Shock Center Protocol

Protocol: Rotarod

Date: 2/8/17

Originator: J. Neal

Note:

Following is the MNBF protocol for Rotarod. Shock Center performs Rotarod in the MNBF with their equipment.

Habituation

1) Before moving mice to MNBF for habituation, open file
2) Save and rename file in appropriate folder
3) Weigh each animal recording animal weight in the new file just saved
4) Number the tail of each animal before placing in box.
5) Load boxes, as they are completed, onto a pallet for transportation to MNBF
6) In Rotarod room, Select RESET/STOP button on the Rotarod equipment panel, the rod will begin rotating at a slow constant speed. Leave it rotating throughout the entire habituation period.
7) Let habituate to testing room for 1 hour.

Setting up Experiment

8) In Rotarod program double click the box that reads Label 1 and name your experiment. You may also use label 2 for additional test information.
9) Test parameters may be set up using the software or using the equipment panel buttons. With software, program changes are viewable on panel display. For example, to set up an accelerating speed test:
    a. Click Accelerated Speed Mode
    b. Rotarod prompt: Set Ramp Duration? Use Up/Down buttons to adjust test duration in minutes. Press ESC to enter.
    c. Rotarod prompt: With Reverse? Select yes/no.
    d. Under Speed, click Set Max Value. Use Up/Down buttons to set max speed value. Press ESC to enter. Repeat for Set Min Value.
        Note: An MNBF trial uses a minimum of 4 rpm to a maximum of 40 rpm over a 5 minute (300 seconds) test period with 3 consecutive trials per mouse.
10) Double click box in bottom right of main menu, and enter animal IDs with respect to lane number. Click OK to save.
11) Select File from menu bar and click Configuration and OK.
12) Select File from menu bar again and click Acquired Data.
13) Delete any previous data using the green burn bucket icon.
14) Check box that says Refresh Each Measure.
15) Create an experiment/treatment excel sheet on your personal laptop or on the desktop in the Rotarod room. There should be at least 5 columns: Animal ID, Test Lane, Trial 1, Trial 2, and Trial 3. You will record latency to fall in this excel file.
16) Pre-plan your testing order in the excel sheet. Up to 5 mice can be tested at one time. Mice are usually housed 4 per side so you may end up using only 4 lanes per run. Do NOT split the cage to save time. For example, if each side of a duplex cage has 3 mice then break this up into 2 runs with

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> 3 mice each, utilizing only 3 lanes. If you split the box you would test 3 from one side and 2 from the other side which is incorrect.

Running Experiment

17) Move the box with test mice from the rack to the counter beside the Rotarod so that they are in close proximity.
18) Rotarod will still be rotating in RESET mode which was set up for habituation. Position counter trip-plate so that it clicks into place and the handle end rises about ¾ inches from base. The panel should also show a 0 (white zero on blue background) for that lane if the trip-plate is properly set.
19) Gently but swiftly place mice on rotor in preassigned lane. When all subjects are loaded press START button on equipment panel and the program will begin. Load mice in sequential order from left to right (lane 1 to lane 5) every time.
20) As the mice fall, they will activate the trip-plate, and data will be sent in real-time to the software. Sometimes a mouse will gently lower itself off or fall off at close range so that the plate does not drop; in this case manually push down on the trip-plate to break the connection and record latency.
21) Also record the latency if your excel sheet. The computer program is used as a supplemental backup.
22) As each mouse falls return it to its home cage. Set trip-plate aside or carefully place back in lane, but do NOT reset the trip plate [or else another latency value will be recorded which may confuse your data collection!]
23) When all mice have fallen off or max time per trial has been reached (300 seconds for MNBF), whichever comes first, press RESET/STOP button to return rod to a constant speed. If any mice remain on the wheel then return them to their home cage.
24) If performing a consecutive trial, reset the trip plates so that they read '0' and reload the same group of mice into their preassigned lane. Press START button to begin another trial. It is not necessary to clean between consecutive trials.
25) If starting another group of mice then click File and select 01-ROTA47600. Repeat steps 7-11: Double click animal ID box and change animal IDs. File-> Configuration-> OK ->File-> Acquired Data. Check box that says Refresh Each Measure.
26) Wipe down rod, plates, and dividers with 70% ethanol between test groups.
27) At the end testing, clear memory from Rotarod by pressing F3-MEM on panel>F3-RES>F3-Yes.
28) Turn off Rotarod by pressing down on RESET/STOP button until rod stops rotating and flicking ON/OFF switch on the back of the equipment.
29) Thoroughly wipe down Rotarod equipment with 70% ethanol and be sure to clean underneath and behind the machine too.
30) All of the data that you need for data analysis is in the excel file that you updated throughout testing. A backup is recorded in the rotarod's memory and can be exported as below.

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Exporting Rotarod Data

31) On Acquired Data page in software, click Export in the menu panel. Name file and save in My Documents folder (already set as default.)

32) Open saved .csv data file. In this file, open a new worksheet tab. Under Data tab, select Get External Data 'From Text' and open saved .csv file again.

33) The Text Import Wizard will appear. Choose a Delimited file type, click next, and check the box next to semicolon. Hit finish and import data into existing worksheet.

34) You may want to rearrange columns but animal ID, time stamps, labels, and latency information are all there. Save file as excel workbook and email to yourself (since laptop is off network).