Protocol & Methods for LRN Study

**BBB**

🡪 DONE

**Ladder Walk**

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**Ledge Test**

The ledge test functions as an indicator to alterations related to coordination and may also show balance alteration. Normal naïve rodents will walk along the narrow ledge with cautious but steady movements, adjusting posture and limb placement to maintain balance. Ataxic rodents will exhibit wobbling or swaying as they attempt to move. Other signs of neurodegeneration include a wide stance or limb splaying for stabilization, difficulty initiating or sustaining movement along the ledge, and a sudden loss of balance or frequent slipping/falling off. The ledge test functions by provoking behaviors that rely on fine motor coordination and cerebellar function, which are compromised in ataxia. Difficulty balancing, abnormal posture, limb misplacement, and falls are key indicators that point to neuromuscular or cerebellar abnormalities. To assess the ledge test, the following protocol will be used:

1. Lift the rodent from its cage and place it on the cage’s ledge. Rodents will typically walk along the ledge and attempt to descent back into the cage. We will be conducting this with progressively smaller in width beams. The decreasing width of these beams will allow for a range of narrow beam tests to be conducted until rodents expressing ataxia are unable to continue to walk along and begin to fall.
2. Observe the rodents as it walks. A naïve rodent will walk along the ledge/beam without losing its balance. If this is the case, **the score assigned is 0**. If the rodent loses its footing while walking along the beam or ledge, but otherwise appears coordinated, it will receive **a score of 1**. If it does not effectively use its hind legs, or lands on its head rather than paws when descending or falling, it receives **a score of 2**. If it falls of the ledge, or nearly so, while walking or attempting to lower itself, or shakes and refuses to move at all despite encouragement, **it receives a score of 3**. Some rodents will require a gentle nudge to encourage walking.
3. Record the score.

**Lever Pull**

🡪 email sent to Megan, waiting response to schedule meeting for Lever Pull test.

**Hunch Behavior/Posture (Kyphosis)**

A distinguishing feature of proximal muscle weakness is exhibited through a curved spine (kyphosis). Kyphosis is an abnormal forward curvature of the spine, typically in the thoracic region, leading to a hunched or rounded back posture. In animals (especially rodents), it often presents as a persistent arching of the back. Neurodegeneration affecting proximal axial muscles is one cause. The lateral reticular nucleus is a brainstem structure in the medulla oblongata and it receives proprioceptive and spinal input; it projects heavily to the cerebellum; and it is involved in modulating motor output, posture, and axial muscle tone via cerebellar loops. Since the LRN helps to transmit spinal proprioceptive information to the cerebellum, if we disrupt these feedback loops, the cerebellum receives incomplete or distorted body position signals which leads to poor postural control. This impairs tone regulation in paraspinal and axial muscles which potentially lead to chronic muscle imbalance.

1. Remove the rodent from its cage and place it on a flat surface.
2. Observe the rodent as it walks. If the rat is able to easily straighten its spin as it walks, and does not have persistent kyphosis, it receives **a score of 0**. If the mouse exhibits mild kyphosis but is able to straighten its spine, it receives **a score of 1**. If it is unable to straighten its spine completely and maintains persistent but mild kyphosis, it receives **a score of 2**. If the mouse maintains pronounced kyphosis as it walks or while it sits, it is assigned **a score of 3**.
3. Place the mouse back into its cage and record its kyphosis score into a laboratory notebook.

**Steady State Locomotion Analysis for Stride Frequency and Length**

Gait is a measure of coordination and muscle function. In subjects exhibiting ataxia, there are significant and observable changes in their everyday gait. Changes in step duration and variability are thought to be a part of the body’s compensatory mechanisms to deal with increased stability and lack of coordination caused by ataxia. By increasing step and stride time, and exhibiting a greater variability, subjects with ataxia may be attempting to increase stability and reduce the risk of stumbling or falling. There are a few notable things that we could see if these rodents are ataxic.

1. Increased step time
   1. Individuals with cerebellar ataxia exhibit a significantly increased step time when compared to healthy individuals; indicating a longer duration for each individual step they take.
2. Increased stride time
   1. The duration of a full gait cycle (two steps) is also significantly increased in individuals with ataxia.
3. Increased step length variability
   1. Beyond just the duration, there is a significant increase in the variability of step length. This means that the length of individual steps can vary significantly, reflecting the lack of coordination inherent in ataxia.
4. Reduced swing phase
   1. The swing phase, where the foot is off the ground, is significantly reduced.

To assess ataxia through gait, we will perform the following:

1. Remove the rodent from its cage and place it on a flat surface with its head facing away from the investigator.
2. Observe the rodent as it walks. If the rat moves normally, with its body weight supported on all limbs, with its abdomen not touching the ground, and with both hindlimbs participating evenly, it receives a score of 0. If it shows a tremor or appears to limp while walking, it receives a score of 1. If it shows a severe tremor, severe limp, lowered pelvis, or the feet point away from the body during locomotion, it receives a score of 2. If the mouse has difficulty moving forward and drags its abdomen along the ground, it receives a score of 3.
3. Place the rodent back into its cage and record its gait score.
4. This step will be completed through kinematic analysis for a notably more sensitive assessment than described by the above scoring guidelines. The above score will still be recorded in a notebook for reference and comparison during statistical analysis.

**Hindlimb Clasping**

Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease is an autosomal dominant neurodegenerative disorder caused by CAG trinucleotide repeat expansion within the coding region of SCA3 gene. Clinical manifestations of the SCA3 gene include cerebellar ataxia, peripheral nerve palsy, pyramidal and extrapyramidal signs. SCA3 neurodegeneration is primarily found in the brainstem, basal ganglia, cerebellum and spinal cord. One method of assessing motor functions in small rodents is to observe if the animal clasps is forelimbs and hindlimbs into its body or sprays its limbs when suspended by its tail. When suspended by their tails, ataxin-3-Q79 transgenic mice at 6 months of age displayed forelimb clasping response instead of normal escape reflexes (limbs spread). Ataxia is caused by a CAG trinucleotide repeat expansion in the ATXN3 gene which results in a build of polyglutamine (polyQ) expansion in ataxin-3 protein. This build up results in a neurodegeneration in previously listed areas of interest (cerebellum, brainstem nuclei, basal ganglia, and spinal cord motor neurons. The hindlimb clasping reflects hypertonia or abnormal reflexes due to loss of inhibitory GABAergic signaling. It further reflects impaired descending corticospinal and cerebellar input, which normallyinhibits excessive muscle contraction during postural adjustments.

1. Grasp the tail near its base and lift the animal clear of all surroundings and objects.
2. Observe the hindlimb position for 10 seconds. If the hindlimbs are consistently splayed outward and away from its abdomen, then it is assigned **a score of 0**. If one hindlimb is retracted toward the abdomen for more than 50% of the time suspended, it receives **a score of 1**. If both hindlimbs are partially retracted toward the abdomen for more than 50% of the time suspended, it receives **a score of 2**. If its hindlimbs are entirely retracted and touching the abdomen for more than 50% of the time suspended, it receives **a score of 3**.
3. Place the animal back into its cage and record its hindlimb clasping score.