NeuroPhys Users' Manual

Version 2.3

Contents

Overview

- This User Manual contains instructions on how to use NeuroPhys software with our hardware JAGA16 device or JAGA penny device.
- JAGA systems (JAGA16 or JAGA penny) are wireless neural recording acquisition devices.
- NeuroPhys is the neural recording acquisition software (NeuroSys, LLC). NeuroPhys is fully integrated with our device JAGA16 and JAGA Penny.
- For JAGA Penny, you will also need to install a USB driver for our receiver once you install NeuroPhys. The instruction on installing the driver is on our website www.jinga-hi.com >Document>Software>Installation NeuroPhys/NeuroSorter/USB driver for JAGA Penny
- The data output from NeuroPhys is .PLX or can be converted to .CVS using NeuroSorter data analysis software (NeuroSys, LLC), or a matlab array based on our matlab codes
- See also NeuroPhys_Error&Questions.pdf
- Please contact us at software@jinga-hi.com or tom.neurosysllc.com with your inquiries.

System Requirement

The current version of NeuroPhys runs on Windows XP, 7, 8, 10. Listed below are the computer systems we tested; they work seamlessly with NeuroPhys and JAGA16.

1. Quad-core CPU Intel E6600, 2.4GHz, 4 GB RAM, Windows 7, 32-bit.
2. Dual-core CPU, 8GB RAM, Windows 7, 64-bit.
3. Intel Core i7-4500U 2 GHz Processor 8 GB DDR3 RAM 256 GB Solid-State Drive 14.0-Inch Screen Windows 8.

Install Software

1. Download the installer from www.neurosysllc.com/Software.html and run it. It should install two programs: **NeuroPhys** - main data acquisition program
   SpikeSorter - offline data analysis program

2. Launch NeuroPhys by clicking the brain icon:

3. At first launch, NeuroPhys will ask you to register the product.

Click **Register Product**. Then select **Import license from XML file**, and choose the license file you were sent.

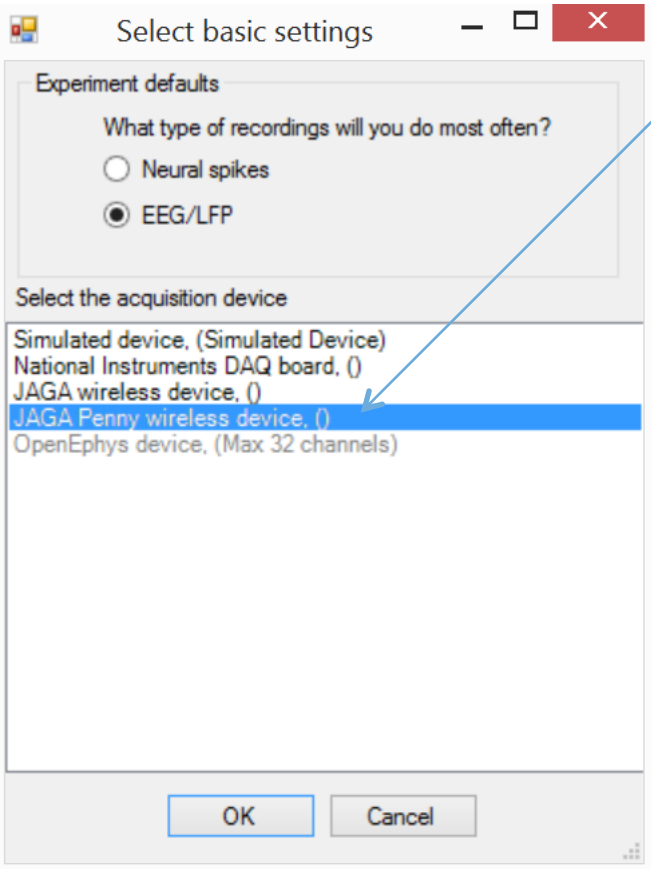
If you do not have the XML file, you can click **Run a FREE version!**. This version will be fully functional without time limit. However, the free version allows only 4 active channels. To activate all channels, you will have to buy a license or obtain a trial license.

Configure Software

The first time you run NeuroPhys on your computer, you will be asked to confirm the configuration. You can change this setting at any later time by going to:

Tools>Basic Setup

1. The program defaults to **Neural Spikes** for the recording type. If you will be conducting EEG/LFP recordings for most of your experiments, select **EEG/LFP**.

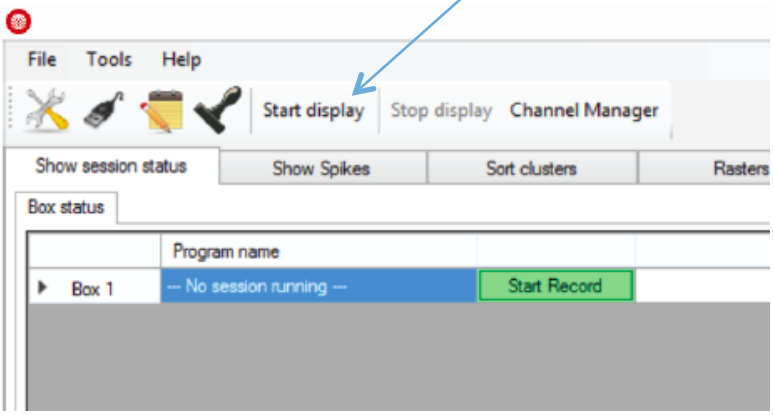


2. Select the hardware you will be using by clicking **JAGA16 wireless device** * or **JAGA penny** **.

3. If you are evaluating the software without any hardware, then select **Simulated device**. This gives demonstration waveforms that you can play with.

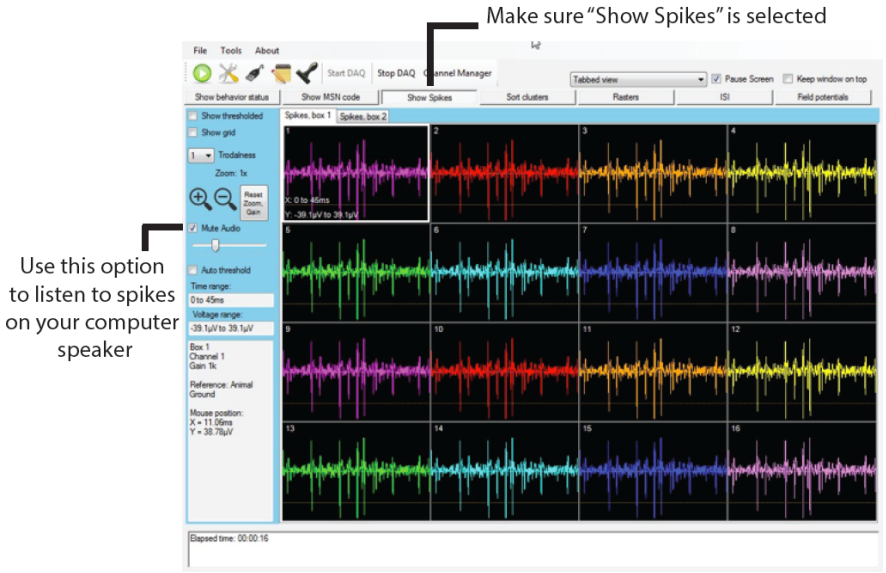
4. Click **OK** when you are done.

5. Once the program launches, click **Start display** to check whether the device and the software are working.



Software Display without any device:

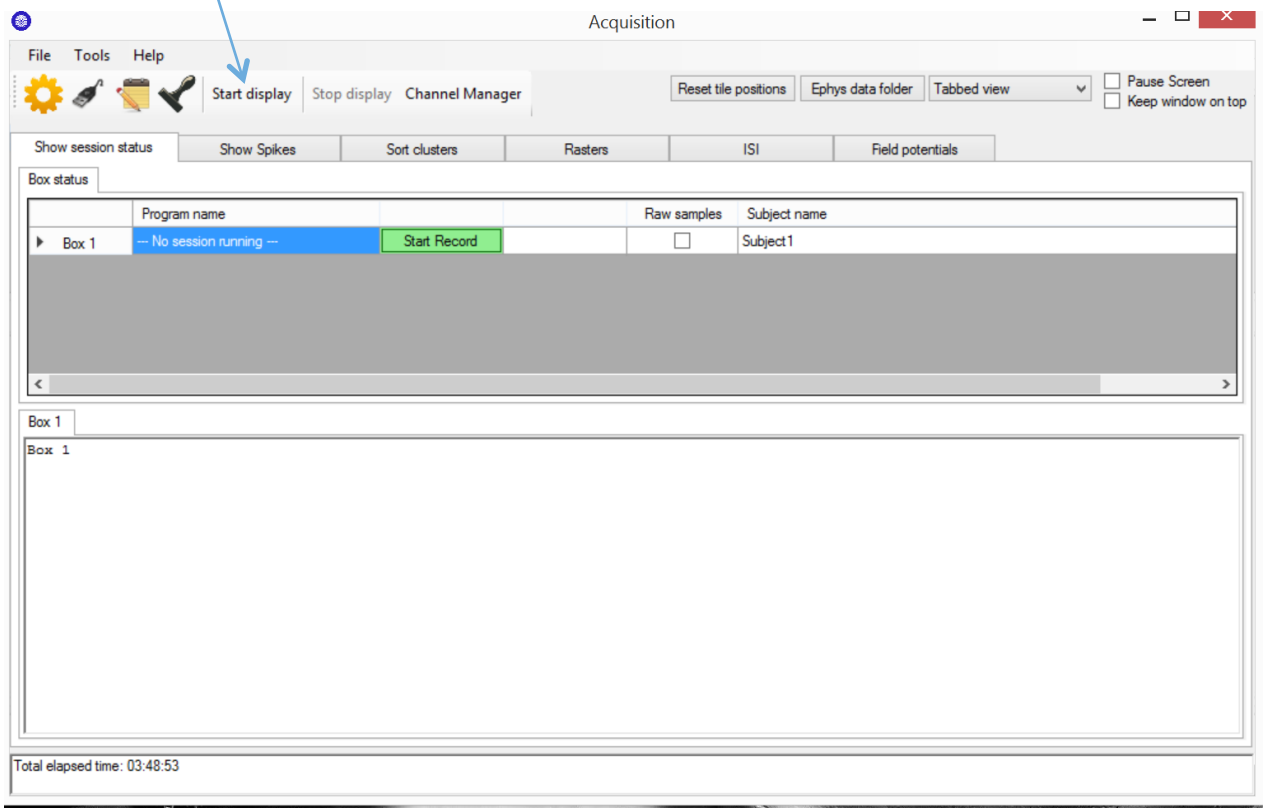
Without a hardware device, the software will automatically generate simulated signals. Select **Show Spikes** tab after clicking **Start display** to see waveforms. The waveform updates every 50 milliseconds like below.



Getting Started

Once you have configured the software and verified the test signals, you are ready to start recording. On your window, you will see the following buttons and tabs:

- **Start display**, **Stop display**, **Channel Manager** (first row)
- **Show session status**, **Show Spikes**, **Sort clusters**, **Rasters**, **ISI**, **Field potentials** (second row)
- Toggle for **Tabbed view**, **Tiled view**, **Paneled view** (top right corner). **Paneled view** allows you to see **Sort clusters**, **ISI**, and **Show spikes** windows simultaneously, and this option is highly recommend, but works best with a widescreen monitor.



Start a Simple Recording Session

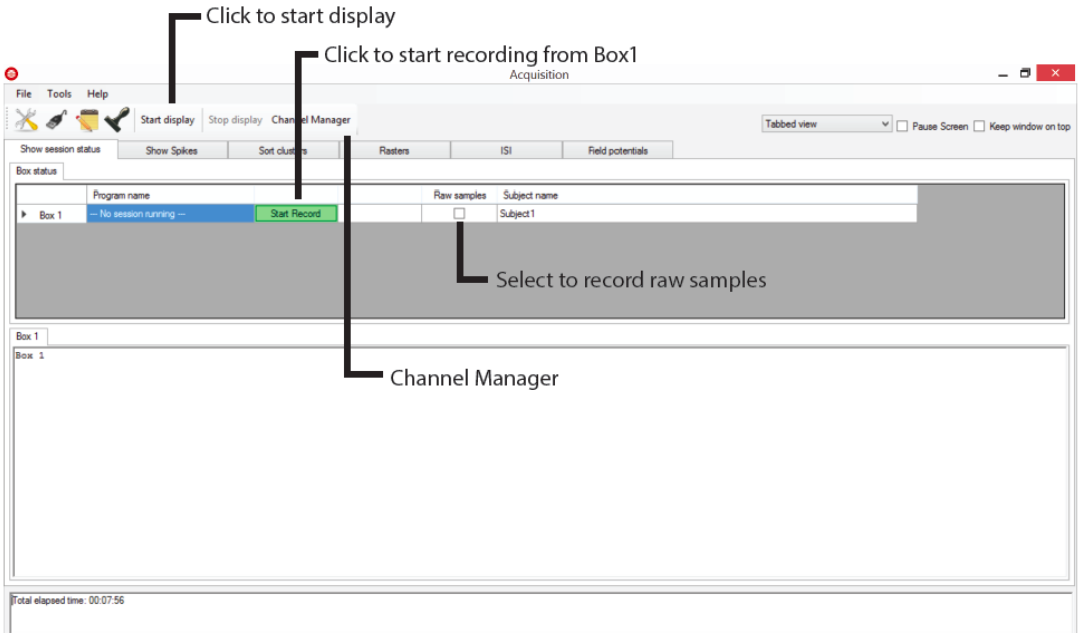
1. Select tab **Show session status**, followed by the tab **Box status**. Here, you will see a list with one row for every recording chamber (only one shown here) with a green button labeled **Start Record**.

   a. When you are ready to record, press **Start Record**. (Make sure you already clicked **Start display**.)

   b. By default, ONLY DETECTED SPIKES will be saved to disk. Waveforms in between detected spikes **will not** be saved by default. If you want to record raw samples (*i.e.* not spike sorted), select **Raw samples** and press **Start Record.**

   c. If you want to save in native format (JAGA format), you will need to double click on Raw samples, the click on the box Record raw data in native format (JAGA.dat) instead of .PLX. Otherwise it will be saved in raw .PLX format

   d. Each channel is referenced to ground by default. However, if you have a dedicated reference wire, you can right-click on that channel and select **Reference all channels to this wire** (See section **Channel Manager** for details.)

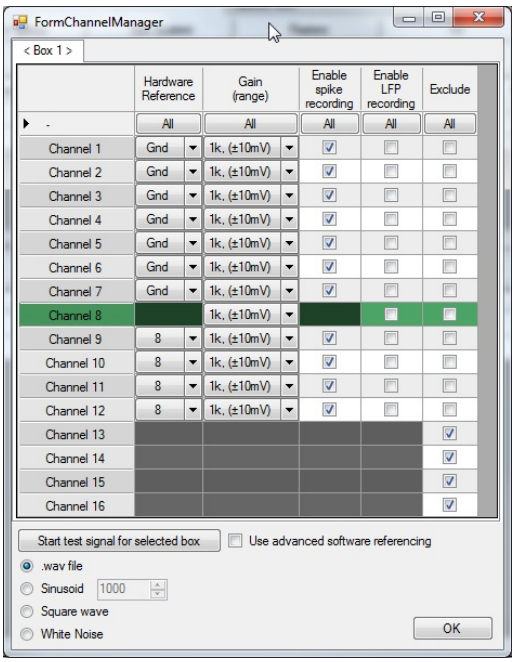


2. Select **Show Spikes** tab to visualize waveforms. The recording in each window represents 50 milliseconds of data.

3. To take a closer look at waveforms in any of the channels, **double-click** on a panel. This will toggle the selected panel to full-screen and hide the rest 15 channels. If you double-click again it goes back to the multi-channel screens

Channel Manager

For more precise control of recording settings, you can use the **Channel Manager** to view and change all settings at a glance. When you click **Channel Manager**, you will see this dialog box:



1. If you have more than one recording chamber configured, you will see one tab for each chamber, with the tabs named **<Box1>**, **<Box2>**, *etc*. Unlike the menu above, Channel Manager allows you to select a separate reference for each channel. In the above example, channels 1-7 are referenced to ground, while channels 9-12 are referenced to channel 8. The differential subtraction is performed prior to amplification, which allows for excellent common mode rejection.

2. Channel that is used as reference will be highlighted in green.

3. You can exclude some channels from processing by checking the very last column "Exclude". These channels will not be displayed, and no calculations will be performed on them, reducing CPU load. This should be done if some wires are not connected on a particular animal, or if a wire has gone bad and carries only noise. Excluding a channel will prevent a user from accidentally recording from it or using it as reference.

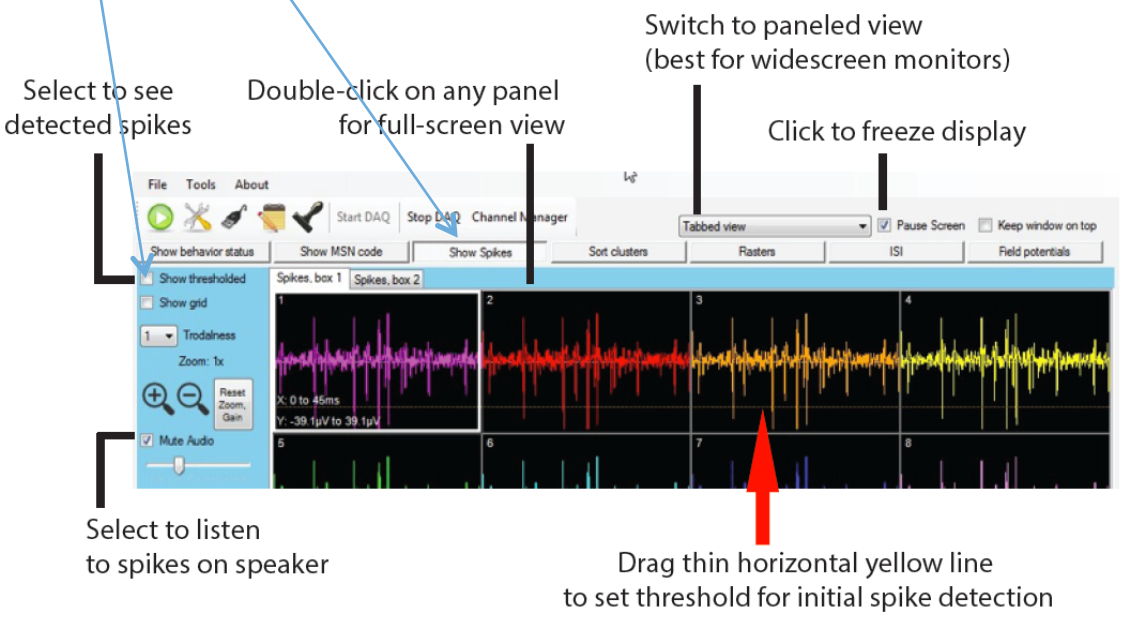
4. Note that excluded channels still contribute to the system's overall sample throughput. Hence, if you want to sample at a higher sampling rate than the default, you should reduce the number of active channels under **Options** rather than simply excluding them.

Show Spikes

For **spike recording**, you will want to selectively view amplitudes that rise above background noise. The program can selectively display and save such waveforms.

1. Select **Show Spikes** tab.

2. Click **Show thresholded** (on the blue left panel) to toggle to the thresholded mode. This mode will display only those waveforms that exceed a set voltage. The threshold appears as a *horizontal yellow line* across each plot (red arrow, look above), which you can drag up and down with the mouse. If the threshold line seems to be missing, it is sometimes at the *top or bottom edge* of the window.

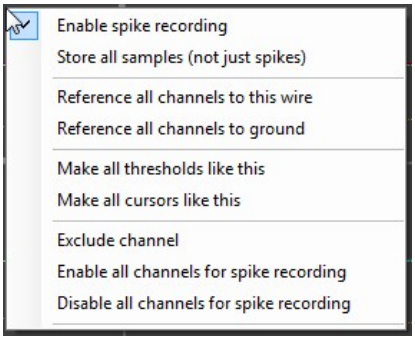


3. To listen for spikes during your recording session, unclick **Mute Audio**.

Note:

- You can also set thresholds automatically by clicking and *holding* the **Auto threshold** button – this will set the threshold to be 2 standard deviations below the mean voltage. You have to *hold down* this button for a second or two for this to take effect. Once you let go, the threshold will lock in place.
- In the thresholded mode, you will see 800µs of each spike waveform, with 200µs shown before and 600µs shown after the threshold is hit. These time settings can be changed in the settings.

More options for Spikes



Here is an explanation of each option:

Enable spike recording: By default, detected spikes are saved for each channel. If you toggle this off, then detected spikes are not saved and waveforms will display in grey instead of in color. You should disable spike recording for channels that do not appear to have any useful neural spikes.

Store all samples: If selected (it is off by default) then all samples, not just detected spikes, are saved. Your file will become HUGE. Most of the time you do not want this.

Reference all channels to ground: This is the default state when program is first launched.

Reference all channels to this wire: If you want some channels to have one reference, and some to have another, you can do this by using the Channel Manager (further described in section "Channel Manager").

Make all thresholds like this: This refers to the threshold used to detect whether a spike has occurred or not. This option makes all channels in this box have the same threshold as the window you just clicked.

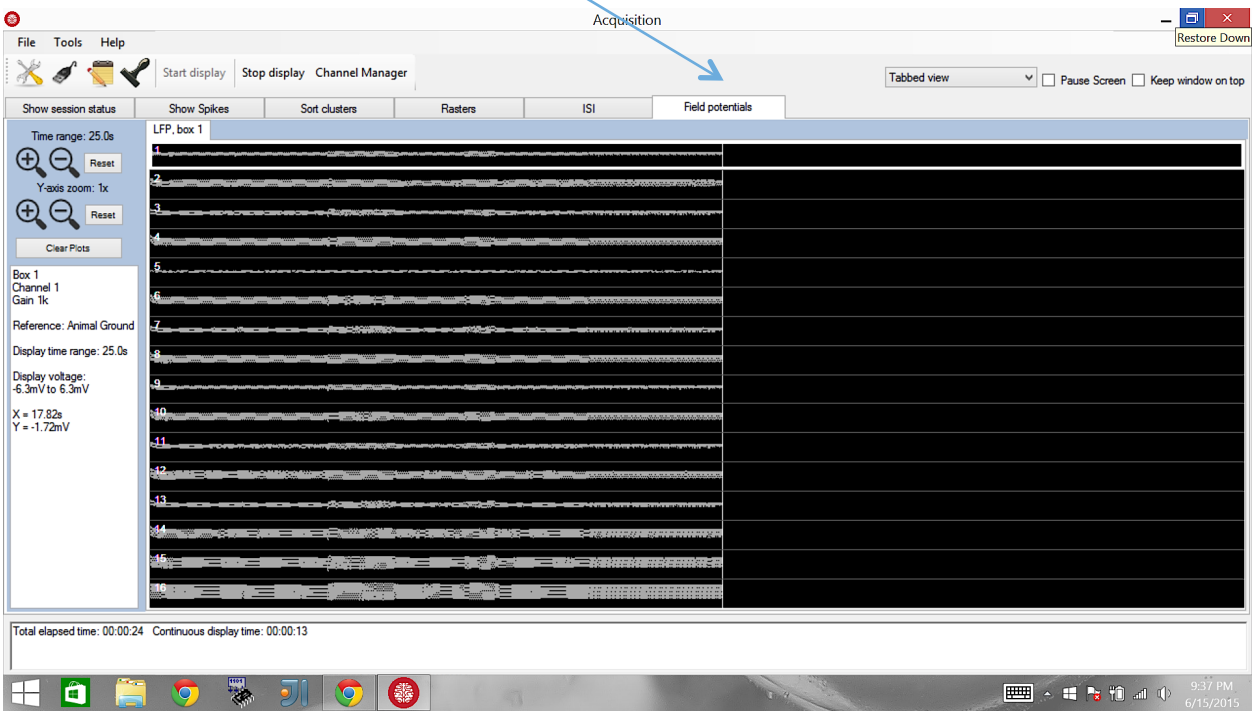
Make all cursors like this: This refers to the vertical cursors used to calculate the voltages in the 2-D cluster plots. This option makes all channels to have the same cursor as the window you just clicked.

Exclude channel: This disables recording for this channel, similar to the effect of disabling "spike recording". However, when a channel is excluded, its waveforms are no longer visible, not even in greyed out form. Also, excluding a channel will significantly reduce CPU load because the computer will no longer have to calculate and display waveforms for this channel.

Field Potentials

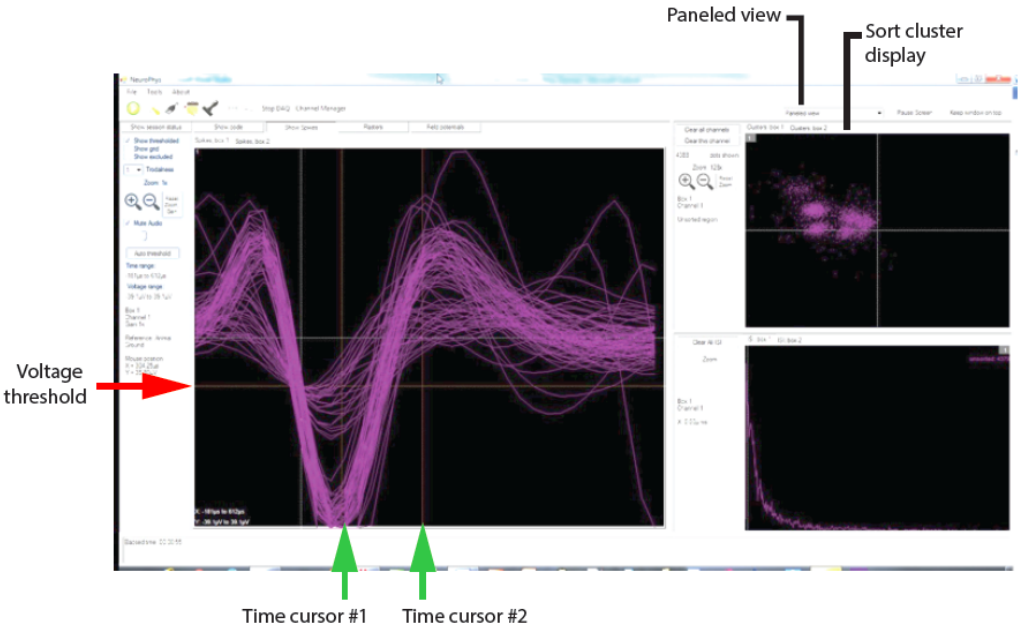
You can also view field potentials, which are the input signals down-sampled to one kilohertz.

1. Select **Field potentials** tab.
2. You will see data displayed in all channels.



Real Time spike Sorting

Neurophys software has the ability to sort (*i.e.* classify) spikes in real time. To demonstrate these capabilities, you can start a self-test session as described above. Select **Paneled View** mode using the toggle menu in the upper right of the main screen. Your screen will look something like this when you are running the self-test:



In the example above, Channel 1 is enlarged by double-clicking on it so that only this channel is displayed. If you look closely, you will notice three cursors on this window, shown by yellow dashed lines: Each of these cursors can be adjusted by dragging it with the mouse.

The yellow lines represent:

1. See red arrow above: This horizontal line sets the voltage threshold of detected spikes.
2. See green arrows above: These two vertical lines define start and end time points.

The "Sort clusters" display panel in the upper right corner shows a scattering of dots. The dots work as follows:

1. Each dot corresponds to one detected spike, *i.e.* a waveform that exceeds the specified voltage threshold.

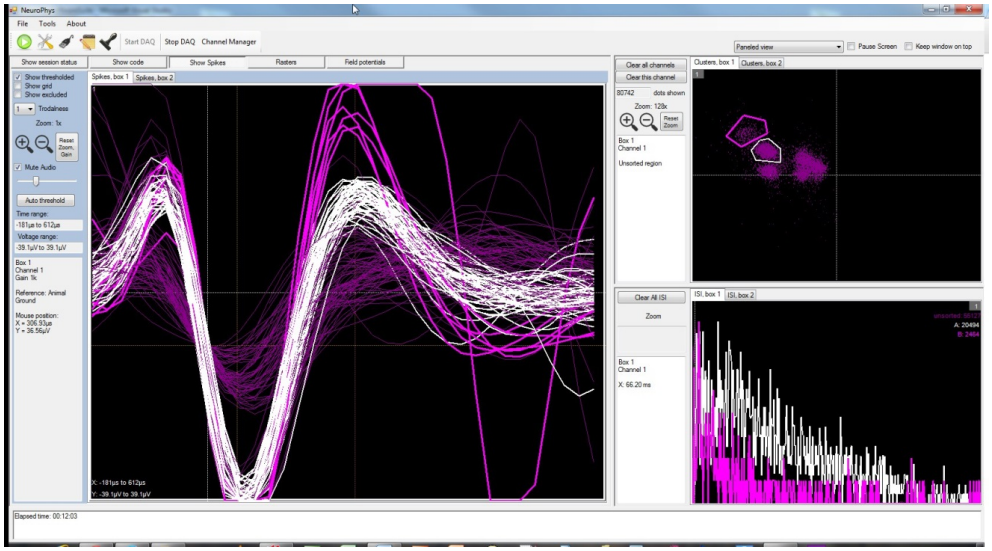
2. The X and Y coordinates of each dot are the *voltages at which each spike waveform intersects time cursor #1 and time cursor #2 respectively.*

3. The dots are mostly in the upper-left quadrant of the screen, because most waveforms intersect the first time cursor at a negative voltage (corresponding to an X value to the left of

center), and the second cursor at a positive voltage (corresponding to a Y value above the center).

4. The time cursor was placed on #1 and #2 where the waveforms reach their approximate minimum and maximum voltages, respectively. This causes the dots corresponding to neural spikes to be as far as possible from background noise.

5. Notice also how the dots segregate into multiple distinct clusters – these clusters correspond to distinct neurons having distinct waveform shapes. You can click and drag the mouse to outline a polygon that encircles one of the clusters. This will cause all the waveforms corresponding to that cluster to display in a distinct color. You can place multiple polygons, and each will show up as a different color, with successive clusters receiving a designated letter “a”, “b”, “c”, etc. This designation is saved along with the spike timestamp and waveform during a recording session. The result should look something like the window below. You’ll see that I have outlined two clusters:



When you complete a session, click on the red **Stop** button under the **Session Status** tab. This button is immediately next to the green button you clicked to start the session.

You can now view your data file in the NeuroSorter program.  Find the icon like this.

JAGA16_CH32

You can connect two JAGA16 devices and record up to 32 channels. If you record in .PLX form, the generated data will have all 32 channels in one file. If you record in native format, there will be two files (channel 1-16 and channel 17-32 files).

Where is my data?

By default, all recorded waveforms are saved to the folder "NeuroPhysData", found in the folder "My Documents". Each session will generate two files, .TXT and .PLX. A third file format .CVS can also be obtained using SpikeSorter software. The files are described below:

1. A text (.TXT) file containing session log details – currently the session log shows the session duration and any text sent by Med Associates programs running concurrently with the recording session.

2. A Plexon-format (.PLX) file containing spike timestamps and waveforms.

3. You can also generate .CVS file format from .PLX using SpikeSorter (See User Guide for SpikeSorter for more details).

You can change the folder options by clicking **Tools**>**Options**.

Appendix 1: Software settings

If you click on this wrench-like symbol , you will get a dialog with four pages of settings, which are detailed below.

Page 1: Data folders, behavior settings

This page has the following settings:

First Box ID. This sets the ID number of the first recording chamber. This is 1 by default. You can change it if you have multiple systems and you want each physical box to have a unique ID number. For example, if you have two 32-channel systems, the first system's recording chambers will have ID numbers 1 and 2 (the default) and you can set the second system to start numbering chambers at 3.

Number of operant chambers. This sets the number of physical boxes in your system. By default this will be the same as the number of recording chambers, i.e. 1 for a 16-channel system, and 2 for a 32-channel system.

Behavior outputs per operant chamber. This is not used in electrophysiology systems, and is intended for controlling additional hardware such as levers, lights, pumps, *etc*.

Timing resolution. This sets the resolution with which behavioral timestamps are recorded. In most cases, these timestamps will be received from external software, such as MATLAB, Labview, Med-PC®, or other software.

Ephys data folder. This is the folder to which standard ephys data (spikes and LFPs) will be saved.

Wideband data folder. This is the folder to which wideband data will be saved. These files will be much larger than the spike and LFP files.

Page 2: Basic ephys settings

ID of first electrophysiology box. By default, this is the same as the first overall box ID. If you change the ID of the first overall box (*e.g.* from 1 to 3, in the example above), then this will change accordingly. This should be changed only if you have some operant chambers that are NOT configured for electrophysiology.

Number of electrophysiology boxes. By default, this is simply the total system channels divided by 16. I.e. it will be 2 for a 32-channel system, and 1 for a 16-channel system. You should change this only if you are not using all available boxes, e.g. if you only want to use one box in a 32-channel system. This will reduce the number of displayed graphs, and also reduce the CPU load.

Active electrophysiology channels per chamber. This sets the number of channels used per recording chamber. By reducing the number, you can achieve higher sampling rates as the total system sampling rate is limited to 1MHz.

Acquisition sample rate: This is a read-only field. It reports the achieved sampling rate.

LFP acquisition rate. This is a read-only field that reports the sample rate for local field potentials (LFP).

The next three radio buttons determine where the program will get its settings whenever it starts up. The choices are:

1. Load same settings that were in use when the program previously closed.
2. Load default settings.
3. Load settings from a particular file.

Filter settings. All spike and LFP channels have optional software filters. These are:

1. **Attenuate low frequencies**. This option turns on a high-pass second-order Butterworth filter that attenuates frequencies below the specified cutoff frequency.
2. **Attenuate high frequencies**. This turns on a low-pass second-order Butterworth filter that attenuates frequencies above the specified cutoff.
3. **Attenuate low/high frequencies LFP**. These options do the same thing, but for local field potentials.

Subtract periodic noise. If checked, this setting activates an averaging algorithm that removes any noise with a periodicity of 60 Hz.

Inverted inputs. If checked, this setting will invert the inputs, *i.e.* make negative voltages positive and vice versa. This should only be used if you have a preamplifier that has a negative gain value.

Test waveform settings. This determines the type of waveform used for self-testing.

Page 3: Advanced electrophysiology settings

(This section will come later.)

Appendix 2: MATLAB integration

If you are controlling behavior from MATLAB®, here is how you can perform behavioral event timestamps to NeuroPhys, for recording alongside electrophysiological data:

Before starting a recording session, you need to run the following code, either from the MATLAB prompt, or within a *.m file.

If running 32-bit Windows, run the following command AFTER you have installed NeuroPhys:

```
>> loadlibrary('c:\Windows\System32\NS_Library.dll', 'c:\Program Files\NeurosysLLC\NeuroPhys\NS_Library.h')
```

If you are running under 64-bit Windows use this line instead:

```
>> loadlibrary('c:\Windows\SysWow64\NS_Library.dll', 'c:\Program Files (x86)\NeurosysLLC\NeuroPhys\NS_Library.h')
```

You may be prompted to selected a compiler by typing “mex –setup”. The default, “lcc-win32” compiler will work fine.

You only have to run the above command once per MATLAB session. It loads the shared DLL that makes the following functions available to MATLAB:

```
Calllib(‘NS_Library’, ‘NS_StartRecording’, boxNumber)
```

This function allows MATLAB to initiate a recording session in NeuroPhys. “boxNumber” is an integer referring to the operant chamber number (the lowest numbered box will be “1”). If you have only one chamber, this should always be “1”.

```
Calllib(‘NS_Library’, ‘NS_SendEvent’, boxNumber, eventNumber, 0)
```

This function sends a timestamped event to NeuroPhys, which will be recorded alongside any ongoing electrophysiological data. The timestamp will be calculated at the exact instant this function is called, even though the data may not be saved to disk until a few

milliseconds later. Hence, the timestamp will be extremely accurate, and will be recorded in the same units (clock ticks) as used for the electrophysiological data. eventNumber is a user-selectable number between 0 and 255, and allows events of different types to be distinguished from each other. The last argument (0) is not used.

Calllib('NS_Library', 'NS_StopBox', boxNumber)

This allows MATLAB to stop a recording session in the specified operant chamber.

Calllib('NS_Library', 'NS_StartRecordingWideband', boxNumber)

This allows MATLAB to start recording wideband signals, *i.e.* it will record the raw voltages present at the A/D board inputs at the full sampling rate available. This will generate very large files that retains all possible information that can be recorded. This is useful if you don't know exactly how you will analyze your data, and wish to process the session off-line at a later time.

Appendix 3: Med-PC® integration

Probably the best way to illustrate Med-PC® integration is with a sample MPC file. After installing NeuroPhys, this file will appear in "..\My Documents\NeuroPhysData", along with a couple of sample data files:

```
\
\  RecordingTest.MPC
\
\  Simple test file to show communication between MedPC and NeuroPhys.
\

^LeftLever = 1       \ Left lever is output 1. If your chamber is wired differently, you should change this.

s.s.1,

s1,
 0": ~sendeventname(BOX, 1, 'ProgramStart');~;
    ~sendeventname(BOX, 2, 'LeverPress');~; ---> S2

S2,
  #start: ~startRecording(BOX);~;
       ~sendtext(BOX, 'Program starting' + #13 + #10);~;
       ~sendevent(BOX, 1, 0);~; ---> S3
```

```
S3,
  #R^LeftLever: ~sendevent(BOX, 2, 0);~; ---> S4

S4,
  5": ~stopBox(BOX);~; ---> StopAbortFlush
```

Copy the above file from “My Documents\NeuroPhysData” into “C:\MED-PC IV\MPC”. Then compile it using TRANS®, and run it from Med-PC®. Make sure that NeuroPhys is already running before you run it.

The command “StartRecording” will cause NeuroPhys to start recording spikes. This program will wait for a single press on the left lever, and then quite 5 seconds later.

Note that all communication with NeuroPhys occurs via functions called from PASCAL, so all such functions begin and end with a tilde symbol.

The command “sendevent” sends an event with the specified ID number (1 or 2 in the above example). The command “sendeventname” is optional, and is used to send a user-friendly name to NeuroPhys, which will then write it into the PLX file header. When you later open your PLX file in the spike sorter, or in Neuroexplorer, these user-friendly names will appear, and will make your life a lot easier.

Just before the Med-PC program ends, call “StopBox” to stop the recording session. If you forget to include this line, NeuroPhys will keep recording, and you will have to stop it manually (the red button on the box status tab).

Appendix 4: Recommended offline spike sorters

NeuroPhys records data in the same format (.PLX) that is used by Plexon Inc. This format has been used widely in the field for many years. You can read and analyze PLX files using a wide range of tools from many sources.

1. **SpikeSorter**
   This software can be downloaded with NeuroPhys. Developed by NeuroSys, LLC.

2. **MATLAB**
   You can use the code provided by Plexon to convert your PLX files into MATLAB compatible format. The file can be downloaded free from Plexon website.

   a. Go to http://www.plexon.com/software-downloads

   b. Under tab "SDKs", click on "Omniplex and MAP Offline (For reading previously recorded data files)".

3. **Plexon's Offline Sorter**

4. **Nex Technology's NeuroExplorer**

Appendix 5: Data output to CSV files

The NeuroPhys output files can be converted to CSV files using SpikeSorter software. For further information, please check the User Manual for