STANDARD OPERATING PROCEDURE
2-Choice Pairwise Visual Discrimination Task (PVD) and Reversal (PVR)
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TITLE: Two-Choice Pairwise Visual Discrimination Task (PVD) and Reversal (PVR)

SOP NO.: mPVD-v1

DATE: January 17, 2019

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1.0 INTRODUCTION

The PVD task with reversal has been designed to measure effects of drugs and other manipulations (ex: genetic) on visual learning and cognitive flexibility. The test is performed in specially designed touchscreen-based automated chambers with 2 response locations (left and right windows) using food reinforcers to maintain performance. The PVD task requires the subject to learn to associate a food reward with a nose-poke response to one image (S+ stimulus) when it appears in one of the windows and ignore a second visually distinct image (S- stimulus) appearing simultaneously in the other location. After the task is learned, reversal learning is attempted where the food reward becomes linked to the former S- stimulus and responses to the former S+ stimulus go unrewarded.

2.0 EQUIPMENT
- Mouse Touch Screen Systems and ABET II
  http://lafayetteneuroscience.com/listing/mice-touch-chambers-components/
- 89540CAM Pairwise (Visual) Discrimination (PD) Task with Cambridge Amendment from the Cambridge University Group, a file run within ABET II during training and evaluation

3.0 PROCEDURE

3.1 General Equipment:
- Best practice to test the hardware prior to every training or testing day. Ensure that the expected inputs and outputs are observed.
- All programs are found in PVD[1] v3 subdirectory in the ABETII software.
- A quick test of the feeder should be done prior to every training or testing day. Manually switch on the feeder pump and make sure the food is delivered and remove clog if necessary[2].
- Make sure the PVD Mask is inserted (2 windows).

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[1] Note that pairwise visual discrimination (PVD) is sometimes referred to simply as pairwise discrimination (PD) or visual discrimination (VD). Your subdirectory and files may have variations of PVD, PD, or VD.
[2] Best practice is to also check that milkshake is still flowing between each animal being run in the touchscreen chamber.

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- Reward provided is Neilson Strawberry milkshake (SM) (Saputo Inc. Montreal Quebec. H1P1X8). This milkshake can be found in most grocery stores (including Loblaws and Superstore).

3.2 Pre-training

- Make sure your mice are food restricted to 85-90% of their free-feeding weight prior to the start.
- Provide strawberry milkshake to the mice in their home cages for 2 days immediately prior to training.
- Divide each group of subjects into 2 counter-balanced sub groups containing both control and test mice to control for the time of day the experiment is performed, and the particular cabinet being used in case of an equipment failure.
- If testing multiple time points during a mouse's life: You may wish to pre-select a pair of images to be used in the discrimination/reversal task for each age point required. Preselecting 5 pairs allows for 5 potential data sets over the life of each cohort and prevents those images from being displayed during the training and maintenance phases. All training schedules should be checked for which images they will display.

3.3 Training Procedures

3.3.1 Basic training schedule

Generally, mice are given 1 session per day.

Stage 1: Habituation1

ABETII program file: 89540 Mouse (VD) Pairwise Habituation 1 v2
Duration: 1 session, 600s (10 minutes).
Trial number: Unlimited
Description: Mouse is left in the chamber for 10 min. All lights are turned off. No stimulus or reward is presented. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: None

Stage 2: Habituation2a

ABETII program file: 89540 Mouse (VD) Pairwise Habituation 2 v2
Duration: 2 sessions, 1200s (20 minutes)
Trial number: Unlimited

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Description: The tray light is initially turned on. A tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivered for 6000ms (150µl). The program waits for the mouse to enter the food tray. When the mouse leaves the reward tray, the reward tray light is turned off. There is a 10s delay before the tray light is turned on, a tone is played and SM is then delivered for 800ms (20 µl)[3]. If the mouse is in the reward tray at the end of the 10s delay, an extra 1s is added to the delay. The procedure is repeated until the session ends. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: Drinks milkshake (none observed in tray).[4]

Stage 3: Habituation2b

ABETII program file: 89540 Mouse (VD) Pairwise Habituation 2 v2
Duration: 1 session, 2400s (40 minutes)
Trial number: Unlimited
Description: The mouse is left in the chamber for 40 min. Reward presentation is the same as described in stage 2. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: No milkshake found in tray at end of session.

Stage 4: "Initial touch"

ABETII program file: 89540 Mouse (VD) Pairwise Initial Touch Training v3
Duration: Number of sessions varies across mice, 3600s (60 minutes)
Trial number: 30
Description: Make sure that "Image Time" is 30s; 'Feed Pulse Time" is 800ms; "tone duration" is 1000 ms, and ITI period is 20s. The stimulus (any image not designated for use in discrimination/reversal trials) is displayed in either the left or right window. The other window is blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. After a delay (Image Time – 30s) the stimulus is removed and a reinforcer is delivered ('Feed Pulse Time – 800ms). Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency is 3 KHz. The tone duration is (1000 ms). Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (20s) another stimulus is displayed. If the mouse touches the screen while the stimulus is displayed the stimulus is removed and a tone will be played and 3 x reward volume is dispensed. Collection of this reward again starts the ITI and then progresses to the next stimulus. Training is performed with the house light off.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

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[3] Note that ABETII has a pre-set standard of 280ms (7µl) of strawberry milkshake delivered. The TCN Lab while at Cambridge increased this volume. The rationale is that animals that get few rewards on challenging tasks may remain more motivated as the reward is larger when it is delivered.
[4] If your mouse does not drink milkshake, you may wish to give the milkshake in the home cage with their food, check the weight of the animal, and give extra sessions.

Stage 5: "Must touch"

ABETII program file: 89540 Mouse (VD) Pairwise Must Touch Training v3
Duration: Number of sessions varies across mice, 3600s (60 minutes)
Trial number: 30
Description: Make sure tone duration is set to 1000 ms (from the 'Tone Duration' variable) and ITI period is set to 20s. The stimulus, an image selected pseudo randomly (no image shown twice in a row) from a list which must not include any of the images to be used in discrimination/reversal trials. The stimulus is presented in only one window at a time. The other windows are blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to receive reinforcement. No reinforcer is delivered if the mouse touches the blank part of the screen. Reinforcer delivery is accompanied by illumination of the tray light and a tone. The tone frequency default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (20s) another stimulus is displayed.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.[5]

Stage 6: "Must initiate"

ABETII program file: 89540 (VD) Mouse Pairwise Must Initiate Training v3
Duration: Number of sessions varies across mice, 3600s (60 minutes)
Trial number: 30
Description: This schedule trains the mouse to initiate after an ITI. Make sure tone duration is set to 1000 ms (from the 'Tone Duration' variable) and ITI period is set to 20s. A free reinforcer is delivered, and the tray light is turned on. The mouse must nose poke and exit the reward tray before a stimulus is displayed randomly on the screen. The stimulus, an image selected pseudo randomly (no image shown twice in a row) from a list which must not include any of the images to be used in discrimination/reversal trials. The stimulus position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to elicit tone/food response. There is no response if the mouse touches the blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period the tray light is again illuminated. The mouse *must* nose poke and exit the reward tray before the next stimulus is displayed.

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[5] If after 7 sessions a mouse does not reach criterion for "must touch", take it back one step; that is, retrain the mouse on "initial touch" again until it reaches criterion and repeat the "must touch" training. If after 7 sessions of the second attempt of "must touch" the mouse does not reach criterion, remove it from the study.

Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.[6]

Stage 7: "Punish incorrect"

ABETII program file: 89540 (VD) Mouse Pairwise Punish Incorrect Training v3
Duration: Number of sessions varies across mice, 3600s (60 minutes)
Trial number: 30
Description: This schedule trains the mouse not to touch an incorrect location. Training is the same s for "Must initiate", except if a mouse touches an incorrect (blank) location the house light is turned ON for 5s (time out, TO) and no reward is given. Once the time out period finishes the house light is turned OFF again and the ITI period begins (20s). There is no time limit on the display of the stimulus (no omissions score) and no correction trials.
Criterion: Completion of 24/30 trials or better within 60 min for 2 consecutive sessions.[7]

3.3.2 PVD task acquisition, baseline and reversal learning

Stage 8: PVD task acquisition

ABETII program file: 89540 (VD) Mouse Pairwise Discrimination v3
Duration: Number of sessions varies across mice, 3600s (60 minutes)
Trial number: 30
Description: The session begins with a priming delivery of reinforcer 800ms (20 µl) and on exiting the food magazine the first trial begins. Following tray exit a S+ image and a S- image are presented in either of the 2 windows. The left/right ordering of the S+ and S- images is pseudo random with no ordering repeated more than 3 times. A correct response, touching at the location in which the S+ stimulus was presented, will trigger the presentation of reinforcer 800ms (20 µl) into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is 1000 ms. The subject collects the food by making an entry at the food magazine. On exiting the food tray the ITI (20s) will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial. An incorrect response, i.e. touching the S- image will cause a time out (TO, 5s) and the house light to be turned ON. After the TO, the house light will be turned OFF and the ITI will begin (20s). After the ITI the tray light will come on and the subject must enter and exit the food tray to start the

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[6] If after 5 sessions a mouse does not reach criterion for "must initiate", take it back one step; that is, retrain the mouse on "must touch" until it reaches criterion and repeat the "must initiate". If after 5 sessions of the second attempt of "must initiate" the mouse does not reach criterion, remove it from the study.

[7] If after 30 sessions (30 days) the mouse does not reach criterion for "Punish incorrect", remove it from study.

correction trial. In a correction trial the left/right ordering of the S+/S- images is repeated from the previous trial and repeated each subsequent trial until a correct choice is made. The results of correction trials do not count toward criteria for completion of the session.
Criterion: 24/30 trials correct within 60 min, for 2 consecutive days.[8]

Stage 9: PVD baseline

ABETII program file: 89540 (VD) Mouse Pairwise Discrimination v3
Duration: 2 sessions, 3600s (60 minutes)
Trial number: 30
Description: Baseline sessions are run either immediately after a mouse reached the PVD acquisition criteria, or once all the mice in the experiment have reached the PVD acquisition criteria.[9] Baseline sessions are identical to the PVD task acquisition ones.
Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Stage 10: Pairwise visual reversal (PVR)

ABETII program file: 89540 (VD) Mouse Pairwise Discrimination v3[10]
Duration: 10 sessions immediately following completion of the PVD baseline, 3600s (60 minutes)
Trial number: 30[11]
Description: A correct response is now defined as touching at the location in which the S- stimulus was presented and will trigger the presentation of reward 800ms (20 µl) into the food magazine. Therefore, for each animal, you must change which image is reinforced compared with non-reinforced in the schedule design. As during the acquisition stage, food delivery is accompanied by illumination of the tray light and a tone. The tone duration is (1000 ms tone). The subject collects the food by making an entry at the food magazine. On exiting the food tray, the ITI (20s) will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial. An incorrect response, i.e. touching the stimulus that was previously the S+ image will cause a time out (TO, 5s) and the house light to be turned ON. After the TO, the house light will be turned OFF and the ITI will begin (20s). After the ITI the tray light will come on and the subject must enter and exit the food tray to start the correction trial. In a correction trial the left/right ordering of the S+/S- images is repeated from the previous trial and repeated each subsequent trial until a correct choice is made. The results of correction trials do not count toward criteria for completion of the session.

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[8] If after 30 sessions (30 days) the mouse does not reach criterion for "Acquisition", remove it from study.
[9] This depends on whether you want to match the mice for touchscreen ability or the age of the mice. If you are studying a neurodegenerative disease, you may wish to use the latter approach. To do this, you would place the mice that reached criterion on a maintenance schedule where they are given 1-2 reminder sessions per week of the PVD task.
[10] You may wish to set up a separate schedule labelled something like 'PD_Reversal_1_v3' to keep better track of which sessions were the reversals compared with acquisition sessions.
[11] You may wish to divide the first session into three days of 10 trials each.

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Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Optional Stages if Multiple Time Points Used:

Stage 11: PVD maintenance

For maintenance, see Stage 7: "Punish Incorrect". Run Stage 7 1-2 times per week until subjects are the desired age for your second time point.
Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Stage 12: PVD and PVR subsequent time points:

Run subjects in the same manner as Stage 8, 9, and 10 with a novel set of stimuli to test acquisition and reversal when the same mice are older. Whenever possible, we recommend counterbalancing all sets of stimuli to be used across groups and time points.
Criterion: See Stages 8, 9, and 10[12].

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[12] However, as subjects age it is possible that acquiring the PVD task will take longer or fail to occur. This may require adjusting subsequent time points or dropping subjects from the study according to previously stated criteria.

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Flow chart of key steps and criterion listed in the SOP.

Habituation 1 one session, 10 min

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Habituation 2a two sessions, 20 min each

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Habituation 2b one session, 40 min

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Initial Touch 30 trials in 1 hr, one session

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Must Touch 30 trials in 1 hr, one session

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Must Initiate 30 trials in 1 hr, one session

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Punish Incorrect 30 trials in 1 hr, choose random image
24/30 or better for 2 consecutive sessions

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PVD Acquisition Choose correct image, 30 trials in 1hr
excluding correction trials
24/30 or better for 2 consecutive sessions

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PVD Baseline Choose correct image, 30 trials in 1hr
excluding correction trials, 2 sessions

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PVR Choose correct image (previously incorrect), 30 trials
in 1hr excluding correction trials, 10 sessions