Neurophenotyping Centre
Determination of behavioral phenotype

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Neurophenotyping is the study of how specified genes, subjected to environmental influences and stressors, modify the brain, behavior and cognitive function. Accurate identification of genetic effects on behavior can require multiple behavioral assessments, as single genetic alteration can evoke multiple phenotypic and/or behavioral changes.

Phenotype and genotype: what is the difference?

The genotype is the totality of an organism's genetic code. Although an animal may possess a specific gene, that gene may or may not be expressed or activated; additionally, the degree to which the gene is activated may have functional significance at the behavioral level. Recent evidence has indicated quite clearly that many genes lie dormant, waiting for the appropriate "trigger" to be activated. Additionally, increasing evidence indicates that these triggers include experiential and environmental factors.

The phenotype can be considered as a portrait of which genes have been activated, and the extent of that activation. More technically, the phenotype is the totality of all the traits and characteristics of an individual. Since, as mentioned above, environmental factors can influence gene activation, the phenotype can be considered as an indication of the interaction between genes and the environment. To illustrate, consider those genes that determine an individual's height. Whether or not the individual will achieve this potential will depend upon such experiential factors as how well nourished this person is during development, whether he/she is raised in a stress-free environment, etc. Thus, star basketball players are not just born; they may need the right genes to have the potential to grow to 7 feet tall, but without the appropriate environmental supports during development, they may only achieve a height of 5 feet.

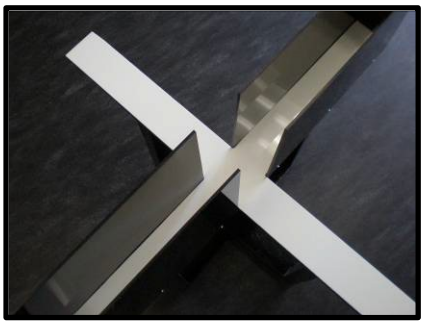
The phenotype is not restricted to physical traits, such as height, but also includes "psychological traits", such as intelligence, emotional profiles, and predispositions to respond in particular ways to particular situations. These latter traits are generally subsumed under the rubric of the "behavioral phenotype", and behavioral scientists have developed and rely upon a number of "gold standard" tests that can be used to determine the behavioral phenotype that results from a given genetic modification in rodents. These are briefly described below.

Tests commonly used to determine behavioral phenotypes

It should be noted, first off, that each of these tests has been in use for an extensive period of time. As such, their use often does not invoke any major ethical challenges. For instance, all tests which we employ within the Douglas institute are conducted according to protocols approved by the Animal Ethics Committee of McGill University, which conforms to the guidelines established by the Canadian Council on Animal Care (CCAC)

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. serotonin-based) anxiolytics. 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. Anxious animals will avoid the open arms, and spend more time in the closed arms.



The Morris Water Maze

This test assesses spatial learning and memory, and has been shown to be very sensitive to both memory-enhancing and memory-impairing manipulations. The animal is placed in a pool of water that has been made opaque and is required to find a submerged platform. To solve the task the animal relies on 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. 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.

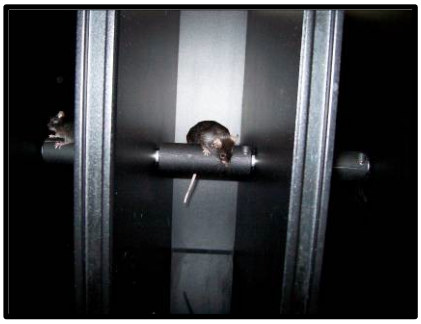
Motor Function tests

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. The animal is placed in a Plexiglas box for habituation. After habituation, the locomotor activity of the animal is measured and recorded as photocell interruptions that are recorded by a computer, as each box is equipped with two lines of photocells at different heights, this system allows for automated recording of horizontal and vertical movement, as well as stereotyped behavior.

Rotarod

This test is generally used to assess the rodent's motor coordination and balance. During training, which generally requires approximately three trials, animals are placed on a rod that rotates at a constant speed. This is done to acquaint the animal with the demands of the task. For test trials, animals are placed on the rotating rod, and the speed of rotation is gradually increased. The latency until the animal falls from the rod onto the padded floor is recorded. The maximum rotating speed attained can also be recorded. The longer the amount of time on the rod, and/or the greater the attained speed, the better the coordination and balance.



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. The advantages offered by this test are that it is easy and rapid to perform and can be repeated several times on the same animal. Animals are placed in a Plexiglas chamber on an elevated glass platform with a controlled light beam located below. Animals need to be habituated to the Plexiglas chamber prior to testing. Test trials involve positioning the radiant heat source under the glass floor directly under the hind paw of animal. The experimenter turns off the beam when the animal lifts the paw or displaces the paw from the light beam. Latency to lift or displace the paw is recorded.

Forced swim test

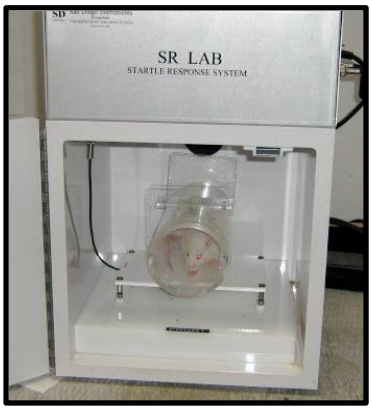
The forced swim test assesses the reaction to an aversive, inescapable situation. Animals are placed into a small container of water, and the latency and total time spent immobile are measured. Whereas the interpretation of the immobility response is unclear, this test has been shown to be very sensitive to pharmacological compounds possessing antidepressant activity. As such, it is a useful, simple and reliable test that can be used to assess novel antidepressants. The animal is placed into a glass cylinder containing water on two occasions (pre test and test sessions). The pretest is applied to teach the animal that escapes from the cylinder is not possible. The test session is conducted on the next day and the time-spent swimming, climbing, diving, and immobile are recorded. Antidepressant compounds generally reduce the amount of time the animal spends immobile.

Differential reinforcement of low rates task

This test assesses "impulsivity", or the animal's ability to withhold a response for a predefined period of time in order to obtain reward. The animal is first acclimatized to a 23 hour food deprivation schedule for a period of days, and is then trained to press a lever for food, gradually; the interval between rewarded 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 food. The number of non-reinforced bar presses is considered as the main measure of impulsivity.

Prepulse Inhibition (PPI)



PPI is the principal test used to measure sensorimotor gating, a pre attentional mechanism that is involved in gating the startle response to a loud, unexpected stimulus. 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. PPI is assessed in startle chambers each consisting of a Plexiglas chamber 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 and both prepulse and pulse stimuli. A piezoelectric strain meter attached to the base transduces the startle response. Stabilimeter readings are rectified 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. Animals are placed into the Plexiglas restrainers, and after 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.

This list of tests is exhaustive and can be adaptable regarding each model and work hypothesis.

Conclusion

The above list is by no means exhaustive, but is rather meant to provide a flavor for the ways in which different behavioral mechanisms can be assayed in the rodent. One final point deserves emphasis, and for this reason has been saved for last: Accurate behavioral phenotyping generally requires the use of multiple tests that span different behavioral mechanisms. For example, a deficit in the Morris Water Maze may indicate impairment in spatial learning and memory, but this is not the only interpretation. Poor performance can arise because animals are less proficient swimmers, or may be more stressed when placed into the pool. Thus, to discriminate between these possibilities, it is necessary to utilize tests which assess motor function and reactivity to stress. If these tests indicate "normal" performance then, by exclusion, it is more likely that a deficit in the water maze is a learning or memory deficit.

For more information, or a request for service please contact:

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