SOF-700RA-29

ZEBRAFISH SHUTTLE BOX PASSIVE AVOIDANCE

MEDSTATE NOTATION^{TM} PROCEDURES

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Table of Contents

CHAPTER 1

Introduction

MED-PC gives researchers the ability to use pre-programmed applications such as the Zebrafish Shuttle Box Avoidance to make data collection easy. These pre-programmed applications can also be modified to meet the evolving demands of a research protocol. This manual provides step-by-step instructions on how to use this pre-programmed application.

In addition to this manual, refer to DOC-010 MED-PC IV User's Manual for the installation of the MED-Associates interface drivers, the MED-PC IV Software, and the Delphi Compiler. Also refer to the Wiring Guide that accompanies the hardware for instructions on hardware configuration. The Hardware Configuration software utility is installed with MED-PC IV and is used to assign the inputs and outputs in the interface cabinet for each task controlled by the program. Figure 1.1 shows the ENV-012Z-X1 Zebrafish Shuttle Box for which this protocol was developed.

Figure 1.1 – ENV-012Z-X1 Zebrafish Shuttle Box
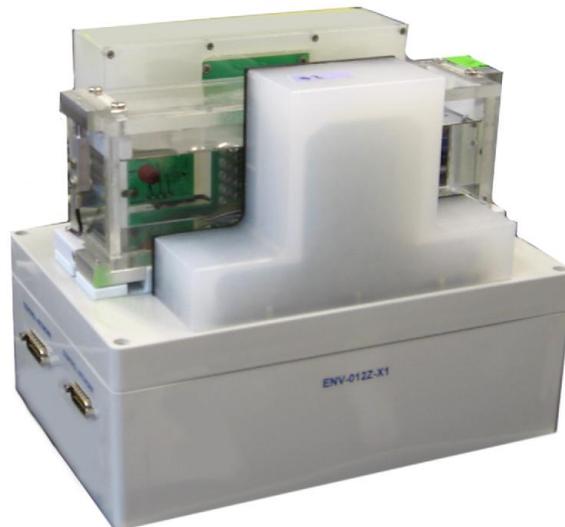
Data file structure, file-saving format, and other related options are also determined by the Hardware Configuration software utility. Refer to DOC-003 MED-PC IV Programmer's Manual if you are unfamiliar with the process of translating and compiling an application.

The staff at Med Associates, Inc. is available to answer any questions that may arise. Please contact us via e-mail at support@med-associates.com with a detailed description of the problem or your desired goals so that we may provide you with concise and detailed information.

CHAPTER 2

Installing Drivers and Software

Please refer to the ENV-012Z-X1 Fish Shuttle Box Wiring Guide for hardware setup.

Before using the software, the necessary drivers and software must be installed. Install the MED-PC software using the instructions in DOC-010 MED-PC IV User's Manual. Drivers for the computer interface are included on the MED-PC installation CD.

The Zebrafish Shuttle Box Avoidance CD contains the Zebrafish Shuttle Box Passive Avoidance Training.mpc MED-PC protocol. To install the protocol, insert the installation CD into the CD-ROM player on the PC. Click on **Install the Zebrafish Shuttle Box software.** When prompted, confirm that MED-PC is installed in C:\MED-PC IV\, and the installation program will automatically copy the Zebrafish Shuttle Box Passive Avoidance Training.mpc to the "C:\MED-PC IV\MPC\" folder.

Translating and Compiling

Programs written in MedState Notation need to be translated using Trans IV before they can be executed in this application. Open Trans IV and select **Translation | Translate and Compile**. Select the Zebrafish Shuttle Box Passive Avoidance Training.mpc, and click **Make**. Click **OK** to start the translator. If any problems are encountered during this process, refer to the on-screen help menu, DOC-010 MED-PC IV User's Manual, or contact support@med-associates.com for assistance.

Figure 2.1 – Trans IV Control Panel for Translating and Compiling MedState Notation Code

Backing Up the Software

Med Associates strongly encourages creating backup copies of the Zebrafish Shuttle Box programs in case of disk failure. When making modifications to the protocol, it is advised to keep original copies.

CHAPTER 3

Using the Software

In Zebrafish Shuttle Box Passive Avoidance, subjects (Zebrafish) learn to avoid the environment where an aversive stimulus was previously delivered. This test is classically used to test short-term or long-term memory capabilities. In this test, an aversive stimulus (shock) is administered to a Zebrafish once the Zebrafish enters a certain side of the shuttle box. This side is typically darkened with a shade or cover. Latency to swim to the side of the shuttle box associated with the aversive stimulus is measured for each subsequent trial, and is taken as evidence of memory retention. The Zebrafish Shuttle Box Passive Avoidance includes both acclimation and testing phases.

The Zebrafish Shuttle Box has 8 columns of 6 I/R photo beam sensors per column (illustrated in Figure 3.1) and separate green, yellow, and red Stimulus Lights on the left and right.

Figure 3.1- IR Beam Numbering as Seen From Front

1	2	3	4		5	6	7	8
	*		*		*		*	
*		*				*		*
	*		*		*		*	
*		*				*		*
	*		*		*		*	
*		*				*		*
	*		*		*		*	
*		*				*		*
	*		*		*		*	
*		*				*		*
	*		*		*		*	
*		*				*		*

 The protocol, by default, starts when the animal is placed in the left side of the chamber. When the animal is detected on the "start" side, a 5 minute acclimation period begins. The procedure then runs a fixed number of 30 trials.

A trial proceeds as follows: When the Zebrafish swims into the shock zone it will be shocked after the shock delay period. The default shock delay is 0 seconds (immediately after crossing into the shock zone). The shock is turned off when the Zebrafish escapes from the shock zone or the maximum shock time is reached. If the Zebrafish escapes from the shock zone, then the next trial starts immediately.

If the Zebrafish fails to escape from the shock zone before the maximum shock duration runs out, then the latency to escape will be recorded as maximum shock duration (default 30 seconds), the shock will be turned off, and the program will turn the Alert signal on.

IMPORTANT If the Zebrafhis fails to escape, it must be moved to the Start/Safe side. When the Zebrafish is back on the Start/Safe side, a START command must be reissued in order for the experiment to continue.

If the Zebrafish fails to cross into the shock zone before the No Response (Limited Hold) time runs out, then the latency to cross will be recorded as the no response time (default 180 seconds) and the next trial will start immediately. The experiment will end when the Max Number of Trials is reached or the Max Session Time is reached.

Below are the variables used with (description), [default value (units)], and <options> listed:

- Duration of **Acclimation Period** [5 (minutes)]

- Number of **Trials to Run** [30 (trials)]

- **Start/Safe side** (where the Zebrafish should be when the trial begins, the side opposite the Shock side) [1] <1=left side;2=right side>

- **Stimuli on Safe side** (setting will correspond to a light that will remain on/flash in the Safe side for the duration of the session) [3] <1=Green; 2=Yellow; 3=Red>

- **Stimuli on Shock side** (setting will correspond to a light that will remain on/flash in the Shock side for the duration of the session) [3] <1=Green; 2=Yellow; 3=Red>

- **Stimuli Flash Rate** (the rate each Stimuli light will flash on and off) [0.5 (seconds)]

- **Delay to Shock Onset on Dark Side** (how much time will elapse between the Zebrafish entrance to the Shock Side and the shock) [0 (seconds)]

- **Max Shock Duration** (maximum time the shock can last if the Zebrafish fails to escape the Shock Side during the shock) [30 (seconds)]

- **No Response (Limited Hold) Time** (the longest allowed duration the Zebrafish can spend on the Safe side before a new trial begins)[180 (seconds)]

- **Shock On Time** (duration of the shock pulse) [0.1 (seconds)]

- **Shock Off Time** (duration of time between shock pulses) [2.4 (seconds)]

- **Session Time** [60 (minutes)]

Default values may be modified using the MED-PC Experiment Loading Wizard, within the protocol, or with the **Change Variables** option. Changes made within the protocol Medstate Notation require the code to be translated and compiled to be applied at runtime.

Notes:

- Start Side = Safe Side.

- With Zebrafish, the shock must be pulsed on and off so that the fish do not become accustomed to the current running through the water. Use the Shock On Time and Shock Off Time variables to set the desired timing for the shock pulsation.

- The program can take measurements as fast as every 10ms for up to eight chambers.

Using the MED-PC IV Load Wizard

Open MED-PC IV and the MED-PC Experiment Loading Wizard Welcome screen (Figure 3.2) is displayed. To proceed with the wizard, click **Next** and the Box Selection screen will be displayed.

Figure 3.2 – Med-PC Wizard Welcome Screen

To avoid this load wizard, deselect the checkbox labeled **Run this experiment automatically when starting MED-PC**. Close this screen by clicking the **Close** button. Closing this screen immediately reveals the MED-PC Run-Time Screen shown in Figure 3.4. To continue using the Wizard, press **Next**.

On the Box Selection screen, the researcher chooses which boxes will be used in the experiment. Select the boxes that will run the experiment by clicking in the radio button next to the box number and click **Next** to proceed.

The next screen is the Select a Procedure screen. This is where the application to be run is selected. The screen displays a list of all the currently compiled procedures. Select Zebrafish Shuttle Box Passive Avoidance, and then click **Next**.

The Enter Experiment Data screen will appear next. The purpose of this screen is to allow annotations to be added to the data file that is produced by MED-PC IV. These annotations will help identify the Subject, Experiment, and Experiment Group upon which data was collected. Comments can be added here as well, and the data file can be given a customized file name. Enter the information desired, and click **Next** (the information on this panel is optional, and can be skipped if so desired).

The next screen to appear is the Review Choices screen. This is a method of confirming that the information entered is correct. If it is not correct, select Previous, and edit the data. If it is correct, select **Next**.

The Alter Session Parameters Screen is the next screen to appear and displays the default parameter settings, as shown in Figure 3.3. The Alter Session Parameters screen allows the researcher to alter the parameters by which a procedure executes. Click **Next** to proceed.

Figure 3.3 – Default Protocol Parameters
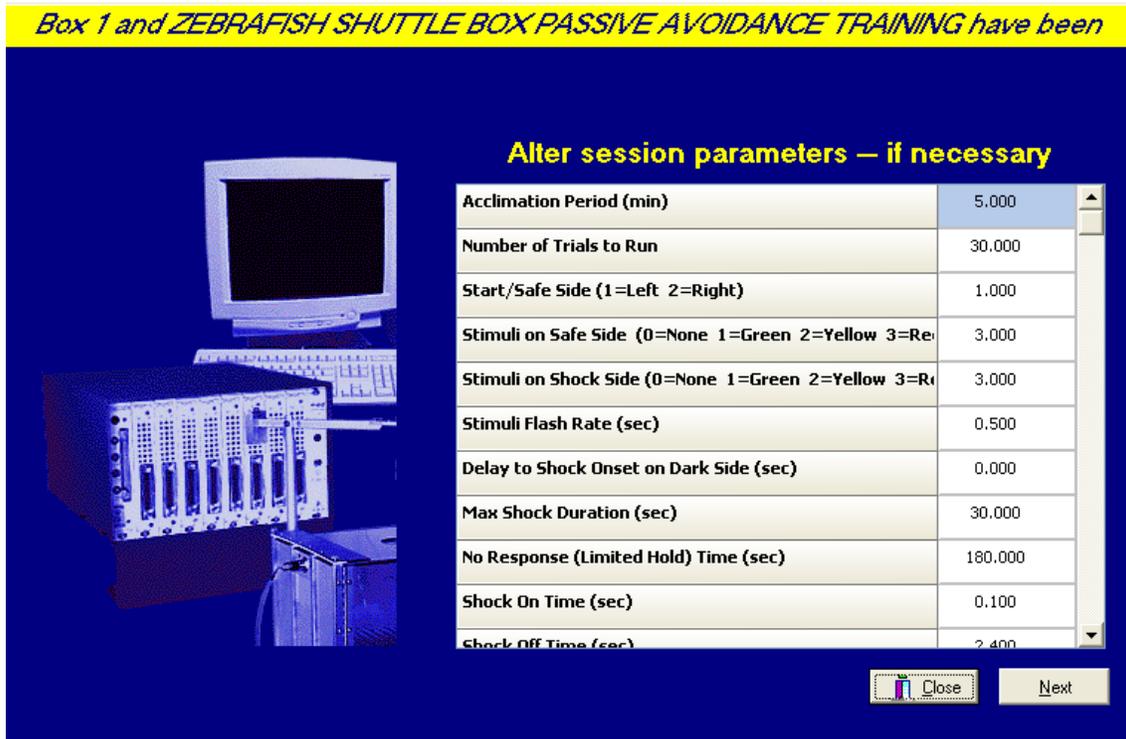
The Send Start Command Screen appears next. The options available on the screen vary depending upon how many boxes are described in the Hardware Configuration. You will be presented with as many boxes as are described in the Hardware Configuration. If more than 1 box is in the Hardware Configuration, then an option to load more boxes is presented.

Next, the researcher decides to either load more boxes, send a start signal to boxes that are already loaded, or enter the MED-PC IV run-time environment without sending a start signal by selecting **I am finished with the wizard**. The Run-Time screen, shown in Figure 3.4, is then displayed.

Figure 3.4 – MED-PC Runtime Screen
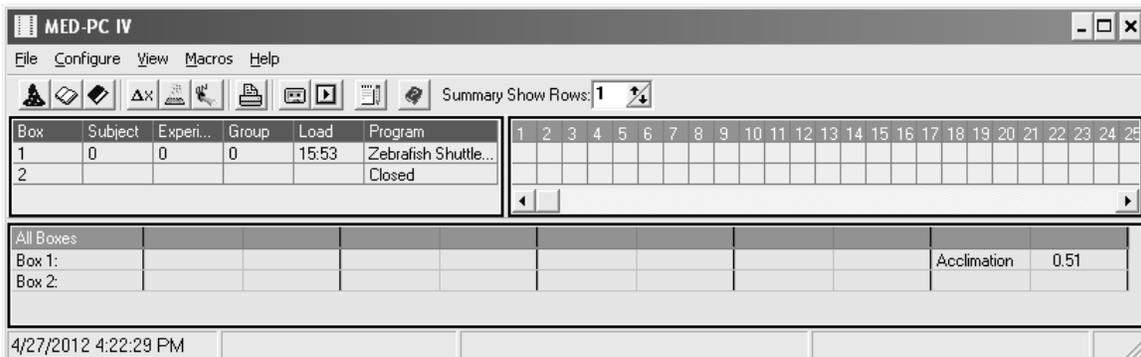
Viewing/Changing Variable Values

A protocol's variables may be changed from their default values, even once a session is in progress. Change variables by selecting **Configure | Change Variables** on the main menu, or click the 4th tool bar item **ΔX**. In the "Display Data from Box" section located in the lower left hand corner of the Displaying Variables window (Figure 3.5), choose the chamber(s) to modify. By clicking additional boxes in the "Additional Boxes to Update" section, changes made to a single box are duplicated on the boxes selected.

Figure 3.5 – Displaying Variables Screen
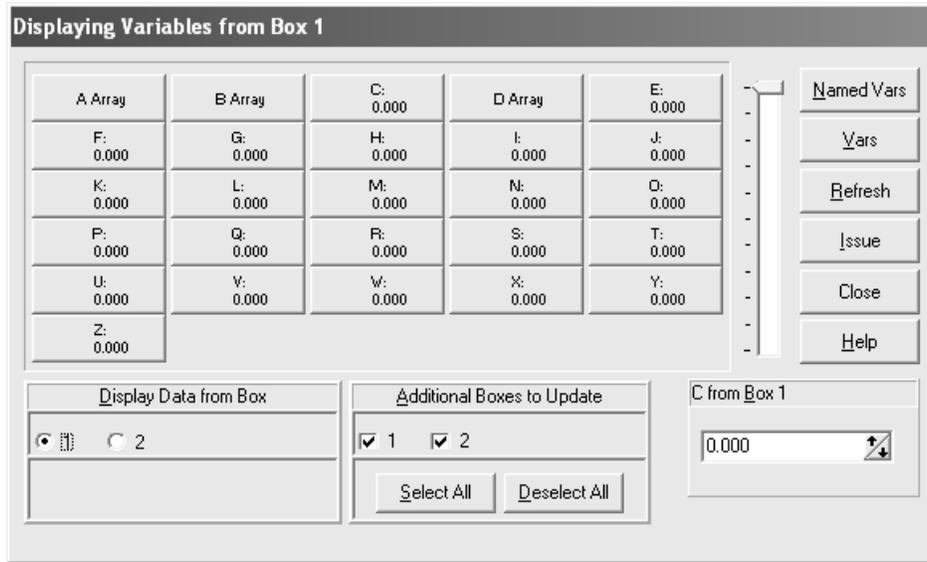
In the Displaying Variables screen click an array on the table, and each element in that array can be viewed. To change a value, select the variable in the upper left section of the dialog, and replace the value in the lower right hand box or use the up/down arrows to increment by 1. Click the **Issue** button for the change to take effect. Click **Named Variables** to produce the display in Figure 3.7. Change variables here as needed.

Figure 3.6 – Displaying Array A
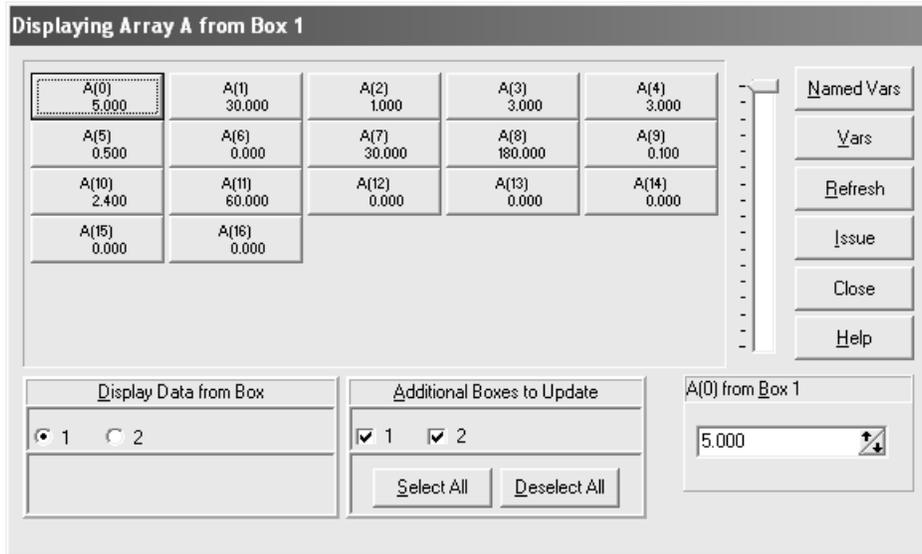
Figure 3.7 – Displaying Named Variables
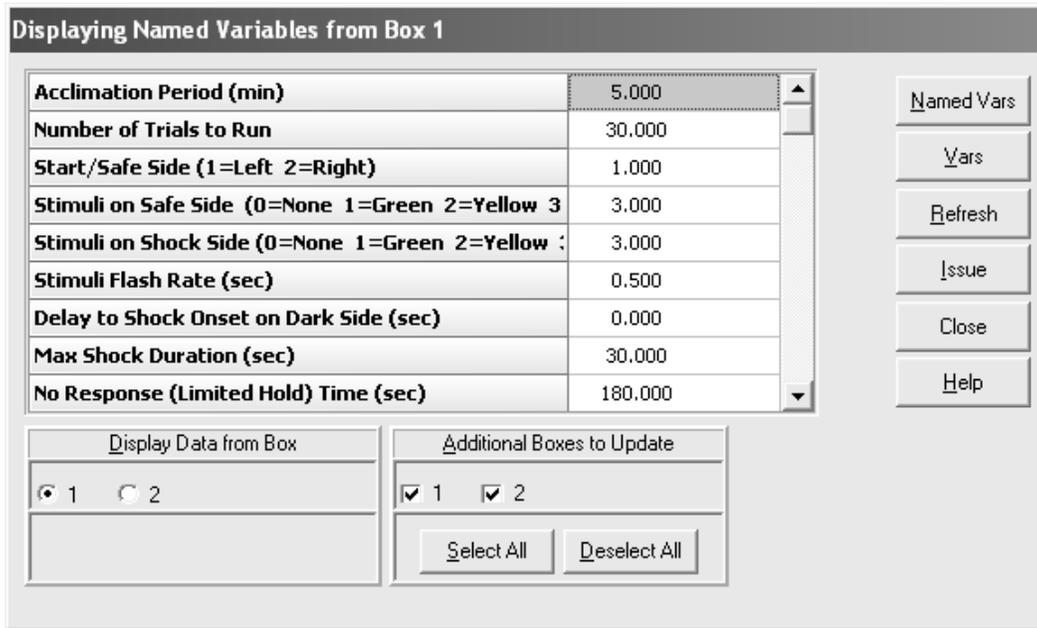
CHAPTER 4

Saving Data & Understanding the Raw Data File

Unless otherwise specified, data will be saved to MED-PC installation directory (default is C:\MED-PC IV\Data). The data file format is specified in the Hardware Configuration Utility. (See DOC-010 MED-PC User's Manual for data file format options.) Data are saved at the end of the protocol or can be saved manually by selecting **File | Save Data Manually** or **File | Save Data (Flush)**. Data files may be opened with Notepad, Wordpad, or any word processor or spreadsheet software; however, ensure they are always saved "unformatted" for use with data extraction utilities such as MED-PC to Excel (MPC2XL). Data file formats are explained in detail in the MED-PC IV User's Manual.

```
File: C:\MED-PC IV\DATA\!2012-04-25_16h18m.Subject 0
Start Date: 04/25/12
End Date: 04/25/12
Subject: 0
Experiment: 0
Group: 0
Box: 1
Start Time: 16:18:45
End Time: 16:27:41
MSN: Zebrafish Shuttle Box Passive Avoidance Training
C:        0.000
E:        0.000
F:        0.000
G:        0.000
H:        0.000
I:       87.000
J:        0.000
K:        0.000
L:        1.000
M:        0.000
N:        0.000
O:        0.000
P:        0.000
Q:        0.000
R:        0.000
S:      194.000
T:        0.000
U:        0.000
V:        0.000
W:        0.000
X:        0.000
```

Session identification information entered when starting the experiment.

I = Subscript for Data Array D

L = Location Flag (1 = Left, 2 = Right)

S = Acclimation/Session Timer

```
Y:        0.000

Z:        0.000

A:
    0:        5.000       30.000        1.000
    3:        3.000        3.000        0.000
    6:       30.000      180.000        0.100
    9:        2.400       60.000        0.000
```

Named Variables Array Defaults:

Acclimation Period = 5 minutes

Number of Trials to = 30

Start/Safe Side = 1 (Left)

Stimuli on Safe Side = 3 (Red)

Stimuli on Shock Side = 3 (Red)

Stimuli Flash Rate = 0.5 seconds

Delay to Shock Onset on Dark Side = 0 seconds

Max Shock Duration = 30 seconds

No Response (Limited Hold) = 180 seconds

Shock On Time = 0.1 seconds

Shock Off Time = 2.4 seconds

Session Time = 60 minutes.

```
B:
    0:       30.000       30.000        5.628
    3:       30.000        0.789
```

Summary Data Array:

Crossings, Escapes, Latency

```
D: (I)
    0:        1.000      151.100        1.460
    3:        2.000        0.340        0.200
    6:        3.000        0.470        6.220
    9:        4.000        3.720        1.890
   12:        5.000        2.710        1.340
   15:        6.000        1.140        1.410
   18:        7.000        0.980        0.900
   21:        8.000        1.060        1.040
   24:        9.000        0.920        0.860
   27:       10.000        0.860        0.780
   30:       11.000        0.450        0.560
```

Trial by Trial Array:

D(I)   = Trial Number

D(I+1) = Latency to Cross into Shock Zone (No Response Time (default 180 seconds) if no Crossing)

D(I+2) = Latency to Escape from Shock (Maximum Shock Duration (default 30 seconds) if no Escape)

CHAPTER 5

Data Analysis – Using MED-PC to Excel

Using a Pre-Formatted Table Profile (.MTP file)

MED-PC to Excel (MPC2XL) is a program that helps to import data from MED-PC (the raw data file format, previous section) to a spreadsheet program such as Microsoft Excel. MPC2XL needs to be installed separately from MED-PC IV. Please refer to DOC-036 User's Manual for MPC2XL for installation instructions.   Follow the step-by-step instructions below for importing data obtained from the Zebrafish Shuttle Box Passive Avoidance.mpc procedure.

Open Microsoft Excel, and select cell A1. Minimize the Excel window. Open **MED-PC to Excel**. The MED-PC to Excel window will be displayed, as shown in the Figure 5.1. In the **Transfer Data** window located in the leftmost file tab, click **Select** in the **Table Transfer** section at the bottom of the dialog window.

Figure 5.1 – Select Table Transfer Profile (.mtp) file
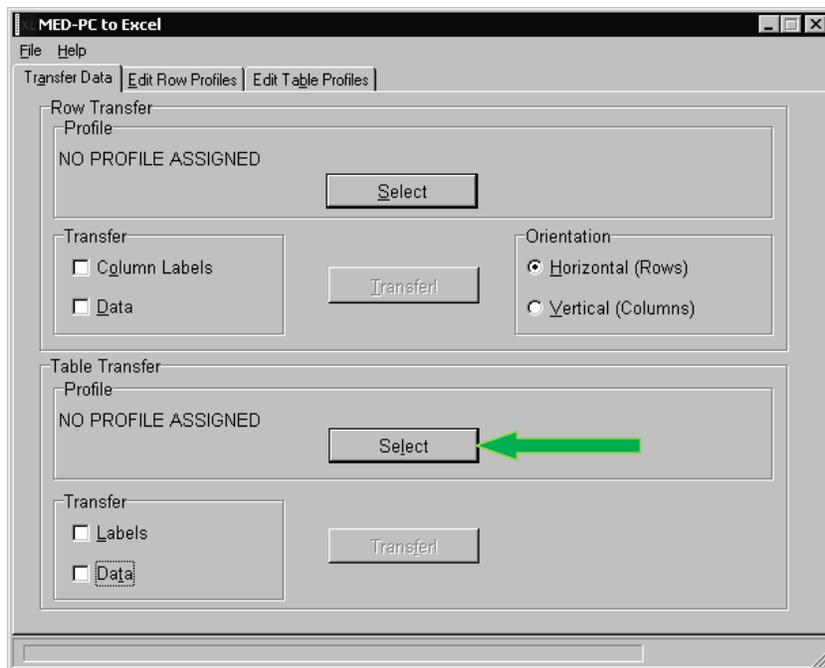
Choose Zebrafish Shuttle Box Passive Avoidance Training.mtp in the C:\MED-PC IV\Data folder.

*Figure 5.2 – Click **Transfer!** to Select Data file to Transfer to Excel*
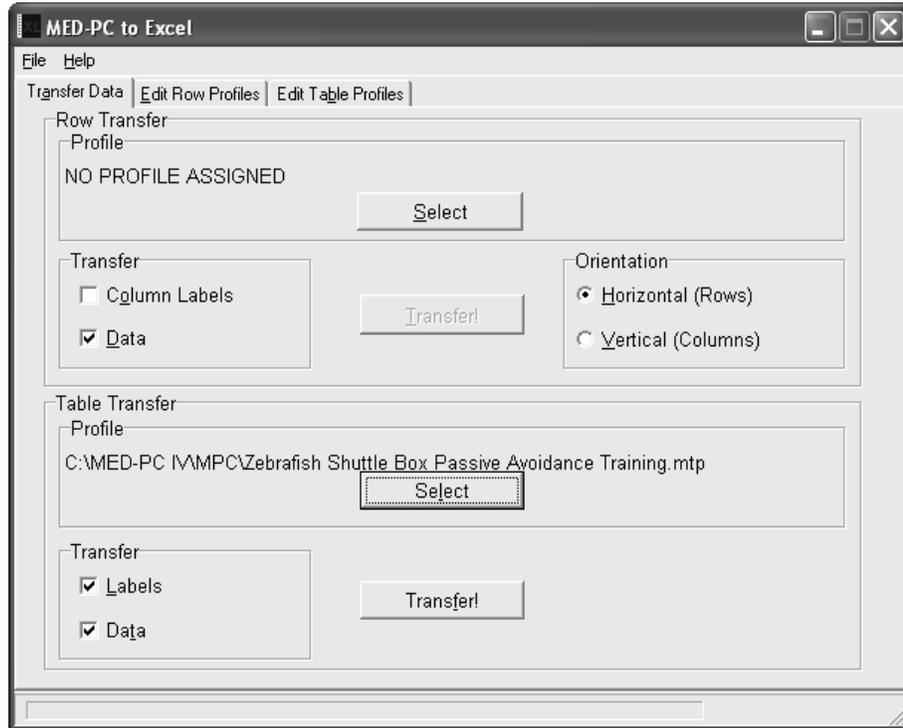
Note that the datapath for the Zebrafish Shuttle Box Passive Avoidance Training.mtp is listed in the **Table Transfer "Profile"** section. Select **Labels** and **Data**, to print data labels and import data. Then click **Transfer!.** Specify the raw data file to transfer, and then click **Open**. The data will transfer to Microsoft Excel spreadsheet.

Refer to DOC-036 MPC2XL User's Manual for more information on transferring MED-PC data files to Microsoft Excel.

Appendix A

Contact Information

Please contact MED Associates, Inc. for information regarding any of our products.

Visit our website at www.med-associates.com for contact information.

For technical questions, email support@med-associates.com.