OVERVIEW This script is intended primarily for identifying sleep spindles in EEG recordings. However, it may also be suitable for detecting seizure activity or other eeg features. Sleep spindles are brief bursts of 12-15Hz oscillations in the EEG during slow wave sleep. They occur in all the mammalian species that have been tested and are thought to be implicated in sensory processing and in the consolidation of long-term memories. Thus, there is significant research interest in the effect of learning, memory retrieval and pharmacological agents on the frequency of sleep spindles.

Sleep spindles are relatively small and somewhat variable and so identifying them automatically is a far from trivial task. This script attempts it using a multi-stage process - although stages 2-4 are optional. Since the script can be used to detect features other than spindles, we refer to the detected features below as *events*.

Stages of Event detection

1. Spectral power Plot EEG spectral power in a user-defined frequency band vs. time and mark bursts that exceed a user-defined threshold (mean plus user-defined multiple of *stdev*). This analysis can be done on the entire data file or be gated to a specific sleep state if the data has been scored. Events shorter than a user-defined duration can be excluded while events that are close together can be amalgamated into a single event. There are also options to exclude events that exceed a user-defined duration or an upper power limit. Detected events are clearly marked with blue bars.

2. Spike count The power spectrum method alone is unlikely to be 100% successful. Stage 1 settings that detect all the target events are likely also to mark a significant number of false positives. Stage 2 attempts to remove at least some false positives by excluding events where too few EEG peaks (or troughs) exceed a user-defined threshold amplitude during the power bursts.

3. Manual Edit The script allows you to review the data event by event or page by page, manually deleting marked events that do not pass the "eyeball test", adding markers for events that were missed and amalgamating events that belong together.

4. Event duration There is an option to adjust the duration of marked events so that they are deemed to start and end when the banded power level crosses a user-defined baseline level.

Prerequisites

Software The script requires Spike2 v.8.18 or higher

Included files It uses several libraries of script functions: *ghutils.s2s* and *ug.s2s*. These should be kept in the folder *Documents/SpikeN/include* or alternatively, in the same folder as the *SleepSpindleN.s2s* script (where N is a serial number).

Channel Naming Conventions The script recognises channels to process, in part by their channel titles. Titles of EEG channels must be unique, as short as practicable and contain the string EEG or eeg. The preferred format for channel titles that the script must process is: EEG 1, EEG 2 etc. To edit channel titles to meet this requirement, double-click on the channel title, enter a new title in the *Channel Information* dialog and click on Apply. You can analyse multiple EEG channels in the same file, one after the other, provided that the channel naming scheme is followed.

Event colours The script sets the colours linked to marker codes 1 and 2 to blue and green respectively. As a result, accepted events are marked with blue bars and rejected events are green. These markers will revert to the colours specified for markers in the Spike2 colour palette when the script closes.

Script Dialogs

Most items in script dialogs can be controlled either by clicking on them with the mouse or via keyboard hotkeys. Using hotkeys such as cursor arrows to navigate and zoom is likely to be quicker and more convenient. Using spinner arrows to adjust the position of horizontal cursors avoids the need to press the *tab* key to force typed-in values to update.

You can move dialogs to convenient screen positions by dragging the title bar. The dialogs remember their settings and positions between trials so it is easy to apply the same detection parameters to multiple EEG channels or multiple data files.

Gated analysis

Sleep stage marker channels for gated analysis must be TextMark channels drawn in *States* mode. The sleep state must be indicated by the marker code for each state, usually 01, 02, 03 etc. with an optional additional text code such as W, N, R etc. The only restriction on the channel title is that is should not be *Epochs* since this script creates a separate epoch channel with that title.

USER GUIDE

When run for the first time, the script will prompt you to create a hotkey *SpSzDet* on the script bar for one -click access to the script. If you do not want a hotkey or reminders to create one then comment the line of script marked with ñ-------ñby adding a single quote mark at the start of the line.

The script toolbar has a toolbar with 11 buttons:

Quit

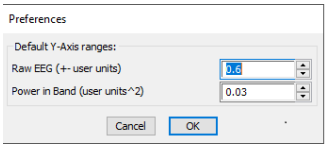
This button simply tidies up and closes the script.

User Guide

Click here to display a text only version of this guide. Click this button again (or any other button) to hide it.

Preferences

Click here to set up the Y-axis ranges for EEG and banded power channels that make it easiest for you to recognise the features you want to identify, sleep spindles, seizure activity etc. During the analysis you will be able to restore this preferred range via dialog buttons labelled Y-Ranges. A certain amount of trial and error will be required to find the optimum Y-ranges for raw EEG and power in band channels. When you have found suitable values, enter them here. They will be used to set the default scaling of data until such time that you change the settings.



Reset

Clicking here will delete *all* additional channels created by this script *whether in the current or previous analysis session*s, returning the data file to its un-analysed state. There is an *Are you sure?* warning in case you have second thoughts. This button speeds up the process of repeatedly analysing a single data file while searching for optimal settings. There is also an option to close any open plots and spreadsheets resulting from a previous analysis.

Open File

The script operates on the current time view and expects only one time view to be open at a time. If there is no current time view or you want to open a new one, click on Open File, browse to and open a file to analyse. The previous data file, if any, will close when the new one opens.

Detect Events

This button will be available if a data file is open. Click to open a Setup dialog to configure stage 1 of event detection based on spectral power. The dialog has several groups of items:

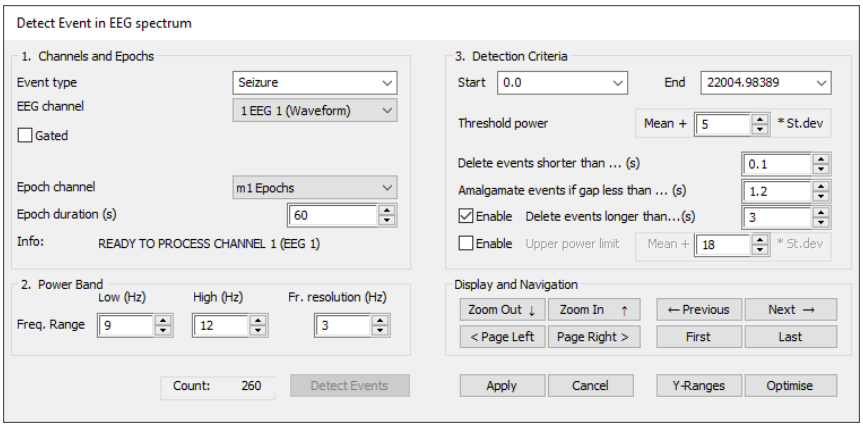
Channels and Epochs

Event type

Select the event type to search for from the drop-down list or type in the name of the event type.

EEG channel Select the EEG channel of interest. Only visible Waveforms and RealWaves with titles matching the naming convention e.g. "*EEG: 1*, *EEG: 2 etc*." will be available for selection. If none are visible or if the selected channel has already been analysed then the Detect Events button will be disabled.

Detect events dialog



Gated by sleep stage Check this box to analyse only data within a specified sleep state. Select the channel containing the sleep states from the drop-down list that appears and enter the Stage code.

Epoch channel The data must be sub-divided into epochs for analysis. If there is a pre-existing channel with the title *Epochs*, it will be used. If not, a new *Epochs* channel will be created. You must specify the required epoch duration. In the case of gated sleep stage analysis, choose an epoch duration equal to the sleep stage duration or a multiple of it. New epoch markers (numbered vertical bars) will be added to the file when you close the dialog by clicking on Apply. You can configure the appearance of the epoch markers later via the Spike2 View menu (*View/Vertical Mark*).

Power Band

Frequency range Here, you define the spectral band limits for the feature you want to detect. A band of 12-15Hz is typical for spindle detection but other limits may be appropriate for seizure detection.

Frequency resolution The script generates an extra channel in the data file showing power in band *vs.* time using the frequency resolution that you select. There is, inescapably, a trade-off between the frequency and time resolution of the plot. Increased frequency resolution leads to a reduction in time resolution. Since sleep spindles are brief events, maximising the time resolution is usually the best option. Do this by setting the frequency resolution to the width of the frequency band.

The frequency resolution and band edge items can be adjusted via the spinner controls after the power-in-band plot has been generated so you can observe the effect of adjusting the band width and resolution to find values that best suit your analysis.

Detection Criteria The main criterion for event detection is: banded spectral power above a threshold defined in terms of $mn + n * stdev$ of power in a user-defined time range.

Time range This is the start and end of the time range to analyse (usually the entire file). You can type in times or select Cursors or other landmarks from the drop-down lists. Means and standard deviations of banded power are calculated over this time range except in the gated option where the calculation is limited to the gated periods. Note that gaps in the EEG data at the start and end of the selected time range will be ignored. However, any other gaps in the data will be treated as zeroes and will thus affect mean and stdev. calculations.

Threshold power Set a default threshold level in terms of *Mean + N standard deviations* over the time range. You can adjust the threshold level later interactively before the results are finalised.

Delete events shorter than Threshold crossings of shorter duration than specified here will be excluded.

Amalgamate gaps less than Supra-threshold episodes with gaps less than specified between them will be combined into a single event.

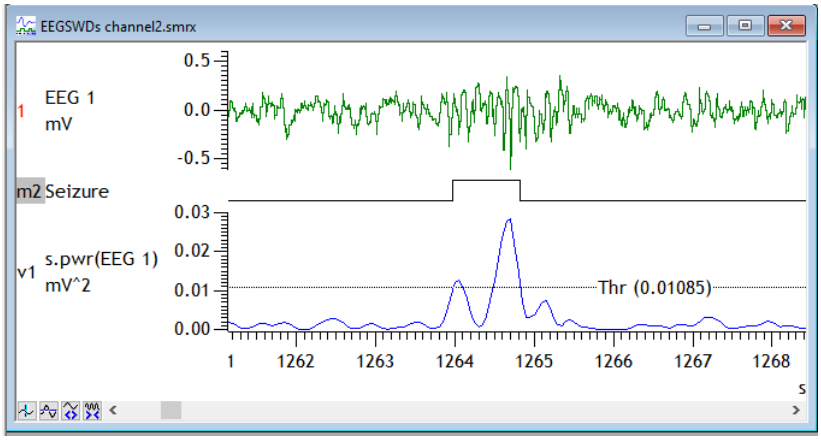
Delete events longer than There is an option selectable via a check box to exclude supra-threshold events longer than a specified duration.

Upper power limit There is an option, selectable by checkbox to exclude events where the power in the selected band exceeds an upper limit.

Detect Events After setting sensible initial values, click this button to mark all the time ranges that meet your criteria in the chosen EEG channel. The dialog will remain open but disabled until the necessary calculations are complete. The display will then change to show:

- the sleep stage marker (if applicable),
- the selected EEG channel,
- A Virtual channel showing power in the chosen band (title: *s.pwr(EEG n)*). This channel will contain horizontal cursors marking the threshold level and upper power limit (if enabled).
- A *Level* channel marking the episodes that meet the criteria set in the dialog.
- a count of events detected displays at the bottom left corner of the dialog.

Example Seizure detection



Channel v1 shows a power surge in the band 9-12 Hz as a result of an event on EEG 1. This crosses the user-defined threshold marked by the horizontal cursor and results in a seizure candidate being marked in channel m2. The detection criteria are those shown in the previous dialog.

Display and Navigation You can now use these dialog buttons to inspect the data and compare the marked episodes with your visual assessment of the data. The usual Spike2 methods for adjusting X- and Y-ranges remain available.

Optimise (Hotkey: Q) adjusts the Y-ranges to show all of the data and keeps threshold levels in the visible range.

Y-Ranges (Hotkey: Y) resets the Y-Ranges to the defaults set in the *Preferences* dialog.

Zoom In / Zoom Out (Hotkeys: Ź ŷ) Zoom in or out by a factor of x2.

Prev. Evt / Next Evt These buttons centre the view on the previous or next detected event on the selected channel (highlighted channel number)

Page Left / Page Right (Hotkeys: < >). Shift the view back or forward by one page.

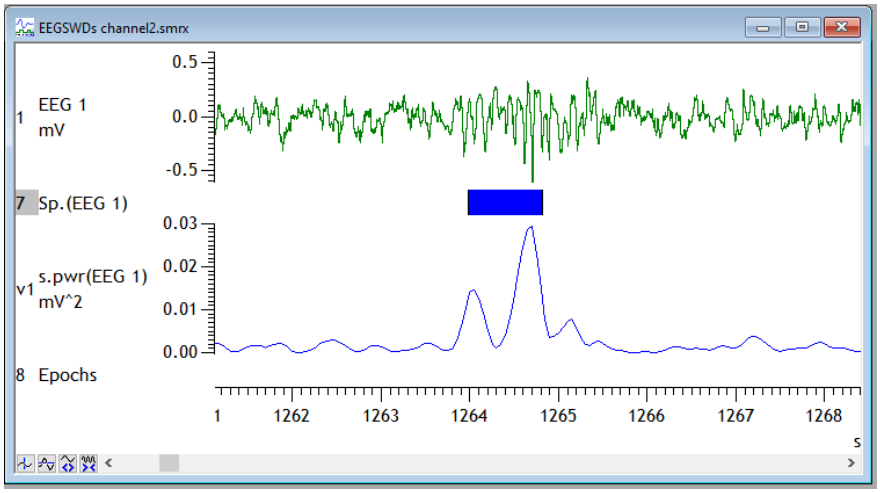
First / Last (Hotkeys: Home / End). Centre the display on the first or last detected event.

If you are not satisfied with the results, you can adjust the event detection criteria in the dialog and the level channel marking episodes meeting the criteria will update. Bear in mind that the re-calculation involved may take a noticeable amount of time when files are large.

You can adjust:

- Power band limits and frequency resolution
- Minimum and maximum event duration and amalgamation of adjacent events
- Threshold power level and upper power limit (if enabled). These thresholds can be adjusted by dragging the horizontal cursors or via spinner arrows of the threshold item in the dialog.

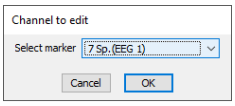
Apply When you are satisfied with the search criteria, click here to save them. The dialog will close and the level channel will be replaced by blue coloured bars in a state channel (title; *Sp.(EEG n)*) next to the power in band channel *see below*. Alternatively, click on Cancel to close the dialog and return the file to the previous un-analysed state.



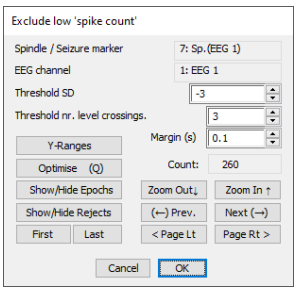
You can now step to the Analysis stage if the results are satisfactory or press one or more of the Refine, Edit and Evt. Duration buttons to refine the results.

Finding the best settings in this dialog will require some trial and error and may be time-consuming. However, once good settings have been found, the hope is that the same or very similar settings will work for other recordings in your study so that the dialog settings will require minimal adjustement in future.

Refine This button provides a method for eliminating marked events where the number of spikes in the raw EEG is below a threshold count. If there is more than one visible event marker then the first step is to choose which Event channel to process in a preliminary dialog.



Next, in the main *Refine* dialog, select a threshold level to detect significant spikes. You can do this either by dragging the horizontal cursor in the EEG channel or via the spinner arrows in the dialog. These shift the threshold in steps of 0.1**stdev*. Drag the cursor below the baseline level or set the *stdev* multiplier in the dialog negative to count EEG troughs rather than peaks. You must also select a count for the threshold number of spikes that cross the threshold during marked events.



The time range for detection of EEG spikes can be extended slightly before and after the marked event by an adjustable *Margin* in the range 0 to 0.5s. The default margin is 0.1s either side of the marker. Spikes that meet these criteria are shown as ticks in a channel labelled *Evts* next to the Event marker channel *Sp.(EEG n).* Marked events that fail the spike count test are coloured green. These channels update whenever you change the detection criteria. Dialog buttons and their hotkeys allow you to zoom in and out, navigate the file and adjust the Y-ranges to facilitate setting the optimal threshold levels.

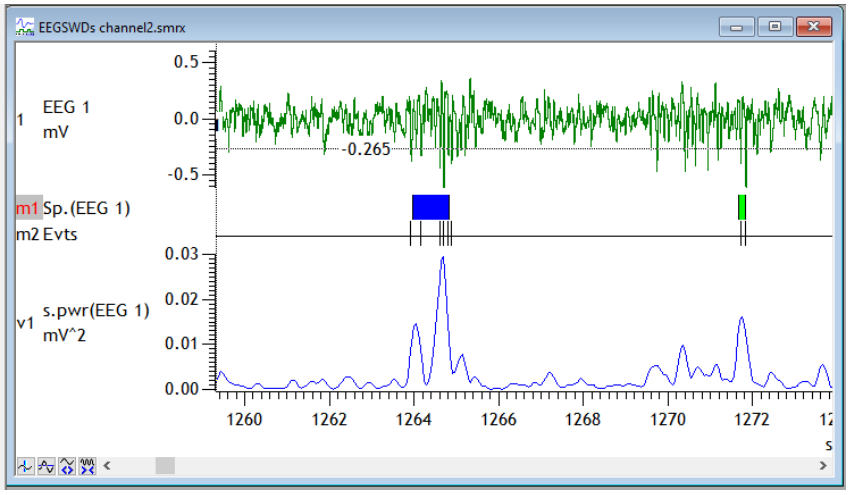
Additional buttons:

Show / Hide epochs This buttons toggles between visible and hidden epoch markers. Hide them if they obscure your view of the data.

Show / Hide rejects Click here to flip between displaying or hiding those events that fail the *Refine* criteria. The count of displayed events changes accordingly.

Click on Cancel to close the dialog and return to the un-refined data or OK to permanently delete the rejected events.

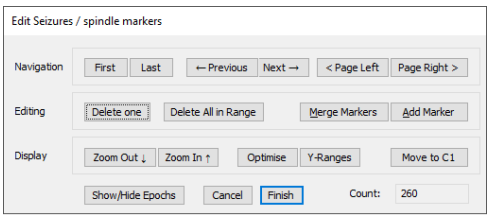
Refining the data



Channel m1 shows candidate events while m2 shows counts of EEG spikes that cross the user-defined threshold (horizontal cursor) during that event. Events marked in blue are accepted because the spike count exceeds the chosen value (3). Candidates that fail this criterion are marked green and will be rejected.

Edit Click this button to inspect and manually edit the events detected in the data file, deleting false positives, amalgamating adjacent markers or adding new events manually via dialog buttons. The dialog has the usual navigation buttons (First / Last, Previous / Next, Page Left / Page Right plus Zoom Out / Zoom In to facilitate easy inspection of events. The current event is centred in the view and marked with a cursor.

The Editing tools are:



Delete one Click here to delete the current event. The cursor and the event to be deleted must both be visible.

Delete all in range Click here to fetch two cursors. Drag them to bracket the time range of events to include and press *Enter* to delete, *Esc* to cancel or press the corresponding buttons on the script toolbar (Do It and Back). Events in progress at the cursor positions will also be deleted.

Merge Markers Click here to merge the current marker with the following one. Both events must be fully visible.

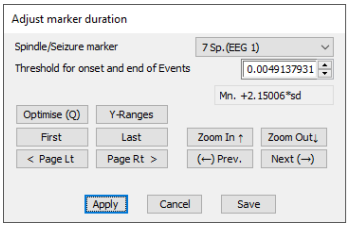
Add Marker

Click here to add an event manually. Drag the cursors to mark the start and end of the new event and click on Do It on the script toolbar or press *Enter*. Press *Esc* or hit Back on the toolbar to return to the Edit dialog

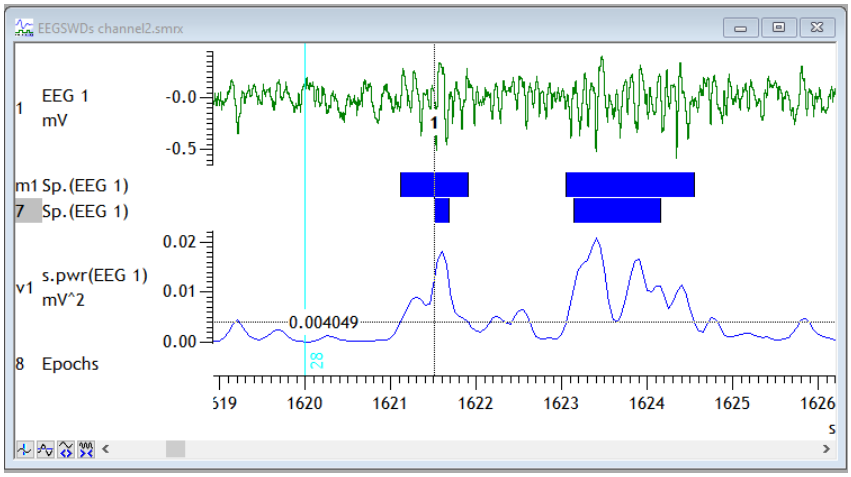
When ready press Finish to save all the edits to disk and close the dialog or press Cancel to discard all the changes.

Evt Duration

The onset and end of events marked by the Detect button are the times when the banded power crossed the detection threshold. This button gives the option to extend the duration of marked events so that they begin and end when the power level crosses a user-defined *baseline* level. You can set the desired baseline power level by dragging a horizontal cursor or by clicking on the spinner arrows in the dialog. The dialog shows both the actual power level and converted to mean power plus a multiple of standard deviation. The dialog has buttons for scaling and navigating the data file to facilitate setting an appropriate baseline level. Click on Apply to create a modified event marker channel next to the original. Press Save to replace the original marker channel with the modified one. Press Cancel to retain the original event marker.



Adjust Event duration



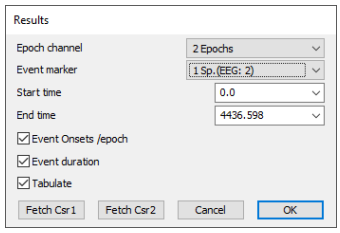
v1 shows power in the band 9-12 Hz in EEG 1. Ch 7 shows the original event markers while m1 shows the markers with onset and end adjusted to times when the power level crossed a user-defined baseline level (horizontal cursor). The blue vertical mark is an epoch boundary.

Analyse

This button will only be available if the data file contains at least one data set.

In the dialog, choose the epoch channel and event channel and then select the time range to process. You can select the time range by entering times or by choosing landmarks from the drop-down lists. You can select 3 types of analysis via check boxes. These are:

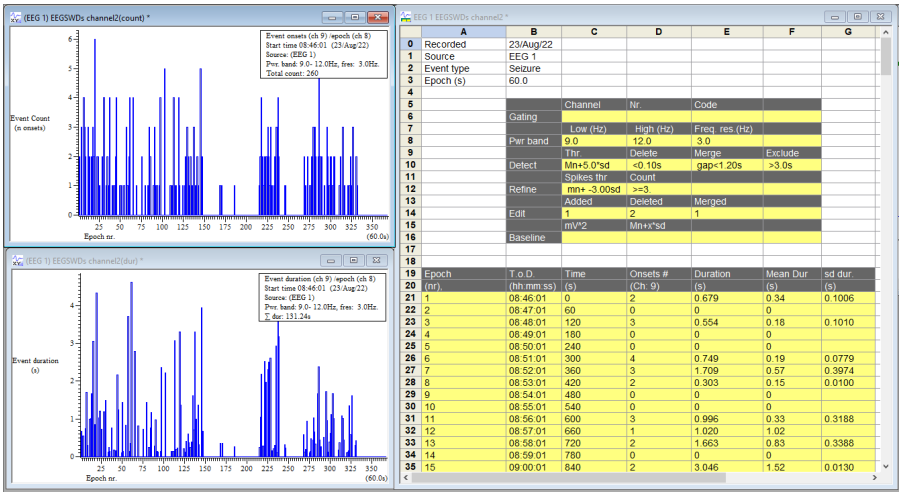
- A plot of Event Count (onsets) *vs.* Epoch nr.
- A plot of total Event Duration vs. Epoch nr.
- A Table of Event counts and durations per epoch.



Window titles are based on the name of the source file and the title of the EEG channel. The table output includes a summary of the settings used in the *Detect*, *Refine*, *Edit* and *Evt Duration* dialogs followed by an epoch by epoch table of event counts and event durations.

Example Results

The plots of Event counts and Event durations are XY views. You can access tools to modify their appearance via the context menu by right-clicking on the views. You can change the colour scheme via the *Colour Palette* item on the main Spike2 toolbar. The keys can be shown, hidden or dragged into a suitable position.



	A	B	C	D	E	F	G
0	Recorded	23/Aug/22					
1	Source	EEG 1					
2	Event type	Seizure					
3	Epoch (s)	60.0					
4							
5			Channel	Nr.	Code		
6		Gating					
7			Low (Hz)	High (Hz)	Freq. res.(Hz)		
8		Pwr band	9.0	12.0	3.0		
9			Thr	Delete	Merge	Exclude	
10		Detect	Mn+5.0*sd	<0.10s	gap<1.20s	>3.0s	
11			Spikes thr	Count			
12		Refine	mn+-3.00sd	>=3			
13			Added	Deleted	Merged		
14		Edit	1	2	1		
15			mV^2	Mn+x*sd			
16		Baseline					
17							
18							
19	Epoch	T.o.D.	Time	Onsets #	Duration	Mean Dur	sd dur
20	(nr)	(hh:mm:ss)	(s)	(Ch: 9)	(s)	(s)	(s)
21	1	08:46:01	0	2	0.679	0.34	0.1006
22	2	08:47:01	60	0	0	0	
23	3	08:48:01	120	3	0.554	0.18	0.1010
24	4	08:49:01	180	0	0	0	
25	5	08:50:01	240	0	0	0	
26	6	08:51:01	300	4	0.749	0.19	0.0779
27	7	08:52:01	360	3	1.709	0.57	0.3974
28	8	08:53:01	420	2	0.303	0.15	0.0100
29	9	08:54:01	480	0	0	0	
30	10	08:55:01	540	0	0	0	
31	11	08:56:01	600	3	0.996	0.33	0.3188
32	12	08:57:01	660	1	1.020	1.02	
33	13	08:58:01	720	2	1.663	0.83	0.3388
34	14	08:59:01	780	0	0	0	
35	15	09:00:01	840	2	3.046	1.52	0.0130

Tables are stored as Spike2 grid views. Only the first few rows are shown in the example. You can copy the contents to spreadsheet software by selecting the required data set and copying and pasting via the clipboard. The table has two main components. The upper table gives a summary of the main dialog settings used to generate the results during each stage of processing (Detect, Refine, Edit and Duration adjustment).

The main table shows the number of event onsets in each epoch and the duration of those events. Where there are multiple events within an epoch the mean and standard deviation of duration is shown. These columns will likely be blank if the epoch duration used was brief. The total event count, total event duration and percentage of time occupied by events is shown at the end of the table.

Note that the colouring of tables is only available for users of Spike2 v10.04 and higher. Earlier version will show the table as black and white.

Save / Delete

Clicking this button provides options to delete the current result files or save them to disk in the same folder as the source data file.