Ethanol Dependence Procedures

Ethanol Dependence and Testing Procedures

Chronic Ethanol Exposure by the Inhalation Route

The inhalation method of chronic ethanol (EtOH) administration offers several advantages over other methods such as feeding liquid diets or repeated intragastric intubation. These include:

(a) physiological dependence can be established in a relatively brief period of time,
(b) loss of body weight and body temperature during intoxication is minimized, so the animals remain healthy even after several cycles of intoxication/withdrawal,
(c) rigorous control may be exerted over the duration of EtOH exposure, as well as timing of initiation and termination of repeated withdrawal periods, and
(d) the level of intoxication, as assessed by blood EtOH concentration (BEC), can be maintained relatively stable during the course of intoxication, as well as from one cycle of intoxication to another.

The design of the inhalation chambers is similar to that previously described (Becker and Hale, 1993), with recent modifications (Becker and Lopez, 2004). Briefly, mice were placed in cutom- designed Plexiglas inhalation chambers (60 X 36 X 60 cm), with the housing conditions identical to that in the colony room. Ethanol (95%) was volatilized by passing air through an air stone (gas diffuser) submerged in the ethanol. The ethanol vapor was mixed with fresh air and delivered to the chambers at a rate of 5 liters/min, which maintained the ethanol concentration in the chamber in the range of 19-22 mg/liter air. These parameters typically yield stable BECs of approximately 150-200 mg/dl in adult male C57BL/6J mice following repeated 16 hr bouts of exposure. Mice are either individually or group housed in pans containing wood-chip bedding, with food and water freely available. At the beginning of each cycle, EtOH intoxication is initiated by administration of 1.6 g/kg EtOH (8% w/v) and BEC is stabilized by injection of the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg). All injections are given by the ip. route in a volume of 0.02 ml/g body weight. At the same time, mice maintained in the control (air) chamber receive an injection of saline rather than EtOH. In order to control for possible pyrazole-related effects, control chamber mice are injected with pyrazole, as well. Thus, handling of mice, as well as the number and timing of pyrazole injections is equated for chronic ethanol treated and control groups of mice.

Ethanol Samples and Measurement

Blood samples are collected from the retro-orbital sinus with heparinized capillary tubes (approximately 60 µl is collected). The blood samples are collected on ice and then an aliquot (10 µl) diluted 50:1 with 3.4% perchloric acid (v/v). The samples are vortexed and centrifuged at 12,000 x g. The supernatant is then used in a modified enzymatic assay based on the Calbiochem-Behring method (La Jolla, CA). Blood EtOH levels are determined at 340 nm on a spectrophotometer. The assay procedure measures the amount of NAD reduced to NADH (NAD is the cofactor in the oxidation reaction: alcohol + alcohol dehydrogenase Æ acetaldehyde). The amount of NAD reduced to NADH is stoichiometrically related to the amount of alcohol available for oxidation. More recently, we have employed a different enzymatic assay procedure for measurement of blood ethanol levels (ANALOX method that employs an acetaldehyde (Clark-type amperometric oxygen electrode). For this procedure, 50 µl of blood is centrifuged and an aliquot (5 µl) of plasma is used to measure the amount of oxygen consumed in the reaction: alcohol + alcohol oxidase Æ acetaldehyde +peroxide (the amount of oxygen consumed in this reaction is stoichiometrically related to the amount of alcohol available for oxidation). Blood ethanol concentrations (BEC) are typically expressed as mg/dl blood.

Air samples from the inhalation chambers (3 ml) are collected with a 5000 µl gas-tight Hamilton syringe through a port in the chamber wall. The air sample is immediately transferred to and stored in a vacu-container until used in the spectrophotometric enzymatic assay procedure described above. Air EtOH concentration is typically expressed as mg/liter air.

Dependence Testing: Handling-Induced Convulsions (HIC)

Withdrawal seizure severity is assessed by scoring handling-induced convulsions (HIC). The HIC scoring scale is modified after Crabbe and Kosobud (1990) and Goldstein (1972) - see Appended Table. Although the neural mechanisms underlying the HIC response are not well understood, the HIC measure has proven to be a sensitive and reliable index of CNS hyperexcitability associated with ethanol withdrawal following either acute or chronic EtOH treatment (Crabbe et al., 1991), including multiple EtOH withdrawal experience (Becker et al., 1997). Additionally, the technique has been shown to be sensitive to both dose and duration of chronic EtOH exposure (Becker and Hale, 1993), it can be performed repeatedly in the same subject (Becker, 1994), and HIC scores appear to correlate with other measures of EtOH withdrawal (Kosobud and Crabbe, 1986).

In chronic EtOH exposure studies, following final withdrawal (removal of animals from ethanol or air inhalation chambers) all mice are scored (in random order) for HIC activity hourly for the first 10 hours, and then at 24, 32, 48, and 72 hours post-withdrawal. Briefly, each mouse is gently picked up by the tail, held in place, and then rotated along a 360° arc. Convulsive signs are rated depending on the severity of the response as well as the extent of the handling manipulation required to elicit the behavioral response. Withdrawal testing is conducted by an investigator who is "blind" to the subjects' experimental history. Data are presented as hourly HIC scores and, as a measure of the overall withdrawal response, area under the HIC curve. The latter measure is calculated by using the trapezoidal formula for integrating area under the HIC curve.