

## **Mouse (WID) Withdrawal-induced Drinking Animal Model**

### **Withdrawal-induced drinking (WID): two bottle choice**

Mice will be housed and acclimated for two weeks prior to testing in a reverse light cycle room (lights off at 9:00am, lights on at 9:00pm). Food and water will be available ad libitum throughout testing. On day 0, mice will be individually housed and two bottles containing water will be presented to the mice to acclimate them to the two bottle choice procedure. On days 1-6, three hours after the lights turn off (i.e. at 12:00pm), one water bottle will be replaced with a bottle containing 15% ethanol for 2 hours. Ethanol and water consumption during these 2 hour periods will be recorded. On day 7, mice will be housed in their original groups and divided, based on equal ethanol and water consumptions, into two groups. The ethanol vapor group will receive injections of a loading dose of ethanol (1.5 g/kg) and the alcohol dehydrogenase inhibitor, pyrazole (68.1 mg/kg in saline) and then placed into the vapor chambers. The control group will receive pyrazole (68.1 mg/kg in saline) before placement into control chambers. Mice will be placed in the chambers at 3:00pm for 16 hours. At 7:00 am on day 8, mice will be removed from the chambers and tail blood will be sampled for blood alcohol determination. Vapor exposure will be repeated on days 8 and 9. The target blood alcohol concentration will be 150-200 mg% and will be obtained by manipulating the flow of air over the ethanol source. On day 10, mice will be removed from the chambers at 7:00 am, blood sampled, and individually housed once again. On day 11, at 12:00 pm, mice will be given access to a bottle containing water and a bottle containing 15% ethanol for 2 hours. On days 12-18 the two bottle choice limited access procedure will be repeated. Mice will then be allowed a 0-2 week period of abstinence and the entire 18 day experiment will be repeated. Previous studies have shown a more robust withdrawal-induced drinking effect following a second bout of vapor exposure. Mice will be weighed every 4-6 days throughout the experiment. Ethanol consumption following ethanol vapor exposure will be compared to the ethanol consumption of control mice as well as with consumption prior to vapor exposure.

### **Withdrawal-induced drinking (WID): operant**

Mice will be housed and acclimated for two weeks prior to testing in a reverse light cycle room (lights off at 9:00am, lights on at 9:00pm). Food and water will be available ad libitum throughout testing. Mice will be trained to lever press for 10% ethanol using operant testing chambers outfitted for lever responding for liquid reinforcement. Each of these clear Plexiglas chambers measures 14.9 x 15.2 x 18.3 cm and is housed within a larger exterior box (Coleman coolers) equipped with an exhaust fan serving to ventilate the chamber and to mask background noise. One wall of each operant chamber is equipped with two levers (2.5 cm in width, 5 cm apart and 2.5 cm from the grid floor). Between the levers there are two plastic drinking cups separated by a clear Plexiglas divider (7.5 x 10 cm). A lever press requires  $5 \pm 1$  g of downward force and results in the disruption of a photocell beam. A continuous reinforcement schedule (FR1) will be used initially, whereby a single lever press will result in the delivery of 0.01 ml of fluid into one of the two drinking cups. The FR requirement will be increased on an individual mouse basis so that responding matches consumption (i.e. no ethanol fluid is left in the drinking cups at the end of the sessions). Fluid delivery and recording of operant responses (photocell beam breaks) are controlled by microcomputer. Mice will be trained in daily 1 hr sessions, 5 days per week.

A saccharin fading procedure used previously in mice (Roberts et al., 2000) to establish ethanol as a reinforcer will be employed. Both levers will be available and responding to one lever will

result in the delivery of saccharin/ethanol and responding to the other resulted in the delivery of water. The progression of saccharin fading training will be as follows: 10 days of saccharin vs. water, 6 days of 5% ethanol + saccharin vs. water, 4 days of 5% ethanol vs. water, 4 days of 8% ethanol + saccharin vs. water, 4 days of 8% ethanol vs. water, and 12 days of 10% ethanol + saccharin vs. water. For the final 20 days prior to vapor chamber exposure, unsweetened 10% ethanol and water will be available. Throughout operant training, the lever associated with saccharin/ethanol and the lever associated with water will be kept constant. Ethanol dilutions (5, 8, and 10% w/v) will be made up using 95% ethyl alcohol and water. Sodium saccharin (Sigma Chemical Co., St. Louis, MO, USA) will be added to water or the ethanol solutions to achieve a final concentration of 0.2%.

Half of the mice of each genotype or group will be exposed to intermittent ethanol vapor (14 hr on, 10 hr off) and the other half will be exposed to air for 3 weeks. Mice will be tested for operant ethanol self-administration (for 60 min) throughout this exposure period, 8 hr into the "off" phase of vapor exposure 5 days per week. For exploratory Core studies, the ethanol vapor exposure paradigm will mimic that of the WID-2BC model (see above) and mice will not be tested in the operant boxes during this exposure time. The target blood alcohol concentration will be 150-200 mg% and will be obtained by manipulating the flow of air over the ethanol source. Following removal from the vapor chambers, mice will be allowed a short period of abstinence prior to retesting in the operant boxes. In the WID-O model described in the Progress Report above, a 1 week period of abstinence was employed with success. However, when the vapor exposure period is shortened and intensified as in the WID-2BC model, we will explore using a 1 day abstinence period prior to retesting.

Mice will be sampled for blood alcohol levels every 4-5 days during the lengthier vapor exposure periods or daily during the shorter periods as in the WID-2BC model. Blood will also be assayed for alcohol content following operant ethanol self-administration sessions during withdrawal. These latter samplings will allow for the determination of whether this model produces excessive ethanol consumption as defined by INIA (resulting in blood alcohol levels in excess of 100 mg% under these conditions).

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