Ethanol Self-Administration Procedures

Home-Cage Limited Access Drinking Paradigm

The following is a description of general procedures used for measuring voluntary EtOH self- administration in the home cage of mice under limited access conditions. Adult C57BL/6J mice are individually housed in pans with wood-chip bedding under a modified 12 hour light-dark cycle (lights on at 0300 hr). The mice are allowed to acclimate to this housing environment for a minimum of one week prior to the start of the study. Mice have free access to food (rodent lab chow) throughout the study. The animals are weighed once per week, at which time the pans are changed. Water bottles are changed as needed (usually twice per week). Mice are not food or water deprived throughout the study.

Using a modified sucrose-fading technique (Samson, 1986), mice are given daily access to EtOH for 2 hr in the home cage, beginning at 0.5 hr prior to the start of the dark cycle (1430 hr). Standard water bottles are removed and replaced with 15 ml bottles containing EtOH/sucrose solutions. Mice will receive 10% EtOH/5% sucrose for 2-3 days, 12% EtOH/5% sucrose for 2-3 days, 15% EtOH/5% sucrose for 2-3 days, 15% EtOH/2% sucrose for 2-3 days, 15% EtOH/1% sucrose for 2-3 days, and then 15% EtOH/0% sucrose as a final solution for the rest of the study. At the end of the 2 hour access period, EtOH/sucrose bottles are removed and standard water bottles are returned to the home cage. (Red lights in the colony room minimize disturbance of animals during their dark cycle). Although C57BL/6J mice will readily drink EtOH (even unadulterated 15% EtOH), the sucrose-fading procedure is employed to further stabilize daily intake. During the 2 hr limited access period mice are given access to water as an alternative fluid (EtOH vs. water), with the position of the bottles alternated on a daily basis.

The different EtOH and sucrose solutions are prepared as v/v and w/v solutions, respectively. The EtOH/sucrose solutions are presented at room temperature. EtOH (2 hr) and water (22 hr) intake are measured daily (to the nearest 0.1 ml). Dependent variables recorded and analyzed include EtOH intake (ml and g/kg), water intake (ml) and total fluid intake (EtOH + water intake).

Operant Ethanol Self-Administration and Reinstatement Paradigm

Training & Maintenance Procedures for Operant EtOH Self-Administration

Adult C57BL/6J mice are individually housed in pans with wood-chip bedding under a reverse

12 hour light-dark cycle (lights on at 1800 hr). The mice are allowed to acclimate to this housing environment for a minimum of one week prior to the start of the study. Mice are given free access to food (rodent lab chow) and water throughout the study. The animals are weighed once per week, at which time the pans are changed. Water bottles are changed as needed (usually twice per week). Mice are not food or water deprived throughout the study.

Mice are tested in standard operant chambers (Med Associates, Inc., St. Albans, VT) configured with two retractable levers, a liquid well, house light, and stimulus light, and tone generator. The chambers are situated in sound-attenuating boxes with exhaust fans. Stimulus events and responses are controlled and monitored by computer-driven Med PC software programs. Initially, mice are trained to lever press to receive a 5% EtOH/10% sucrose reinforcement (20 µl) delivered to the well by an infusion pump. Mice are trained to respond under a FR-1 schedule of reinforcement. A stimulus light above the well and a tone is presented while the house light is turned off for 1.5 sec, coinciding with activation of the infusion pump (delivery of the

reinforcer). Additional responses during this time have no consequences. At the start of each daily 15 min session, both levers are extended into the chamber (one lever is designated as the "active" lever, with the position being counterbalanced within groups). Responses on the "inactive" lever l have no consequences at any time during the sessions.

A sucrose-fading technique (Samson, 1986) is employed to gradually introduce EtOH into the solution (reinforcer). That is, the sucrose concentration will be gradually faded out as the EtOH concentration is gradually increased, until the animals are responding for 15% EtOH/0% sucrose during the 15 min daily test sessions. More specifically, mice are presented with the following sequence of solutions: 5% EtOH/10% sucrose, 10% EtOH/10% sucrose, 12% EtOH/10% sucrose, 15% EtOH/10% sucrose, 15% EtOH/5% sucrose, 15% EtOH/2% sucrose, 15% EtOH/1% sucrose, 15% EtOH/0% sucrose. The number of sessions mice receive a particular EtOH/sucrose reinforcer varies depending on response rates generated (tailored to individual mice). Once stable responding is established for 15% (v/v) EtOH (FR-1 schedule of reinforcement), the number of responses required to earn a reinforcer is increased from one to four over several test sessions (FR-4 schedule of reinforcement). The animals are maintained under this testing regimen for daily 15 min sessions to establish stable baseline responding and EtOH intake. Dependent variables recorded and analyzed include number of lever responses, number of EtOH reinforcers delivered, and number of licks at the well. Responses on both the "active" and "inactive" levers will be monitored throughout the sessions. The latter serves as a measure of non-specific responding (i.e., unrelated to EtOH reinforcement). Monitoring licks at the well is useful for ensuring that the mice consume the EtOH reinforcers delivered (earned) during the course of each session. All data will be collected in 15 one-minute bins. This enables more precise analysis of response topographies.

Extinction & Reinstatement Testing

After stable baseline responding (FR-4) is established for 15% (v/v) EtOH reinforcement, operant responding is assessed under extinction conditions (i.e., responding on either the "active" or "inactive" lever has no consequences in terms of changes in stimulus cues, pump activation, or EtOH delivery). Daily testing sessions (15 min) under extinction conditions continue until response rates are no more than 20% of baseline levels for three consecutive sessions. Typically, 5-7 days of extinction testing is necessary to achieve this criterion, although this can be easily extended if necessary.

Once stable extinction responding is established, reinstatement testing is conducted. Presentation of conditioned cues, stress, or EtOH itself may be used for assessing reinstatement of EtOH seeking behavior. For EtOH "priming" studies, a select dose of EtOH is typically injected 5 min prior to the start of the operant test session. EtOH also may be presented in the well of the operant chamber (response non-contingent) during the test session. Testing for conditioned cue-induced reinstatement involves presentation of the stimulus cues (those previously associated with EtOH reinforcement; i.e., light and tone) during the test session. Presentation of the conditioned cues may be response-contingent (e.g., FR-1 or FR-4 schedule) or independent of responding.

In studies designed to assess stress-induced reinstatement of EtOH seeking behavior, mice receive intermittent footshock in the operant chambers (according to standardized procedures). At 10 min following exposure to footshock stress, both levers are extended into the chamber, signaling the beginning of the 15 min operant session. The effects of acute stress (or conditioning cues) on reinstatement of EtOH-appropriate responding can be examined under either extinction or reinforcement conditions. The former condition is more akin to typical stress- induced reinstatement studies conducted in rats (Le et al., 1998; Martin-Fardon et al., 2000). During reinstatement testing, responses are recorded on the "active" as well as "inactive" levers. Following this test session, similar daily operant sessions are continued (under extinction or reinforcement conditions), but none of the animals receive footshock stress. These sessions continue until response rates are stable for at least three consecutive days. An additional group of mice that are maintained on operant EtOH self-administration throughout the study (daily sessions without interruption with extinction sessions) may be included in the study design. This group serves as a critical control for comparison of effects related to extinction testing, as well as subsequent acute stress effects on EtOH responding under either extinction or reinforcement conditions.