Feeding Behavior and Chemistry

9/05

Behaviors: Larry Tecott, UCSF: tecott@itsa.ucsf.edu
Chemistries: Peter Havel, UCD: pjhavel@ucdavis.edu

General
Mice will be group-housed (4-6 mice per cage) in standard polycarbonate mouse cages (29 x 18.5 x 13 cm) with free access to standard chow (PicoLab Mouse Diet 20, Purina Mills) and water under a 12 hr light/dark cycle. Behavioral assays were performed using male mice between the ages of 3 to 6 months. Unless otherwise indicated, naïve mice were used for each behavioral assay, and cohorts of size *n*=8 were used for each group. Between subjects, testing apparatus will be cleaned with a 0.25% bleach solution, wiped down with water, and then dried. All experimental conditions will be counterbalanced by genotype. For all behavioral tests, investigators will be blind to genotype and drug treatment.

Open field (OF) assay will be used to examine locomotor responses to a novel aversive environment. Behavior in this test is believed to reflect a conflict between an aversion to open, brightly lit areas and the drive to explore a novel environment. In the open field, decreased % time in the center, relative to the periphery of the field in similarly active mice is interpreted to indicate increased anxiety-like behavior. Mice will be exposed to a 50 x 50 cm open field enclosure for 5 minute period, according to our standard procedure (Heisler et al., 1998). Mice will be removed from their home cage and placed at a corner of the open-field. Exploratory activity will be videotaped for 5 minutes, during which distance traveled, proportion of time spent in the center versus the periphery, and the number of entrances into the center will be quantified using automated video-tracking software (Ethovision 3.2, Noldus, Inc.).

Elevated zero maze (EZM). In this pharmacologically-validated variant of the elevated plus maze, animals are placed on a raised annular platform divided into 4 quadrants. Two non-adjacent quadrants are walled. In similarly active mice, increased time in the walled quadrants of the maze relative to the open quadrants maze is interpreted to indicate increased anxiety-like behavior. An advantage of the EZM assay relative to the elevated plus maze is the absence of ambiguity in the interpretation of time spent in the central square of the plus maze. Animals will be subjected to a 5 minute elevated zero maze test according to our previously published procedures (Heisler et al., 1998). Mice will be removed from their home cage, placed in the closed arm of the zero maze and videotaped for 5 minutes. The number of open quadrant entrances, time spent in the open arm and total activity will be quantified using Ethovision. Similar activity levels, but altered open quadrant time will be interpreted as a change in anxiety-like behavior.

Motor Coordination. Motor coordination will be assessed with an Accurotor rotarod (Accuscan Instruments). The rotation rate will be accelerated from zero to 30 rpm over 5 minutes, and continued at 30 rpm for 5 more minutes until the end of one trial. Four animals will be tested concurrently in separate 11 cm-wide compartments on a rod approximately 3 cm in diameter and elevated 35 cm. Each animal will be assessed over 7 trials with 20-min intertrial intervals. In each trial, the latency to fall from the rod was recorded. (Bonasera et al., in press).

Home Cage Activity and Food Intake. Animals will be housed individually in rat cages (48 x 27 x 13 cm) with bedding, food and water, under a 12 hr light/dark cycle. To assess activity, beam breaks will collected each hour for seven days with a photobeam activity system (FlexField, San

Diego Instruments). Both horizontal locomotor activity (as monitored by a 4 x 8 array of infra-red photobeams) and rearings (as scored by an elevated set of 8 infra-red photobeams) will be recorded. Food pellets and water bottles will be weighed daily to determine daily consumption. (Nonogaki et al 2003; Bonasera et al., in press).

Morris Water Maze. Mice will be trained using a visible platform/hidden platform version of the water maze task. Prior to each training session, mice will be removed from their group cages and individually housed in holding cages for the duration of that day's training (approximately 6 hrs). An 8 cm x 8 cm clear Plexiglas platform with an attached 7.5 x 7.5 x 7.5 cm^3 black Plexiglas cube (visible platform) will be centered in a randomly selected quadrant of a circular, 92 cm inner diameter polyethylene tank filled with warm water made opaque using white tempura-based paint (DryTemp powder tempura). Multiple objects will be placed in the testing room to act as distal cues; these cues will remain constant through all experiments. In one training block, a mouse will be gently transported from its holding cage and released into the water facing the maze wall. Swimming paths will be videotaped and analyzed using a commercially available software package (EthoVision, Noldus Inc.). The trial will end when the mouse mounts the platform with all four paws or if the mouse does not mount the platform after 60 seconds of swimming. All mice will spend 20 seconds on the platform before returning to the holding cage. Following a one minute rest interval, the mouse will be returned to the maze at a different starting quadrant and the above process repeated until all four quadrants have been tested over four trials. Each mouse will undergo two blocks of training per day. On day 3 (block 5), the visible platform will be replaced by an 8 cm x 8 cm clear Plexglas platform submerged 0.5 to 1 cm below water level (hidden platform). All mice will receive five days (10 blocks) of training to the hidden platform. Once all mice complete the final training block, the hidden platform will be removed from the maze, and all mice receive a one minute probe trial starting from the maze location opposite the old platform position. Probe trials will be performed immediately after the final block of hidden platform testing. (Tecott et al 1998; Ruan et al., 2001; Bonasera et al., in press).

Measurement of serum chemistries

Dr. Peter Havel's laboratory at UC Davis (pjhavel@ucdavis.edu) is collaborating with us to measure serum levels of the following molecules: insulin, leptin, corticosterone, free T4 and TSH, growth hormone, glucose, free fatty acids, and triglycerides.

References

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