# BEHAVIORAL PHENOTYPING

Mice engineered to express a transgene, whether to overexpress or underexpress/ knockout an endogenous gene, or to express a mutant gene, for any given purpose may display changes in behavior as compared to wild-type control mice. The behavioral change(s) may have arisen due to impact at one or more levels of the nervous system and requires careful testing in order to determine the nature of the change. For example, a gross change in locomotion may, in fact, be solely due to a sensory defect.

## Group size and selection

One of the first considerations in behavioral testing is the number of mice to use. Typically, fairly large group sizes are needed, often 8-10 mice/condition, if not more. In spite of great care in minimizing extraneous variables (light-dark cycle, temperature, noise, etc.) and in maximizing standards (handling, timing, etc.), sufficient group sizes are essential to provide proper statistical power in the face of inherent variability. (Avoiding mixed background mice or backcrossing onto a single background can also help.) Comparison to carefully matched controls, such as littermates, is also key. (NB: Gender may be an important contributing factor.)

## Initial Screen

An initial battery of behavioral tests conducted on mice in their home cages can provide important clues. The first set of tests should involve simple assessment of the neurological status of the mouse: eyeblink, whisker and ear twitching, basic locomotion, rearing, visual cliff, righting reflex, hanging, toe pinch, acoustic startle. Though simple, these tests can be nonetheless conducted in a quantitative manner (events/unit time; distance traveled/unit time; time to right; etc.). They should be performed over several days at different times of the day, to avoid any 'false-positives'. (Remember that mice are primarily nocturnal, and hence may be sleeping when you go to test them.) The tests are recommended to proceed from least to most stressful (see Crawley, 2008), as listed.

## Test sequences

The next series of behavioral tests are more involved, but significantly more sensitive. Even if there does not appear to be a gross change in locomotion, the first general test should be the open field. Mice do not like to be out in the open and so this test will reflect locomotion, anxiety, exploration and habituation. Though somewhat oversimplified, mice that are highly anxious will hug the walls (thigmotaxis), whereas mice with below normal anxiety will run around the center of the open field. Normal mice will start along the wall, but then explore. This test can be combined with novel object recognition, which provides for a measure of memory and learning.

The next tests will be predicated on whether there is an indication of a sensory, motor, or learning deficit, though all three possibilities may need to be considered. If there is an apparent learning deficit, it must be assured that there are no sensory or motor deficits underlying or perhaps even accounting for a difference observed in a learning task. As learning tasks, two of the most widely used are fear conditioning and the Morris water maze.

-Contextual fear conditioning uses an inescapable, mildly aversive (unconditioned) stimulus associated to the context (environment: cage, room) as the conditioned stimulus. Freezing (period of immobility in the cage) is a common response of rodents when afraid and is a highly sensitive means to measure fear learning. The use of context engages the hippocampus and contextual fear conditioning is strongly modulated upon pharmacological or genetic manipulation of hippocampal function. The task is highly relevant to diseases involving the hippocampus, such as Alzheimer's disease.

-Cued fear conditioning is distinct from contextual fear conditioning in that a precisely timed tone is tightly paired (associated) with the aversive (unconditioned) stimulus. This task engages the amygdala and is highly relevant to models of emotional or anxiety disorders.

-Trace fear conditioning is similar to cued fear conditioning, but the unconditioned stimulus and the conditioned stimulus are separated by an interval ('trace interval') of variable length. This task engages both the hippocampus and prefrontal cortex.

-Morris water maze exploits the natural aversion of mice for water. The mice must learn to find a hidden platform in a circular tub filled with opaque water using spatial cues placed around the room. This task thus engages the hippocampus for both spatial orientation while navigating and learning. For this task, it is essential to demonstrate that the mice have no deficit in swimming. Even if the mice are capable of normal swimming, there are conditions/strains/transgenic lines where the mice simply will not undergo training. (They float in the tub, rather than swim.) In that case, the Barnes mice may be a more appropriate alternative.

-Barnes maze exploits the aversion of mice to a brightly lit open area, while allowing them a place to hide. The 'maze' consists of a round surface containing a large number of holes around the periphery. One of the holes contains a little compartment where the mice can hide; the other holes are simply open. Like the Morris water maze, the mice learn to navigate to the hole with the hidden compartment using spatial cues. An advantage of the task is that it is weakly aversive as compared to the Morris water maze and certainly the fear conditioning test.

Alternative mazes include the radial arm maze and the T-maze, which each have their own advantages.

If locomotor deficits are suspected, then the Rotarod test is a reliable first choice. If, however, the locomotor deficits appear to be more a problem of balance and/or coordination, then the vertical rod inversion test is a good choice.

-In the Rotarod test, the mice must first balance and then walk on a rotating rod. It can be modified to test for motor learning, as well. Its use is highly relevant to

models of motor dysfunction and disease, such as ataxias, dystonias, Parkinson's disease, Huntington's disease, etc.
-Vertical Rod Inversion test is simple but sensitive test for motor function, coordination and balance. A mouse is placed nose up near the top of a long rod. The time for the mouse to turn and climb down is assessed. This test is also highly relevant to Parkinson's disease, etc.

As for sensory deficits, the choice of task would depend on whether vision, hearing, touch, or pain is suspected. Interestingly, deficits in detecting odors may be early sign in certain diseases (Parkinson's; Alzheimer's) and can also be tested. Mice normally rely heavily on olfaction while navigating their environment. (Odors can also be used in the learning tasks.) Visual deficits will likely preclude any further behavioral testing.
-The Startle Test and Pre-Pulse Inhibition (PPI) can be measure hearing deficits and sensory gating deficits. Deficits in PPI are of particular relevance to schizophrenia and also to Alzheimer's disease, autism and attention deficit disorders.
-The Hot Plate test is a simple measure of nociception.

Tests for anxiety or depression-like states have been constructed. Though human anxiety or depression (or any other psychiatric disorder) cannot be truly modeled in rodents, these tests are notable in that anti-anxiety drugs or anti-depressants show efficacy in reducing apparent anxiety or depression-like states, respectively. For anxiety, the open-field test is, as mentioned previously, a means to measure anxiety, as mice are aversive to the open, at least during the early phase of the test. Another test for anxiety is the elevated plus-maze, which presents open and closed arms to a mouse in an apparatus that it is high off of the floor. (There are several other tests for anxiety, again each with their own advantages.) As for depression-like states, the forced swim test is one of the more common tasks used. In this test, the mice are place in a small, water-filled cylinder. Though the mice try to swim and/or escape, after a period of time they stop swimming. With successive trials, the mice undergo 'learned helplessness'.

Finally, social tests exploit basic behaviors utilized by rodents on a daily basis. Social interaction with another mouse or mouse odor can be used to test for novelty, exploration and even learning. Aggression in this setting can measure social dominance, among other parameters.

References

Crawley, J.N. (2008) Behavioral phenotyping strategies for mutant mice. *Neuron* 57, 809-818.