

**Instruction Manual**

**80614-LD - Location Discrimination (LD) Task  
for Mouse Touch Screen Systems  
and ABET II (2 x 6 Mask version)**



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The purpose of this manual is to allow the user to achieve expertise in the use of the software.

Please read and understand the information contained in this manual before using the software. Only competent and capable personnel should use the software.

This document should be retained for future reference as it contains the name and address of the manufacturer within the EC

# CONTENTS

<b>RECOMMENDED READING .....</b>	<b>4</b>
<b>CHANGES TO MOUSE LD TASKS V1 – V2.....</b>	<b>5</b>
<b>INTRODUCTION.....</b>	<b>7</b>
<b>1 THE TASK.....</b>	<b>9</b>
BOX POSITION NUMBERS AS DESIGNATED BY ABET.....	9
1.1. BASIC TASK PROTOCOL.....	10
<b>2 INSTALLING THE LD SOFTWARE.....</b>	<b>13</b>
2.1. ACCESSING THE LD TOUCH SOFTWARE.....	13
<b>3 SETTING-UP THE HARDWARE .....</b>	<b>14</b>
3.1. CONNECTING THE HARDWARE: .....	14
TABLE 1 .....	15
3.2. TESTING THE HARDWARE: .....	15
TABLE 2 .....	16
3.3. CLEANING, PRIMING AND CALIBRATING THE FEEDER (LIQUID PUMP).....	17
<b>4 RUNNING SCHEDULES .....</b>	<b>19</b>
4.1. PREPARING SCHEDULES .....	19
4.2. RUNNING SCHEDULES .....	20
4.3. CHANGING VARIABLE DEFAULTS. ....	23
4.4. FINALIZE/LOCK SCHEDULES.....	24
<b>5 TRAINING PROCEDURES.....</b>	<b>25</b>
5.1. FOOD DEPRIVATION/HANDLING (3 DAYS) .....	26
5.2. HABITUATION .....	27
5.3. INITIAL TOUCH TRAINING (LOCATION DISCRIMINATION) 6 USUALLY 1 DAY .....	29
5.4. MUST TOUCH STIMULI (LOCATION DISCRIMINATION TRAINING) (~5-10 DAYS)) .....	30
5.5. MUST INITIATE (LOCATION DISCRIMINATION TRAINING) (~2 DAYS).....	31
5.6. PUNISH INCORRECT (LOCATION DISCRIMINATION TRAINING) (~2 DAYS) .....	32
5.7. LOCATION TRAINING: ACQUISITION (~ 1 WEEK) .....	33
TABLE 3: SESSION VARIABLES FOR TRAINING SCHEDULES .....	36
<b>6 TESTING SCHEDULES / TASK MANIPULATIONS.....</b>	<b>37</b>
6.1. LOCATION DISCRIMINATION: REVERSAL.....	37
6.2. 2-CHOICE LOCATION DISCRIMINATION: PROBE SESSIONS .....	38
6.3. 3-CHOICE LOCATION DISCRIMINATION: FULL TEST.....	38
6.4. CHANGING VARIABLES .....	38
TABLE 4: SESSION VARIABLES FOR LD TASKS .....	40
TABLE 5: SESSION VARIABLES FOR LD TASKS .....	40
6.5. CHANGING LIST VALUES.....	41
TABLE 6: LIST VARIABLES.....	42
<b>7 ANALYZING RESULTS.....</b>	<b>43</b>
7.1. RAW DATA.....	43
7.2. FILTERING DATA .....	43
7.3. REPORTS .....	43
7.4. DATA ELEMENTS AVAILABLE WITH THE STANDARD APPLICATIONS.....	43
<b>8 RUNNING SCHEDULES VIRTUALLY.....</b>	<b>49</b>

## **ABET II LOCATION DISCRIMINATION TASK FOR THE MOUSE**

Campden Instruments and Lafayette Instrument Company would like to thank Dr Lisa Saksida, Dr Timothy Bussey and Dr Carola Romberg (Cambridge University, Cambridge, UK) for their help in preparing this task and manual.

### **Recommended Reading**

This manual should be used in conjunction with the ABET II manual. For additional information on the tests see:

*Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science. 2009 Jul 10; 325(5937):210-3.*

*McTighe SM, Mar AC, Romberg C, Bussey TJ, Saksida LM. A new touchscreen test of pattern separation: effect of hippocampal lesions. Neuroreport. 2009 Jun 17;20(9):881-5.*

## Changes to Mouse LD Tasks v1 – v2

The Mouse Location Discrimination Tasks have been amended to fit in with standard parameters recently agreed upon by the Cambridge University Group. The changes from previous releases of the task are detailed below. These are the default parameters used by the schedules and can all be changed either from the ABET Schedule Designer or from the Execution Manager.

One parameter that has not been changed, and does not conform to what is done at Cambridge, is having the house light off and turning it on only during the time-out period. This is opposite to the procedure followed at Cambridge. Feedback from customers indicates that there is no disadvantage to doing it this way and there may be some advantage. No direct study to compare the two conditions has been done, however. Again, you can easily change these parameters via the `House Light Normally On` variable.

**Mouse LD Habituation 1 v1** ó No changes

**Mouse LD Habituation 2 v1** ó No changes **House light still on.**

**Mouse LD Initial Touch Training v2** ó

- ITI extended from 0 to 10s.
- **House light still on**
- Criterion changes from `completion of 30 trials in 30 min` to `completion of 30 trials in 60 min`

**Mouse LD Must Touch Training v2** ó

- ITI extended from 5 to 10s.
- **House light still on**
- Criterion changes from `20 trials in 30 min on two consecutive days` to `completion of 30 trials in 60 min`

**Mouse LD Must Initiate Training v2** ó

- ITI extended from 5 to 10s.
- **House light still on**
- Criterion changes from `30 trials in 30` to `completion of 30 trials in 60 min`

**Mouse LD Punish Incorrect Training v2** ó

- ITI extended from 5 to 10s.
- **House light still on**

- Criterion changes from 30 trials in 30 minutes at >85% correct to 23/30 trials correct (77%) in 30 minutes on two consecutive days and increase the maximum schedule time to 60 min to train to this level.

#### **Mouse LD 1 v2; 2-choice and 3-choice tasks v2**

- These schedules are not used as standard in the Cambridge labs.
- ITI extended from 5 to 10s.
- **House light still normally off**

#### **Analysis:**

- Corrected the count of Blank Touches to not include touches to the correct and incorrect windows (whether lit or not).

#### **Mouse LD 1 choice reversal v3**

- ITI extended from 5 to 10s.
- Now reverses when x out of y trials completed rather than when an overall %correct achieved
- **House light still normally off**
- Added option to end schedule after x reversals have been completed.
- Criterion changed see manual for details.

#### **Analysis:**

- Added measure to End Summary data to record the number of reversals completed to criteria (including the first set of trials)
- Added a measure to record the number of trials required to reach criteria, for every reversal performed.
- Corrected the count of Blank Touches to not include touches to the correct and incorrect windows (whether lit or not).

## **Introduction**

These are the specifications for the mouse Location Discrimination Task (LD) currently used at Cambridge University, Cambridge, UK. 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. After pre-training the mice begin training of the Touch LD 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 LD task:

### **For cleaning and calculating the feeder:**

SM FeederCal Mouse Touch

### **For checking equipment**

12-TouchMouseTestLines

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

Mouse LD Habituation 1, Mouse LD Habituation 2

### **Training for the task:**

Mouse LD Initial Touch Training, Mouse LD Must Touch Training, Mouse LD Must Initiate Training, Mouse LD Punish Incorrect Training

### **The tasks:**

Mouse LD 1 choice, Mouse LD 1 choice reversal.

Mouse LD block 2 choice EH, Mouse LD block 2 choice EI, Mouse LD block 2 choice IH, Mouse LD block3 choice.

Mouse LD random 2 choice EH, Mouse LD random 2 choice EI, Mouse LD random 2 choice IH, Mouse LD random3 choice.

This manual will take you through:

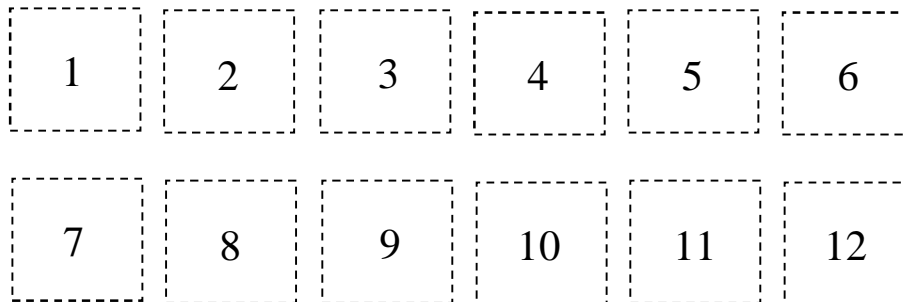
1. The Task
2. Installing the software
3. Setting up the hardware for the task.
4. Checking the equipment by running the test schedule.
5. The habituation procedures that introduces the mice to the equipment.
6. The training procedures
7. Running the task
8. Analyzing the results.
9. Running the schedules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



## 1 The Task

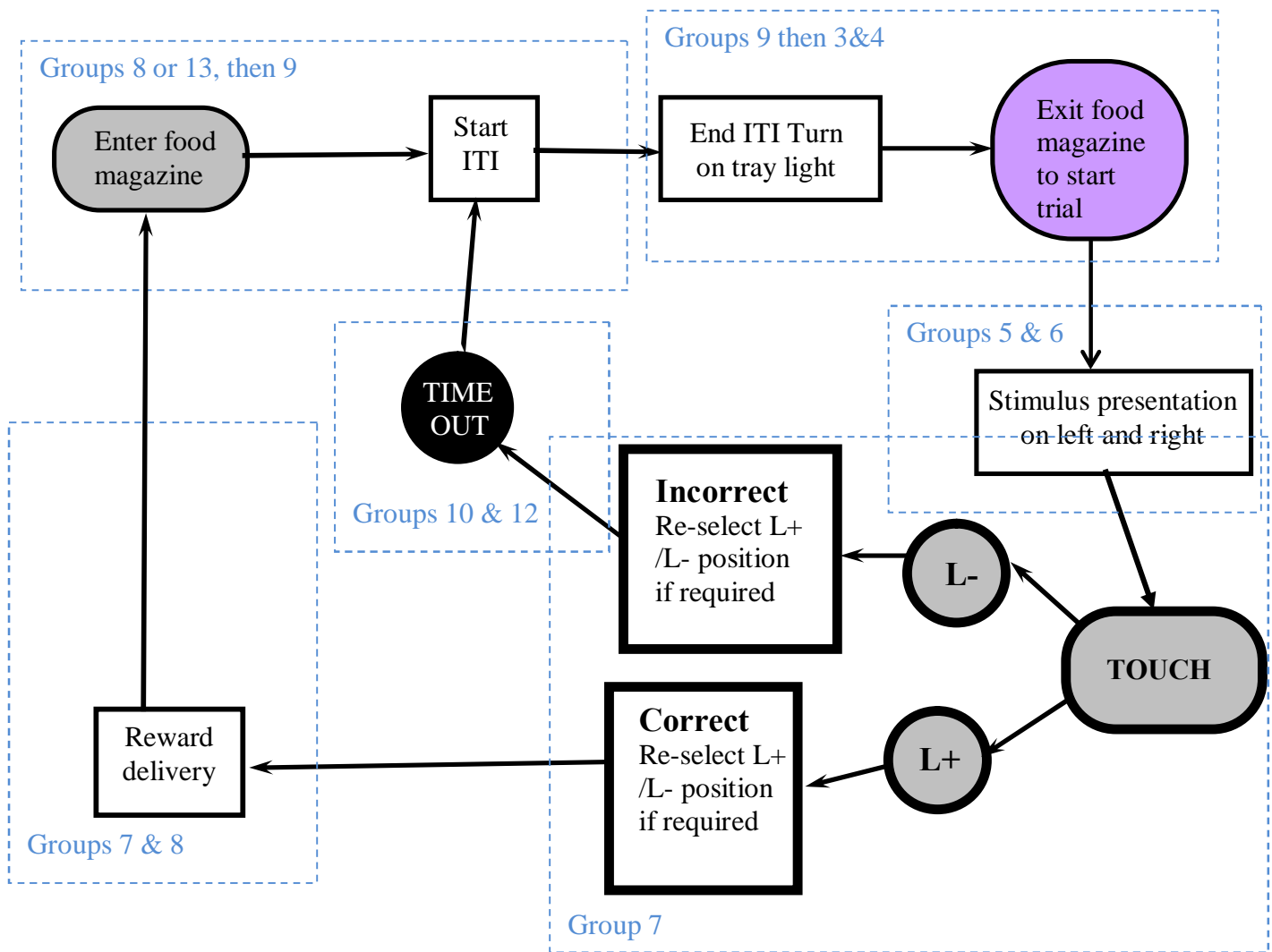
The task is illustrated below. The schedule uses the 6 x 2 mask, but only the bottom row (7-12) of windows is used for this task. A trial begins with the presentation of two white square images positioned on the left and right of the screen, so they are an equal distance from the centre of the screen. There are three levels of difficulty that can be incorporated into this task ó Easy (positions 7 & 12); Intermediate (positions 8 & 11); Hard (positions 9 & 10). One square is programmed as being correct (L+) and one as being incorrect (L-). Whether the L+ is on the right or left is determined by the user and is counter balanced across animals. Until a designated reversal takes place, L+ will remain the same side throughout the experiment, even when several levels of difficulty are used within the same task.

Usually animals are trained to the intermediate level and other levels are used for further task manipulations. Reversal within an experiment has been restricted to one choice (one level of difficulty) only.



### Box position numbers as designated by ABET

The mouse must nose poke the correct stimulus to elicit the tone/reward tray light and food delivery response. This is followed by an ITI (inter trial interval) before the mouse is given a chance to nose poke the food tray to start another trial. If the mouse nose-pokes the incorrect stimulus, no reward will be delivered and a time out will follow, followed by the ITI period. The tray light will then illuminate. The mouse must nose poke the food tray to start another 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.)]



\*\* Text in this Color denotes Conditional Statements within the ABET II Schedule in their associated Groups.

## 1.1. Basic Task Protocol

### 1) Setting Variables

- Maximum session length and the maximum number of trials are set from the -Execution Managerø (We recommend 1 hr and 60 trials.) When either has been exceeded then step 10.
- The tone frequency is set via the A1Sound1, A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) The tone duration is set from the -Tone Durationø variable (default 1000 ms).
- Which side will be the correct position is set from the -Correct Sideø variable (1 for left, 2 for right - default is 1).

- d. For the 1-choice experiments the difficulty level is set from the 'Difficulty' variable (default 2 ó intermediate)
  - e. Whether the house light is normally on set from the variable (default False).
  - f. Inter trial interval set from 'ITI' variable (default 10 s).
  - g. Time-out set from 'Time Out' variable (default 5 s).
  - h. Amount of food to be delivered is set via the 'Feed Pulse Time' (default 280ms / 7µl).
  - i. 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 activated and then released.
  - c. Then step 3.
- 3) Images Displayed**
- a. Turn off the tray light.
  - b. Determine L+ and L- position according to test criteria (1 choice, 2 choice reversal etc)
  - c. Display the white square image in both the L+ and L- images positions.
  - d. Then step 4.
- 4) Image response**
- a. Response to the L+ image, then step 5.
  - b. Response to the L- image, then step 6.
- 5) Reinforcement**
- a. Images are removed.
  - b. Liquid food is delivered (usually pump turned on to deliver 7µl), 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).
  - 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 10s)
  - c. Then step 9
- 9) Trial initiation**
- a. Turn on tray light
  - b. Wait for tray-report / feed-tray to be activated and then released.
  - 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

## **2 Installing the LD 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 LD Schedules and Analysis files may be on the same or a separate disk. 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 *õEditö* menu.

Schedules:

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

Analysis Sets:

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

### **2.1. Accessing the LD Touch software**

Some familiarity with the ABET II Software product is necessary for running the LD Touch task package. The recommended approach would be to work through the Tutorials in the ABET II help/manual. Since you do not need to create schedules for the LD 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 II manual whenever you have any questions or concerns.

### 3 Setting-up the hardware

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

To help you configure and test the hardware we have a testing schedule 03-Touch MouseTestLinesö. 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 6 x 2 Mask
4. Before starting the system, note the order of power up should be:
  - a) Power up the system\*\*
  - b) Power up the PC
  - c) Start up the Whisker Server Software
  - d) Start the ABET II software.

\*\* The system, other than the computer, can be powered up after the software has been started. With our easy install system, however, all the system (including the PC) is turned on from one power switch for your convenience.

The Mouse Touch Screen box should be configured as shown on the following table (Table 1). You will probably have been given an environment file to install. If not, or if you need to make changes, then go to the Environment Designer and type the name as written below (case sensitive) in the box under -Inputsø or -Outputsø and tick the relevant line. Do not type the # sign. This is automatically entered and the associated number is increased if the name is used more than once. For the -Soundø lines write -Soundø in the -Outputø box and then tick lines 13, 14 and 15 in order.

**Table 1**

Standard Touch Screen Environment					
Outputs			Inputs		
Line (WS *)	Identifier	Which schedules require this identifier	Line	Identifier	Which schedules require this identifier
1 (32)	HouseLight	ALL	1 (0)	Tray	ALL
2 (33)	TrayLight	ALL	2 (1)	BIRBeam	None
3 (34)	Feeder	ALL	3 (2)	FIRBeam	None
4 (35)	Sound_On	ALL	4 (3)	RightFIRBeam	None
5 (36)	White_Noise_Off	ALL	5 (4)	FeederReport (if pellet drop detector purchased)	None
6 (37)	Sound #1	ALL	6 (5)	FeederFault (if pellet drop detector purchased)	None
7 (38)	Sound #2	ALL	7	Spare	
12 (39)	Sound #3		8	Spare	

\* (WS ó line number for box 1 when viewed from the Whisker Server. Lines for other boxes will be this number + ((box no. -1) \*8))

### 3.2. Testing the hardware:

It is recommended that this quick test of the hardware be done prior to every days training or testing:

12-Touch MouseTestLines 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 Scheduleø icon and select -12-Touch MouseTestLinesøand click -Openø
- 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**

<b>Schedule</b>	<b>Inputs to activate</b>	<b>Output response</b>
12-Touch MouseTestLines	Simulate a Touch in Grid x (where x equals grid positions 1 to 12)	Image appears Grid x as a full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Enter the feed-tray	House-light and tray-light illuminate and pulses feeder - 800ms. Pulses 3 KHz Tone (Sound_On) 500ms
	Exit the feed-tray	Houselight and tray light extinguish
	Block Front IR activity beams	House-light illuminates
	Block Back IR activity beams	Tray-light illuminates
	Block Right Front IR activity beams **	House-Light and Tray-light illuminates, Pulses 3 KHz Tone (Sound_On) 500ms

\*\* This line is only used when the feeder is in the Autoshaaping 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 LD 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 ó for water deprived mice) or Yazoo strawberry milkshake (SM ó 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 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.)
- 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.

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. 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 Reconnect the tubing to the reward tray.

Each liquid feed system will vary a little. This small difference is not usually of concern for the LD 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 (a-d).
- b Set the end of the feed-tube and needle to deliver food into a weighed container.
- c From the -Execution Managerø set the touch box(es) to be primed to run the protocol -SM FeederCal-Mouse Touchø for SM [or -FeederCal-Mouse Touchø for CM] with the variable öPrimeö in the **Properties** tab set to Falseø
- d Run ( ▶ ) the schedule. The schedule will pump CM/SM through the tubes for 10 x 280ms [5 x 800ms] bursts
- e Weigh the liquid (should weigh (10 x 7µl) = 0.07g [(5 x 20µl) = 0.1g]).

- f If necessary adjust the :CMPulseøtime in the :FeederCal-Mouseø **Properties** tab. Reload the schedule, :FeederCal-Mouseø 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 :CMPulseøtime.
- h You will need to enter this new :CMPulseøtime into the appropriate schedules, default variable value, using the :Schedule Designerø Again see the ABET II 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 by ABET II.

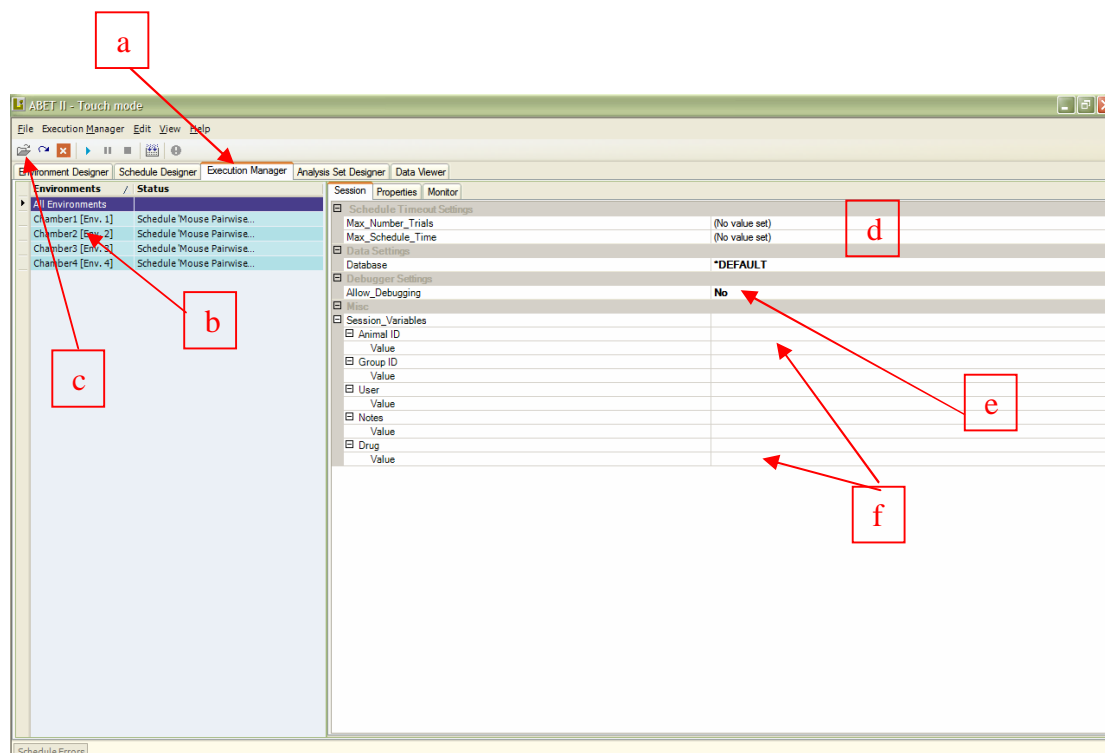
## 4 Running Schedules

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.

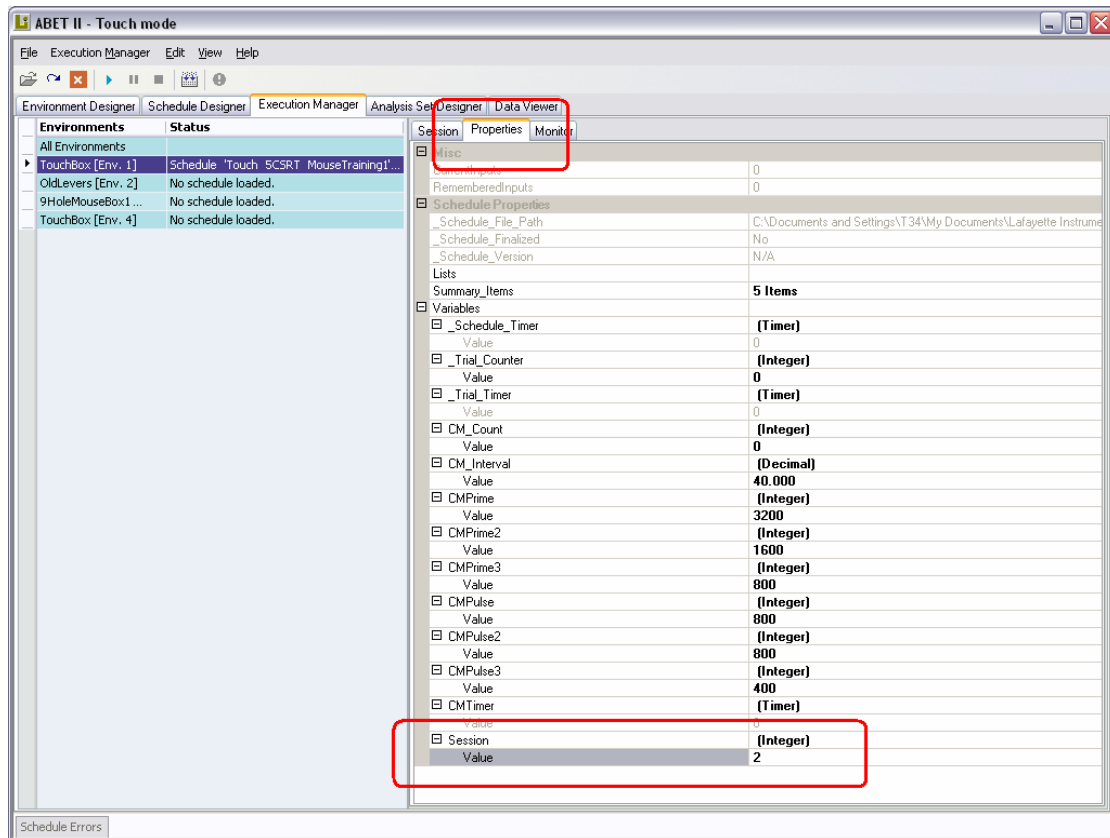
### 4.1. Preparing Schedules

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.



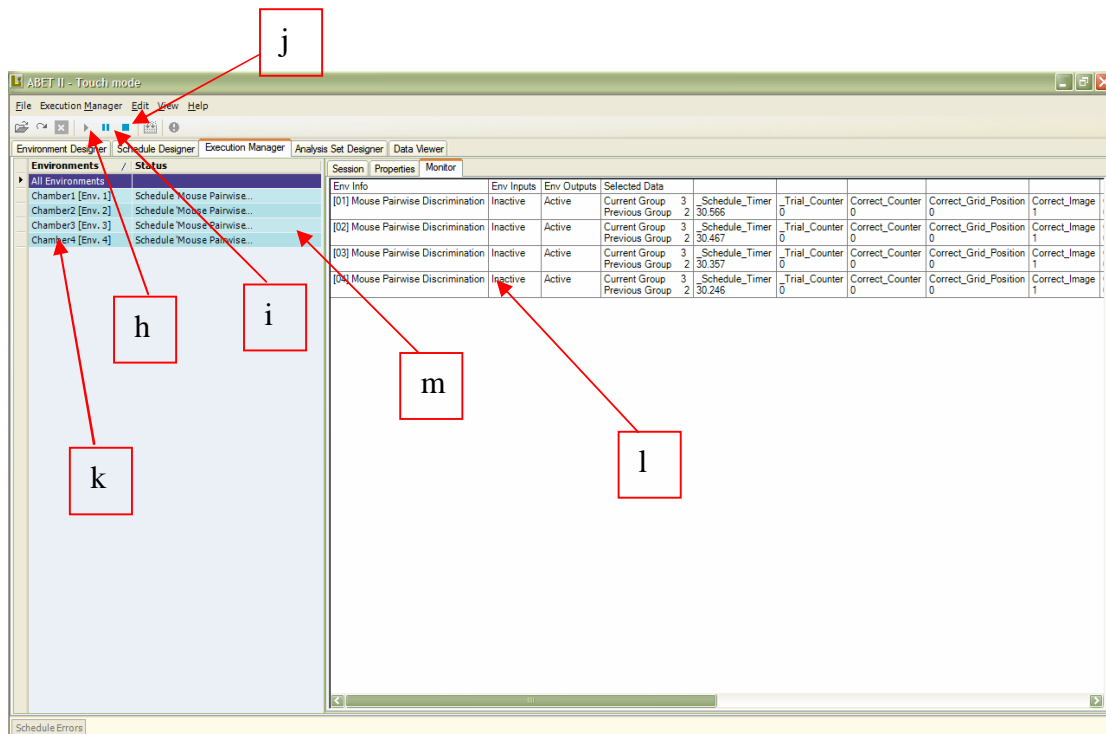
- g. Click on the Properties tab and change any variables that need changing from their default value. (Values that can be changed are indicated in chapters 5 and 6.) Changing the Default value for these variables will be covered in section 4.3 below.



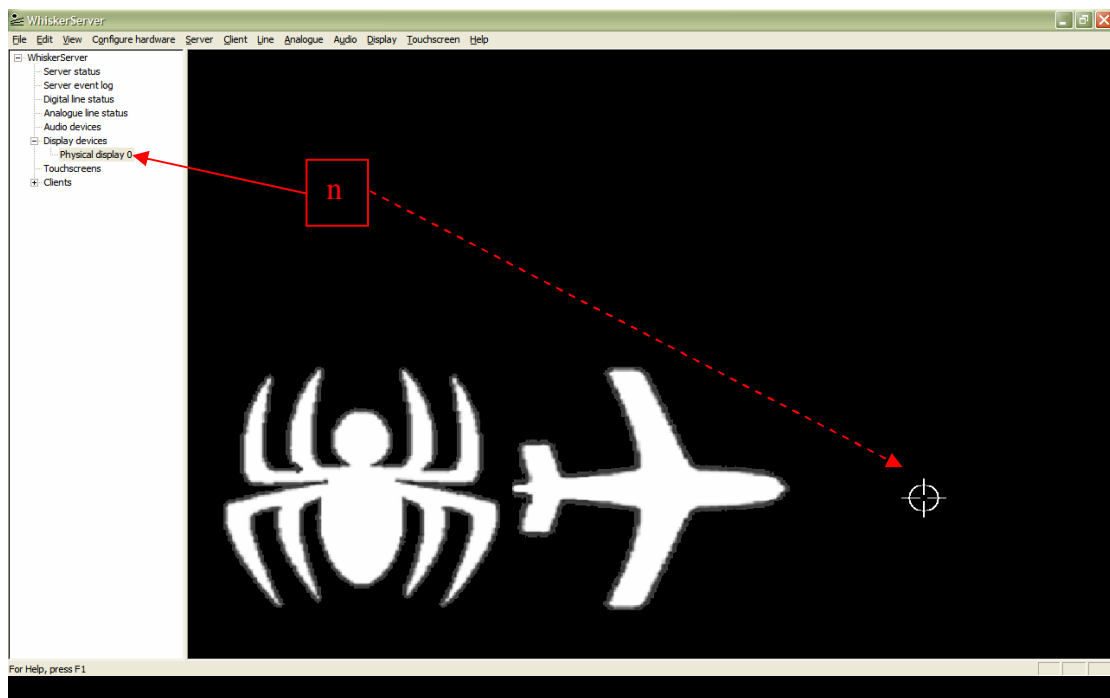
The Schedule is now ready to play:

## 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. What is appearing on the screens the animal sees and the last place the animal touched can be viewed from the Whisker server, by opening the 'Display devices' tree and clicking on each 'Physical display' in turn. (Note: Box 1 has Physical display 0, Box 2 has Physical display 1, etc)
- o. If you want 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)



The screenshot shows the WhiskerServer application window with the 'Line' menu open. The 'Line details...' option is selected, and a table of line configurations is displayed. A red box labeled 'O' highlights the 'Owner name' column. A red arrow points from the 'Line details...' menu option to the table.

Line number	Free (unpeg) all lines	Owner name	First alias	ON event	OFF event	Safety timer	Reset state	Hardware description
0	Free line on/off...	ABET II	B111	InputON:B111	InputOFF:B111			Fake input
1		ABET II	B112	InputON:B112	InputOFF:B112			Fake input
2		ABET II	B113	InputON:B113	InputOFF:B113			Fake input
3		ABET II	B114	InputON:B114	InputOFF:B114			Fake input
4		ABET II	B115	InputON:B115	InputOFF:B115			Fake input
5		ABET II	B116	InputON:B116	InputOFF:B116			Fake input
6		ABET II	B117	InputON:B117	InputOFF:B117			Fake input
7		ABET II	B118	InputON:B118	InputOFF:B118			Fake input
8								Fake input
9								Fake input
10								Fake input
11								Fake input
12								Fake input
13								Fake input
14								Fake input
15								Fake input
16								Fake input
17								Fake input
18								Fake input
19								Fake input
20								Fake input
21								Fake input
22								Fake input
23								Fake input
24								Fake input
25								Fake input
26								Fake input
27								Fake input
28								Fake input
29								Fake input
30								Fake input
31								Fake input
32		5	ABET II	B101			Off	Fake output
33		5	ABET II	B102			Off	Fake output
34		5	ABET II	B103			Off	Fake output
35		5	ABET II	B104			Off	Fake output
36	###	5	ABET II	B105			Off	Fake output
37		5	ABET II	B106			Off	Fake output
38	###	5	ABET II	B107			Off	Fake output
39		5	ABET II	B108			Off	Fake output
40							Leave	Fake output
41							Leave	Fake output
42							Leave	Fake output
43							Leave	Fake output
44							Leave	Fake output
45							Leave	Fake output
46							Leave	Fake output
47							Leave	Fake output

Further information can be found in the section 'Starting the Schedule' in the main manual.

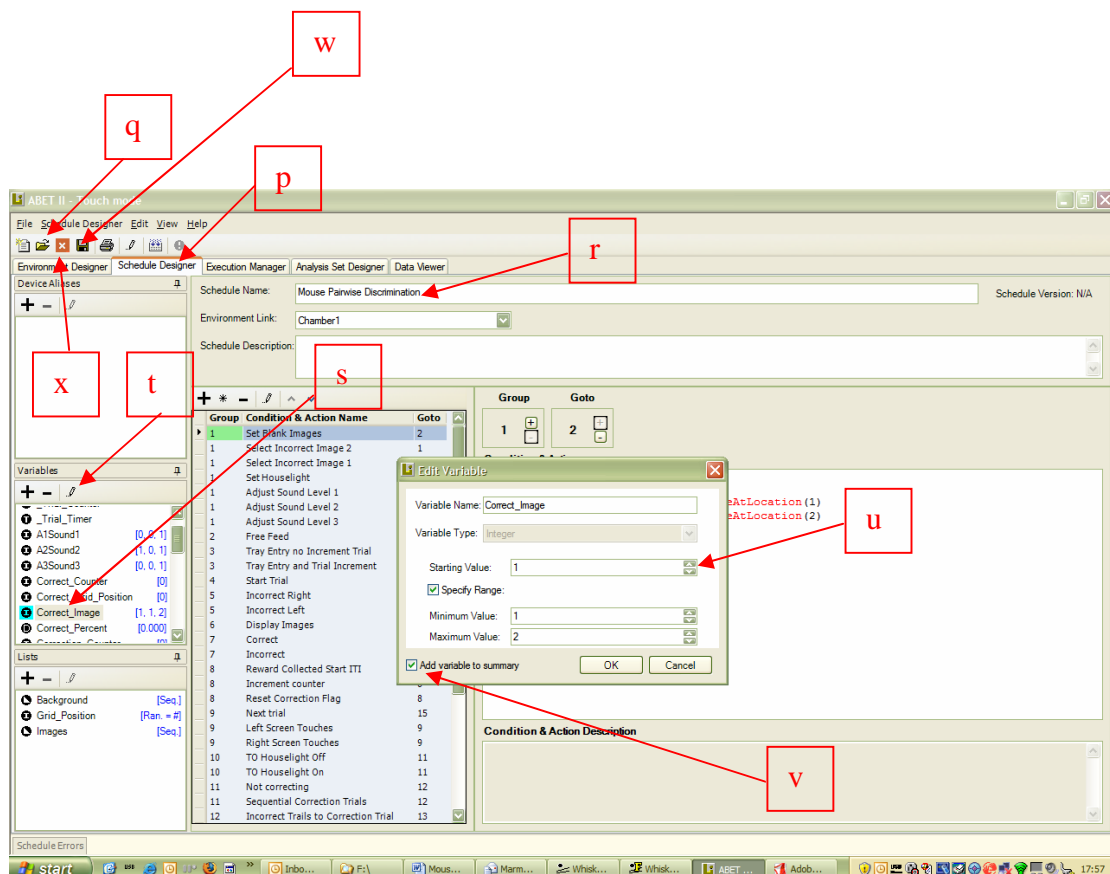
### 4.3. Changing Variable Defaults.

To change the default values of the variables that can be changed in the program (see section 6.1 and table 3):

*Note: Be careful not to change any variables not given in as changeable in the manual, as you may effect 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 6.2) 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 cannot be changed in the future and you will always know exactly what version of a schedule was used.

To lock a schedule go to the Schedule Designer menu and select Finalize Version



## 5 Training Procedures

Prior to the habituation and training procedures, make sure that the Touch Screen box has been set-up to run the Mouse LD schedules (see section 3). Reward area opposite the screen, 6x2 Mask inserted.

**The basic training schedule is illustrated below:**

The training program illustrated assumes food deprivation and using strawberry milkshake as a reward. We believe 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.

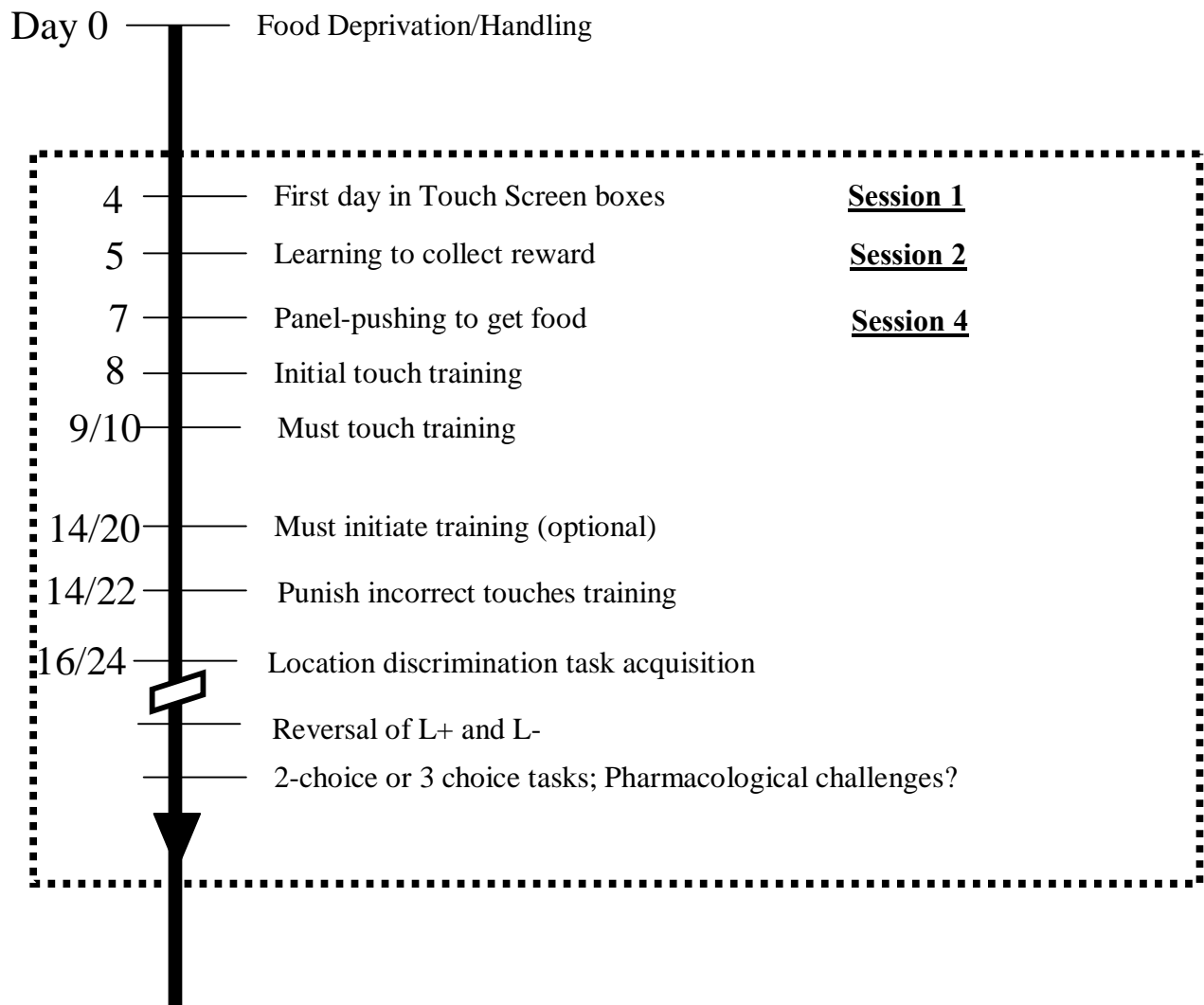
This training schedule shown below details the training based on:

Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ. **A functional role for adult hippocampal neurogenesis in spatial pattern separation.** *Science*. 2009 Jul 10;325(5937):210-3.

And

McTighe SM, Mar AC, Romberg C, Bussey TJ, Saksida LM. **A new touchscreen test of pattern separation: effect of hippocampal lesions.** *Neuroreport*. 2009 Jun 17;20(9):881-5.

Introduction of the liquid reward is as described in Trueman RC, Brooks SP, Dunnett SB. (2005) **Implicit learning in a serial choice visual discrimination task in the operant 9-hole box by intact and striatal lesioned mice.** *Behav Brain Res*. 159 pp 313-322



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

- i. Day1-3 weigh mice and handle for 1 minute.
- ii. Calculate 85% of baseline weight.
- iii. 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:

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

#### Post-protocol Steps:

- É Ensure box power pack and computers are turned off.
- É Replenish food as required.

- É 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.

## 5.2. Habituation

The original work at Cambridge used pellets for reward and simply left the mouse in the chamber for 15 min with 5 pellets in the reward tray. Once the mouse had found and eaten all the pellets, they then proceeded to the initial touch training / pavlovian stage. Below is given the procedure used at Cardiff to habituate to a 9-hole box and strawberry milkshake reward and train the animals to panel push for food. You may prefer (particularly as the standard chamber have no panel to push) to add the strawberry milkshake reward to the reward tray during the Habituation 1 stage and run this for 15 min and once all the reward has been collected, proceed straight to the Initial Touch Training stage.

**Habit 1:** The Mouse is left in the chamber for a 10 min session, with all lights turned off. No stimulus or reward presentation. Tray door is fixed back, if present. The activity of the mouse is monitored.

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 frequency is set via the A1Sound1, A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) 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, with the tray-light turned on. A Tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivery 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 turned on, a tone is played and SM is then delivered for 280ms (7µl). 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. The food-tray panel (if present) should be fixed back for this session.

**Habit 2b:** The Mouse is left in the chamber for a 40 min session, with the tray-light turned on. A Tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivery 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 turned on, a tone played and SM is then delivered for 280ms (7µl). 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. The food-tray panel (if present) should be fixed back for this session.

**Habit 2c:** The Mouse is left in the chamber for a 40 min session, with the tray-light turned on. A Tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivery 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 turned on, a tone is played and SM is then delivered for 280ms (7µl). 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. If you have a food-tray panel, this can now released if the mouse is to be trained to push the panel to gain access to the SM reward.

Repeat habit 2c until mice are collecting all reward.

**Procedure for Habituation 1:**

- a Insert the 6 x 2 blank.
- b Introduce food into the reward tray.
- c From the -Execution Managerø set the Mouse touch screen box(es) to run the schedule -Mouse LD Habituation 1ø Set the Session: Max\_Schedule\_Time to 10 or 15 minutes, i.e. 10:00/1500.
- 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 -Editø menu, selecting -Preferencesø and clicking on -Session Fieldø prior to loading a program
- e Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule. The schedule will monitor IR beam breaks, foodtray entries and touches to the screen
- f At the end of 10/15 minutes the -Execution Managerø will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**Tip:** Save the -Execution Managerø configuration for the Habituation 1 training by clicking -File/Save Experimentø This way experiment and its settings for Habituation 1 trainings can be easily loaded using -File/Open Experimentø 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.

**Procedure for Habituation 2a:**

- a Insert the 6 x 2 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 -Mouse LD Habituation 2-. Set the Session: Max\_Schedule\_Time to 20 minutes, i.e. 20:00.
- 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.

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

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

#### **Procedure for Habituation 2c:**

Same as for 2b, except this time the tray door can be released if panel pushing is required.

Note: Nose pokes into the tray will be detected whether the door is present or not.

### **5.3. Initial Touch Training (Location discrimination) – Usually 1 day**

The stimulus (a white square) is displayed randomly in one of the bottom row windows (positions 7 to 12). The stimulus is presented in only one window at a time. The other windows are left 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 (variable Image Time ó default 30s) the image is removed and food is delivered (variable -Feed Pulse Time ó default 280ms). Food delivery is accompanied by illumination of the tray light and a tone. [The tone frequency is set via the A1Sound1, A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) 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 10s) 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`

**Criterion:** 30 trials in 60 min

### Procedure for Initial Touch Training:

- a Insert the 6 x 2 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 `Mouse LD Must Initiate Training`. Set the Session: `Max_Schedule_Time` to 60 minutes, i.e. 30:00. 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.

### 5.4. Must Touch Stimuli (Location discrimination training) (~5-10 days)

The stimulus (a white square) is displayed randomly in one of the bottom row windows (positions 7 to 12). The stimulus is presented in only one window at a time. The other windows are left 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 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 frequency is set via the `A1Sound1`,

A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) The tone duration is set from the -Tone Durationø variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default 10s) another image is displayed.

**Criterion:** Completion of 30 trials in 60 min

### **Procedure for Must Touch Training:**

- a Insert the 6 x 2 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the -Execution Managerø set the same touch screen box(es) to run the schedule -Mouse LD Must Touch Trainingø Set the Session: Max\_Schedule\_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 20.
- 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 -Editø menu, selecting -Preferencesø and clicking on -Session Fieldø 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 Managerø will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

### **5.5. Must Initiate (Location discrimination training) (~2 days)**

Note: Work at Cambridge has found that this stage is not necessary and we would recommend going straight to the Punish incorrect stage, which will also train the mouse to initiate. If however, you find you strain of mice are struggling with the -punish incorrectø stage, you can use this stage to train the initiation separately.

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. [The stimulus (a white square) is displayed randomly in one of the bottom row windows (positions 7 to 12). The stimulus is presented in only one window at a time. The other windows are left 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 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 frequency is set via the A1Sound1, A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) The tone duration is set from the -Tone Durationø variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period

(default 10s) the tray light is again illuminated. The mouse must nose poke and exit the reward tray before the next image is displayed.

**Criterion:** Completion of 30 trials in 60 min

**Procedure for Must Initiate Training:**

- a Insert the 6 x 2 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the -Execution Managerø set the same touch screen box(es) to run the schedule -Mouse LD Must Initiate Trainingø 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 -Editø menu, selecting -Preferencesø and clicking on -Session Fieldø 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 Managerø will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

**5.6. Punish Incorrect (Location discrimination training) (~2 days)**

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. 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 has been done successfully at Cambridge.] (There are options whether to have correction trials or to only started a correction trial when x incorrect trials in a row are made.)

**Criterion:** 23/30 trials correct (77%) in 30 minutes on two consecutive days. No correction trials

**Procedure for Punish Incorrect Training:**

- a Insert the 6 x 2 blank.
- b Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- c From the -Execution Managerø set the same touch screen box(es) to run the schedule -Mouse LD Punish Incorrect Trainingø 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 -Editø menu, selecting -Preferencesø and clicking on -Session Fieldø 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 Managerø will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

### 5.7. Location Training: Acquisition (~ 1 week)

*This task, where there is only one correct side during the schedule, is provided if you wish to use it. The Cambridge labs, however, have gone straight from the 'Punish Incorrect Training' to the 'LD I choice Reversal' task (see section 6.1).*

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. A trial begins with the presentation of two identical white square images, displayed in the intermediate level positions (8 and 11) in the Bussey Mouse Operant Mode 6 x 2 high grid - bottom row). One side (L+) is designated as correct (counter balanced across animals). The mouse must nose poke the correct stimulus (L+) to elicit the food delivery response. Food delivery is accompanied by illumination of the tray light and a tone. [The tone frequency is set via the A1Sound1, A2Sound2 and A3Sound3 variables (default 3 KHz). The volume can be adjusted by means of turning a screw in the Chamber Interface Module (see 80626-I37 manual) The tone duration is set from the -Tone Durationø variable (default 1000 ms).] Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (default is 10s) 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 (L-), no pellet will be delivered and a time out will follow before the mouse is given the opportunity to start another 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.)] Once the time out period finishes the house light is inverted again and the ITI period begins.

**Criterion:** Session criterion: 40 trials per daily session. Each day the starting correct location is changed.

### Procedure for Location Discrimination:

- h Insert the 6 x 2 blank.
- i Prime the feeder with the Yazoo strawberry milk (see 3.3 above).
- j From the -Execution Managerø set the same touch screen box(es) to run the schedule -Mouse LD 1 choiceø Set the Session: Max\_Schedule\_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 40.
- k 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 -Editø menu, selecting -Preferencesø and clicking on -Session Fieldø prior to loading a program
- l Change any user changeable variables as required (see table 3 below).
- m Introduce the subject, Mouse (Mice), and Run ( ▶ ) the schedule.
- n At the end of 60 minutes or 40 trials completed (which ever happens first) the -Execution Managerø will indicate that the session has ended. The Mouse (Mice) should now be removed from the chamber.

User changeable variables in the LD schedules – default/recommended values							
Schedule-Variable	Habit 1	Habit 2 a(b)	Initial Touch	Must Touch	Must Initiate	Punish Incorrect	LD 1 choice
<b>**Session Length</b>	10/15 min	20(40) min	60 min	60 min	60 min	60 min	60 min
<b>**Max Trials</b>	NVS	NVS	30	30	30	30	40
<b>A1Sound1</b>	-	0	0	0	0	0	0
<b>A2Sound2</b>	-	1	1	1	1	1	1
<b>A3Sound3</b>	-	0	0	0	0	0	0
<b>Acclimatisation time</b>	-	0s	-	-	-	-	-
<b>Delay Time</b>	-	10s	-	-	-	-	-
<b>Feeder Pulse Time</b>	-	280ms	280ms	280ms	280ms	280ms	280ms
<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>Image Time</b>	-	-	30s	-	-	-	-
<b>ITI</b>	-	-	10s	10s	10s	10s	10s
<b>Time Out</b>	-	-	-	-	-	5s	5s
<b>Correction Trials Set</b>	-	-	-	-	-	False	-
<b>No. Incorrects before Correction Trial</b>	-	-	-	-	-	-	-
<b>Difficulty</b>	-	-	-	-	-	-	2
<b>Correct Side</b>	-	-	-	-	-	-	1 (Left)

\*\* 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 ó Time before the first priming feed

Delay Time ó Time between food deliveries.

Image Time ó Time for which image is displayed

Tone Duration ó This can be set to 0 if no tone is required.

Correction Trials Set ó If false then no correction trials will take place

No. Incorrects before Correction Trial ó the number of incorrect trials to be made in a row before a correction trial ensues

Correct Side ó choice of **1** (Left) or **2** (Right). Selection of the correct image side should be counterbalanced across the study and then changed for the reversal trial.

Difficulty - There are three levels of difficulty that can be incorporated into this task ó **1**. Easy (positions 7 & 12); **2**. Intermediate (positions 8 & 11); **3**. Hard (positions 9 & 10).

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

## 6 Testing Schedules / Task Manipulations

Once criteria has been reached then the subject can be tested on a reversal trial and/or 2-choice or 3-choice tasks

### 6.1. Location Discrimination: Reversal

As for location discrimination acquisition, except once x out of y trials (usually 7 out of 8 trials) have been correctly responded to (on a rolling basis), then the correct position is reversed.

There is also an option to end the schedule once the schedule has reversed x times.

#### Criteria:

##### 1. Intermediate separation training

- Counterbalance -Correct Side L/R (1/2) start (according to performance rankings, in each group)
- 60 trials per 60 min session (can split first session into 2 of 30 trials each to ensure animal completes all trials)
- Difficulty 2 (separation 3 windows)
- ITI 10s
- Unlimited reversals allowed
- Criterion for each acquisition/reversal is 7/8 consecutive trials correct
- Criterion for this intermediate training phase is 1+ reversals in each of 3 out of 4 consecutive sessions.
- Once criterion reached, either put them on maintenance (alternate sessions as long as they reverse in every one, otherwise consecutive until they reverse again), or take them off completely.
- Wait until all mice have reached criterion. Then rebaseline for 2 days. Ideally, all mice get a reversal (or more) on both days. If there are only a few cases of mice that don't reverse both days, and especially if they at least acquire on one day and reverse on another, probably move those mice on to use judgement.

##### 2. Probes

- Counterbalancing (for each group separately): rank animals in terms of days to criterion, and secondarily reversals to criterion, and then assign L1(left, difficulty 1, separation 5 windows), R1, L3 R3(right, difficulty 3, separation 1 windows) down the list.
- ITI 10s
- Criterion (for acquisition or reversal): 7 out of 8 consecutive trials correct
- Unlimited trials in 60 mins, but limited to 2 reversals (acquisition + one reversal). Ideally all mice complete 2 reversals - this allows us to compare trials to acquisition and to a single reversal for each mouse in each session. Unlimited trials gives them a better chance of doing this.
- Each mouse gets double alternating sessions of each separation e.g. for a mouse starting on L1, session 1: sep 1, session 2: sep 1, session 3: sep 5, session 4: sep 5, repeat. Every two days, when you switch to the other separation, mice should start on the side they were assigned in the counterbalancing e.g. Left for the above mouse. The correct side for the

second day of each pair of probe sessions should be determined according to the side that was correct at the end of the previous session, as normal.

## **6.2. 2-Choice Location Discrimination: Probe Sessions**

Once criterion on the training has been reached the mice can be given 4 probe blocks of trials, two at maximum (easy) separation (7 and 12 in the Bussey Mouse Operant Mode 6 x 2 high grid - bottom row) and two at minimum (hard) separation (9 and 10 in the Bussey Mouse Operant Mode 6 x 2 high grid - bottom row). The order that the sessions were given and the starting correct side was counterbalanced such that each mouse received a maximum separation starting left and a minimum separation starting left in one session, and in another session a maximum separation starting right, a minimum separation starting right. No reversal is available within the schedule.

The above can be achieved by running the -Mouse LD block 2 choice E Hø

We have also provided tasks to compare the easy with intermediate separation -Mouse LD block 2 choice E Iø and intermediate and hard separation -Mouse LD block 2 choice I Hø

In addition 2 choice schedules have been provided where the difficulty is presented randomly. Each trial the difficulty is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. Three schedules are available to you for this random presentation: -Mouse LD random 2 choice E Hø -Mouse LD random 2 choice E Iø and -Mouse LD random 2 choice I Hø

**Criterion:** Session criterion: 80 trials (2 blocks at each difficulty) within 60 min. Each day the starting correct location is changed.

## **6.3. 3-Choice Location Discrimination: Full Test**

In the full test all three difficulties are presented, either in blocks (Mouse LD block 3 choice) or pseudo randomly (Mouse LD random 3 choice - each trial the difficulty is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row.).

## **6.4. Changing Variables**

The variable that can be changed are shown above in table 4 and 5. 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 effect the running of the schedule.**

Schedule-Variable	LD 1 choice	LD 1 choice reversal	LD 2 choice block EH	LD 2 choice block EI	LD 2 choice block IH
<b>**Session Length</b>	60 min	60 min	60 min	60 min	60 min
<b>**Max Trials</b>	40	60	80	80	80
<b>A1Sound1</b>	0	0	0	0	0
<b>A2Sound2</b>	1	1	1	1	1
<b>A3Sound3</b>	0	0	0	0	0
<b>Feeder Pulse Time</b>	280ms	280ms	280ms	280ms	280ms
<b>Houselight Normally On</b>	False	False	False	False	False
<b>Tone Duration</b>	1000ms	1000ms	1000ms	1000ms	1000ms
<b>ITI</b>	10s	10s	10s	10s	10s
<b>Time Out</b>	5s	5s	5s	5s	5s
<b>Difficulty</b>	2	2	-	-	-
<b>Starting Difficulty</b>	-	-	1	1	1
<b>Correct Side</b>	1 (Left)	1 (Left)	1 (Left)	1 (Left)	1 (Left)
<b>Reversal Block</b>	-	8	-	-	-
<b>Reversal Threshold</b>	-	7	-	-	-
<b>Block Size</b>	-	-	20	20	20
<b>End After x reversals</b>	-	False	-	-	-
<b>Max Reversals</b>	-	2	-	-	-

\*\* 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:

Tone Duration ó This can be set to 0 if no tone is required.

Correction Trials Set ó This must be set to false. No correction trials take place with the LD scdules

Correct Side ó choice of **1** (Left) or **2** (Right). Selection of the correct image side should be counterbalanced across the study and then changed for the next session. In the case of the reversal trial, this is the starting correct side for the experiment.

Difficulty - There are three levels of difficulty that can be incorporated into this task ó **1**. Easy (positions 7 & 12); **2**. Intermediate (positions 8 & 11); **3**. Hard (positions 9 & 10).

Starting Difficulty ó (2 choice block) Decides whether the easier separation (1) or whether the harder separation (2) starts the test.

Reversal Block ó minimum number of trials to be completed with a particular correct side, before reversal is considered via the Reversal Threshold, also the number of recent trials to be assessed for reversal via the Reversal Threshold.

Reversal Threshold ó No. of correct trials to be completed within the reversal block, before the correct side is reversed. i.e. for 7/8 trials correct resulting in reversal Reversal Block = 8 and Reversal Threshold

=7.

End\_After\_x\_reversals ó Select ÷Trueø to have the schedule end when x reversals are complete. (x is set from ÷Max\_Reversalsø (default 2 ó which includes the first set of trials. i.e. after 7/8 trials have been responded to correctly the correct location will reverse. When 7/8 trials with this reversed location have been responded to correctly the schedule will end,)

**Table 4: Session variables for LD tasks**

Schedule- Variable	LD 2 random choice EH	LD 2 random choice EI	LD 2 random choice IH	LD 3 block choice EH	LD 3 random choice EH
<b>**Session Length</b>	60 min	60 min	60 min	60 min	60 min
<b>**Max Trials</b>	80	80	80	48	48
<b>A1Sound1</b>	0	0	0	0	0
<b>A2Sound2</b>	1	1	1	1	1
<b>A3Sound3</b>	0	0	0	0	0
<b>Feeder Pulse Time</b>	280ms	280ms	280ms	280ms	280ms
<b>Houselight Normally On</b>	False	False	False	False	False
<b>Tone Duration</b>	1000ms	1000ms	1000ms	1000ms	1000ms
<b>ITI</b>	10s	10s	10s	10s	10s
<b>Time Out</b>	5s	5s	5s	5s	5s
<b>Starting Difficulty</b>	-	-	-	1	-
<b>Correct Side</b>	1 (Left)	1 (Left)	1 (Left)	1 (Left)	1 (Left)
<b>Block Size</b>	-	-	-	8	-
<p><b>** 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)</b></p> <p>÷÷Not applicable to this schedule</p> <p><b>Explanations:</b>  Tone Duration ó This can be set to 0 if no tone is required.  Correction Trials Set ó This must be set to false. No correction trials take place with the LD scedules  Correct Side ó choice of <b>1</b> (Left) or <b>2</b> (Right). Selection of the correct image side should be counterbalanced across the study and then changed for the next session. In the case of the reversal trial, this is the starting correct side for the experiment.  Starting Difficulty ó (3 choice block) There are three levels of difficulty that can be incorporated into this task ó <b>1</b>. Easy (positions 7 &amp; 12); <b>2</b>. Intermediate (positions 8 &amp; 11); <b>3</b>. Hard (positions 9 &amp; 10). Difficulty will cycle 1, then 2, then 3. This figure decides where in that cycle the test begins.  Reversal Block ó minimum number of trials to be completed with a particular correct side, before reversal is considered via the Reversal Threshold  Reversal Threshold - % correct that must be achieved before the correct side is reversed.</p>					

**Table 5: Session variables for LD Tasks**



## **6.5. Changing List Values**

Lists create values which can change between trials. 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:

<b>LD Task</b>	<b>List</b>	<b>Default Value</b>	<b>Selection Criteria</b>	<b>Comment</b>
All	Background	1x Plain black image	NA	Not advised to change this
All LD tasks (not training)	Images	1 white square	NA	Image can be replaced to introduce a new stimulus
All training tasks	Training Images	1 white square	NA	Image can be replaced to introduce a new stimulus
All training tasks	Grid position	5 of each grid position nos. 7 through to 12	Random equal number. No value selected more than 3 times in a row	Used to select the position the image is displayed. Could change the numbers present to eliminate some grid positions or introduce the top row (nos. 1 to 6), the number of times each occurs in the list (e.g. to deliberately introduce a side bias) and the selection criteria can change.
All LD tasks (not training)	Grid position	List of 7, 12, 8, 11, 9 & 10	Pairs selected by variable -Difficultyø	Not advised to change this, unless you want to change the pairings of which windows are displayed at the same time.
All 2- choice block tasks and 3-choice block task	Difficulty Selection	List of either 1&3(EH), 2&3(IH), 1&2 (EI) or 1&2&3 (3-choice)	Sequential	Used to select the difficulty level for the current block Do not change
All 2- choice random tasks and 3-choice random task	Difficulty Selection	5 of each difficulty level no. - 1&3(EH), 2&3(IH), 1&2 (EI) or 1&2&3 (3-choice)	Random equal number. No value selected more than 3 times in a row	Used to select the difficulty level for the current trial. Could change the number of times each occurs in the list (e.g. to deliberately introduce a bias), and the selection criteria can change.

**Table 6: List variables**

## 7 Analyzing Results

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 LD 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.

#### LD 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 Touches to the bottom windows (i.e. the windows 7 to 12)
- f. No. of Touches to the top windows (i.e. the windows 1 to 6)
- 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 Touches to the bottom windows (i.e. the windows 7 to 12)

- m. No. of Touches to the top windows (i.e. the windows 1 to 6)
- n. No. of times the Food Tray Entered

## **LD 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 Touches to the bottom left windows (i.e. the windows 7 to 9)
- e. No. of Touches to the bottom right windows (i.e. the windows 10 to 12)
- f. No. of Touches to the top windows (i.e. the windows 1 to 6)
- g. No. of times the Reward IR Beam broken
- h. No. of times the Screen IR Beam broken

## **LD Initial Train**

### **End of experiment summary:**

- a. Session length (condition column)
- b. Total no. of images displayed
- c. No. of correct responses
- d. No. of times the blank window is touched (while and image is displayed)
- e. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- f. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- g. % left correct (% of the images displayed on the left that were touched - i.e. the windows 7 to 9)
- h. % right correct (% of the images displayed on the right that were touched i.e. the windows 10 to 12)

### **Across the whole experiment**

- i. Correct touch latency (latency to touch an image after it is displayed)
- j. Correct Left touch latency (i.e. to image displayed in windows 7 to 9)
- k. Correct Right touch latency (i.e. to image displayed in windows 10 to 12)
- l. Blank touch latency (Latency to the first touch of the blank image after image in other window displayed)
- m. Latency to collect reward resulting from a correct touch

## **LD Must Touch**

### **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 (i.e. the windows 7 to 9)
- e. No. of Right touches during the ITI period (i.e. the windows 10 to 12)

**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.e. to image displayed in windows 7 to 9)
- i. Correct Right touch latency (i.e. to image displayed in windows 10 to 12)
- j. Latency to collect reward resulting from a correct touch

**LD 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 (i.e. the windows 7 to 9)
- e. No. of Right touches during the ITI period (i.e. the windows 10 to 12)

**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.e. to image displayed in windows 7 to 9)
- i. Correct Right touch latency (i.e. to image displayed in windows 10 to 12)
- j. Latency to collect reward resulting from a correct touch

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

- a. Session length (condition column)
- 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 (i.e. the windows 7 to 9)
- f. No. of Right touches during the ITI period (i.e. the windows 10 to 12)

**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)
- i. Correct Left touch latency (i.e. to image displayed in windows 7 to 9)
- j. Correct Right touch latency (i.e. to image displayed in windows 10 to 12)
- k. Latency to collect reward resulting from a correct touch

## **LD 1 choice**

### **End of experiment summary:**

- a. No. of trials completed
- a. Session time
- b. % correct responses
- b. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- c. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- d. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- e. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- f. Total number of time the top windows touched (i.e. the windows 1 to 6)

### **Blocks of 10 trials analysis**

- g. No of correct trials within the block of trials
- h. % correct within the block of trials
- i. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- j. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- k. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- l. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- m. Total number of time the top windows touched (i.e. the windows 1 to 6)

### **Trial by trial analysis**

- n. Correct grid position this trial
- o. No. Correct (i.e. 1 for correct trial; 0 for incorrect trial)
- p. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- q. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- r. Total number of time the top windows touched (i.e. the windows 1 to 6)
- s. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- t. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- u. Reward collection latency
- v. Correct image response latency (if correct trial)
- w. Incorrect image response latency (if incorrect trial)

## **LD 1 choice reversal**

### **End of experiment summary:**

- a. No. of trials completed
- c. Session time
- d. % correct responses
- b. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- c. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- d. Total no. of left touches to a blank window (i.e. the windows 7 to 9, but not including the correct or incorrect window)

- e. Total no. of right touches to a blank window (i.e. the windows 10 to 12, but not including the correct or incorrect window)
- f. Total number of time the top windows touched (i.e. the windows 1 to 6)
- g. Times to criteria - records the number of reversals completed to criteria (including the first set of trials)

#### **Blocks of 10 trials analysis**

- h. No of correct trials within the block of trials
- i. % correct within the block of trials
- j. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- k. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- l. Total number of time the left windows touched (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- m. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- n. Total number of time the top windows touched (i.e. the windows 1 to 6)

#### **Trial by trial analysis**

- o. Correct grid position this trial
- p. No. Correct (i.e. 1 for correct trial; 0 for incorrect trial)
- q. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- r. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- s. Total number of time the top windows touched (i.e. the windows 1 to 6)
- t. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- u. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- v. Reward collection latency
- w. Correct image response latency (if correct trial)
- x. Incorrect image response latency (if incorrect trial)

#### **Overall analysis**

- y. No of trials to criteria - records the number of trials required to reach criteria, for every reversal performed.

#### **LD 2 or 3 choice (for all 2 choice or 3 choice Location Discrimination tasks)**

##### **End of experiment summary:**

- a. No. of trials completed
- b. Session time
- c. % correct responses
- d. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- e. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- f. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- g. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)

- h. Total number of time the top windows touched (i.e. the windows 1 to 6)
- i. No of corrects at trial difficulty -Easyø
- j. Total number of -Easyøtrials
- k. % correct for -Easyøtrials
- l. No of corrects at trial difficulty -Intermediateø
- m. Total number of -Intermediateøtrials
- n. % correct for -Intermediateøtrials
- o. No of corrects at trial difficulty -Hardø
- p. Total number of -Hardøtrials
- q. % correct for -Hardøtrials

### **Blocks of 10 trials analysis**

- r. No of correct trials within the block of trials
- s. % correct within the block of trials
- t. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- u. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- v. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- w. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- x. Total number of time the top windows touched (i.e. the windows 1 to 6)

### **Trial by trial analysis**

- y. Correct grid position this trial
- z. No. Correct (i.e. 1 for correct trial; 0 for incorrect trial)
- aa. No. of Left touches during the ITI period (i.e. the windows 7 to 9)
- bb. No. of Right touches during the ITI period (i.e. the windows 10 to 12)
- cc. Total number of time the top windows touched (i.e. the windows 1 to 6)
- dd. Total no. of left touches to a blank window (i.e. the windows 7 to 9 but not including the correct or incorrect window)
- ee. Total no. of right touches to a blank window (i.e. the windows 10 to 12 but not including the correct or incorrect window)
- ff. Reward collection latency
- gg. Correct image response latency (if correct trial)
- hh. Incorrect image response latency (if incorrect trial)



## 8 Running schedules virtually

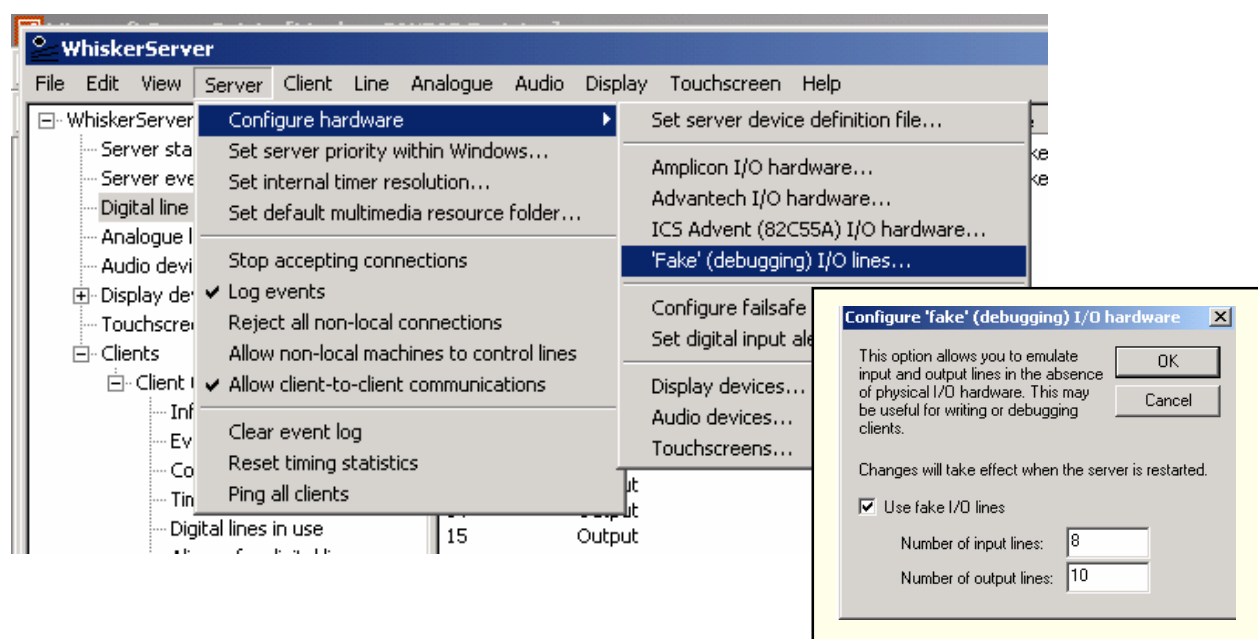
You may find it convenient to be able to look at and change schedules away from the lab. Your licence allows you to load both the ABET and Whisker software onto another computer for this purpose. This chapter will take you through the set-up to run the schedules manually.

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.

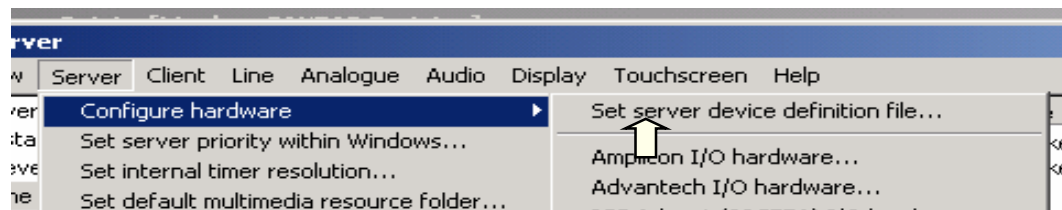
Because you have no real hardware attached, we will need to create some fake lines on the Whisker server.

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.

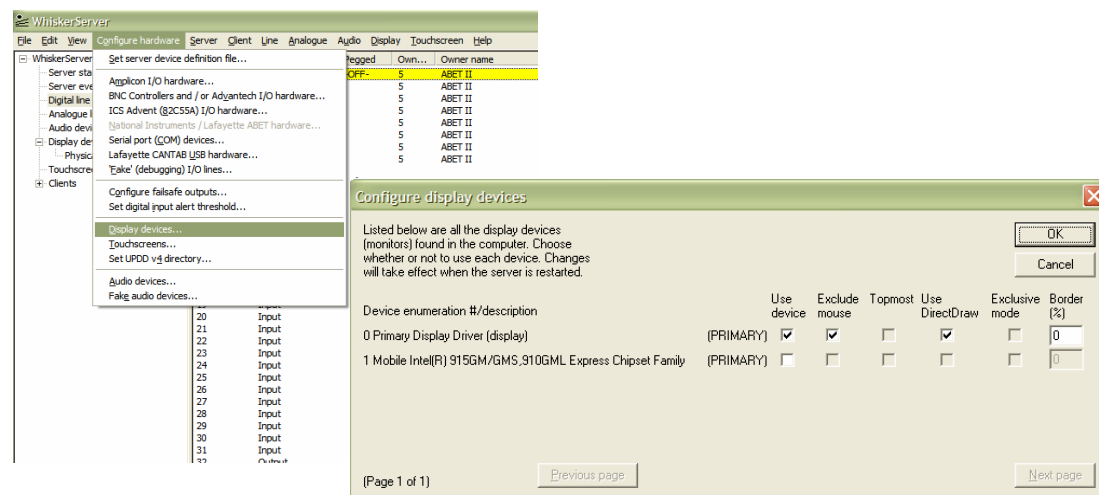
From the Whisker Server window click **Server\Configure hardware\ Fake (debugging) I/O lines** as shown below. Select 32 input and 32 output lines.



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:



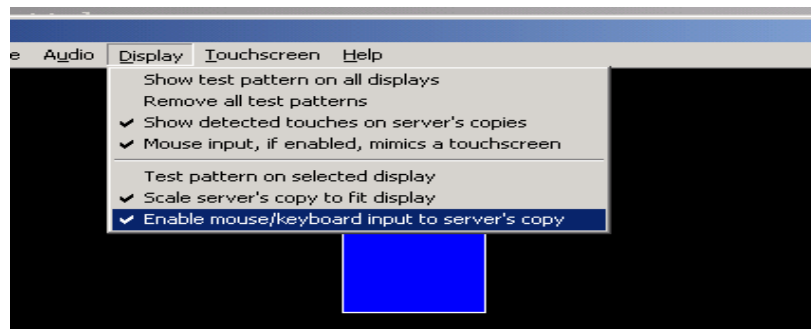
*You will need to close Whisker and restart for these changes to take effect.*

You will also need to set-up the ABET environment. Copy the `Environments.abetEnvsø` 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 statusø` selecting the required line (see Table 1 for line numbers) and pegging the lines on and off using the `-Iø` and `-Oø` keys on your keyboard. (To release the lines to the program control click `-Line/Free(unpeg all lines)ø`) Also, by clicking on the top `-Lineø` menu and selecting `-Line Detailsø` 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 lineø` box and click `-Updateø` Click `-Force onø` and `Force offø` 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..ø`



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