ERG (Electroretinography)

The electroretinogram of anesthetized, dark-adapted mice is recorded in response to stimuli of each of 10 luminances. The maximal value and luminance for half-maximal response are determined for the STR, a-, b- and c-waves and displayed with respect to the distribution for the entire population. These functions are performed automatically.

Fundus Photography

The fundus of an anesthetized mouse is photographed while its body temperature is maintained at 38C with a small animal fundus camera with a 2.4 Mpix resolution. The image is sent by FTP to the University of Iowa for analysis. Interesting mice are given secondary screening with histology.

VEP (Visually Evoked Potentials)

The responses from the visual areas of the cortex to flashing stimuli and checkerboards of alternating bright and dark are recorded from an anesthetized mouse. The response amplitude is compared with the distribution for all animals to determine the spatial frequency required for a half maximal response.

(ERG) Electroretinography

Mice are dark adapted for 2 hours before testing occurs, and all ERGs are recorded in the dark with the aid of IR goggles. First off, mice are injected with a mixture of Ketamine (1 □g/g) and Xylazine (0.4 □g/g). After they are anesthetized, mice are placed in separate aluminum chambers for stimuli presentation where they rest on 1-watt heaters to maintain the normal body temperature of 38°C. Their eyes are moistened, and thin silver/nylon electrodes are laid vertically across each. Next, a clear lens is placed on one eye and an opaque one on the other.

Ten different stimuli are presented using digital outputs wired to 526 nm green LEDs from Agilent Technologies, which are controlled by a Labview program. Three different digital outputs are utilized to control LEDs, which gives a range of 5 log units for luminance. By varying the amount of time an LED is in the on state, perceived brightness changes in a linear relationship. Using a 200 □m pinhole, a normal LED is made 1000 times dimmer. This is used for the first 3 luminances, a normal LED for the next 5, and a bank of 10 for the brightest one. The last stimulus presented is accomplished with a dim LED constant adapting light while a normal LED is flashed.

Data is acquired using the same Labview program. Signals from the two electrodes are fed through a differential amplifier in which the one from the opaque eye is subtracted from the signal from the clear one. This negates any effects the ECG may have on the ERG. The differential amplifier has filter settings of 0.1 Hz high pass and 10 kHz low pass, while the signal is amplified by a factor of 10,000. Ten different ERG recordings are made with each one an average of between 2 and 20 light flashes, more flashes for the dimmer luminances. Once a mouse has seen all luminances, the program alerts the user. The contact lenses and electrodes are then removed from the mouse, and it is laid down to rest. The anesthetic wears off in 45 minutes to an hour.

The program saves the data to a text file, which is imported into an analysis program written in Matlab. Time course responses are plotted upon opening the file. Then, minimal values for the A waves and maximal values for the B and C waves are

plotted versus the log of their respective luminances automatically. Sigma, log luminance at half maximal response, is found for each component and plotted on a distribution containing all animals for a given generation. The STR amplitude is divided by the maximal B wave and the amplitude of the light adapted B wave response is divided by the normal B response. These are also plotted on distributions. Any mice tested whose values lie outside of three standard deviations of the mean are retested, and the animals are bred if the value is repeated.

Visual Evoked Potential (VEP)

Mice are tested one at a time for the VEP test. They are first injected with a mixture of Ketamine (1 □g/g) and Xylazine (0.4 □g/g). Next, a slit is made in the scalp between the ears of the mice (see figure 1). This allows access to the portion of the skull above the visual cortex. The area is moistened with saline solution, and an array of 12 electrodes is placed over it using a micromanipulator. Then a reference electrode is placed near the array, but not over the cortex in order to negate any effects the ECG may have on the ERG. The mouse is placed 6" from a 15" computer monitor, on which stimuli are presented.

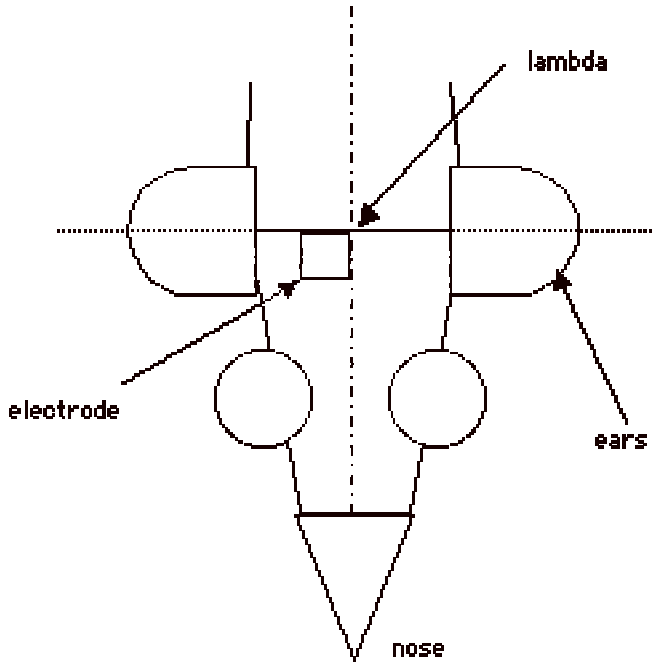

Figure 1: The electrode array is placed as shown in the diagram at the corner of lambda. Embedded within the 4 mm by 5 mm Delrin coating, are 12 stainless steel electrodes spaced 1 mm apart.

A flash and a reversing black and white checkerboard pattern are shown to the mouse using a Matlab program. This computer communicates with a second computer that acquires the data. The communication takes place over the serial ports and allows the presenting computer to tell the acquiring computer when it is about to present, which sets off a trigger to begin data acquisition. Wires running from the electrode array to a differential amplifier are then sent to an analog data acquisition board. The differential amplifier has filter settings of 0.1 Hz high pass and 10 kHz low pass, while the signal is

amplified by a factor of 10,000. Data from the board is sent to a Labview program, which saves the data from the flash and pattern reversal stimuli in two different files, with each having 12 columns, one for each channel.

The user is then able to choose which channel delivered the VEP response with the least noise and largest amplitude. A new file is created with the best channel data from each stimulus in a separate column. The minimum value of the VEP is found by Labview and plotted against a distribution of all animals of that same generation. Any mice tested whose values lie outside of three standard deviations of the mean are retested, and the animals are bred if the value is repeated.

After the channel is chosen, the electrode is raised above the skull, and the mouse's skin is pinched back together. Vetbond™ is then applied to reseal the scalp. The mouse wakes up 45 minutes to an hour later.