

Withdrawal Induced Drinking (WID) Rat

(*Note: animals should be on reverse lights cycles so that all drinking occurs in the dark phase)

WID Rats (two-bottle choice)

Adult Wistar rats (≥ 60 days of age) are trained during the dark to self-administer alcohol or water in a 2-bottle choice situation in the home cage using a variation on the previously used saccharin fading procedure (Roberts et al., 1996). This procedure produces pharmacologically relevant blood alcohol levels (BALs) in non-dependent animals with limited access to alcohol in an operant situation (Roberts et al., 1999). The present procedure utilizes a sweetened solution ("supersaccharin") containing 3% glucose and 0.125% saccharin (Sigma Chemical) to train the animals to drink during limited access sessions. Animals drink this solution during the first training session, thus circumventing the need for water restriction to initiate alcohol drinking.

Self-administration sessions during training are conducted 5 days per week. Prior to each session, animals are weighed, bottles are weighed, and the animals are then each placed in separate cages and presented with two bottles (experimental solution vs. water) for a 30 min daily session. The position of the bottles on the cage tops is alternated for each session. Bottles should be carefully handled and attached to cage lids with stainless steel springs to reduce spilling. Also, an ethanol and water bottle should be placed daily on an extra empty cage during the 30-min sessions; the amount of fluid lost from each of those bottles should be subtracted from the daily fluid intake amounts for each rat. Self-administration sessions are 30 min long, except during the initial stages of supersaccharin training in which animals are allowed to drink for several hours per session or until they learn to drink the sweetened solution. Ethanol (10% w/v) is then added to the sweetened solution, and once drinking stabilizes (around one week), the glucose is removed from the solution so that it only contains 0.125% saccharin and 10% w/v ethanol. Animals are kept at this stage until behavior once again stabilizes (around one week) and they are then given 10% w/v ethanol alone. Following maintenance on 10% w/v EtOH, stable drinkers ($\pm 25\%$ across three consecutive sessions) are evenly divided into two groups and exposed for 4-8 wks to air (*Nondependent*) or intermittent ethanol vapors (*Dependent*, 14 h on / 10 h off; e.g., on at 6 PM and off at 8 AM). Self-administration sessions during post-dependence testing are conducted every 2-3 days between 2-8 h into withdrawal from vapors.

WID Rats (two-lever operant)

Adult Wistar rats (≥ 60 days of age) are trained during the dark to self-administer 10% (w/v) ethanol vs. water in 30 min operant sessions with an FR1 schedule of reinforcement (each lever press is reinforced) using a variation on the previously

used saccharin fading procedure (Roberts et al., 1996). This procedure produces pharmacologically relevant blood alcohol levels (BALs) in non-dependent animals with limited access to alcohol in an operant situation (Roberts et al., 1999). The present procedure utilizes a sweetened solution (“supersaccharin”) containing 3% glucose and 0.125% saccharin (Sigma Chemical) to train the animals to drink during limited access sessions (Funk et al., 2006). Animals drink this solution during the first training session, thus circumventing the need for water restriction to initiate alcohol drinking.

Operant self-administration sessions during training are conducted 5 days per week. Prior to each training session, animals are weighed, placed individually into the operant boxes, and the levers are extended for a 30 min operant session. A drop of experimental solution will be placed on the assigned lever prior to the session to aid in the initial learning process. Self-administration sessions are 30 min long, except during the initial stages of supersaccharin training in which animals are allowed to drink for several hours per session or until they learn to drink the sweetened solution. Ethanol (10% w/v) is then added to the sweetened solution, and once operant responding stabilizes (around one week), the glucose is removed from the solution so that it only contains 0.125% saccharin and 10% w/v ethanol. Animals are kept at this stage until behavior once again stabilizes (around one week) and they are then either given 10% w/v ethanol alone. Following maintenance on 10% w/v EtOH, stable drinkers ($\pm 25\%$ across three consecutive sessions) are evenly divided into two groups and exposed for 4-8 wks to air (*Nondependent*) or intermittent ethanol vapors (*Dependent*, 14 h on / 10 h off; e.g., on at 6 PM and off at 8 AM). Self-administration sessions during post-dependence testing are conducted every 2-3 days between 2-8 h into withdrawal from vapors.

Mouse Animal Models Core

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