Time-Dependent Negative Reinforcement of Ethanol Intake by Alleviation of Acute Withdrawal

Supplemental Information

Subjects

Male DBA/2J and C57BL/6J mice were shipped at 8-9 weeks of age from the Jackson Laboratory (Sacramento, CA). To minimize complications, food was removed from the home cage overnight before surgery. Mice were injected subcutaneously (SC) with the non-narcotic analgesic (NSAID) Meloxicam (0.2 mg/kg; M3935, Sigma, St. Louis, MO) 30 min before they were fully anesthetized with isoflurane gas (5% loading dose, 1.5-3% maintenance). Mice were then injected SC with 0.5 ml of 2.5% w/v dextrose and 0.5 ml saline followed by surgical implantation of a chronic SilasticÒ intragastric (IG) catheter using procedures previously described in detail elsewhere (1-3). All mice received an additional dose of Meloxicam via the IG catheter 24 h after surgery. After surgery, mice were housed individually on a 12-h light-dark cycle (lights on at 0700) with free access to water and Rodent Diet 5001 (Lab Diet, Richmond, IN) except as noted in the Procedure. The Oregon Health & Science University Institutional Animal Care and Use Committee approved all procedures, which followed the National Institutes of Health “Principles of Laboratory Animal Care.”

Apparatus

Detailed descriptions of the test apparatus have already been reported (2,3). Briefly, the apparatus consisted of 24 acrylic and aluminum chambers (20 x 20 x 22.5 cm) housed separately in sound-attenuating enclosures with ventilating fans. The IG catheter back mount (313-000BM-10/SPC, Plastics One, Roanoke, VA) was connected via polyethylene tubing to a 22 ga. fluid swivel (375/22, Instech Solomon, Plymouth Meeting, PA) mounted above the chamber on a counterbalanced lever arm (SMCLA, Instech Solomon). Ethanol and water were delivered to the swivel from 12-ml syringes mounted on syringe pumps (Model A or Model R-E Razel Scientific

Instruments Inc., St. Albans, VT). Each chamber was equipped with two retractable drinking tubes (ENV-252M, Med Associates, St. Albans, VT) mounted 9.5 cm apart on one wall, 3.5 cm above the stainless steel mesh floor. To accurately measure oral ethanol intake in Experiment 4, the standard drinking bottles were replaced with tubes created by attaching double ball bearing stainless steel sippers (TD-101, Ancare, Bellmore, NY) to polystyrene serological pipettes (13-678-12E, Fisher Scientific, Pittsburgh, PA), which allowed volume measurements to the nearest 0.05 ml (adapted from Ford *et al.* (4)). The tubes were connected to lickometers (ENV-250B, Med Associates) interfaced to a computer that stored lick totals automatically every 5 min using LabVIEW 6.1 software (National Instruments, Austin, TX).

Supplemental References

1. Cunningham CL, Clemans JM, Fidler TL (2002): Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice. *Pharmacol Biochem Behav* 72:659-668.
2. Fidler TL, Dion AM, Powers MS, Ramirez JJ, Mulgrew JA, Smitasin PJ, *et al.* (2011): Intragastric self-infusion of ethanol in high- and low-drinking mouse genotypes after passive ethanol exposure. *Genes Brain Behav* 10:264-75.
3. Fidler TL, Powers MS, Ramirez JJ, Crane A, Mulgrew J, Smitasin P, Cunningham CL (2012): Dependence induced increases in intragastric alcohol consumption in mice. *Addict Biol* 17:13-32.
4. Ford MM, Nickel JD, Phillips TJ, Finn DA (2005): Neurosteroid modulators of GABA(A) receptors differentially modulate Ethanol intake patterns in male C57BL/6J mice. *Alcohol Clin Exp Res* 29:1630-40.