**DNMTS Training Protocol (Outer Sample)**

Reward sizes:

Sample – 1 μL

Delay – 1 μL

Choice – 5 μL

Task timing:

ITI – 10 seconds (with scan punishment – a punish light illuminates and resets the ITI every time they poke a port during this time. Helps discourage scanning during the task)

Delay – 5 seconds (see **EARLY WITHDRAWAL** in notes)

Early withdrawal timeout – 30 seconds

Punishment timeout – 20 seconds

* **Habituation**
  + The water reward is dispensed at sample, delay, and reward ports sequentially, without the animal first needing to poke to dispense the reward. This is only done for one day to familiarize them with the reward locations.
  + The second day of habituation follows the first, with the only difference being the animal must poke the port to cause water to be delivered. The only light that illuminates during the choice phase is the correct port, and there are no punishments or timeouts for incorrect choices or pokes. This habituation is also done for only one session.
* **Training**
  + The original sample light will illuminate in addition to the correct choice light during the choice phase.
  + Animals are now punished for incorrect pokes:
    - Incorrect pokes during the sample reset the trial to the ITI after a brief timeout
    - Early withdrawal during the delay resets the trial to the ITI after a lengthy timeout
    - Incorrect pokes during the choice triggers an intermediate-length timeout, followed by the end of the trial. (see **REPEAT TRIALS** in notes)
  + In the earliest days of training, the delay hold time is set to .05 seconds for the first trial, and set to increase by .05 seconds each trial. As animals become accustomed to an increasing delay, the amount that the delay increases per trial is increased, up to an eventual 1 second per trial (so that they are doing the full delay by the 6th trial – the first 5 trials of a testing session are ignored)

Notes

**SETUP**

The 5 port box from Sanworks is used in this task, with the back wall retrofitted to accommodate an extra port used as a fixation port during the delay. Ports 2 and 4 are covered with 3D-printed port caps, leaving ports 1, 3 and 5 and the delay port available for the task. Trial types where the middle port is the sample port are omitted for simplicity of training. Approximate time to proficiency is 4 weeks.

If you wish to train a more comprehensive task with more trial types, do not use the port caps on ports 2 and 4. The suggested training protocol is as follows:

1. Train the trial types with the sample port on one of the two outer ports until the mouse has reached ~70% proficiency.
2. Introduce the trial types with ports 2 and 4 as the sample until the mouse has reached ~70% proficiency.
3. Incorporate the trial types with the middle port as the sample.

**EARLY WITHDRAWAL**

A global timer is used to manage the transition between the delay and the delayReward states, allowing for the use of two states in the delay – DelayOnHold and DelayWaitForReentry. DelayOnHold activates when the animal has entered the port, and when a port out occurs, it goes to DelayWaitForReentry, a state with a .75 second duration. If the animal has still not reentered the delay port at the end of this short grace period, it is considered an early withdrawal. This permits the animal to break the photogate any number of times during the fixation without prematurely ending the trial due to a false early withdrawal.

**REPEAT TRIALS**

During training, performance of each trial types is calculated over a moving window. If an animal has made less than 50% of correct guesses on a certain trial type, trials of that type will be repeated until the animal has made a correct guess.