DOUGLAS HOSPITAL RESEARCH CENTRE
McGill UNIVERSITY ANIMAL CARE COMMITTEE

Standard Operating Procedures January 2007 version

Behavioural tests - RODENTS (Rats, mice)

1. INTRODUCTION

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form (AUP). Approval of the protocol indicates approval of the deviation from the SOP for that project only. A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOPs must be referred to in the Procedures section.

2. INFORMATION REQUIRED

Species and strain: _____
Age and or genetic modification and or genotype (if applicable): _____
Approximate weight: _____

3. BEHAVIOURAL TESTS: Put an **X** next to the test(s) to be performed:

3.1 Anxiety-related tests	
☐	1. Elevated plus maze
☐	2. Open field
☐	3. Tatcher Britton novelty conflict paradigm
3.2 Memory and learning tests	
☐	1. Morris water maze
☐	2. Object recognition
☐	3. Radial arm maze
☐	4. Fear potentied startle
3.3 Motor function tests	
☐	1. Open field locomotor activity
☐	2. Rotarod
3.4 Nociception and drugs effects tests	
☐	1. Tail flick
☐	2. Von Frey hair
☐	3. Thermal sensitivity test
3.5 Stress tests	
☐	1. Tail pinch
☐	2. Forced swim test
☐	3. Restrain test
3.6 Impulsivity test	
☐	1. Differential reinforcement of low rates task
3.7 Screening test for drug side effects	
☐	1. Conditioned taste aversion
3.8 Test of Sensorimotor Gating	
☐	2. Pre-pulse inhibition

3.9 Are there changes to this SOP indicated in the AUP form?

Yes ☐ No ☐

If yes, specify changes: _____

PI signature: _____ **Date:** _____

PLEASE ATTACH <u>ONLY</u> THIS SIGNED COVER SHEET TO THE BACK OF EACH RELEVANT AUP FOR ANIMAL CARE COMMITTEE APPROVAL.

3. Description of the behavioural tests

3.1 Anxiety-related tests

3.1.1 Elevated plus maze

This is a "gold-standard" test to measure the fear and anxiety in the rodent. The test creates a conflict between the animal's natural tendencies to explore new environments with its fear of open, unprotected spaces. The test is very sensitive to both traditional (i.e. benzodiazepines) and novel (i.e. 5-HT based) anxiolytic.

Method and interpretation of the test

The apparatus consists of a maze formed in the shape of a **+** sign, two of the arms have walls (closed arms), whereas the remaining two arms are without walls (open arms). The animal is placed in the middle of the elevated plus maze apparatus and the number of entries into and total time spent in the close and open arms is recorded over a 5 minutes period. Anxious animals will avoid the open arms, and spend more time in the closed arms.

3.1.2 Open field

Open-field behavior is used as a measure of reaction to novelty. Animal are placed for 10 minutes into a 1X1m open field and behavioral measures of exploration are recorded.

Method and interpretation of the test

The degree of "stress" can be manipulated by varying the intensity of the overhead lighting. The animal is placed in the middle of 1X1m open field at the start of the trial. The open field is divided in 16 squares, and the number and location of square entries is calculated. Anxious animals will spend more time in thigomotaxis (i.e., entering squares located along the walls of the open field.

3.1.3 Tatcher Britton novelty conflict paradigm

The Thatcher Britton test is a variant of the open field test. It creates a conflict between the animal's desire to satisfy a biological need (hunger) and its fear of open spaces.

Method and interpretation of the test

The animal is first food deprived for a 24-hour period. It is then placed into a 1X1m open field for 10 minutes. Five pellets of standard chow are laced in the middle of the open field. The time the animal takes to begin eating is measured. Anxious animals will take longer to begin eating.

3.2 Tests of Learning and Memory

3.2.1 The Morris Water Maze

This test is considered a "gold-standard" for assessing spatial learning and memory in both rats and mice. This test has been shown to be very sensitive to both memory-enhancing and memory-impairing manipulations, including lesions and pharmacological interventions.

Method and interpretation of the test

The animal is placed in a pool of water at 24-25^{0}C that has been made opaque by the addition of No toxic paint (gouache). The animal is required to find a submerged platform and solve the task using only distal spatial cues provided in the testing room (rats) or cues affixed to the walls of the pool (mice). Each animal undergoes 4 trials a day for 5 successive days. The distance and the time animals take to find the platform are recorded by an automated tracking system. The longer the latency to find the platform, the poorer the learning. Following acquisition, two types of probe trials are administered. In the first, the platform is removed and the animal is permitted to explore the pool for a fixed period (typically 30 or 60 seconds). These trials permit measurement of the memory trace, by analyzing the amount of time the animal spends in close proximity to where the platform was located previously. In the second, the

platform is reinserted, and is made visible by lowering the water level. This allows assessment of whether poor performance may be attributed to visual deficits. Animals need to be dried before being returned to their home cage with a blanket or a heating lamp.

3.2.2 Object recognition

This test provides a rapid and reliable method for assessing working memory in the rodent. Unlike other methods that assess working memory, the object recognition test does not require extensive pretraining, but rather exploits the rodents' natural tendency to explore novel objects.

Method and interpretation of the test

Animal is placed in an open field for three days of habituation (10 minutes per day), to dissipate any fear of the new environment that may interfere with memory performance. On day 4, the animal is placed for 10 minutes in the center of the field with two identical objects placed in two corners of the field. The animal is then returned to its home cage for a retention interval ranging between 5-60 minutes. Subsequently, the animal is replaced into the open field for 10 minutes; one of the objects is familiar (i.e., identical to the objects in the first exposure to the open field), the other is novel (one that the animal has never seen before). The time that the animal spends exploring the novel and familiar objects is recorded. A greater cumulative amount of time spent exploring the novel object reflects improved working memory function.

3.2.3 Radial arm Maze

Like the Morris water maze, the radial arm maze assesses spatial memory function in the rodent. The advantage of this test is that it can be used to assess both working and reference memory.

Method and interpretation of the test

The radial arm maze consists of a 33 cm wide octogonally-shaped central platform, from which extend eight 10.5 cm wide, 50 cm long arms. A 5-cm diameter food cup is recessed into the end of each arm. Each arm and the central platform are enclosed by 18.5 cm high Plexiglas walls. The floors of the central platform and the arms are made of stainless steel and are painted flat black. Animals are first acclimatized for a 7 day period to a 23-hour food deprivation schedule. Weights are monitored daily to insure that they do not fall below 90% normal free-feeding weight (adjusted for age). During the 1-hour feeding period, animals are given access to standard rodent chow. In addition, animals are given access to Kellogg's Froot Loops (which subsequently serves as the reinforcer for radial arm maze training). Habituation training to the arm maze occurs over three days. Animals are placed individually in the center of the platform and are permitted to explore the entire maze for 10 min. On the initial day of habituation, pieces of Froot Loops are scattered in the top half of each arm of the maze. The following day, pieces are located only in outer quarter of each arm and in the food cups. On the last day of habituation, a single Froot Loop piece is placed in the food cup. By the end of habituation training, all animals readily entered each arm, and have consumed most of the Froot Loops within a ten-minute time limit. Radial arm maze training is then initiated. Animals are tested on consecutive days, one trial per day. Before each trial the maze is wiped with an antiseptic cloth and one-third to one-half of a piece of Froot Loops is placed in the food cup of each arm. At the beginning of each trial, animal is placed inside the central platform and a computer-controlled timer is started. An arm choice is recorded when the animal enters an arm with all four paws. Since food is not replaced within a trial, only the first entry in each arm is rewarded. Repeated entries, therefore, constitute memory errors. The trial continues until the rat enters all eight arms, makes a total of 15 errors, or ten minutes elapse. Training generally requires 30 days.

3.2.4 Fear potentiated startle

Fear potentiated startle is a simple and reliable test for assessing fear acquired through Pavlovian conditioning in the rat (the validity and reliability of this test in the mouse is questionable).

Method and interpretation of the test

On day 1, half of the animals (conditioned group) are first exposed to pairings of a light and shock (0.4 mA to 0.5 mA electric current is applied to the paws through a grid floor during 0.5 s). The remainder of the animals (control group) are placed in the chambers, but are exposed to the light alone (i.e., no shock is presented to these animals). On day 2, all animals are exposed to two types of trials: (1) startle-

alone trials in which an acoustic startle stimulus is presented alone, and (2) light-startle trials in which the light is presented prior to presentation of the acoustic startle stimulus. The degree of conditioned fear to the light can be assessed both within subject (i.e., greater startle to the light-startle trials relative to the startle-alone trials in the conditioned group), and between subject (i.e., greater startle in the conditioned group to the light-startle trials relative to the control group).

3.3 Motor Function tests

3.3.1 Open field locomotor activity

This test is used to assess general locomotor activity. It is also ideal for assessing drugs (e.g. dopaminergic compounds) that influence central nervous system substrates involved in motor output.

Method and interpretation of the test

The animal is placed in a plexiglass box for 60 minutes for habituation. After habituation, the locomotor activity of the animal is measured and recorded for 30 minutes by using the Versamax computer program, which permits automated recording of horizontal and vertical movement, as well as stereotyped behavior.

3.3.2 Rotarod

This test is generally used to assess the rodent's sensorimotor coordination. Motor coordination and equilibrium were tested on an accelerating Rotarod.

Method and interpretation of the test

Animals are placed on a rotaring rod and were first pretrained on the apparatus for five trails at an interval of 10 minutes on a constant speed at 4 rpm during 2 minutes. After the trained, animals are replace on the Rotarod for the first trail test. The speed of the rotation is gradually increased from 4 to 40 rpm. The latency until the animal fell from the rotating rod was recorded for a maximum of 5 minutes. Both the length of time that the animal remains on the rod and the speed are recorded for each trial. Normal animal will try to keep is equilibrium on the Rotarod.

3.4 Nociception and drugs effects tests
(Sensory systems)

3.4.1 Tail flick test

There are variants of the tail-flick test. In the first, a focused beam of hot light is applied to the distal end of the animal's tail. In the second, the distal 5 cm of the animal's tail is submerged into heated water. In both instances, the latency to tail withdrawal form the heat source is recorded. The maximum time the heat source is applied or tail is submerged is 10-15 seconds depending on the temperature applied, which ranges from 46◦C to 53◦C. The tail flick is spinally-mediated; as such it is ideal to assess spinal mechanisms involved in nociception. It is also sensitive to all known analgesic compounds.

Method and interpretation of the test

Both tests provoke a vigorous withdrawal movement of the tail. It is the reaction time of this movement that is recorded (tail flick). Longer latencies are interpreted as antinociception.

3.4.2 Von Frey hair test

Von Frey hair assay is used to measure the tactile sensitivity of the animal.

Method and interpretation of the test

The animal is placed in a Plexiglas box with a grid under it. Different sizes of metal stem can be inserted through the netting of the box under the animal to pinch the lower part of its leg. The reaction time, the size of the stem compared to the reaction and the withdrawal are measured.

3.4.3 Thermal sensitivity test

This test determines the acute thermal sensitivity of the plantar surface of an animal's hind paw to a high intensity focused light beam. It is considered to be a test of acute thermal pain, not producing tissue damage. A variation of this test is the "hot plate test". This test measures nociceptive sensitivity via a spinal response primarily. The advantages of this test are that it is easy and rapid to perform and can be repeated several times on the same animal.

Method and interpretation of the test

Animals are placed in a plexiglass chambers placed on an elevated glass plateform with a Controlled light beam source (ITTC Sciences), timer and thermometer. Prior to testing, the light beam needs to be calibrated to determine the time-related increament in temperature. Animals need to be habituated to the plexiglass boxes (5 min sessions, 3 days in a row) prior to testing. On the day of testing, animals are placed in the boxes for 5 min prior to starting the test. The test is started when the radiant source is positioned under the glass floor directly under the hind paw of animal. The beam is turned off by the experimenter when the animal lifts the paw or displaces the paw from the light beam. Latency to lift or displace the paw is recorded. Left and right paws are tested alternatively and an average of 3 testing per paw and per animal is performed. If there is no significant difference in the latencies between left/right paws, these can be pooled. The maximum time the heat source is applied or tail is submerged is 10-15 seconds depending on the temperature applied, which ranges from 46◦C to 53◦C

3.5 Stress tests

3.5.1 Tail pinch

This test is used to measure stress hormones levels (e.g., corticosterone) in the animal or to measure effect of analgesic drugs.

Method and interpretation of the test

Stress is induced by placing a wooden clothespin at 2 or 3 cm from the base of the tail for 10 minutes one time a day. The clothespin was immediately replaced if the animal removed it. This is sufficient to significantly raise stress hormones levels, as assessed by radio-immunoassay on blood samples drawn at different times during and after the tail pinch test. To measure a nociceptive response the same method was use. The analgesic response was indicated by the time (latency) required for the animal to respond to this pressure by vocalizing or biting at the clip.

3.5.2 Forced swim test

The forced swim test assesses the rodent reaction to an aversive, inescapable situation. Animals are placed into a small container of water, and the latency and total time spent immobile are measured. Whereas the interpretation of the immobility response is unclear, this test has been shown to be very sensititive to pharmacological compounds possessing antidepressant activity. As such, it is a useful, simple and reliable test that can be used to assess novel antidepressants.

Method and interpretation of the test

The animal is placed into a glass cylinder containing 7.5 cm of water at 25^0C on two occasions (pre test and test sessions). The pretest is applied to teach the animal that escape from the cylinder is not possible, and lasts for 15 minutes for the rat, and 5 minutes for the mouse. The test session, conducted on the next day, is 5 minutes in duration for both rat and mouse, and the time spent swimming, climbing, diving, and immobile are recorded. Antidepressant compounds generally reduce the amount of time the animal spends immobile.

3.5.3 Restraint test

This test is used to measure stress hormones levels (e.g., corticosterone) in the animal.

Method and interpretation of the test

Restraint stress test is imposed using plastic tubular restrainers. This procedure does not completely immobilize the animal, in that movement is limited but not eliminated. Restraint test is imposed for 10 minutes, which is sufficient to significantly raise stress hormones levels, as assessed by radio-immunoassay on blood samples drawn at different times before, during and after restraint. The blood samples are obtained in a small amount a maximum of 500 µL for rat and a maximum of 120 µL for a mouse from the tail. A maximum of 6 ml/kg of blood can be collect every 3 weeks.

3.6 Impulsivity test

3.6.1 Differential reinforcement of low rates task

This test assesses the animal's ability to withhold a response for a predefined period of time in order to obtain reward. Failure to withhold responding for the time interval is considered an indication of impulsivity.

Method and interpretation of the test

The animal is first acclimatized to 23-hour food deprivation and is then preexposed to sweetened milk, which is used as the reinforcer in the test. The animal is then trained to press a lever for sweetened milk reinforcement (20 seconds), and each reinforced bar press is accompanied by the onset of a light located above the lever. Gradually, the interval between reinforced lever presses is increased. For instance, after one reinforced bar press, the animal must wait 30 seconds before pressing in order to receive a second reinforcement. Responses within the 30 second interval reset the timer, so that the animal must wait a full 30 seconds before pressing a third time in order to receive reinforcement. The number of non-reinforced bar presses is considered as the main measure of impulsivity.

3.7 Screening tests for drug side effects

3.7.1 Conditioned Taste Aversion

The conditioned taste aversion test can be used to explore 2 options: 1- For determining drugs with gastrointestinal side effects. 2- To study the behavior of the animal about association of the pain versus the ingestion of the saccharin solution.

Method and interpretation of the test

This test requires several stages conducted over several days. In stage 1, animals are acclimatized to water deprivation, in which water is available in the home cage for a period of 60 minutes only. After the 60 minutes of free drinking, the water is removed from the cage. In the second stage, beginning on the sixth day, the animals are divided into two groups: the first receives a water-saccharin solution followed by an injection of tested drug: 2% body weight (for example: Lithium Chloride or other drugs) for option 2 above; the other group receives the water-saccharin solution alone. If the tested drug induces gastro-intestinal distress, which the animal should associate with ingestion of the saccharin solution. Accordingly, from day 8 to 10, when access is restricted to the saccharin solution, animals previously receiving saccharin-drug pairing should avoid consumption of saccharin.

3.8 Test of sensorimotor gating

3.8.1 Prepulse Inhibition (PPI)

PPI is the "gold standard" test to measure sensorimotor gating, a preattentional mechanism that assesses the reactivity to an acoustic stimulus that induces a startle response. PPI refers to the finding that a weak pre-stimulus (referred to as the prepulse), presented prior to a loud acoustic stimulus that causes the animal to startle (known as the startle stimulus), reduces the amplitude of the response elicited by the startle stimulus. It is assumed that the pre-pulse stimulus reduces startle amplitude by reducing the processing of the startle stimulus. In other words, the pre-pulse stimulus filters or gates processing of the startle stimulus. In the human, PPI is impaired in psychotic disorders, in particular, schizophrenia.

Method and interpretation of the test

PPI is assessed in 4 startle chambers each consisting of a Plexiglas chamber (8 cm diameter, 16 cm long) mounted on a Plexiglas base within a lit, ventilated sound-attenuating chamber. A speaker

located in the ceiling of the chamber provides the background noise (70 dB) and both prepulse and pulse stimuli. A piezoelectric strain meter attached to the base transduces the startle response. Stabilimeter readings are rectified, digitized on a 4095 scale, and recorded by a computer. An average of 100 1-msec readings, beginning at stimulus onset, is used as the measure of startle amplitude for each trial. To assess startle magnitude in the absence of the prepulse, a 120 dB, 30 msec stimuli is presented alone. To assess PPI, this startle stimulus is preceded by a 30 msec prepulse stimulus. The intensity of the prepulse ranges between 3-15 dB above the background noise, in increments of 3 dB (this, given that the background noise level is 70 dB, prepulse intensities range between 73-85 dB). Animals are placed into the Plexiglas restrainers, and after a 5 min. acclimatization period are exposed to a total of 37 trials. The first two trials are startle trials (no prepulse is presented). Over the next 35 trials, animals received 10 further startle trials and 5 trials at each of the 5 prepulse intensities. These trials are randomly generated, with the restriction that no more than 2 trials of the same type can occur in succession. The intertrial-interval is a variable-interval schedule with an average of 15 sec (range = 5 -30 sec).

4. Disinfection of apparatus used for behavioural tests

4.1 Product to be used to disinfect all behavioural apparatus

Peroxygard® is commonly used to clean and disinfect animal apparatus and they can be used on hard surfaces such as floor, walls, metal surfaces, stainless steel surfaces, porcelain and plastic surfaces. Peroxygard® contains Accelerated Hydrogen Peroxide, a new cleaning and disinfecting patented technology. This product reduces or eliminates pathogenic organisms with broad spectrum sanitizing in just 30 seconds. Virucidal and bactericidal disinfection is accomplished in just five minutes. This product does not release odours and it is not toxic. This is why it is largely used for animals. It is really important for behavioural tests, as odours can affect the behaviour of the animals.

4.2 How use this product?

4.2.1 Preparation

Dilute 1:16 that is 256 mL Peroxygard® Concentrate added to 4 litres of tap water or 8 oz Peroxygard® Concentrate added to 1 Gallon of tap water.

4.2.2 Protective Barriers

Disposable gloves and protective clothing should be worn when we using Peroxygard®.

4.2.3 Cleaning, Disinfecting and Supplies Preparation

1. Remove soil and body materials.
2. Spray Peroxygard® 1:16 on the apparatus and wait 5 minutes.
3. Rinse and wipe dry

All Behavioral apparatus need to be disinfecting after each use.