¹EPINETLAB USER MANUAL



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EPINETLAB: A Software for Seizure-Onset Zone Identification From Intracranial EEG Signal in Epilepsy



EpiNetLab is a post-processing analysis platform for automatic identification and quantification of High Frequency Oscillations (HFO) contained in EEG signal. The tools are accessible from within EEGLab, an interactive MATLAB toolbox for processing continuous and event-related EEG, MEG and other electrophysiological data incorporating independent component analysis (ICA), time/frequency analysis, artefact rejection, event-related statistics, and several useful modes of visualization of the averaged and single-trial data. EEGLAB runs under Linux, Unix, Windows, and Mac OS X (https://sccn.ucsd.edu/eeglab/index.php).

While EpiNetLab was designed with intracranial EEG (iEEG) data in mind, the platform can in principle be used to analyze scalp EEG as well both in signal and source space, provided the dataset can be imported in its higher order framework EEGLab. MEG data import for Elekta Oy ".fif" format has also been developed as part of the project.

The toolbox can be requested from Lucia Quitadamo (<u>l.quitadamo@aston.ac.uk</u>); enquiries on clinical application can be directed to Stefano Seri (<u>s.seri@aston.ac.uk</u>)

The software can be also be downloaded from <u>https://github.com/quitadal/EPINETLAB.</u>

Installation tips: In order for EpiNetLab software to be visible within the EEGLab GUI, the folder containing all its functions has to be copied to the folder "plugins" of EEGLab. When this is done, starting EEGLab from the MATLAB prompt delivers the following screen with "EPINETLAB (HFO)" menu item visible, see Fig.1:

							EEGLAB	v14.1.2		
File	Edit	Tools	Plot	Study	ERPLAB	ERPsets	Datasets	Help	EPINETLAB (HFO)	
		No curi	rent da	ataset					Preprocessing Functions	►
		-							Seizure Inspection	
			- (Create a	new or lo	ad an exis	ting datase	t:	Wavelet-based Time-Frequency Analysis Kurtosis-based Statistics HFOs Detection	
				Use "Fi	le > Impor	t data"	(ne	w)	HFOs Visualization (in time) HFOs rates plot SOZ Identification	•
				Or "Fi	le > Load (existing d	ataset" (ol	.d)	HFOs detection on multiple files	
			- :	If new,					Export HFOs to Micromed Single Pulse Electrical Stimulation (SPES) analysis	•
				"File >	Import epo	ch info" (data epochs) else	EPINETLAB Help	►
				"File >	Import even	nt info" (continuous	data)		
			,	"Edit >	Dataset in	fo" (add/e	dit dataset	info)		
				"File >	Save datas	et" (save	dataset)			
			- 1	Prune da	ta: "Edit :	> Select d	ata"			
			- I	Reject d	ata: "Tool:	s > Reject	continuous	data"		
			- I	Epoch da	ta: "Tools	> Extract	epochs"			
			- I	Remove b	aseline: "	Tools > Re	move baseli	ne"		
			- 1	Run ICA:	"Tools	> Run ICA	"			

Figure 1: EEGLAB with EPINETLAB installed.

Getting Started

We will assume here that you have been able to export from your clinical EEG system the iEEG data you wish to analyze in a format that EEGLab is able to read.

The preliminary clinical validation was performed on peri-ictal data and on interictal as described in the 2 initial publications:

- <u>Quitadamo, LR, Foley, E</u>, Mai, R, De Palma, L, Specchio, N<u>& Seri, S</u> 2018.
 <u>'EPINETLAB: a software for seizure-onset zone identification from intracranial EEG</u> signal in epilepsy' <u>Frontiers in Neuroinformatics</u>. DOI:10.3389/fninf.2018.00045
- <u>Quitadamo, LR</u>, Mai, R, Gozzo, F, Pelliccia, V, Cardinale, F<u>& Seri, S</u> 2018, '<u>Kurtosis-based detection of intracranial high-frequency oscillations for the</u> <u>identification of the seizure onset zone</u>' <u>International Journal of Neural Systems</u>, vol. In press, 10.1142/S0129065718500016, pp. 1850001. DOI:<u>10.1142/S0129065718500016</u>

In particular, we tend to analyze:

- 1. Peri-ictal data: two seizure episodes. An EEG segment containing 10 min before and 2 min after the electrographic onset of each seizure was extracted and was considered as the period of interest for HFOs identification.
- 2. Interictal data were extracted from 10 min of iEEG signal collected during stage III sleep of the second night of the monitoring period.

The ultimate choice however rests with the user. We also remind users that the tool is not intended as a clinical device lacking any regulatory approval from appropriate agencies. We made this available to users for research purposes only. Its use outside of this context is done exclusively under the user's responsibility.

PRE-PROCESSING

1. Data Import: extensions for EEGLAB

These extensions allow to import various type of data. EEGLAB contains native function to import some data formats (Fig.2).

- **BIOSIG data import:** Import/export data in a wide variety of data formats, developed by <u>Alois Schloegl</u>, the creator of the EDF+ data format. For more information about BIOSIG toolbox visit <u>this page</u>.
- FileIO: toolbox allowing data import in multiple data formats.
- CTF data import: Import CTF MEG data. Available from Darren Weber's <u>EEG</u> <u>sourceforge</u> project, this extension imports MEG data (plus concurrent EEG, if any) plus sensor locations and data events from data in the CTF (Vancouver, CA) data format.
- ANT data import (v1.03): Import data files in the EEP format. Contributed by <u>ANT Software</u> (Netherlands) to import data in their format. Email contact: <u>info@ant-software.nl</u>.

- **BVA data import/export:** Import/export files from/to the Brain Vision Software Analyser suite. Contributed by Andreas Widmann of the University of Leipzig (Germany) with Arnaud Delorme.
- **Neuroimaging 4D:** Christian Wienbruch of the University of Konstanz (Germany) has an extension available for loading Neuroimaging 4-D data into EEGLAB.
- **TDT data import:** Adam Wilson at the NITRO Lab at the University of Wisconsin Madison (USA) offers an extension available for loading Tucker-Davis Technology format data into EEGLAB.
- NeurOne data import: EEGLAB extension for reading the file format of NeurOne system.
- **Micromed data import:** Micromed (Italy) has an extension available for loading their data format into EEGLAB. Contact <u>Cristiano Rizzo</u> (cristiano.rizzo@micromed-it.com) for details.

The import menu from EEGLab with appropriate converters installed is shown below:



Figure 2: "Import data" functionality in EEGLAB.

2. iEEG channel selection

If your dataset contains a mix of iEEG and scalp EEG or if service channels are present, this is the time to "clean" the dataset from any channel which is not iEEG. In our clinical practice we only connect contacts located in the white matter to be used as reference, after having selected them in the Neuro-inspire planning software from the post-implantation CT co-registered with the patient's MRI. If you connect all the SEEG contacts you can remove the channels you don't wish to analyze.

REMEMBER: the statistical thresholding is a critical part of the automated detection; including non grey-matter contacts might affect the final result (please see the paper to understand how/if).

You can exclude contacts using an EEGLab function available from the GUI (Fig.3)

							EEGLAB	v14.1.2					0 6 0	Q Search Documen	itatio
File	Edit	Tools	Plot	Study	ERPLAB	ERPsets	Datasets	Help	EPINETLAB (HFO)						
											Community				
_										 • • • •	Request Support	lect()			
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											input desired range			1 - IR1-G2	
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										Point range (ex: [1 10])			x10	4 - IR8-G2 5 - IR9-G2	
		Channel:	s per f	rame				113		Epoch range (ex: 3:2:10)		• • <u>·</u>	x1 :	6 - IR10-G2 7 - IB11-G2	
		Framos	oer eno	ch				1955	128	Channel range		.	j	8 - IR12-G2	
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													×1 :	13 - BR11-G2	
		Events						none					1	15 - CR1-G2	
										Help		Cancel Ok	_ /	16 - CR2-G2 17 - CR8-G2	
		Samplin	g rate	(HZ)				2048			will require th		x1	18 - CR9-G2	
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		Dataset	size (MD)				899.	4						-11
														Cancel Ok	
										 	_				21
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Figure 3: "Select data" functionality in EEGLAB.

Channel range option in the [Edit>Select Data] menu allows you to remove multiple channels from your dataset (command+click for non-continuous or shift +click for contiguous channel selection in the Mac environment).

When you are finished, we advise saving the new file with different name (Fig. 4); it is advantageous to do the same for each step of the GUI-led analysis process to be able to go back to specific intermediate steps in case an error occurs.

Nha	t do you want to do w	ith the new dataset?	
	Name it:	HB 2018SEP22 .TRC File	Edit description
~	Save it as file:	/Users/seris/Documents/HB/Analysis_	Browse
Som	e changes have not b	een saved. What do you want to do wit	h the old dataset?
som	e changes have not b Overwrite it in memo	een saved. What do you want to do wit ry (set=yes; unset=create a new dataset)	h the old dataset?
Som 2	e changes have not b Overwrite it in memo Save it as file:	een saved. What do you want to do wit ry (set=yes; unset=create a new dataset) /Users/seris/Documents/HB/Analysis_	h the old dataset? Browse

Figure 4: EEGLAB GUI for saving a new dataset.

In this case 12 channels have been removed (113 to 101), Fig. 5.

• •					EEGLA	B v14.1.2			
File	Edit	Tools	Plot	Study	ERPLAB	ERPsets	Datasets	Help	EPINETLAB (HFC
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	Chai	nnels pe	er fra	me		101			
	Fra	mes per	epoch			19551	28		
	Epo	chs				1			
	Eve	nts				none			
	Sam	pling ra	ate (H	z)		2048			
	Epo	ch star	t (sec)		0.00	0		
	Epo	ch end	(sec)			954.6	52		
	Refe	erence				unkno	wn		
	Chai	nnel loo	cation	s		No (l	abels only	7)	
	ICA	weight:	s			No			
	Data	aset si:	ze (Mb)		805.6			

Figure 5: The EEGLAB main window is updated after channel removal.

It is assumed here that the dataset is acquired against a physical reference (in this example against G2 input of the amplifier).

In line with current clinical practice we advise performing iEEG data analysis in bipolar montage using sequential inputs for each electrode or against white matter contacts.

3. Montage Creation

At this stage you will want to create the "montage" in which the analysis will be performed. A bipolar chain option tends to be the preferred option in the clinical literature on sEEG data visualization and we have consequently offered this option. To do this you will have to prepare the montage labels using some text editing functions available in EpiNetLab.

Labels formatting (Fig.6):

Typically, from the example used here, you would want to remove the last 3 characters from the labels (-REF), to allow the bipolar montage label to contain only one – character. This makes it easier for the program to identify channels when processing. EpiNetLab doesn't deal well with the ' (prime) symbol often used in French practice to denote left-side implanted electrodes, therefore replacing the "prime" symbol with the letter "L" for left is advised.

	101				
SR3-G2 SR4-G2 SR6-G2 SR7-G2 SR8-G2 SR8-G2		Remove last n characters		3	Do
SP9-G2 RF1-G2 RF2-G2 RF3-G2 RF3-G2		Remove first n characters			Do
RR7-G2 RR8-G2 RR9-G2 RR10-G2 KR2-G2		Remove space characters			Do
KP3-G2 KP4-G2 KP8-G2 KP9-G2 JR1-G2		Remove special characters			Do
JR2-G2 JR3-G2 JR6-G2 JR7-G2 JR8-G2		Replace	with		Do
JP9-62 JR13-62 JR14-62 JR15-62 GR1-62 GR2-62		Replace channel labels with			Do
GR5-G2 GR6-G2 GR8-G2 GR9-G2 GR10-G2 GR11-G2		Load labels from external file			
GN11-G2 PMR1-G2 PMR2-G2 PMR5-G2 PMR5-G2		Load .txt			
PMR7-G2 PMR9-G2 PMR10-G2 PMR11-G2 PMR12-G2					Do
	Select all				Clara
					01030

Figure 6: EPINETLAB channel labels formatting GUI.

To do this: [Select all > Remove last n Characters =3 > DO].

Remember that EEGLab asks you to save new file offering you option to give it a different name and/or overwrite in memory the data vs keep both the modified and original. This has memory implications. Please refer to the EEGLab manual for detailed information.

4. BAND-PASS FILTERING

EpiNetLab doesn't require iEEG data to be filtered in the HFO interval of choice. Timefrequency analysis can be performed on the broadband signal, albeit with computational cost. A relatively recent paper has shown that sharp transients and harmonics of non-sinusoidal signals can produce spurious oscillations after filtering and that these could be confounded with actual oscillatory activity. The aa. advise that high-pass filtering of EEG traces for detection of oscillatory activity should be performed with great care (C.G. Bénar, L. Chauvière, F. Bartolomei, F. Wendling. Pitfalls of high-pass filtering for detecting epileptic oscillations: A technical note on "false" ripples. Clinical Neurophysiology 121 (2010) 301–310).

However, the user can perform re-filtering of the data if they are so inclined by using the EEGLab GUI command [Tools>Filter the data] (Fig.7) and choose the most appropriate filter type and interval (e.g. 80 250 or 250 500).



Figure 7: EEGLAB "filtering data" functionality.

5. CREATING BIPOLAR MONTAGE

At this stage you will want to create the montage in which your data will be analyzed. If you chose to work with data referenced to one or more white matter electrodes or any other combination, this can be done from the EEGLab interface [Tools>Re-Reference], see Fig. 8.



Figure 8: EEGLAB "Re-referencing" GUI.

or from EpiNetLab GUI command [Preprocessing Functions>Average References Creation], in which you will have to select contacts on the left panel and define the number of contacts participating the average reference and their labels. This function was developed for users acquiring data from subdural grids . A full description of the process is in Appendix 1 at the end of this manual.

If bipolar montage is what you want, then the EpiNetLab GUI command: [Preprocessing functions>Bipolar Montage Creation] is available to you (Fig. 9).

#1. UP 20198ED22 TD) Eile	Preprocessing Functions	•	Labels Formatting	
#1: HB 20163EP22.1RC	, rile	Seizure Inspection		Bipolar Montage Creation Average References Creation	
Filename:lysis_SZ1/sz	l_chan_sele.set	Wavelet-based Time-Frequency Analysis Kurtosis-based Statistics		Label-based channels Ordering File Cutting	
Channels per frame	101	HFOs Detection		Compute MEG virtual sensors correlation	
Frames per epoch	1955128	HFOs Visualization (in time)			
Epochs	1	HFOs rates plot SOZ Identification			
Events	none	UEOs datastian an usubiala filas			
Sampling rate (Hz)	2048	HFOS detection on multiple files	_		
Epoch start (sec)	0.000	Export HFOs to Micromed Single Pulse Electrical Stimulation (SPES) analysis	•		
Epoch end (sec)	954.652				
Reference	unknown	EPINET LAB Help	•		
Channel locations	No (labels only)				
ICA weights	No				
Dataset size (Mb)	805.6				

Figure 9: EPINETLAB "Bipolar Montage Creation" functionality.

The menu will offer you the list of the G1 and G2 contact to create your bipolar chain (Fig. 10).



Figure 10: EPINETLAB "Bipolar Montage Creation" GUI.

At the end of this process you should save the bipolar montage as text file in an appropriate location and create the new ".set" (EEGLab format) file in bipolar format. The number of channels will have obviously decreased accordingly.

Again, the EEGLab menu will ask you to save the new file and if you want the old one to be overwritten in memory.

6. FILE PARSING/CUTTING

Now you have a bipolar file; to expedite wavelet coefficient computation and avoid running out of memory on your computer you will want to split the file in smaller segments (2' chunks in our validation dataset). The example in Fig.11 uses a 954" long file (= ~ 10 '); we decided to create 5 consecutive files of 2' duration.

E E	EGLAB v14.1.2				Linisei	🕽 🕐 🔍 QSearch D		
File Edit Tools Plot Study ERF	LAB ERPsets Datasets Hel	Preprocessing Fu	nctions >	Labels Formatting				
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Filename:is_S21/sz1_cha	n_sel_bip.set	Wavelet-based Ti Kurtosis-based St	me-Frequency Analysis tatistics	Label-based channels Orde File Cutting	ring			
Channels per frame	68	HFOs Detection	HFOs Detection		rs correlation	Value		
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Epoch end (sec)	954.652	Single Pulse Elect	rical Stimulation (SPES) analysis 🕨		EEG	1x1 struct		
Reference	unknown	EPINETLAB Help	•		ERP	[]		
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New to MATLAB? See resources for Ge	tting Started.							
<pre>pop_loadset(): loading file Reading float file '/Users/S eeg_checkset warning: 3rd di eeg_checkset warning: number eeg_checkset note: upper tim Done</pre>	/Users/seris/Documents/H eris/Documents/HB/Analys mension size of data (1) of columns in data (195 he limit (xmax) adjusted	HB/Analysis_SZ1/s sis_SZ1/sz1_chan_ does not match 55128) does not m so (xmax-xmin)*s	Output Files					

Figure 11: EPINETLAB "File Cutting" functionality.

The menu offers the option of file duration; the seizure file will be divided in N files of the chosen length, in this case 120", see Fig.12.

	FileCutting2
File Cutting	
Load .set	/Users/seris/Documents/HB/Analysis_SZ1/sz1_chan_sel_bip.set
Epoch [s]	120
Epoon (5)	
Output Format	
o.set	
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Output Files	
/Users/seris/Doc	uments/HB/Analysis_SZ1/sz1_chan_sel_bip_Split1.set
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Figure 12: EPINETLAB "File Cutting" GUI.

I

HFO DETECTION

This stage can be approached by analyzing single or multiple files at the same time. The latter is the most obvious choice; files can be selected from the GUI and

parameters for the estimation must be defined in this menu (Fig. 13).

For the full description and scientific bases for each menu choice, please refer to the paper in *Frontiers in Neuroinformatics* mentioned at the beginning of this manual.

Ripples (80-250 Hz) and Fast Ripples (250-500 Hz) must be analyzed separately but the frequency interval can be also set arbitrarily by the user. Other items to choose are:

- Spectral Estimation method: Staba vs Wavelet and if wavelet is chosen as option, which wavelet will be used.
- Detection parameters: Staba method has been implemented here only due to its wider recognition at the time of project inception. More methods have since been published and are not implemented in our software.

If wavelet method is chosen, the user can fine-tune the criteria used by the algorithm to discard as Artefacts events with spectral profile in the chosen HFO window if they meet criteria such as:

- 1) Present in more than N channels at the same time: this behavior is typical of external artefacts, e.g. muscular artefacts.
- 2) Power of the event spreading in all the frequency window: this behavior is typical of spikes.

Also the minimum duration of the candidate events can be decided, e.g. 20ms for ripples and 10ms for fast ripples. The default parameters have been used for the validation study, which was focused on the 80-250 Hz interval, and are set as default.



Figure 13: Multiple file selection.

Selecting the Process command, the computation starts and its duration is dependent among others on number of electrodes, sampling rate, number and size of files, frequency interval.

To give a very approximate idea, processing one unfiltered 2' file sampled at 2 KHz with 68 bipolar channels on a Macbook Pro 3.5 GHz Intel Core i7, 16Gb RAM laptop has taken 27 minutes.

7. DISPLAY ANALYSIS RESULTS WITH HFO RATES

After having computed HFO rates for each channel and each epoch file, you will want to produce a figure of HFO rates for each iEEG electrode and display the electrodes as color bars.

- If your dataset contains only stereo-EEG electrode data, select [EPINETLAB(HFO)>HFO rates plot->Plot HFO rates on sEEG channels]. The toolbox will ask you to consecutively load the following objects in this precise sequence:
 - All the files containing HFOs for each channel (text files contained in the WaveletHFO folder) (Fig 14).

Select HFO detection	n results files				
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Organize • Nev	v folder				# • 1
👉 Exocites	Name	Date modified	Туре	Size	
	EEG 34 1024Hz clean bipolar cl 80-250Hz Solit1 WaveletHFO.txt	10/07/2017 15:24	Text Document	14 KB	
词 Libraries	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Split2_WaveletHFO.txt	10/07/2017 15:34	Text Document	13 KB	
Documents	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Split3_WaveletHFO.txt	10/07/2017 15:45	Text Document	12 KB	
Music	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Splin4_WaveletHFO.txt	10/07/2017 15:58	Text Document	12 KB	
Pictures	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Split5_WaveletHFO.txt	10/07/2017 16:11	Text Document	14 KB	
Videos	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Split5_WaveletHFO.tst	10/07/2017 16:24	Text Document	13 KB	
	EEG_34_1024Hz_clean_bipolar_cl_80-250Hz_Split7_WaveletHFO.txt	10/07/2017 16:31	Text Document	7 KB	
P Computer	PercRes.txt	10/07/2017 16:53	Text Document	2 KB	
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Figure 14: Selection of the files containing detected HFOs in multiple epochs.

- The bipolar montage.
- The unipolar montage: this is a text file you can create yourself using any text editor that contains for each row the letter corresponding to each electrode present in the dataset followed by CR/LF.
- If you have SEEG channels and grids (or just grids), select [EPINETLAB(HFO)
 > HFO rates plot- > Plot HFO rates on sEEG channels AND grids].

The program will ask you to consecutively load the following objects:

• All the files containing HFOs for each channel or grid contacts (text files contained in the WaveletHFO folder). You need to select them all from the folder. Please note that the program will automatically recognize

grids if the labels start with Gd or Gr (if you need more labels to be recognized we can just add new ones).

- The bipolar montage. In case of grids, referencing each contact of the grid to the *Average Reference* can be performed within EEGLAB.
- The unipolar montage.
- Two figures constitute the output of these procedures: one containing the color bars with the HFO rates on each contact (Fig.15) and one containing the distribution of HFO rates in each of the epochs chosen for analysis (Fig. 16).



Figure 15: HFO distribution on each contact: black means no HFOs detected, white means maximum HFO rate.



Figure 16: Distribution of HFO rates in each analysed epoch.

We advise saving the two figures both as bitmap/tiff/jpeg and in the MATLAB format (.fig) if you wish to visualize the figures outside the MATLAB environment. Also, after you create the figures, a new file **PercRes.txt will be created** in the "WaveletHFO" folder. The file contains the list of the percentages of HFO rates corresponding to each channel.

APPENDIX 1

AVERAGE REFERENCE IN EPINETLAB

When you have GRIDS besides stereo-EEG electrodes, the best approach for data pre-processing is to refer each contact of the grid to the average reference (AR) of the grid itself, while using bipolar montage just for SEEG.

A new functionality was added to EPINETLAB, under the "Preprocessing Functions" tab, which is called "Average Reference Creation" and allows you to add to the signal a new channel which contains the average reference of the grid. This channel is added at the end of the channel list. If you have many grids, you can add many average references to the file, the important thing is to call them with different names, e.g. AR1, AR2, AR3, REF1, REF2, etc.

Below you find an example of how to use this new functionality (patient O.S., file EEG_407176).

First thing to do with Micromed files is to format labels in order to remove the "–G2" string from each channel label.



You select the channels after having removed scalp EEG and EMG/EKG/service channels) and then choose 3 as number of characters to delete from the end of the channel labels. Then press "Do" and the last three characters will be removed from each label.

Labels formatting	60		×	\mathbf{X}	
Gd02-G2	80				
Gd03-G2 Gd04-G2 Gd05-G2		Remove last n characters		-3	Do
Gd06-G2 Gd07-G2 Gd08-G2		Remove first n characters			Do
Gd00-G2 Gd09-G2 Gd10-G2	=	Remove space characters			Do
Gd11-G2 Gd12-G2 Gd13-G2		Remove special characters			Do
Gd14-G2 Gd15-G2 Gd16-G2	1	Replace	with		Do
Gd17-G2 Gd18-G2 Gd19-G2		Dealers showed labels with			
Gd20-G2 Gd21-G2 Gd22-G2		Replace channel labels with			Do
Gd23-G2 Gd24-G2 Cd25-G2		Load labels from external			
Gd26-G2 Gd27-G2 Gd27-G2		Load .txt			
Gd28-G2 Gd29-G2 Gd30-G2					Do
G031-G2	Select all				
					Close
elsFormatting					
elsFormatting				_	
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abels formatting	68			-	
elsFormatting	68	Remove last n characters		3	()
absformatting abels formatting Gd01 Gd02 Gd04 Gd05 Gd05 Gd05 Gd06 Gd05 Gd06 Gd05 Gd06 Gd0	68	Remove last n characters Remove first n characters		3	
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AsFormatting	68	Remove last n characters Remove first n characters Remove space characters Remove special characters Replace Replace	with	3	Do Do Do Do Do Do Do
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About the second	68	Remove last n characters Remove first n characters Remove space characters Remove special characters Replace Replace channel labels with	with		Do Do Do Do Do Do
Ediformatting Ca01 Ca02 Ca01 Ca02 Ca04 Ca04 Ca04 Ca04 Ca04 Ca04 Ca04 Ca04	68	Remove last n characters Remove first n characters Remove space characters Remove special characters Replace Replace Replace channel labels with	with	3	Do Do Do Do Do Do
abels formatting abels formatting Gall	68	Remove last n characters Remove first n characters Remove space characters Remove special characters Replace Replace Replace channel labels with Load labels from external	with	3	Do Do

Then we can create the AR channel. We select the "Average References Creation" functionality.



In the GUI that appears, in the channels list we select the contacts on which to perform the AR. In this case from Gd1 to Gd32. Then we press Create and the AR channel is created.

Average References (A	Rs) creation	
Channels	Average reference	
68	Nr. of channels/contacts	
00		
Gd25 🔺		Create
Gd26		
Gd27	Label	N
Gd28	(e.g.: AR1, Ref1)	\ \
Gd29		Add to file
Gd30		
Gd31	Output	
Gd32 ≡		*
SGd1		
SGd2		
SGd3		
SGd4		
SGd5		
SGd6		
SGd7 💦		
SGd8 T		
Select All		*
op_AverageReferences		
Average References (A	ARs) creation	
Average References (A	ARs) creation	
Average References (A	ARs) creation Average reference Nr. of channels/contacts	32
Channels 68	ARs) creation Average reference Nr. of channels/contacts	32
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Average References (A Channels 68 Gd25 Gd26 Gd27 Gd27 Gd28	ARs) creation Average reference Nr. of channels/contacts Label (e.o.: AR1. Reff)	32 Create
Average References (A Channels Gd25 Gd26 Gd27 Gd28 Gd29	Area creation Average reference Nr. of channels/contacts Label (e.g.: Ar1, Ref1)	32 Create
Average References (A Channels 68 Gd25 Gd26 Gd27 Gd28 Gd29 Gd29 Gd29	ARs) creation - Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1)	32 Create Add to file
Average References (# Channels 68 6425 6426 6427 6428 6429 6430 6429 6430	Area creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1)	32 Create Add to file
Average References (A Channels 68 6225 6326 6327 6328 6329 6330 6331 6331 6331	Area creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create Add to file
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Average References (/ Channels 68 Gd25 68 Gd26 Gd26 Gd27 Gd28 Gd30 Gd31 6 Gd31 6 Gd32 5 Gd4 5 SGd4 5 SGd4 5 SGd5	Area creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create Add to file
Werage References (/ Channels 68 6d25 642 6d26 6 6d27 6 6d28 6 6d29 6 6d30 6 6d31 6 5G31 5 5G34 5 5G34 5 5G35 5 5G36 5 5G36 5 5G36 5	ARs) creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create
Werage References (/ Channels 68 Gd25 6 Gd26 6 Gd27 6 Gd28 6 Gd29 G Gd30 6 Gd31 6 Gd32 5 SGd1 5 SGd2 5 SGd3 5 SGd4 5 SGd5 5 SGd6 5 SGd7 5	Area creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create Add to file
Average References (A Channels 68 Gd25 Gd26 Gd29 Gd29 Gd30 Gd31 Gd31 Gd31 SGd1 SGd2 SGd3 SGd4 SGd4 SGd4 SGd5 SGd6 SGd7 SGd8	Area creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create
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werage References (/ Channels 68 Gd25 Gd26 Gd27 Gd28 Gd29 Gd30 Gd31 Gd32 SGd1 SGd2 SGd3 SGd4 SGd5 SGd6 SGd7 SGd7 SGd8 SGd7	Area contraction Average reference Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output	32 Create Add to file
Average References (/ Channels 68 6425 6426 6427 6428 6430 6431 6432 6432 6432 6433 6431 5644 5644 5644 5644 5644 5644 5644 564	Ars) creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output Save file Save file	32 Create Add to file
Average References (A Channels 68 Gd25 Gd26 Gd27 Gd29 Gd29 Gd29 Gd30 Gd31 Gd31 Gd31 SGd1 SGd2 SGd4 SGd4 SGd5 SGd6 SGd7 SGd7 SGd7 SGd8	ARs) creation Average reference Nr. of channels/contacts Label (e.g.: AR1, Ref1) Output Save file	32 Create Add to file

Then we insert a label for the new channel, for example AR and add it to the file. You will see that in the list of channels on the left, AR will appear at the end of the list and the number of channels will change from 68 to 69.

pop_AverageReferences		
Average References (AR	s) creation	
Channels	Average reference	
69	Nr. of channels/contacts	32
IF6 A		Create
IF8	l abel	
OF1	(e.g.: AR1, Ref1)	AR 🔨
OF2		Add to file
OF4	Output	
AST1	AR was created from char	nnels/contacts G(_
AST2		
AST4		
PST1		
PST2		
PST4		
AR -		_
Select All	4 III	•
	Save file	Close

In the Output window, a summary of the operations is reported: "AR was created from channels Gd01, Gd02, ..., Gd32".

Last thing to do is to save the new file.

	69	Nr. of channels/contacts	22			
F6		N. or chambla contacta	32			
F7 F8			Cleate			
DF1		(e.g.: AR1, Ref1)	AR			
DF2			Add to file			
DF3 DF4		0.15.1				
AST1		AR was created from channels/contacts G(
AST2 AST3						
AST4						
PST1						
PST3	E					
PST4						
	-		-			
S	elect All	•	•			

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The new file will be automatically added to the EEGLAB interface.



Now the we have the AR relative to the grid, when we create the bipolar montage, each contact of the grid can be referred to the same AR channel, while for the contacts of the SEEG electrodes we still use the classical subtraction between adjacent contacts.





In the end you just need to save the montage in the TXT file and then create the .set file containing the AR/bipolar contacts.