ASR User manual

Spike2 scripts auditory stimulation and single unit recording

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ASR v7 .s2s

This Spike2 script (`AudioStimRecSU`$_n$`.s2s`) allows you to generate acoustic stimuli via one or two speakers or vibratory stimuli using a mini-shakers. You can record single unit responses to these stimuli and analyze them online. The script is still under development and comes with no guarantees. If you encounter a problem when using them report it to CED and we will do our best to fix it

Features

These include:

- One or two channels of auditory stimulation e.g. left and right (*not both simultaneously*)
- Tone-pips, tone-bursts and noise-bursts generated internally by the CED1401 interface. These stimuli have a trapezoid envelope, that is, a linear ramp rising phase followed by a plateau and then a linear fall. Rising and falling ramp duration can be set independently to generate asymmetric envelopes. You can select a wide range of ramp and plateau durations, stimulus repetition rates and number of stimuli to present. The envelope can be filled with noise (pass band DC ï 1kHz) or sinusoids in the frequency range from 1Hz to 10kHz.
- Trigger an external stimulus generator and record externally generated TTL pulses for synchronizing online phase ï response analysis
- Play back pre-recorded waveforms as auditory stimuli
- Stimulus intensity is adjustable by means of CED3505 programmable attenuator(s). See separate data sheet for the attenuation range and step size.
- Internally generated stimuli are adjusted so that equal sound intensities can be achieved over the full range of carrier frequencies by compensating for the frequency characteristic of the speaker(s).
- Intra- or extracellularly recorded auditory responses are displayed on-screen along with a stimulus monitor. Spikes are detected online by means of a virtual window discriminator. Unit activity is displayed as an event channel and also optionally as instantaneous spike frequency.
- You can also perform the following analyses online:
- post-stimulus time histogram
- inter-spike interval histogram
- Phase locking, i.e., a phase histogram shows the phase lag of each spike with respect to internally or externally generated synchronization pulses. Results are also shown on a polar plot. Mean vector, vector length (r) and the Z statistic indicating significance of the distribution (relative to uniformity) are displayed online
- Automatic auditory threshold determination using an automated sequence of stimuli.
- You can specify the frequency range and intensity range of stimuli to use and the order of presentation
- Responses are displayed as Intensity-Response functions for each frequency
- These intensity response functions are then used to generate a threshold tuning curve.

System Requirements Power 1401 interface and 1 or two CED 3505 programmable attenuators.

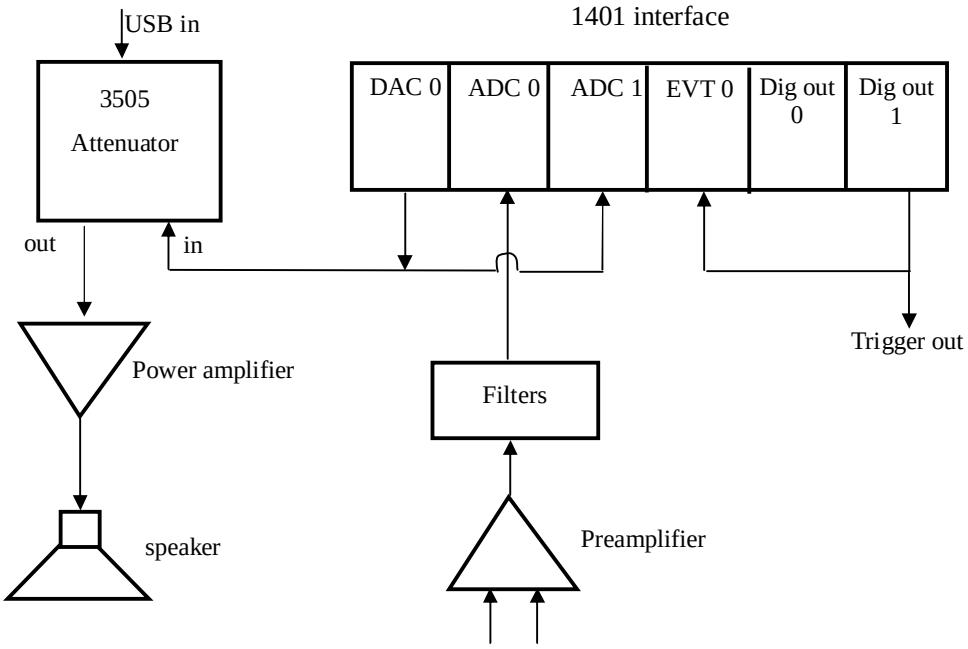
Spike 2 version 7.07 or higher.

Script and related files: The single unit recording software consists of 5 files:

- `AudioStimRecSU`$_n$`.s2s` the main script for auditory stimulation and recording
- `SoundCalSU`$_n$`.s2s` . a script for performing a sound intensity calibration
- `SU.s2c` or `SU.s2cx`, the sampling configuration
- `SU7.pls` a sequencer file that controls stimulus presentation
- `SUsettings.txt` this text file contains your current stimulus set up preferences.
- Up to 4 additional text files holding calibrations for left and right speakers (`SpkcaldataSU`$_n$`.txt`)and or vibration stimulators (`MScaldataSU`$_n$`.txt`) will be created by the calibration script.

Save the files to a folder named ***AudStimRec7*** inside your *Spike2* folder. The script will not work unless the files are stored at this location

Connections



Internal stimulus generator setup

The diagram shows the connections needed to generate one channel of acoustic stimulation via DAC0 and record one channel of neural responses via ADC0 and a single stimulus monitor on ADC1. For a two channel system, use, DAC 1 as input to the second attenuator.

Stimulus waveforms generated by the DAC outputs have a time resolution of 25εs (40kHz). The resolution can be increased to 10εs on request. You could use a ΌTô piece to connect DAC0 to the input of the attenuator and the stimulus monitor channel, ADC1 in order to monitor an un-attenuated version of the stimulus waveform. As delivered, the sampling configuration is set up to record 1 channel of neural data and 3 stimulus monitor channels, for example *x-, y-* and *z-* components of particle velocity. You can add more channels to the sampling configuration or remove those that you donôt need. If you do, we recommend that you review the parameters in the *Resolution* tab of the sampling configuration to ensure that you achieve sufficiently high sample rates per channel. As delivered the sample rate on each of the 4 recorded waveform channels is 25kHz.

Record single unit activity on ADC0 via a suitable preamplifier and filters. The default sampling rate of 25kHz will give good resolution of spike shape and thus gives ample scope for off-line spike sorting if required.

`Digital outputs 0` and `1` both generate signals that can be used to trigger an external device. `Digital Output 0` goes high at the onset of a display sweep and goes low again at the end of the selected latency. `Digital output 1` goes high at the onset of an internally generated stimulus and goes low again when the stimulus ends. Either of these outputs can be connected to `Event input 0` to record an additional monitor of stimulus timing.

External Stimulus generator setup.

Should you wish to use an external stimulator, then connect its output to the attenuator and stimulus monitor channel instead of DAC0. Connect `digital output 1` to the trigger or gate input of the stimulator. If the stimulator has a Ό*sync*ô output that generates a TTL pulse at the onset of each cycle of waveform output, you can connect this to `Event input 1` of the 1401. You will then have the option of triggering period histograms based on the external synchronization pulses rather than peaks/troughs in the stimulus monitor channel identified by the software.

Calibration

Before running this script for the first time you must calibrate the intensity of internally generated stimuli using the `SoundCalSU`$_n$`.s2s` script. See the separate `SoundCalSU` data sheet for how to do this. The `AudioStimRecSU`$_n$ script will close with an error message if the file containing the relevant calibration is missing.

Responses to internal stimuli are automatically labelled with the actual stimulus intensity based on the calibration table. The script treats external stimuli as uncalibrated. No correction factors are applied and results are labelled with the attenuator setting rather than the absolute intensity.

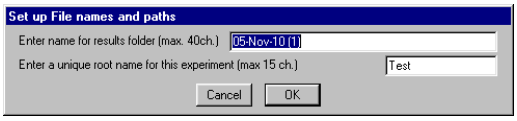
Running the ASR script

The script will add a hotkey labelled *AudStim* to the script bar when you run it for the first time. Click on this hotkey to run the script in future. The script then performs a self-test to ensure that all necessary files are found and that the equipment is switched on and communicating. The serial numbers of the attenuator(s) will be displayed along with the USB virtual COM ports used to communicate with them. It also reports the voltage range that the 1401 has been set to. Note that the peak to peak output voltage for un-attenuated waveforms will be adjusted automatically to ±5V irrespective of the 1401 voltage range.

If the system passes the self-test then a script toolbar with 14 buttons, each with a keyboard shortcut, will display.

Main Toolbar

Start by clicking on Record ON. A dialog will open for you to specify the folder where the results will be stored based on the current date and a serial number. You can also specify a root file name (default: `Test`) for all the results generated during the current trial. The serial number ensures that the results of separate trials performed on the same day are stored in different folders. All the result folders are stored in a sub-directory of the Spike2 folder called SUdata. Press OK to continue.



A data window will open in the lower half of the screen and sampling will start in *paged* mode, that is analogous to an oscilloscope with free running sweeps. The background colour is grey indicating that the results are not being saved to disk. The time view includes the following channels (some of which may be hidden*):*

- 1: the raw neuronal recording
- m2: an event channel marking spikes detected on channel 1.
- m2a: (optional) this channel records the instantaneous frequency of detected spikes.
- 2-5: Monitors of the stimulus waveform (sound and optional particle velocity monitors).
- 6: a level channel recording of `Event input 0`. Connect `digital output 1` to `Event input 0` to use this as an auxiliary stimulus monitor channel.
- 7: This channel, labelled *sync,* is available for recording TTL synchronization pulses from an external stimulator. You can select this channel as the trigger for generating period histograms
- m1: Event channel recording troughs in the stimulus monitor waveform. This channel, labelled *cyc,* serves as an internal trigger for period histograms showing the degree of phase locking of spikes to the stimulus waveform.
- 30: This is a TextMark channel It will hold Textmarks storing additional data on internally generated stimuli, for example attenuation level and intensity.
- 32. This is the digital marker channel used to trigger display sweeps during stimulation.

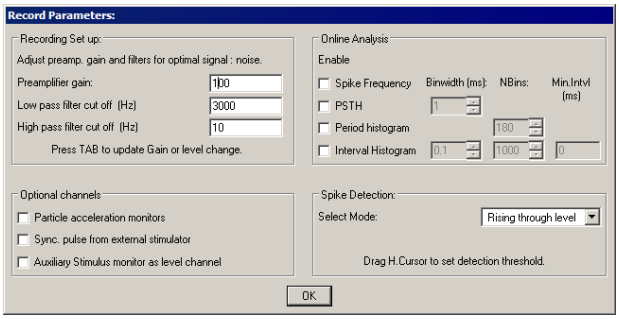
Record parameters dialog

Click on Record SetuP to customize the settings. The first step is to adjust the gain and filter settings on your preamplifier to achieve the best signal to noise ratio and a signal range extending over a significant portion of the input range of the 1401

interface. You can check this by double clicking on the Y-axis of channel 1 and selecting Show All from the *Y-Range* pop-up dialog.

When you enter the preamplifier gain in the dialog and press tab, the time view will update to show the data with the appropriate Y-scaling. The low and high pass filter cut-off values that you enter in the dialog will be added to the Channel Comment of your data files for future reference.

Now choose the method of spike detection. Four options are available: peak or trough detect and threshold crossing -either rising or falling. If you select peak or trough detection, you must also enter a level change value. This is the minimum level change that must occur before the next peak/trough.



However, the recommended detection method is spikes crossing a threshold. Choose rising through level or falling through level depending on the polarity of the spikes in your recording. A horizontal cursor will appear on channel 1. Drag the cursor to set the spike detection threshold. A major advantage of the level crossing method is that you can adjust the threshold level during recording if the amplitude of the spikes changes.

Finally, select the required online analysis options by checking the appropriate boxes.

- Check *Spike frequency* to show a dot display of instantaneous frequency of each detected spike in the time view.
- Check *PSTH* to show a post-stimulus time histogram. You will need to specify the bin-width for the display.
- Check *Interval histogram* to display the distribution of intervals between detected spikes. You must specify the range and resolution of the plot in terms of the bin-width, number of bins and the offset, that is the minimum interval to be included.
- Select *Period histogram* to show the distribution of spikes as a function of the phase of the stimulus wave at which they occur. You need to select the number of bins in the plot. For example 360 bins will give a resolution of 1° in phase angle. Bear in mind that the phase will differ slightly depending on whether spikes are marked at their peaks or at some threshold level on their rising or falling phase.

Your selections in the *Record parameters* dialog will be stored between experiments so after the initial set up, you only need to call up this dialog if you want to change the default set up.

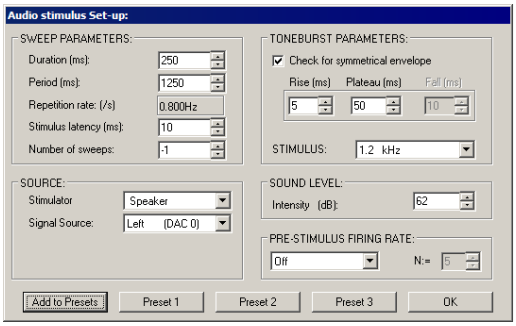
Click OK to close the dialog but continue to view the incoming data. At this point, you can try out the Scroll Mode button. This button toggles the display between oscilloscope and chart recorder display modes. You can adjust the time-base in scrolling mode using the usual Spike2 tools. Click the button again to revert to paged mode. The display switches automatically to triggered sweeps during auditory stimulation.

Record Off Click here to stop recording data. In this case, the time view will close automatically, because there is no data to save. If the data were being written to disk, sampling would stop and the file would remain open. The Save As and Delete All buttons would be enabled for closing the files.

Record On This button restarts recording of incoming data into a new data file.

Write On / Write Off. These buttons control whether or not the incoming data is saved to disk. The trace colour is green when the data is being saved and red when it is being discarded. You have the option to turn writing to disk off while setting up an experiment and searching for a suitable unit. This avoids cluttering your hard drive with potentially large but unimportant data files.

Stimulus Click here to configure an auditory stimulus. The *Auditory Set Up* dialog has five sections. As with other Spike2 script dialogs, you can navigate through the various items by selecting them with the mouse or by pressing *Tab* or *Shift+tab* to move to the next/previous item. You can change the value of numeric items by typing in a new value or using the spinner arrows to increment or decrement the current value. The keyboard cursor arrows act as shortcut keys for the spinner controls of the current dialog item. You can also move through drop-down list items using the arrow keys on the keyboard.



Sweep parameters

- *Duration:* This is the duration of the display sweep. The minimum value has been set at 20ms.
- *Period:* this is the time from the onset of one sweep to the onset of the next. Thus you must set it longer than the sweep duration. The stimulus repetition rate corresponding to the chosen sweep period will update as soon as you click on the next item or press *tab*.
- *Latency:* This is the time from sweep onset to the onset of the stimulus. You will not be allowed to set a latency that would cause the stimulus to extend beyond the end of the sweep.
- *Number of sweeps:* determines the number of stimuli to present before sampling stops automatically. You can set this item to ó-1ô to set the current stimulus as the *search stimulus*, that is, to repeat indefinitely until you hit the Stim OFF button.

Source:

Here, you can select the stimulation device (*Speaker / Mini-shaker*), which channel to use (*DAC0 / DAC1*) for internally generated stimuli (*tone bursts / playwaves*) or you can trigger an external stimulus generator (*External*). The script will load the calibration table for the selected device. The script assumes that the dac outputs are connected to the same attenuators and output devices that were used during the calibration procedure. Note that when you change the stimulus channel from dac0 to dac1 or vice versa, the current data file will be closed and discarded and a new one will open.

If *External* is chosen, there will be no output from the dacs, only trigger pulses from `digital output 0` and `digital output 1`. Additional selection boxes appear in the dialog:

- *Carrier Frequency*: This will be built into the title of the result views that are generated and is used to set up an appropriate period histogram.
- *Sync source*: This can be internal or external. If you select *Internal,* then channel m1, based on discrimination of troughs on the stimulus monitor waveform, will be used as the trigger for period histograms. *External* selects sync pulses on channel 4, as the source of triggers for period histograms.
- *Phase:* You must specify the phase relationship between the sync pulse and the stimulus waveform. For example, select ï90° if the trigger coincides with troughs of the waveform, select 0° if it coincides with signal rising through zero *etc*. Period histograms will be automatically corrected for any phase difference between the trigger pulses and the phase of the stimulus itself.

Tone-burst parameters:

This section is available if you select internal stimulus generation. The characteristics of the tone-burst are set up here, that is, the duration of the linear rise, fall and plateau phases of the stimulus envelope. If the checkbox is checked the tone-burst will have a symmetrical envelope with a fall time identical to the rise time. You will not be allowed to use a combination of values for sweep duration, latency and stimulus duration that would result in the sweep ending before the stimulus had finished.

You can choose the carrier frequency of a tone burst within the envelope from a drop-down list of available pure tones, filtered noise or stored waveforms. The list of stimuli available is restricted to those which were calibrated using the *SoundCal* script.

Sound level:

In this section, you select the initial sound level from a drop-down list. In the case of internally generated tone bursts, the corresponding intensity, taking into account the calibration of the sound generation system, is shown in the selection box. If you chose an external stimulus source this section shows attenuation. It will be up to you to calculate the intensity based on the intensity of the external source minus the attenuation.

Pre-stimulus firing rate:

This section provides several options for measuring the level of spontaneous neuronal activity prior to delivering a train of stimuli. You can switch this feature off, measure the rate over a fixed time (default: 5s) or wait for a chosen number of impulses to be detected (default: 500). The pre-stimulus activity level is printed below the X-axis of the PSTH.

Setting a preferred stimulus type by adjusting multiple dialog settings takes time that may be precious during a recording of uncertain duration. The dialog therefore gives you the option to set up the parameters for 3 types of stimulus in advance and then switch to these favourite settings with a single mouse click. To do this, set up your the stimulus parameters in the dialog and click on Add to Presets.

In the dialog, select the preset button to use, enter an appropriate button label and press OK to add the current stimulus setting to that preset buttons. Clicking the preset button then sets up the stored stimulus. The stored stimulus settings are saved to the computerôs registry and so will remain available until you decide to modify them (or change computer!).

Click on OK to close the Stimulus dialog. You will get an error message if you have chosen an invalid combination of stimulus parameters. You will need to re-open the dialog and make the necessary adjustments. If all is well, then the selected stimulus will be loaded you will be ready to stimulate and record.

Playing auditory stimuli

Click here to start playing the stimulus that you set up in the stimulus dialog and view responses as triggered sweeps. If you press Stim ON without explicitly setting up a stimulus then the most recently used stimulus (or a default stimulus) will play. The current stimulus parameters will be displayed in the title bar of the time view and in a small text window that you can drag to a convenient position on the screen. If you selected other forms of online analysis in the *Record Setup* dialog then additional result view windows will also appear.

If using threshold crossings to detect nerve impulses in response to auditory stimuli then you can drag the horizontal cursor to a suitable level at any time. If you scrolled the horizontal cursor out of range then you can fetch it into the visible range using the Fetch HC button. The Stim ON button label changes to Stim OFF while stimuli are playing. Click this button or its hotkey (*Space bar*) to stop stimulating and revert to a paged display (untriggered sweeps). You can switch to a chart recorder-type display by switching to Scroll Mode. You can use the usual Spike2 tools to adjust time ranges in scroll mode or paged mode.

If you selected a search stimulus (-1 as number of sweeps in the Stimulus dialog) then the stimulus will repeat indefinitely. You can then use the dB-UP and dB-DWN buttons (hotkeys: ↑ ↓) to adjust the stimulus intensity step by step. The step size is determined by the characteristics of the 3505 attenuator (typically steps are 3dB).

Similarly, you can press the Freq DWN or Freq UP buttons (or their hotkeys: ← →) to switch to the next or previous stimulus in the list of available carrier frequencies. Clicking on the Frequency Up / Dwn or Intensity Up / Dwn buttons or their hotkeys while the stimulus is switched *off* allows you to press the buttons multiple times in order to set the next frequency and intensity that you need without actually *playing* all the intermediate stimulus frequencies / intensities.

In addition, if you change frequency or intensity using these buttons then any additional result view windows will be reset with the previous results discarded. If you want to save these results then you must press Stim Off followed by SaVe As... You can press the Save Asé or Delete All buttons during recording but with the stimulus switched *off* in order to process Result views such as PSTH or INTH views. However, you must press Record OFF to stop sampling before pressing Save Asé or Delete All in order to save or discard the raw data.

Record Auditory Responses

When you find a unit that you want to characterise, a typical sequence of actions might be:

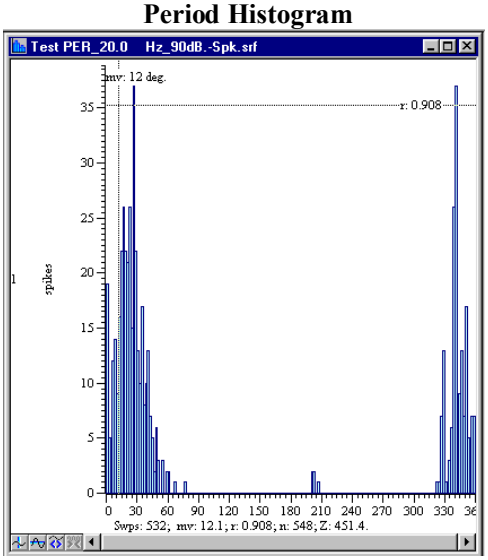
- Make final adjustments to the spike detection threshold to ensure that all of the spikes of interest will be picked up.
- Click Write On to start saving the raw data to disk.
- Stim On to begin stimulation. If you opted to measure resting firing rate, this will start now and a message to that effect will appear on the screen. When this is complete, stimulation begins and if selected, PSTH, interval histogram, period histogram and polar phase plots will display. Stimulation continues until you click on Stim Off or the required number of sweeps is reached.
- At the end of a stimulus train, you can, use the Save Dataé and/or Delete All buttons to store or discard the result views. You can then continue with other stimuli Sampling into the same data file continues until you click on Record Off followed by Save As... to save the raw data.
- Click Record On if you need to re-start sampling into a new data file. You can use the dB UP and dB Dwn buttons (hotkeys: Ŷ Ÿ) before or during a stimulus sequence. A TextMark will be added to the data file to indicate the new intensity/ frequency. Alternatively, click on Stimulus to change other stimulus parameters or choose a preset stimulus before pressing Stim On.

Period Histogram and Polar phase diagrams

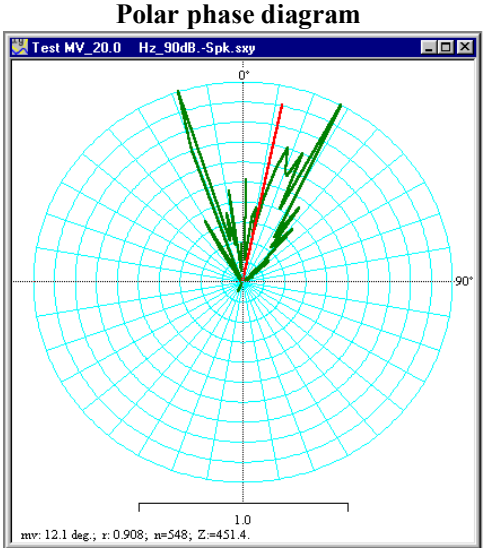
The period histogram features a vertical cursor marking the mean vector and a horizontal cursor indicating vector length (`r`) which can vary between 0 and 1. These circular statistics indicate the degree of phase locking of detected events. The sweep count shown in the legend is the number of cycles of the stimulus waveform that were included in the analysis. The `Z` statistic (`Z = r²*n` where `n` is number of events detected) indicates the likelihood that the distribution differs significantly from uniformity. The probability of observing `Z > 4.5` by chance is `< 1%`. The polar plot shows the mean vector as a pointer of length `r`. A normalized version of the raw data is shown on the same plot for comparison.

For further information on circular statistics see*:*
Edward Batschelet, Circular statistics in biology*, Academic Press, London, 1981. ISBN 0120810506.*

Period Histogram



Polar phase diagram

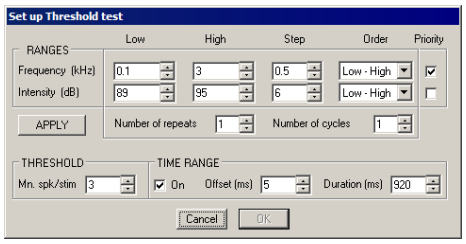


Auditory Intensity-response plots and tuning curves

Click on Thr Test in order to set up an automated sequence of stimuli to test auditory responses over a user-defined frequency and intensity range. The basic stimulus parameters such as tone burst duration and repetition rate are as defined in the Stimulus dialog. The *Threshold Set up* dialog has two sections: In the *Ranges* section, you set the limits of the frequency range and intensity range to test *preferably* using the spinner arrows on the dialog items. The step values specify the size of the intermediate frequency and intensity steps. Available frequency steps are multiples of 100 Hz. Intensity steps are multiples of the attenuation steps available from your attenuator, typically, 3dB. The upper intensity limit is set by the intensity achieved during the calibration procedure at 1 kHz. The actual upper intensity set in the dialog must be achievable by the attenuator, that is, it must differ from the upper intensity limit by a multiple of the attenuator step size (typically 3 dB). If the chosen frequency range is not exactly divisible by the chosen step size, then the frequency steps will be calculated starting at the low edge of the range and the upper limit of the range will be added even though it not on a step boundary. For example, with the settings as shown below, the list of frequencies tested would be: 0.1, 0.6, 1.1, 1.6é .4.6, 5.0 kHz

The order and priority items control presentation order. The options for both frequency and intensity are: *Low to High*, *High to Low* and *Random*. The priority checkboxes are not applicable when one of the parameters is changed randomly.

When you have chosen your settings in the dialog, press the Apply button. You will get warning messages if your settings are invalid and tips on what items to adjust in order to get avalid combination. The OK button will only enable when you have achieved valid settings.



Example settings might be*:*

Frequency: *High to Low,* *Intensity:* High to Low , Priority: *Intensity*.

This means tests start at the highest frequency and intensity. Intensity is then reduced stepwise over the selected range before the frequency is decreased by one step and repeating the intensity sweep.

If we changed to frequency priority, then the test would start at the highest frequency and intensity and sweep downward through the selected frequencies before reducing the intensity by one step and repeating.

If *Random* is selected, then we step randomly through all the available intensity steps at a given frequency before changing intensity or *vice versa*.

You can achieve full randomisation by selecting *Random* frequency and *Random* intensity.

Number of repeats controls the number of successive stimuli presented before the frequency or intensity changes. *Number of cycles* controls the number of times that the entire sequence of stimuli is repeated.

The second part of the dialog allows you to specify the threshold level in terms of mean spike count per stimulus. You can also set a peri-stimulus time window during which spikes will be counted. If this is disabled all detected spikes will be

included. If enabled, only spikes in the time window are counted. Here offset is time after the onset of the stimulus (not the sweep) and duration is the width of the required time window. The default duration is 10ms longer than the stimulus duration. When you are satisfied with the settings, press OK to start the test.

The mean spike count in response to each frequency-intensity combination is added to a family of intensity-response plots. The response to each frequency is plotted in a different colour. Each data point is the mean spike count in response to all the consecutive *repeats* of each stimulus. Additional points are added if several *cycles* of stimulation are applied.

When the sequence of stimuli ends then related data points (i.e. same frequency are joined with straight lines. If more than one cycle of stimulation was performed then the lines join the mean response over the different cycles. The intensity-response plots are then used to construct a threshold response curve using the threshold criterion entered in the dialog.

You can save the intensity ï response functions and threshold curve via the Save Asé button or discard them via Delete All. The time view will continue to sample raw data while you do this. You must turn recording off in order to save the time view.

Testing the system

An easy way to test the threshold /intensity ï response plot functions without a preparation is to split the output of the attenuator via a T-piece so that you can record its output on ADC0 instead of the neural input. We can then record level crossings on the stimulus waveform as if they were nerve impulses. DAC0 connects to the attenuator input and ADC1, the stimulus monitor, as usual.