03±0.47**		p<0.01	
Exercise Round 2							
Weight Loss (g)		0.5±1.254*		11.29±3.05*		p<0.05	
Percent change in body weight (%)		0.07±0.30*		2.38±0.63*		p<0.05	
Overall Weight Loss							
Weight Loss (g)		1.88±2.63**		21.14±1.93***		p<0.01	
Percent change in body weight (%)		0.05±0.55**		4.43±0.32**		p<0.01	

Table 1. Body weight measurements during the forced exercise regimen. In this paradigm, body weights are measured on a weekly basis and before (first session) and after (last session) each phase of the exercise regimen to provide a measure of the physiological effects of this forced exercise regimen. Values are represented as mean±SEM, and a paired two-tailed t-test was used to analyze differences between exercise and nonexercise groups after each phase of the regimen. In total, exercise rats had a mean weight loss of 21.14±1.93 g and a 4.43±0.32% decrease in body weight. Comparatively, nonexercise rats have a mean weight loss of 1.88±2.63%, and a 0.05±0.55% decrease in body weight. Thus, there is a significant difference in total weight loss (n=7 per group, p=0.0016, t=5.417, paired two-tailed t-test) and percent decrease in body weight (n=7 per group, p=0.0021, t=5.158, paired two-tailed t-test) throughout this forced exercise regimen between exercise and nonexercise rats. Significant differences in weight loss (n=7 per group, p=0.0036, t=4.633, paired two-tailed t-test) and percent decrease in body weight (n=7 per group, p=0.0037, t=4.603, paired two-tailed t-test) were observed during round 1. Similar results were observed during round 2 of forced exercise in that there was as significant difference in weight loss (n=7 per group, p=0.0163, t=3.305, paired two-tailed t-test) and percent decrease in body weight (n=7 per group, p=0.0174, t=3.255, paired two-tailed t-test) between exercise and nonexercise groups. However, no significant differences were observed in either parameter during the baseline or acclimation periods, demonstrating that only the forced exercise training periods exert physiological effects on rats in the exercise group.

Discussion

There is evidence to suggest that lifestyle strategies, such as exercise, may reduce the risk for chronic age-related diseases and their cardinal symptoms, including those of the neurodegenerative diseases like Alzheimer's disease and PD[20]. The benefit of forced exercise on a treadmill, as opposed to voluntary exercise on a running wheel, is that the investigator can determine the frequency, the rate, and/or intensity of exercise, and duration of the exercise session. Thus, specific post-exercise effects, such as increased locomotor or cognitive performance, may be predicted or expected following the employment of a given regimen. Furthermore, the translation of results from forced treadmill exercise to the human condition is very probable. In fact, recent studies in PD patients demonstrate that when the patient complies with an exercise regimen set forth by a trainer on a tandem bike, these patients exhibit improved locomotor scores in comparison to PD patients who only exercise at their own pace[4].

The incidence of neurodegenerative diseases significantly increases with aging. Thus, age is an important consideration when designing studies that aim to translate any potential results to the human condition. For example, studies that utilize adolescent or adult animal models to study neurodegenerative disease do not take into consideration that aging is the neurobiological background of such diseases. This potentially confounds the translation of any observable effects clinically because the physiological factor of age has not been fully considered in such studies. Therefore, studies examining the effects of exercise on either locomotor or cognitive performance, should consider using aged animal models since cognitive and locomotor impairments are also associated with age-related deficits in neurochemical indices within the central nervous system[10,12,16]. This would allow for a more germane understanding of the neurobiological effects of exercise on locomotor and cognitive function with aging in either the presence or absence of disease pathology.

Finally, in order to ascertain the impact of exercise alone on post-exercise measures in cognitive or locomotor performance, three practices are essential to obtain nearly 100% compliance to an exercise regimen. First, it is important to maintain a reverse light-dark cycle throughout all phases of the forced exercise regimen. This ensures that test subjects are training during their active (wake) cycle. Second, it is vital that the test subjects are slowly acclimated to both the treadmill environment and the equipment used to determine their locomotor or cognitive performance. Here, the investigator should follow the procedures outlined in Figure 1 in the timeline recommended. Finally, the electric footshock apparatus should never be used during any phase of this forced exercise regimen. Instead, as highlighted in the protocol, a plexiglass backstop can be utilized to encourage subjects to continue exercising during the treadmill exercise sessions. At this juncture, the exercise regimen described should be considered a starting point for any laboratory, with the expectation that the frequency and duration of the exercise regimen can be varied according to the investigator's paradigm. The limits to the longevity of this forced exercise regimen certainly have yet to be realized, but one may assume that barring injury, compliant rats should be able to maintain adherence to the exercise regimen for a number of months. However, it should be noted that the speed at which test subjects may execute treadmill exercise within a given time frame (30 min in this regimen) may require adjustment and maximum speeds have not exceeded 15 m/min the aforementioned studies. The best compliance is likely to be realized at speeds that do not exceed 11 m/min. Indeed, if we are to translate the exercise findings to the middle-aged and aging population, we might expect the most representative animal protocol is one that focuses on longevity and compliance, rather than speed.

To summarize, this forced exercise protocol is one that can be employed in virtually any laboratory, particularly for those with a focus on neurodegenerative disease and aging. The test subjects used in this report were all 18 months of age at the start of the study. Furthermore, the essential steps needed to obtain virtually 100% compliance in the test subjects have also been discussed. Obviously acquiring 100% compliance is a significant benefit to the investigator, primarily because one will only have to use the number of subjects required to test a given hypothesis at the desired power. Moreover, achieving 100% compliance in this regimen does not require the employment of electric footshock. Indeed, elimination of electric footshock is imperative in order to interpret the impact of exercise on behavioral measures that depend upon catecholamines, especially dopamine, given the long-known impact of stressors like footshook upon catecholamine biosynthesis[17-18].

Disclosures

The authors declare no conflicts of interest.

Acknowledgements

Funding for this work was provided in part by a research grant awarded to M. F. Salvatore from the National Institute on Aging (AG040261). The authors would also like to acknowledge Victoria Fields for her assistance with the behavioral experiments associated with the work presented and Mackenzie Orchard for his assistance with construction of the backstop for the treadmill.

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