#### Procedure Type: Behavior Study Assay

Procedure Title: (Choose one below)

Acoustic Startle Response Bar Holding Grip Strength Elevated Zero Maze Elevated Plus Maze Foot Placement Test Open Field Test Passive Avoidance Test Rota Rod Cylinder Test Climbing Test

Species: Rat or Mouse

#### Pain/Distress Category: C

Please note that the timing and frequency of testing sessions must be outlined in the Procedure Relationships tab of the AUP for each assay. This information may also be included within the procedure description (e.g., "This procedure is performed a maximum of \_\_\_\_\_\_ times per session. The animal will be tested no more than \_\_\_\_\_\_ sessions in their lifetime with a rest period of at least \_\_\_\_\_\_ days between sessions."), however, because this information will differ between AUPs, it has not been included in the standard verbiage below.

#### Procedure Title: Acoustic Startle Response

#### **Procedure Description:**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

The rodent is placed in the testing apparatus and given 5 minutes to acclimate to the environment. After acclimation period, acoustic stimuli (65dB – 120 dB) are administered for 30-40 ms. Acoustic stimuli are repeated for no more than 80 times in one session. Animals are in chamber for no more than 30 minutes. Chambers will be cleaned with 70% ethanol between animals.

#### How does this procedure fit into or address your overall research goals?

This procedure assesses anxiety levels and sensory-motor gating. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from the normal health are expected.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for any signs of distress (e.g., hunched posture, ruffled fur), immediately after completion of the procedure and up to 30 minutes thereafter. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal.

#### **Procedure Title: Bar Holding**

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

The animal is allowed to hang by the forelimbs from a wire or small bar. Padding is placed under the wire or bar so that the animal is not injured when releasing from the wire or small bar.

#### How does this procedure fit into or address your overall research goals?

This procedure is used to assess limb strength and coordination. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Because the area below the bar is padded no injuries are expected. However, animals will be examined closely to make sure they do not sustain injuries after releasing from the wire or small bar.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for any signs of injury after releasing from the wire or small bar immediately after completion of the procedure. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal.

#### Procedure Title: Grip Strength

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

A rodent is held by the tail and lowered toward the grip strength apparatus. Once allowed to grab the handle with the forelimbs, the animal will be pulled back until it releases the grip from the handle. The equipment measures the grams of force required to remove the rodent from the apparatus.

#### How does this procedure fit into or address your overall research goals?

This procedure is used to assess limb strength and coordination. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

De-gloving of the tail (i.e., removal of skin) may occur if the tail is held too close to the tip. Though not life threatening, if this occurs, the animals will be euthanized immediately, or an OLAC veterinarian will be consulted to see if the animal can be spared for future testing. Releasing the bar could potentially result in injury to the feet or toe nails. These would not be life threatening.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for any signs of tail de-gloving, immediately after completion of the procedure. If any abnormal signs are noted, an OLAC veterinarian will be contacted. Animals will be examined for tail injury and scrapes to the feet or injury to toe nails.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized.

#### Procedure Title: Elevated Zero Maze

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

Animals will be allowed to acclimate to the testing room for one hour or more prior to beginning test. An individual rodent will be placed on a donut-shaped platform that has two open areas and two closed areas and is elevated above the floor. Padding is placed under the maze so that the animal is not injured if the animal falls from the maze. This test measures the willingness of animals to enter the open areas. The animals will be placed in a closed quadrant on the testing apparatus and allowed to roam freely for a maximum of 45 minutes. Time spent in different portions of the maze will be monitored by video recording. Maze will be cleaned with 70% ethanol after each use.

#### How does this procedure fit into or address your overall research goals?

This procedure is used to assess anxiety. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

Animals that fall from the maze will be monitored for any signs of injury.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized.

#### **Procedure Title: Elevated Plus Maze**

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

Animals will be allowed to acclimate to the testing room for one hour or more prior to beginning the test. Individual animals will be placed in an elevated plus maze compromised of a cross shaped structure with two open arms and two closed arms elevated above the floor. Padding is placed under the maze so that the animal is not injured if the animal falls from the maze. This test measures the willingness of animals to enter the non-walled areas. The animals will be placed in a closed quadrant on the testing apparatus and allowed to roam freely for about 5-45 minutes. Time spent in walled versus open portions of the maze will be monitored by video recording. Maze will be cleaned with 70% ethanol after each use.

#### How does this procedure fit into or address your overall research goals?

This procedure is used to assess anxiety. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Anxiety is assessed by the animal's interest in entering an open area and is not an induced stress, thus, no clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

Animals that fall from the maze are monitored for any signs of injury. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized.

#### Procedure Title: Foot Placement Test

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

The hind paws are dipped in a water based non-toxic ink that can be easily washed off. Animals are then allowed to walk on a narrow piece of white paper and the pattern of foot placement while walking is analyzed. All ink is washed off of feet before being returned to their home cage.

How does this procedure fit into or address your overall research goals? This procedure is used to assess gait abnormalities. (Insert protocol-specific rationale here.)

# Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for cutaneous reaction to ink application immediately after completion of the procedure. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal.

#### Procedure Title: Open Field Test

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

Animals are allowed to habituate to the testing room for 30-60 minutes. Animals are placed in a large open space consisting of a solid floor and surrounded by 4 walls. Animals are recorded for analysis while exploring the environment. The time spent against the walls vs away from the walls. Open Field apparatus will be cleaned with 70% ethanol after each use.

How does this procedure fit into or address your overall research goals? This procedure assesses anxiety. Mice naturally spend most of their time near a wall. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

Monitoring is not generally required after completion of this procedure. (If anxiolytic compounds are utilized, additional monitoring may be required.) If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal.

#### Procedure Title: Passive Avoidance Test

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

The lowest shock intensity possible will be used in this task to motivate the animal.

Animals are trained in one trial. Animals that demonstrate signs of locomotor disabilities or pain perception will not be tested. The passive avoidance testing apparatus consists of two compartments. One compartment is lit with no aversive stimulation. The second compartment is dark with ability to administer a shock. A sliding door separates the two compartments. Rodents prefer the dark compartment.

Training procedure: The animal is placed in the lit/white compartment. The door is opened to allow the animal to go into the dark compartment. The latency to enter the dark compartment is measured. When the animal enters the dark compartment with all four paws, the door is closed and a 1-2 second foot shock is administered (0.2-0.5mA shock). The animal will then remain in the dark compartment for 10 additional seconds after the shock is discontinued. The animal will then be placed back in the home cage.

Testing: At the time of testing (1-7 days after training), the animal is placed in the white/lit compartment. The door is opened, and the latency to re-enter the dark compartment is recorded. No shock is administered when entering the dark compartment.

Testing apparatus will be cleaned with 70% ethanol after each use.

#### How does this procedure fit into or address your overall research goals?

This procedure assesses learning and memory. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

This procedure is not invasive and will not result in the need to euthanize an animal.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal, if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized.

#### Procedure Title: Rota Rod

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

The rota rod consists of a rod partitioned into segments by walls. One mouse can be tested in each segment. Padding is positioned below the rotating rod apparatus to catch animals in the event they fall from the apparatus. Animals are placed on the rota rod, which is either stationary or rotating. If animals are undergoing the fixed speed version of the test, the rod continues to turn at a speed of 4-16 rpm for the entire trial, which is no more than 10 minutes. Animals may also undergo the accelerating version of the test in which the rod starts from a stationary point, and then accelerates from 4 rpm to 20-80 rpm. The test ends when the animal falls off the rod or 5 minutes of time has passed. The apparatus is cleaned with 70% ethanol in between animals.

#### How does this procedure fit into or address your overall research goals?

This test is used to screen for motor deficits, coordination of movement, muscle weakness, or rate of fatigue (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Because the area below the device is padded, no clinical effects or changes from normal health are expected. However, animals will be examined closely to make sure they do not sustain injuries after falling from the device.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for any signs of distress, as described below, immediately after completion of the procedure. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

This procedure is not invasive and will not result in the need to euthanize an animal.

#### **Procedure Title: Cylinder Test**

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

An opaque open top plastic cylinder is placed over a rodent. The number of left vs right forepaw touches are recorded as the animal rears up on to its hind legs. Animals remain in apparatus for no longer than 15 minutes. Device will be cleaned with 70% ethanol after each use.

How does this procedure fit into or address your overall research goals? This procedure is used to assess locomotor asymmetry in rodents. (Insert protocol-specific rationale here.)

## Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

This procedure is non-invasive and not expected to result in injury, exhaustion or overt distress. Animals will be monitored for 15-30 min once in its home cage.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized.

#### Procedure Title: Climbing Test

#### **Procedure Description**

The testing apparatus utilized will be one that is commercially available for this purpose. If the testing apparatus will be developed by the lab, an OLAC veterinarian will inspect the apparatus prior to its first use with live animals.

To test rearing and climbing, a closed top wire mesh cylinder no taller than 20 cm is placed over the animal on a flat sanitizable surface. Climbing will be recorded in 5-minute testing sessions videotaped for analysis. Device will be cleaned with 70% ethanol after each use.

#### How does this procedure fit into or address your overall research goals?

This procedure assesses rearing and climbing abilities. (Insert protocol-specific rationale here.)

### Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes from normal health are expected.

#### Describe post procedure monitoring that will be performed.

Animals are monitored for any signs of distress, as described below, or injury immediately after completion of the procedure. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

### What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

Unless an animal is severely injured or breaks a limb from a fall, this procedure will not result in the need to euthanize an animal before the study is completed.

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