

Drinking in the Dark (DID): Theme and Variations

This document summarizes the “standard” Drinking-in-the-Dark method as used with selectively bred alcohol-preferring (P) rats.

DID Multiple Scheduled Access (DID-MSA)

1. P rats are acclimated to the facility for at least 2 weeks. When the rats reach 75 days old (post-adolescence), the animals are single-housed in stainless steel hanging cages, with an internal food hopper, in vivaria that are on a reverse dark-light cycle (0900 lights off). Work in the vivarium is conducted with indirect lighting from the computer screen and a single 40-watt red incandescent light bulb. The vivaria are temperature (21° C) and humidity (50%) controlled. The DID-MSA procedures are conducted in these cages, which serve as the rats' home-cage throughout the DID-MSA protocol. The rats are habituated to the hanging stainless steel cages and the reverse-dark light cycle for 2 weeks. Food and water are available *ad libitum*. The stainless steel cage-bank has 30 cages each side of the bank and is replaced with a clean, sterile bank at least every 2 weeks. Absorbent paper pan liners under the cages are changed at least 3 times a week. The drinking bottles, which hold approximately 300 mls of fluid each, are rinsed and filled with new solutions twice a week and cleaned every 2 weeks. The drinking bottle positions are randomly switched twice a week (i.e., when the old solutions are replaced with new solutions). The solution bottles are standard glass bottles holding approximately 300 ml of fluid, with a rubber stopper (no. 10) holding an angled (~135°) stainless steel sipper tube. The sipper tubes do not have a ball-bearing tip. We have found very low spillage, which is monitored by 2 ghost-cages, using this set-up. Bottles are attached to the wire mesh front of the cages using stainless steel springs. Vitamin and mineral fortified food (Harlan Teklad Rodent Diet, 7017) is freely available.

The rats also experience the body and bottle weighing regimen during the habituation phase. Animal body and initial bottle weights for each of the 5 DID-MSA days are recorded by a personal computer during the half hr prior to lights off. Bottle weights are also recorded at the end of each 1 hr session. Therefore, the control rats also experience disturbances associated with bottle weights for the DID-MSA animals.

2. After the habituation phase (2 to 3 weeks), multiple concentrations of ethanol are presented to the animals. In general, 15% and 30% concentrations are presented concurrently with water. The “standard” protocol involves giving each rat three 1-hr access periods starting at lights out, with each access period separated by 2 hrs without ethanol (i.e., 0900—1000, 1200—1300, and 1500—1600 hr). These three 1 hr access days are limited to 5 days a week, with a 2 day weekend separating each 5 day block.

Our laboratory has found that starting the first access session sometime after lights off results in lower BACs at the end of the 1 hr session. This appears to be due to increased food in the stomach, compared with rats that initiate their first 1 hr session at lights off.

Additionally, our laboratory has found that starting the rats off with 24-hr access, and then reducing this incrementally each week (i.e., 24 hr for week1, 16 hr for week 2, 8 hr for week 3, and 4 hr for week 4, before limiting their access to three 1 hr sessions, results in higher hourly intakes during the 1st and 2nd weeks. However, P rats will achieve ethanol

intakes between 5 and 7 g/kg/day (three 1 hr sessions) by the end of the 4th week using the “standard” DID-MSA procedure outlined above.

3. Because maximal daily intakes are achieved by the end of the 4th week, the rats are given 4 weeks of access beyond this point. Therefore, the animals have 8 weeks of DID-MSA within our “standard” DID-MSA procedure.

4. Two control conditions are employed: 1 group remains ethanol naïve, but experiences all of the handling and weight measurements experienced by the DID-MSA animals. A second control group serves as a continuous access condition. Within the continuous access condition, the rats receive 24 hr access to the same multiple concentrations of ethanol (15% and 30%) 7 days a week. Therefore, these animals never experience a protracted withdrawal, whereas the DID-MSA animals do.

5. On the Monday of the 9th week (after the DID-MSA animals have had 8 weeks of 5 days access to ethanol per week), ethanol is removed from the DID-MSA rats and the continuous rats at 1000h. For the Micro Array study of Bill McBride et al., animals are live decapitated and the brains quickly removed and flash frozen in isopentane on dry ice at 1, 4, 8, and 24 hrs after the ethanol is removed.

The utility of the DID-MSA procedure stems from the fact that P rats self-administer approximately 2 g/kg/1 hr session repeatedly, with BACs exceeding 120 mg% readily attained. Note that these BACs may not reflect peak BACs, because our standard procedure for measuring BACs is conducted at the end of their 1 hr session. And, because over 95% of the P rats’ ethanol intake occurs within the first 12-min, peak BACs probably occur before the end of the 1 hr session.

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