The NIH Neurogenomics Project

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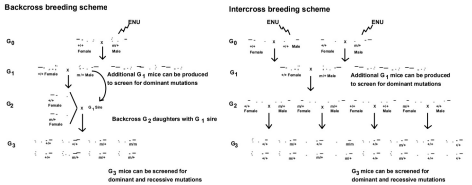
Introduction

The NIH Neurogenomics Project at the Northwestern University Center for Functional Genomics is focused upon identifying genes involved in a number of Neuroscience-relevant systems and behaviors. We are using a forward-genetic strategy of combining chemical mutagenesis with high-through phenotypic screening, allowing the altered phenotypes of mutants to lead to their identification. Identification of mutants, in turn, allows for the association of genes with functions, as well as providing model systems for studying the molecular and genetic mechanisms underlying the phenotype affected.

The Project at Northwestern is using the chemical mutagen N-ethyl-N-nitrosourea (ENU) to produce mutations throughout the genome, followed by three generations of breeding to produce homozygous mutants. By screening third-generation (G3) progeny of mutagen-treated mice, dominant, semidominant, and recessive mutations may all be recovered. Phenotypic screens used at Northwestern focus on five domains: Learning and Memory, Neuroendocrine Responses to Stress, Response to Psychostimulants, Circadian Rhythmicity, and Vision.

Breeding Scheme

We use two different breeding schemes to produce G3 mice for our recessive screens. The backcross scheme is outlined at the left of the figure while the intercross scheme is outlined on the right.



In both schemes G1 mice are produced as follows: ENU (100 mg/kg body weight) is injected intraperitoneally into wild-type C57BL/6J (JAX # 000664) male mice once a week for three weeks, starting at 6-8 weeks of age. On average the G0 male mice recover fertility at 14 weeks post injection, but only 50% of all ENU treated mice ever regain fertility to sire G1 offspring. Each G1 mouse produced from the ENU treated mice represents one mutagenized G0 gamete. After this point, the two breeding schemes diverge.

In the backcross scheme, male G1 mice are mated to wild type C57BL/6J females to produce G2 offspring. The G2 females are then backcrossed to their G1 fathers to produce G3 progeny.
In the intercross scheme, two G1 mice are bred together so that two mutagenized G0 gametes are represented in each kindred. G2 sibs are then intercrossed to produce G3 progeny.

Our target rate of production and screening is 10,000 G3 mice/year.

Phenotypic Testing

We have selected five phenotypic domains on which to focus our screens. In addition, a set of "preliminary assessment" tests are conducted to provide supporting information to aid interpretation of other assays. All mice go through all the tests (unless there are concerns over the animal's ability to perform a test, or it is to be bred); the sequence of tests is designed to minimize carry-over influences of one test to the next.

Preliminary Assessment

The Preliminary Assessment consists of recording the animal's body weight at two ages (6 weeks and 10 weeks), a hearing screen (IHR Clickbox, Preyer reflex), and two behavioral assays: the elevated plus maze and the open field behavior.

Neuroendocrine Response to Stress

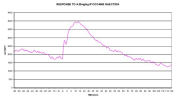
A standardized, acute stress (10 min restraint) is presented. The tip of the tail is cut and blood samples are collected at the beginning and end of the stress using capillary tubes. Serum samples are radioimmunoassayed for Corticosterone and for Thyroid Stimulating Hormone (TSH) to assess the responsiveness of the hypothalamic-pituitary-thyroid and hypothalamic-Pituitary-adrenal axes to stress.

Learning and Memory

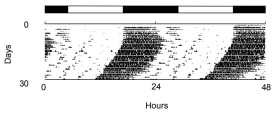
We use fear conditioning as our screening test for learning and memory. Mice are trained to fear the test chamber with a series of four 0.75 mA footshocks, one minute apart. 24 hours after training, the mice are returned to the same test chamber for 5 min and fearful behavior (freezing) is recorded as a measure of context-dependent learning or memory. A second contextual memory test is done 2 weeks following the training to assess long-term memory.

Response to Psychostimulants

The hyperlocomotion response to administration of 20 mg/kg cocaine is recorded. Drug-naïve wild-type C57BL/6J mice exhibit a robust induction of activity in response to this dose; hyper- or hypo-responsive mice are selected.



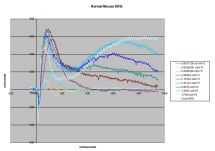
Circadian Rhythmicity



The circadian rhythm of wheel-running is monitored. Animals are maintained first in a light-dark cycle, then constant darkness (to remove time cues). The period and persistence of a free-running rhythm, and phase angle of entrainment are studied.

Vision

Two assays of the visual system are used. The fundus of the eye is examined and photographs are taken of all mice. These are reviewed by the opthalmology team at the University of Iowa. Electroretinograms (right) are recorded. We expect to begin recording visually evoked potentials (VEP) in the near future .



Lines Produced

Mice with unusual phenotypes are classified as putative mutants and must demonstrate genetic transmission of the altered phenotype to be considered mutants. Testing of heritability is typically accomplished by breeding the affected individual, but may also be done by breeding siblings in cases in which the affected individual has reduced fertility or poor health.

To date, 106 putative mutants (in all domains) are being bred to test for heritability of the phenotype. Two circadian rhythm mutations have been confirmed heritable and are being genetically mapped. One visual system mutation has been confirmed to be heritable, and the mode of inheritance and phenotypic characteristics are being determined.

Personnel

In addition to researchers at Northwestern University, follow-up detailed phenotypic characterization of mutants and putative mutants involves expert research groups at three other universities: Columbia University, Duke University, and the University of Iowa.

Joseph S. Takahashi	Northwestern University	Neurogenomics Project Director
Lawrence H. Pinto	Northwestern University	Vision Screen
Fred W. Turek	Northwestern University	Circadian Screen
Jon E. Levine	Northwestern University	Neuroendocrine Screen
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Kazuhiro Shimomura	Northwestern University	Primary Screens
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Marc Caron	Duke University	Psychostimulant follow-up
Raul Gainetdinov	Duke University	Psychostimulant follow-up
Val Sheffield	University of Iowa	Vision follow-up
Edwin Stone	University of Iowa	Vision follow-up
Gregory Hageman	University of Iowa	Vision follow-up

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