Supplement 1

METHODS AND MATERIALS

Subjects

Male ethanol-preferring inbred (iP) rats were derived from a line provided by Indiana University. This stock of inbred P rats (5B substrain) was derived from breeders of the selected line of P rats originally provided in 1999 by Indiana University (courtesy of Dr. T.K. Li) and has been bred on-site at the University of North Carolina at Chapel Hill. The rats were housed in pairs before surgical implantation of the cannulae. After surgery, rats were singly housed for the remainder of the study. Rats were housed in Plexiglas cages with water and food available continuously unless otherwise mentioned. The colony room was maintained on a 12-h light/dark cycle and experiments were conducted during the light portion of the cycle. At the beginning of testing, rats weighed 459 ± 10 g (mean ± S.E.M.). All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, National Academy Press, 1996) and institutional guidelines.

Apparatus

Operant conditioning chambers. The self-administration chambers (30.5 cm x 24.1 cm x 21.0 cm; Med Associates, Georgia, VT) were located within sound-attenuating cubicles. Each cubicle was equipped with an exhaust fan that provided ventilation and masked external sounds. The left and right wall of each chamber contained one liquid receptacle and a response lever (Med Associates). Lever press responses activated a syringe pump (Med Associates) that delivered 0.1 ml of solution into the receptacle across a 1.66-s period. A stimulus light located above each response lever was illuminated during pump activation. Lever presses during reinforcer delivery were recorded, but produced no programmed consequence. The chambers were interfaced (Med Associates) to a computer that was programmed to control sessions and record

data at 100-ms intervals.

Locomotor Activity Chambers. Four clear Plexiglas chambers (43.2 cm x 43.2 cm; Med Associates) were used to assess locomotor activity. Horizontal distance traveled (cm) was determined from the number of photobeam breaks and collected via computer interface in 2 min time intervals.

Procedure

Detailed ethanol self-administration training. On the first day of training, rats were placed in the self-administration chambers for a 16-h training session to establish reliable lever pressing behavior. No food was available during this session. During this session, the schedule of reinforcement gradually increased from a concurrent fixed ratio one (CONC FR1 FR1) to CONC FR4 FR4. That is, once 4 reinforcers were delivered on a lever, the schedule on that lever increased to FR2. Once the total reinforcers received on that lever was 10 the schedule increased to FR4 (i.e., four lever responses resulted in the presentation of 0.1 ml of the solution paired with that lever) and that response requirement remained for the duration of the session. Water was removed from the home cage 24 h before this session and returned following the training session. After this initial training session, the rats received one 30-min self-administration session each day (M-F) and both levers were maintained on a CONC FR4 FR4. From this point forward, water was continuously available in the home cage. A sucrose-fading procedure (1) was used such that ethanol was gradually added to the 10% sucrose solution and the sucrose was gradually faded out so that ethanol (15% v/v) alone maintained lever pressing. The exact order of exposure was as follows: 10% sucrose/2% ethanol (10S/2E), 10S/5E, 10S/10E, 5S/10E, 5S/15E, 2S/15E. There were 2 sessions at each concentration. After sucrose fading, self-administration sessions with ethanol (15% v/v) and water as the reinforcers continued for the remainder of the study. Ethanol reinforcement was paired with the left lever for half of the rats and with the right

lever for the other half. After completion of 28 self-administration sessions with 15% ethanol, rats underwent surgery for cannulae implantation.

Cannulae implantation surgeries and microinjections

Rats were anesthetized with an isoflurane/oxygen combination and bilateral injector guide cannulae (26 gauge; Plastics One Inc., Roanoke, VA) were stereotaxically (Kopf Instruments, Tujunga, CA) implanted to terminate 2 mm above the targeted brain region. The nucleus accumbens stereotaxic coordinates were anterior/posterior (AP) = +1.6, medial/lateral (ML) = +1.5, dorsal/ventral (DV) = -5.0 mm from dura. The dorsomedial caudate putamen coordinates were AP = +1.6, ML = +1.5, DV = -2.6 mm from dura. The mPFC coordinates were AP = +3.2, ML = +0.6, DV = -1.3 mm from dura (2). The cannulae were secured to the skull with dental cement (Duralon, Henry Schein, Melville, NY; Cerebond, Plastics One Inc.) and anchor screws (Plastics One Inc.). Dummy cannulae that did not extend past the end of the guide cannulae were inserted into the guides to protect the brain tissue. Rats were allowed at least 7 days of recovery before resuming self-administration training and had one week of self-administration training before testing began.

Injections were made with Hamilton syringes (Hamilton, Reno, NV) connected to 33-gauge injectors (Plastics One Inc.). Rats were placed in a holding cage (48 x 26 x 20 cm) that allowed free movement. Dummy cannulae were removed, and the injectors were inserted bilaterally to a depth 2 mm below the guide cannulae. A pump was used to deliver a volume of 0.5 µl/side over a 1 min period. The injectors were left in place for an additional 90 seconds to allow for drug diffusion.

Histological verification

After completion of the experiment, rats were deeply anesthetized and perfused transcardially with 0.1 M PBS followed by 4% formaldehyde (pH = 7.4). Brains were extracted and sliced into 40-µm coronal sections and stained with cresyl violet.

Cannulae placement was verified using an Olympus CX41 light microscope (Olympus America, Center Valley, PA) and only the data from rats with cannulae determined to be in the target brain regions were used in the analyses.

Histological verification showed that 2 rats in the LY379268 group, 1 rat in the dorsomedial caudate group, and 1 rat in the sucrose group had cannulae placements outside of the target region. These rats were excluded from the analyses and data presentation. Anatomical location for all rats in each group with accurate placements and representative photomicrographs are shown in Figure 1.

1. Samson HH (1986): Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. *Alcohol Clin Exp Res* 10:436-442.

2. Paxinos G, Watson C (1998): *The rat brain in stereotaxic coordinates, 4th ed*. San Diego, CA: Academic Press.