

EPhys Matlab Toolbox

Electrophysiology Experiment Software for TDT

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last updated on 1/22/2013

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Introduction

The EPhys toolbox for Matlab integrates with Tucker-Davis Technologies (TDT) OpenEx software package and real-time processing hardware to facilitate the design and control of electrophysiology experiments. The toolbox includes a control panel which runs the experiment, a protocol design utility for generation parameters for an experiment, and a utility for the automated calibration of simple acoustic stimuli. Some familiarity with the Matlab environment and basic principles of RPDs circuit design are important for implementing the EPhys toolbox. Creating new experiment protocols, however, requires no programming by the user; however, advanced dynamic Matlab scripts can be written to create more complex experiments.

There were several motivations for the designing the EPhys toolbox to extend OpenEx's capabilities. One motivation was to facilitate the design of complex multiparametric experiment protocols in a simple but powerful graphical user interface (GUI). This is accomplished with the Protocol Design Utility (**ProtocolDesign**) (p. 11).

A second motivation was to enable the use of high sampling rates (~200 kHz on some hardware) required for producing ultrasonic stimuli on the TDT real-time modules. The

Function column

The **Function** column can take one of three options selected from a drop-down list. This field indicates whether the parameter tag is updated before each trial ("Write") or the value is read by EPhys Control Panel after each trial ("Read"). If both functions are required, then the "Write/Read" option should be selected. While most parameters will simply be used to update the parameter tags on the RPDs circuit, the "Read" functionality is useful for dynamic scripts.

Values column

The **Values** column is the primary focus of the parameter table. This field is used to specify the values that the parameter (specified in the **Tag** column of the same row) will have for a protocol. In the example parameter table above, the Frequency parameter tag is associated with the values: 1000 2000 4000 8000 16000 (separated by spaces). These values will be used to update the Frequency parameter tag in the RPDs circuit example above. The same goes for the Duration parameter tag for the values: 25 50 100 (**specified in** milliseconds according to the Schmitt component requirements).

Instead of manually specifying a list of variables, the **Values** column will accept any Matlab code which evaluates to a numeric scalar or vector. Any code which runs as a line item, including

function calls, in the Matlab command window will work in this field. For example, instead of specifying the values manually as in Figure 6, we could have also entered: `2000*2.^(0:3)` with the same result. Another example of valid input: `logspace(log10(1000),log10(42000),40)`.

When using the \$ flag with a parameter tag, the value entered will be the default values when starting an experiment (see page 15 for details).

Clicking the View Compiled button (located below the table) brings up a new window with all permutations of specified parameter (Figure 7). In this Compiled Protocol table, each row starts with a trial number with each subsequent column a parameter value. Note that the total number of trials displayed in the table will be truncated if over 2000 trials are generated. The figure caption will indicate the total number of trials.

Buddy column

The **Buddy** column of the parameter table is used to have the values of more than one parameter co-vary. In other words, making a buddy variable will make the values of two or more parameters to always be the same. One stipulation is that the parameters with a selected buddy variable must have the same number of values. Multiple buddy variables can be specified.

Rand column

When activated, the Rand field works in conjunction with the **Values** field to provide a random value, selected at run time, between two values specified. For example, if a parameter tag is to be updated with a random value between 50 and 200, then enter `50 200` in the **Values** field and check the **Rand** field. Before the parameter is updated during the experiment, a random value will be selected from a uniform distribution (using the Matlab `rand` function) with the lowest possible value of 50 and a highest possible value of 200. This behavior is indicated on the compiled table (click View Compiled) as the two scalar values `[50 200]`.

WAV column

Activating the WAV field will launch a new window. Use this window to add WAV format files to a list. The file names, sampling rate, and duration of each WAV file will be displayed in the table in the new window. The order in which the WAV files are listed in the table are the order they will be specified for the parameter for which the WAV field was clicked. Please note that the sampling rate of the WAV file should be the same as the TDT real-time

module will be running. If this is not followed, then aliasing will occur during playback resulting in unintended stimulus artifacts.

Calib. (calibration) column

The Calib. field is used to select a calibration file to be associated with a parameter tag. Calibration files are generated using the Calibration Utility (CalibrationUtil) (p. 21). Clicking the drop-down box in this column will launch a Windows dialog requesting the user **to locate** a calibration **file** (*.cal) which **was** created using the CalibrationUtility. Select the calibration file to be associated with the parameter in the same row. When compiled (after saving **or** clicking the 'View Compiled' button), an additional parameter will be automatically generated which will contain the calibrated value. This associated parameter will have the same name as the parameter but will **be prefixed** with a tilde (~).

For example: The circuit in Figure 5 (page 14) demonstrates a basic gated tone function. The Frequency parameter tag is linked to the Freq input of the Tone component and a second parameter labeled ~Frequency is linked to the Amp input of the Tone component. This associated parameter should not be explicitly added to the parameter table in the Protocol Design Utility, but will be automatically generated when adding a calibration to the Frequency parameter. The values generated for the ~Frequency parameter will be selected or calculated from the normalization curve in the calibration file. The result of this will be that each tone frequency presented during an experiment will be generated at a voltage which could produce a sound level (for example, 80 dB SPL) set during the calibration procedure. A PA5 programmable attenuator can then be used to attenuate the voltage to the intended sound level during an experiment.

Note: Values **which** are not explicitly calibrated are calculated from the the voltage normalization curve using a piecewise cubic hermite interpolating polynomial (the PCHIP function in Matlab).

A calibration file can be used in conjunction with parameters using the \$ flag in the same manner as with the normal parameters as described above. For example, \$Frequency would be associated with the calibrated ~\$Frequency parameter in RPvds.

Protocol Design Options

A few options (Figure 8) go along with each protocol file which control presentation of trials. The Randomize option will reorder the sequence of trials in a pseudorandom fashion.

By default the order of trials is compiled (meaning the actual trial sequence is generated) when the protocol file is saved. This will ensure that the same sequence of trials (even if randomized order) will be presented each time the experiment is run. This behavior can be altered by

enabling the **Compile at Run-Time** option. When enabled, the protocol is compiled (the trial sequence generated) each time the experiment is run. This has some advantages if using a custom script.

The # Reps field accepts a scalar value which indicates how many times to present each combination of parameters. A value of 5 would repeat all combinations of parameters 5 times.

The inter-trigger **interval** (ITI) field accepts either a single scalar value or two scalar values separated by a space (eg. 200 500). If a **single** value is specified, then a constant time interval will be used between stimulus triggers. If two values are entered, then a random time interval will be used between trials within the specified range. The field is in milliseconds.

Finally, the name of a custom written function can be specified in the Trial Select Function. This function supplants and should extend the default trial selection functionality. This is an advanced topic which should be tested thoroughly before running an experiment. If a custom function is specified then it must be somewhere on the Matlab path.

EPhys Control Panel (EPhysController) (p. 15) accomplishes this by offloading stimulus triggers to the host computer. This approach, which has certain considerations discussed later, reduces the overhead (number of circuit components) required on to run an RPDs circuit which frees up additional processing power for circuit components required for various stimulation paradigms. An additional advantage of using the host computer to control stimulus triggering is the added capability for using custom Matlab scripts to dynamically select the next stimulus parameters based on previously acquired data (such as spike counts, field potential power, button presses, etc).

Thirdly, a Calibration Utility (**CalibrationUtil**) (p. 21) automates calibration of acoustic sources and creates standardized calibration files which can be used to normalize sound levels of parameters (such as stimulus frequencies).

Finally, the use of high-channel electrode arrays has dramatically increased the size and complexity of electrophysiological datasets. Efficiently maintaining, managing, and accessing these very large datasets is greatly facilitated by the use of a common database structure. To address this, the EPhys toolbox includes GUIs which can be used to upload acquired data and stimulus parameters to an SQL server as well as GUIs to navigate, access, and visualize stimulus-evoked responses.

Hardware Recommendations, Software Requirements & Installation

Hardware recommendations

The minimum requirements of EPhys toolbox are those of the installed version of Matlab and TDT software. The EPhys toolbox was developed on a standard quad-core processor personal computer with 4 GB of memory running Windows 7 64-bit operating system.

Required software

The EPhys toolbox was designed in Matlab version R2011a and higher. The EPhys toolbox does not require any additional toolboxes to function; however, EPhys uses the OpenDeveloper ActiveX controls which can be obtained from TDT. OpenDeveloper is designed to interface with electrophysiology data structures and the OpenEx software suite to run an experiment.

If installing Matlab for the first time, it is recommended to Matlab install to a directory such as “C:\MATLAB\R2012b” (or whatever the current version) and not to the default “Program Files” directory. Installing into the default “Program Files” directory may cause problems with permissions when updating with TortoiseSVN on Windows Vista and later.

Currently, the EPhys toolbox is distributed using a software versioning (svn) system hosted by GoogleCode and additional software will be required to obtain the latest releases of the EPhys toolbox software (see next section).

Obtaining and Using SVN

The software versioning system (svn) requires third-party software to be installed locally on the computer which will be used with the EPhys toolbox. TortoiseSVN is a free software package and can be downloaded here: <http://tortoisesvn.net/downloads.html>. Download and install the latest TortoiseSVN software version for your operating system (32-bit or 64-bit) using default options. Once installed, follow these steps to obtain the EPhys software:

1. Create a new folder called “work” in the Matlab root directory such as “C:\MATLAB\work”
2. Open the work folder in a Windows Explorer, right-click in the white space to open a context menu, and click the “SVN Checkout...” menu item.

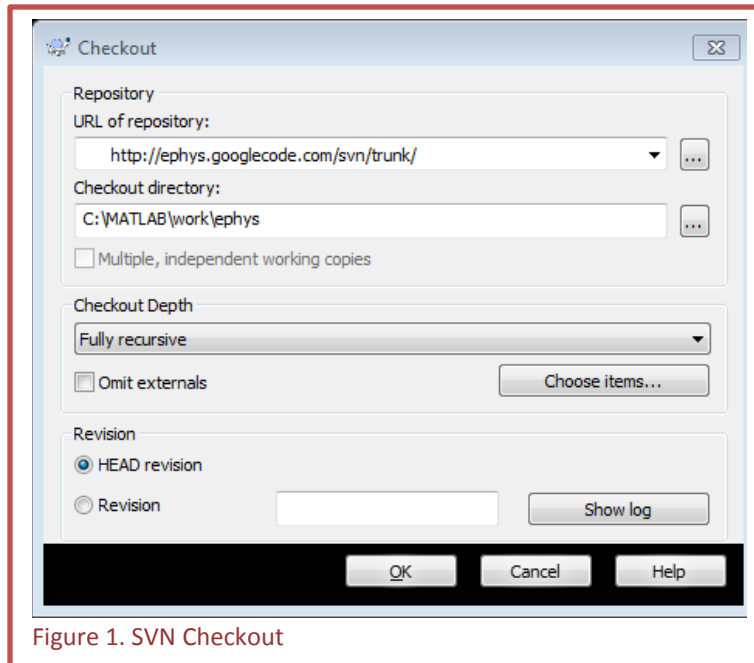


Figure 1. SVN Checkout

3. Enter the following information into the Checkout prompt and click OK.
 - a. URL of repository: <http://ephys.googlecode.com/svn/trunk/>
 - b. Checkout directory: C:\MATLAB\work\ephys
4. A dialog should appear as the EPhys software is downloaded.

The versioning system allows those using the software to easily update files for updates and bug fixes by right-clicking the parent folder (such as: “C:\MATLAB\work\ephys”) and selecting “SVN Update” from the context menu. This system also provides the opportunity for distributed collaboration on future software developments.

A wiki page can be found at <https://code.google.com/p/ephys/wiki/Intro>. This wiki provides a place where issues can be raised, discussed, and addressed with software fixes. This requires a Google account.

EPhys Toolbox Overview

The EPhys toolbox for Matlab is designed to integrate with the OpenEx software package provided by TDT. The OpenWorkbench program, included in the OpenEx package, should be used to define which TDT real-time hardware will be used during an experiment. Please refer to the TDT OpenWorkbench documentation for details on how to do this.

Creating a New Experiment

The procedure for designing new experiments with EPhys begins with assigning labels and RPvds files to the TDT hardware modules using the Device Navigator of OpenWorkbench (TDT). While labels are arbitrary in reality, descriptive labels should be used in practice. One example would be to use the label **Stim** for a module (such as an RP2.1, RX6, RZ6, or other module) which will be used to control stimulation during the experiment, such as a speaker or light. If multiple stimulus modules are to be used in an experiment then names such as **Stim1** and **Stim2**, or **SpeakerStim** and **LightStim** can be used (**Error! Reference source not found.**). One or multiple data acquisition modules (such as an RX5, RZ5, or other module) can be assigned a label in a similar manner (**Acq**, for acquisition device, is used in Figure 2). Note that not all modules need to be populated. The labels assigned in the Device Navigator of OpenWorkbench will be used to direct parameters during protocol design using the EPhys toolbox.

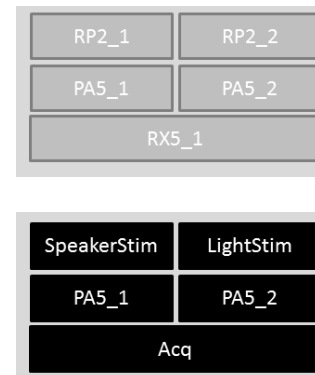


Figure 2. Unpopulated (top) and populated (bottom) device navigator

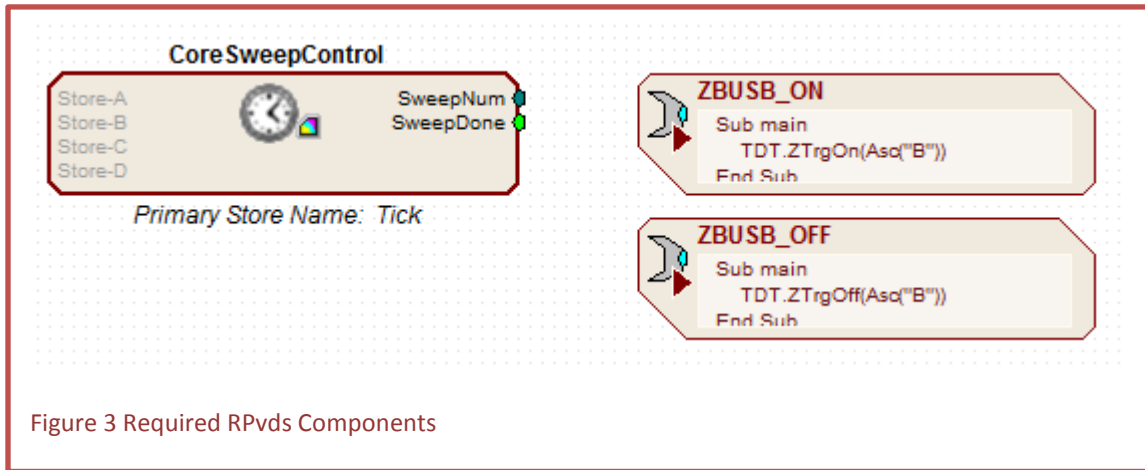
NOTE: Programmable Attenuator PA5 modules should be labeled PA5 followed by a suffix if desired as is in the example in Figure 2.

An RPvds file (with the extension .rcx) should be assigned to each real-time module which is defined in the Device Manager (see TDT documentation for details). The conventions for designing RPvds files for use with OpenWorkbench are outlined in the TDT documentation and should be followed. There are a few requirements for RPvds circuit designs when using EPhys to control experiments that will be discussed next.

RPvds Requirements

For the majority of experiment designs, the EPhysController (discussed on page 19) controls parameter values and timing of stimulation of the TDT hardware modules (defined in the OpenWorkbench Device Navigator; see page 9). EPhysController first updates all parameter

tags on real-time modules as well as any PA5 programmable attenuators, and then uses the **zBus B** trigger issue a synchronised trigger pulse across all real-time modules. In order to accomplish this from Matlab two custom scripts must be included on one of the RPvds modules



in use. The two script components in Figure 3 must be titled **ZBUSB_ON** and **ZBUSB_OFF** and may be copied from an example RPvds document.

The **CoreSweepControl** macro (left component in Figure 3) handles module synchronization and is required to be included in every RPvds file. This macro is available in RPvds by clicking the Components menu and selecting Circuit Macros.

Note: If using multiple RPvds circuits, each CoreSweepControl macro must have a unique primary store name. This can be modified by double clicking the macro object in RPvds, clicking the Setup tab, and then Change button. Any four letter name can be used (eg, Tock).

Protocol Design Utility (`ProtocolDesign`)

The Protocol Design Utility is a graphical user interface which is used to specify the values of parameters for an experiment. A simple example of this would be to specify all frequency and sound level combinations for a tone-evoked receptive field experiment. Instead of manually specifying every frequency/level combination, of which there may be hundreds or thousands of combinations, one can use the Protocol Design Utility to specify which frequencies and which sound levels should be presented and the utility permutes these parameters to create a list of stimuli for the experiment.

Creating a New Protocol

Before creating a new protocol, an OpenWorkbench project should be created as described above (see Creating a New Experiment on p. 9). The Protocol Design Utility can be launched in two ways. The command `ProtocolDesign` can be entered into the Matlab command window directly. Alternatively, clicking the “P” icon on the

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with the same result. Another example of valid input: `logspace(log10(1000),log10(42000),40)`.

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For example: The circuit in Figure 5 (page 14) demonstrates a basic gated tone function. The **Frequency parameter tag is linked to the Freq input of the Tone component and a second parameter labeled **~Frequency** is linked to the Amp input of the Tone component. This associated parameter should not be explicitly added to the parameter table in the Protocol Design Utility, but will be automatically generated when adding a calibration to the **Frequency** parameter. The values generated for the **~Frequency** parameter will be selected or calculated from the normalization curve in the calibration file. The result of this will be that each tone frequency presented during an experiment will be generated at a voltage which could produce a sound level (for example, 80 dB SPL) set during the calibration procedure. A PA5 programmable attenuator can then be used to attenuate the voltage to the intended sound level during an experiment.**

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Finally, the name of a custom written function can be specified in the Trial Select Function. This function supplants and should extend the default trial selection functionality. This is an advanced topic which should be tested thoroughly before running an experiment. If a custom function is specified then it must be somewhere on the Matlab path.

EPhys Control Panel (EPhysController) (p. 15) toolbar will launch the utility.

Adding/Removing Modules

Creating modules in OpenWorkbench was covered in an earlier section (see Creating a New Experiment, p 9). The labels for modules defined in OpenWorkbench can be added to or removed from the protocol design by clicking the “+” or “-” buttons, respectively. Clicking the “+” button will launch a dialog box in which the user should enter the label (capitalization matters) of one module at a time.

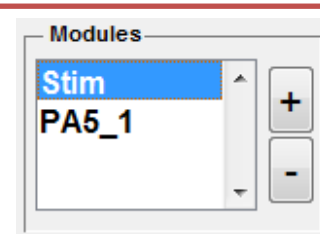


Figure 4 Add/Remove module

Working With Parameter Tags

Once a module has been added to the protocol, parameters can be specified using the table on the right of the Protocol Design Utility.

The R Pvds circuit of the right is a simple example of an R Pvds circuit which plays tones of various frequencies and durations. The R Pvds circuit to the right is one realization of such a design. The parameter tags (ParTag components) called **Frequency** and **Duration** are linked to the **Freq** parameter

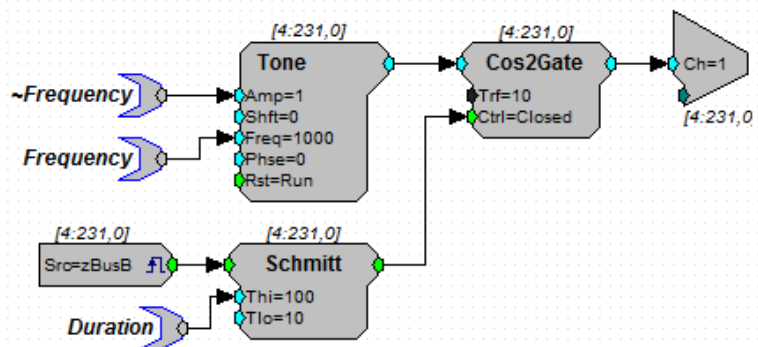


Figure 5 Example R Pvds circuit using parameter tags (ParTag component)

of the Tone generating component and the **Thi** parameter of the Schmitt logic component, respectively (the **~Frequency** tag is discussed on page 17).

The **Frequency** and “Duration” parameter tags are entered (case sensitive) into the “Tag” column in the Protocol Design Utility parameter table. The other columns of the table are used to specify the values and how the parameter functions.

Tag	Function	Buddy	Values	Rand	WAV	Calib.
Frequency	Write/Read	< NONE >	1000 2000 4000 8000 16000	<input type="checkbox"/>	<input type="checkbox"/>	< NONE >
Duration	Write/Read	< NONE >	25 50 100	<input type="checkbox"/>	<input type="checkbox"/>	< NONE >

Figure 6 Protocol Design Utility parameter table with example of parameter values controlling “Frequency” and “Duration” parameter tags (ParTag) specified in the R Pvds circuit.

The \$ flag

Some experiment designs require the values of parameters to be specified when beginning the recording. This can be done by prepending a **\$** (dollar sign) to a parameter tag name (a **\$** should also prepend the parameter tag name in the corresponding R Pvds file); for example, **\$Frequency** or **\$Duration**. The **compile at runtime** option must be checked to enable this functionality. A prompt will automatically appear for the user to enter a value for the parameters with the **\$** flag when clicking the **Record** button on the EPhysController GUI (see page 19 Figure 9) to begin an experiment.

Function column

The **Function** column can take one of three options selected from a drop-down list. This field indicates whether the parameter tag is updated before each trial (“Write”) or the value is read by EPhys Control Panel after each trial (“Read”). If both functions are required, then the “Write/Read” option should be selected. While most parameters will simply be used to update the parameter tags on the R Pvds circuit, the “Read” functionality is useful for dynamic scripts.

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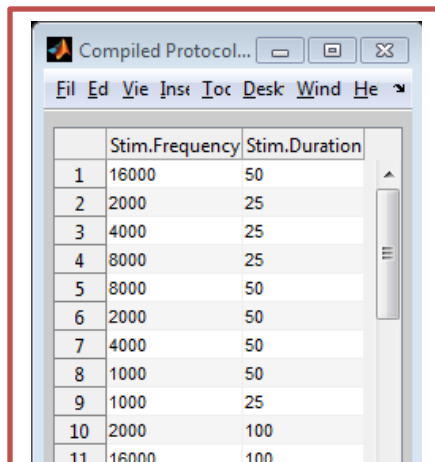
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WAV column

Activating the **WAV** field will launch a new window. Use this window to add WAV format files to a list. The file



	Stim.Frequency	Stim.Duration
1	16000	50
2	2000	25
3	4000	25
4	8000	25
5	8000	50
6	2000	50
7	4000	50
8	1000	50
9	1000	25
10	2000	100
11	16000	100

Figure 7 Example of a compiled

names, sampling rate, and duration of each WAV file will be displayed in the table in the new window. The order in which the WAV files are listed in the table are the order they will be specified for the parameter for which the WAV field was clicked. Please note that the sampling rate of the WAV file should be the same as the TDT real-time module will be running. If this is not followed, then aliasing will occur during playback resulting in unintended stimulus artifacts.

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Protocol Design Options

A few options (Figure 8) go along with each protocol file which control presentation of trials. The **Randomize** option will reorder the sequence of trials in a pseudorandom fashion.

By default the order of trials is compiled (meaning the actual trial sequence is generated) when the protocol file is saved. This will ensure that the same sequence of trials (even if randomized order) will be presented each time the experiment is run. This behavior can be altered by enabling the **Compile at Run-Time** option. When enabled, the protocol is compiled (the trial sequence generated) each time the experiment is run. This has some advantages if using a custom script.

The **# Reps** field accepts a scalar value which indicates how many times to present each combination of parameters. A value of **5** would repeat all combinations of parameters 5 times.

The inter-trigger interval (ITI) field accepts either a single scalar value or two scalar values separated by a space (eg. **200 500**). If a single value is specified, then a constant time interval will be used between stimulus triggers. If two values are entered, then a random time interval will be used between trials within the specified range. The field is in milliseconds.

Finally, the name of a custom written function can be specified in the **Trial Select Function**. This function supplants and should extend the default trial selection functionality. This is an advanced topic which should be tested thoroughly before running an experiment. If a custom function is specified then it must be somewhere on the Matlab path.

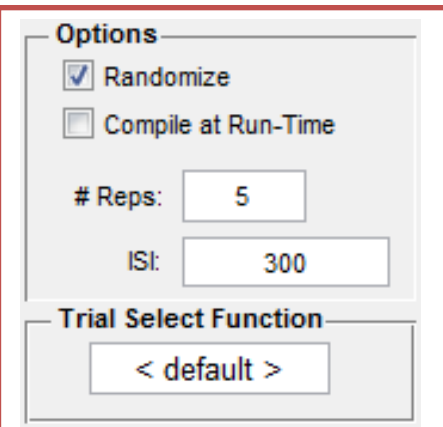


Figure 8 Protocol Design options

EPhys Control Panel (EPhysController)

The EPhys Control Panel controls physiology experiments in conjunction with OpenEx software. To launch the program, enter **EPhysController** into the Matlab command window and hit enter. To begin an experiment:

1. OpenProject must be open on the same computer and the OpenWorkbench Device Manager (see on page 9) should be set for the intended experiment.
2. Create a new tank or select an existing tank using the left panel of the EPhys GUI.
3. Click the **Locate Protocols...** button and select a directory containing one or multiple protocol files.
4. Click **Record** to begin an experiment.
5. Progress of the experiment will be displayed at the bottom of the EPhysController and the software will automatically stop after finishing and reset for the next protocol.

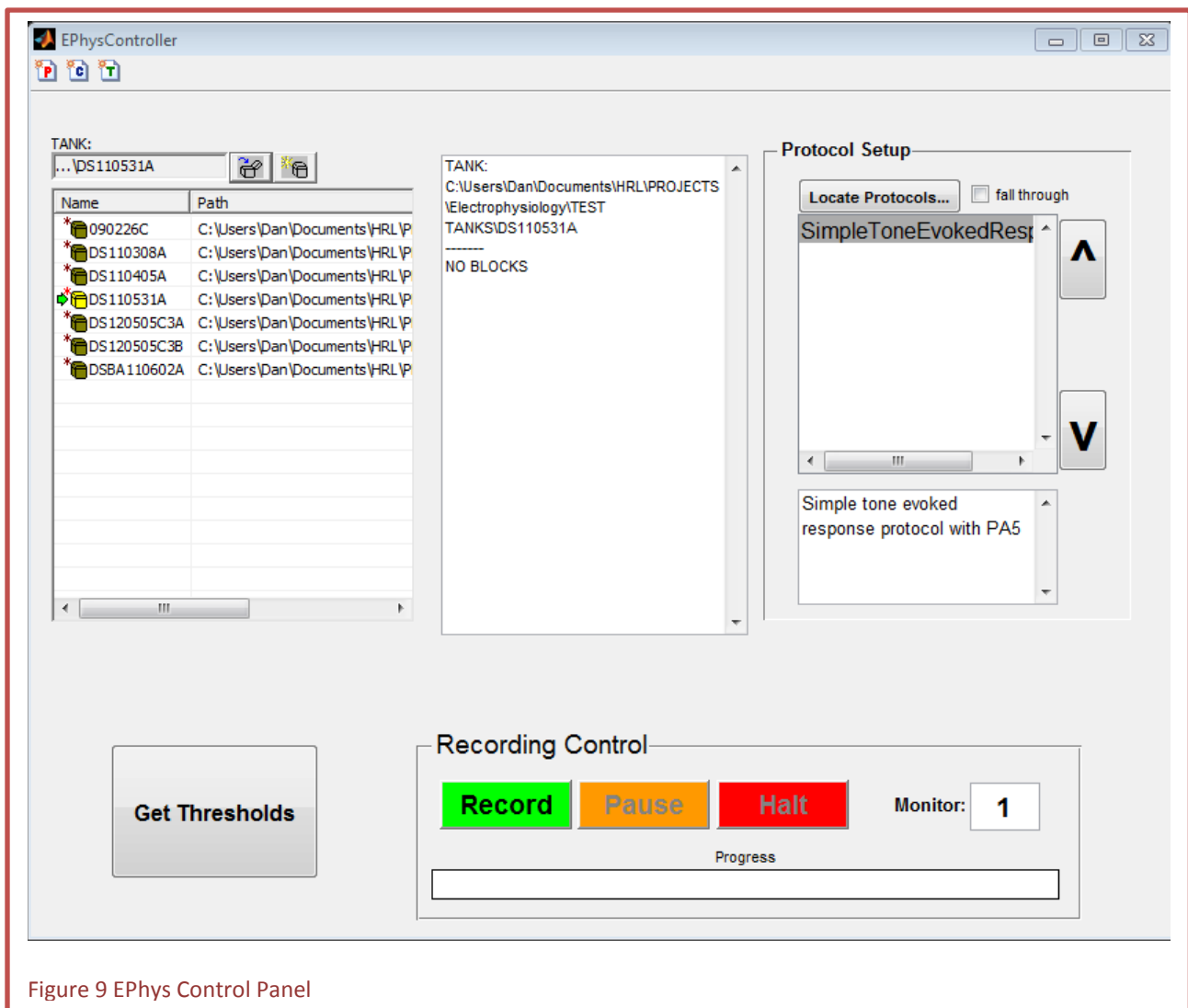


Figure 9 EPhys Control Panel

Calibration Utility ([CalibrationUtil](#))

The screenshot shows the 'Calibration Utility' window. It has a title bar with the MATLAB logo and the text 'Calibration Utility'. The interface is divided into several sections: 'Hardware', 'Stimulus', and 'Calibration'. The 'Hardware' section contains a 'Stim' group with 'GB', 'RX6', '1', and '100 kHz' dropdowns, and an 'Acq' group with 'RP2' and '1' dropdowns. The 'Stimulus' section has a 'Tone' dropdown. The 'Calibration' section shows '57.4 mV RMS = 114 dB SPL' and 'Ref. Piston Norm = 100 dB SPL'. A large 'Run' button is on the right.

Calibration Utility

Hardware

Stim

GB RX6 1 100 kHz

Acq

RP2 1

Stimulus

Tone

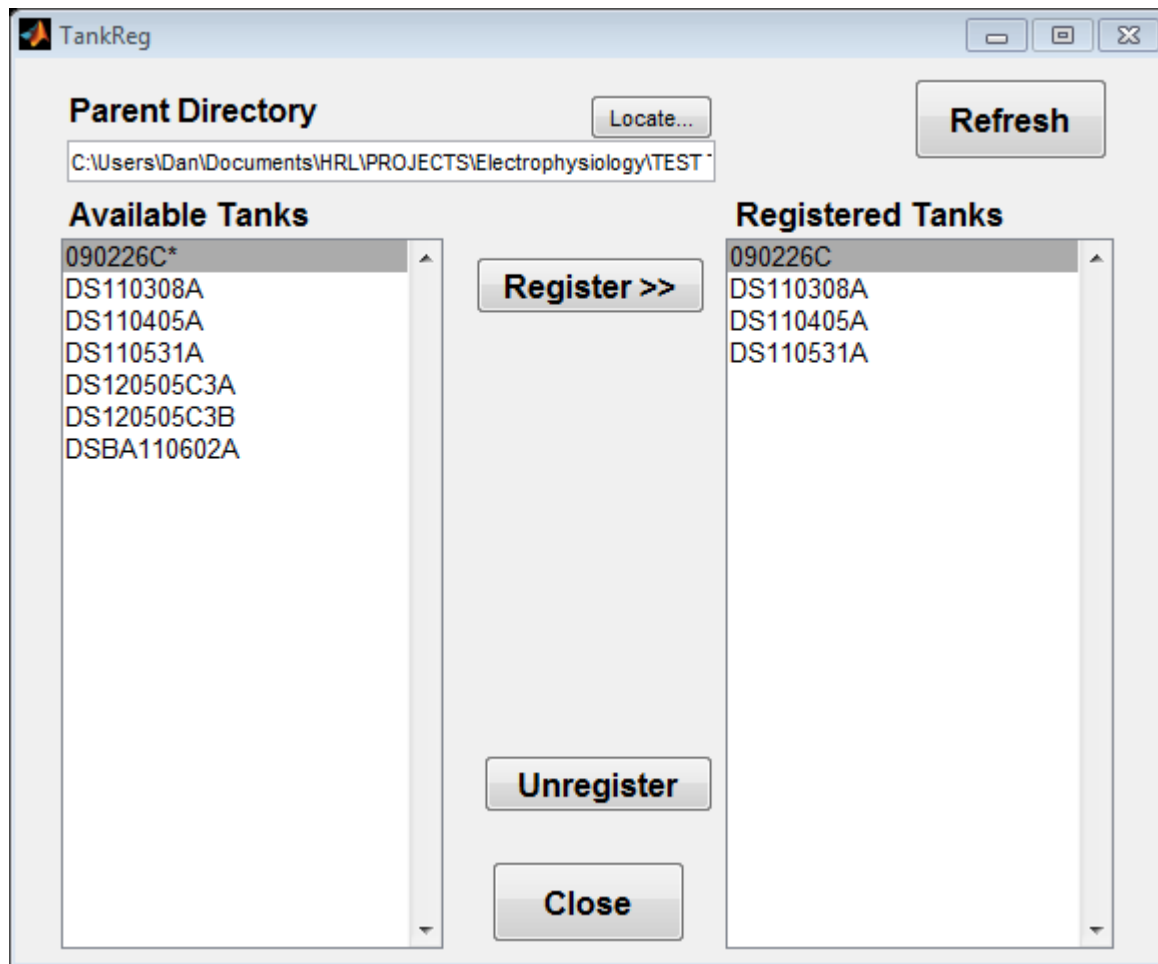
Calibration

57.4 mV RMS = 114 dB SPL

Ref. Piston Norm = 100 dB SPL

Run

Tank Registration (TankReg)



Database Browser (DB_Browser)

The screenshot displays the DB_Browser application window. At the top, a 'Settings' tab is active, showing a dropdown menu for 'Databases' set to 'ac_hp_salicylate_acute'. To the right are 'Parameters' and 'Plot' buttons. Below this, the interface is divided into five main sections: Experiments, Tanks, Blocks, Channels, and Units.

- Experiments:** A list of 18 experiments. Experiment 7 (090908) is selected and highlighted.
- Tanks:** A list of 4 tanks. Tank 40 (3h-SS [090908E]) is selected and highlighted.
- Blocks:** A list of 3 blocks. Block 117 (eFRA [1]) is selected and highlighted.
- Channels:** A list of 17 channels. Channel 1986 (AC014) is selected and highlighted.
- Units:** A list of 2 units. Unit 3360 (SU1 (5113)) is selected and highlighted.

Each of the five sections has a 'show all' checkbox and an 'Exclude' button at the bottom. At the bottom of the window are two buttons: 'Get Protocol' and 'Get LFP'. On the right side, there is a plot area showing a graph of a signal (likely LFP) over time, with a scale factor of $\times 10^{-4}$. The plot shows a sharp negative deflection followed by a slower positive recovery. Below the plot are 'show all' and 'Exclude' checkboxes, and a 'Get Spiketimes' button.

