Small Animal Treadmill Protocol
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Location and Specifications

The treadmill, model Eco 3/6 from Columbus Instruments, is located in room 7614 of Medical Science Building II. It has the capability of exercising up to three rats or six mice simultaneously in individual lanes. The user can adjust the running speed from $\sim 7$ to 70 m/min with a resolution of 0.1 m/min, and the running surface can be inclined from $0^\circ$ to $25^\circ$ above horizontal in $5^\circ$ increments. A stimulus can be created using the electrical shock grids, and grids can be enabled or disabled individually for each lane. The intensity and repetition rate of the stimulus is user controlled. All data collection and analysis must be done manually.

Recommended Protocol

Acclimation:

There are two basic categories of treadmill exercise: exercise training, where mice are trained over a period of time to run faster and farther, and acute exercise, where mice are simply run to exhaustion. Despite having different aims, both systems of exercise share many steps, especially at the beginning.

NOTE: Log in on the provided sheet and check that the treadmill is inclined to the correct angle before placing any animals on it.

Mice should always be properly acclimated to the treadmill prior to any experimentation. Place the mice on the treadmill in their respective lanes with the belt unmoving and shock grids off but with the belt motor on (it makes enough noise to be a potential problem). Leave the mice undisturbed for 15 minutes.

Warm-up:

The next step is to turn the shock grids on and start the belt. However, like a human runner, the mice must be warmed up before running at high speeds to avoid injury and potential data artifacts. The length of the warm-up, the initial speed, and the rate of acceleration are dependent on the desired final speed and
the purpose of the experiment. The warm up procedure is identical for both exercise training and acute exercise regimens: turn on the shock grids for each lane to be used and then start the belt at a slow speed (7 m/min is the slowest possible speed and is recommended in the beginning of an experiment). Slowly ramp up the speed until a desired setting or result is reached. A common acceleration used for both acute exercise and exercise training regimens is 1 m/min$^2$. The acceleration can be tailored to each experiment and experimenter.

**Exercise Training Regimens:**

Exercise training regimens are designed to train mice to run faster and farther over a period of time to determine long term effects of stress. Running the mice to exhaustion is generally avoided. In this type of experiment, the acceleration begun during the warm-up is continued until a predetermined final speed is reached. At this point, the mice are run until a user selected amount of time has elapsed and then the belt and shock grids are deactivated and the mice are returned to their cages. If at any point during the experiment a mouse becomes exhausted, the shock grid for that lane must be turned off and the mouse allowed to rest.

**Acute Exercise Regimens:**

Acute exercise regimens are designed to run mice to exhaustion and to determine the short and long term effects of extreme stress. In this type of experiment, there is no final speed and the acceleration is continued until the mice reach exhaustion. Often, the inclination of the treadmill is increased as well, but this may be impractical with the current setup as it could significantly disrupt the mice. As before, once a mouse exhibits signs of exhaustion, the shock grid must be turned off and the mouse allowed to rest.

**Exhaustion:**

Often, mice will become exhausted after running for long periods and/or at high intensity on the treadmill. It is imperative that the shock grid for that lane be deactivated and the mouse be allowed to rest, or damage to the mouse and to
There are multiple ways to define exhaustion, and some of the most common are as follows:

- greater than 5 consecutive seconds on the shock grid without attempting to reengage the treadmill
- spending greater than 50% of its time on the shock grid
- the third time a mouse is willing to sustain 2 seconds or more of shocking rather than return to the treadmill

Ultimately, it will be up to each investigator to use common sense and define exhaustion in his or her own terms. **NOTE:** Experimenters should be aware that there are some mice that simply cannot keep up with the majority of similar mice. These are fairly rare, and they should be culled from the experiment whenever possible. In addition, there are significant variations in exercise performance between strains of inbred mice (for example, several investigators have reported C57bl/6 mice as being poor treadmill performers). See the reviews by David Bernstein¹ and Hintze and Shesely², as well as the article by Lightfoot et.al³ for more information.

**Cleanup:**

It is possible to clean almost every part of the treadmill with a mild solution of detergent and water. In particular, there is a plastic tray underneath the treadmill that collects feces and urine. It should be removed and cleaned after every use of the treadmill. In addition, care should be taken to prevent urine or other liquids from damaging the control surfaces of the treadmill. If there are any concerns not addressed here, consult the blue instruction manual near the treadmill or contact Janet Hoff (coordinator of the CIG).

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² Hintze, T and E. Shesely. Is a Mouse Like Any Other Mouse? *J Mol Cell Cardiol*. 34: 1283-1286, 2002