



Pairwise (Visual) Discrimination  
(PD/PVD) Task for Mouse Touch  
Screen Systems and ABET Cognition  
**89540A**

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## I Recommended Reading

This manual should be used in conjunction with the 'ABET Cognition' manual.

**Bussey TJ, Saksida LM, Rothblat LA.** (2001) Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. *Behav Neurosci.*;115(4):957-960. <https://doi.org/10.1037//0735-7044.115.4.957>.

**Brigman JL, Bussey TJ, Saksida LM, Rothblat LA.**( 2005) Discrimination of multidimensional visual stimuli by mice: intra- and extradimensional shifts. *Behav Neurosci.*;119(3):839-842. <https://doi.org/10.1037/0735-7044.119.3.839>.

Campden Instruments and Lafayette Instrument Company would like to thank Prof Lisa Saksida and Prof Timothy Bussey (Western Ontario University, Canada) for their help in preparing this task and manual.

## II Introduction

These are the specifications for the mouse Pairwise (Visual) Discrimination Task (PD). They contain general details about initial shaping, training to baseline and the basic task manipulations performed at baseline.

The basic training schedule is detailed in chapter 5. Briefly, mice should be thoroughly habituated to being picked up, handled, and weighed prior to the onset of testing. Once the animals can be handled with ease, then they need to be water or food deprived to provide an incentive to work in the task. This schedule should be implemented gradually and in accordance with Home Office procedures (if applicable). The first few days of testing in the touch screen boxes involve habituating to the new environment, learning where the food is presented. After pre-training the mice begin training of the Touch PD task. Finally, once baseline performance has been reached various task and neural manipulations can be carried out.

You have been provided with the following schedules for running a Mouse Touch PD task:

**For cleaning and calculating the feeder:**

FeederCal Mouse Touch

**For checking equipment:**

A MouseTestLines-2 x1

**For habituating the Mouse to the Touch Chamber:**

A Mouse Pairwise Habituation 1, A Mouse Pairwise Habituation 2

**Training for the task:**

A\_Mouse Pairwise Initial Touch Training, A\_Mouse Pairwise Must Touch Training, A\_Mouse Pairwise Punish Incorrect

**The task:**

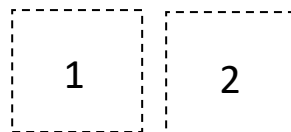
A\_Mouse Pairwise Discrimination

This manual will take you through:

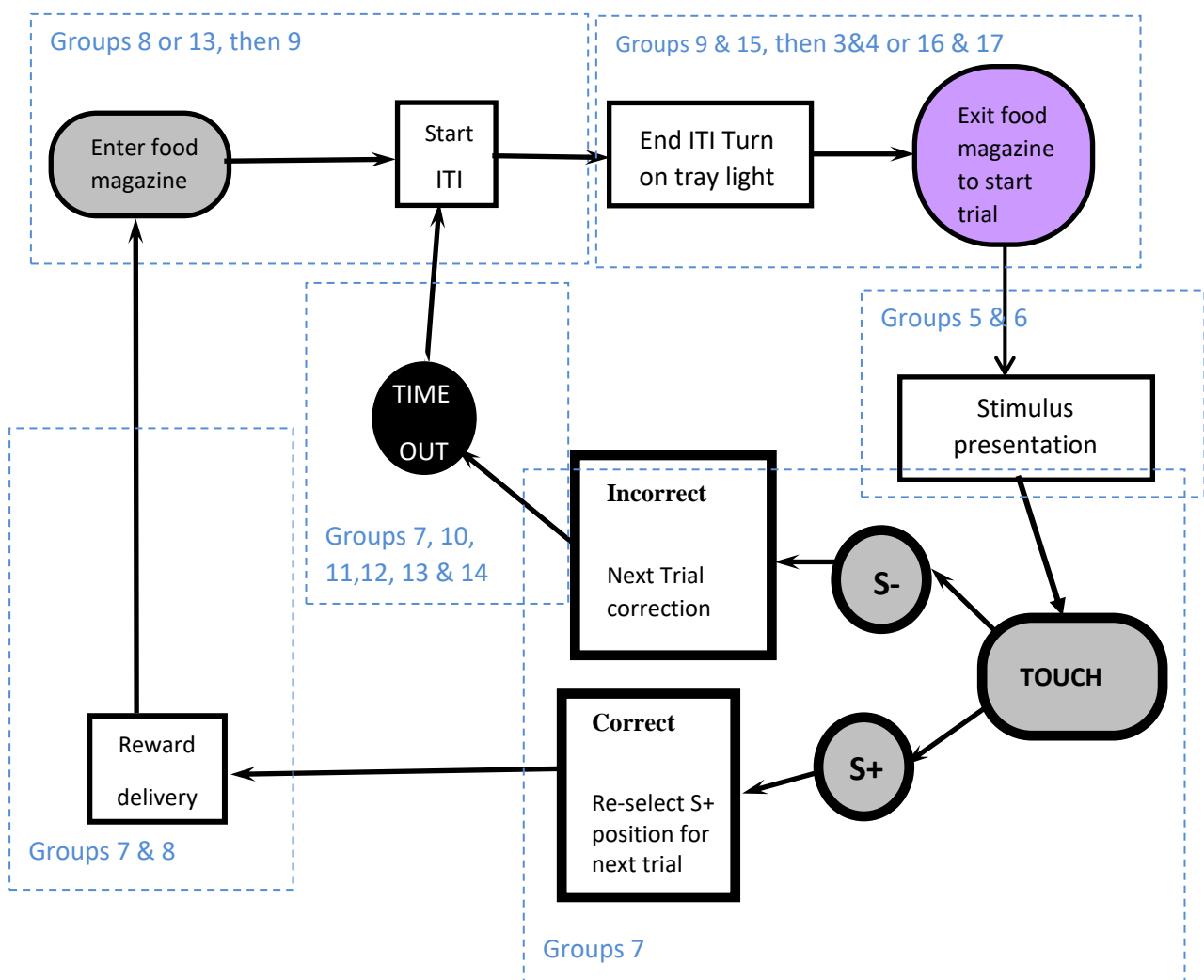
1. The Task
2. Installing the software
3. Setting up the hardware for the task.
4. Checking the equipment by running the test schedule.
5. The habituation procedures that introduce the mice to the equipment.
6. The training procedures
7. Running the task
8. Analyzing the results.
9. Running the schedules on another virtually on another computer

## 01 The Task

The task is illustrated below. A trial begins with the presentation of two novel stimuli on the screen; one is programmed as being correct (S+) and one as being incorrect (S-). Whether the S+ is on the right or left is determined pseudo-randomly. The mouse must nose poke the correct stimulus to elicit the tone/reward tray light and food delivery response. If the mouse nose-pokes the incorrect stimulus, no reward will be delivered, and a time out will follow before the mouse is given the opportunity to complete a correction trial. [During the time out period the house light is inverted (if mice have been trained with the house light on, then it is turned off, if the mouse has been trained with the house light off, then most of the trial is conducted with the house light off and it will be switched on for the timeout period.)] Correction trials will ensue until the correct stimulus is chosen. A correction trial consists of re-presentation of the stimulus array in the same L-R configuration. Correction trials are not included in the % correct figure.



Box position numbers as designated by ABET.



## 1.1. Basic Task Protocol

### 1) Setting Variables

- a. Maximum session length and the maximum number of trials are set from the 'Execution Manager'. (We recommend 1 hr and 30 trials.) When either has been exceeded then step 10.
- b. The correct tone is set from the 'Audio' List 'Correct Tone' (default 3 KHz). The volume can be adjusted by means of 'Tone Intensity' variable. The tone duration is set from the 'Tone Duration' variable (default 1000 ms).
- c. A tone to accompany an incorrect response is set from 'Wrong Tone Duration' variable (default 500 ms) and from 'Wrong Tone On' (default '0' i.e. will not be played). The incorrect tone frequency is set from the 'Audio' List 'Incorrect Tone' (default 250 Hz). Intensity from the 'Wrong Tone Intensity' variable.
- d. A click noise is set to accompany initiation of the trials from the 'Click Time' variable (default 200 ms) and also from the 'Initiate Click On' (default '0' i.e. will not be played). The click noise is set from the 'Audio' List 'Click'. Intensity from the 'Click Intensity' variable.
- e. Which image will be the correct image is set from the 'Correct Image' variable (default image 1).
- f. Whether the house light is normally on set from the variable (default False).
- g. Inter trial interval set from 'ITI' variable (default 20 s).
- h. Time-out set from 'Time Out' variable (default 5 s).
- i. Amount of food to be delivered is set via the 'Feed Pulse Time' (default 800ms /20µl).
- j. Whether correction trials will take place is set from the 'Correction Trials Set' variable (default True). If set, then the number of consecutive errors before a correction trial begins is determined via the 'No Incorrects before Correction Trial' variable (default 1).
- k. Then Step 2.

### 2) Session Start

- a. Free food is delivered by the liquid pump and the food-tray light is turned on.
- b. Wait for tray-report / feed-tray to be entered (accompany by a click if selected) and then exited.
- c. Then step 3.

### 3) Images Displayed

- a. Turn off the tray light.
- b. If normal trial then the S+ and S- images are displayed and the positions are chosen pseudo randomly, such that the same image will not be displayed on the same side more than 3 times in a row (not including correction trials).
- c. If a correction trial, then the S+ and S- images are displayed in the same positions as last trial.
- d. Then step 4.

### 4) Image response

- a. Response to the S+ image, then step 5.
- b. Response to the S- image, then step 6.

### 5) Reinforcement

- a. Images are removed.
- b. Liquid food is delivered (usually pump turned on to deliver 20µl), a tone is played (usually 3 KHz for 1000 ms) and the tray light is turned on.
- c. Wait for tray entry.
- d. Then step 8.



- 6) Time-out**
  - a. Images are removed.
  - b. House light is inverted (usually turned on) and wrong tone is played if selected.
  - c. Wait for the time-out duration (usually 5s)
  - d. Then step 7
  
- 7) Time-out end**
  - a. House light is inverted (usually turned off).
  - b. Then step 8
  
- 8) ITI**
  - a. Turn off tray light.
  - b. Wait for ITI period (usually 20s)
  - c. Then step 9
  
- 9) Trial initiation**
  - a. Turn on tray light
  - b. Wait for tray-report / feed-tray to be entered (accompany by a click if selected) and then exited.
  - c. Then step 3
  
- 10) Finish**
  - a. All lights are turned out.
  - b. All images are removed.
  - c. Data recording ceases.
  - d. Data is transferred for analysis

## 02 Installing the PD Software

If the ABET II Software and Hardware Drivers have not been installed, please do so at this time using the Installation Manual provided with that product.

The Mouse Touch PD Schedules and Analysis files may be on the same or a separate disk or sent electronically. Simply copy these files to the appropriate folders on your computer. The default locations are given below. These can be verified in the Preferences Window that is accessible from the “Edit” menu.

Schedules:

C:\.....My Documents\Lafayette Instrument\ABET II\ABET Schedule Folder

Analysis Sets:

C:\.....My Documents\Lafayette Instrument\ABET II\ABET Analysis Folder

### 2.1. Accessing the PD Touch software

Some familiarity with the ABET II Software product is necessary for running the PD Touch task package. The recommended approach would be to work through the Tutorials in the ABET help/manuals. Since you do not need to create schedules for the PD tasks, once you are comfortable with loading, running and saving experiments, you could proceed with this manual. Feel free to revert back to the ABET manuals whenever you have any questions or concerns.

## 03 Setting Up the Hardware

This manual will take you through the steps needed to get the touchscreen boxes up and going with the PD task.

To help you configure and test the hardware, we have a testing schedule “A\_MouseTestLines-2x1”. This will allow you to quickly test all the available lines on a standard Touch chamber.

### 3.1. Connecting the hardware

1. Follow the hardware manual provided with your touchscreen boxes to install and connect your boxes to the controlling computer.
2. Locate the feeder so it is opposite the screen (as opposed to in front of the screen).
3. Insert the 2 x 1 Mask
4. Before starting the system, note the order of power up should be:
  - a) Switch the system power on and start up the controller PC
  - b) Switch on the 81430 ‘CONTROL’ switch for all the units on the system. A green LED indicator will flash whilst the 81430 Core unit initializes. When fully green the system is ready.
  - c) Start the ABET Cognition Software
  - d) Switch on the 81430 ‘CHAMBER’ switch to apply power to the chamber peripherals. A green light indicates that the chamber 24V power is switched on and OK. Note, the chamber power is also enabled by the 81430 control system. It will not switch power to the chamber until the 81430 ‘Control’ is running. It is possible to leave the Chamber power switched On and the power be managed by the CONTROL system. If at any time the green chamber power indicator switches to Red, then there is an overcurrent fault somewhere in the chamber which must be investigated. When the red light indicates, power to the chamber will be switched off and must be recycled (via the Chamber power switch or otherwise) to reset this fault. If the fault persists check the chamber wiring with a process of elimination: disconnect peripherals one by one, recycling the power each time, until the red light no longer appears, thus isolating the problematic part of the system.

The Mouse Touch Screen box should be configured as shown on the following table (Table 1).

**Note:** The PD schedule will use Houselight#1 as the houselight in the experiment. In the configuration shown in Table 1 this is the new under lid light. If you would prefer, for backwards compatibility, to use the overhead LED change the names so Houselight #1 becomes Houselight #2 and Houselight #2 becomes Houselight #1.

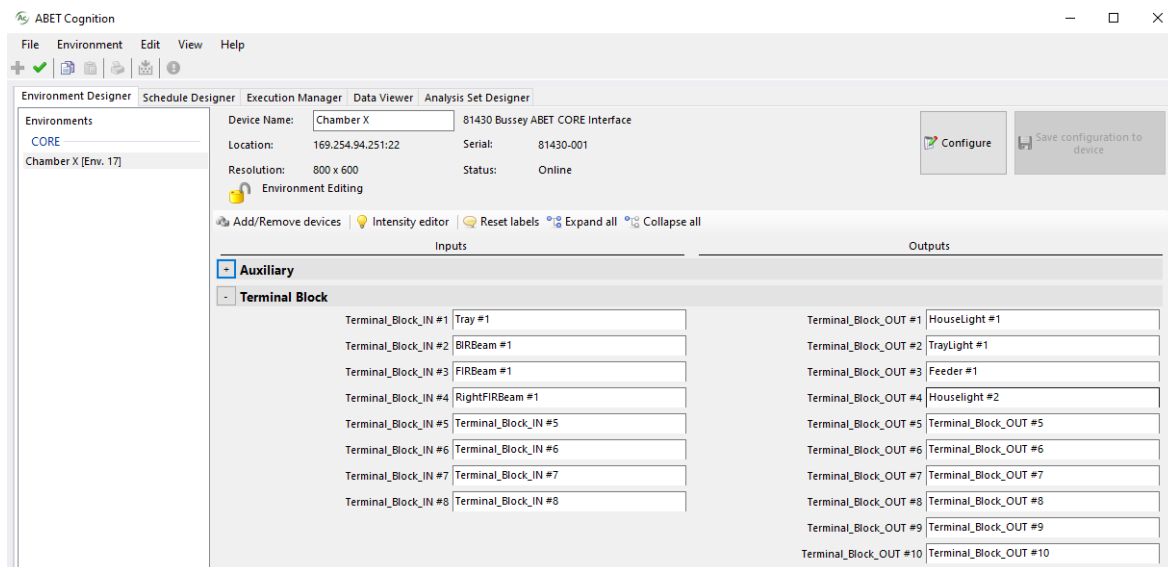


Table 1

### 3.2. Testing the hardware

It is recommended that this quick test of the hardware be done prior to every day's training or testing.

A\_MouseTestLines-2x1 will allow you to test all grid spaces and I/O, without a need to be near the computer.

- From the main the Execution Manager, select the boxes you wish to test.
- Click the 'Open/Load Schedule' icon and select 'A\_MouseTestLines-2x1' and click 'Open'.
- Click the play icon. The boxes are now ready to test. Table 2 shows the response you should achieve by activating the various input lines.

Table 2

Schedule	Inputs to activate	Output response
A_MouseTestLines-2x1	Simulate a Touch in Grid 1 (touch in the left white frame)	Toggles on/off a white square image in Grid 1
	Simulate a Touch in Grid 2 (touch in the centre white frame)	Toggles on/off a white square image in Grid 2
	Push the feed-tray door	House-light and tray-light illuminate. Feeder is turned on and a 3Hz sound is pulsed for 1 second
	Release the feed-tray door	Houselight, tray light and feeder are turned off.
	Block Back IR activity beams	House-light illuminates
	Release Back IR activity beams	House-light turns off
	Block Front IR activity beams	A 250Hz sound is turned on
	Release Front IR activity beams	250Hz sound is turned off
	Block Right Front IR activity beams **	Tray-light illuminates
	Release Right Front IR activity beams **	Tray-light turned off

\*\* This line is only used when the feeder is in the Autosshaping position in front of the screen and the beam switch is moved to the two front beam position (see hardware manual). This is not required for the PD Task.

### 3.3. Cleaning, priming and calibrating the feeder (liquid pump)

Depending on whether you choose to water or food deprive your mice to increase motivation for the task, we would recommend using a liquid reward of either 10% condensed milk (CM – for water deprived mice) or Yazoo (or an equivalent premade milkshake available to you) strawberry milkshake (SM – for food deprived mice). Both rewards are sticky and the feeding tubes should be cleaned after each operant session. A prime / clean override switch has been provided on the pump to make this easier.

**Cleaning Procedure:**

- a For each box to be cleaned place warm distilled water in the pump reservoir. Disconnect the feed-tube and needle from the reward tray and place the end in a collecting vessel. (Pliers and a slight twisting motion is required to remove the needle from the guide tube.) Alternatively hold the reward tray facedown over a container to catch the flushed through water.
- b Power up the interfaces and boxes then toggle the Prime/Clean switch on the pump and allow it to pump water through the tubes for at least 15s or until the tubing appears to be clean. Wipe out the reward tray.

Prior to the start of an experiment, the feeder will have to be primed to ensure that there is food delivered on the first feed.

**Priming Procedure:**

- a Power up the interfaces and boxes.
- b For each box to be primed place the reward liquid (CM or SM) solution in the pump reservoir. Optionally disconnect the feed-tube and needle from the reward tray and place the end in a collecting vessel.
- c Toggle the Prime/Clean switch on the pump and allow it to pump the CM solution until it is dripping from the tube.
- d Either reconnect the tubing to the reward tray or wipe away the excess food from the reward tray.

Each liquid feed system will vary a little. This small difference is not usually of concern for the PD task, but if it is vital that the same amount of liquid is dispensed to each box, a calibration schedule has been set-up for you.

**Feeder Calibration Procedure:**

- a Prime each system as described above, removing the feed-tube and needles from the reward tray (a-d).
- b Set the end of the feed-tube and needle to deliver food into a weighed container.
- c From the 'Execution Manager' set the touch box(es) to be primed to run the protocol 'FeederCal-Mouse Touch' with the variable "Prime" in the Properties tab set to False'. Set the CMPulse to the amount you want to deliver for each reward (usually 800ms).
- d Run (F5) the schedule. The schedule will pump the liquid through the tubes for 10 x CMPulse bursts
- e Weigh the liquid (should weigh  $(10 \times 7\mu\text{l}) = 0.07\text{g}$  [ $(5 \times 20\mu\text{l}) = 0.1\text{g}$ ]).
- f If necessary adjust the 'CMPulse' time in the 'FeederCal-Mouse' Properties tab. Reload the schedule, 'FeederCal-Mouse', and modify the variable then re run the schedule with the new variable. See example below.
- g Repeat c-f until 0.07g [0.1g] is delivered with a resolved 'CMPulse' time.
- h You will need to enter this new 'CMPulse' time into the appropriate schedules, default variable value, using the 'Schedule Designer'. Again see the ABET manual on how to edit or set the default values for variables.

**Calculation Example:**

Default CMPulse is 800 ms (x5) = 2000ms

Result: measured volume by weight = 0.13g or 130µl based on the default CMPulse value.

So determine the desired pulse length based on actual delivery rate...

$130\mu\text{l (actual)} \div (2000\text{ms}) = 0.065\mu\text{l/ms (delivery rate)}$

$\text{CMPulse (desired)} = (100\mu\text{l} \div 0.065\mu\text{l/ms}) \div 5 = 308\text{ms (rounded up)}.$

Use 308ms for the value of CMPulse and retest the calibration.

**Note:** The accuracy of the feeder(s) is dependent on the viscosity of the reward liquid, age, diameter and plasticity of the tubing in the pump as well as the accuracy of the pulse delivered.

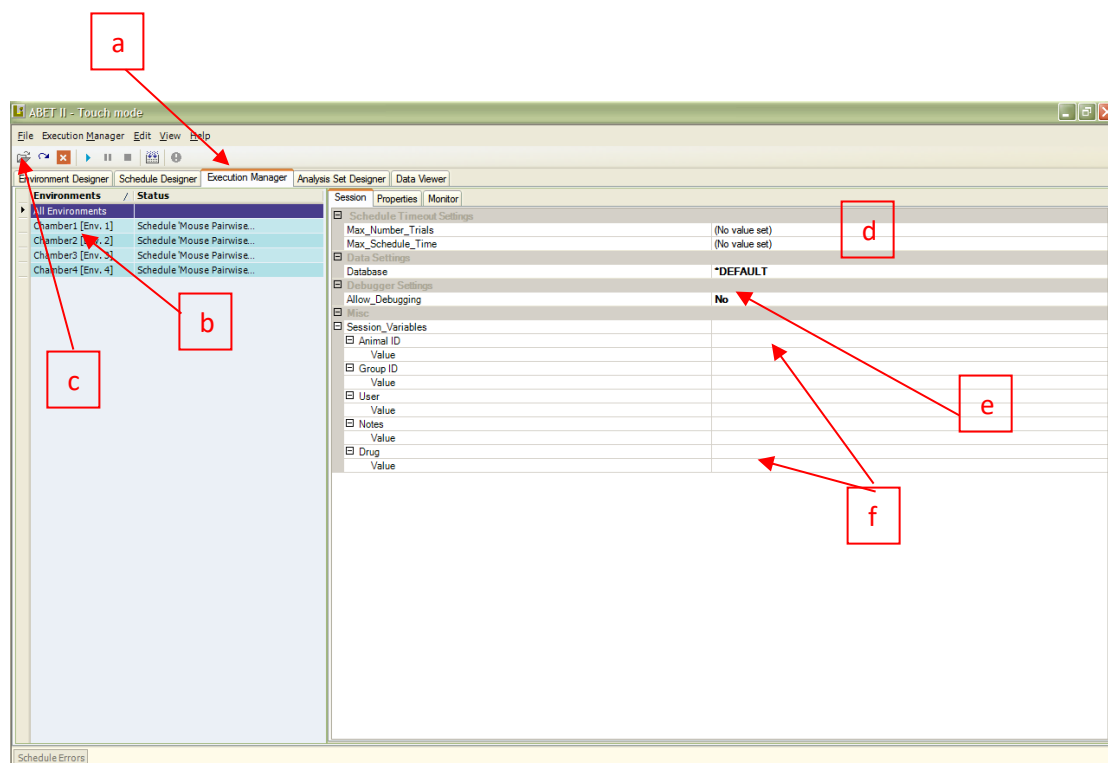
## 04 Running Schedules

This chapter will cover in general how to run and view the schedules provided and also how to change the variable parameters. Details of the individual schedules provided and how to use them are in the following chapters.

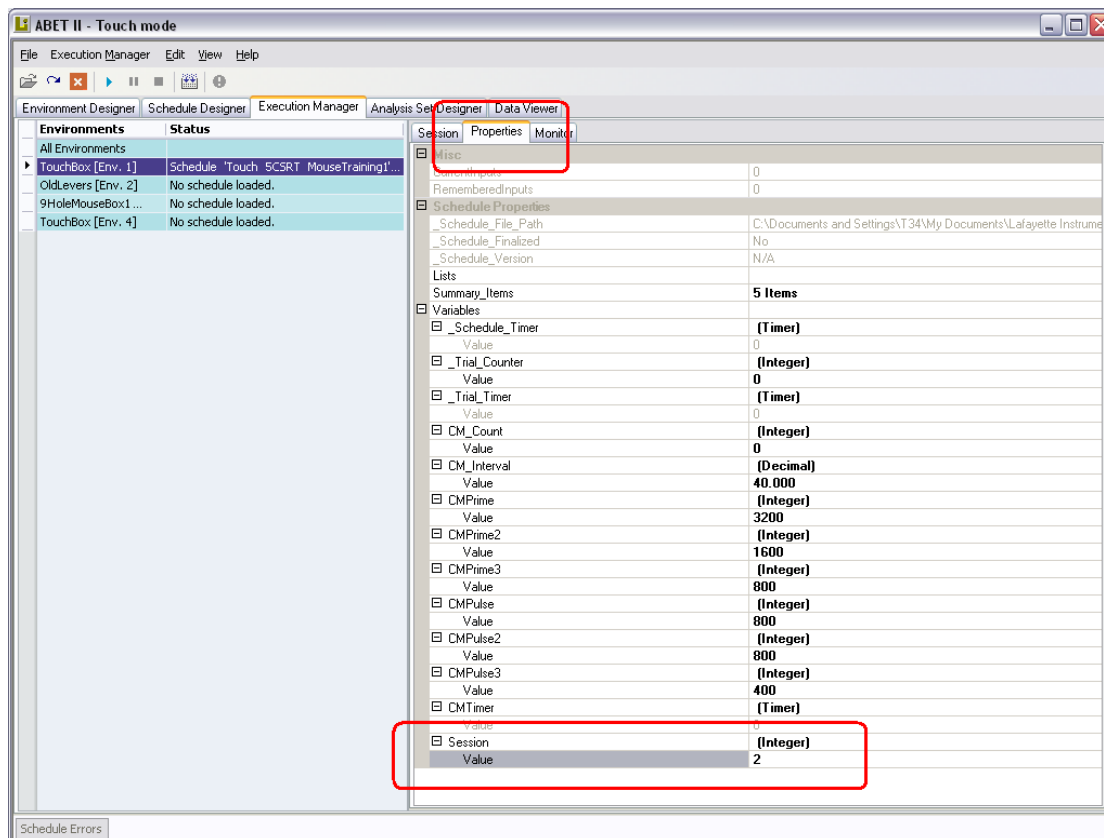
### 4.1. Preparing Schedules

Power-up the system as described in section 3.1 above.

- Click on the Execution Manager Tab
- Select the boxes required (all boxes can be selected by clicking on 'All Environments').
- Load the schedule required (Note: different schedules can be loaded onto different boxes to run at the same time. Simply select each box and load a schedule in turn.)
- Fill in the Maximum trials and maximum schedule time if required (if either is not required as an end criteria, then leave as 'No value set')
- Note: The 'Allow Debugging' is meant to aid you debug any schedules you have written or altered. It should be left as 'No' when running an actual experiment.
- Add any experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program.



- Click on the 'Properties' tab and change any variables that need changing from their default value. (Values that can be changed are indicated in chapters 5 and 6.) Changing the Default value for these variables will be covered in section 4.3 below.



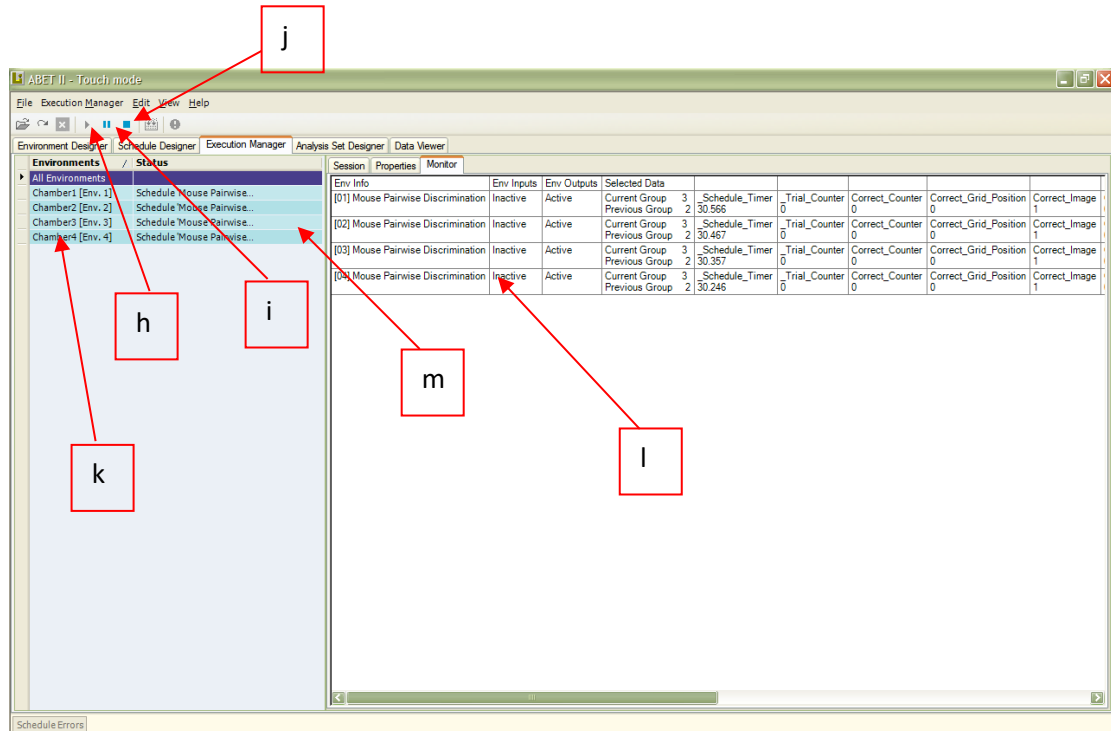
**Note:** Experiments can be set in advance using the 'Experiment' Tab.

The Schedule is now ready to play.

## 4.2. Running Schedules

- h. Select the boxes you want to start and click on the play button. (Boxes can be started individually or together).
- i. You can temporarily pause the schedule by clicking the 'pause' button.
- j. You can terminate the program by clicking the 'stop' button. (Make sure you have only selected the boxes you want to stop and do not have 'All Environments' selected.)





- k. You see a detailed view of what is happening in each box by selecting the individual boxes.
- l. If 'All Environments' is selected an overview of the experiment progress is shown. How to change what is shown here is explained in section 4.3.
- m. The Status window will indicate 'finished' once the experiment is complete. Once the data has finished downloading, click x to unload the schedule.
- n.

#### 4.3. Changing Variable Defaults.

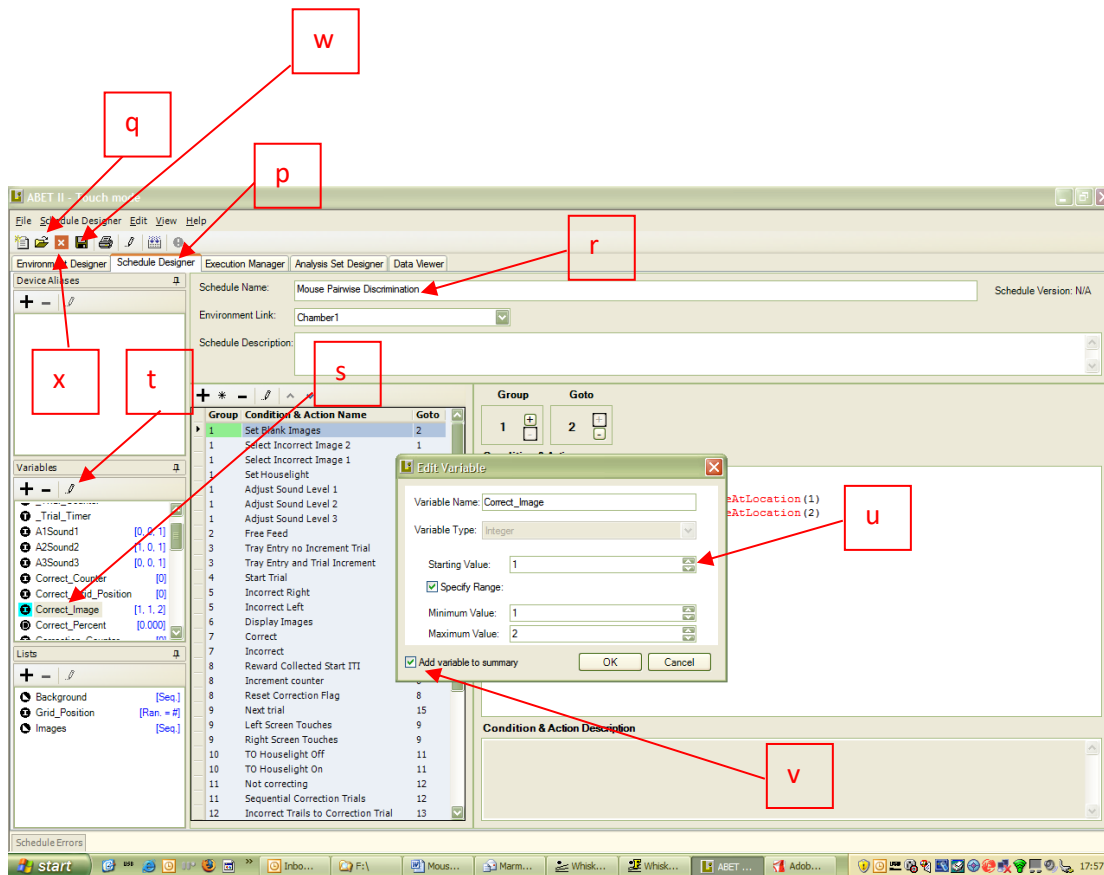
To change the default values of the variables that can be changed in the program (see section 6.4 and table 3):

**Note:** *Be careful not to change any variables not given in as changeable in the manual, as you may affect how the program runs.*

- p. Go to the Schedule Designer
- q. Open the relevant schedule
- r. If you want to save this as a separate schedule then type in a new name for the schedule. (Alternatively, you can create a new version of the same schedule name from the 'Schedule Designer' menu by selecting 'Add/Change Version').
- s. Find the relevant variable
- t. Click edit
- u. Change the value
- v. You can also change whether this is shown in the summary 'monitor' while the schedules are running
- w. When you have made all the required changes save the amended schedule

x. Unload the schedule

Values in the 'List' (see 6.4) can be similarly changed.



#### 4.4. Finalize/Lock Schedules

All the schedules have been provided to you in an open format for you to change to your own requirements. It is, however, a good idea to 'lock' the programs before using them for experiments, so they can't be changed in the future and you will always know exactly what version of a schedule was used.

To lock a schedule, first give it a version number by going to the 'Schedule Designer' menu and select 'Add/Change version'. Next lock that version by going to the 'Schedule Designer' menu and selecting 'Finalize Version'

## 05 Training Procedures

Prior to the habituation and training procedures, make sure that the Touch Screen box has been set-up to run the Mouse Pairwise schedules:

- Reward area opposite the screen.
- 2x1 Mask inserted.
- Best practice to test the hardware prior to every training or testing day (see section 3.2). Ensure that the expected inputs and outputs are observed.
- All schedules are found in the C \Documents\Lafayette Instrument\ ABET II\ABET Schedule Folder\80540A folder.
- 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. Best practice is to also check that milkshake is still flowing between each animal being run in the touchscreen chamber.
- Reward is usually pre-made strawberry milkshake.

### The basic training schedule is illustrated below:

The training program illustrated assumes food deprivation and using strawberry milkshake as a reward. Performance will be improved using liquid reward over pellet reward for two reasons:

- i. A very small reward can be given each time, reducing risk of satiation, and associated reduced motivation.
- ii. Mice are quick to consume the reward and are ready to attend to the test as soon as exiting the reward tray.

This training schedule shown below details the training as described by

**Horner AE, Heath CJ, Hvoslef-Eide M, Kent BA, Kim CH, Nilsson SR, Alsiö J, Oomen CA, Holmes A, Saksida LM, Bussey TJ.** (2013) The touchscreen operant platform for testing learning and memory in rats and mice. *Nat Protoc.* Oct;8(10):1961-84. DOI: [10.1038/nprot.2013.122](https://doi.org/10.1038/nprot.2013.122)

### 5.1. Food Deprivation/Handling (3 days)

- i. Day1-3 weigh mice and handle for 1 minute.
- ii. Make sure your mice are food restricted to 85-90% of their free-feeding weight prior to the start.
- iii. Provide strawberry milkshake to the mice in their home cages for 2 days immediately prior to training.
- iv. 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.
- v. 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.

- vi. Day4 start session1. Weigh/handle daily & ensure mice do not fall below 85% (or according to limits defined by HO project license).

Pre-protocol Steps:

- Ensure dispenser contains enough food.
- Ensure correct mask is in place.
- Ensure box power pack and computers are turned on.
- Test hardware and touchscreen are operational

Post-protocol Steps:

- Ensure box power pack and computers are turned off.
- Replenish food as required.
- Clean box on a regular basis i.e. replace paper tray liners, clean tray + bottom of cage, wipe clean Perspex box, pump water through liquid dispenser tubes.

Generally, mice are given 1 session per day.

**NOTE:** Previous versions of this task have 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. Consequently, our recommended delivery of milkshake is now 800ms (20 $\mu$ l).

## 5.2. Habituation

This procedure is to familiarise the mouse with the chambers and where food will be rewarded. The schedule will also monitor the activity of the mouse.

**Habit 1:** The Mouse is left in the chamber for a 10 min session, with all lights turned off. No stimulus or reward presentation. The activity of the mouse is monitored. It is critical that the mouse is removed from the chamber as soon as the habituation is complete.

In the following Habit 2 training schedules the mouse is trained to associate reward being delivered with the tray light illuminating and (optionally) a tone being played. The tone played is set from the 'Audio' List 'Correct Tone' (default 3 KHz). The volume can be adjusted by means of 'Tone Intensity' variable. The tone duration is set from the 'Tone Duration' variable (default 1000 ms).

**Habit 2a:** The Mouse is left in the chamber for a 20 min session. The tray-light is initially turned on. A Tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivery 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 turned on, a tone is played and SM is then delivered for 800ms (20 $\mu$ l). 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 chamber as soon as the habituation is complete.

**Habit 2b:** The Mouse is left in the chamber for a 40 min. Reward presentation is the same as described in Stage 1b. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

**Procedure for Habituation 1:**

- a Insert the 2 x 1 blank.
- b Introduce food into the reward tray.
- c From the 'Execution Manager' set the Mouse touch screen box(es) to run the schedule 'A\_Mouse Pairwise Habituation 1'. Set the Session: Max\_Schedule\_Time to 10 minutes (600s)
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule. The schedule will monitor IR beam breaks, food-tray entries and touches to the screen
- f At the end of 10 minutes the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Tip:** Save the 'Execution Manager' configuration for the Habituation 1 training by clicking 'File/Save Experiment'. This way experiment and its settings for Habituation 1 trainings can be easily loaded using 'File/Open Experiment'. This experiment can also be used as a basis for subsequent training and test configurations, which can also be saved individually following modification.

**Note:** Each chamber can be run independently from each other, therefore each Mouse can be progressed through the training at its own speed.

**Duration:** 1 session, 600 s (10 minutes)

**Trial number:** Unlimited

**Criterion:** None

**Procedure for Habituation 2a:**

- a Insert the 2 x 1 blank.
- b Prime the feeder with the strawberry milk (see 3.3 above).
- c From the 'Execution Manager' set the same touch screen box(es) to run the schedule "A\_Mouse Pairwise Habituation 2". Set the Session: Max\_Schedule\_Time to 20 minutes (1200s)
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e If, as a result of your calibrations (section 3.3 above) you want to change the pulse times, change the values of 'Prime Feed Time' and the 'Feeder Pulse Time' either from the 'Properties' tab. (Alternatively you can change the default values by opening the program in the 'Schedule Designer' and changing the values from the 'Variable list'. Change any other user changeable variables as required (see table 3 below).
- f Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule. The schedule will deliver food as described above and monitor IR beam breaks, food tray entries, the latency to collect the food and the time spent eating.
- g At the end of 20 minutes the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

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

**Trial number:** Unlimited

**Criterion:** Drinks milkshake (none observed in tray).\*

*\*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.*

#### **Procedure for Habituation 2b:**

Same as for 2a, except the schedule time is increased to 40 mins.

**Duration:** usually 1 sessions, 2400 s (40 minutes)

**Trial number:** Unlimited

**Criterion:** Drinks milkshake (none observed in tray).

### **5.3. Initial Touch Training/ Pavlovian Training (Pairwise discrimination)**

Stimuli are displayed randomly on the screen one at a time. (Stimuli consist of various shapes. One stimulus is presented at a time, on one side of the screen. The other side of the screen is left blank. The left or right position is chosen pseudo randomly, such that any image will not be displayed on the same side more than 3 times in a row.) After a delay (variable Image Time – default 30s) the image is removed and food is delivered (variable 'Feed Pulse Time – default 800ms). Food delivery is accompanied by illumination of the tray light and a tone. [The tone played is set from the 'Audio' List 'Correct Tone' (default 3 KHz). The volume can be adjusted by means of 'Tone Intensity' variable. The tone duration is set from the 'Tone Duration' variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default 20s) another image is displayed. If the mouse touches the screen whilst the image is displayed (where the image is displayed), the image is removed, a tone will be played and 3 x food delivered immediately. Collection of this reward again starts the ITI and then progresses to the next image.

Touch training is by default performed with the house light off. This can be changed by setting the variable 'House Light Normally On' to 'True'.

#### **Procedure for Initial Touch Training:**

- a Insert the 2 x 1 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the 'Execution Manager' set the same touch screen box(es) to run the schedule 'A\_Mouse Pairwise Initial Touch Training'. Set the Max\_Schedule\_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30.
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e If, as a result of your calibrations (section 3.3 above), you want to change the pulse times, change the values of 'Prime Feed Time' and the 'Feeder Pulse Time' either from the 'Properties' tab.

(Alternatively you can change the default values by opening the program in the 'Schedule Designer' and changing the values from the 'Variable list'.) Change any other user changeable variables as required (see table 3 below).

- f Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule.
- g At the end of 60 minutes or 30 trials completed (which ever happens first) the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Duration:** Number of sessions varies across mice, 3600 s (60 minutes)

**Trial number:** 30

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

#### 5.4. Must Touch Stimuli Pairwise discrimination training

Stimuli are displayed randomly on the screen one at a time. (Stimuli consist of various shapes. One stimulus is presented at a time, on one side of the screen. The other side of the screen is left blank. The left or right position is chosen pseudo randomly, such that any image will not be displayed on the same side more than 3 times in a row. The 'images' List must not include any of the images to be used in discrimination/reversal trials.) The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. [The tone played is set from the 'Audio' List 'Correct Tone' (default 3 KHz). The volume can be adjusted by means of 'Tone Intensity' variable. The tone duration is set from the 'Tone Duration' variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default 20s) another image is displayed.

##### Procedure for Must Touch Training:

- a Insert the 2 x 1 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the 'Execution Manager' set the same touch screen box(es) to run the schedule 'A\_Mouse Pairwise Must Touch Training'. Set the Session: Max\_Schedule\_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e Change any user changeable variables as required (see table 3 below).
- f Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule.
- g At the end of 60 minutes or 30 trials completed (which ever happens first) the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Duration:** Number of sessions varies across mice, 3600 s (60 minutes)

**Trial number:** 30

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

### 5.5. Must Initiate Pairwise discrimination training

A free delivery of food is made 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. Optionally this initiation of trials can be accompanied by a click on tray entry, to give the mouse positive feedback. (Stimuli consist of various shapes. One stimulus is presented at a time, on one side of the screen. The other side of the screen is left blank. The left or right position is chosen pseudo randomly, such that any image will not be displayed on the same side more than 3 times in a row.) The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches the blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. [The tone played is set from the 'Audio' List 'Correct Tone' (default 3 KHz). The volume can be adjusted by means of 'Tone Intensity' variable. The tone duration is set from the 'Tone Duration' variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default 20s) the tray light is again illuminated. The mouse must nose poke and exit the reward tray before the next image is displayed.

#### Procedure for Must Touch Training:

- a Insert the 2 x 1 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the 'Execution Manager' set the same touch screen box(es) to run the schedule 'A\_Mouse Pairwise Must Initiate Training'. Set the Session: Max\_Schedule\_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e Change any user changeable variables as required (see table 3 below).
- f Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule.
- g At the end of 60 minutes or 30 trials completed (which ever happens first) the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Duration:** Number of sessions varies across mice, 3600 s (60 minutes)

**Trial number:** 30

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

### 5.6. Punish Incorrect (Pairwise discrimination training)

As for previous training described above, except if a mouse touches the opposite side of the screen to the stimulus (blank side) the house light will be inverted for a time out period (default 5s) and no reward is given. There is also an option to play a low tone to indicate a wrong response (default off). [To switch on make 'Wrong Tone On' = 1. Duration and Intensity are set from 'Wrong Tone Duration' and 'Wrong Tone Intensity' respectively].] Once the time out period finishes the house light is inverted again and the ITI period begins. [Optionally the mouse must then complete a correction trial (the image and position from the previous trial are kept the same) and must repeat the same trial until a



correct response to the image is made, at which point it will receive a tone and reward. However, training without this correction is more usual and is the default – ‘Correction Trials Set’ - False] (There is also an option to only start a correction trial when x incorrect trials in a row are made.) The ITI before the correction trial is determined from the Corr ITI variable and can be shortened if a correction trial is used).

**Procedure for Punish Incorrect Training:**

- a Insert the 2 x 1 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the ‘Execution Manager’ set the same touch screen box(es) to run the schedule ‘A-Mouse Pairwise Punish Incorrect Training’. Set the Session: Max\_Schedule\_Time to 60 minutes( 3600s). Set the Max No. Trials to 30.
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the ‘Edit’ menu, selecting ‘Preferences’ and clicking on ‘Session Field’ prior to loading a program
- e Change any user changeable variables as required (see table 3 below).
- f Introduce the subject, Mouse (Mice), and Run (🏃) the schedule.
- g At the end of 60 minutes or 30 trials completed (which ever happens first) the ‘Execution Manager’ will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Duration:** Minimum 2 sessions, 3600 s (60 minutes)

**Trial number:** 30

**Criterion:** Completion of 24/30 trials or better within 60 min for 2 consecutive sessions. If after 30 sessions (30 days) the mouse does not reach criterion for “Punish incorrect”, remove it from study.

## 5.7. Discrimination Training: Acquisition with correction trials

A free delivery of food is made and the tray light is turned on. The mouse must nose poke and exit the reward tray to begin the first trial. Optionally initiation of trials can be accompanied by a click on tray entry, to give the mouse positive feedback. A trial begins with the presentation of two novel stimuli on the screen; one is programmed as being correct (S+) [default image 1] and one as being incorrect (S-). Whether the S+ is on the right or left is determined pseudo-randomly, such that the image will not be displayed on the same side more than 3 times in a row. The mouse must nose poke the correct stimulus to elicit the food delivery response. Food delivery is accompanied by illumination of the tray light and a tone [The tone played is set from the ‘Audio’ List ‘Correct Tone’ (default 3 KHz). The volume can be adjusted by means of ‘Tone Intensity’ variable (default 20). The tone duration is set from the ‘Tone Duration’ variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default 20s) the tray light is again illuminated. The mouse must nose poke and exit the reward tray to start the next trial and cause the images to be displayed again.

If the mouse nose-pokes the incorrect stimulus, no pellet will be delivered, and a time out will follow before the mouse is given the opportunity to complete a correction trial. There is also an option to play a low tone to indicate a wrong response (default off). [To switch on make ‘Wrong Tone On’ = 1. Duration and Intensity are set from ‘Wrong Tone Duration’ and ‘Wrong Tone Intensity’ respectively.] [During the time out period the house light is inverted (if mice have

been trained with the house light on, then it is turned off, if the mouse has been trained with the house light off then most of the trial is conducted with the house light off and it will be switched on for the timeout period.)) Once the timeout period finishes the house light is inverted again and the ITI period begins. Correction trials will ensue until the correct stimulus is chosen. A correction trial consists of re-presentation of the stimulus array in the same L-R configuration. Correction trials are not included in the % correct figure. (There are options to not have correction trials or to only started a correction trial when x incorrect trials in a row are made.) The ITI before the correction trial is determined from the Corr ITI variable and can be shortened (Corr ITI –default 20s).

**Note:** You might wish to change the default images to better matched pairs – see sections 6.4 and 6.5.

#### **Procedure for Pairwise Discrimination:**

- a Insert the 2 x 1 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the 'Execution Manager' set the same touch screen box(es) to run the schedule 'A\_Mouse Pairwise Discrimination'. Set the Session: Max\_Schedule\_Time to 60 minutes, i.e. 3600s. Set the Max No. Trials to 90.
- d Add any other experimental details that should be recorded. Fields have already been provided for Animal ID, Drug, Group ID and User. You can create further fields by going to the 'Edit' menu, selecting 'Preferences' and clicking on 'Session Field' prior to loading a program
- e Change any user changeable variables as required (see table 3 below).
- f Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule.
- g At the end of 60 minutes or 30 trials completed (which ever happens first) the 'Execution Manager' will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Duration:** Number of sessions varies across mice, 3600 s (60 minutes)

**Trial number:** 30

**Criterion:** 24/30 trials correct within 60 min, for 2 consecutive days. If after 30 sessions (30 days) the mouse does not reach criterion for "Acquisition", remove it from study.

### **5.8. PD baseline**

Baseline sessions are run either immediately after a mouse reached the PD acquisition criteria, or once all the mice in the experiment have reached the PD acquisition criteria. (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 PD task.) Baseline sessions are identical to the PD task acquisition ones.

**Duration:** 2 sessions\*, 3600 s (60 minutes)

**Trial number:** 30

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

\* Typically, only 2 sessions are required during baseline. However, you should run statistics and ensure that your groups do not differ. If they do, continue running your mice until their performance is stable and

the same. If your groups remain significantly different across 10+ sessions, stop your experiment on this step. With a few exceptions, you cannot draw conclusions on the reversal stage if the performance of your groups differ during the baseline sessions

User changeable variables in the PD schedules – default/recommended values							
Schedule- Variable	Habit 1	Habit 2 a(b)	Initial Touch	Must Touch	Must Initiate	Punish Incorrect	PD
<b>**Session Length</b>	10 min	20 (40) min	60 min	60 min	60 min	60 min	60 min
<b>**Max Trials</b>	NVS	NVS	30	30	30	30	30
<b>Acclimatisation time</b>	-	0s	-	-	-	-	-
<b>Delay Time</b>	-	10s	-	-	-	-	-
<b>Feeder Pulse Time</b>	-	800ms	800ms	800ms	800ms	800ms	800ms
<b>Houselight Normally On</b>	-	False	False	False	False	False	False
<b>Prime Feed Time</b>	-	6000	-	-	-	-	-
<b>Pulse Tone</b>	-	True	-	-	-	-	-
<b>Tone Duration</b>	-	1000ms	1000ms	1000ms	1000ms	1000ms	1000ms
<b>Tone Intensity</b>	-	20	20	20	20	20	20
<b>Image Time</b>	-	-	30s	-	-	-	-
<b>ITI</b>	-	-	20s	20s	20s	20s	20s
<b>Time Out</b>	-	-	-	-	-	5s	5s
<b>Correction Trials Set</b>	-	-	-	-	-	False	True
<b>No. Incorrects beforeCorrection Trial</b>	-	-	-	-	-	(1)	1
<b>Correct Image</b>	-	-	-	-	-	-	1
<b>Corr ITI</b>	-	-	-	-	-	(5)	20
<b>Initiate Click On</b>	-	-	-	-	0(off)	0(off)	0(off)
<b>Click Intensity</b>	-	-	-	-	15	15	15
<b>Click Time</b>	-	-	-	-	200 ms	200 ms	200 ms
<b>Wrong Tone On</b>	-	-	-	-	-	0(off)	0(off)
<b>Wrong Tone Intensity</b>	-	-	-	-	-	52	52
<b>Wrong Tone Duration</b>	-	-	-	-	-	500 ms	500 ms

\*\* Schedule Time and Maximum Trials have to be set from the Execution Manager before each program run. By default they are unlimited. Other variables can be changed from the Execution Manager or from the Schedule Designer (see chapter 4)  
'-' Not applicable to this schedule

**Explanations:**

Acclimatisation time – Time before the first priming feed  
Delay Time – Time between food delivery.  
Image Time – Time for which image is displayed  
Tone Duration – This can be set to 0 if no tone is required.  
Correction Trials Set – If false then no correction trials will take place  
No. Incorrects before Correction Trial – the number of incorrect trials to be made in a row before a correction trial ensues  
Correct Image – choice of 1 or 2. Selection of the correct image should be counterbalanced across the study and then changed for the reversal trial.  
Corr ITI – the ITI length used before a correction trial, following the timeout period.  
Initiate Click On and Click Time – control whether a click accompanies entry into the food try to initiate a trial  
Wrong Tone On and Wrong Tone Duration – control if a 250Hz tone is played when a wrong response is made, and for how long it is played.

**Table 3:** Session variables for the training schedules

## 06 Task Maintenance and Task Manipulations

### 6.1. Discrimination Training: Reversal

Immediately following completion of the PD baseline the subject can be tested on a reversal trial.

As for discrimination training/acquisition, but S+ and S- are reversed as follows:

Previously correct image, now incorrect

Previously incorrect image, now correct

This is achieved by changing the 'Correct Image' variable from 1 to 2 (if 1 was used for the task acquisition) or from 2 to 1 (if 2 was used for the task acquisition). You may wish to set up a separate schedule labelled something like 'PD\_Reversal\_1' to keep better track of which sessions were the reversals compared with acquisition sessions

**Duration:** 10 sessions immediately following completion of the PVD baseline, 3600s (60 minutes)

**Trial number:** 30 - You may wish to divide the first session into three days of 10 trials each.

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

#### *Tips and Tricks- When to reverse*

There are several different protocols for moving animals from the initial discrimination on to reversal; each has its advantages and disadvantages, depending on the needs of the experimenter:

- i. Once a mouse reaches criterion, move it on to reversal the next day. Advantages: All mice are at the same level, and no mice are over trained (given further training past criterion), when reversed. Disadvantages: Mice experience different numbers of training days before reversing. Mice are staggered, and so it is difficult to make decision on a day-to-day basis based on a group's mean performance levels. Because each mouse has a different number of training days, complete group learning curves for acquisition cannot be drawn, but trials/sessions/errors to criterion can be used for analysis.
- ii. Keep training all animals until all the animals in a group are at criterion level of performance. Then move onto reversal stage. Advantages: all mice are 'in synch'; all mice have same number of training days prior to reversal. Can draw and analyse full learning curves for acquisition. Disadvantages: some mice will be over trained. This and the protocol below are useful for, e.g., pharmacological studies on reversal learning, as all mice reverse at the same time and so can all be dosed on the same day(s).
- iii. We favour a compromise between the above two protocols, in which when a mouse reaches criterion, training stops and it is put 'on rest'. Then once the slowest mouse has reached criterion, all mice are baselined for two or three days to ensure they are all still performing at criterion. Then reverse. This has the advantages of protocol 1 but the mice are kept 'in synch' during reversal.
- iv. Rarely we use a fourth protocol which is to train all mice for XX sessions and then reverse irrespective of performance. Although all mice are kept 'in synch', this approach has mostly disadvantages, e.g., mice are all at different performance levels when reversed, and most seriously, some mice may not have acquired the initial discrimination to a level at which reversal is meaningful. However, we have used it in studies of, e.g., rapid progressive disease models where the top priority is that all mice are reversed at exactly the same age.

Choosing a protocol from the above is especially relevant to studies of discrimination and reversal, but of course the same principals can be applied to moving animals on from any task to any other task.

Session 1 of reversal.

When animals reverse, they suddenly find that the stimulus they thought was correct is no longer correct, and their performance levels drop to 20% or below. This can be demotivating, and it is often difficult to get animals to complete all trials in a session. This can be problematic, as you want the same number of trials in each point on your graph (cell in your analysis). Two tricks are 1. Feed an animal less the day before reversing, 2. Decide a priori to break session 1 of reversal into, say, 3 separate days. So if you usually give 30 trials/day, for the first 3 days of reversal give 10 trials/day. On day 4 you can probably give 30 trials again. Then combine days 1-3 into one 30-trials 'session' for analysis.

Calibrating performance levels

We have found that in some studies, for example pharmacological studies where we are attempting to improve performance, it is useful to be able to make the task harder. In visual discrimination/reversal and other tasks like PAL, we do this by 'morphing' (blending) the stimuli together. This works very well.

## 6.2. PD Maintenance

For maintenance, see Stage 5.6: "Punish Incorrect". Run this stage 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.

## 6.3. PVD and PVR subsequent time points

Run subjects in the same manner as stages 5.7, 5.8 and 6.1 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:** As described stages 5.7, 5.8 and 6.1. 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.

## 6.4. Changing Variable Values

The variable that can be changed are shown above in table 3. How to change these variables is covered in section 4.1 and 4.3.

**Note:** Be careful not to change variables other than those listed in table 3, as others may affect the running of the schedule.

## 6.4. Changing List Values

Lists create values which can change between trials. They are also used to set images and audio files. To access the lists, open the relevant task in the 'Schedule Designer'. Select the list and click the edit icon to edit as required. The 'Selection Mode' can be used to change the way the list is accessed. The following lists are available for change:

PD Task	List	Default Value	Selection Criteria	Comment
All	Background	1x Plain black image	NA	Not advised to change this
All	Grid Position	5x '1' (left window) and 5x '2' (right window)	Random equal number. No value selected more than 3 times in a row	Used to select the position of the correct image. Values of '1' and '2' must stay, but the number of each in the list (e.g. to deliberately introduce a side bias) and the selection criteria can change.
Pairwise Discrimination (PD)	Images	1. 'Marbles' image 2. 'Fan' image (see section 6.5 for alternatives)	NA	Variable 'Correct Image' selects which of these will be the correct image. Images can be replaced to introduce new stimuli.
All except PD, Habit1 and Habit2	Training Images	40 random images	Random equal number.	Individual images, total number of images and selection criteria can all be changed
All except Habit1	Audio	Correct Tone (3000Hz) Incorrect Tone (250Hz) Click	NA	Selects the sound played when a correct response, incorrect response (optional) and initiation nosepoke (optional) is made. Double click on the 'Details' to change to another tone/noise.

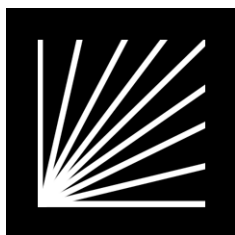
**Table 4:** List variables

## 6.5 Matching pairs for the mouse Pairwise/Visual Discrimination Task

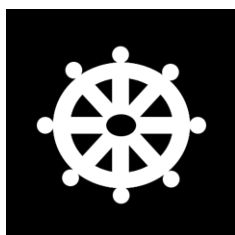
Although the images provided for the mouse pairwise discrimination task are reported in the literature as being a matched pair, with the Campden boxes mice have shown a bias towards the 'marbles' image, demonstrating the importance of defining the exact image size, resolution and position of the image. Work has been done by our customers to find alternative matched pair for the mouse pairwise discrimination task in the Campden Touchscreen chambers which you may prefer to use.

The 4 images immediately below can be found in the \Documents\Lafayette Instrument\ABET II\ABET Multimedia Folder\PD Training\New 2x1 Mouse.

Work done at Pfizer has shown the following pairs to match



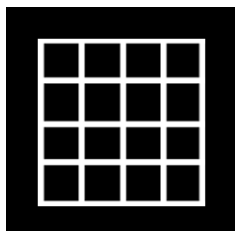
800x600-19-240x240



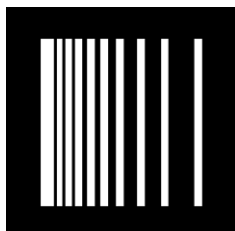
800x600-35-240x240

Work reported in a poster (available on request):

The Bussey/Saksida lab have also found the following are a good match (also located:



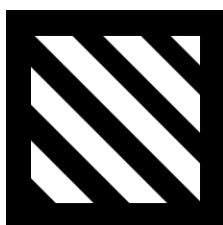
800x600-24-240x240



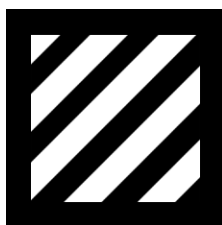
800x600-25-240x240

Ref: Horner AE, Heath CJ, Hvoslef-Eide M, Kent BA, Kim CH, Nilsson SR, Alsiö J, Oomen CA, Holmes A, Saksida LM, Bussey TJ. The touchscreen operant platform for testing learning and memory in rats and mice. Nat Protoc. 2013 Oct;8(10):1961-84.

Also frequently used by the Bussey/Saksida lab are these images:



800x600-left-diagonal  
-partial-228x228



800x600-right-diagonal  
-partial-228x228

Images located in the \Documents\Lafayette Instrument\ABET II\ABET Multimedia Folder\Image CPT\mouse\partial folder).

Ref: Luo, J., Tan, J., & Nithianantharajah, J. (2020). Associative Learning and Motivation Differentially Requires Neuroligin-1 at Excitatory Synapses. BioRxiv, 2020.01.01.890798. <https://doi.org/10.1101/2020.01.01.890798>



## 07 Analyzing Results

The Results from the experiments can be analyzed by using the ABET II Data Viewer Tab.

### 7.1. Raw Data

Viewing raw data is covered in some detail in Tutorial #2 of the ABET II manual beginning on page 42. Additional Data Tools are described in Tutorial #3 beginning on page 54. Many of the data elements listed below may be obtained from the Event Totals or by using the Reduced Data feature.

### 7.2. Filtering Data

One or more analysis sets have been provided with the PD Applications. To run an analysis set see Running the PRP measure on a single test session in the ABET II manual starting around page 80 and substitute the appropriate analysis.

### 7.3. Reports

All data screens, raw, event totals, reduced data, and analysis may be exported or copied for easy insertion in reports.

### 7.4. Data Elements Available with the standard applications

Individual Analysis by schedule is given below. See the ABET II manual on Executing Analysis routines for individual or group data sets.

#### PD Habit 1

##### For each 5 minute block:

- a. No. of times the Reward IR Beam broken
- b. No. of times the Screen IR Beam broken
- c. No. of times the animal Crossed from reward to screen (as detected by the chamber activity beams)
- d. No. of times the animal Crossed Screen to reward screen (as detected by the chamber activity beams)
- e. No. of Left Touches (i.e. the left screen window touched)
- f. No. of Right Touches
- g. No. of times the Food Tray Entered

##### End of experiment summary:

- h. No. of times the Reward IR Beam broken
- i. No. of times the Screen IR Beam broken
- j. No. of times the animal Crossed from reward to screen (as detected by the chamber activity beams)
- k. No. of times the animal Crossed Screen to reward screen (as detected by the chamber activity beams)
- l. No. of Left Touches (i.e. the left screen window touched)
- m. No. of Right Touches
- n. No. of times the Food Tray Entered

**PD Habit 2****End of experiment summary:**

- a. Session length (condition column)
- b. No. of trials completed
- c. No. of times the Food Tray Entered
- d. No. of Left Touches (i.e. the left screen window touched)
- e. No. of Right Touches
- f. No. of times the Reward IR Beam broken
- g. No. of times the Screen IR Beam broken

**PD Initial Train****End of experiment summary:**

- a. Session length
- b. Total no. of images displayed
- c. No. of correct responses
- d. No. of times the blank flanker window is touched (while and image is displayed)
- e. No. of Left touches during the ITI period
- f. No. of Right touches during the ITI period
- g. % left correct (% of the images displayed on the left that were touched)
- h. % right correct (% of the images displayed on the right that were touched)

**Across the whole experiment**

- i. Correct touch latency (latency to touch an image after it is displayed)
- j. Correct Left touch latency
- k. Correct Right touch latency
- l. Blank touch latency (Latency to the first touch of the blank window after image in other window displayed)
- m. Latency to collect reward resulting from a correct touch

**PD Must Touch****End of experiment summary:**

- a. Session length
- b. No. of correct responses
- c. No. of times the blank flanker window is touched (while and image is displayed)

- d. No. of Left touches during the ITI period
- e. No. of Right touches during the ITI period

**Across the whole experiment**

- f. Correct touch latency (latency to touch an image after it is displayed)
- g. Blank touch latency (Latency to the first touch of the blank image after image in other window displayed)
- h. Correct Left touch latency
- i. Correct Right touch latency
- j. Latency to collect reward resulting from a correct touch

**PD Must Initiate****End of experiment summary:**

- a. Session length (condition column)
- b. No. of correct responses
- c. No. of times the blank window is touched (while and image is displayed)
- d. No. of Left touches during the ITI period
- e. No. of Right touches during the ITI period

**Across the whole experiment**

- f. Correct touch latency (latency to touch an image after it is displayed)
- g. Blank touch latency (Latency to the first touch of the blank image after image in other window displayed)
- h. Correct Left touch latency
- i. Correct Right touch latency
- j. Latency to collect reward resulting from a correct touch

**PD Punish Incor(rect)****End of experiment summary:**

- a. Session length
- b. No. of trials completed
- c. Total number of correction trials
- d. % correct responses (not including correction trials)
- e. No. of Left touches during the ITI period
- f. No. of Right touches during the ITI period

**Across the whole experiment**

- g. Correct touch latency (latency to touch an image after it is displayed)
- h. Blank touch (incorrect) latency (Latency to the to touch of the blank image after image in other window displayed)
- j. Correct Left touch latency
- k. Correct Right touch latency
- l. Latency to collect reward resulting from a correct touch

**PC analysis (for Pairwise Discrimination task)****End of experiment summary:**

- a. Session length
- b. No. of trials completed
- c. Total number of correction trials
- d. % correct responses (not including correction trials)
- e. No. of Left touches during the ITI period
- f. No. of Right touches during the ITI period

**Blocks of 10 trials analysis**

- g. No of correct trials within the block of trials
- h. % correct over the whole experiment to end of that trial block
- i. Number of trials completed in the block
- j. % correct within the block of trials
- k. Total number of correction trials within the block

**Across the whole experiment**

*The following include responses during the correction trials:*

- l. Correct touch latency (latency to touch a correct image after it is displayed)
- m. Incorrect touch latency (latency to touch an incorrect image after it is displayed)
- n. Correct Left touch latency
- o. Correct Right touch latency
- p. Latency to collect reward resulting from a correct touch

## 08 Running Schedules Virtually

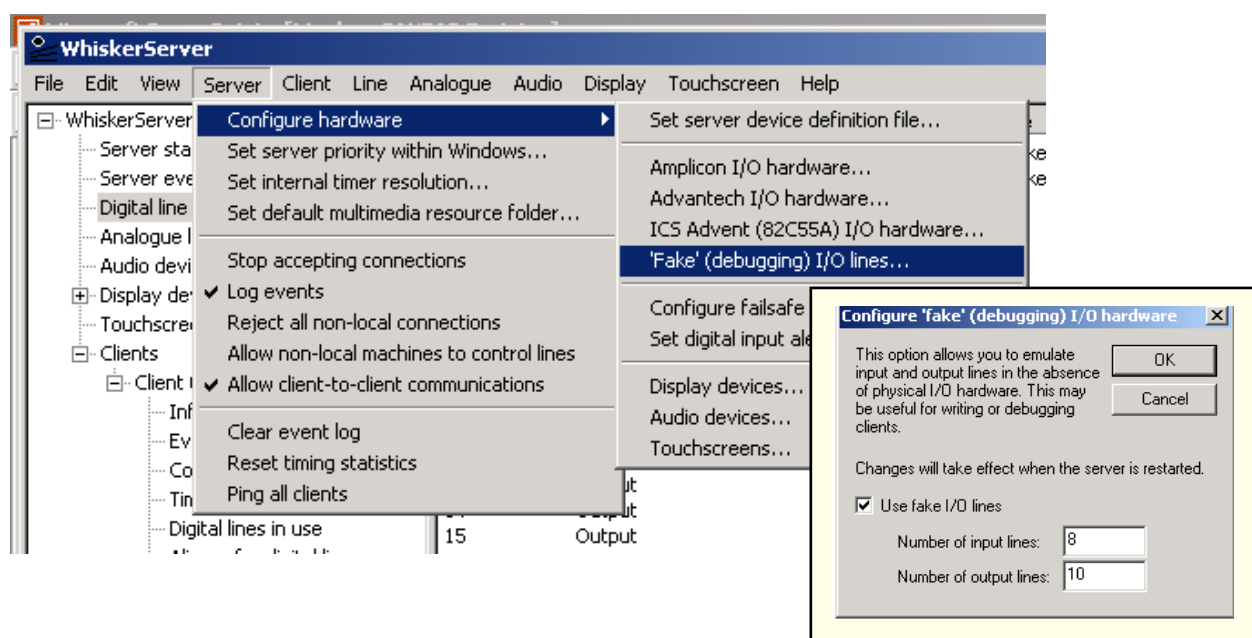
You may find it convenient to be able to look at and change schedules away from the lab. Your licence allows you to load both the ABET and Whisker software onto another computer for this purpose. This chapter will take you through the set-up to run the schedules manually.

Install the Whisker Multimedia and ABET II TOUCH software. If you don't have an install for these, please contact Lafayette Instrument Company or Campden Instruments for a download.

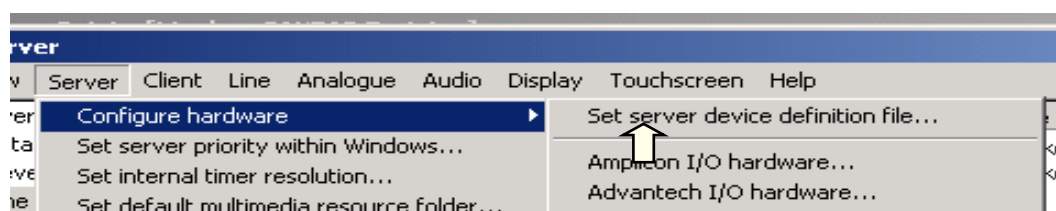
Because you have no real hardware attached, we will need to create some fake lines on the Whisker server.

**Note:** Once you have configured the display, when you launch Whisker the screen will go blank, as the primary screen has been selected for display. 'Alt-tab' will allow you to scroll round the programs you have opened, including the Whisker server. 'Alt-Esc' will allow you to access the 'Start' menu.

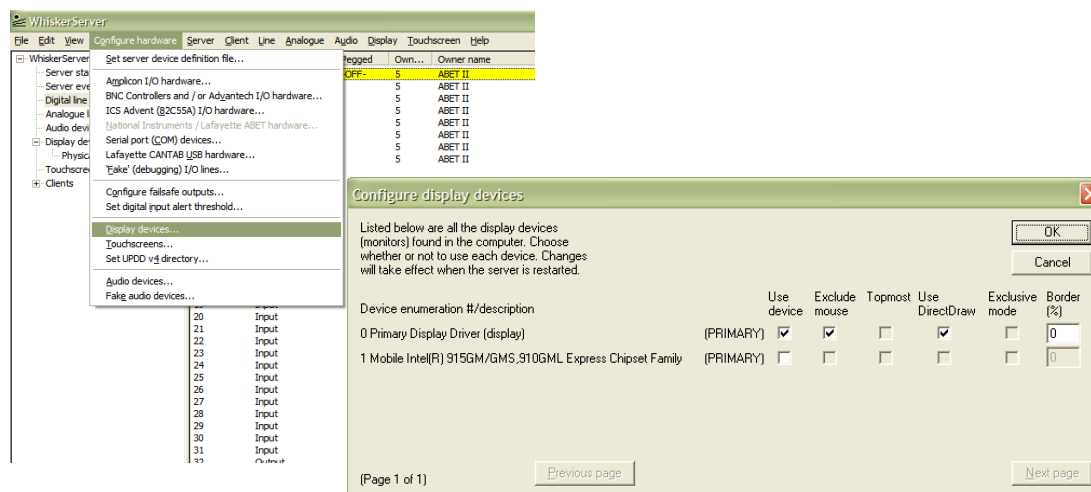
From the Whisker Server window click 'Server\Configure hardware\ 'Fake' (debugging) I/O lines...' as shown below. Select 32 input and 32 output lines.



You will then need to load a definition file as shown below. The definition file you require is 'Whisker device definitions - Advantech1756.txt'. This will probably be located on your touch screen system PC under 'C:\ProgramFiles\WhiskerControl\Device Definition Files'. You should copy this into the same file on your PC for virtual testing. Definition files are generally stored in C:\Program Files\WhiskerControl\Device Definition Files. They tell Whisker what hardware is attached. Select this file as the definition file to use by clicking on 'Set server deice definition file' as shown below.



Configure the 'Display' as shown:



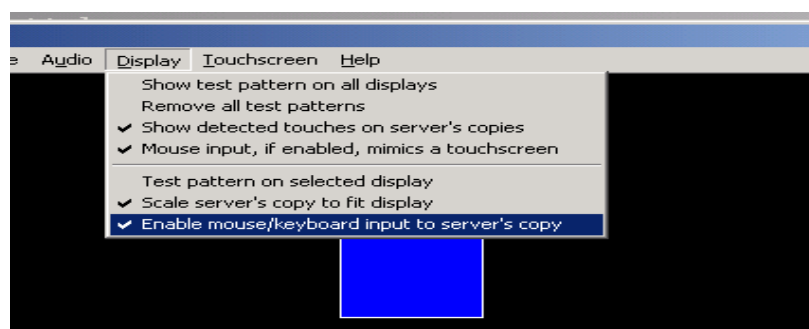
You will need to close Whisker and restart for these changes to take effect.

You will also need to set-up the ABET environment. Copy the 'Environments.abetEnvs' file in your touchscreen PC (found in My Documents\Lafayette Instrument\ABET II\ABET System Folder) into the same folder on your virtual testing PC.

You will now be ready to simulate experiments. Open and start the experiment as you would normally.

You can manually control any of the input or output lines by clicking on 'Digital line status', selecting the required line (see Table 1 for line numbers) and pegging the lines on and off using the '1' and '0' keys on your keyboard. (To release the lines to the program control click 'Line/Free(unpeg all lines)'). Also, by clicking on the top 'Line' menu and selecting 'Line Details' you can toggle inputs (this will allow you to simulate lever on and off for virtual testing of the 5-choice program). To fake tray input enter 0 in the 'Choose a line' box and click 'Update'. Click 'Force on' and 'Force off' as required.

To get past not having a touch screen available, you can respond to the physical display in the Whisker Server Window by selecting 'Enable mouse/keyboard input..'



You can now simulate touches using your mouse in the Whisker display window.



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