

INTRODUCTION

The “***Multi-Analyses_Macros.xls***” file contains a number of macros that allow the MED64 user to rapidly plot potentials and analyze data generated in the Conductor software. These macros ALSO illustrate the use of various subroutines in the MEDWrapper DLL utility¹; these subroutines can also be called from a number of other commercially available programs (e.g. Igor, Matlab).

In addition to documenting the use of these macros, this manual describes the use of various MS excel files which aid in graphing potentials and summarizing data generated using these macros. The OUTPUT IS DESIGNED TO BE EASILY IMPORTED into programs that are commonly used to generate figures for manuscripts or presentation software (e.g. Powerpoint).

The main files of interest are contained in the directory “EXCEL Macros-Conductor”. The MEDWrapper DLL file, instructions for installation are located in the “MedWrapper” directory. In addition, many of these utilities, as well as macros developed for use in Igor and Matlab, can be obtained from the Users Group at the MED64 Website.

Overview of the “Multi-Analyses_Macros.xls” macros.

There are two macros in the “***SOLO Macros***” worksheet, which illustrate the use of the MEDWrapper.dll file. The *first* of these, **Measure Amp & Slope**, will allow you to measure the amplitude or slope of a series of potentials collected in Conductor. Unfortunately, it is rather tedious to use this macro because you have to enter all of the variables for each analysis. Nonetheless, it will give you an overview of some of the routines which can be used to analyze slope and amplitude measures for potentials collected from a single channel. (*Note*- the use is documented in the pdf file – “**MANUAL - Measure Amp & Slope (SOLO) Macro**”).

The second “SOLO Macro”, **DISPLAY EPs**, is quite useful because it allows you to, in conjunction with the GRAPHS excel template files, rapidly export individual traces or averages to an EXCEL spreadsheet, Graph and display the potentials (single or Paired-Pulse traces), and then (using copy and paste) export the traces as vector-based output to your favorite program (e.g. – Corel Draw, Adobe PhotoIllustrator) to make figures for presentations. (Examples are shown in the file zPaired-Pulse Examples.ppt). The use of this macro will be further documented in the upcoming “**MANUAL - Display EPs (SOLO) Macro**”.

In addition, the highly flexible “**Multi-Analyses**” **Worksheet/Macro** will allow you to rapidly analyze the results from paired-pulse experiments, from I/O curve runs, or other experiments run in conductor that utilize multiple files. The major focus of this manual is to document the use of the “Multi-Analyses” Worksheet/Macro .

The basic idea behind this macro is to rapidly analyze the data for a single channel from multiple files. Unlike the “Measure Amp & Slope” SOLO macro (described above), this macro outputs pEPSP slope and Amplitude, and FV amplitude with each pass to the “Multi-Data” worksheet, and data from multiple files (such as I/O curve or Paired-Pulse analyses), as well as multiple analyses for each trace (e.g. Paired-pulse, 0 vs 50 ms), can be done very quickly, by changing a minimum number of variables in the macro worksheet (i.e. Paired-Pulse Delay and/or FileName). I’ve also included two TEMPLATE files, which will allow you to rapidly obtain an analyses of Paired-pulse and/or I/O curves.; the use of these templates is also described in this manual.

¹ In order to run these macros, you need to install the MEDWrapper.dll in the Windows/System32 directory. The MedWrapper.dll is contained in the MEDWRAPPER directory, along with installation instructions and an Html file (Medwrapper Manual), which documents the many routines in the DLL file that can be accessed in Visual Basic and other programs.

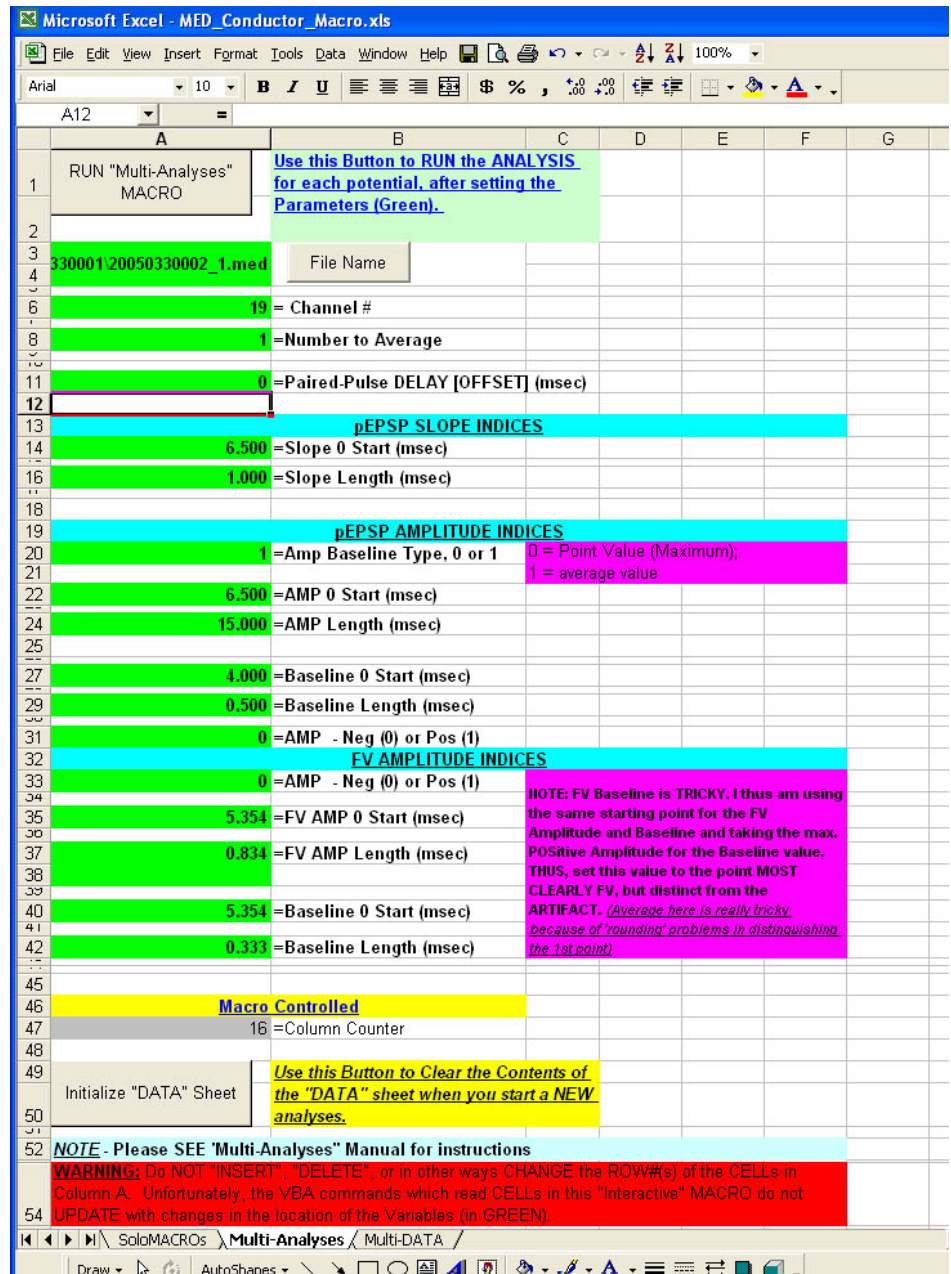
The “MULTI-ANALYSES” Worksheet/Macro

The “**MULTI-ANALYSES**” **Worksheet/Macro**” is a powerful macro that is designed to very quickly analyze the fiber volley amplitude, as well as the slope and amplitude of the dendritic pEPSP from a number of files recorded using MED64 CONDUCTOR. It is ideally suited for examining I/O curves or Paired-pulse experiments where several files are generated. This is because the cursor values and other variables need only be entered/or changed as needed AND the data is output into a single file that contains all of the results organized in a way that allows the use of standard templates to immediately generate a summary of the results.

The screenshot on the right illustrates the “**MULTI-ANALYSES**” **Worksheet**. The worksheet/macro contains sections used to enter basic parameters (e.g. filename, channel #), as well as parameters necessary for measuring pEPSP slope, pEPSP amplitude, and FV amplitude. It also contains the RUN “Multi-Analyses” Macro Button, which uses the parameters to analyze one data file and output the results (pEPSP slope & Amplitude, & FV amplitude) to the **Multi-Data Worksheet**.

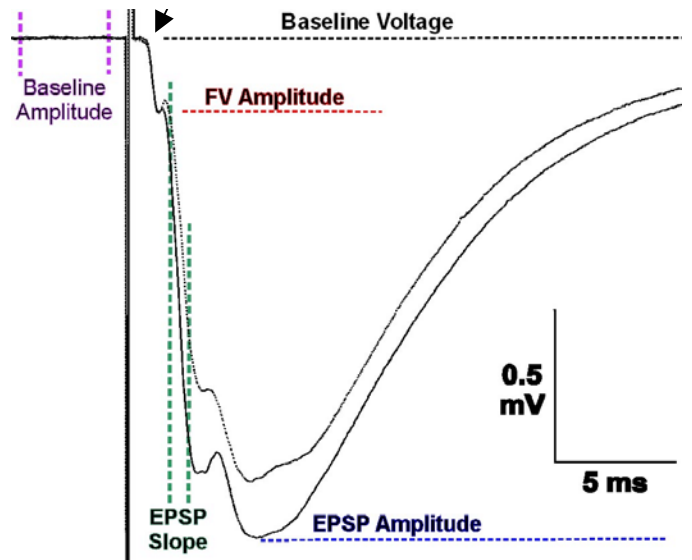
The first thing one must do to use this macro effectively is to enter the appropriate parameters needed to reliably measure pEPSP slope, pEPSP amplitude, and FV amplitude across a series of paired pulse runs OR varying stimulus intensities used to generate and I/O curve. The basic idea is to then generate the output for a single run (and/or file) and then rapidly move to the next analyses by simply changing the Paired-Pulse delay and/or the filename.

The example given below will illustrate the use of this macro to measure pEPSP slope & amplitude, and FV amplitude from a paired-pulse run using an interval of 0 (pre-pulse) and 50 (facilitation) msecs from channel #19 of file “20050033002_1. However, as will be discussed below, this can easily be expanded to include multiple paired pulse runs that, for example, generate several files with paired-pulse delays of 25, 50, 100, 150, 200, 300, 400 & 800 msec (8 files in this case).



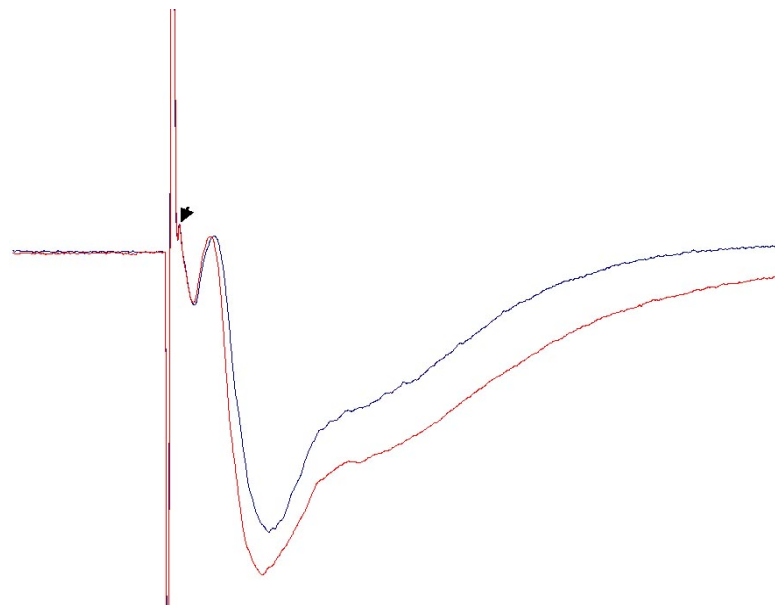
Setting up the Measurement Parameters

Before describing the use of this macro, it is necessary to briefly describe the parameters needed to successfully measure pEPSP slope and amplitude, as well as the fiber volley amplitude. The figure at right illustrates pEPSPs (taken 0 and 50 msec in a paired-pulse experiment) recorded from a standard slice chamber and shows the parameters needed to measure pEPSP slope & amplitude, and FV amplitude under ideal conditions. As shown in this idealized case, the amplitude values for the FV and pEPSP are easily calculated by simply measuring the minimum voltage at the peaks of the FV and pEPSP. This is because the baseline amplitude is zero for both responses and the stimulus artifact returns to a value of zero prior to the onset of the fiber volley. Thus, in this example, one simply needs to define a single start time value just after the stimulus artifact, and two range (or stop values) – one just after the peak of the FV & one after the peak of the pEPSP (e.g. 15 msec) - to calculate FV and pEPSP amplitude, respectively. Although the baseline amplitude is zero in this example, separate start and stop times could be used to calculate a Baseline value, and FV and pEPSP amplitude could also be calculated as the difference from the average 'baseline amplitude value'. (Note- the **MULTI-ANALYSES** **Worksheet/Macro** provides separate baseline start/range times for calculating both FV amplitude and pEPSP amplitude – see below).



It is noteworthy that, if one is careful, you can use the same time indices to measure FV and pEPSP amplitude, as well as pEPSP slope across several delays in a paired-pulse experiment, or across several stimulus intensities in an I/O run if these (start & range) indices are chosen appropriately. Setting the start and range (stop) times for pEPSP amplitude is simple since a long range (e.g. 15 msec) can be used once an appropriate start time has been chosen. Also, since the FV amplitude is expected to be constant in a paired-pulse experiment, one can set the start time and range (or stop time) for the largest (facilitated) response; this is because (as shown) the facilitated pEPSP often reduces the later (positive-going) portion of the FV. A single start and range (or stop time), which is usually adjusted to optimally measure the largest (facilitated) response, can also be used to calculate pEPSP slope.

The following figure illustrates an example of where, unlike the idealized response illustrated above, analyses of FV amplitude and pEPSP amplitude are much more complicated. In the figure at right, the Fiber volley is clearly not separated from the stimulus artifact, and subsequently the amplitude of both the FV and the pEPSP need to be calculated from the inflection point (arrowhead) that is visible at the start of the FV. In this example, a single maximal value is calculated from a start point (& range) used for calculating a baseline value, which differs from zero as shown in the 'idealized' case above (see illustration below). This is often the case at recording sites immediately adjacent to the stimulating electrode; as the short distance involved does not allow for a clean separation of the fiber volley from the stimulus artifact. Since the start of the FV is



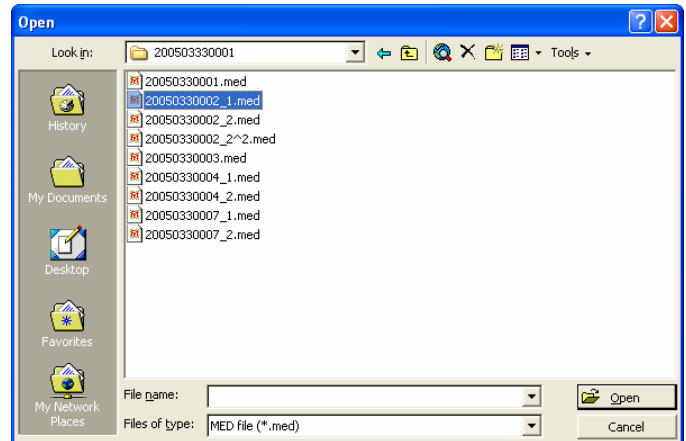
clearly identified, this value (well above zero) can be used as a baseline value for calculating FV and pEPSP amplitude, whose minimal (negative) values are calculated as described above.

USING the MULTI-ANALYSES” Worksheet/Macro:

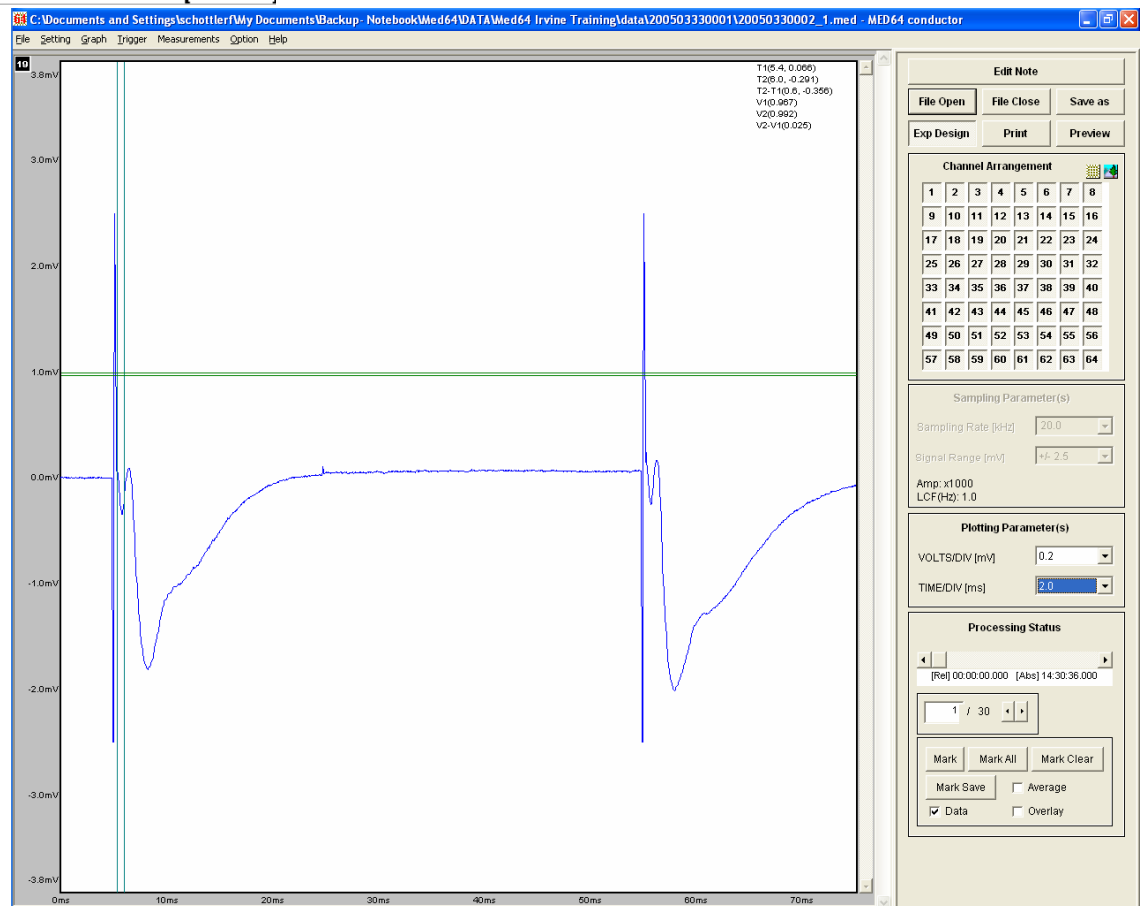
In this section I will illustrate the use of the **MULTI-ANALYSES” Worksheet/Macro** using a paired-pulse file with an interval of 0 (pre-pulse) and 50 (facilitation) msec from channel #19 of file “20050033002_1.

These parameters have been entered into the worksheet as illustrated in the first figure of this manual. The “File Name” button (macro) was used to select the file using standard WindowsXP utilities (picture at right) and the other parameters were entered manually (as shown below).

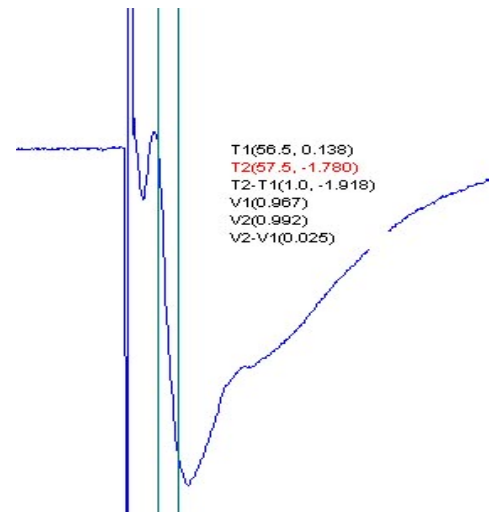
330001\20050330002_1.med	File Name
19	= Channel #
1	=Number to Average
0	=Paired-Pulse DELAY [OFFSET]



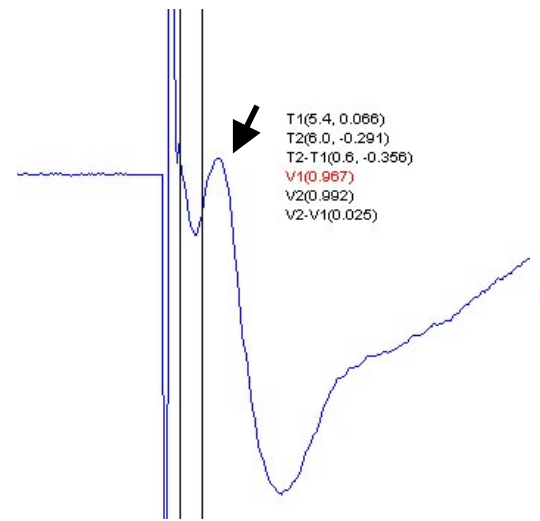
The next, and most important items are the start & range (stop) parameters for the pEPSP slope and amplitude, FV amplitude, and their associated baseline(s). This discussion will utilize the paired-pulse example presented above, to illustrate setting up these parameters for a response in which the FV inflection point is visible, but is clearly above the zero value. As shown in the illustration at right, the start and range (Stop) points for each are determined using the cursors in conductor.



The first cursors set are the start and range (stop) values for calculating pEPSP slope, which are determined from the largest response (in this case, the facilitated response, seen at a delay of 50 msec). In this case, the start cursor for measuring pEPSP slope is set at 6.5 msec (56.5-50 msec) with a range of 1.0 msec (T2-T1). It is very noteworthy that the cursors can be moved precisely to the same point of each potential by simply entering an offset value that is equal to the paired pulse interval (e.g. 50 msec). This makes an analyses of all measures (i.e.- pEPSP slope & Amplitude, FV amplitude) very easy in a paired-pulse analyses, since for each file containing a different paired-pulse delay (e.g. 25, 50, 100, 200 msec), the experimenter could use the same cursor (start & range) values to analyze the pre-pulse (0 time) and paired-pulse differences by simply changing the file name and/or the paired pulse-interval (see below).



The next set of parameters needed are the start/stop indices (cursor values in Conductor) needed to calculate pEPSP amplitude, which include the start and range (stop) values for the baseline and Amplitude (minimal or maximal). Since the start of the Fiber volley is clearly separated from the stimulus artifact in this example, the inflection point is used to calculate a baseline value for the pEPSP. (*Note, in this example, it is also used to calculate the amplitude of the Fiber volley*).



As shown in this example (picture at right), the inflection point of the FV is clearly seen at 5.4 msec (left cursor). Since an average value cannot obviously be used, the BASELINE will be calculated using a Maximum Point Value (0 value for Amp Baseline Type). This value, 5.4 msec, is thus used as a start point for calculating a baseline value. Choosing a range (or stop point) is also very simple here as the given range of 0.5 msec (or 5.9 msec) occurs well before the start of the pEPSP (and would likely do so if this were the maximum response in an I/O curve experiment).

This same start index can very often be used as a start point for determining the peak amplitude of the pEPSP, because the amplitude of the peak negativity associated with the pEPSP will generally not get smaller than the peak negativity of the fiber volley. This would likely be the case also with I/O curves. However, in cases where this is possible, it would likely be more advisable to use a later start point (arrow) for determining the peak negativity associated with the pEPSP. Note that since the maximal value is used for the baseline (Amp Baseline Type = 0), one can set the baseline cursors so that they capture the maximum at the peak indicated by the arrow. The range (or stop point) used to find the peak negativity of the pEPSP can generally be set to 12 or 15 msec.

pEPSP AMPLITUDE INDICES		
1	=Amp Baseline Type, 0 or 1	0 = Point Value (Maximum); average value
5.400	=AMP 0 Start (msec)	1 =
15.000	=AMP Length (msec)	
5.400	=Baseline 0 Start (msec)	
0.400	=Baseline Length (msec)	
0	=AMP - Neg (0) or Pos (1)	

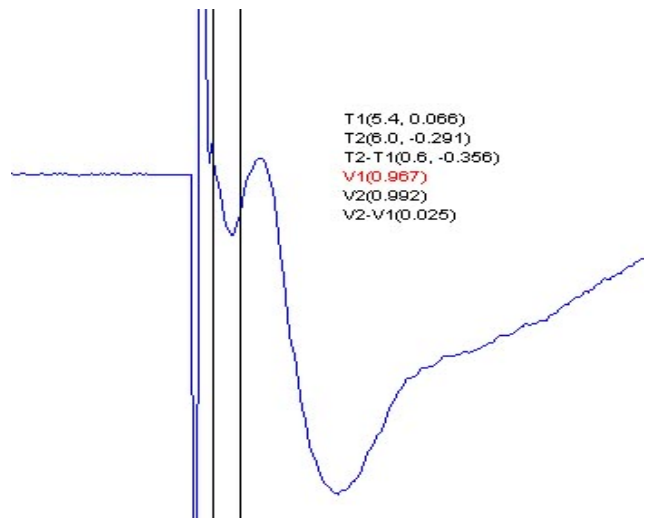
The values for pEPSP slope and amplitude are shown at right.

The parameters used to calculate the FV amplitude are illustrated in the picture at left. As shown, the start point (left cursor) is set at the inflection point that clearly separates the FV from the stimulus artifact. The second cursor (stop point) is placed at a point just after the peak negativity of the FV where the potential is again positive going (again, this is set for the largest response in a I/O or Paired-pulse run).

As noted for the pEPSP baseline calculation (above), this value (5.4 msec) is used as the start point to calculate a baseline (maximum) value. The range is 0.6 msec, as it is the difference between the stop point minus the start point (6.0-5.4 msec).

Unlike the ideal case where the baseline value is calculated from the pre-stimulus period, the baseline value from the fiber volley is calculated from the inflection point. As a result the start & Stop (range) points for both the FV baseline calculation, as well as for finding the peak negativity associated with the FV are identical. Moreover, the amplitude of the FV is simply the peak voltage of the FV minus the maximal value at the inflection point.

The parameters used for calculating FV amplitude are shown below.

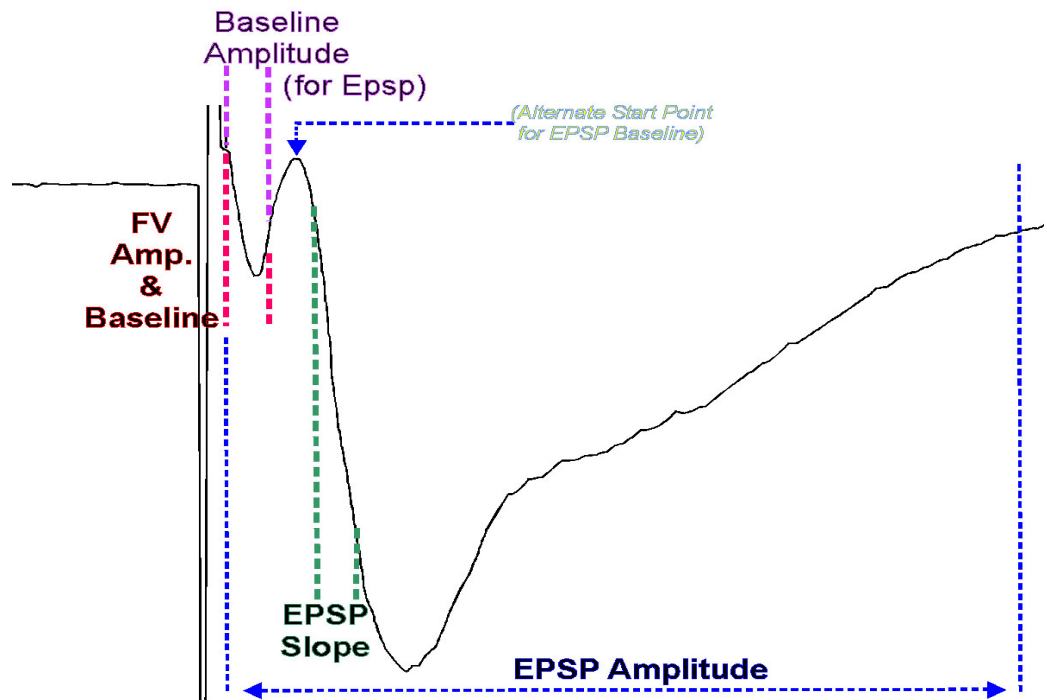


FV AMPLITUDE INDICES	
0	=AMP - Neg (0) or Pos (1)
5.400	=FV AMP 0 Start (msec)
0.600	=FV AMP Length (msec)
5.400	=Baseline 0 Start (msec)
0.600	=Baseline Length (msec)

NOTE: FV Baseline is TRICKY. I thus am using the same starting point for the FV Amplitude and Baseline and taking the max. POSitive Amplitude for the Baseline value. THUS, set this value to the point MOST CLEARLY FV, but distinct from the ARTIFACT. (Average here is really tricky because of 'rounding' problems in distinguishing the 1st point)

This figure (right) summarizes the placement of the Baseline as well as the Amplitude & Slope indices (cursor time pts) for the pEPSP and FV (fiber volley). These indices work well with potentials in which the Fiber volley is clearly distinct from the stimulus artifact (example above).

Note – the maximum value is used to determine the Baseline values for the pEPSP and FV; thus the start point and ranges are identical, as are the indices for FV baseline and FV amplitude.



OTHER POTENTIALS:

In addition to the potentials described above, several MED64 users have used the “**MULTI-ANALYSES**” **Worksheet/Macro**” to analyze smaller potentials recorded from other CNS or PNS regions, or from hippocampal slices in which the tissue and/or experimental conditions are suboptimal.

The example below illustrates the flexibility in using this macro to analyze what are likely non-publishable potentials obtained from the CA1 region of a ‘poor’ slice. It should be noted that ALPHA MED SCIENCES does not promote the use of data that is likely sub-optimal for publication, and recommends that our users improve the experimental conditions used in the preparation and/or maintenance of hippocampal slices to meet standards required by the field of Neuroscience.

The figures below illustrate dendritic recordings from *stratum radiatum* of CA1 following Schaeffer/commissural stimulation, and presents pEPSPs obtained as part of an I/O curve using 50, 100, & 200 μ Amps of current. These potentials are substantially different from those discussed in the previous section in that **1) the fiber volley cannot be clearly separated from the stimulus artifact, and 2) onset of the EPSP is at a higher voltage than the pre-stimulus baseline.**

As shown in the figure (right), the MED64 user(s) who analyzed this data had to adjust the settings for measuring pEPSP

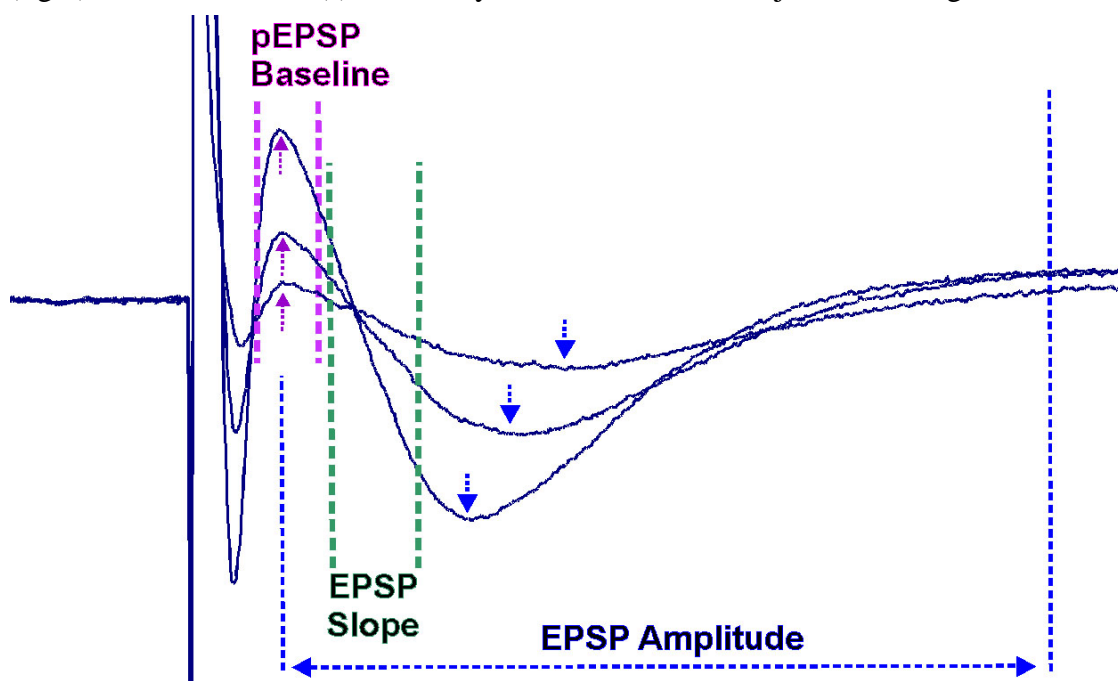
amplitude. Thus, the indices (cursor values, **purple lines**) used to calculate the Baseline values for the amplitude calculation were chosen to detect the maximum voltage (**purple arrows**) at the start of the pEPSP.

The start time index (cursor; **blue vertical line on left**) was also adjusted to reflect what appeared to be the presumed starting point (maximum) of the pEPSP seen just after the peak negativity of the fiber volley. The range (cursor; **blue vertical line on right**) was adjusted to calculate the minimum (negative) voltage.

PEPSP amplitude was thus calculated as the difference between the peak positivity (**purple arrows**) at the end of the Fiber Volley (FV) and the peak negativity (**blue arrows**).

The indices for used to calculate pEPSP slope are chosen identically to that used for the other potentials described above. Again, the major concern here is to choose the start and range values (**green lines**) to accurately measure the slope of the largest potential recorded, making sure that the population spike or the peak negativity (as discussed above) does not enter into the calculation.

(note- although linear regression is used to calculate slope in this macro, other calculations of slope, e.g. – 20 & 80% values, would also be aberrant if a population spike were present and affected the perceived amplitude).

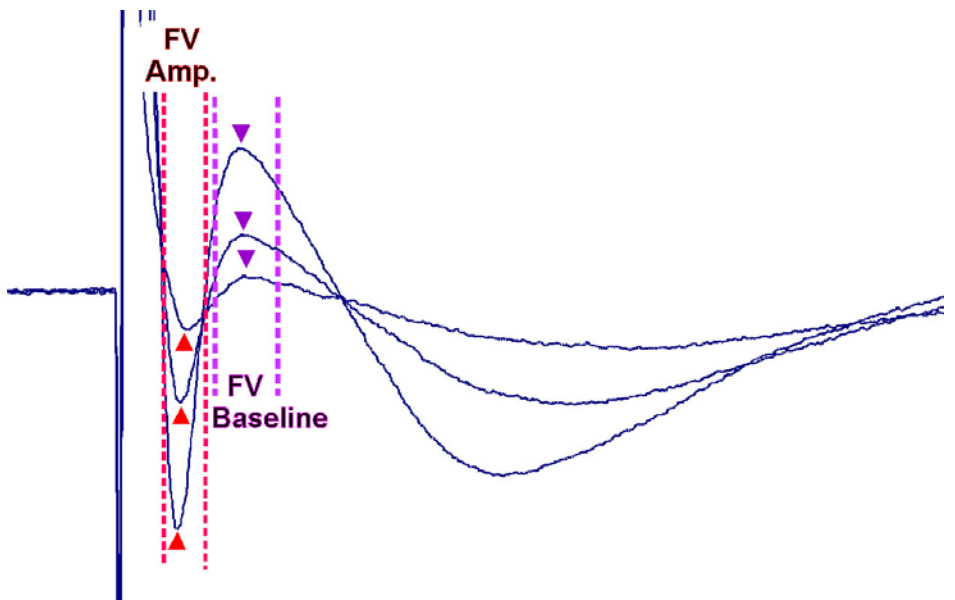


As mentioned above, it is impossible to separate the Fiber Volley (FV) from the stimulus artifact in these recordings (i.e. the I/O run). Thus, some MED users have chosen to measure the FV voltage as the difference between the peak negativity of the FV and the perceived start of the EPSP.

One way to calculate Fiber Volley (FV) amplitude in this I/O example would be to use SEPARATE start and range (or stop cursor) indices for calculating the baseline (maximum) and peak negativity (minimum) values. As shown in the figure at

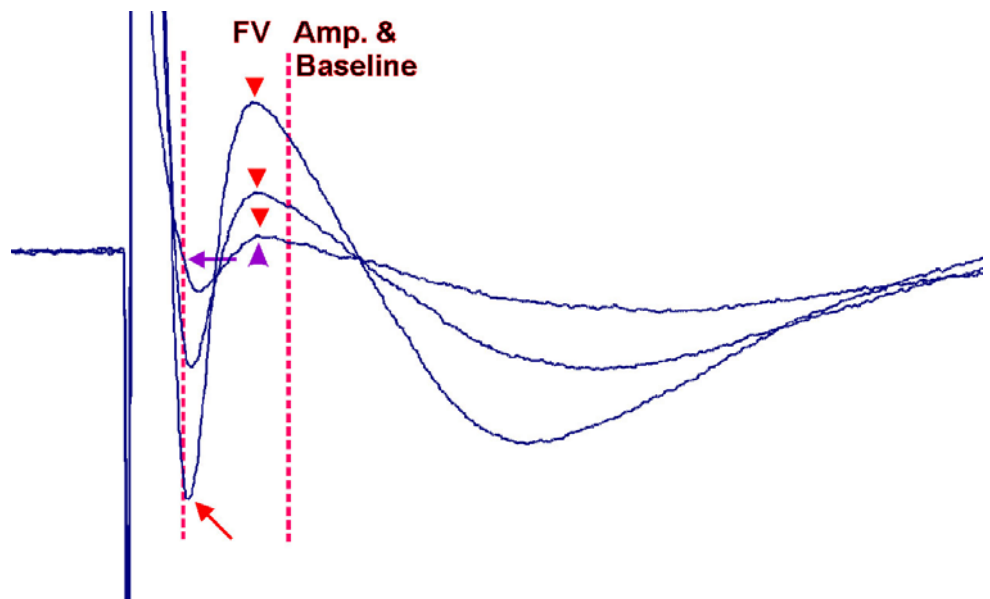
right, the start & range values used for the FV Baseline (**purple lines**) could be chosen identically to that used for pEPSP Baseline described in the paragraph above. This would restrict the calculation for the FV Baseline to the peak positivity (maximum voltage, **purple arrowheads**) associated with the start of the pEPSP.

The minimum voltage (**Red Arrowheads**) associated with the FV amplitude is calculated using the start and range indices indicated by the **vertical RED lines**. Again, the size of the FV is calculated by subtracting the maximum Baseline voltage from the peak negative voltage (FV Amp.)



Alternatively, one can often use the SAME start and range (or stop) indices for calculating FV baseline (maximum) and peak negative amplitude (minimum). As illustrated in the figure below, a single set of start

and range indices (indicated by the **vertical RED lines**) can be used if they are chosen to appropriately measure the positivity associated with the baseline (e.g.– start of pEPSP). One thus has to take great care to ensure that the start value (**left Red cursor LINE**) is placed to guarantee that the stimulus artifact does not come into play in calculating the FV baseline (maximum). As shown, this is more problematic with the smallest potentials, and one has to set the start index (**left Red cursor LINE**) at a point where the maximum positivity is measured at the start of the EPSP (**purple arrowhead**) and not the stimulus artifact (**purple arrow**).



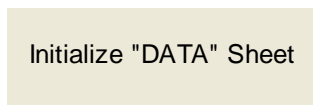
It is also extremely important to set the start index (cursor, **left Red LINE**) so that it accurately captures the peak negativity associated with the FV itself. This is particularly true for the largest FVs (**Red Arrow**), which have the shortest latency and are least clearly separated from the stimulus artifact.

Running the “MULTI-ANALYSES” Worksheet/Macro

Once the parameters have been set up as described above, it is very easy to utilize the “**MULTI-ANALYSES**” **Worksheet/Macro** to analyze your results. This manual will document a simple case of a single file containing paired-pulse data at one paired-pulse interval of 50 msec.

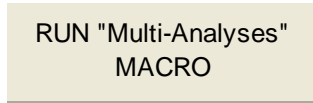
(Note- The parameter defined for responses in this file are described in the section above entitled “**Setting up the Measurement Paramaters**”)

The **FIRST** step is to use the INITIALIZE “DATA” Sheet macro to clear the “Multi-DATA” worksheet that will be used to collect all data from these analyses. To do this simply right click on the ”Initialize “DATA” Sheet BUTTON



The **SECOND** step is to make sure the parameters you entered are correct (e.g. Filename, Paired-Pulse Delay)

The **THIRD** step is to then use the RUN “Multi-Analyses” MACRO to calculate the data.




The **FOURTH** step is, in this example, to change the Paired pulse delay to 50 msec, as shown below and then again use the RUN “Multi-Analyses” MACRO to re- calculate the data.

50 =Paired-Pulse DELAY [OFFSET] (msec)

(The results are output to the “Multi-DATA” worksheet (as shown in the Appendix - final page).

FINALLY, this macro can be adapted to analyze the results from multiple files in a paired pulse analyses (or an I/O curve). For example, to use this macro for file 20050330002_2, 20050330002_3, 20050330002_4, 20050330002_5 (with paired-pulse delay of 100, 200, 400, & 800 msec, respectively) all the user would have to do is change the FILE Name and/or the paired-pulse interval and run the macro again (**BY REPEATING THE SECOND, THIRD AND FOURTH STEPS ABOVE**).

The use of this macro for I/O curves would be identical, except that the paired pulse delay would always be set to a value of 0, the fourth step would be omitted. The user would simply change the file name and press the “Multi-Analyses” MACRO to analyze the data for each file in the I/O run.

In addition, the TEMPLATE files, “Template- Paired Pulse Analysis-Conductor.xls” and “Template-I-O Analysis-Conductor.xls” can be used in conjunction with the **“MULTI-ANALYSES”** **Worksheet/Macro** to very simply and rapidly produce a summarized analysis of Paired-pulse or I/O curve data. This can be accomplished by analyzing the results using the **“MULTI-ANALYSES”** **Worksheet/Macro** as described above and copying the data generated from the “Multi-DATA” worksheet into the “pEPSP Multi-Data (Template) or the “I/O Multi-Data (Template) worksheets of the “Template- Paired Pulse Analysis-Conductor.xls” and “Template-I-O Analysis-Conductor.xls” files as needed.

To do this, first select rows 1-35 of the “pEPSP Multi-Data (Template) or the “I/O Multi-Data (Template) worksheets and clear the contents of the cells in these rows (**Note- do not use the DELETE function, as the formulas in the summary templates will get corrupted**). Next, copy the contents of the Multi-data worksheet in the “MED_Conductor_Macro.xls” file. The worksheets and graphs linked to these Templates provide a summary of the experimental results.

