

**MOUSE: STANDARD OPERATING PROCEDURE**  
**Paired-associate Learning Task (PAL)**

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**TITLE: Paired-Associate Learning Task (PAL)**

**SOP ID.: mPAL-v1**

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## **1.0 INTRODUCTION**

In humans, the visual-spatial PAL task has been used to establish the early onset of cognitive impairment due to Alzheimer's disease. The test is performed in mice with specially designed touchscreen-based automated chambers with 3 response locations (left, right and centre windows) and 3 distinct images using food reinforcers to maintain performance. Each image is assigned a unique correct location (and 2 incorrect locations). The PAL task requires the subject to learn to associate a food reward with a nose-poke response when an image appears in its correct location.

## **2.0 EQUIPMENT**

- Mouse Touch Screen Systems and ABET II  
<http://lafayetteneuroscience.com/listing/mice-touch-chambers-components/>
- 89541CAM Paired-Associate Learning Task (Visual) 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 PAL 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.
- Make sure the PAL Mask is inserted (3 windows).
- 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.



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- Divide each group of subjects into 2 counter-balanced subgroups 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 3 images to be used in the PAL task for each age point required. Preselecting 3 sets (3 images per set) allows for 3 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:** PAL\_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:** PAL\_habituation\_2\_v2

**Duration:** 2 sessions, 1200s (20 minutes)

**Trial number:** Unlimited

**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 (150 $\mu$ l). 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  $\mu$ l)<sup>1</sup>. 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.

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<sup>1</sup> Note that ABETII has a pre-set standard of 280ms (7 $\mu$ l) 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.



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**Criterion:** Drinks milkshake (none observed in tray).<sup>2</sup>

#### **Stage 3: Habituation2b**

**ABETII program file:** PAL\_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:** PAL\_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 (white square, which is not designated for use in dPAL/sPAL trials) is displayed in either the left, right or centre window. 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. 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. Touch training is performed with the house light off.

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

#### **Stage 5: “Must touch”**

**ABETII program file:** PAL\_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 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

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<sup>2</sup> 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.



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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.<sup>3</sup>

#### **Stage 6: “Must initiate”**

**ABETII program file:** PAL\_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 (white square) 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.

**Criterion:** Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.<sup>4</sup>

#### **Stage 7: “Punish incorrect”**

**ABETII program file:** PAL\_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 as 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.

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<sup>3</sup> 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.

<sup>4</sup> 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.



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**Criterion:** Completion of 24/30 trials or better within 60 min for 2 consecutive sessions.<sup>5</sup>

#### **3.3.2 dPAL (or sPAL) test**

##### **Stage 8: dPAL (or sPAL) test**

**ABETII program file:** mouse dPAL\_1\_v3 (or sPAL)

**Duration:** 45 sessions, 3600s (60 minutes)

**Trial number:** 36

**Description:** Make sure tone duration is set to 1000 ms, ITI period is set to 20s, Food/CM pulse time [800ms (20  $\mu$ l SM), time out (TO, 5s). The session begins with a priming delivery of reinforcer and on exiting the food magazine the first trial begins. Following tray exit a S+ image/location pair and a S- image/location pair are presented in 2 windows and the third window is blank. A correct response, touching at the location in which the S+ stimulus was presented, will trigger the presentation of a reinforcer into the food magazine. Reinforcer 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/location pair 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 mouse must enter and exit the food tray to start the correction trial. The next trial does not begin until a response is made, ie. no time-out/no omissions. In a correction trial the same S+/S- image/location pairs are 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:** 36 trials within 60 min, for 45 days.<sup>6</sup>

##### **Optional Stages if Multiple Time Points Used:**

##### **Stage 9: dPAL (or sPAL) 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 10: dPAL (or sPAL) time point 2:**

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<sup>5</sup> If after 30 sessions (30 days) the mouse does not reach criterion for "Punish incorrect", remove it from study.

<sup>6</sup> In some cases, subjects may fail to complete all 36 trials in a session. You may wish to split the first 5 sessions in half.



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Run subjects in the same manner as Stage 8 with either 1) a novel set of stimuli to test acquisition or 2) the same (familiar) set of stimuli to examine retention.

**Criterion:** 36 trials within 60 min, for 45 days.

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

