Conditioned Avoidance Response (CAR) Boxes

See print manual for further information

Setup:

1) Turn on all control consoles by flipping the green switches.
   - In the room on the right side as you enter the lab, there are **4 consoles**. The 2 on the right side control the shock grids.
   - In the room on the left side, there are **3 consoles**. The large one on the left and the small one on the top shelf control the shock grids.

Figure 1 - MED-PC icon

2) Turn on the computer(s).

3) Run the MED-PC IV program by double clicking its icon on the desktop. (Fig. 1)

4) Click *File* → *Open Session*.

Figure 2 - Trans IV icon

5) Enter subject information and choose a program for each CAR box.[1]

6) Once the information is entered for the boxes you are running, click *Configure* → *Signals* and select the boxes you are running. (Fig. 3)

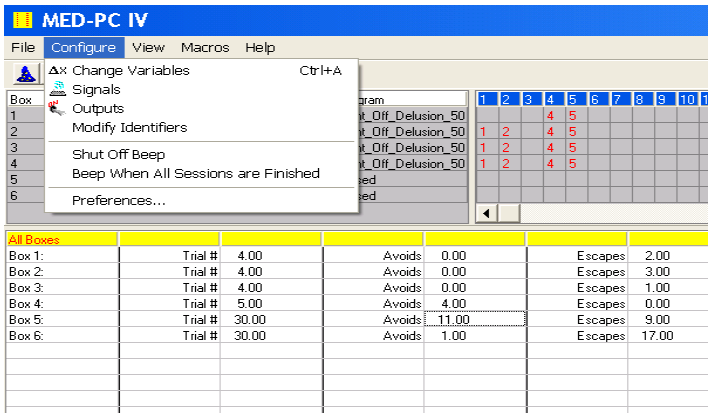

Figure 3 – The MED-PC IV Application

7) Put the rats into the boxes. Click *Issue* in the signals dialog box.

8) When you are finished with the boxes for the day, remember to turn off all control consoles and computer(s).

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[1] If your program is not listed, boot **Trans IV** (Fig. 2) from the desktop and select *Translate* → *Translate and Compile*, highlight your program from the list, click "No Trans" to include a program and "Exclude" to exclude a program from the list

Data Extraction:

1) Open MED-PC to Excel from the desktop. (Fig. 4)

2) Open Excel.

3) Click “Select” under “Row Transfer” and choose a template file (i.e. “Avoidance.mrp”) from the desktop. (Fig. 5)

Figure 4 – MED-PC to Excel icon

4) Check the box labeled “Column Labels” to include them in the excel file.

5) Click “Transfer!” and open “Shortcut to Data” on the desktop.

6) Based on the present date, select the data file you wish to view (i.e. !x-xx-2006).

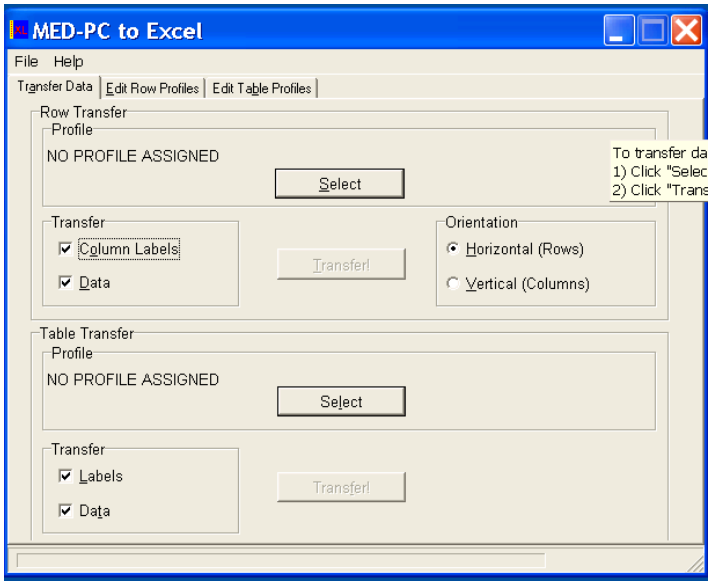

Figure 5 - The MED-PC to Excel Application

Clean up:

1) Shut down all control consoles.

2) Obtain 2 cloth towels and a spray bottle of 30% isopropyl alcohol. Soak one of the towels with water and wring it out so that it is moist.

3) Spray the inner Plexiglas walls and shock grid of the first CAR box with alcohol. Wipe them down first with the moist towel and then with the dry towel until the walls and grid are dry. Remove any feces on the walls or grid with a paper towel. **Do not use cloth towels to remove feces.**

4) Remove the lower tray and dump any feces and other solid debris into a waste bin.

5) Spray the tray with alcohol and wipe away urine with a wet paper towel, then dry it with a dry paper towel. **Use caution when handling the tray as the edges may be sharp and cut your fingers, even if you are wearing gloves (which you should be).**

6) Once it is clean and dry, return the tray to the CAR box and continue on to the next box. Re-soak the moist towel when necessary. Leave the boxes open so that any odors can dissipate.

Training Protocol

1) Upon arrival, rats are left to habituate to their home cages and colony room for 5 days.

2) For 2 days, rats are handled by experimenters for approximately two minutes each and habituated to the CAR boxes for 20 or 30 minutes each, wherein no stimuli are presented except house lights.

3) Rats undergo 20 or 30 trials of CAR training daily for at least 10 days, though not necessarily consecutively. Parameters of CAR training are as follows:
    - Each trial begins with the presentation of white noise (maximum duration=10s, CS), followed by a continuous scrambled footshock (maximum duration=5s, 0.8mA, US) through the grid floor.
    - If the subject moves to the opposite compartment within the 10s of CS presentation, then it turns off the CS and prevents the shock from occurring. This response is recorded as *avoidance*.
    - If the rat remains in the same compartment for more than 10s and crosses upon receiving the footshock, then the shock is turned off. This response is recorded as *escape*.
    - If the rat does not cross during the entire presentation of shock, then the trial is terminated and *escape failure* is recorded.
    - Trials are separated by intervals ranging from 30s to 60s with an average of 45s.

4) Rats who make at least 70% avoidances (14 avoidances out of 20 trials or 21 avoidances out of 30 trials) on each of the last two training days are included in subsequent testing. Rats who do not meet this criterion are excluded from the remainder of the study.[*]

5) Rats are given 5-7 days of drug testing using the same CAR training parameters, depending on the experimental procedure.

[*] 66-75% of subjects typically reach the criterion

MED-PC Programming Basics

The general structure of a MED-PC program is organized as such:

Code	*Description*
^LeftLight = 1	Variable declarations
S.S.1,	State sets
S1,	States
0.001”:	Input section
SET A(^Trials) = 30	Output section
---> S2	Transition

Variables

The first part of the program consists of variable declarations, whereby the values that will be used in the program—i.e. CS length, number of avoidances, number of trials, etc—are initialized. *In practically any computer language, the creation of a variable must consist of both naming the variable and setting it to an initial value.* In MED-PC, named variables must begin with the “^” sign, after which the variable is named and assigned an initial value using the equals sign.

Variables identified with a single letter (A-Z) do not require the “^” sign and can also serve as lists of variables, known as arrays. The following code defines an array “A” with space for 10 variables:

```
DIM A = 10
```

The DIM command creates an empty array that can be assigned values later. Most variables in our MED-PC programs will be part of arrays, with each variable in the array given a name for easy identification. An array with preset values can also be created using the LIST command, as follows:

```
LIST B = 1, 2, 3
```

This creates an array “B” containing three values that have been preset to 1, 2, and 3, in that order. Lists are extremely useful in our work as values can be randomly selected from the list that may dictate exactly how the program runs.

State Sets

Following variable declarations, the “functional” part of the program begins, beginning with state sets. Each program can have a maximum of 32 state sets (S.S.1-S.S.32). For all intents and purposes, state sets do not run in numerical order and in fact can run simultaneously. They are in place merely for the purpose of dividing up the program into various sub-procedures, such as initial setup, animal tracking, and stimulus presentation.

States

Each state set can be divided up into several states that must run in a sequence, though not necessarily in numerical order. There can be a maximum of 32 states (S1-S32) in each state set. The first state that runs is always S1, after which, the order depends upon the transition statement occurring at the end of each state.

Input/output sections

States can be further divided up into several input sections with corresponding output sections. Input and output sections are separated by colons (:), such that the general meaning of the code is:

Input (If this happens): Output (Then do this);

The input section may be a length of time, as in the following example:

1": SHOW 1,Trials,A(0);

This produces a SHOW command after a delay of 1 second. Time intervals as short as one one-thousandth of a second (0.001") can be used. Z-pulses, which are a special type of program signal in MED-PC, can also be used in the input section:

#Z^Start_CS: ---> S3

When used as input, Z-pulses must be preceded by "#Z^", followed by its name. When used as output, the pound sign (#) is removed. Z-pulses are important in that they allow state sets to instantly communicate with one another. If one state set produces a Z-pulse as output, any other state set can detect that Z-pulse as input as soon as it is transmitted and execute a section of code as a result. This is useful, for example, in ensuring that the CS shuts off immediately after the rat makes an avoidance and that the shock shuts off immediately after it makes an escape.

Transition

The end of every output section must be concluded with a transition. This instructs the program whether to go to a different state or to make a "null transition" (SX), which means to stop execution of the current state set until further input is received. If an output section consists of multiple lines, each line except for the last must end with a semicolon (;). Semicolons can also be inserted within individual lines of code to separate different commands, as in the following example:

SHOW 1,Trial #,D(K); RANDD X = S; RANDD V = C; Z^Start_CS ---> S4

This line of code contains 4 commands – a SHOW command, 2 RANDD commands, and a command to send a Z-pulse. Each command is separated by a semicolon, with the last command ending with a transition statement to go to state 4.

How to set basic parameters

At the very least, you should know how to change the program setup parameters, including number of trials to be run, CS length, US length, and session length. This code is located in S1 of S.S.1 and consists of the following:

```
0.001": SET A(^Trials)          = 30;  \ Trials to Run
        SET A(^StimTag)         = 1;   \ Stimulus Tag 1 = Tone
        SET A(^CS_Time)         = 10;  \ CS or Avoid Interval (sec)
        SET A(^UCS_Time)        = 5;   \ UCS or Escape Interval (sec)
        SET A(^OverlapTag)      = 0;   \ CS/UCS Overlap 0 = No
        SET A(^ITIShockTag)     = 0;   \ Punish ITI Crossings 0 = No
        SET A(^SessionTime)     = 90;  \ Session Time (min)
        ---> S2
```

To modify any of these settings, simply change the number that follows the equals sign. For instance, changing the "30" in the first line to "20" will decrease the total number of trials to 20.

Backslashes (\) indicate the beginning of a comment, which is text that is ignored by MED-PC. Comments are helpful in programming in that they can be used to leave explanations of code, highlight sections that need to be changed, or other programming purposes.

Program Descriptions

10xCS1US_10xCS2US_10xCS2

- Shock: Yes
- Trials: 30
- CS: 10s pure, white
- US: 5s
- Layout:
    - 10 white → shock
    - 10 pure → shock
    - 10 pure → no shock

2sec DelayedCS Training

- Shock: Yes
- Trials: 30
- CS: 10s white noise
- US: 5s
- CS terminates 2 sec after avoidance

5sec DelayedCS Training

- Shock: Yes
- Trials: 30
- CS: 10s white noise
- US: 5s
- CS terminates 5 sec after avoidance

Aberrant_Tone

- Shock: Yes
- Trials: 30
- CS: 10s pure, white
- US: 5s
- Layout:
    - 5 pure tone → no shock
    - 5 pure tone → shock
    - 20 noise → shock

Anxiolytic

- Shock: Yes
- Trials: 20
- CS: 10s white noise
- US: 5s

CARTraining100

- Shock: Yes
- Trials: 100
- CS: 10s white noise

- US: 5s

CAR Training
- Shock: Yes
- Trials: 30
- CS: 10s white noise
- US: 5s

CAR_10 trials
- Shock: Yes
- Trials: 10
- CS: 10s white noise
- US: 5s

CAR_20 NoShock
- Shock: No
- Trials: 20
- CS: 10s white noise
- US: 5s

CAR_20 trials
- Shock: Yes
- Trials: 20
- CS: 10s white noise
- US: 5s

D_test
- Shock: Yes
- Trials: 50
- CS: 5s white noise
- US: 2s
- 25 non-avoidable shocks follow CS after 5-30s

DC_test
- Shock: Yes
- Trials: 50
- CS: 5s white noise
- US: 2s
- Avoidable shock follows every CS after 5-30s

DP_test
- Shock: Yes
- Trials: 50
- CS: 5s white noise
- US: 2s
- Non-avoidable shock immediately follows every CS

DPC_test
- Shock: Yes
- Trials: 50
- CS: 5s white noise
- US: 2s
- Avoidable shock immediately follows every CS

DelayedOnset Training
- Shock: No
- Trials: 30
- CS: 10s white noise
- US: n/a
- CS offset 5s after avoidance

Delusion_0
- Shock: Yes
- Trials: 30
- CS: 10s 2.8kHz pure tone, light
- US: 5s
- Layout:
    - 10 pure tone → no shock
    - 20 light → shock

Delusion_50
- Shock: Yes
- Trials: 30
- CS: 10s 2.8kHz pure tone, light
- US: 5s
- Layout:
    - 10 pure tone → no shock
    - 10 light → shock
    - 10 pure tone+light → shock

Light_Delusion
- Shock: Yes
- Trials: 30
- CS: 10s light
- US: 5s

Flooding

- Shock: No
- Trials: 20
- CS: 10s white noise
- US: n/a
- ITI ranges 10”-50”

Habituation_6_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 6 min
- Measures locomotor activity in 5” blocks

Habituation_10_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 10 min

Habituation_20_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 20 min

Habituation_30_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 30 min

Habituation_60_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 60 min

Jean

- Shock: Yes
- Trials: 30
- CS: 10s white noise
- US: 5s
- Layout:
    - 8 pure tone → no shock
    - 8 pure tone → shock
    - 14 white noise → shock

Light_Off_Delusion_50

- Shock: Yes
- Trials: 30
- CS: 10s pure/lights off
- US: 5s
- Layout:
    - 10 tone alone → no shock
    - 10 lights off → shock
    - 10 tone+lights off → shock

NoLights_Hab_20_min

- Shock: No
- Trials: n/a
- CS: n/a
- US: n/a
- Length: 20 min
- Lights off

Pav-fear control

- Shock: No
- Trials: 2
- CS: 30s 5-kHz pure tone
- US: n/a
- Measures locomotor activity in 5” blocks

Pav-fear testing

- Shock: No
- Trials: 3
- CS: 30s 5-kHz pure tone
- US: n/a
- Measures locomotor activity in 5” blocks

Pav-fear conditioning

- Shock: Yes
- Trials: 2
- CS: 30s 5-kHz pure tone
- US: 2s
- CS-US overlap
- Measures locomotor activity in 5” blocks

Pav-fear reactivation
- Shock: No
- Trials: 1
- CS: 30s 5-kHz pure tone
- US: n/a
- Measures locomotor activity in 5” blocks

QuickShock
- Shock: Yes
- Trials: 6
- CS: n/a
- US: 0.5s
- ITI ranges 10”-70”

Simultaneous_cond
- Shock: Yes
- Trials: 10
- CS: 15s pure+light
- US: 2s
- ITI: 180”
- Simultaneous onset of CS and US

Simultaneous_test
- Shock: No
- Trials: 25
- CS: 60s pure+light
- US: N/A
- ITI: 180”, 30”

SS_simulcond
- Shock: Yes
- Trials: 10
- CS: 15s pure+light
- US: 3s
- ITI: 180”
- Begins w/ ITI

SS_unpairedcond
- Shock: Yes
- Trials: 10
- CS: 15s pure+light
- US: 2s
- ITI: 180”
- CS is separated from US by 75s

TONE-Delusion
- Shock: Yes
- Trials: 30
- CS: 10s pure
- US: 5s

Training NoShock
- Shock: No
- Trials: 30
- CS: 10s white noise
- US: n/a

Troubleshooting

If the detector DOES NOT display "Downloading program. Please wait...." then it WILL NOT record your data.

To fix the problem, close the USV Detector program and the MED-PC IV program if it is running. Then switch the right control panel (the one with the light brown serial cables and the audio cables) off and then on again. Then restart your programs.

If you encounter a run time error or a *.dll error, use the same procedure.

If the USV Detector flashes between "Found ANL937" and "Searching for ANL937", or stays minimized on the toolbar close the program and turn off the right control panel. Then restart the computer. Once the computer is booted up, turn the control panel back on. Then boot up the MED USV Detector program. That should solve the problem.

When you boot up the USV Detector and it stays minimized with no way to maximize it, even though you click on it in the toolbar, then it is flashing between “Found ANL937” and “Searching for ANL937”. Follow the same procedure as above, and that should solve the problem.

In the event the program does not close upon completion, go to *File → Close session* and elect to “STOPABORTFLUSH” all boxes. **Do not shut down MED-PC IV or your data will not be saved.**

If anymore problems are encountered, and you find a way to solve the problem, save an explanation in the “READ BEFORE USING USV DETECTOR.txt” file on the desktop and be as specific as possible.