
**Standard Operating Procedure
Center for Systems Neurogenetics of Addiction
(CSNA)**

Reversal Learning Assay

[CSNA-RL v1.1]

Reversal Learning

Area:	JAX-CSNA-BPC
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Controls:	
Superseded Document	N/A, New
Reason for Revision Major or Minor changes	New reward Food reward changed from Sweetened condensed milk to Boost.
Effective Date	September 1,2017

Signatures:	
Author	I indicate that I have authored or updated this SOP according to applicable business requirements and our company procedure: Preparing and Updating Standard Operating Procedures. Name: <u>Ashley Olson, Rainy Dodd, Mikayla Bolduc</u> Signature: _____ Date: <u>September 11, 2018</u>
Approver	I indicate that I have reviewed this SOP, and find it meets all applicable business requirements and that it reflects the procedure described. I approve it for use. Name: _____ Leona H. Gagnon _____ Signature: _____ Date: _____

1. PURPOSE

This SOP addresses the routine procedures used for conducting impulsivity and reversal learning operant conditioning paradigms in mice, including methods for analysis of data, and quality monitoring procedures.

2. SCOPE

The SOP applies to laboratories within the CSNA Behavioral Phenotyping Core.

3. RESPONSIBILITIES

3.1. Laboratory Staff

- 3.1.1. Remain up to date in training with this SOP
- 3.1.2. Comply with this SOP

3.2. Principal Investigator/Core Manager of JAX-CSNA-BPC

- 3.2.1. Ensures that all personnel involved running this SOP are trained to comply with this SOP

4. GLOSSARY/DEFINITIONS

4.1. Definitions

Food Restriction	Mice are provided daily rations of food in order to maintain body weights at 80-90% of free feeding pre restricted weight (base weight). Food restriction begins a minimum of 7 days prior to exposure to the testing chamber. Food is provided at the end of the testing day at least 1 hour, but no more than 2 hours, after testing for each subject.
Habituation	Acclimation of the mouse to the chamber. This is the first exposure for the mouse to the chamber. Each mouse is placed into a pre-assigned chamber for 30 minutes with the house light, white noise, and fan turned on. No reinforcers or stimuli are used in this stage. This must occur for 2 consecutive days. The mouse body weight must remain less than or equal to 90.0% of the base weight for both habituation days.
Reinforcer	A reward that is distributed into the food well in the operant chamber. In the present paradigm, the reinforce is defined as a 20±1µL liquid aliquot of Original Strawberry Boost (Nestlé HealthCare Nutrition, Inc., Florham Park, NJ 07932 USA) delivered via a calibrated pump. The presentation of the reinforcer is paired with illumination of the food well. Upon retrieval of the reinforcer, the food well light is extinguished.
Fixed-Ratio Response	This is defined as the schedule of reinforcement of which the number of responses results in a reinforcer. In the present paradigm, an FR-1 (fixed-ratio 1) schedule is designated which provides a reinforce for every 1 correct response.
Limited Hold	This is the duration of the nose-poke response (0, 0.1, or 0.2 seconds) for which the subject's nose must be maintained in the designated nosepoke aperture to result in the presentation of the reinforcer.
Stage 1	This is the initial training stage. During this session, the house light, white noise, and fan are turned on. Upon the start of the program, a reinforcer is delivered to the food well. Upon detection of a nose-poke into the food well the computer

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	<p>automatically delivers another reinforcer after 15 seconds. The session ends after 60 minutes or 50 reinforcers have been earned. The criterion for passing this stage is greater than or equal to 30 reinforcers for 2 consecutive days, with at least one day earning 50 reinforcers.</p>
Stage 2	<p>This is the second stage of training. During this session, the house light, white noise, and fan are turned on. Upon the start of the program, the center nose-poke aperture is illuminated. Subjects must nose-poke into the center aperture to earn the reinforcer (FR-1). The limited hold for stimulus duration is 0, 0.1, or 0.2 seconds (varied randomly trial to trial). The number of reinforcers earned must be checked every 15 minutes (e.g 15, 30, 45, ect) up to the completion of the test. If no reinforcers are earned in a 15 minutes interval, baiting (see below) must occur. For the first day of Stage 2 testing, each subject is baited after 15 minutes no matter the number of reinforcers earned. The session ends after 120 minutes or 30 reinforcers have been earned. The criterion for passing this stage is 30 reinforcers for 1 test day. If the mouse does not meet this criterion after 10 days, they are regressed (see below) to Stage 1 (see above). If a subject fails to meet Stage 2 criteria after a second round of 10 consecutive test days, they are removed from the study due to regression failure (see below).</p>
Stage 3	<p>This is the third stage of training. During this session the house light, white noise, and fan are turned on. Upon the start of the program, the center nose-poke aperture is illuminated. Subjects must nose-poke into the center aperture to earn the reinforcer (FR-1). The limited hold for stimulus duration is 0.1, 0.2, or 0.2 seconds (varied randomly trial to trial). The number of reinforcers earned must be checked every 15 minutes (e.g 15, 30, 45, ect) up to the completion of the test. If no reinforcers are earned in a 15 minutes interval, baiting (see below) must occur. The session ends after 60 minutes or 50 reinforcers have been earned. The criterion for passing this stage is greater than or equal to 30 reinforcers earned in one testing day. If the mouse does not meet this criterion after 10 consecutive days, they are regressed (see below) to Stage 2 (see above). If a subject fails to meet Stage 3 criteria after a second round of 10 consecutive test days, they are removed from the study due to regression failure (see below).</p>

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Stage 4	<p>This is the fourth stage of training. During this session, the house light, white noise, and fan are turned on. Upon the start of the program, the center nose-poke aperture is illuminated. Subjects must nose-poke into the center aperture to earn the reinforcer (FR-1). The limited hold for stimulus duration is 0.1, 0.2, or 0.3 seconds (varied randomly trial to trial). The number of reinforcers earned must be checked every 15 minutes (e.g. 15, 30, 45, ect) up to the completion of the test. If no reinforcers are earned in a 15 minutes interval, baiting (see below) must occur. The session ends after 60 minutes or 50 reinforcers have been earned. The criterion for passing this stage is greater than or equal to 30 reinforcers earned for one test day. If the mouse does not meet this criterion after 10 days, they are regressed (see below) to Stage 3 (see above). If a subject fails to meet Stage 4 criteria after a second round of 10 consecutive test days, they are removed from the study due to regression failure (see below).</p>
Stage 5	<p>This is the fifth stage of testing. During this stage mice are randomly pre-assigned a nose-poke hole (either “left” or “right”) as correct. During this session, the house light, white noise, and fan are turned on. Upon the start of the program, the center nose-poke aperture is illuminated. Once the mouse nose-pokes the center aperture to start the trial, the center aperture light extinguishes and the two flanking holes immediately adjacent to the center hole (e.g. “left” and “right”) are illuminated. The limited hold for stimulus duration is 0.1 or 0.2 seconds (with a 1:2 ratio, respectively). Nose-pokes into the correct hole are reinforced (FR-1), while nose-pokes into the incorrect hole results in the lights turning off for 1 second and the trial ending. Nose-pokes to the center aperture with no nose-poke to the correct flanking aperture for 30 seconds results in the lights turning off for 1 second and the trial ending. Subjects must nose-poke the pre-determined aperture in order to earn the reinforcer. The session ends after 60 minutes or the PAST20 value is greater than or equal to 0.8 (80% correct). Failure to achieve greater than or equal to 10 trials per day for 3 consecutive days results in regression (see below) to Stage 4 (see above). Failure to achieve a PAST20 value greater than or equal to 0.8 for a total of 300 Trials results in a stage failure and the mouse is removed from the study due to stage 5 failure.</p>

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Stage 6	<p>Stage 6 is the reversal learning stage of testing. During this stage the correct nose-poke hole (either “left” or “right”) is the opposite to that of the previously completed Stage 5. During this session, the house light, white noise, and fan are turned on. Upon the start of the program, the center aperture is illuminated. Subjects must nose-poke into the center aperture to initiate the trial. Once a response in the center aperture is made, the center aperture light extinguishes and the two flanking apertures immediately adjacent to the center hole (e.g. “left” and “right”) are illuminated. The limited hold for stimulus duration is 0.1 or 0.2 seconds (with a 1:2 ratio, respectively). Nose-pokes into the correct hole are reinforced (FR-1), while nose-pokes into the incorrect hole result in the lights turning off for 1 second and the trial ending. Nose-pokes to the center aperture with no nose-poke to the correct flanking aperture for 30 seconds results in the lights turning off for 1 second and the trial ending. Subjects must nose-poke the pre-determined aperture in order to earn the reinforcer. The session ends after 60 minutes or the PAST20 value is greater than or equal to 0.8 (80% correct). Failure to achieve a PAST20 value greater than or equal to 0.8 for a total of 400 Trials or a total testing time of 8 weeks results in a failure and the mouse is removed from the study due to stage 6 failure.</p>
Regression and Regression failure	<p>Regression is when a subject fails to meet stage criterion (see above) and is returned to the previous stage. Regression failure is the exclusion of a subject due to failure to pass stage criterion (see above) after a regression. For example, if a subject fails to meet criteria for stage 5, it regresses to stage 4. If the subject then passes stage 4, it moves back into stage 5. If the subject fails to meet stage 5 criteria for a second time, the subject is excluded due to regression failure.</p> <p>NOTE: Regression can only occur ONCE during testing. For example, if a mouse is regressed from stage 5 to stage 4, then fails to meet stage 4 criteria, it cannot be regressed to stage 3 and therefore is excluded from the study.</p>
Baiting	<p>The act of the technician opening the chamber during testing to apply ~1µL of the reinforcer with a cotton swab to the illuminated nose poke hole. This is used to facilitate training of</p>

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	the mice to hole poke and associate the response with the reward.
Trial Initiation	During Stage 5 and 6 a trial is initiated when the mouse nose-pokes into the center hole resulting in illumination of the left and right flanking holes.
Correct Response	In Stages 5 and 6, responses (nose-pokes) into the assigned hole (either left flanking of center or right flanking of center) resulting in a reinforcer.
Incorrect Response	In Stages 5 and 6, responses (nose-pokes) into the non-assigned hole (either left flanking of center or right flanking of center) resulting in all lights turning off for x seconds (house light and stimulus lights). In the data file, incorrect responses are calculated in "Omit" (see below).
Np Time	Nose-poke time; refers to the limited hold for stimulus at the varying durations programed for each stage during stages 2, 3, and 4. This changes throughout the test to indicate which hold is necessary to complete the task.
Past20	The percentage of correct responses made over the course of 20 consecutive trials during stages 5 and 6. This is a sliding scale such that any error within a window of twenty trials resets the scale back to the first correct response.
Omit	This is defined in the data as an omission trial where the mouse fails to respond. In Stages 5 and 6, the mouse initiates the trial by nose-pokes in the center hole which illuminates the flanking left and right holes from center. Failure to respond (nose-poke) in either left or right hole after 30 seconds is considered an omit.
Stage Placement (Tech) Error	An error occurring due to the technician erroneously placing the subject on the wrong stage for a single day. The subject can continue testing on the correct stage the following day without exclusion. This is limited to a two time human error throughout testing at any stage. This error should be recorded in the data output sheet as a Tech Error.

4.2. Procedure Inputs

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Animal ID

Experiment ID

Run ID

Experimenter ID

Comment

4.3. Procedure Outputs

4.3.1. The procedure outputs are based on the learning and reversal stages (i.e. stages 5 and 6).

Output	Defintion
Correct	The running accuracy of the last 20 trials of the current session.
Omits	The total number of omissions, defined by the mouse successfully responding in the central nose poke hole but does not make a response in a flanking hole within 30 second, limited hold.
Trial Init Lat	Total trial initiation time (in deciseconds). Total time between the illumination of the central hole (hole 3 of 5) and the initiation of the trial; this value must be divided by the number of trials initiated to get the average trial initiation time.
Pellet Ret Lat	Total time to retrieve reward (in deciseconds). Time from successful nosepoke in correct flanking hole and entry to the food magazine; this value must be divided by the number of pellets earned to get the average pellet retrieval time.
Correct Speed	Total correct response time (in centiseconds). Time from nosepoke in central hole and nosepoke in correct flanking hole; this value must be divided by the number of correct trials to obtain the average correct response time.
Incorrect Speed	Total incorrect response time (in centiseconds). Time from nosepoke in central hole and nosepoke in incorrect flanking hole; this value must be divided by the number of incorrect trials to obtain the average incorrect response time.
Left Anti	Total number of anticipatory responses on left side. Response in the left flanking hole without a prior successful nosepoke in the central hole.

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Right Anti	Total number of anticipatory responses on right side. Response in the right flanking hole without a successful nosepoke in the central hole.
Num trials time req 0	Total number of trials at nose poke hold time 1. Nose poke hold time 1 refers to the limited hold for stimulus duration of 0.1.
Num trials time req 1	Total number of trials at nose poke hold time 2. Nose poke hold time 2 refers to the limited hold for stimulus duration of 0.2.
Num trials time req 2	Total number of trials at nose poke hold time 3. Nose poke hold time 3 refers to the limited hold for stimulus duration of 0.2.
Num successes time req 0	Total number of correct trials at nose poke hold time 1. Nose poke hold time 1 refers to the limited hold for stimulus duration of 0.1. A correct trial being one which occurs when the mouse responds in the correct flanking hole without a successful nosepoke in the central hole.
Num successes time req 1	Total number of correct trials at nose poke hold time 2. Nose poke hold time 2 refers to the limited hold for stimulus duration of 0.2. A correct trial being one which occurs when the mouse responds in the correct flanking hole without a successful nosepoke in the central hole.
Num successes time req 2	Total number of correct trials at nose poke hold time 3. Nose poke hold time 3 refers to the limited hold for stimulus duration of 0.2. A correct trial being one which occurs when the mouse responds in the correct flanking hole without a successful nosepoke in the central hole.
Num failures time req 0	Total number of trial failures at nose poke hold time 1. Nose poke hold time 1 refers to the limited hold for stimulus duration of 0.1. An incorrect trials occurs when the mouse responds in the incorrect flanking hold after initiating a trial.
Num failures time req 1	Total number of trial failures at nose poke hold time 2. Nose poke hold time 2 refers to the limited hold for stimulus duration of 0.2. An incorrect trials occurs when the mouse responds in the incorrect flanking hold after initiating a trial.
Num failures time req 2	Total number of trial failures at nose poke hold time 3. Nose poke hold time 3 refers to the limited hold for stimulus duration of 0.2. An incorrect trials occurs when the mouse responds in the incorrect flanking hold after initiating a trial.

5. MATERIALS

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5.1 Instrumentation

5.1.1. Operant Conditioning Chamber Apparatus: The operant conditioning chamber apparatus is Med Associates (St Albans VT, USA) Model ENV-307W-CT. Each chamber is fitted with a horizontal array of 5 nose-poke apertures on one side of the box and a photocell-equipped food delivery magazine on the opposite side. Each nose-poke aperture is equipped with infrared photocell beams which detect entry (via nosepoke) into the apertures. Twelve chambers are currently in operation and run simultaneously.

5.1.2. Environmental Control Chamber: Each operant conditioning chamber is placed within a ventilated (by electric fan), sound attenuated, cabinet (Model ENV-022MD; Med Associates, St Albans VT, USA).

5.1.3. Variable Speed Pump: Each chamber has a variable speed pump for the administration of the reinforcer (Model PHM-122-18 ; Med Associates, St Ablans VT, USA). Pumps are calibrated to deliver a 20uL liquid aliquot reward as the reinforce.

5.1.4. Control software: Med-Associates MedPC IV version 4.2 software.

5.1.5. Scale: A scale for weighing animals and food with 0.1 gram resolution.

5.2. Consumables

5.2.1. 70% ethanol in water solution in a spray bottle: used to sanitize the chambers between subjects

5.2.2. Paper towels

5.2.3. Cotton Swabs

5.2.4. 5 mL luer lock syringes

5.2.5 Strawberry Creamy Boost (Nestlé HealthCare Nutrition, Inc., Florham Park, NJ 07932 USA): Boost is stored at room temperature and is refrigerated after opening. Boost may be used for 24 hours after opening.

5.2.6. Virkon sanitization agent (Lanxess Corp., Pittsburgh, PA)

6. PROCEDURE

6.1. Environment

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- 6.1.1. Procedure Room. The dimensions of the procedure room are approximately 10 feet in length x 10 feet in width.
- 6.1.2. Temperature. The temperature range in the testing room is 70 ± 3 F.
- 6.1.3. Humidity. The humidity range in the procedure room is $50 \pm 20\%$.
- 6.1.4. Lighting. Room lighting measures 550 lux. Room lighting is turned off at any point that the chamber doors are opened (e.g. for baiting as in 6.3.5. below) and measures 6 lux. Lighting within the behavioral chambers is provided by a house light (ENV-227M, Test 1217; Med Associates, St Albans VT, USA) that is mounted on the back of the input/output control tower within the chamber and measures an average of 7 lux at the testing floor. Light lux measures are validated weekly during testing period.
- 6.1.5. Noise. The ambient background noise level in the procedure room is 56dB. The running fan and the white noise generating amplifier within the environmental control chambers produce white noise at 65-70 dB. (ENV-303HDW, Med Associates). Background noise levels are validated and recorded monthly in the room log book.
- 6.1.6. Time of day. The test is conducted during the light phase of the circadian cycle; beginning at least 60 min after the lights on and concluding at least 60 min before lights off.

6.2. Subjects

6.2.1. Species. Mice

6.2.1.1. Study specific animals (e.g, strain, sex, date of birth)documented.

6.2.1.2. Receipt of animals logged (e.g., date of arrival)

6.2.2. Sex. Males or females

6.2.3. Age. The test is initiated in adult mice beginning at 9-13 weeks of age.

6.2.4. Housing. Subjects are individually housed for this test.

6.2.4.1. Subjects are maintained on pine wood chip bedding.

6.2.4.2. Subjects are housed in the housing room for a minimum of 5 days prior to behavioral testing.

6.2.4.3. Enrichment. Cages are provided a nestlet (ScottPharma Solutions, Marlborough, MA, USA, product# NE3600) and disposable dome-shaped shack (Shepherd Specialty Papers, Inc., Watertown, TN, USA) as standard enrichment for individually housed mice.

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- 6.2.5. Diet. LabDiet Specially Formulated Autoclavable Laboratory Animal Diet, PMI Nutrition International, LLC; Brentwood, MO. 6.0% fat content.
- 6.2.6. Food Restriction. Mice are restricted to 80-90% of free feeding body weight prior to beginning this test. Food restriction begins at least 7 days before the habituation phase of the task. On the first and second day of food restriction, mice are provided 2mL of reinforcer and 3.5g of diet. The amount of reinforcer is cut in half (e.g 2mL to 1mL to 0.5mL, ect) daily until the amount given is 0.25mL. The amount of diet is adjusted each day in order to maintain a restricted weight of 80-90% of pre-restricted (base) body weight. Once mice begin earning rewards in the test sessions, reinforcer is removed as part of the daily ration and only diet provided.
- 6.2.7. Daily pre-testing welfare assessment. If a mouse appears lethargic (e.g hypoactive, shaking) and body weight is greater than 80% of pre-restricted weight, 0.2g of food should be provided prior to testing and subjects should be provided a heat source. If a mouse appears lethargic and body weight is less than 80% of pre-restricted weight, 0.5g of food should be provided prior to testing and subjects should be provided a heat source. If lethargy resolves, mice can be tested after a minimum 1 hour recovery. If lethargy does not resolve in 2 hours the mouse is fed their daily ration and not tested that day.
- 6.2.8. Husbandry. Cage change occurs once a week post testing for that day. The cages are changed on the same day every week. The food rations for that day should be provided after the cage change. In the current protocol, cage changes occur on Fridays.
- 6.2.9. Body Weights. Body weights are collected each day before testing. This occurs until the subject passes all criteria or is excluded from the study.
- 6.2.10. Subject Identification. Mice within a cage are marked by ear punches. Ear punches are performed at 6 weeks of age, at the same time that subjects are individually housed.
- 6.2.11. Randomization and counterbalancing. Subjects are tested in the same chamber throughout the entire testing period. Every attempt is made to test mixed batches of strains and/or sexes such that each session does not include only animals from a single strain or sex. A list of subjects IDs, date of test, date of birth, weight, sex, and genotype when available should be prepared in the laboratory notebook prior to testing. Any comments or unexpected observations regarding the session should be noted either generally or in reference to a specific animal, in the laboratory notebook.

6.3. Testing

6.3.1. Mice are tested daily.

6.3.2. Pre-testing preparation.

6.3.2.1. Body weights are recorded for each mouse at the start of the day. Mice are assessed for any welfare concerns as in 6.2.7

6.3.2.2. Pump set-up.

6.3.2.3. Equipment check. Prior to any test session, the operant conditioning chambers should be tested to confirm that all infrared sensors are working properly. System is powered on and all inputs and outputs for each box are operating properly as confirmed by manually breaking the beam in each hole-poke aperture that is illuminated. Any box that fails this step is not used for testing until repaired and mice assigned to this box are not tested for that day.

6.3.2.4. Sanitization. Prior to the placing a mouse into the operant chamber, the chamber is cleaned with 70% ethanol solution (in water) and is then wiped dry with clean paper towels. Virkon is used at the end of the day of testing. Virkon is applied with a 10 min contact time and then wiped off with 70% ethanol solution.

6.3.2.5. Data entry. Prior to placing mice into the chambers, experimental details for each test day are entered into the ancillary notebook (e.g. Google sheet or Excel in a shared location). This includes Subject ID, Weight, Group, Date, Experiment, Stage, Comment, and Experimenter. In addition the program assigned to each subject and subject ID is loaded into the Med-PC software. Specifically on the first day of Stage 1, the pre-restricted weight is placed in the Med-PC comments.(e.g //MJB/COMMENTS:26.6)

6.3.3. Transport to procedure room. Subjects are moved in their home cages from the housing room to the procedure room on a wheeled cart. The procedure room is directly adjacent to the housing room. Cages are not removed from the procedure room until testing for that mouse is completed.

6.3.4. Placement in chambers. Mice are placed individually in assigned chambers. Immediately upon placement into the chambers, the doors are closed and the technician manually starts the testing program.

6.3.4.1. Observe. The technician observes the software to ensure mice are performing the task (e.g number of responses and reinforcers earned). If a specific mouse is not performing, it should be observed from the peephole in the front of the environmental control chamber to confirm stimuli are active (e.g lights on) and

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responses are reinforced. If the subject is responding and the software is not recording, then the test is terminated, subject is removed, equipment is assessed for repairs, and data are excluded for that day. All incidents and observations including technical issues that require exclusion of data are recorded in the comments section of the Google sheet and Lab notebook and are flagged for IGGC review.

6.3.5. Baiting. A cotton swab saturated with the liquid reinforcer. Prior to opening the chambers to present the cotton swab into the illuminated hole, the room lights are turned off. The cotton swab is carefully placed into the stimulus hole under the following criterion:

Stage 2, Day 1: Baiting occurs @ the 15 minute timepoint on Day 1 of Stage 2 for every mouse. An additional assessment for baiting occurs at every 15 min timepoint. If a mouse does not respond (e.g. 0 responses) within each 15 min time period, then the stimulus hole is baited.

Stage 2, Day 2 through Stage 4: Assessment for baiting occurs at every 15 min timepoint. If a mouse does not respond (e.g. 0 responses) within each 15 min time period, then the stimulus hole is baited.

Stages 5 and 6: There is no baiting in stage 5 or 6.

6.3.6. When the mouse reaches the pre-programmed criterion for any stage as in 4.1 above, the program will automatically end. If the mouse does not reach criterion during the session, the program will end after the test duration as in 4.1 above. The mouse should be removed from the apparatus immediately (within 2-5 minutes) of the session ending. The mouse is removed from the chamber and placed into its home cage on the transport cart.

6.3.7. When all sessions are complete, the subjects are returned to the housing room and the chambers are sanitized as in 6.3.2.4.

6.3.8. At least one hour but no more than two hours after being returned to the homecage, subjects are provided their daily food ration.

6.3.9. Repeat steps 6.3.3 through 6.3.8 for all subsequent sets of subjects.

6.4. Data Analysis and QC

6.4.1. Quality Control. Data are reviewed for accuracy of data entry and to ensure any technical errors which lend data excluded are flagged for IGGC review.

6.4.2. Data transfer. Data are imported into an excel file using MPC2XL transfer program. This should be done on a different computer than

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the one used for testing to eliminate chances of corrupting the original files.

6.4.3. Data evaluation.

6.4.3.1. Determine whether any mouse met performance criterion during its daily session as per 4.1 above. Subjects that meet criterion for each stage of the test are moved to the next stage as 4.1 above.

6.4.4. Results and data storage.

6.4.4.1. Data Archived in several locations at minimum: 1) files are saved on the testing computer; 2) files are saved on at least 2 separate external hard drives and 3) files are saved on a shared drive.