EEG Re-referencing： Common Average Referencing Should Not Be Your Default？

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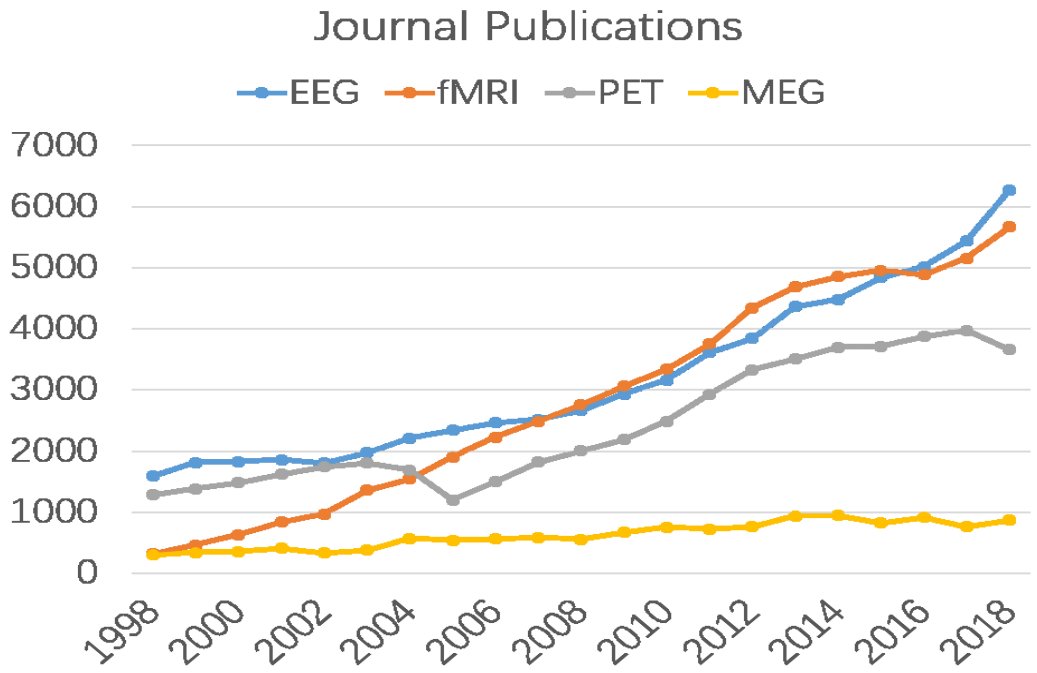
- **Background**

Publication of EEG is No.1

among the main non-invasive tools(Web of Science)



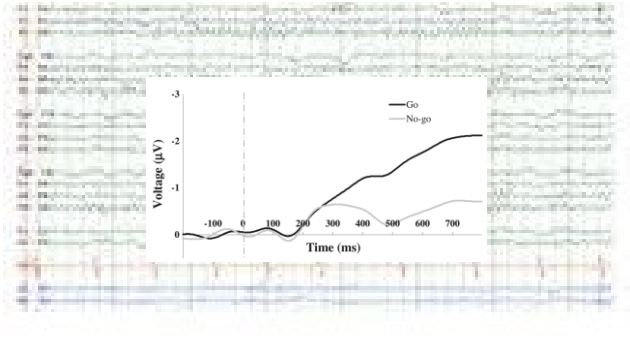
Only 2009-2016, EEG was overwhelmed by fMRI (fMRI has gone down)

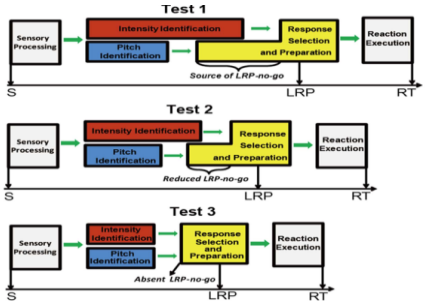
The EEG will have a renaissance!

Procedure of EEG study

Data collection

Data analysis





Inferring the brain

"*the reference*

is an absolutely fundamental aspect of EEG/ERP recordings. If you don't fully understand referencing, you won't understand the signal that you are recording"(Luck 2014)

History of EEG Reference

Bipolar and Unipolar references

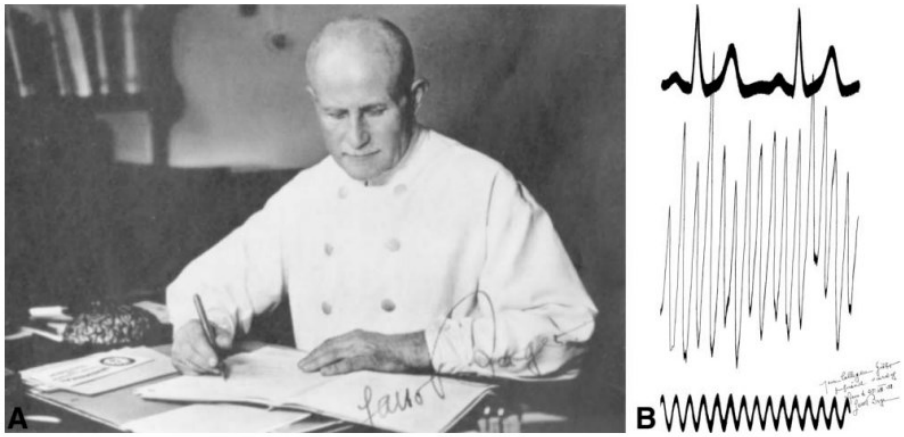


FIG. 1. **A**, Hans Berger. **B**, EEG tracing presented "To my colleagues the Gibbses with warm regards, Hans Berger, 8/25/1938." Simultaneous EKG tracing at top, EEG middle, and 0.10-second marker at bottom. (Courtesy of Frederic A. Gibbs to John R. Hughes.)

Human EEG found in 1924
and formally reported in 1929

1929-1938, Berger published 14 primary papers

He examined bipolar vs. unipolar referential recordings

--- reference effect was known at the beginning

Linked ears, 1935

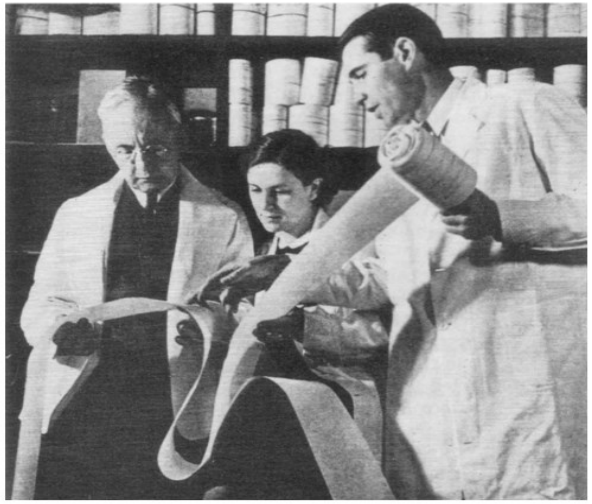


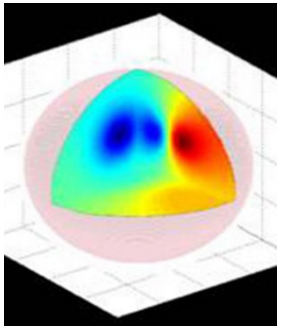
FIG. 6. William Lennox, Erna Gibbs, and Frederic Gibbs examining EEGs on paper rolls in 1937.

In 1930s, the Gibbs and Lennox team studied the EEG patterns of seizures

- They failed to localize the origin of the psychomotor seizure because the reference electrode **linked to the ears** distorted temporal activity (Gibbs etal., **1935**; Feindel et al., 2009).
- Using this ear reference, most of their ‘‘localized dysrhythmias’’ were found in the frontal and occipital regions.

Average reference, 1950

- **For dipoles in a sphere, the integral of potential (v) over the surface is**

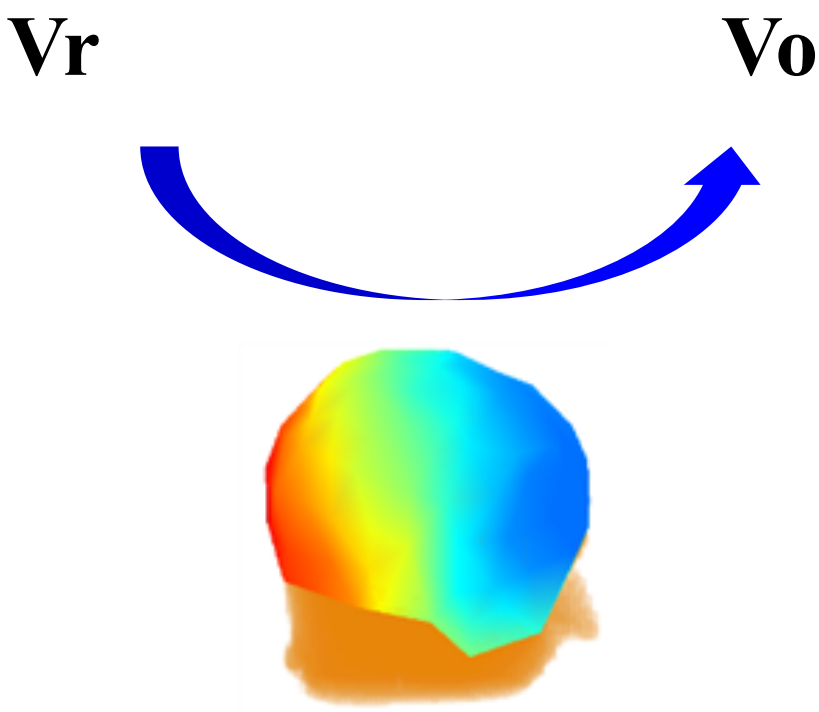


$$S = \int_0^{2\pi} \int_0^{\pi} v(r, \theta, \phi) r^2 \sin\theta \, d\theta \, d\phi$$

=0

- Goldman D. The clinical use of the "average" reference electrode in monopolar recording. Electroenceph Clin Neurophysiol. **1950**; 2: 211-214.
- Offner FF. The EEG as potential mapping: the value of the average monopolar reference. Electroenceph Clin Neurophysiol. **1950**; 2: 215-216

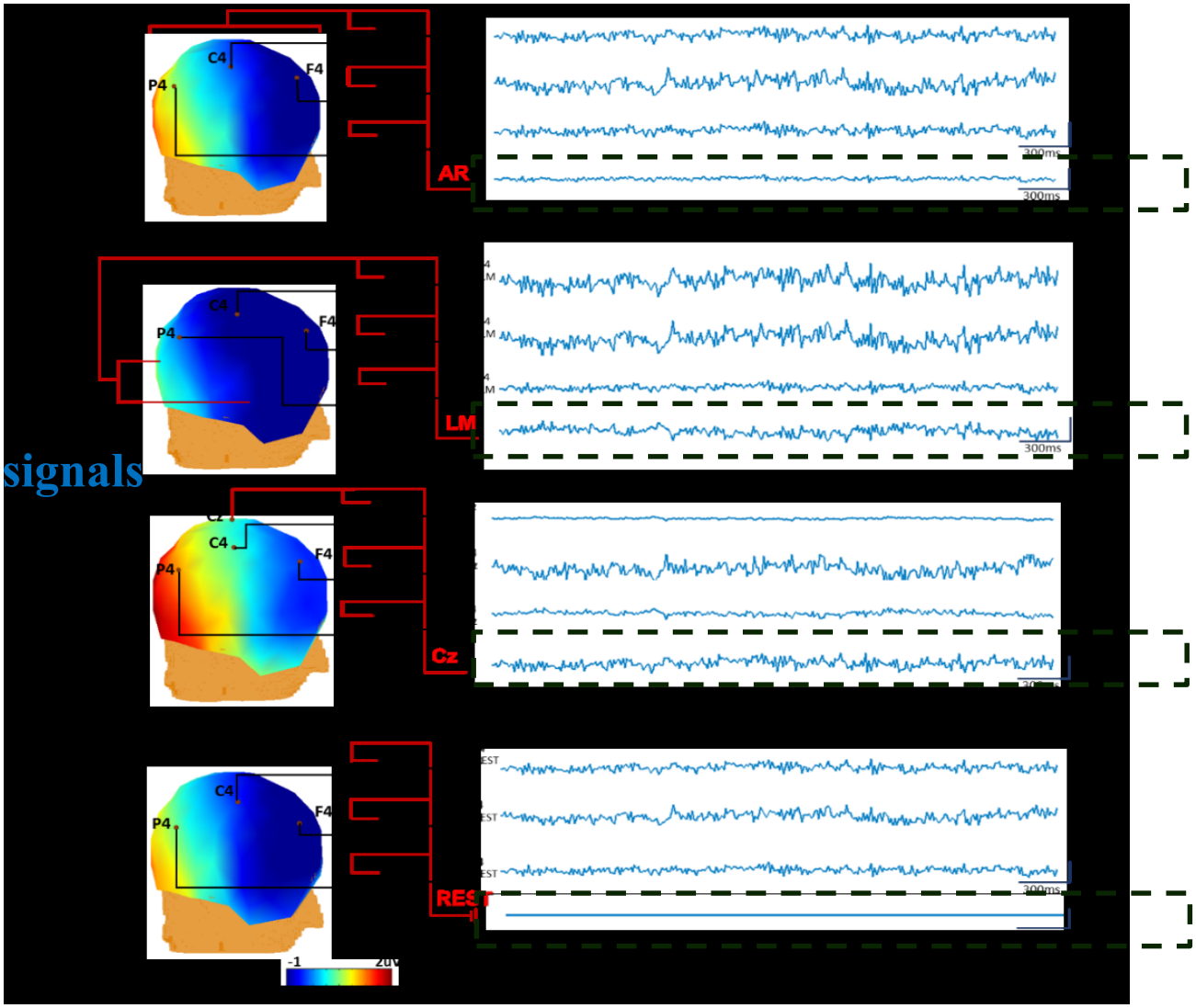
Zero reference by REST, Yao, 2001



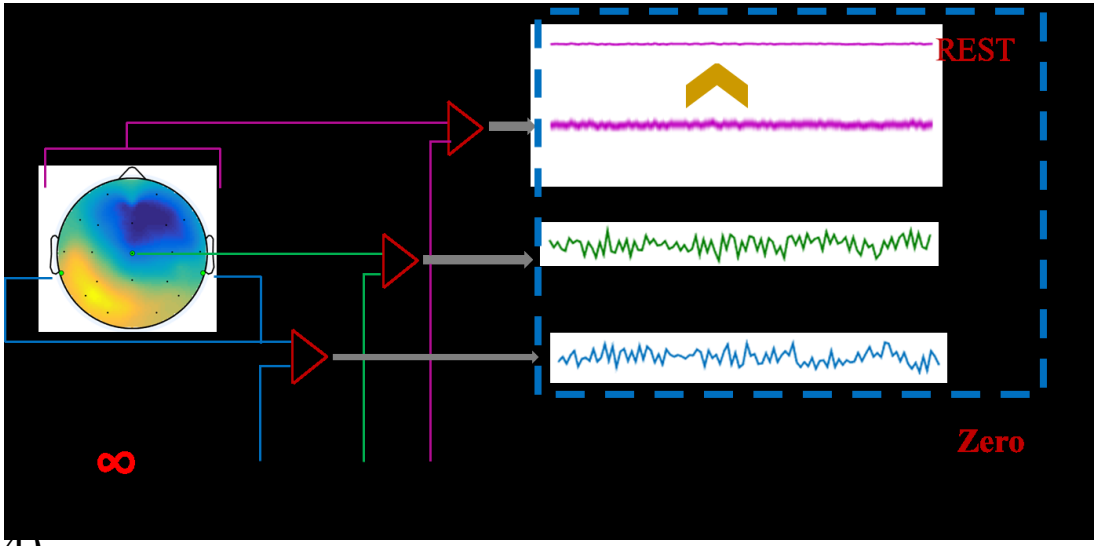
Yao D (2001) A method to standardize a reference of scalp EEG recordings to a point at infinity. Physiol Meas 22:693–711

Waveforms and patterns are different for different references

- Different referencing signals
- Different recordings



Lost signal in unipolar reference recordings



Luck (2014)

"The first things I look for in a Methods section are the filter settings and the location of the reference electrode. Until you know how the data have been filtered and referenced, you can't know how to interpret the ERP waveforms. "

"ERP waveform as the active site, so it's hard to evaluate an ERP waveform if you don't know which sites were used to create the reference portion of the signal"

Electroencephalography and clinical Neurophysiology, 91 (1994) 1–5

EEG 00006

IFCN guidelines for topographic and frequency analysis of EEGs and EPs. Report of an IFCN committee

Marc R. Nuwer (Chairman) (*Los Angeles, CA, USA*), Dietrich Lehmann (*Zurich, Switzerland*), Fernando Lopes da Silva (*Amsterdam, The Netherlands*), Shigeaki Matsuoka (*Kitakyushu, Japan*), William Sutherling (*Los Angeles, CA, USA*) and Jean-François Vibert (*Paris, France*)

- **" … EEG data should be examined in several different montages,….**
- **References… should include the possibilities of linked-ears, linked-mandible, nose, chin, neck or other non-cephalic single site references.**
- **The choice of which references to use should be made after an initial inspection of the data on a bipolar polygraph display, identifying 'active' areas and choosing references that are relatively inactive"**

No "standard", no " recommendation"

Psychophysiology, *37* (2000), 127–152. Cambridge University Press. Printed in the USA.

COMMITTEE REPORT

Guidelines for using human event-related potentials to study cognition: Recording standards and publication criteria

T.W. PICTON,[a] S. BENTIN,[b] P. BERG,[c] E. DONCHIN,[d] S.A. HILLYARD,[e] R. JOHNSON, JR.,[f] G.A. MILLER,[g] W. RITTER,[h] D.S. RUCHKIN,[i] M.D. RUGG,[j] AND M.J. TAYLOR[k]

- **“ The experimenter must specify the reference. A variety of references can be used depending on the type of ERP …”**
- **“ When comparing waveforms.. to literature, it is essential to consider differences in the reference”**
- “…Recordings can be made using either referential montages or bipolar montages… However, they (bipolar) are often very difficult to interpret in ERP studies”
- **“It is often helpful when comparing waveforms with those in the literature that use another reference to plot the waveforms using both references”**

No “standard”, no “ recommendation”

Reference problem in a sense of Algebra approach

Reference problem in sense of algebra

- **Suppose there are n electrodes, we actually will have n-1 channel recordings $y_{i(i=1,\ldots,n-1)}$, they are potential differences between active and reference electrodes**

$$x_1 - x_n = y_1$$

$$x_2 - x_n = y_2$$

$$\ldots\ldots$$

$$x_{n-1} - x_n = y_{n-1}$$

- **what we need are potentials at $x_{i(i=1,\ldots,n)}$, the n recordings with zero reference**

- The problem: we have only n-1 equations, but our unknowns are n
- It is a un-determined problem
- Needs a supplementary equation

Reference problem in sense of algebra

- **Special case 1: if we know that there is a electrode, where the potential is zero ($x_n = 0$), then we actually have n equations**

$$x_1 - x_n = y_1$$
$$x_2 - x_n = y_2$$
$$\cdots\cdots$$
$$x_{n-1} - x_n = y_{n-1}$$

$$x_n = 0$$

- **then the equation set is solvable**

$$x_i = y_i, \qquad i = 1, \ldots, n-1$$

- **The practical on-line unipolar reference assumption:**

Nose/Mastoid/Chin/Cz? Where $x_n = 0$

Reference problem in sense of algebra

- **Special case 2: if we know that the sum of the all channels is zero, then we actually have n equations**

$$x_1 - x_n = y_1$$
$$x_2 - x_n = y_2$$
$$\cdots\cdots$$
$$x_{n-1} - x_n = y_{n-1}$$

$$\sum_{i=1}^{n} x_i = 0$$

- **then the equation set is solvable, we can find $x_{i(i=,1\ldots,n)}$**

- **the practical average reference assumption:**
- **Average of the all channels over the scalp surface is zero**

Reference problem in sense of algebra

- **Special case 3: if we know that the sum of the two ears is zero , then we also have n equations**

$$x_1 - x_n = y_1$$
$$x_2 - x_n = y_2$$
$$\cdots\cdots$$
$$x_{n-1} - x_n = y_{n-1}$$

$$x_{i(left)} + x_{j(right)} = 0$$

- **then the equation set is solvable, we can find $x_{i(i=,1\ldots,n)}$**

- **The linked-ears or mastoids assumption:**
- **Average of the two ears' potential is zero**

Reference problem in sense of algebra

- **Special case 4: based on the fact that the potential difference recordings $y_{i(i=1,\ldots,n-1)}$ are produced by sources S inside the brain**

$$x_1 - x_n = y_1$$
$$x_2 - x_n = y_2$$
$$\ldots\ldots$$
$$x_{n-1} - x_n = y_{n-1}$$

$$y = G_r S$$
$$S = G_r^+ y$$
$$x_n = g_n S = g_n G_r^+ y = Ty$$

- **After we get x_n , we get the other $x_i(i = 1, \ldots, n-1)$ from the known $y_{i(i=1,\ldots,n-1)}$**
- **The REST zero reference approach:**
- **the supplementary equation is based on the fact, the potential is generated by sources S**

Reference problem in sense of algebra

Mini-summary

- In general, the scalp reference problem is un-determined problem

  it does need a supplementary equation

- The supplementary equation for each of Cases 1-3 （online unipolar reference, AR, LM）is assumption

- The challenge: these assumptions are un-reasonable in general.

  Who know where the potential is zero?

  Why should the average over the scalp be zero?

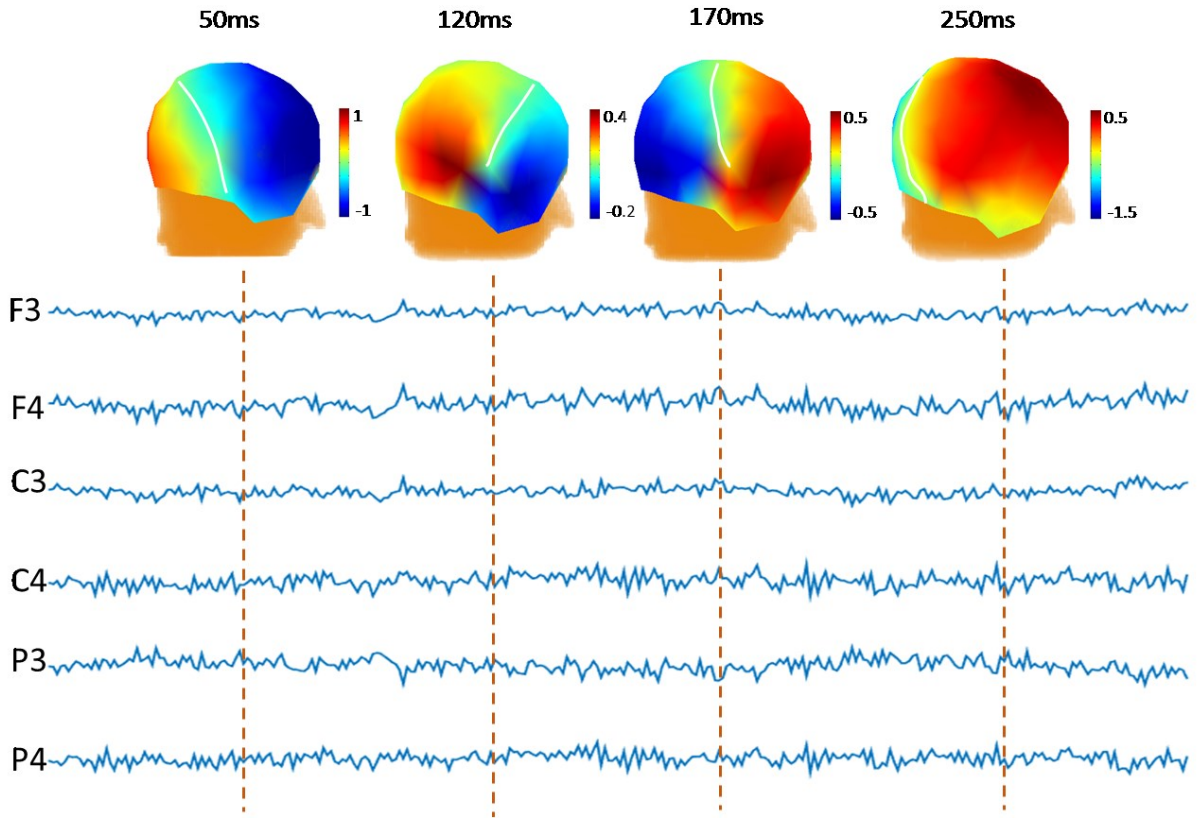
  Why should the average of the two ears be zero?

- REST: the supplementary equation is based on the fact that scalp potential is produced by sources inside the brain, its reasonable

Reference problem in a sense of Physical approach

Physical reason

- **Dynamic brain activities in the volume conductor**



- **No point where potential is constant**

Yao, Pedro etal. BT, 2019

Physics behind AR

Theory/assumption: the surface potential integral = 0?

$$\sum Vi = 0$$

surface potential integral of a spherical head = 0

Bertrand et al 1995

$$\sum Vi \neq 0$$

surface potential integral of a hemi-spherical head model is not zero

Yao, Brain Topor，2017

***RE* of AR:**

① **electrode density (Number)**

② **coverage**

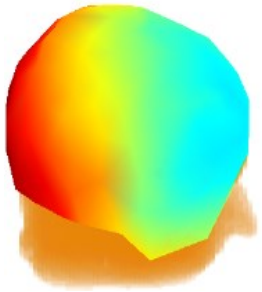
③ **head shape**

Physics behind AR

Luck (2014) "If the study **uses the average** of all sites as the reference, you should check to see whether the electrodes are **broadly and evenly** distributed over the scalp. If not, then the average reference may be inappropriate. And if the manuscript uses the average reference and doesn't **provide a list of all** the electrode sites, then that's a problem. "

"If you wish to use the average reference, you should use a **large number** of evenly spaced electrodes that **cover more than 50%** of the surface of the head, and you absolutely **must report all electrodes** that contributed to the average reference in the methods sections of your papers"

REST (reference electrode standardization technique) (Yao, 2001)



- With theoretical zero reference

  X=GS (1)

- With an actual reference r

  Y=G_rS (2)

- From eq.(2), we have

  S= $G_r{}^{+}Y$ (3)

- Combining eqs.(1) and (3), we have

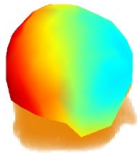
  X=GS= $GG_r{}^{+}Y$ =TY

  X=TY (4)

- source S is adopted as a bridge to link Y and X, actually we do not need to know S, but to know G and G_r, which are determined by the positions of sources and the electrodes
- The inverse problem of source is very difficult ?

REST (reference electrode standardization technique) (Yao, 2001)

- **Non-uniqueness of EEG inverse (Helmholtz,1853)**



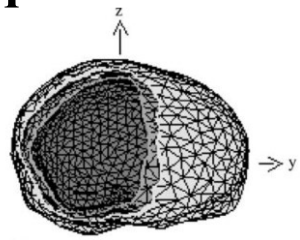
$$Y = G(1)S(1)$$
$$= G(2)S(2)$$
$$\ldots.$$

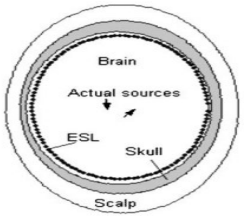
- **Principle of equivalent sources**

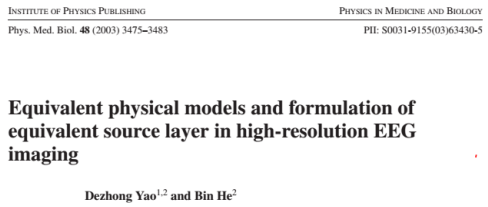
  S(1) and S(2) are equivalent sources of each other in producing the same scalp potential

- **Dipole layer on the cortical surface**

  may be the equivalent sources of arbitrary sources in brain (Yao et al 2013)






INSTITUTE OF PHYSICS PUBLISHING — PHYSICS IN MEDICINE AND BIOLOGY

Phys. Med. Biol. **48** (2003) 3475–3483 — PII: S0031-9155(03)63430-5

Equivalent physical models and formulation of equivalent source layer in high-resolution EEG imaging

Dezhong Yao[1,2] and Bin He[2]

- **Based on equivalent dipole layer on the cortical surface**

  G, G_r^+, *and* T=GG_r^+ are known

  X=TY (4) is realizable

Reference problem in sense of Physics

Mini-summary

- The assumption of AR is un-reasonable

  spherical head， whole surface recordings， dense recordings

- The assumption of REST is reasonable

  The potential is generated by sources inside the brain– physical fact

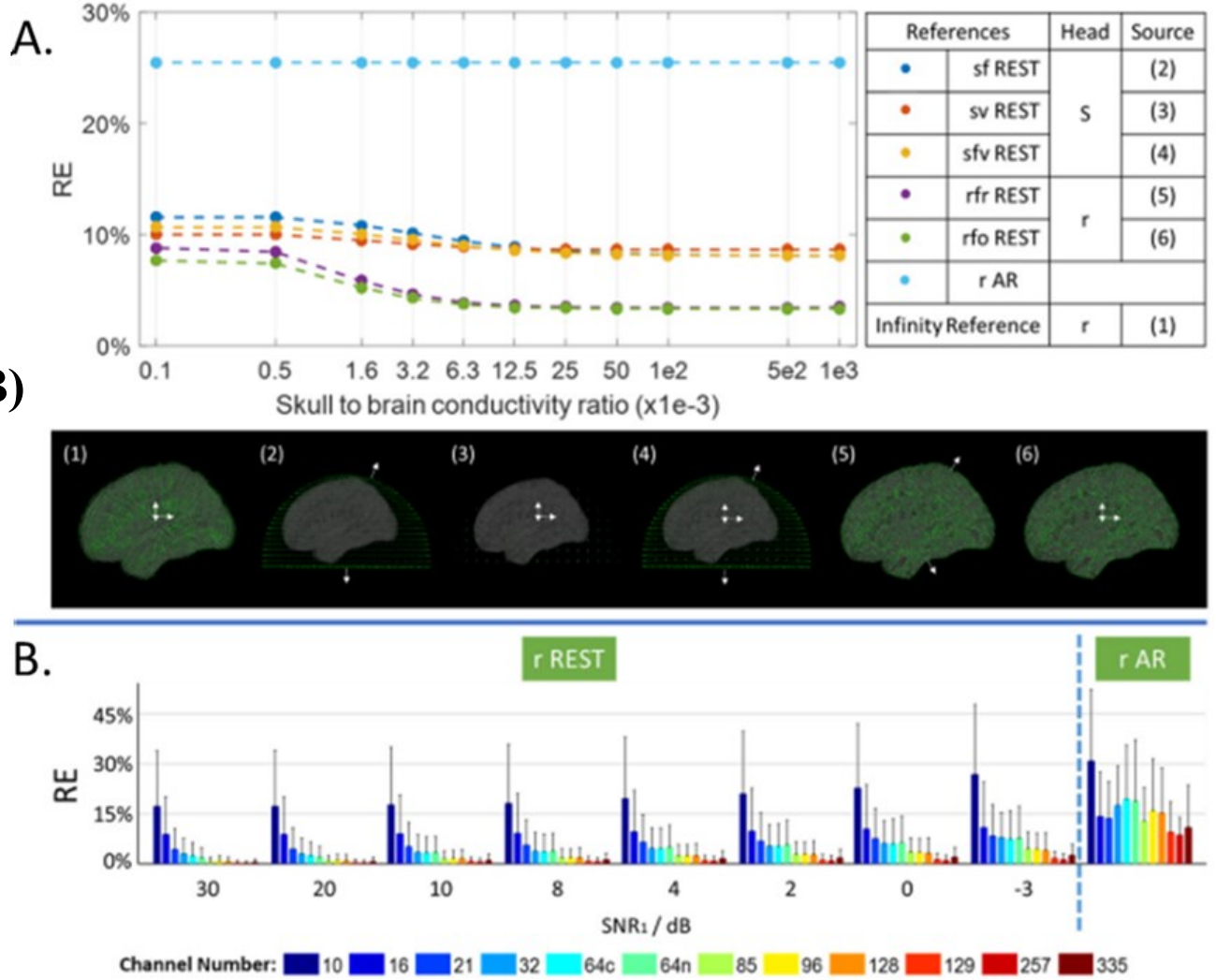
  The sources may be equivalent sources --- solvable

- REST is more physically reasonable

Simulation Evaluation

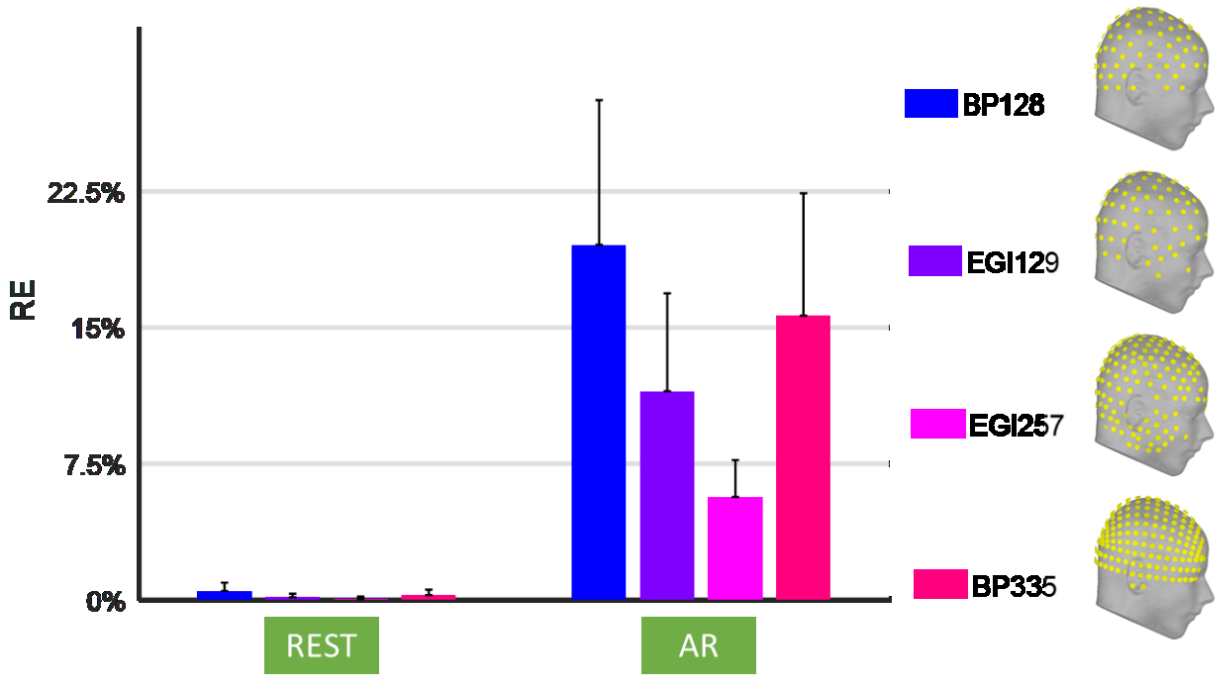
Sensitivity to errors in the head model

REST is not sensitive to Head model (A) and perturbed head model (B)



Hu Yao Pedro etal. JNE，2017

Sensitivity to Coverage of Electrode Cap

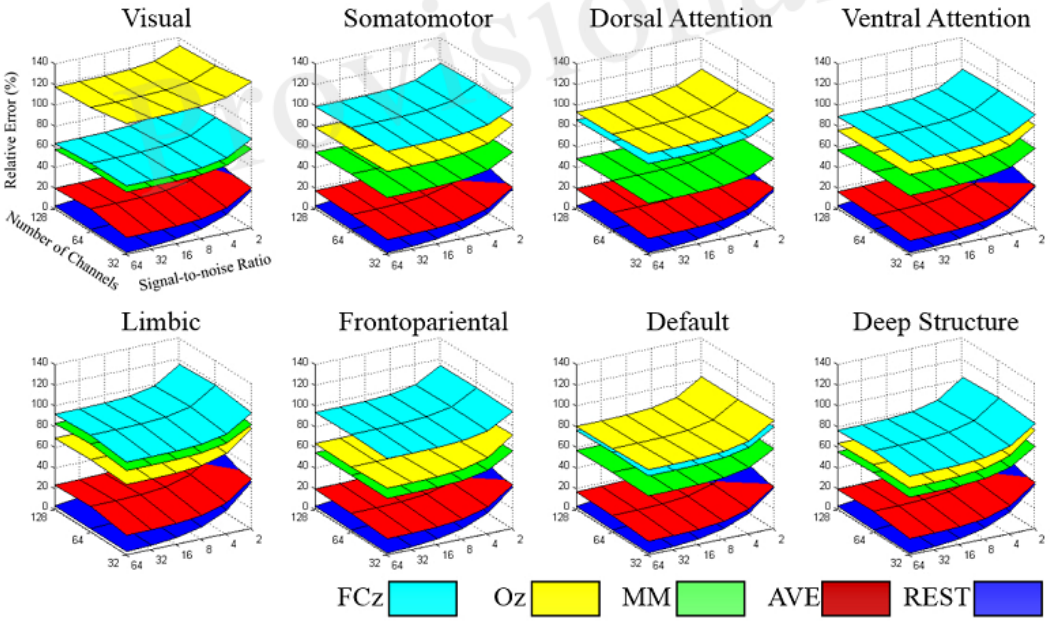


- Wider coverage is better

Hu Yao Pedro et al. JNE, 2017

Sensitivity to neural source positions

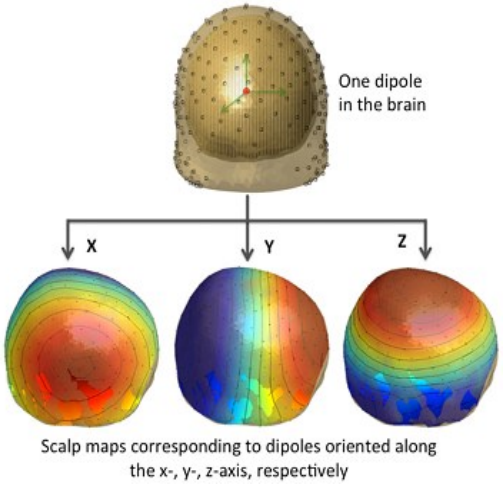
--- Typical brain networks



"Relative error： REST<AVE<MM<(FCz, Oz), regardless of the number of electrodes and SNR"

Lei et al. Front Neurosci 2017

Sensitivity to scalp surface area



Scalp maps corresponding to dipoles oriented along the x-, y-, z-axis, respectively

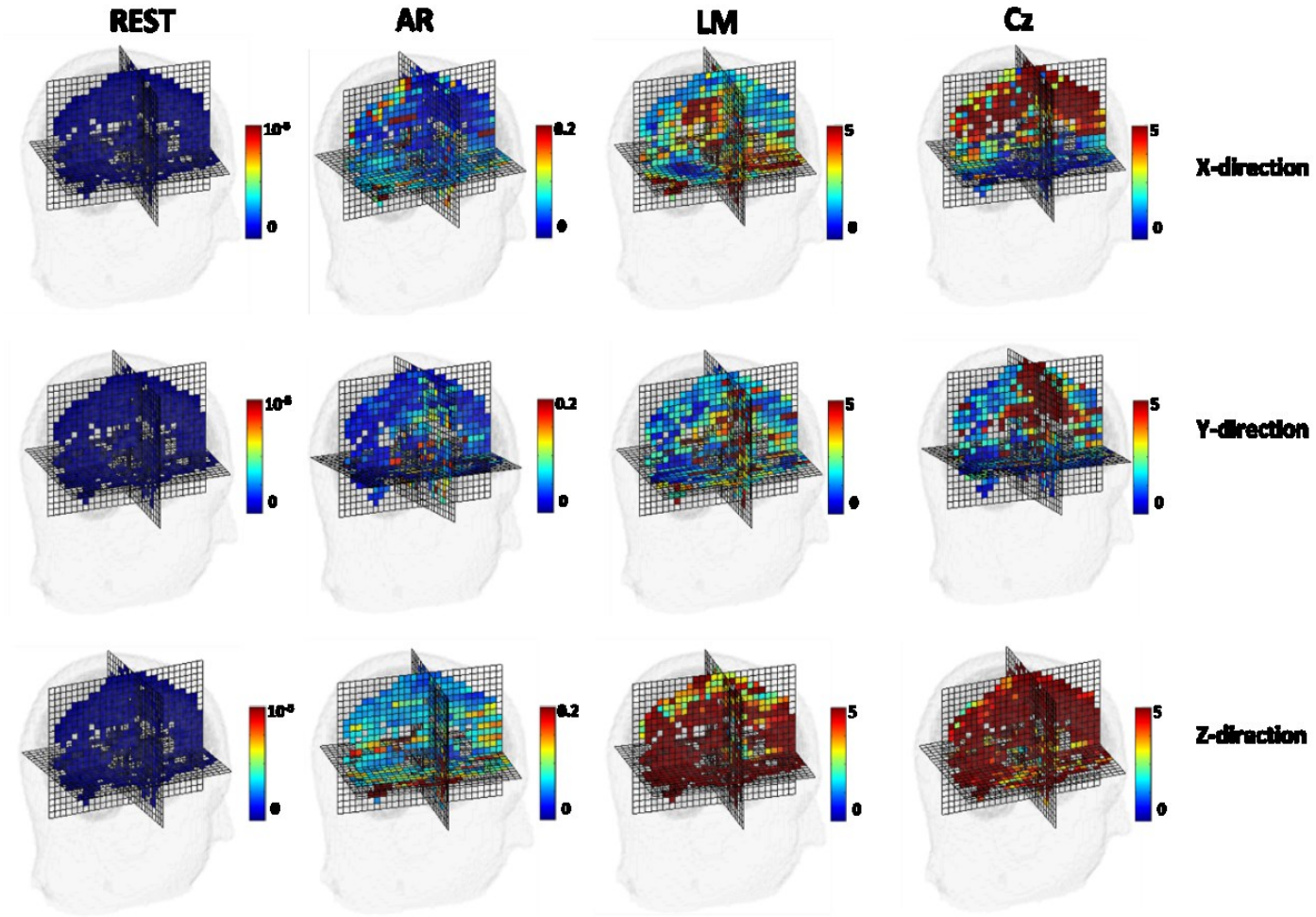
		X	*Y*	*Z*	*XYZ*
Frontal	AR	2.32% (0.75%)	7.14% (0.63%)	−5.53% (1.75%)	1.31% (0.73%)
	REST	0.48% (0.21%)	0.10% (0.08%)	−0.32% (0.21%)	0.09% (0.08%)
Central and parietal	AR	1.98% (0.56%)	−2.15% (1.26%)	−2.09% (0.71%)	−0.75% (0.53%)
	REST	0.23% (0.18%)	0.033% (0.12%)	−0.16% (0.10%)	0.03% (0.10%)
Occipital	AR	−2.92% (4.20%)	−5.50% (0.41%)	−5.13% (1.92%)	−4.52% (1.54%)
	REST	−0.18% (0.45%)	0.01% (0.07%)	0.49% (0.49%)	0.11% (0.22%)

Realistic head model, 256 channels

- **Relative error of REST is much smaller than Average reference (AR)**
- **"the most commonly used EEG re-referencing techniques, such as REST, AR and LMR,…"**

Liu, Mantini etal JNE ,2015

Sensitivity to neural source position



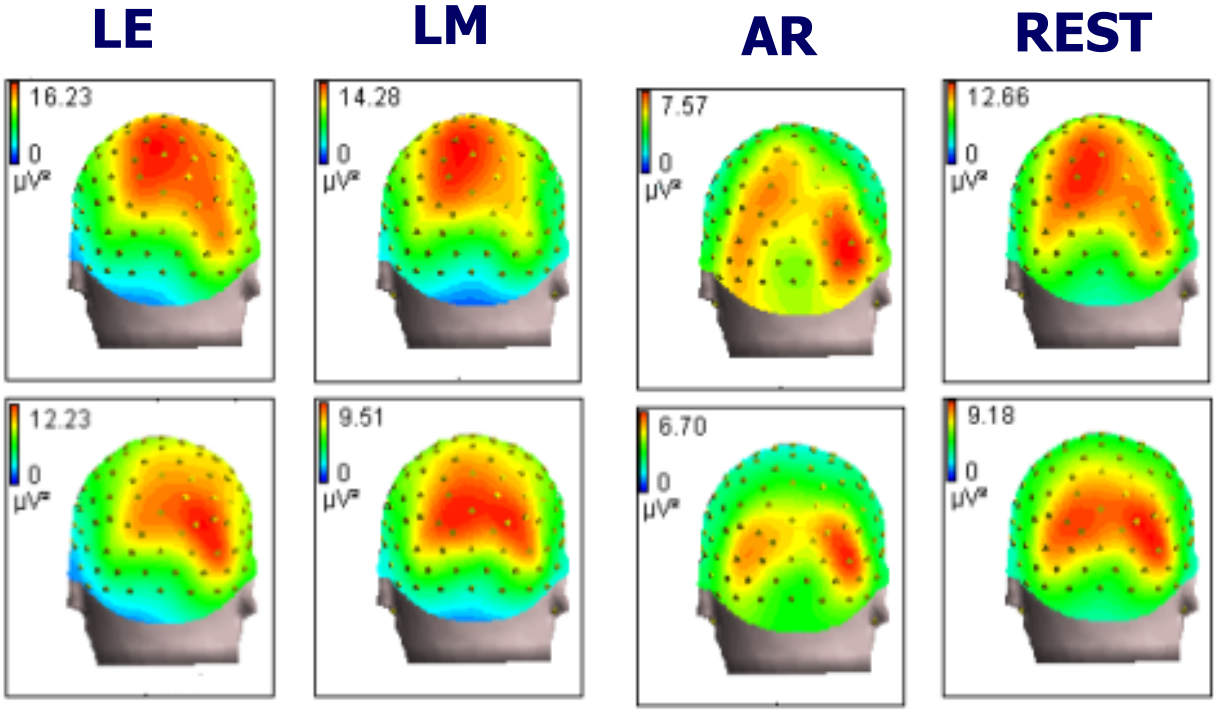
Simulated data with a single source shows the REs of potentials due to referencing. RE at each source is plotted at its position oriented to x-, y-, z- axis, respectively.

REST is the best for almost the all cases

Yao, Pedro et al. BT,2019

Real Data Reference Effects

Power Spectra of EEG (qEEG)



Alpha-1

7.5 - 9.5Hz

Alpha-2

10.0-12.5Hz

Power maps of the EEG recordings with different references. The power is analysed by FFT with 2 s epochs free of artefacts.

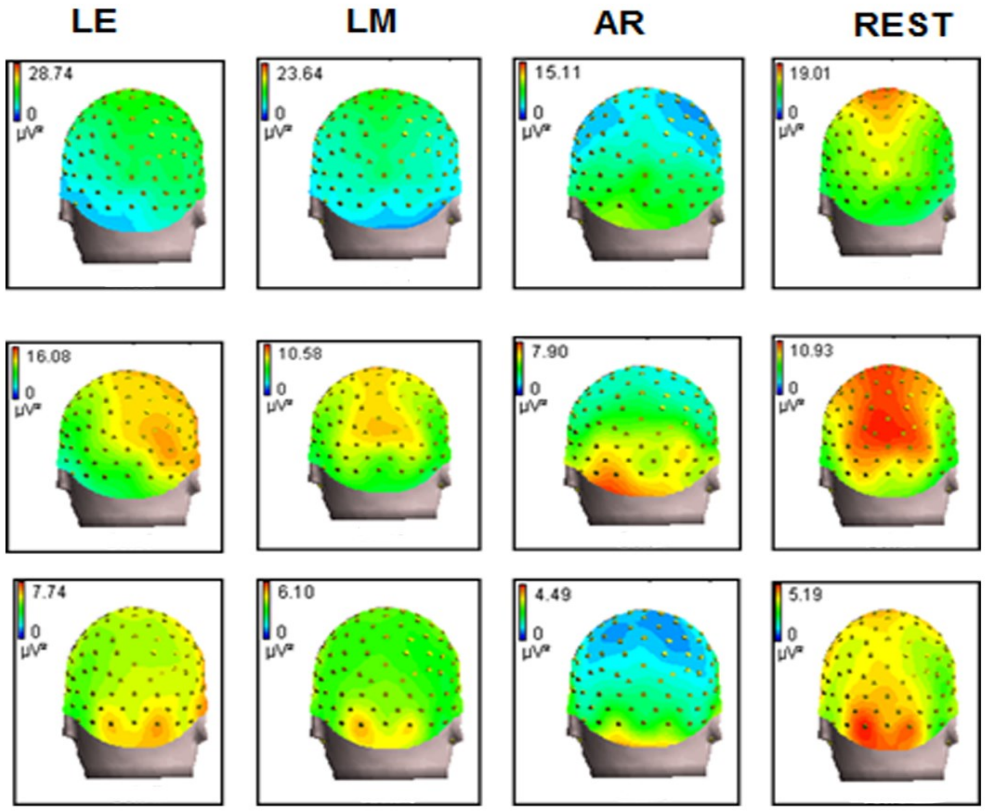
Current practices:

Clinic: linked ears(LM) are common in qEEG

Psychology: LM and AR are widely adopted

Yao, et al, Physiol Meas, 2005, 26:173

Power Spectra of EEG (qEEG)



Current practices:

Clinic: linked ears(LM) are common in qEEG

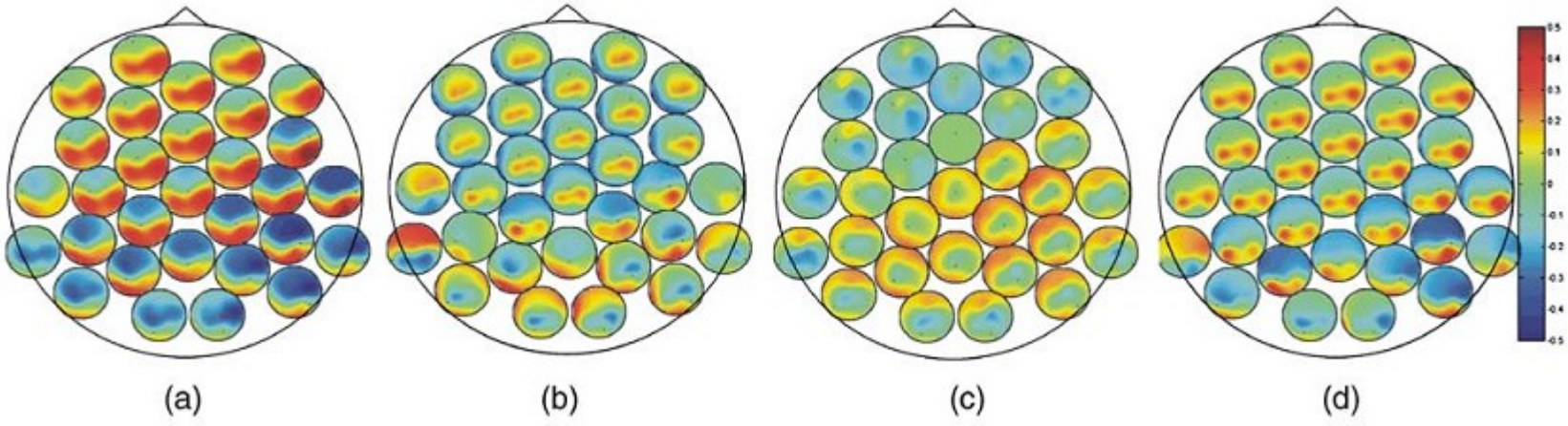
Psychology: LM and AR are widely adopted

Yao Pedro etal BT 2019

Coherency Map of EEG

Eyes closed Alpha（10Hz）

REST **AR** **FCz** **LM**



REST better reflect the parietal-occipital interaction in alpha band

imaginary part of complex coherency visualized as head-in-head plots

Marzetti etal NeuroImage, 2007

Amplitude of ERP

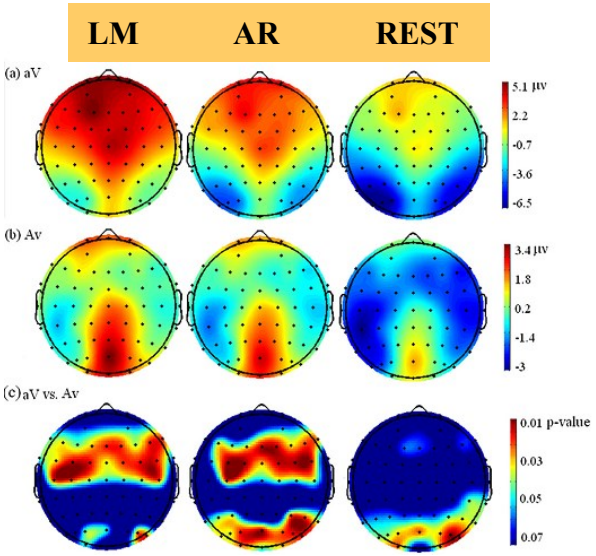
investigate the influence of the reference on experimental effects (between two conditions).

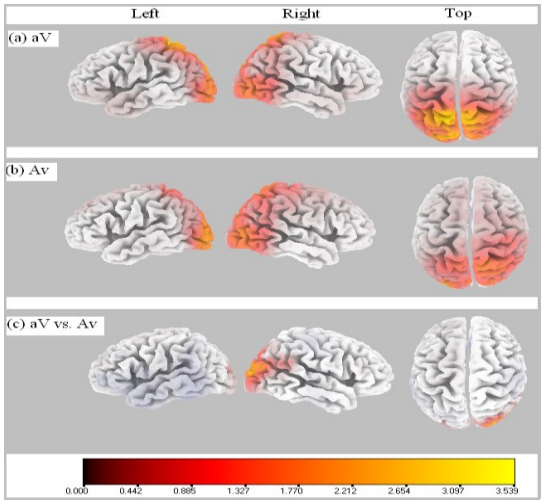
Simultaneous audio-visual stimuli N1 Peak 170-190ms

Visual attention

Audio attention

Statistic difference





Different references may induce quite different results

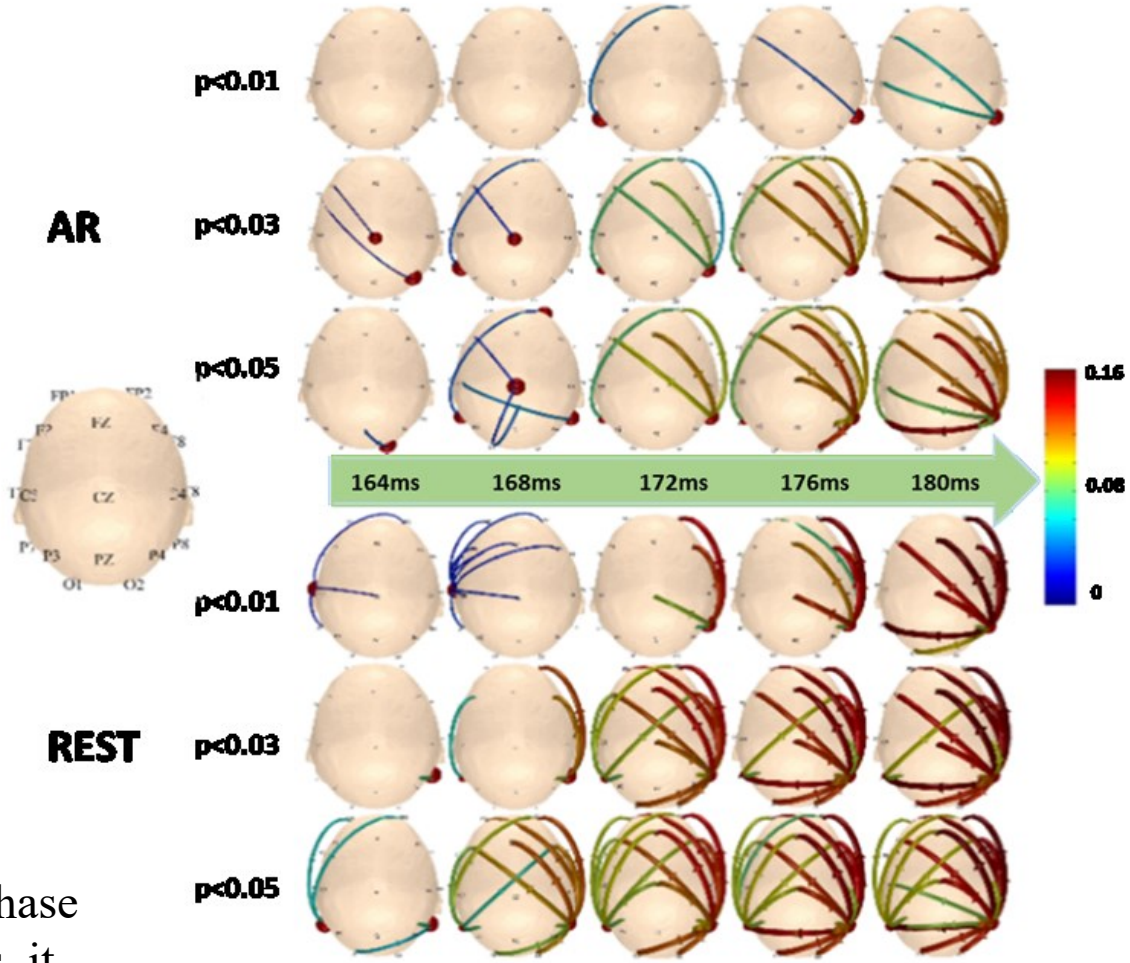
LORETA: the source of the difference wave is at the occipital, support REST

Tian Yao et al 2013 Psychophysiology

Latency of ERP

Latency of scalp time-varying network

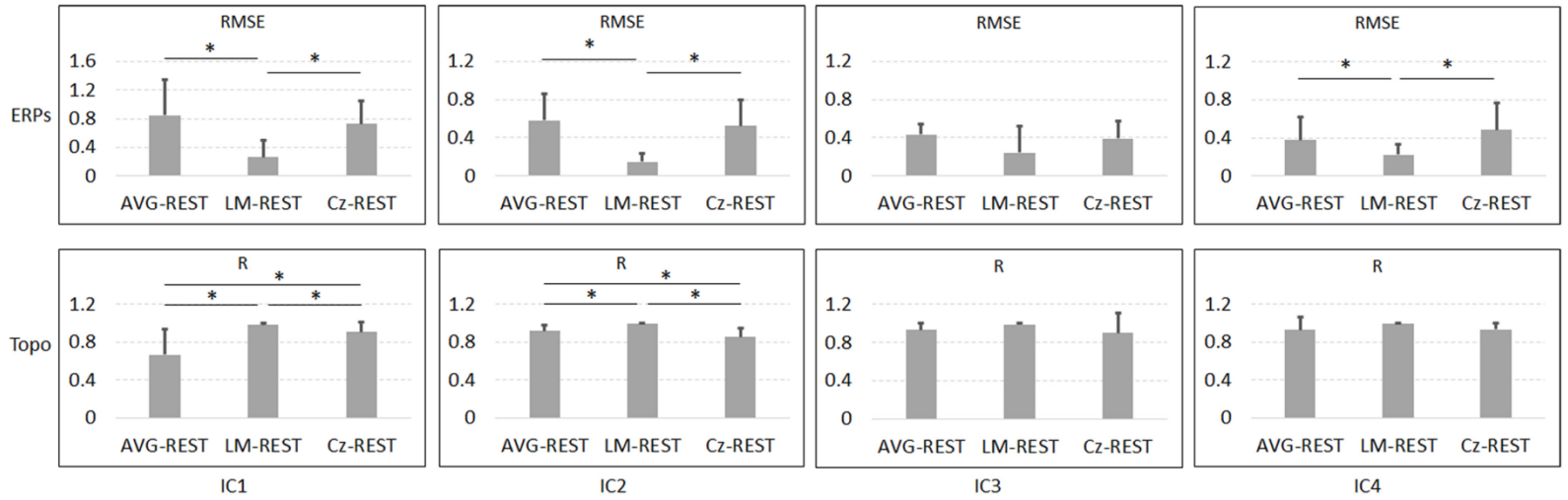
N170 - a negative component elicited by human face

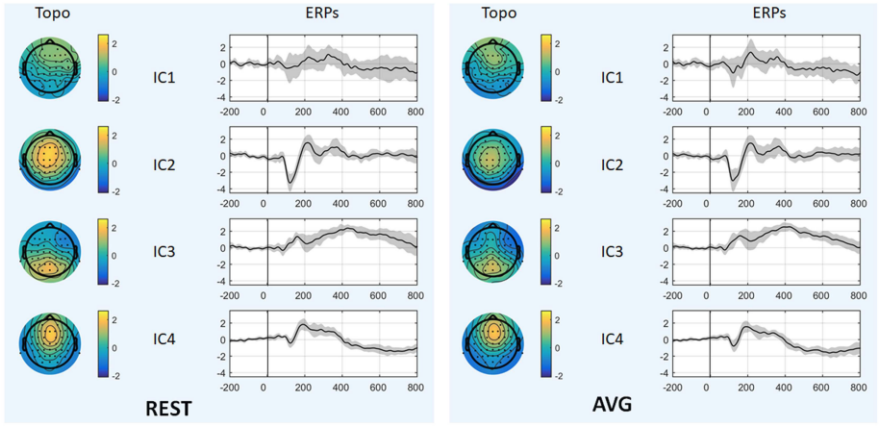


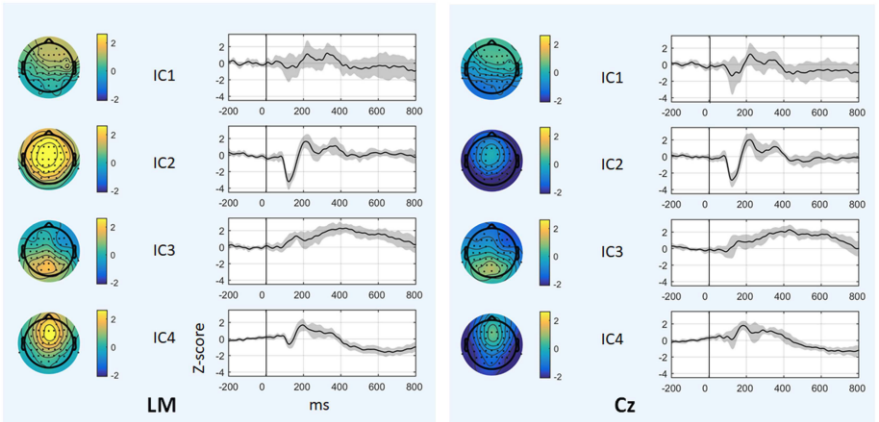
If the reference signal is nonzero, the subtraction would distort the amplitude;

If the reference signal has the delayed phase compared with the other active channels, it would affect the latency as well.

Tian etal. Front Neurosci, 2018







Different EEG Reference Choices for ERPs Extracted by ICA

- There were non-negligible effects of different reference methods on both temporal ERPs and spatial topographies of some ICs
- Compared to Cz, LM, and AR, considering both the performances of temporal ERPs and spatial topographies, the REST reference had overall superiority.

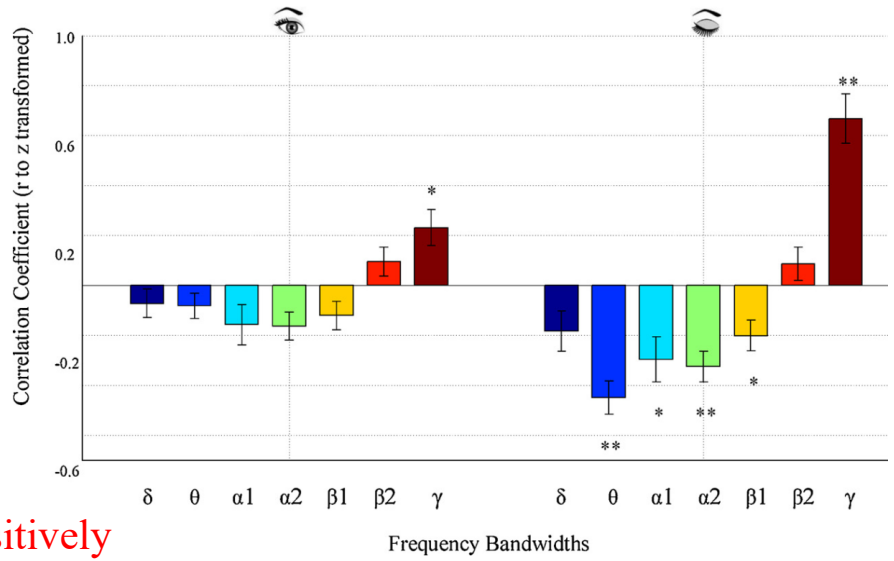
Dong et al. Front Neurosci, 2019

- Global Brain EEG signal

  ---- EEGrest-EEGar

Global EEG and fMRI

- The global signal of rfMRI
- The global signal of EEG = EEG(REST)-EEG(average)



EO :

- γ correlated positively
  r = 0.232, p = 0.003
- α2 correlated negatively
  r = -0.163, p = 0.008

EC:

- γ positively correlated
  r = 0.667, p < 0.001
- θ, α1, α2, and β1 negatively correlated
  r = -0.4450, p < 0.001
  r = -0.296, p = 0.003
  r= -0.325, p < 0.001
  r = -0.201, p = 0.003

Lei etal Journal of Neuroscience Methods ,2019

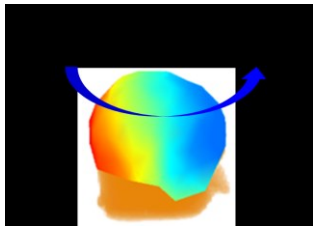
Summary

- **Reference has distinct effect on waveform related parameters:**

  amplitude/ latency/coherence/network/spectra

  /symmetry/covariance/statistic test..

- **REST is the best one to approach to the idea zero (unipolar reference)**
- **REST has been adopted by more 100 papers**
- **Integrated into EEGLAB and FieldTrip**
- **Recommended by IFCN Guidelines (2019)**
- **Recommended OHBM （https://cobidasmeeg.wordpress.com/)**



REST Usage

- **Offline REST ?**

- **Nice coverage , at least upper hemi-head surface**
- **Enough density (>16)**
- **Head shape is approximately known**
- **and you want to have a result more close to truth**

Best choice for most cognitive study and clinic EEG

Demo of EEG REST

Install REST (EEGLAB Plugin version)

- To install REST, download the zip file (http://www.neuro.uestc.edu.cn/rest/ or
- https://sccn.ucsd.edu/eeglab/plugin_uploader/plugin_list_all.php)

- Unzip and place the folder in the 'plugins' folder of your existing EEGLAB installation (so something like ~/eeglab14_1_0b/plugins/REST_reference_v1.1_20190818/eegplugin_rest.m exists).

Dong, L., et al. (2017). "MATLAB Toolboxes for Reference Electrode Standardization Technique (REST) of Scalp EEG." Front Neurosci 11.

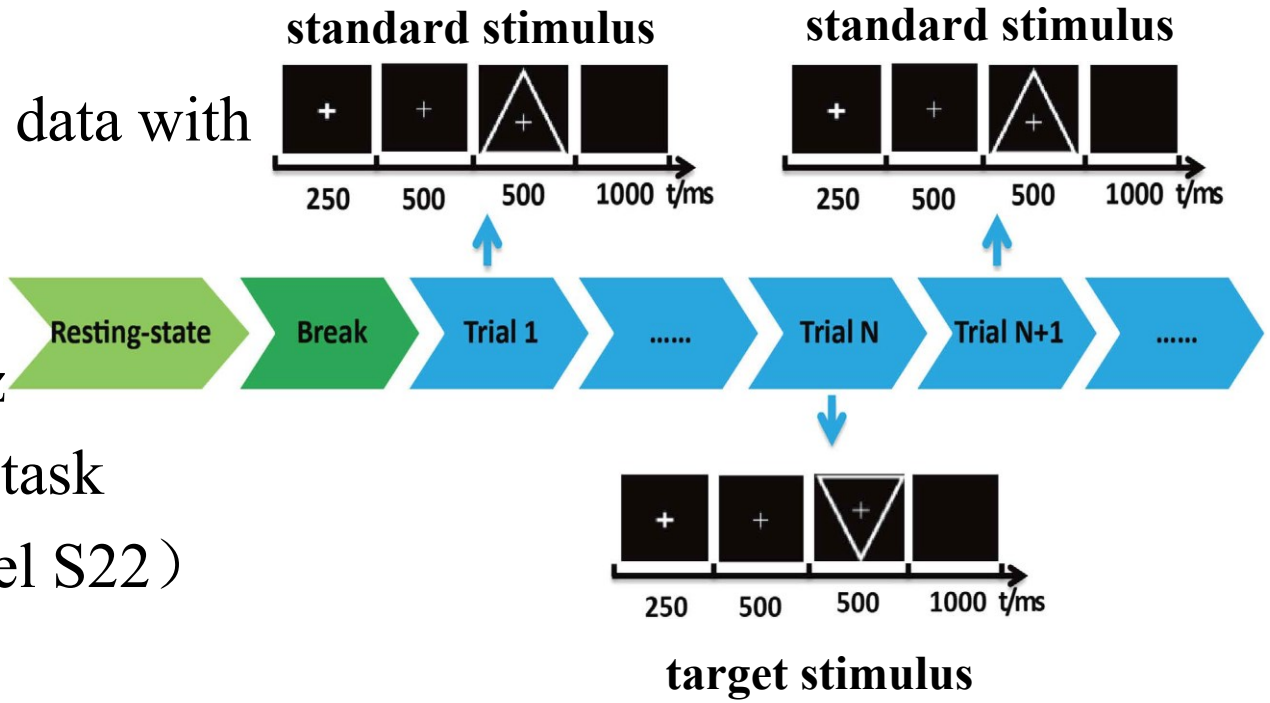
Run REST (EEGLAB Plugin version)

- To run REST, ensure that the correct EEGLAB folder is in your current Matlab path, and run 'eeglab' as a command from the Matlab Command Window.

- Then, load data using EEGLAB, click 'REST'--> 'Re-referencing to REST'

Example data

- A preprocessed EEG data with average reference
- 62 channels
- Sampling rate 500Hz
- Visual oddball P300 task
- 30 target trials （label S22）

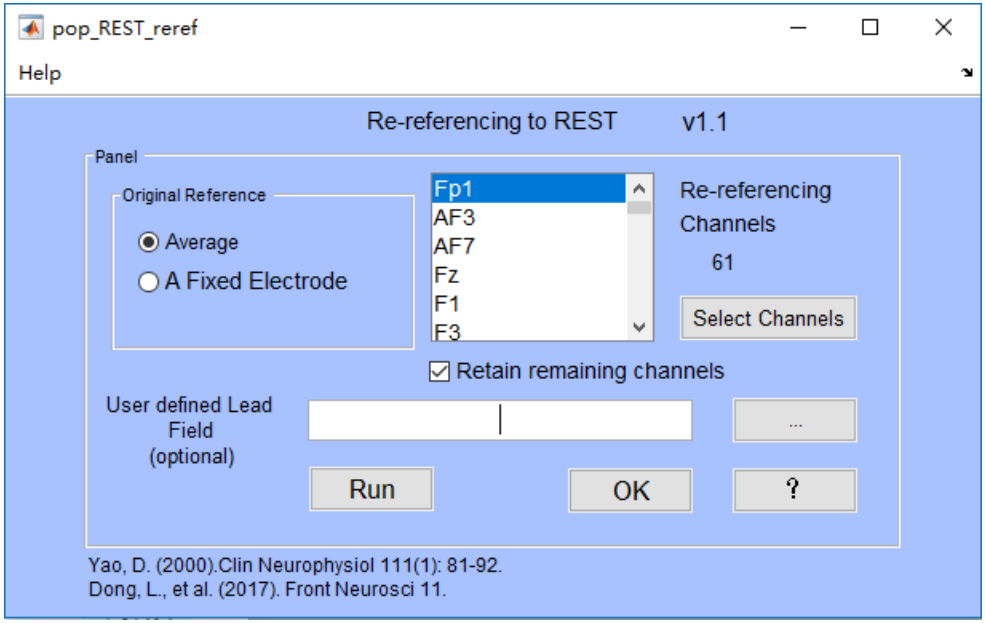


Li, F. L., et al. (2015) Scientific Reports 5.

Demo (step by step)

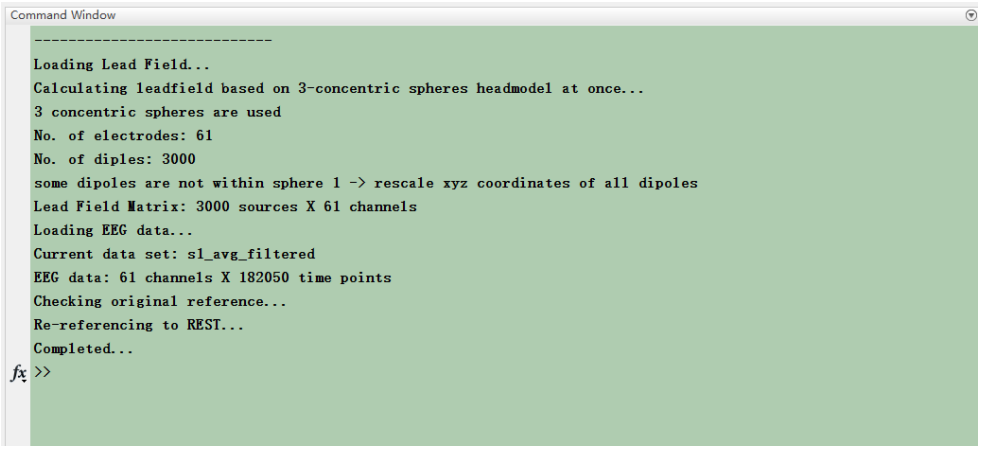
- [1] Select original reference of your EEG data (default is average).
- [2] Select Channels: Select EEG channels you want to re-reference (EEG channels 1-31, 33-62);
- Retain remaining channels?: if you want to keep un-selected channels in the data, check the box;

DO NOT select non-EEG channels!!!

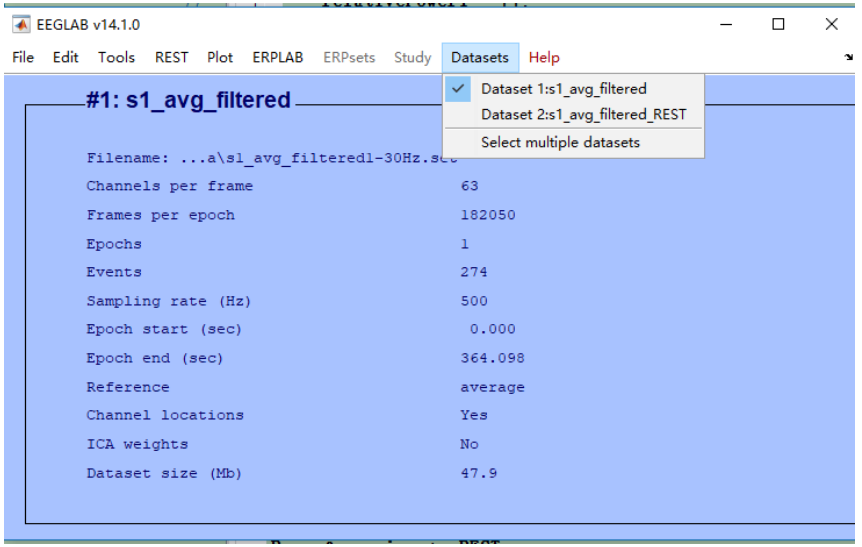


Demo (step by step)

- [3] If channel location has been imported in EEG.channlocs, press button "Run" directly; OR you can select a user defined lead field file, and then Press button "Run";

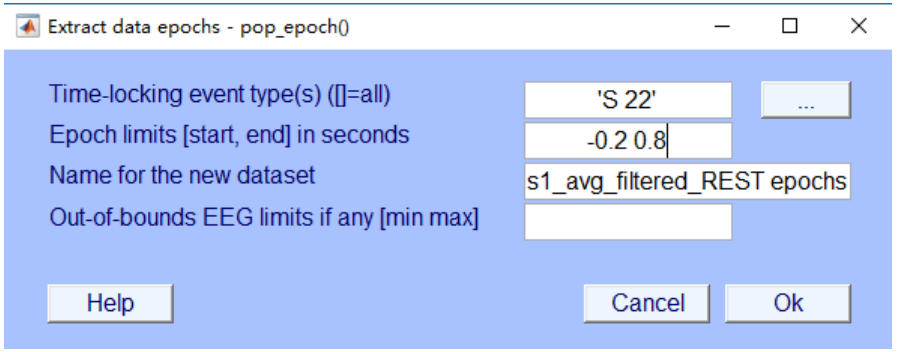


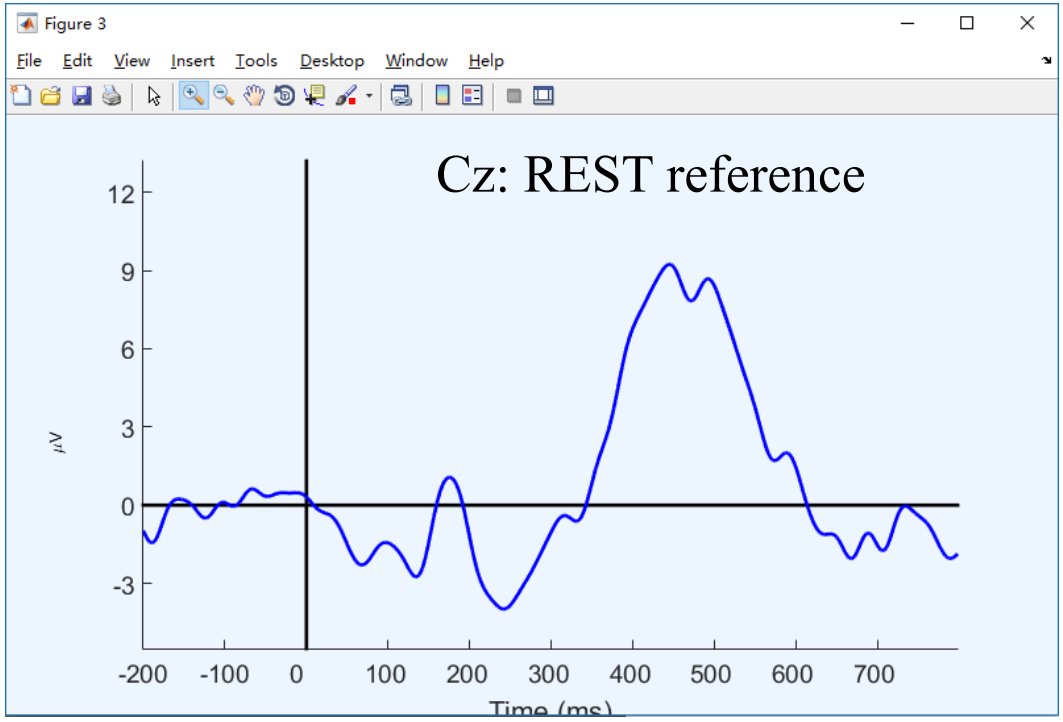
- [4] Press button "OK" to save the re-referencing data to workspace (ALLEEG).In EEGLAB, click 'Datasets'-->'*_REST";



Demo (step by step)

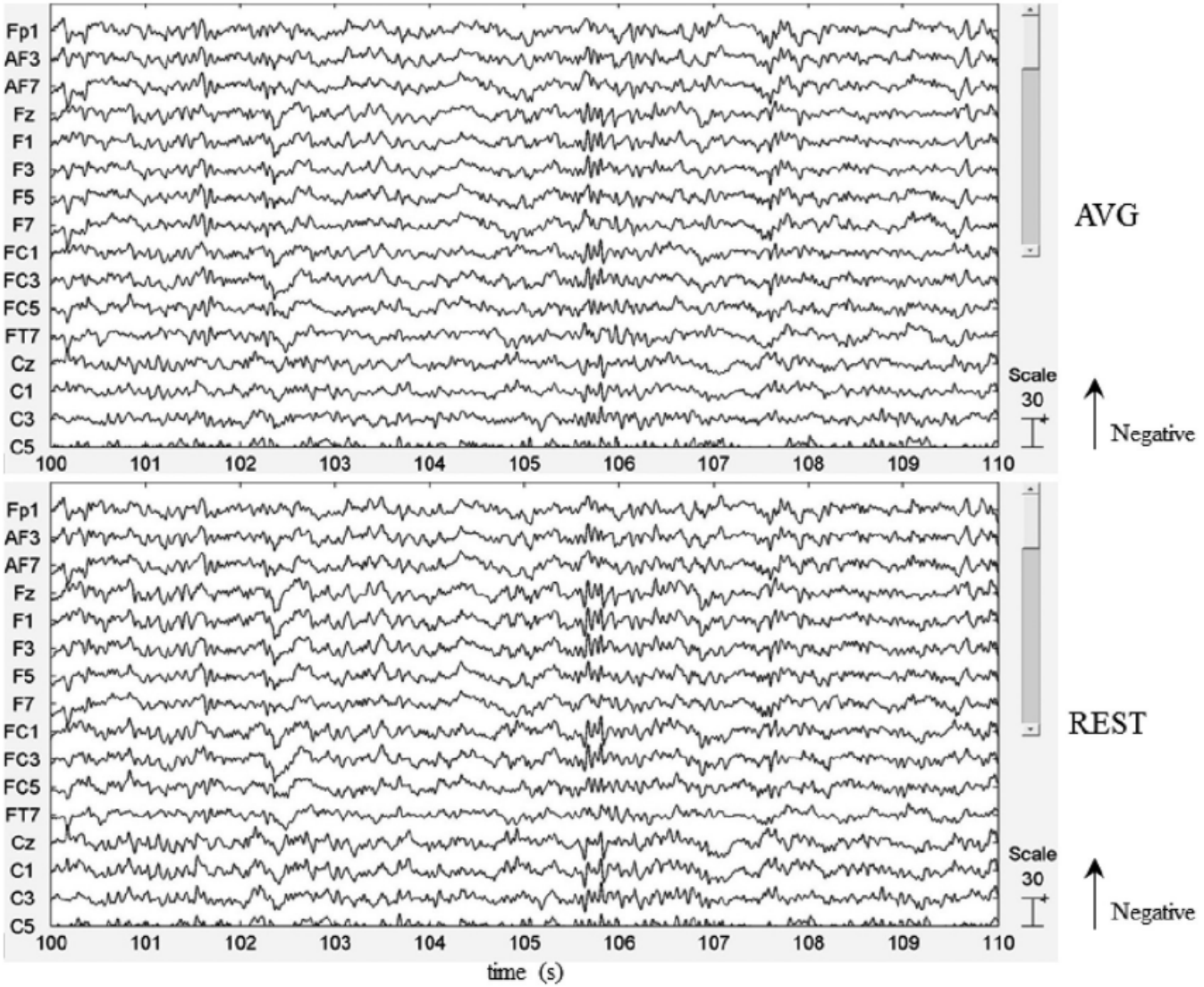
- Show results
- Tools->Extract epochs





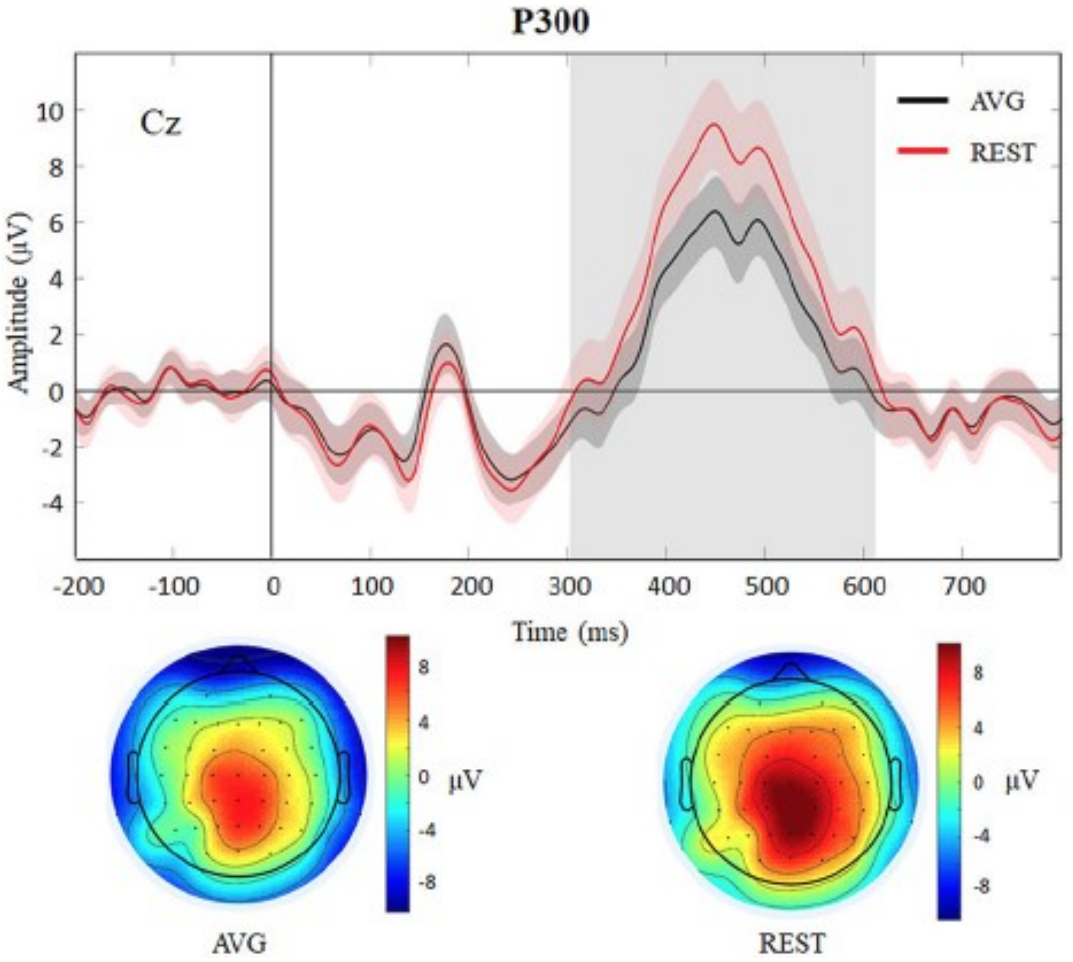
REST vs AVG

Figure shows that by visually inspecting the EEG figures of AVG and REST references, the similar EEG waves were observed, roughly.



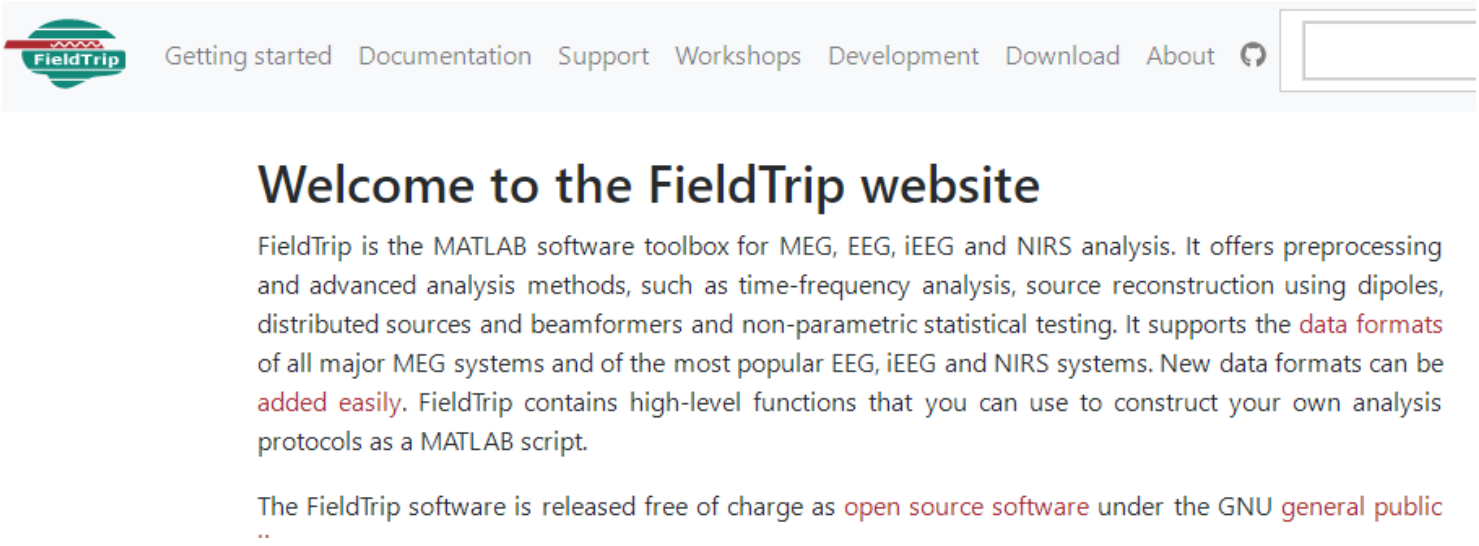
REST vs AVG

- P300 waves (with standard error) of AVG and REST references for Cz are showed and topographies of AVG (peaking at 450ms) and REST (peaking at 448ms) references are displayed.
- The gray region represents significantly (paired t-test, $P < 0.05$) larger signal intensity of P300 for REST than AVG.



REST in FieldTrip

- REST was integrated in the FieldTrip



http://www.fieldtriptoolbox.org/

REST in FieldTrip

See test_ft_preprocessing_REST_574

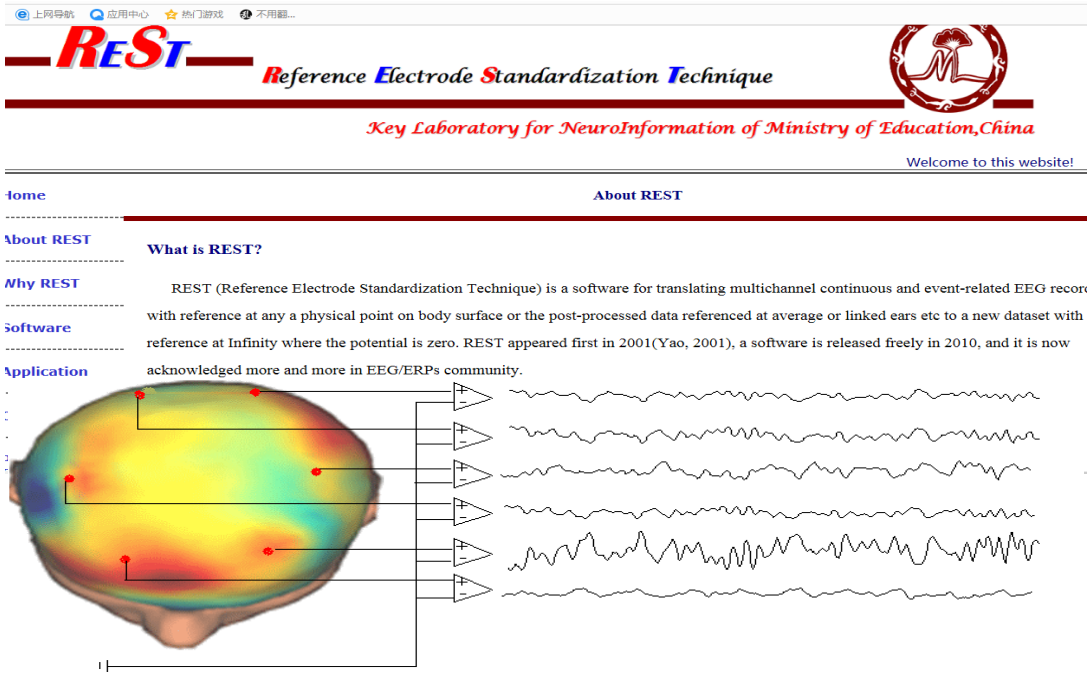
- Usage

```
ft_defaults;

load test_pr574.mat % load data and leadfield calculated by FieldTrip
% --------------------------------------------------------------------
% rest re-referencing
cfg = [];
cfg.reref       = 'yes';
cfg.refmethod     = 'rest';     %  if select 'rest','leadfield' is required.
cfg.leadfield = lf;
%               The leadfield can be a matrix (channels X sources)
%               which is calculated by using the forward theory, based on
%               the electrode montage, head model and equivalent source
%               model. It can also be the output of ft_prepare_leadfield.m
%               (e.g. lf.leadfield) based on real head modal using FieldTrip.

% cfg.refchannel = data.label([1:3,5:60],1); % use first 60 channels
cfg.refchannel     = {'all'};    % vector with indices of the selected channels
%                                  %  (re-referenced channels), or 'all'.
data_eeg_rest        = ft_preprocessing(cfg,data);
% --------------------------------------------------------------------
```

Summary



www.neuro.uestc.edu.cn/REST

Free REST are available

Acknowledgements

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Thanks to the international Prof Pedro Valdes Sosa, Fernando Lopes da Silva , Robert Ostenveld, PL Nunez, AC Chen for cooperations and discussions.

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