BENEFRI Workshop 2019

Methods in Experimental Neurosciences:
From Animal Models to Humans

UNIVERSITÄT BERN

fMRI in Neuroscience

Andrea Federspiel

UNIVERSITÄRE PSYCHIATRISCHE DIENSTE BERN

Psychiatric Neuroimaging Unit
Translational Research Center
University Hospital of Psychiatry
University of Bern

andrea.federspiel@upd.unibe.ch

roadmap

UNIVERSITÄT BERN

9:15-10:00 **Basic concepts of functional Neuroimaging**

fMRI Signal, task-dependent fMRI, resting state fMRI, Functional Network Analysis, processing pipeline, statistical testing, Random Effects, General Linear Model and MRI physics.

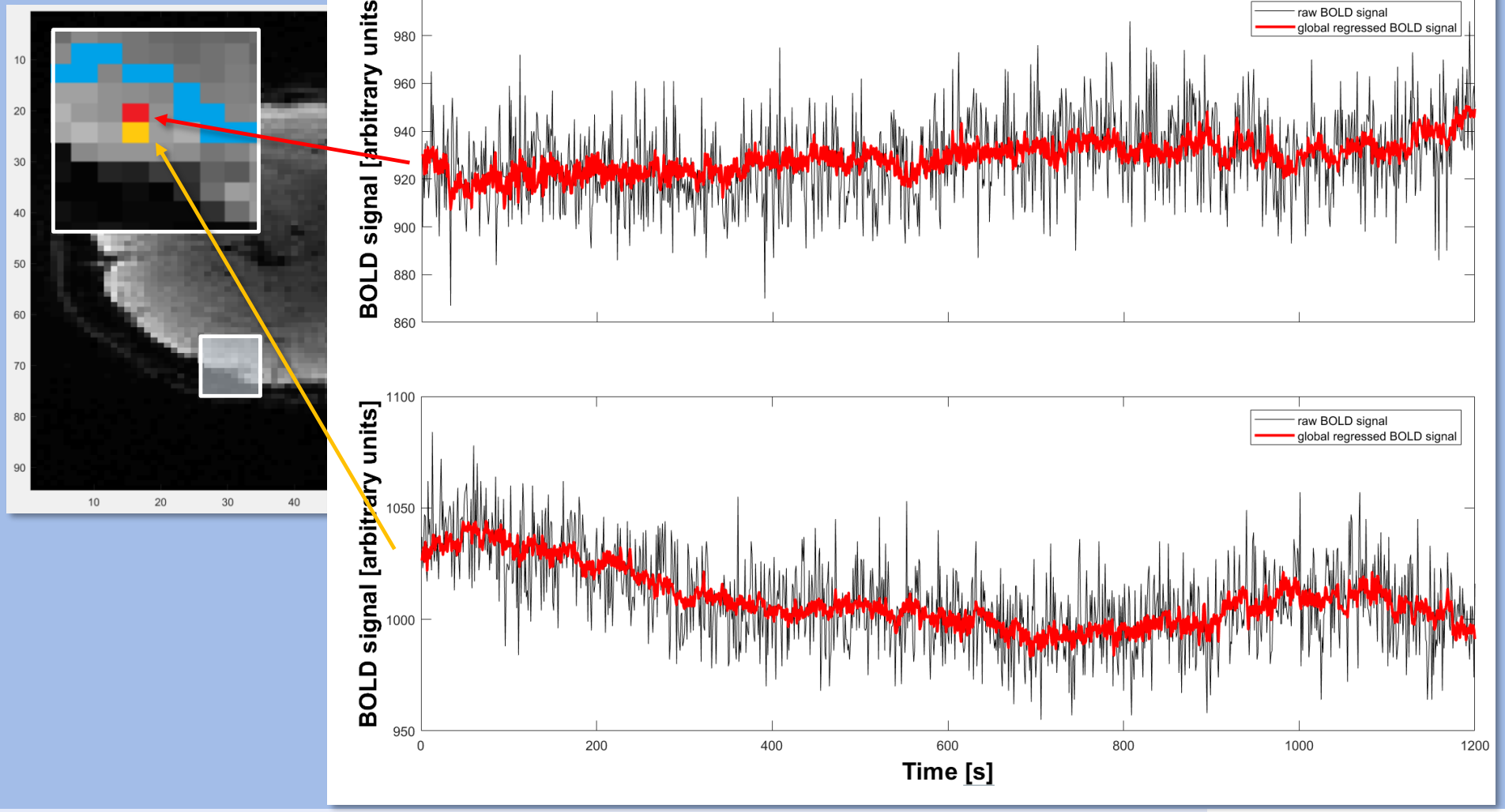
10:15-11:00 **Basic concepts of structural Neuroimaging**

Voxel Based Morphometry, Cortical Thickness, Cortex based inter-subject alignment, Diffusion Tensor Imaging, Tract-Based Spatial Statics.

11:15-12:00 **Advanced Neuroimaging Methods in Neurosciences**

Non-BOLD fMRI, Cerebral Blood flow (CBF), calibrated fMRI, Multimodal Imaging.

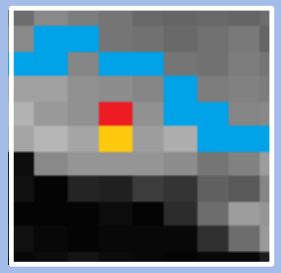
Variance in the neigbourhood

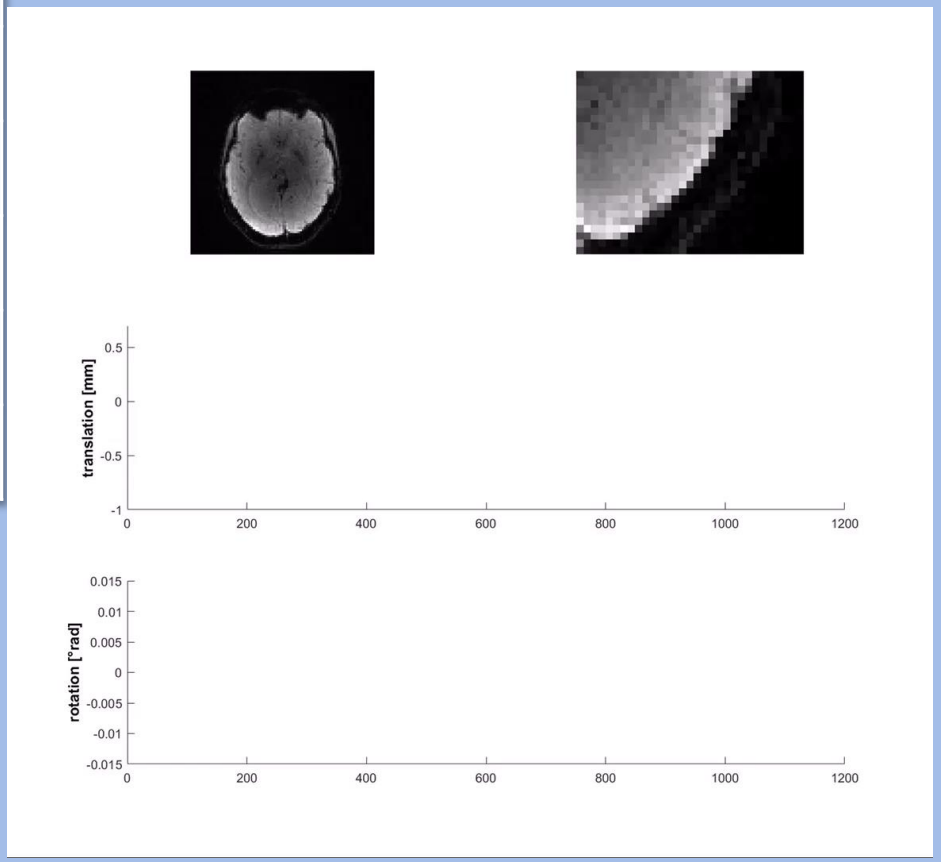


Closer lock at BOLD signal

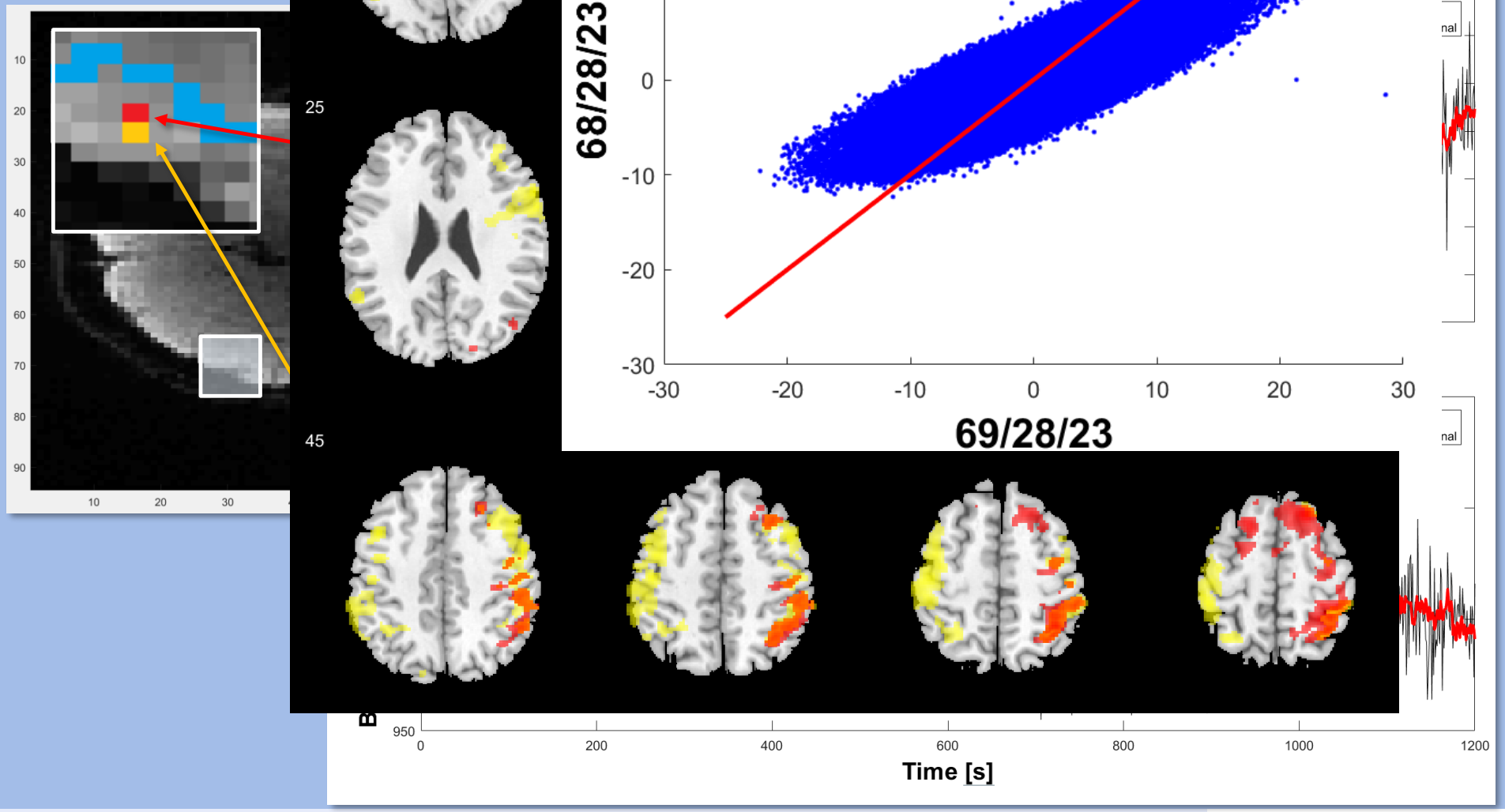
UNIVERSITÄT BERN

Variance in:		68/28/23	69/28/23
gm		301.18	415.69
BOLD (fit)		238.26	250.76
gm - BOLD(fit)		50.41	177.43
wm	298.35		
csf	273.24		
motion	0.06		





Variance


5
25
45
68/28/23
69/28/23
30
20
10
0
-10
-20
-30
-30
-20
-10
0
10
20
30
B
950
0
200
400
600
800
1000
1200
Time [s]

roadmap

UNIVERSITÄT BERN

9:15-10:00 **Basic concepts of functional Neuroimaging**

fMRI Signal, task-dependent fMRI, resting state fMRI, Functional Network Analysis, processing pipeline, statistical testing, Random Effects, General Linear Model and MRI physics.

10:15-11:00 **Basic concepts of structural Neuroimaging**

Voxel Based Morphometry, Cortical Thickness, Cortex based inter-subject alignment, Diffusion Tensor Imaging, Tract-Based Spatial Statics.

11:15-12:00 **Advanced Neuroimaging Methods in Neurosciences**

Non-BOLD fMRI, Cerebral Blood flow (CBF), calibrated fMRI, Multimodal Imaging.

fMRI “roadmap”

- Slice Time correction
- Coregistration (2D fmri → 3D anatomy)
- Segmentation (3D anatomy)
- Normalisation (3D anatomy)
- 1. and 2. level statistics

UNIVERSITÄT BERN

White paper on fMRI

https://www.humanbrainmapping.org/files/2016/COBIDASreport.pdf

Committee on Best Practices in Data Analysis and Sharing (COBIDAS)

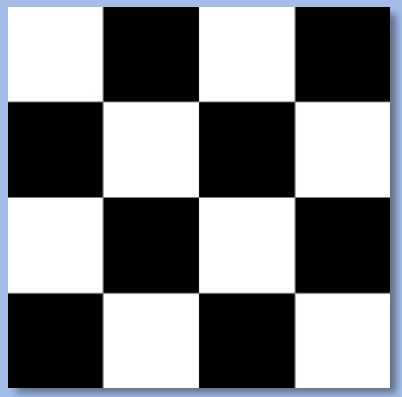
Organization of Human Brain Mapping (OHBM)

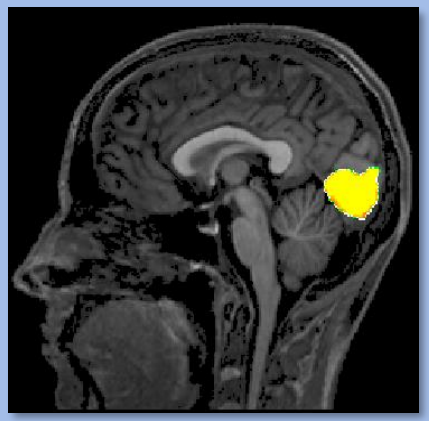
Suggestions and recommendations on how to deal with fMRI Data

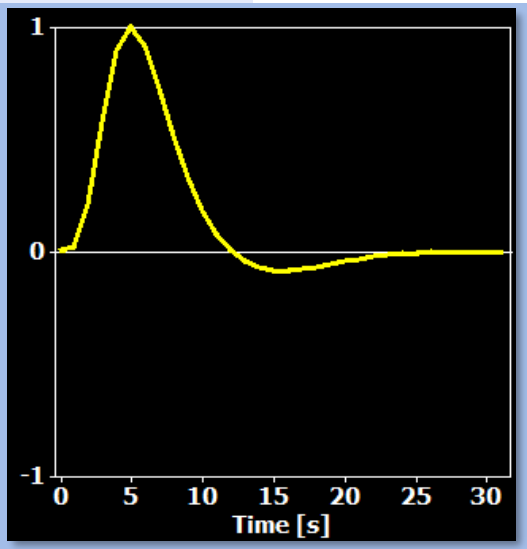
Data acquisition, Design, Data analyisis, etc.

UNIVERSITÄT BERN

Functional Magnetic Resonace Imaging
fMRI: measure of neuronal activity?







Stimulus

Reaction

Measure
BOLD = Blood Oxygen Level Dependent

Concentration between Oxyhemoglobin (diamagnetic) and Deoxyhemoglobin (paramagnetic) in the veins

If neuronal activity high → **BOLD contrast high**

* fMRI (engl. functional magnetic resonance imaging)

Paramagnetic: Magnetic field lower

Image Orientation

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9868–9872, December 1990
Biophysics

Brain magnetic resonance imaging with contrast dependent on blood oxygenation

(cerebral blood flow/brain metabolism/oxygenation)

S. Ogawa, T. M. Lee, A. R. Kay, and D. W. Tank

Biophysics Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974

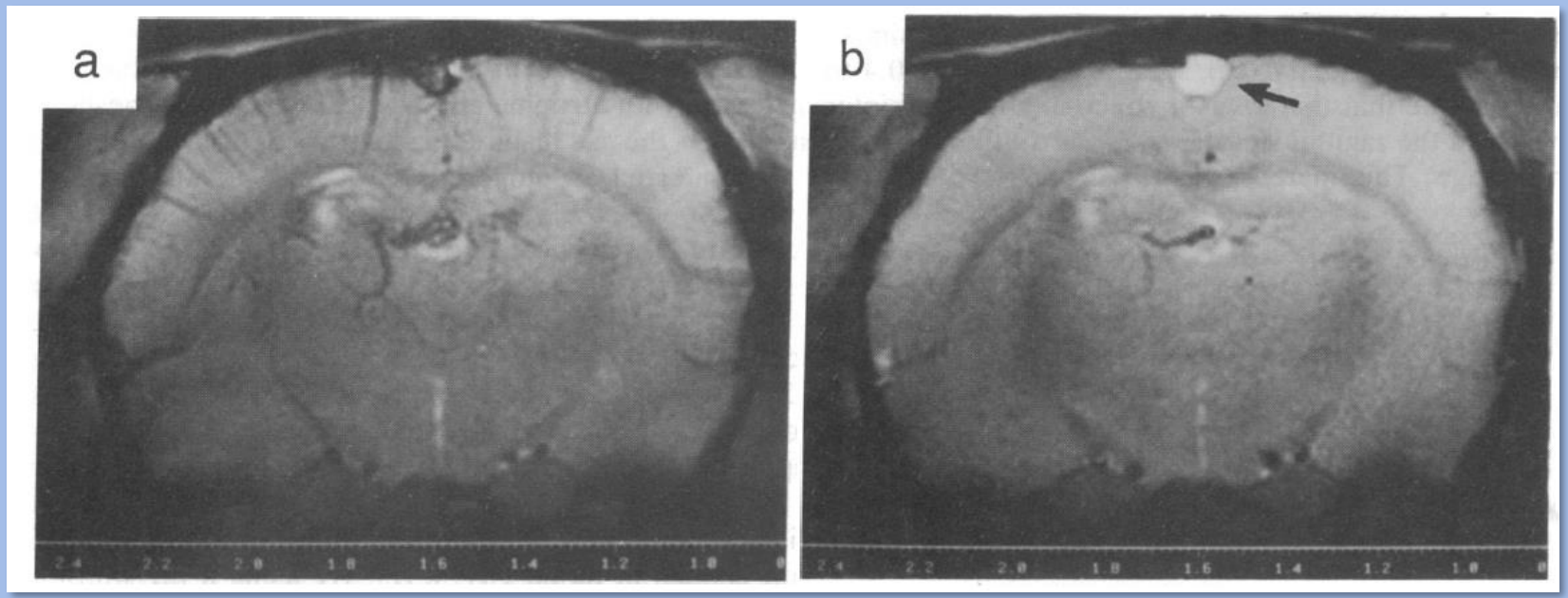
Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5951–5955, July 1992
Neurobiology

Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging

(cerebral blood flow/blood oxygenation/visual cortex/positron emission tomography/magnetic susceptibility)

Seiji Ogawa†, David W. Tank†, Ravi Menon‡, Jutta M. Ellermann‡, Seong-Gi Kim‡, Hellmut Merkle‡, and Kamil Ugurbil‡

What does Neuroimaging means?

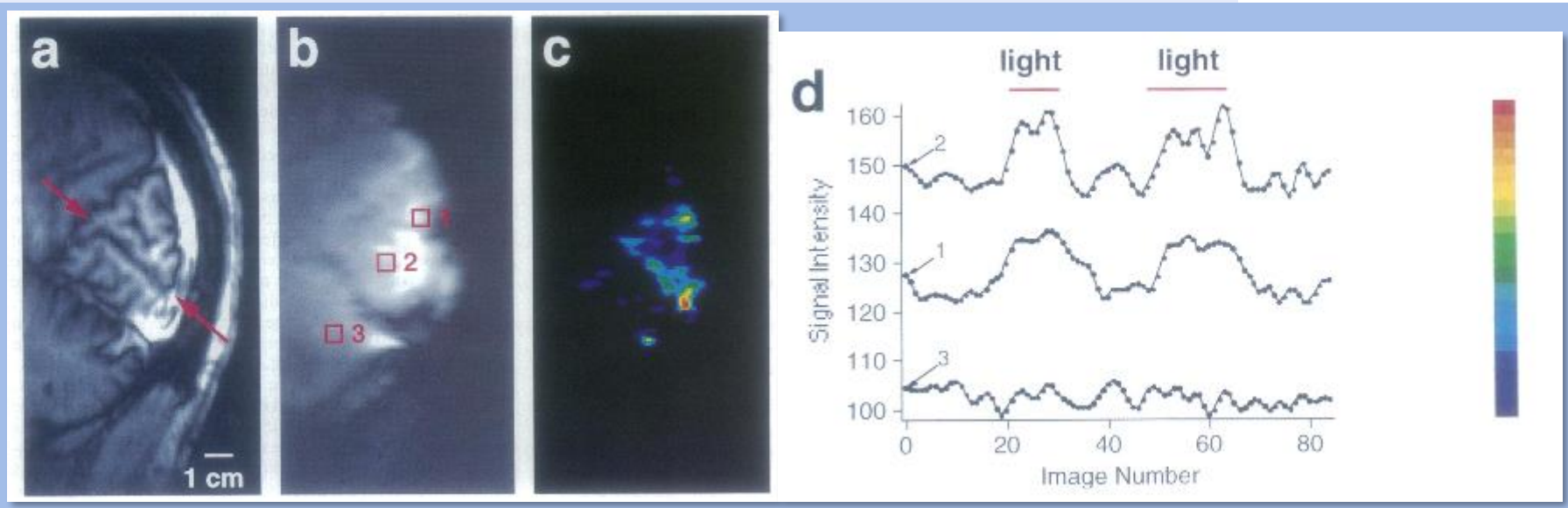


100% O2

90% O2 / 10% CO2

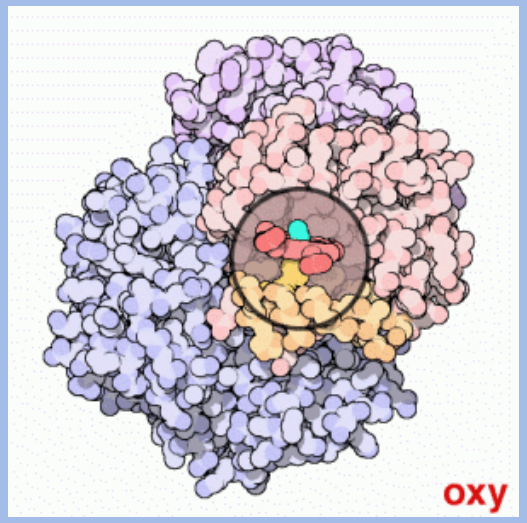
Ogawa S. et al. *PNAS* 1992 89: 5951-5955

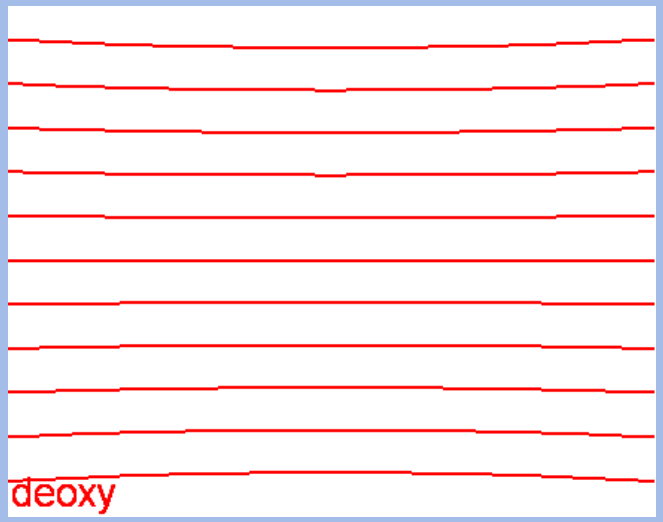
Contrast signal in different regions



Ogawa S. et al. *PNAS* 1992 89: 5951-5955

Active agent responsible for fMRI





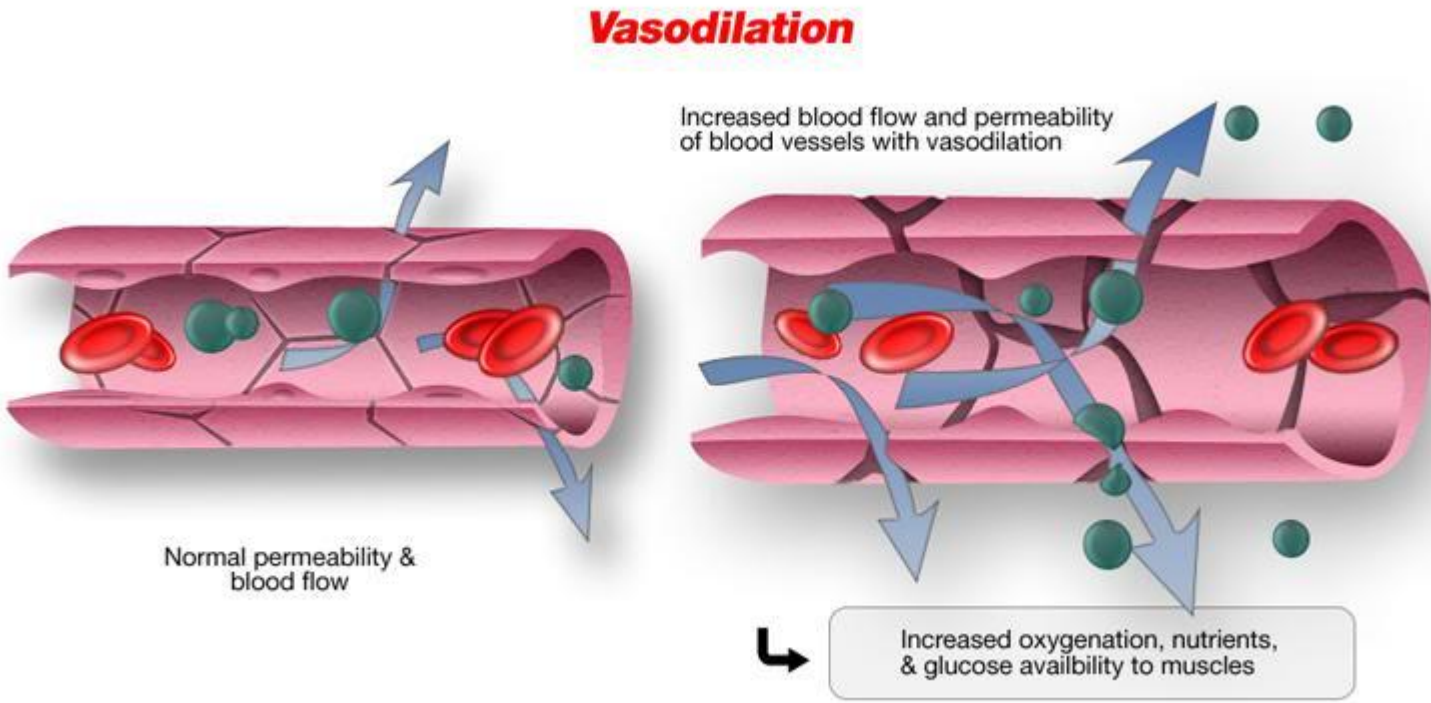
Deoxyhemoglobin: paramagnetic ($\chi > 0$) T_2^* ↓

Hb (4 unpaired e^- ⟶ S=2)

Oxyhemoglobin: diamagnetic ($\chi < 0$) T_2^* ↑

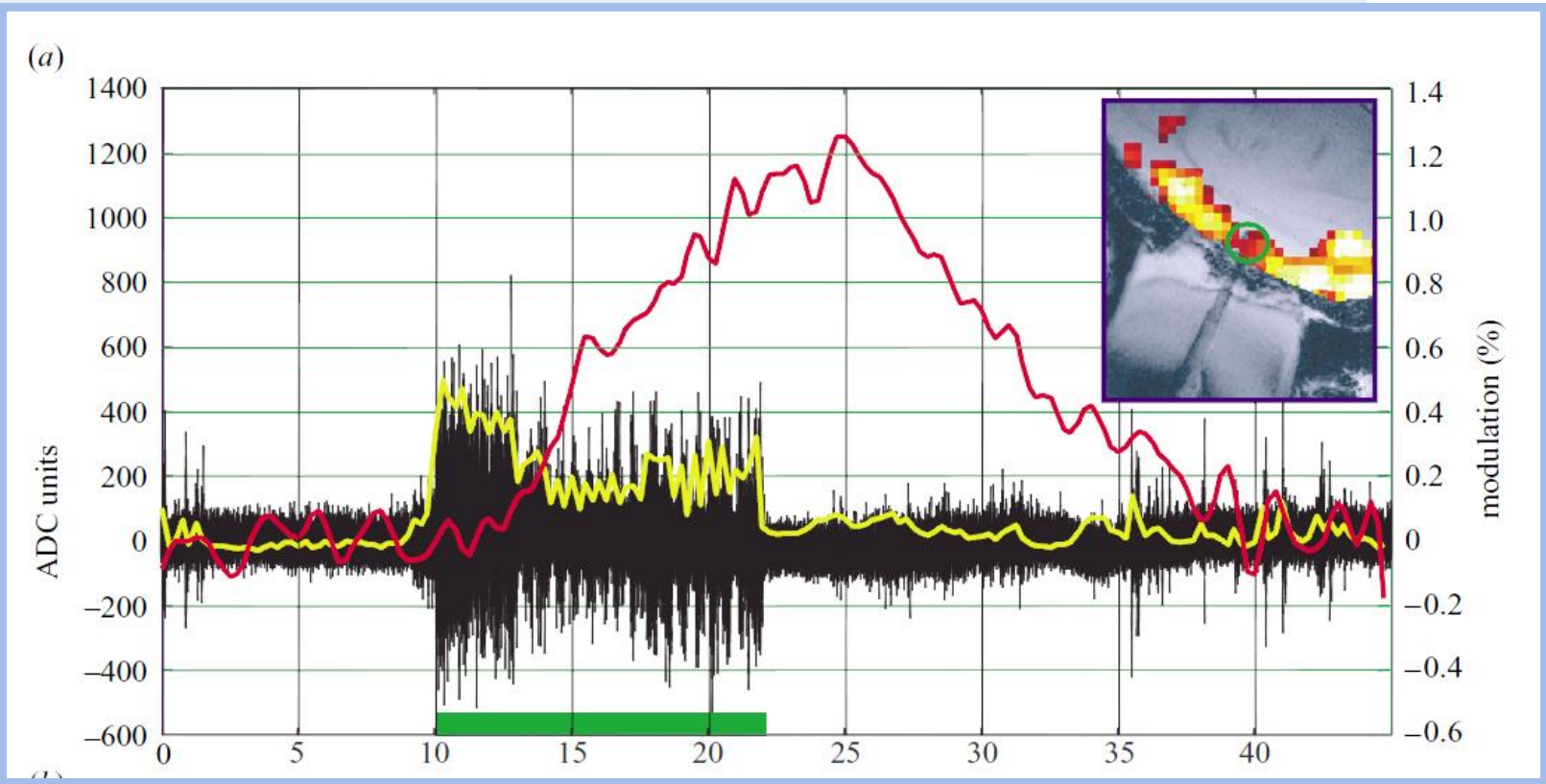
HbO_2 **S=0**

fMRI: vasodilatation



http://www.lookfordiagnosis.com

fMRI: Neuronal Activity



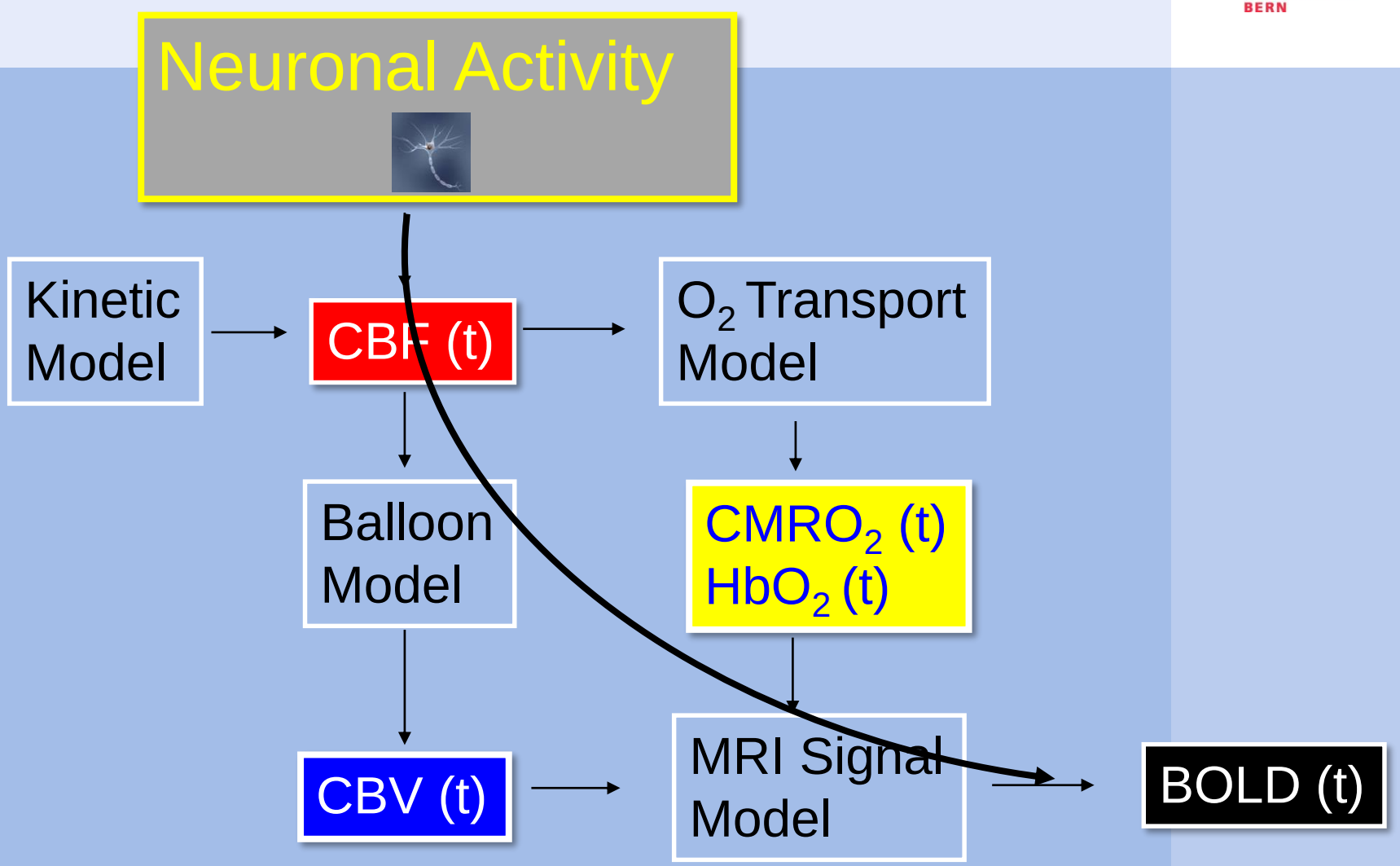
Spike Activity (black / yellow)

BOLD Activity (red)

(Simultan Intracortical / BOLD Measure on wake animal)

Logothetis N.K. et al. *Phil. Trans. R. Soc. Lond. B* 2002 357: 1003-1037

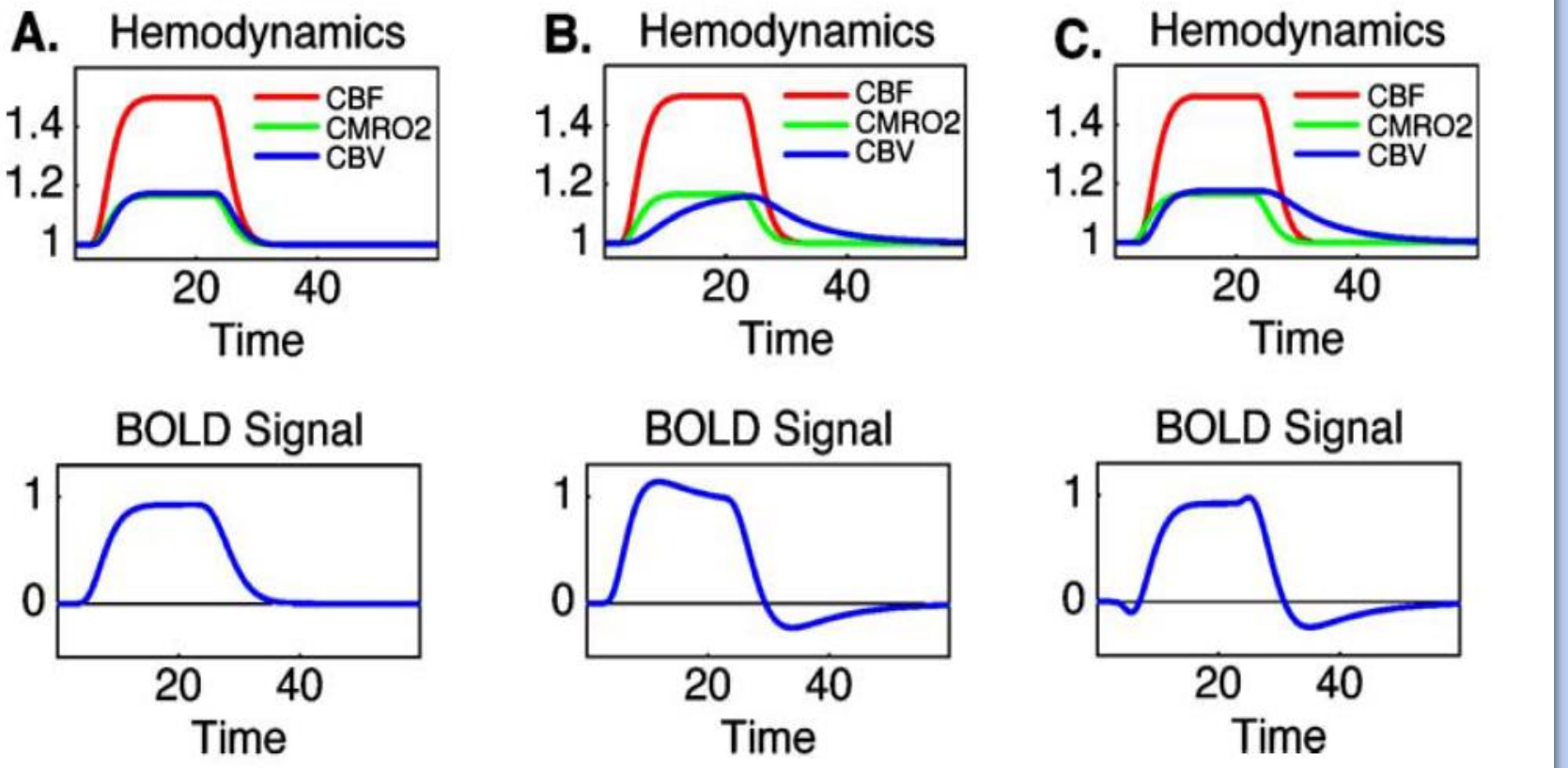
Understanding BOLD



Buxton, R.B. et al 1998 *Magn Reson. Med.* 40:383-396.

Obata, T. et al 2004 *Neuroimage.* 21:144-153.

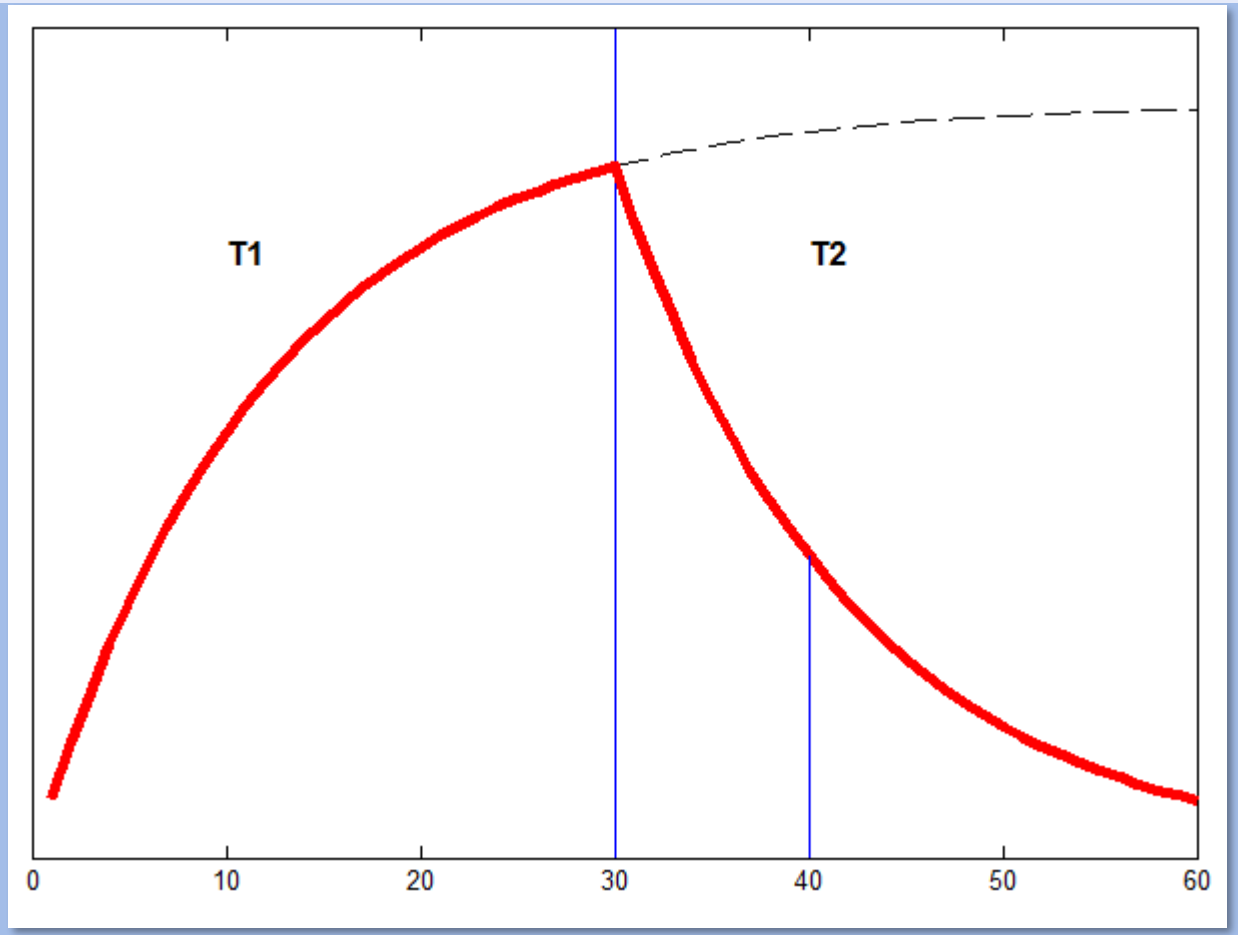
Balloon Model for understanding BOLD



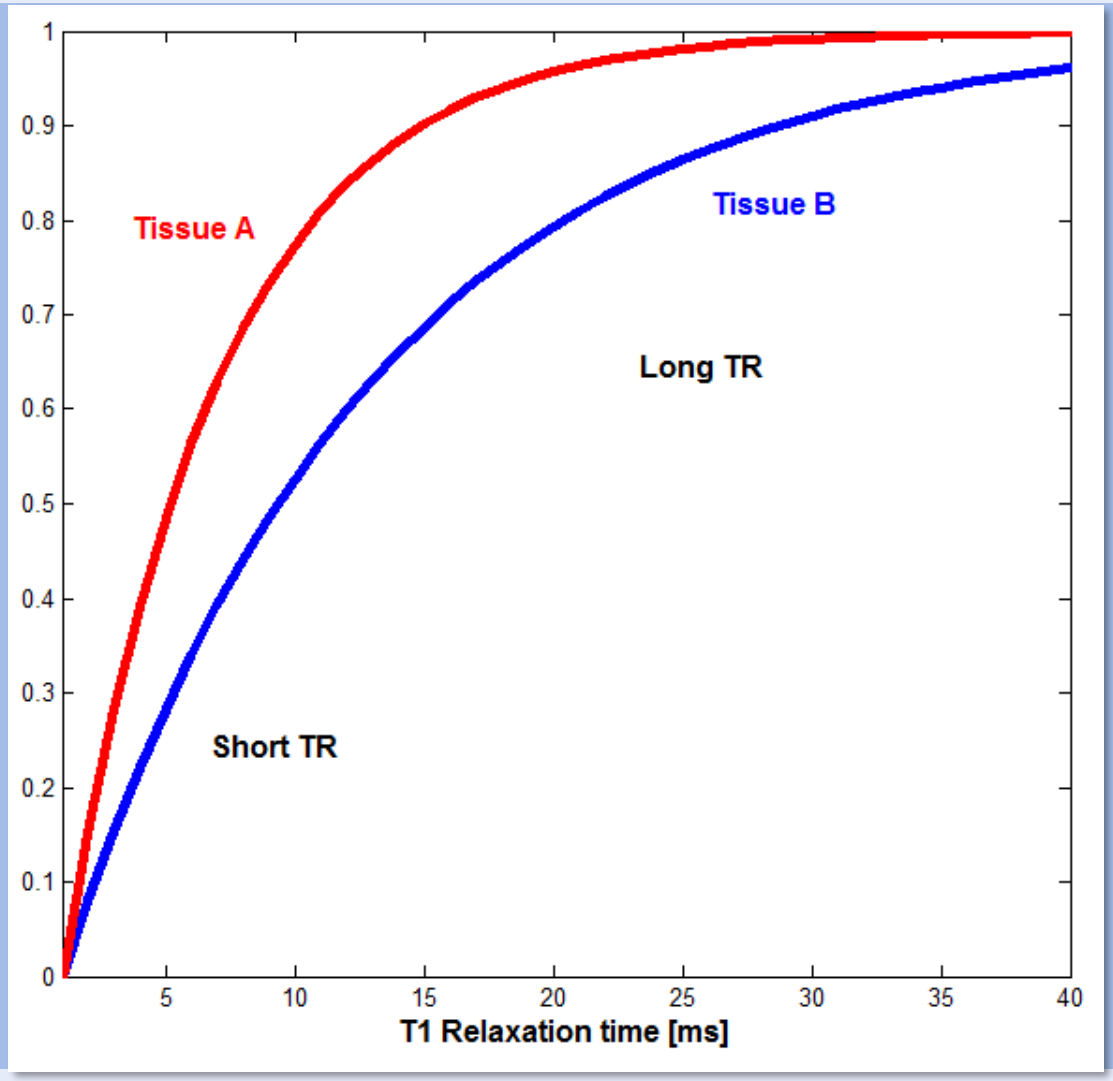
Buxton, R.B. et al 1998 *Magn Reson. Med.* 40:383-396.

Buxton, R.B. et al 2004 *Neuroimage.* 23:220-233.

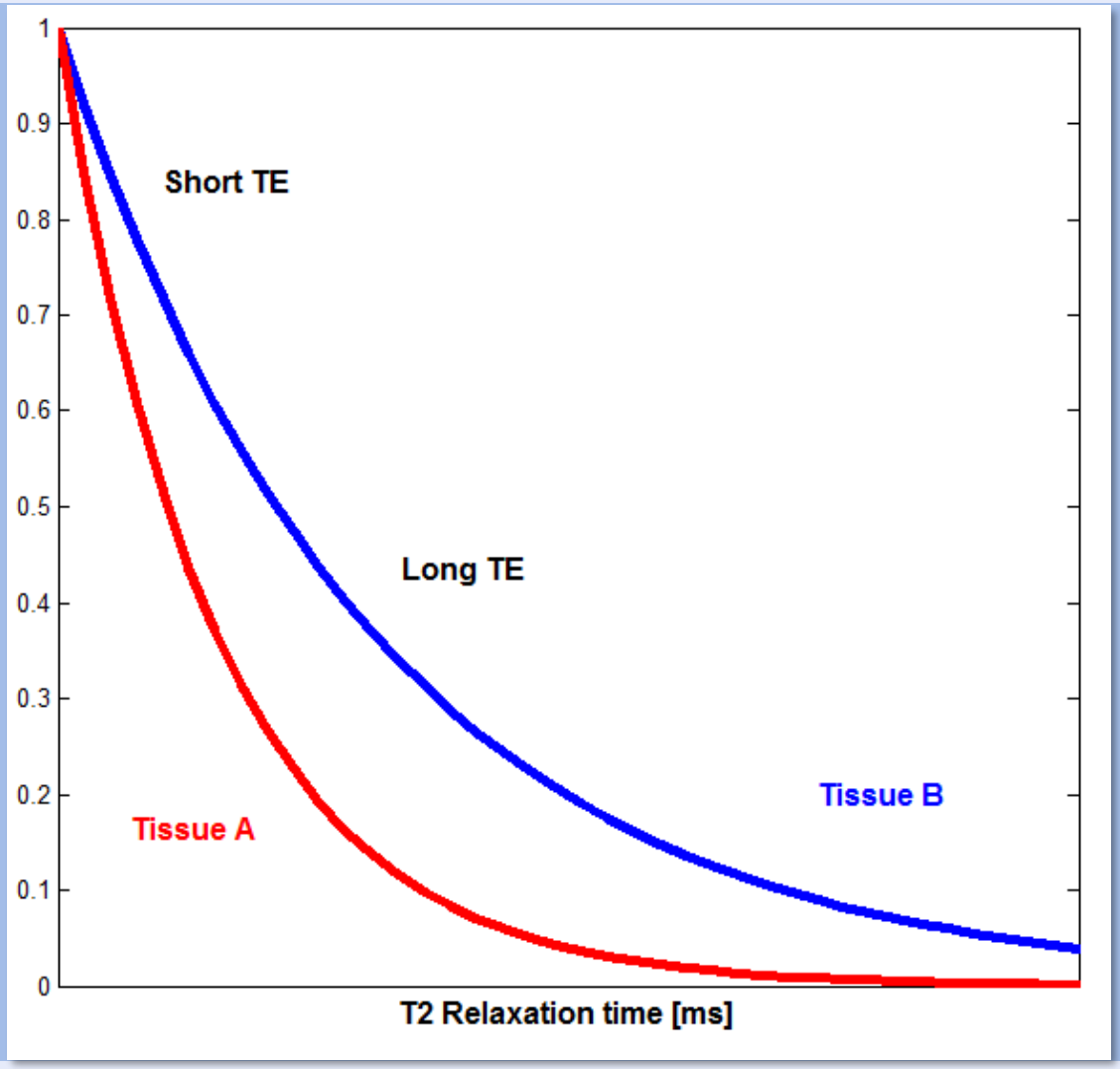
TR, TE (repetition- and echo time)



TR, T_1 relaxation



TE, T_2 relaxation



T_1 and T_2 relaxation times in tissue

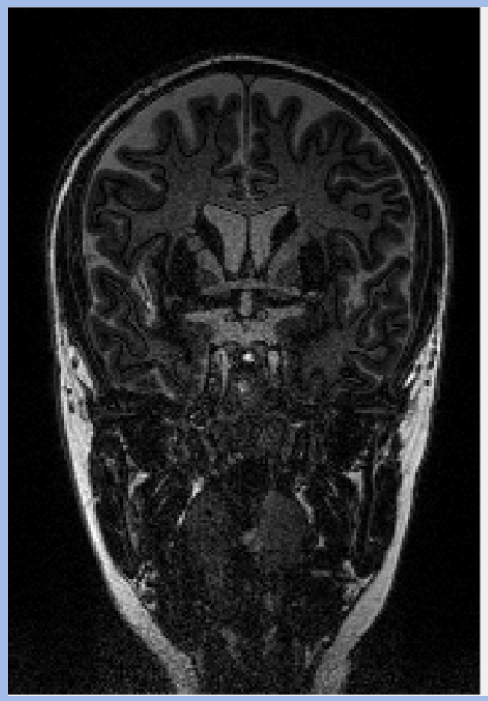
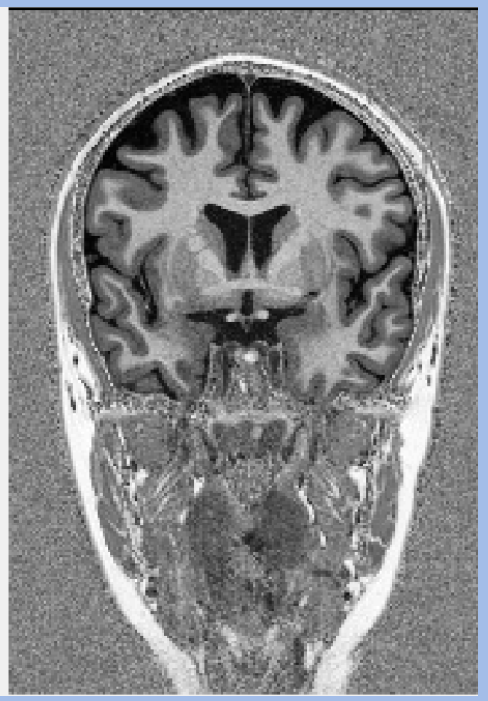
UNIVERSITÄT BERN

Tissue	T_1 [ms]	T_2 [ms]
Gray matter	600	80
White matter	950	100
Blood @ 3T	1450	275
Cerebro Spinal Fluid (CSF)	4500	2200
Fat	250	60

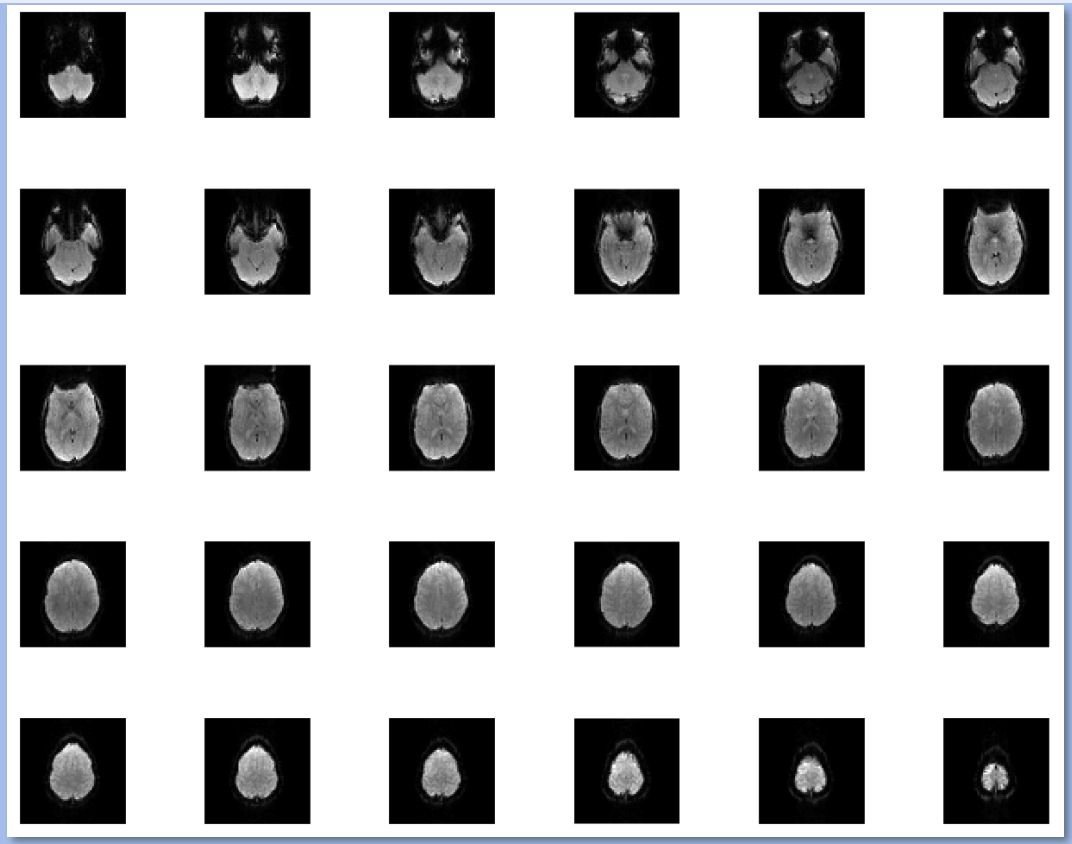
Stanisz G.J. et al. Mag Reson Med 2005: 54 507-512

Typical T1 sequence: mp2rage (TA: 8 min 22 sec)

UNIVERSITÄT BERN

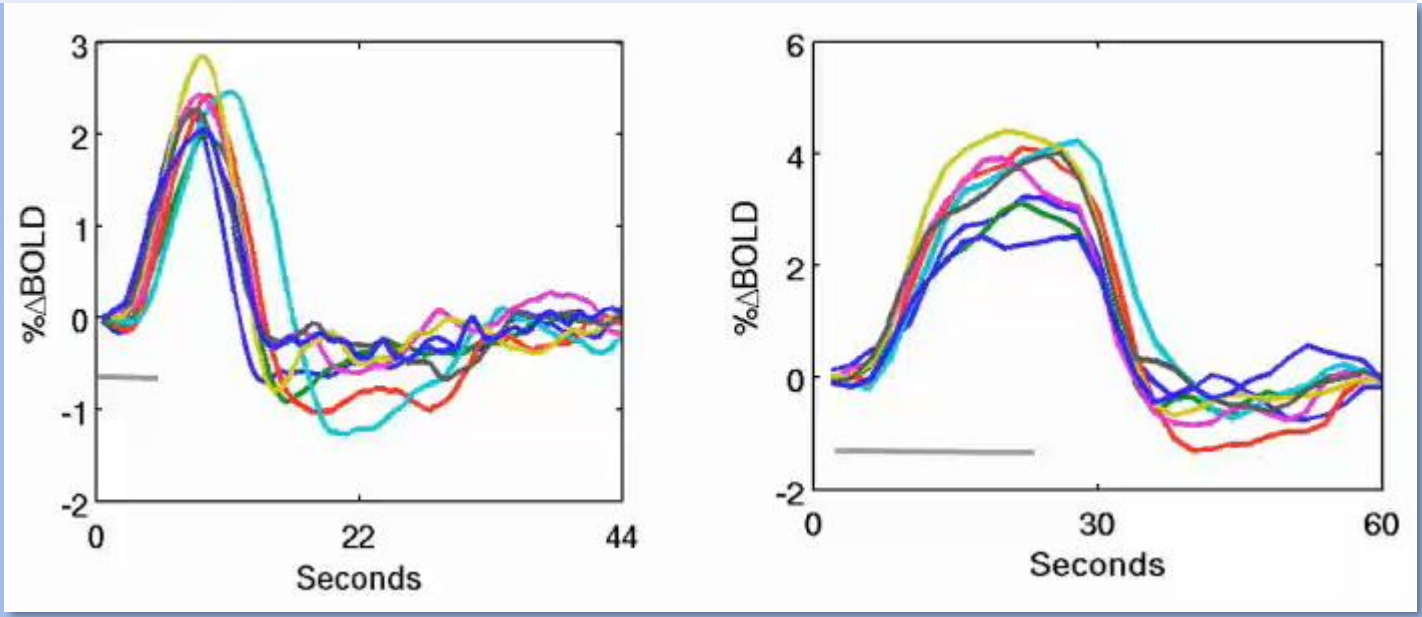
1st inversion	2nd inversion	combined
		
T_{i1}=700 ms Flip.angl.=4°	T_{i2}=2500 ms Flip.angl.=5°	

Typical T2 sequence: multi-band



TR=300 ms
TE=30ms
64 x 64; 3.6 mm^3

Variability of BOLD signal

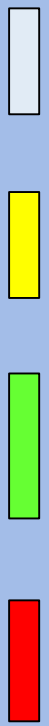


Healthy subjects/ same acquisition time/same age/male/same……

- Vascular origin of variability ?
- Origin of variability due to different Neuronal Activation ?
- etc.

Liau J, Liu TT. 2009 *Neuroimage*. 1;45(2):420-30.

fMRI sources of variance



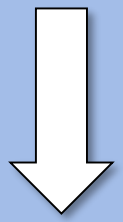
Sequence
Susceptibility

Drug/Coffee/Nicotine/ etc.
Circadian rhythm/Time

Respiration
Cardiac pulsatility in brain
3D Motion

Age
Healthy/Patient

(list not complete)



Task-related variability
Trial-to-trial variability

UNIVERSITÄT BERN

Understanding fMRI signal

$$BOLD_{signal} = \frac{Signal}{Noise} = \frac{\sum_{i=1}^{\infty} S_i}{\sum_{i=1}^{\infty} N_i}$$

$$BOLD_{signal} = \frac{\sigma_{Signal}}{\sigma_{Noise}}$$

Maximize Signal

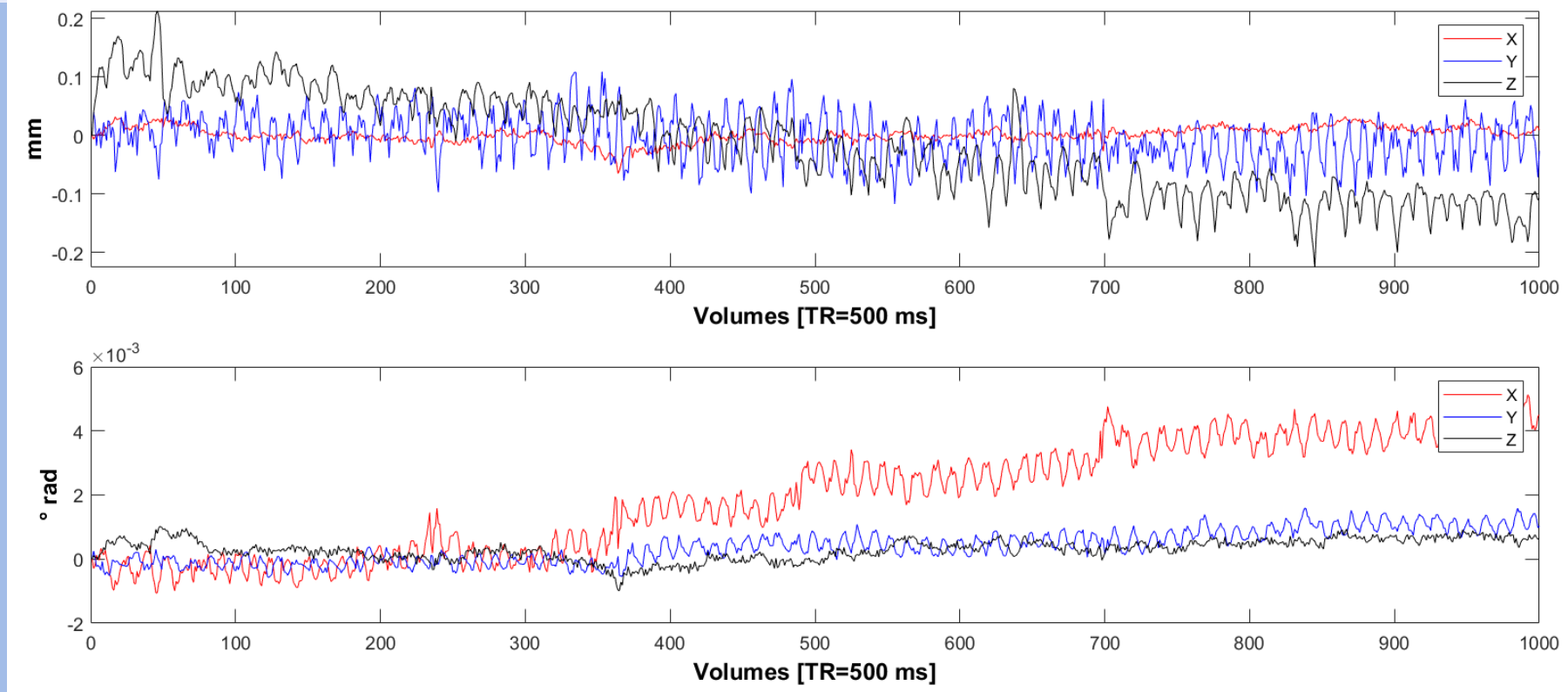
Minimize Noise

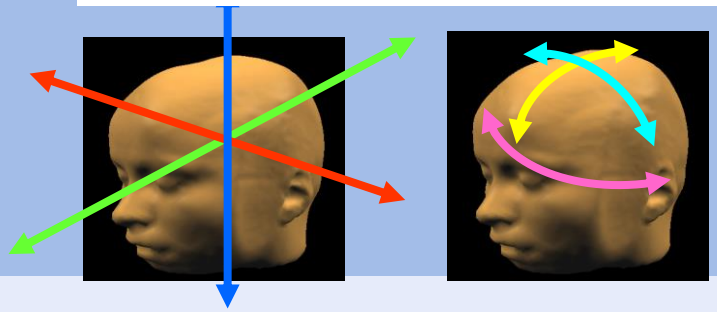
Signal to Noise Ratio (SNR)

$$SNR \propto \rho \frac{FOV_x FOV_y}{\sqrt{N_x N_y \, bw}} \rho_s \sqrt{N_{average}} B_0 \, f$$

ρ, ρ_s:	proton density, slice thickness
FOV:	Field of view in x,y
N:	Number of points in x,y
bw:	sampling bandwidth
B_0:	static magnetic field
f:	sequence parameter (TR, TE, coil, etc...)

3D Head Motion



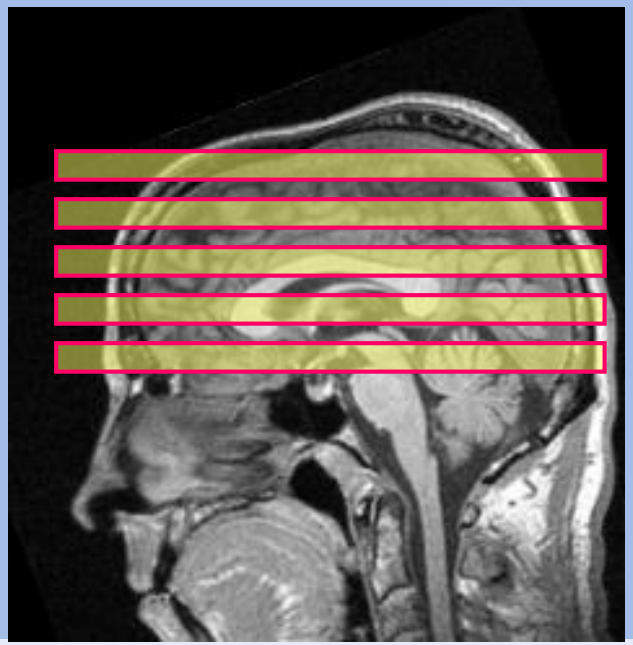


Difficulties

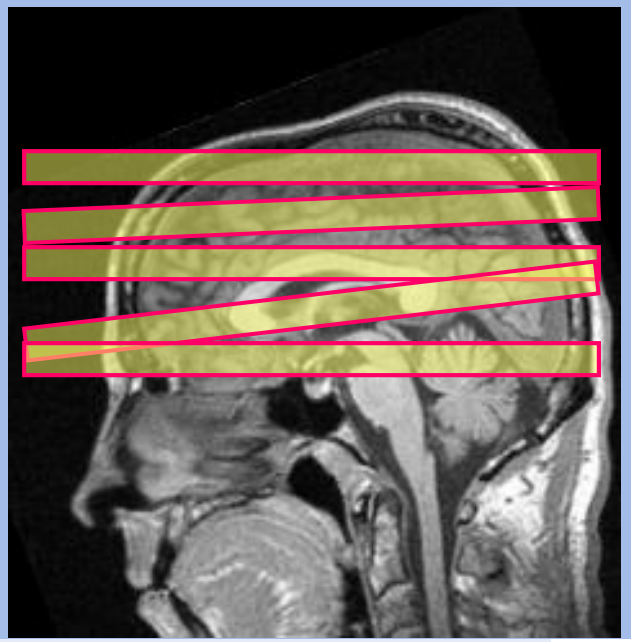
UNIVERSITÄT BERN

>Motion is 3-dimensional

expected acquisition:



slices are actually acquired like this…



Estimating the motion parameters – from data

Function to minimize

- Coefficient of variation of Ratio

$$E \equiv \frac{\sigma_R}{\mu_R} \qquad R \equiv \frac{T(image_i)}{image_{base}}$$

Woods, et al.

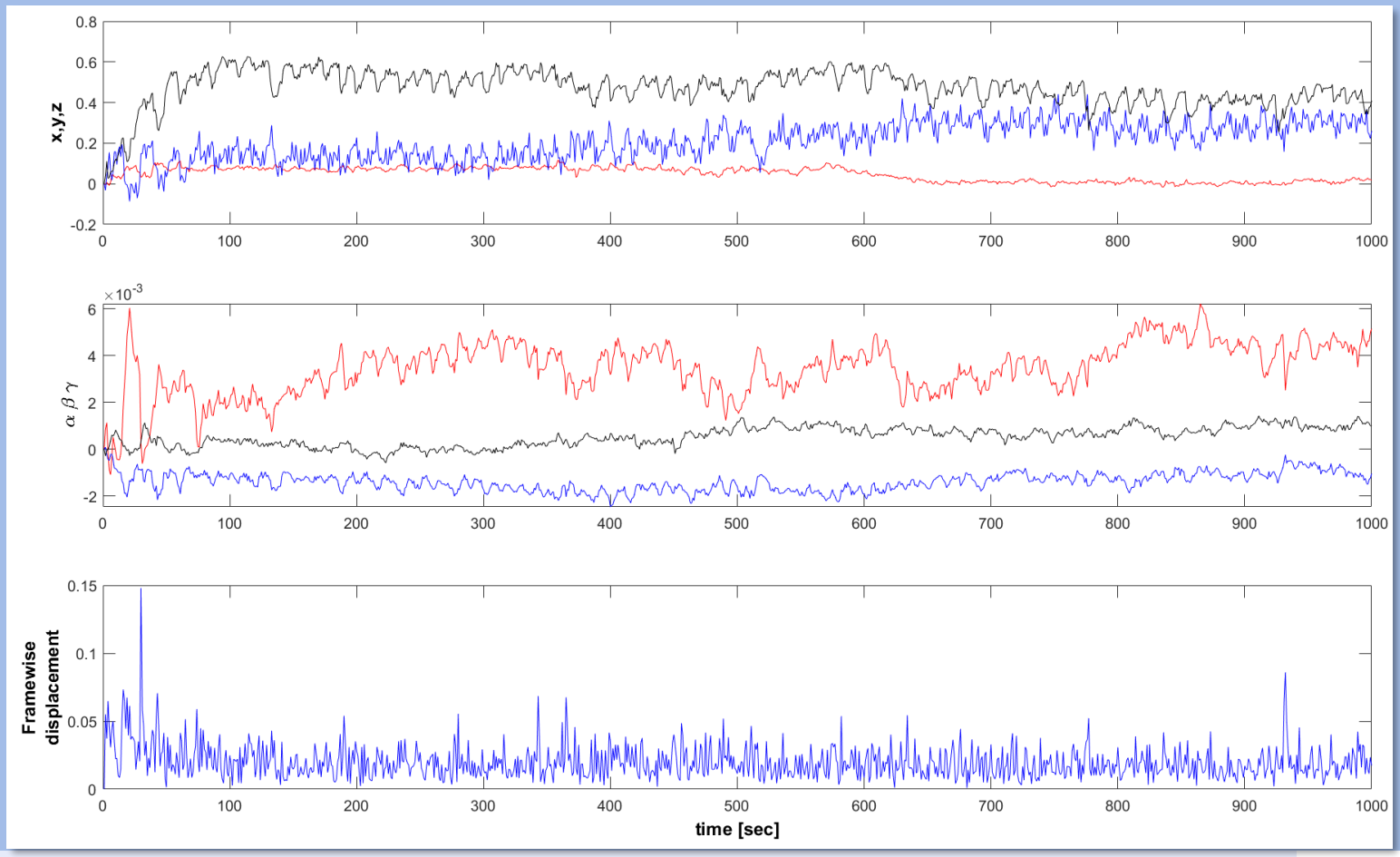
- Squared difference

$$E \equiv \sum \left[T(image_i) - image_{base} \right]^2$$

Hajnal, et al.; Eddy, et al.

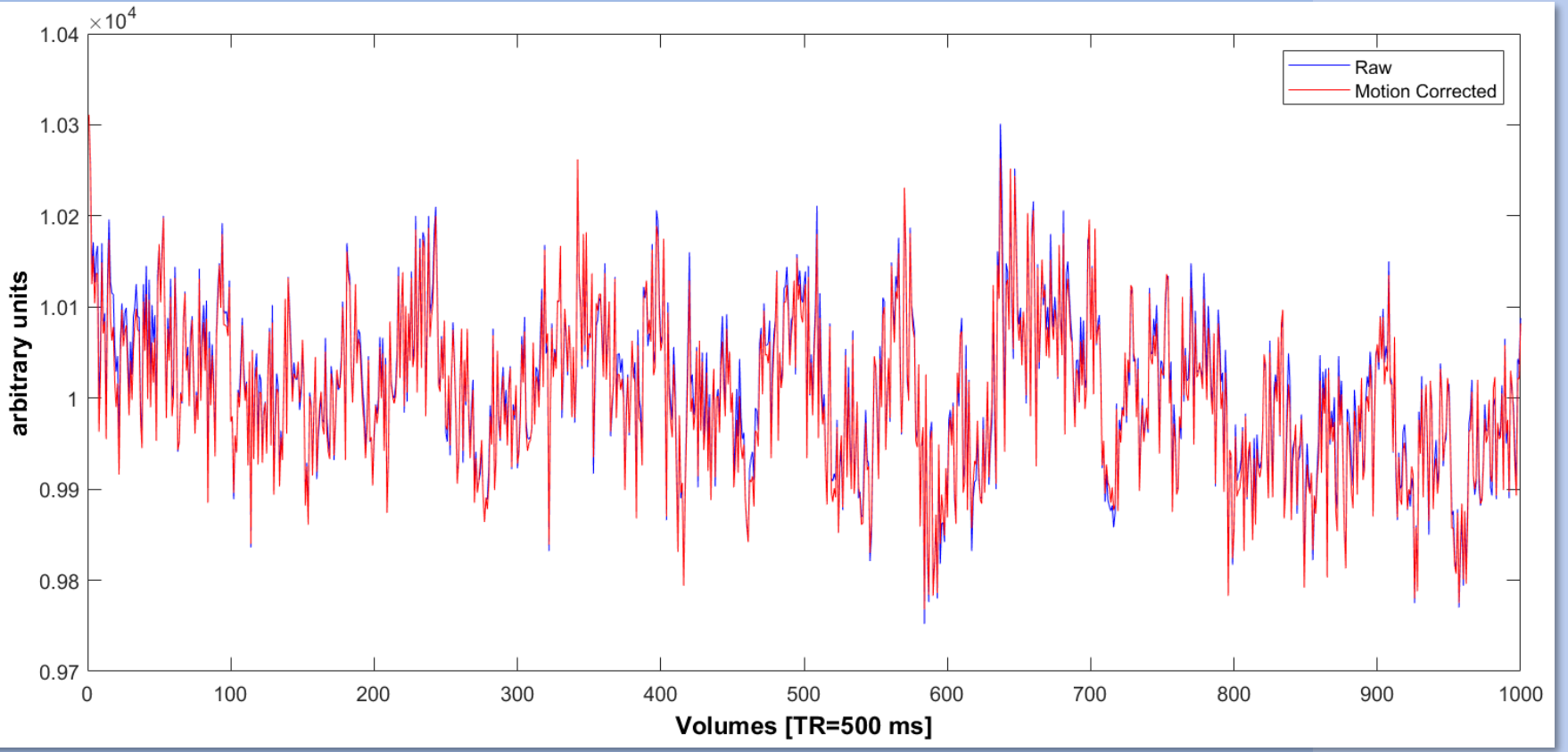
Frame wise displacement

$$FD = \left|\frac{\partial x}{\partial t}\right| + \left|\frac{\partial y}{\partial t}\right| + \left|\frac{\partial z}{\partial t}\right| + \left|\frac{\partial \alpha}{\partial t}\right| + \left|\frac{\partial \beta}{\partial t}\right| + \left|\frac{\partial \gamma}{\partial t}\right|$$



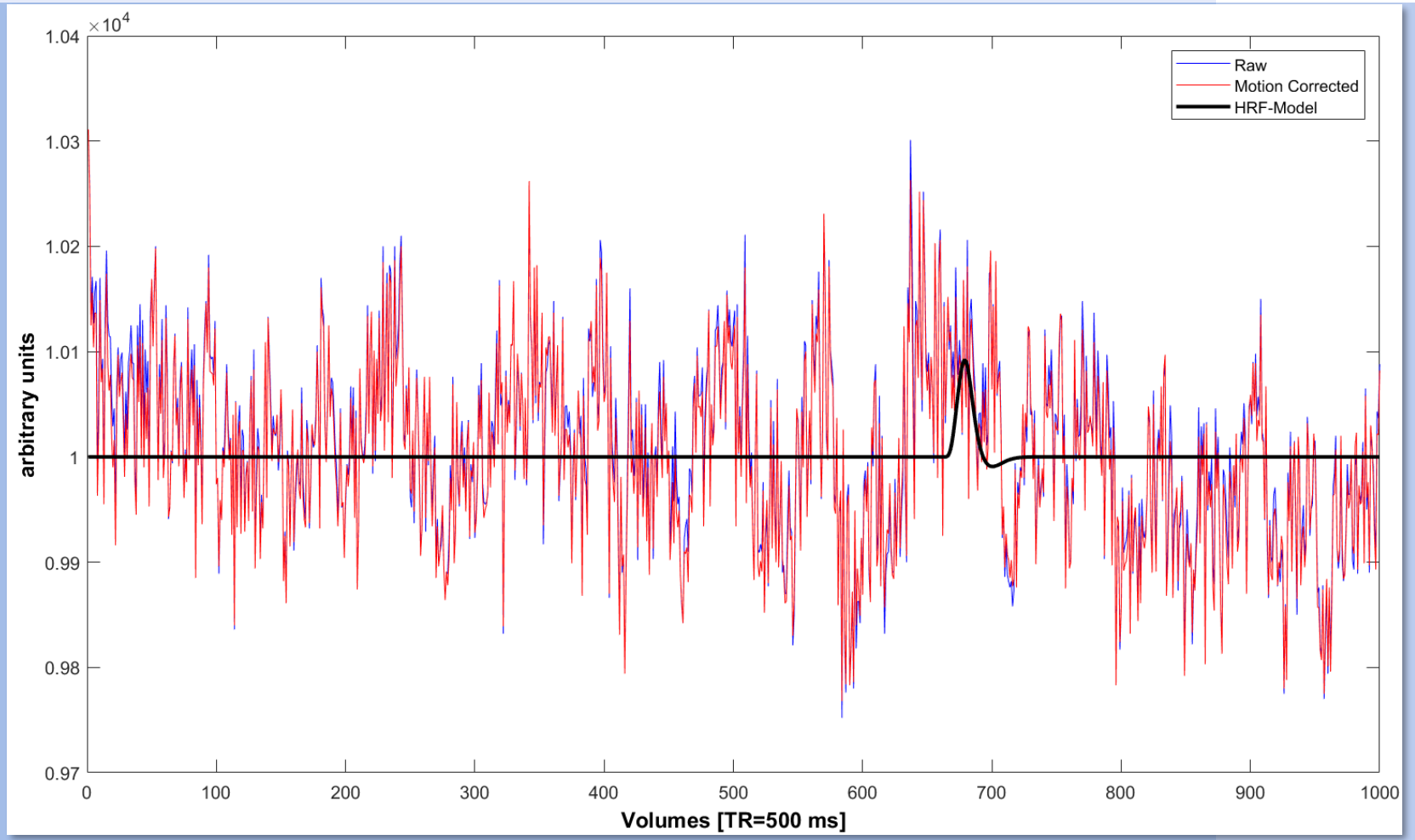
Power JD, et al. 2012; Neuroimage 59: 2142-54

Understanding fMRI signal



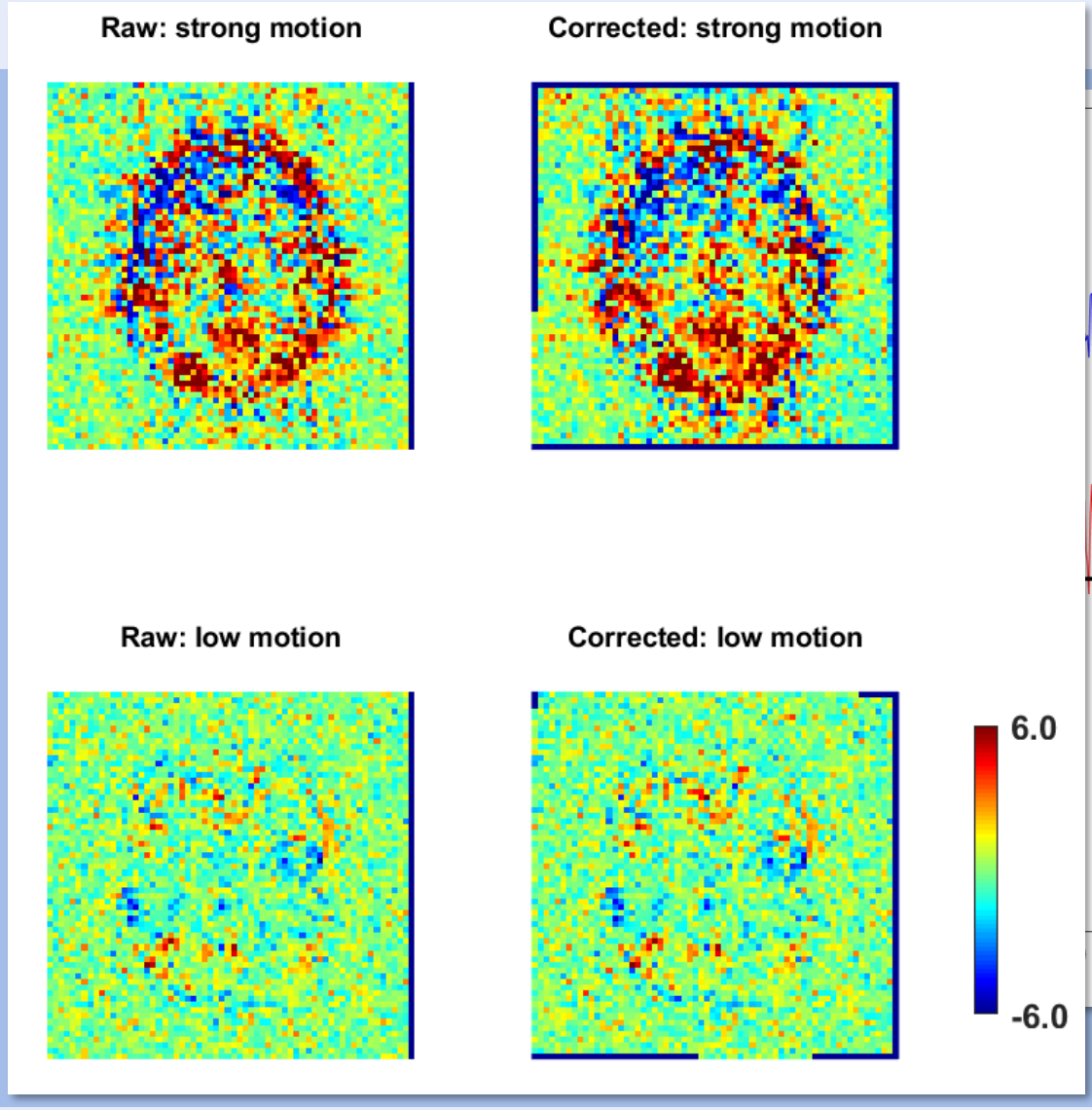
Small motion

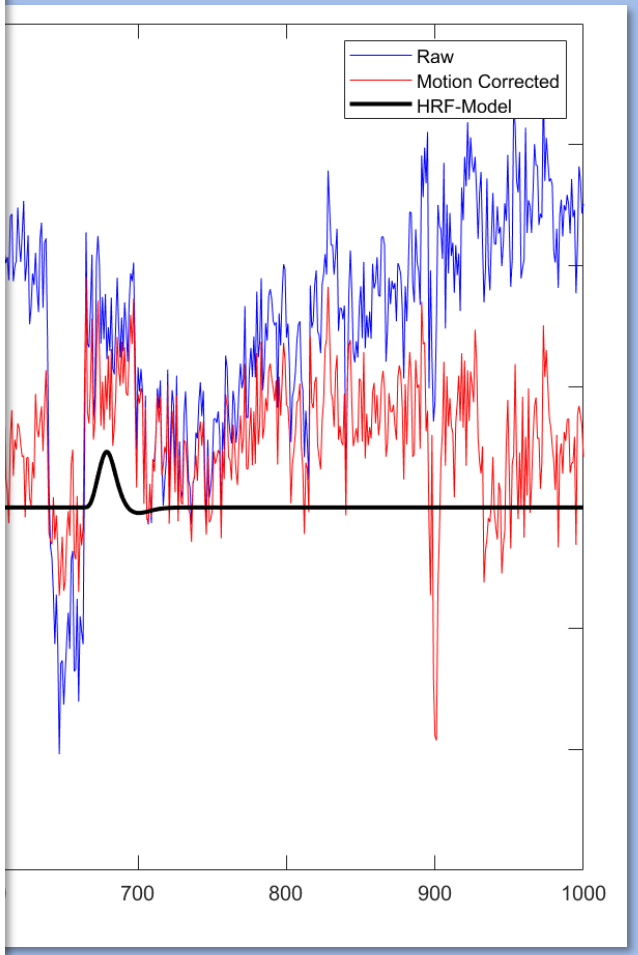
Understanding fMRI signal



Small motion

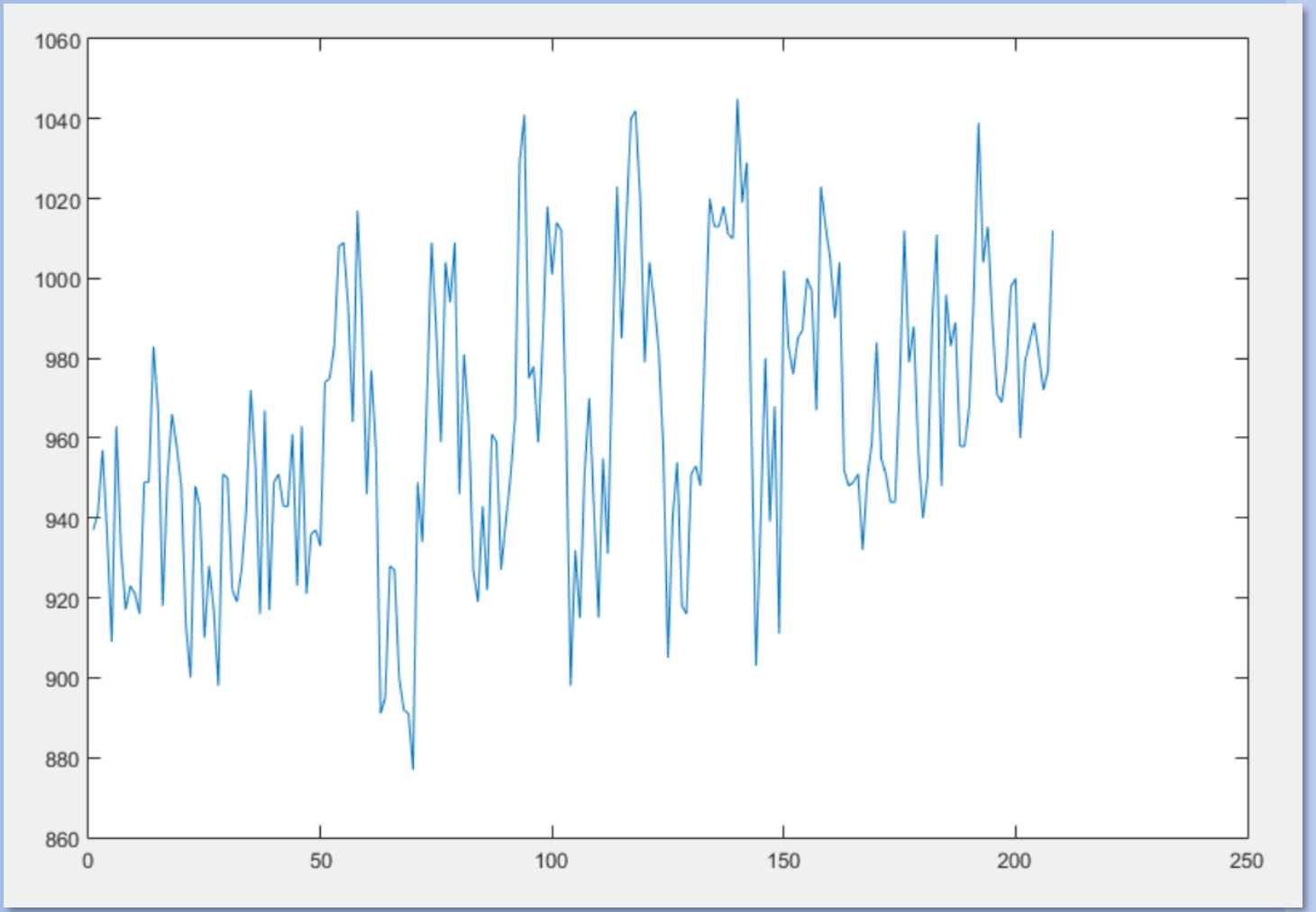
3D Head Motion



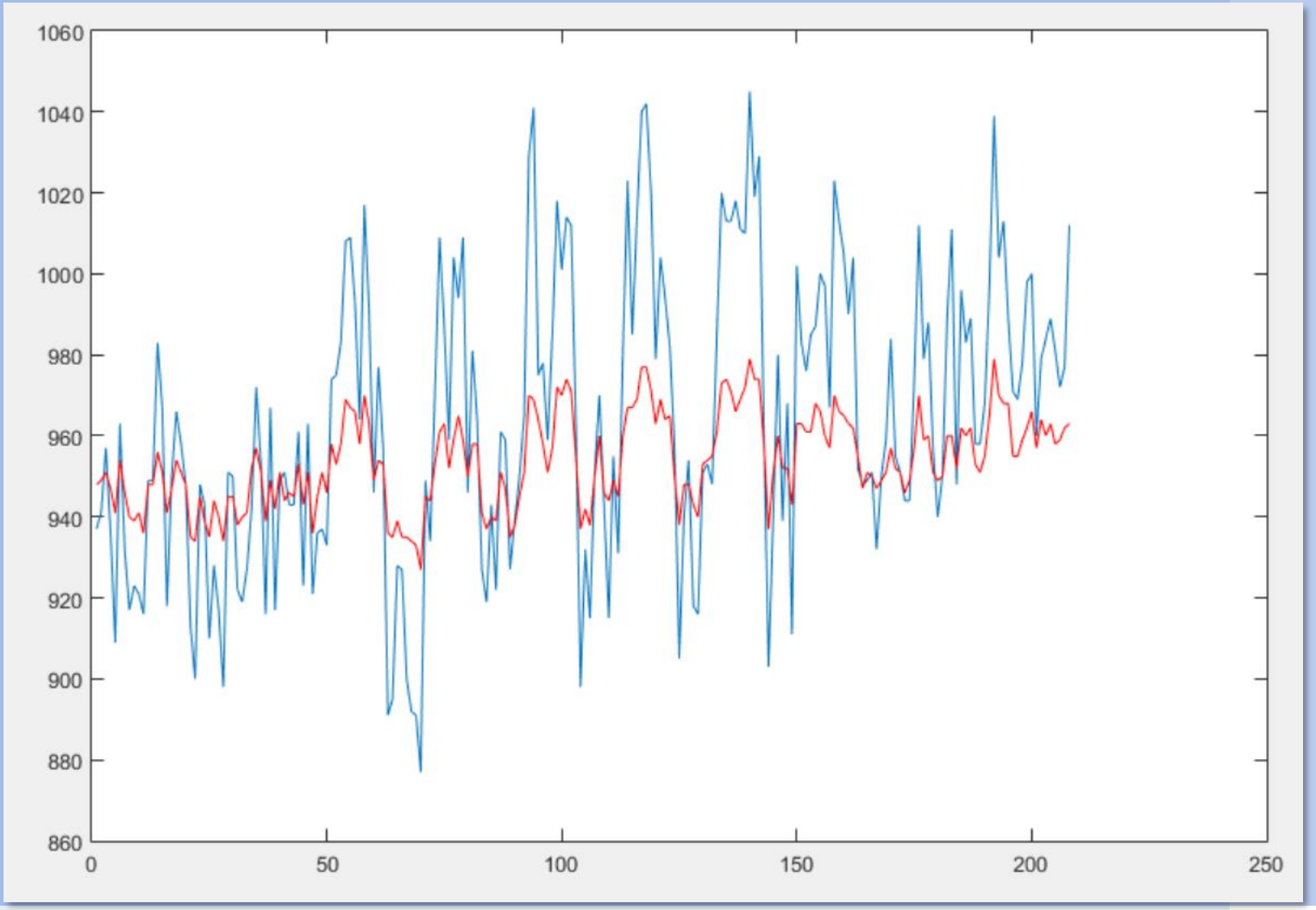


large motion

Understanding fMRI signal

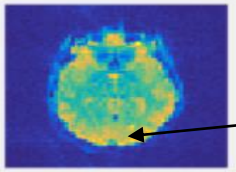


Understanding fMRI signal

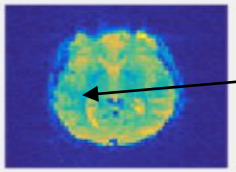


Global Signal regression

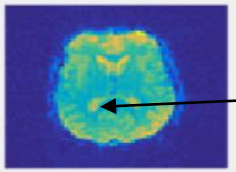
Understanding fMRI signal



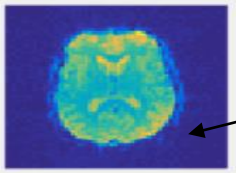
V1



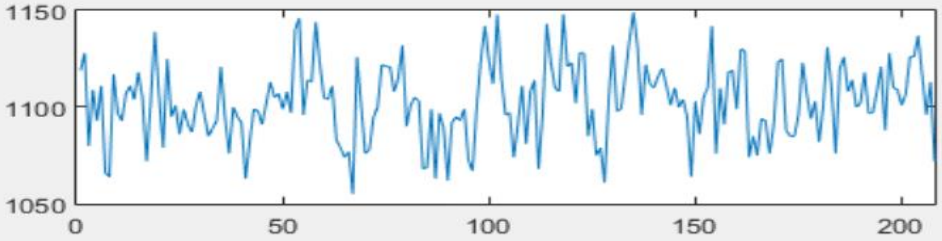
WM

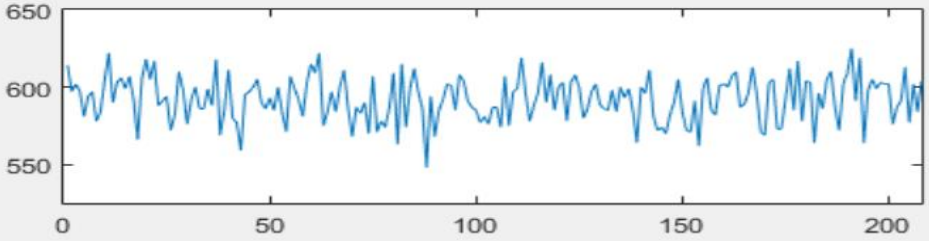


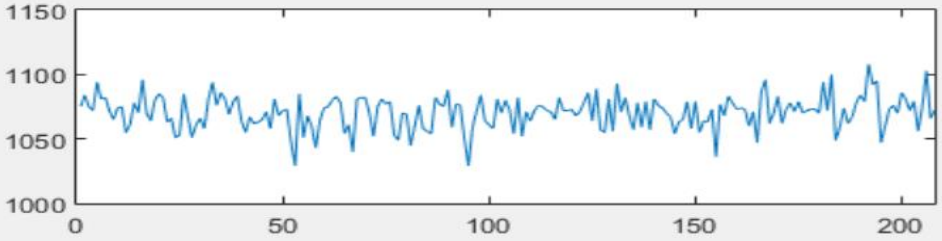
CSF

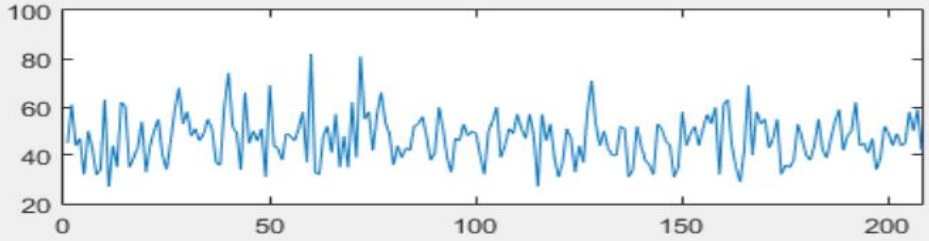


Outside brain









Understanding fMRI signal

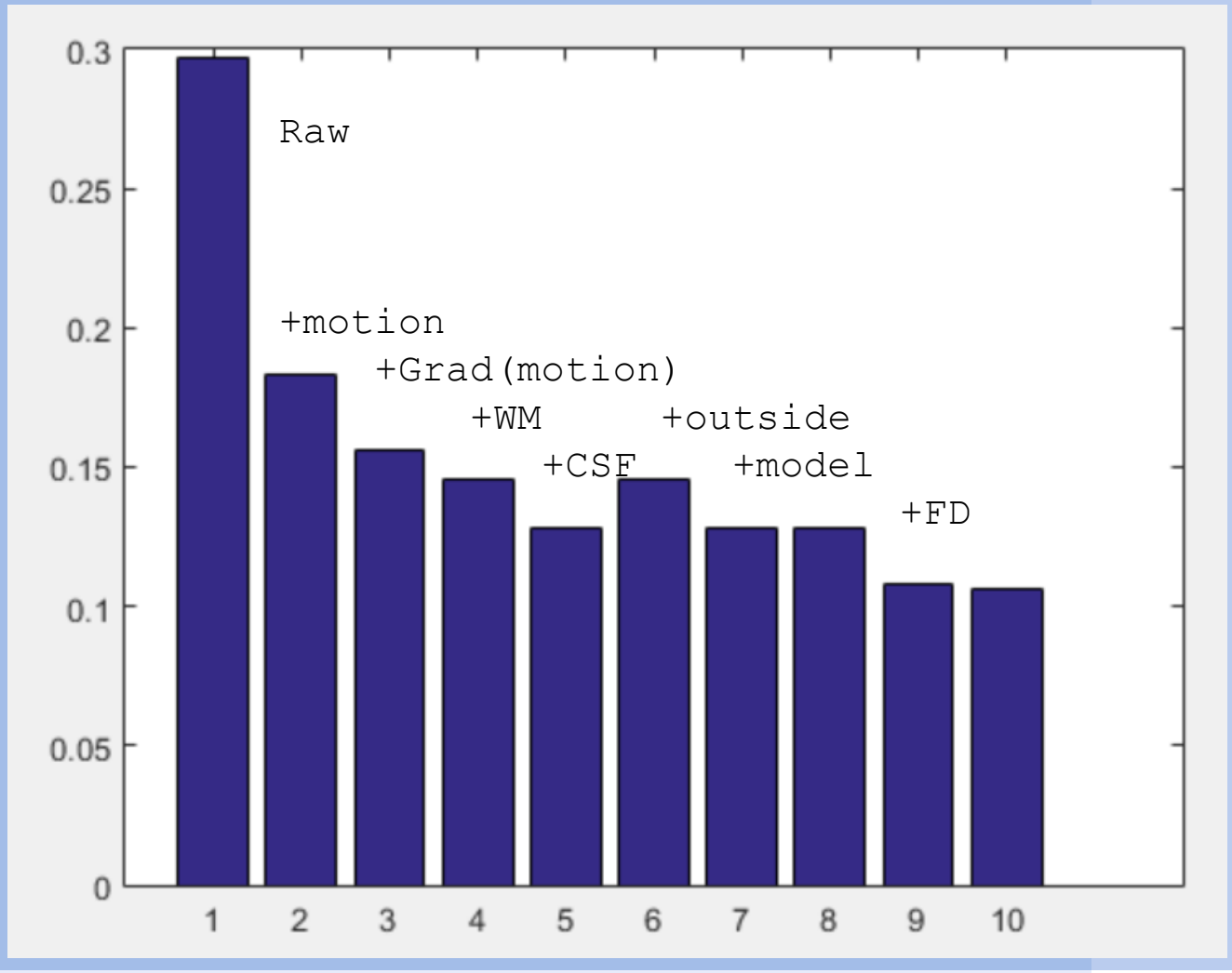
$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + \varepsilon$$

```
[b,dev,stats1] = glmfit(Bold_th,Bold_measure);
```

$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + S_{WM} + S_{CSF} + S_{GM} + \ldots + \varepsilon$$

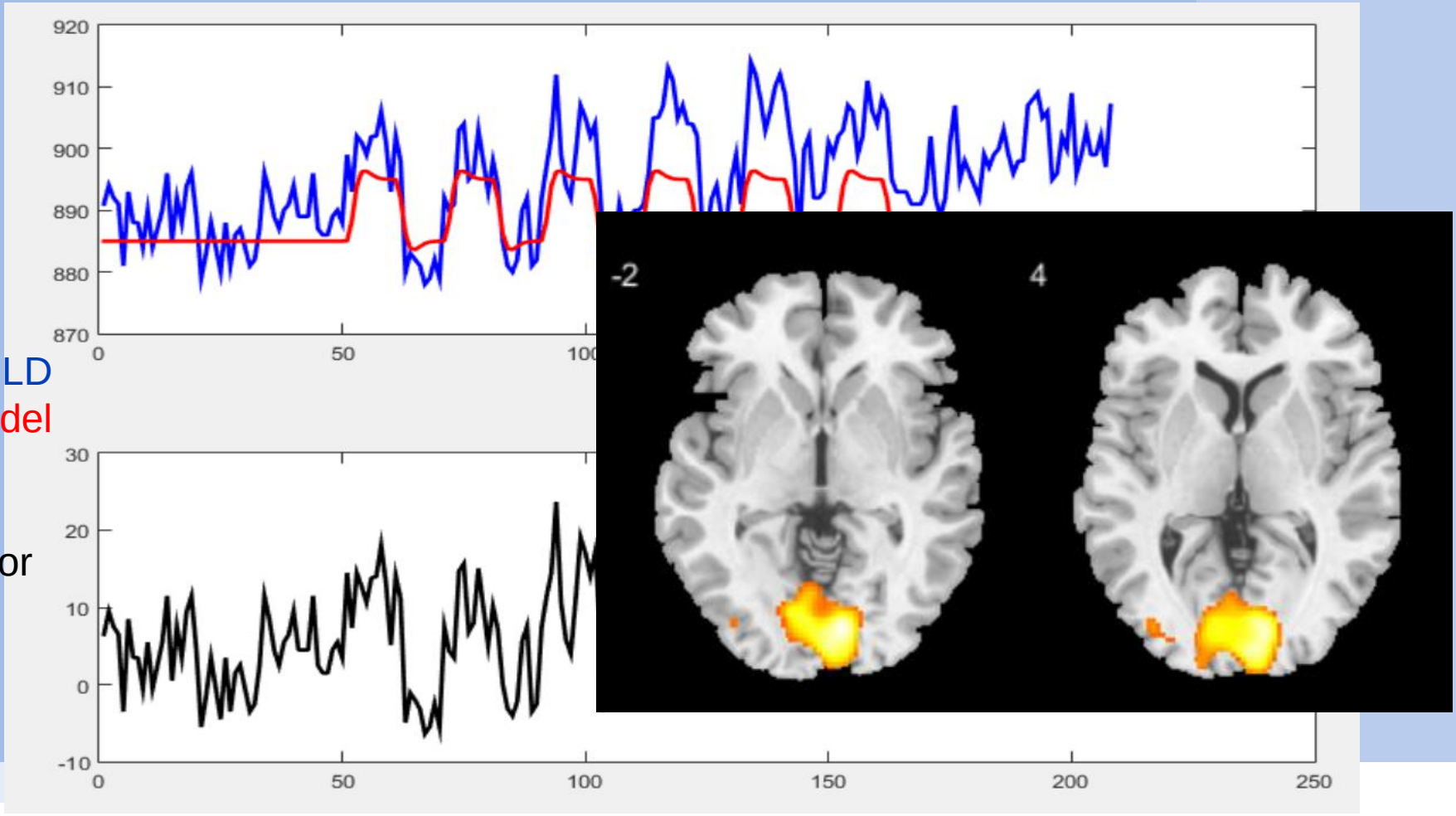
```
[b,dev,stats2] = glmfit(
        [mot dmot fd wm csf n0 Bold_th],Bold_measure);
```

Understanding fMRI signal



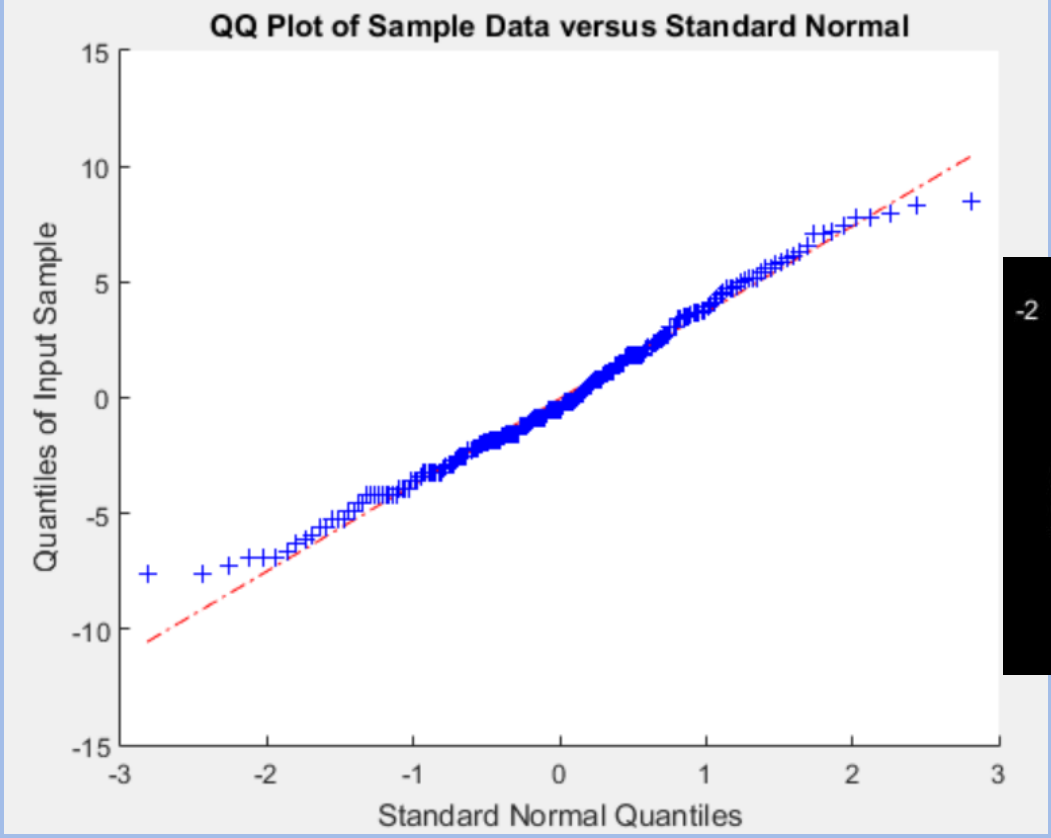
Understanding fMRI signal

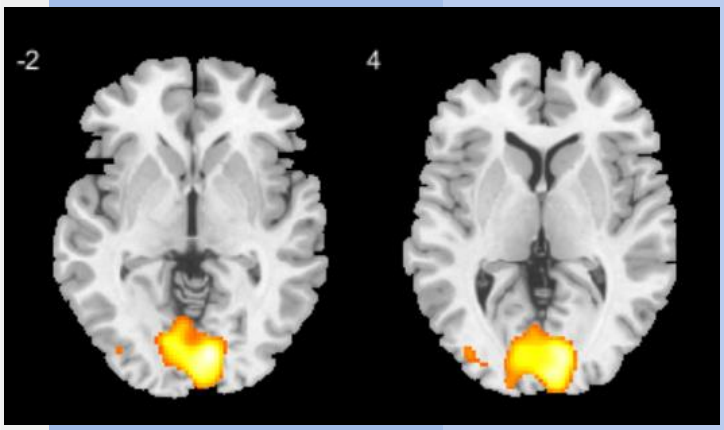
$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + S_{WM} + S_{CSF} + S_{GM} + \ldots + \varepsilon$$



Understanding fMRI signal

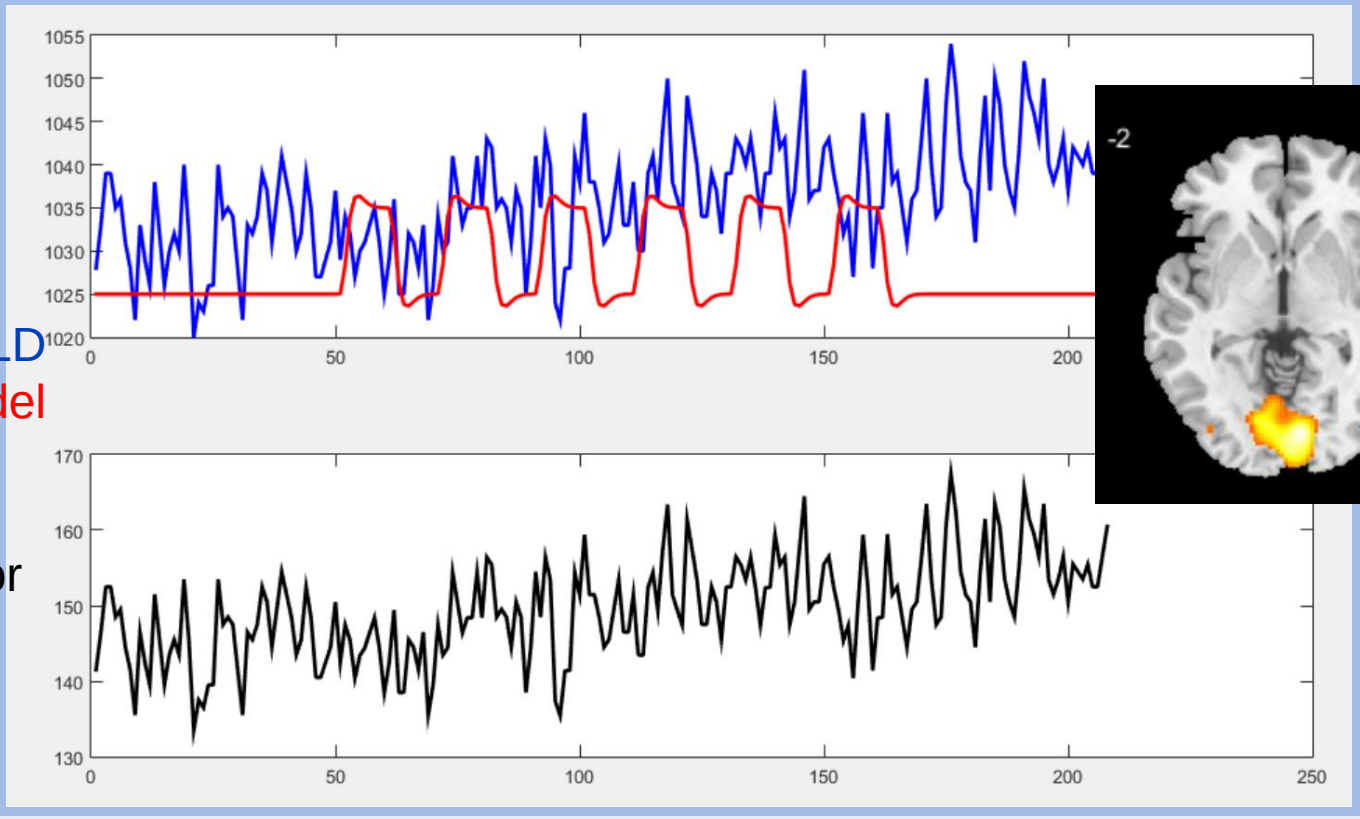
$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + S_{WM} + S_{CSF} + S_{GM} + \ldots + \varepsilon$$

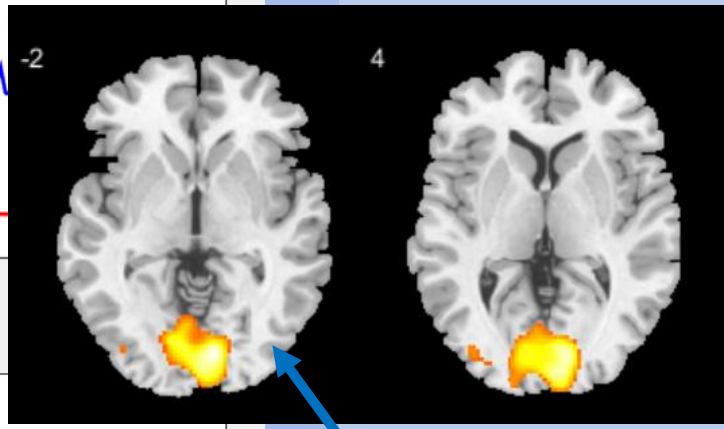




Understanding fMR signal

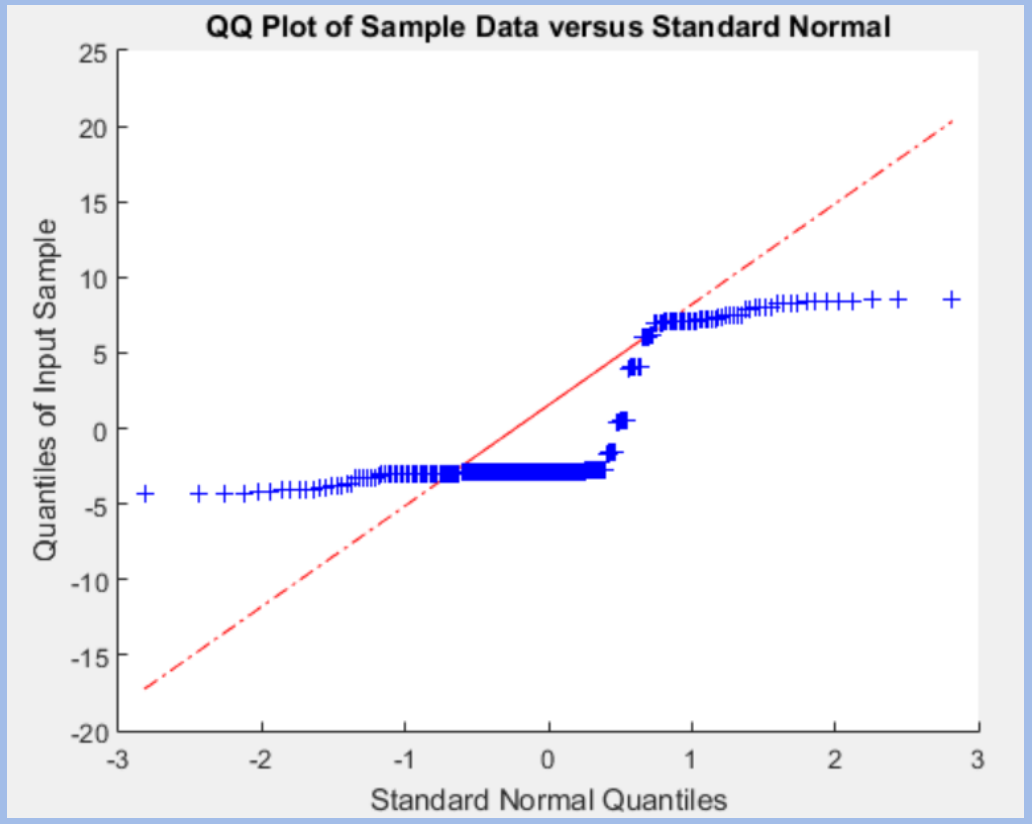
$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + S_{WM} + S_{CSF} + S_{GM} + \ldots + \varepsilon$$

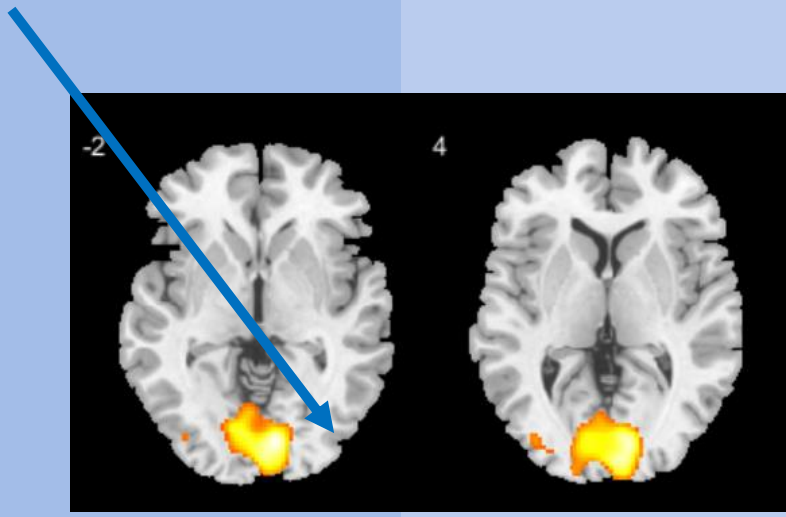




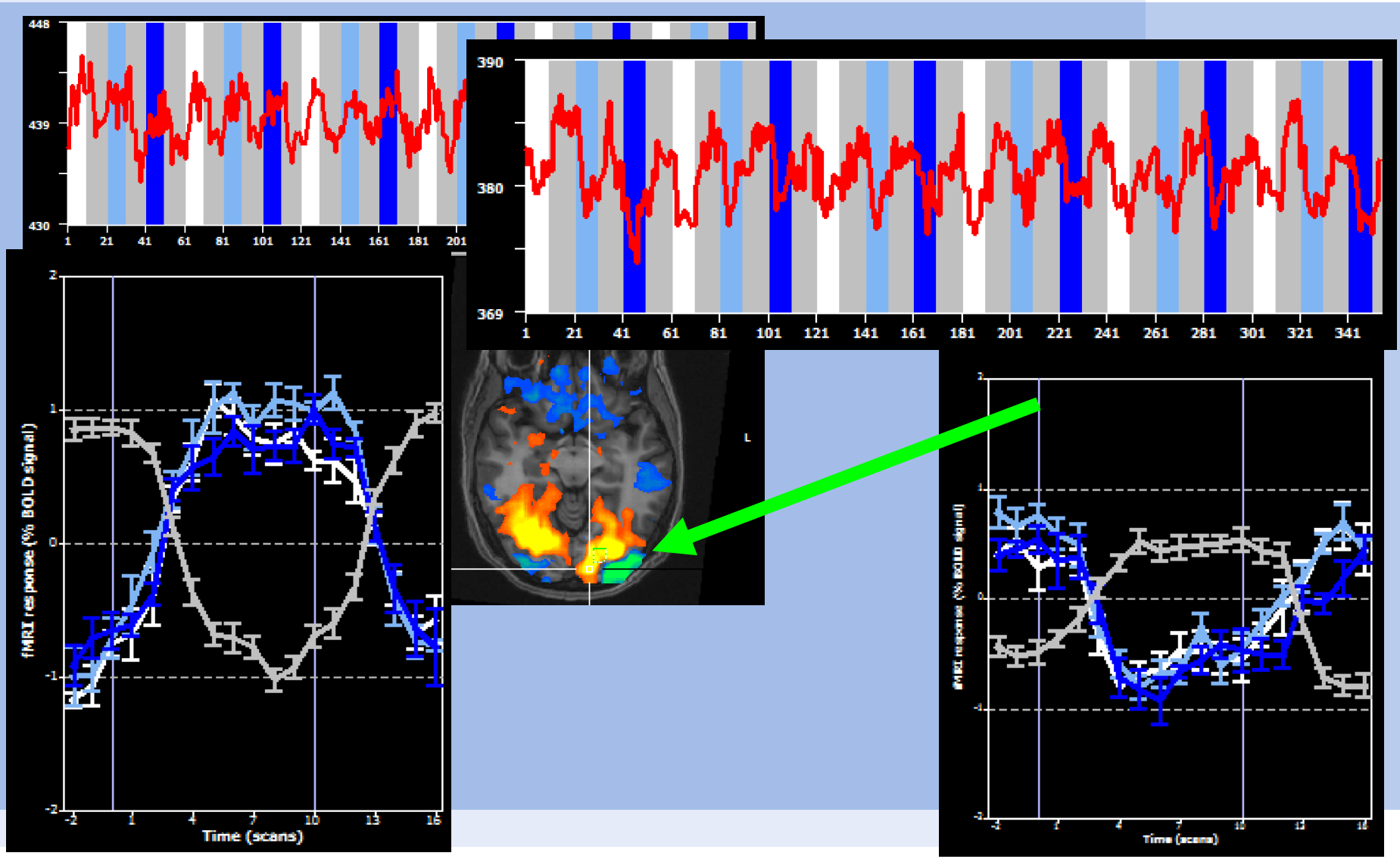
Understanding fMRI signal

$$BOLD_{signal} = \beta_0 + X_{hrf} * \Theta + S_{WM} + S_{CSF} + S_{GM} + \ldots + \varepsilon$$

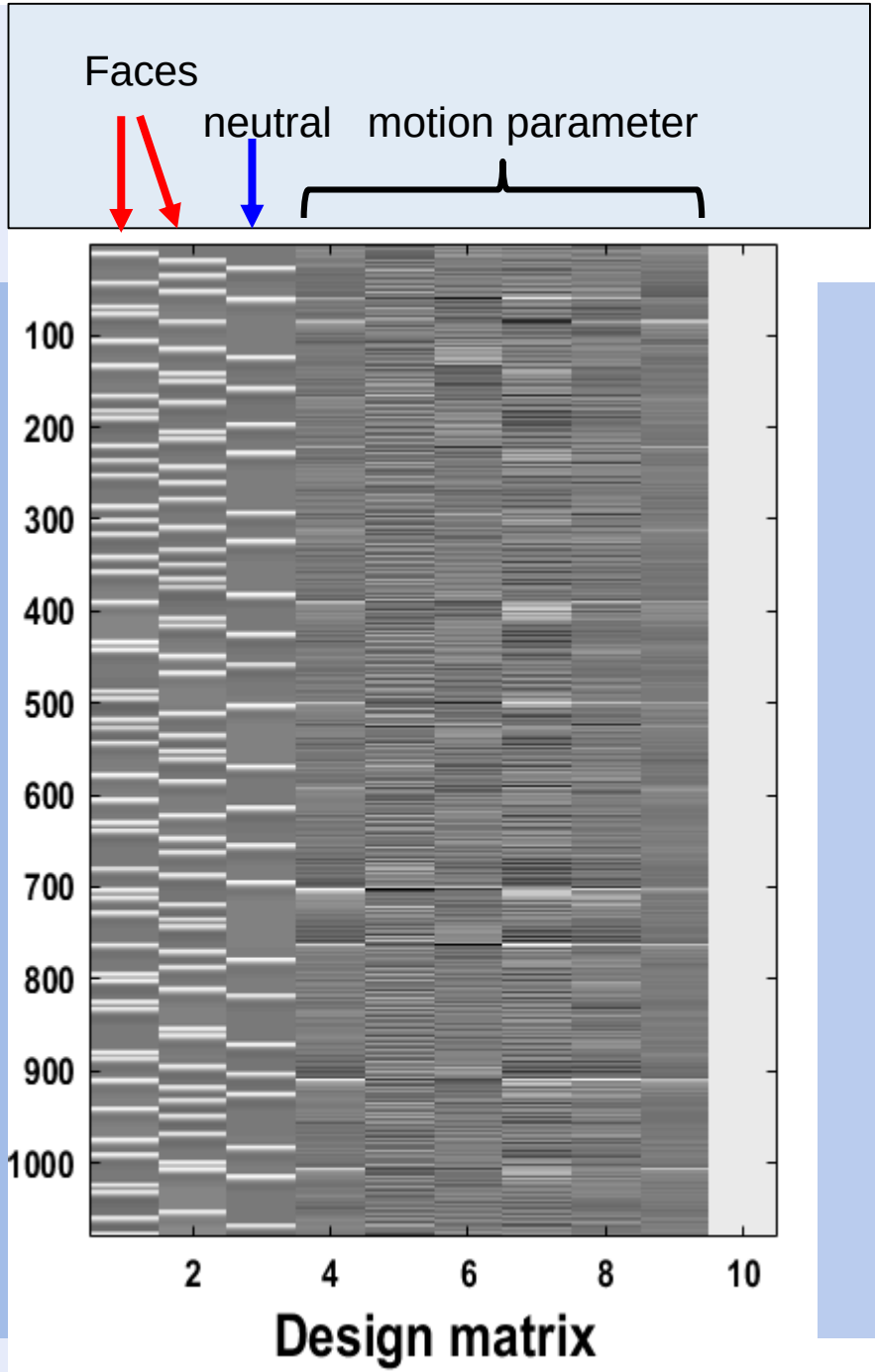




Baseline in fMRI signal



Event related fMRI

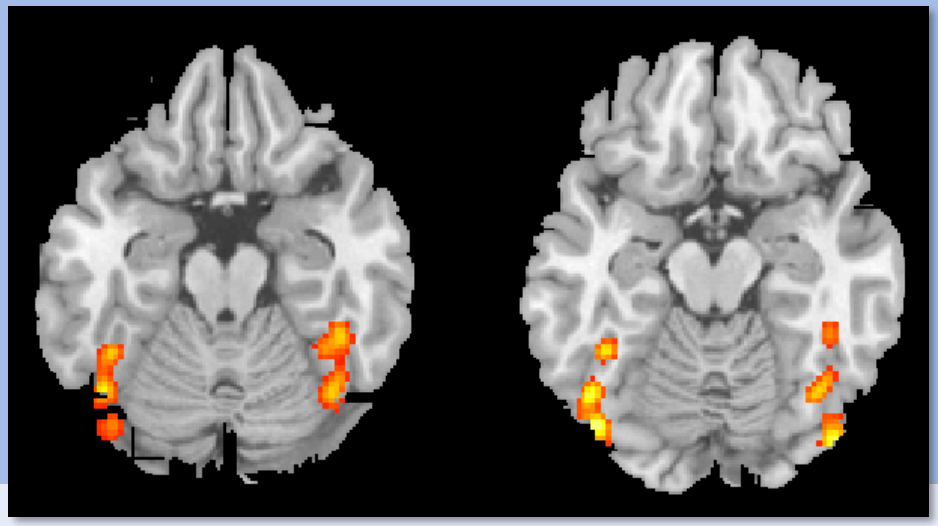


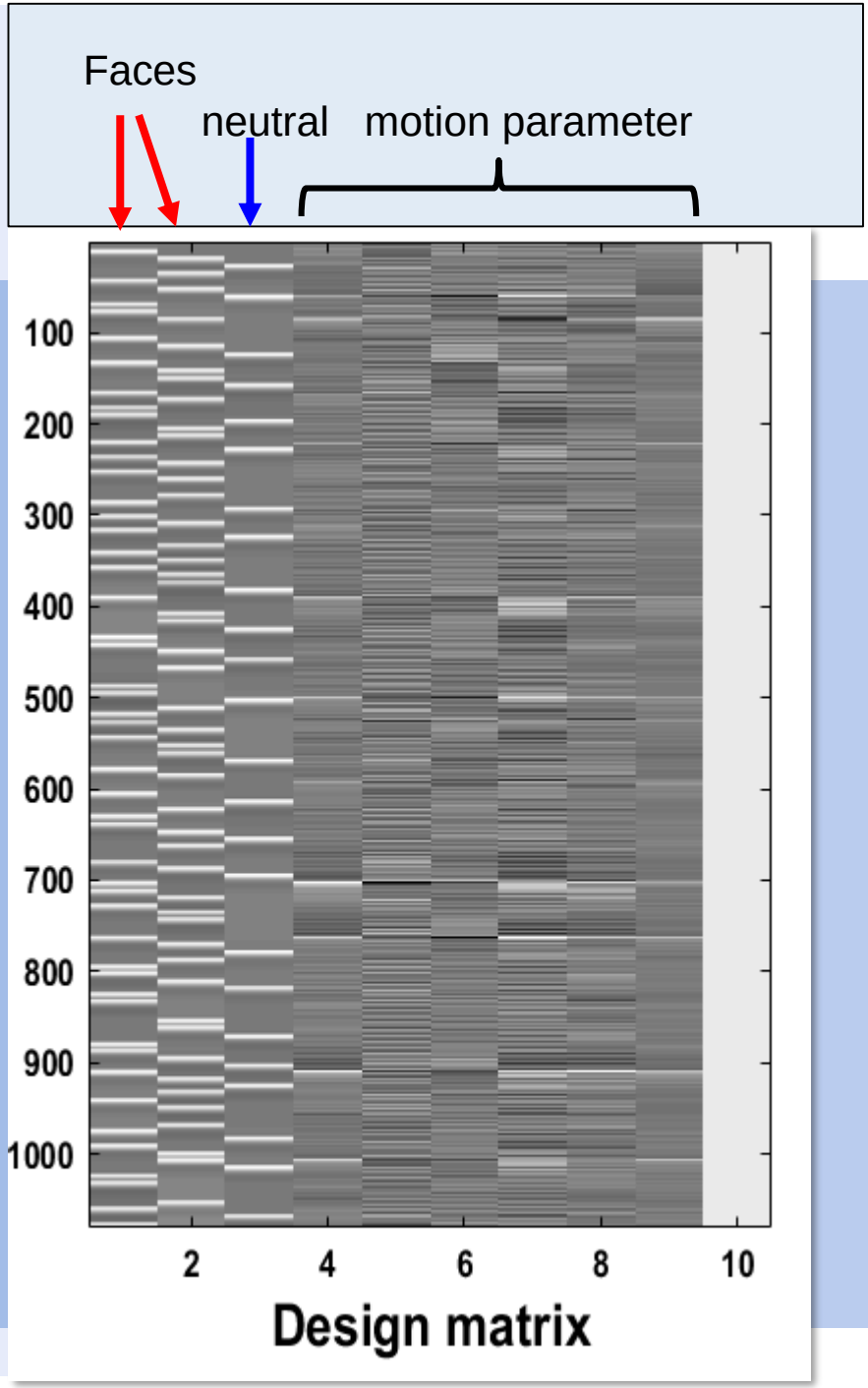
Event related fMRI

Contrast: faces vs neutral

Question: **Where ?**

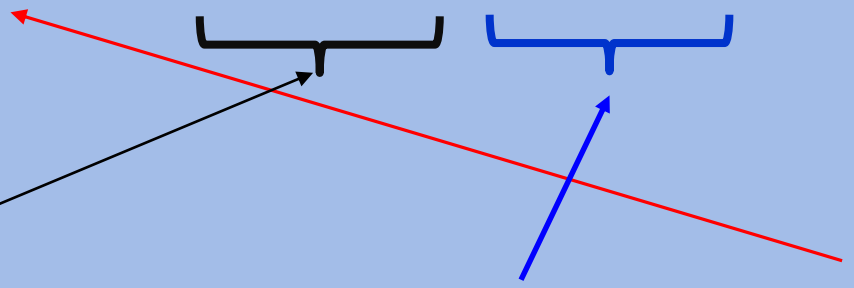
Expectation: fusiform gyrus /fusiform face area





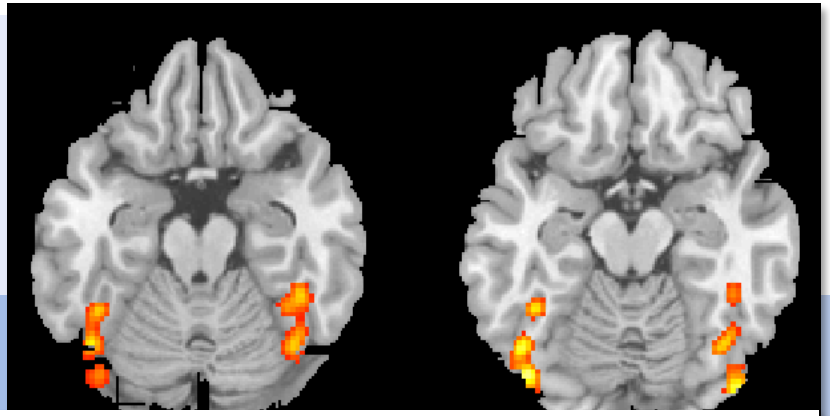
General Linear Model

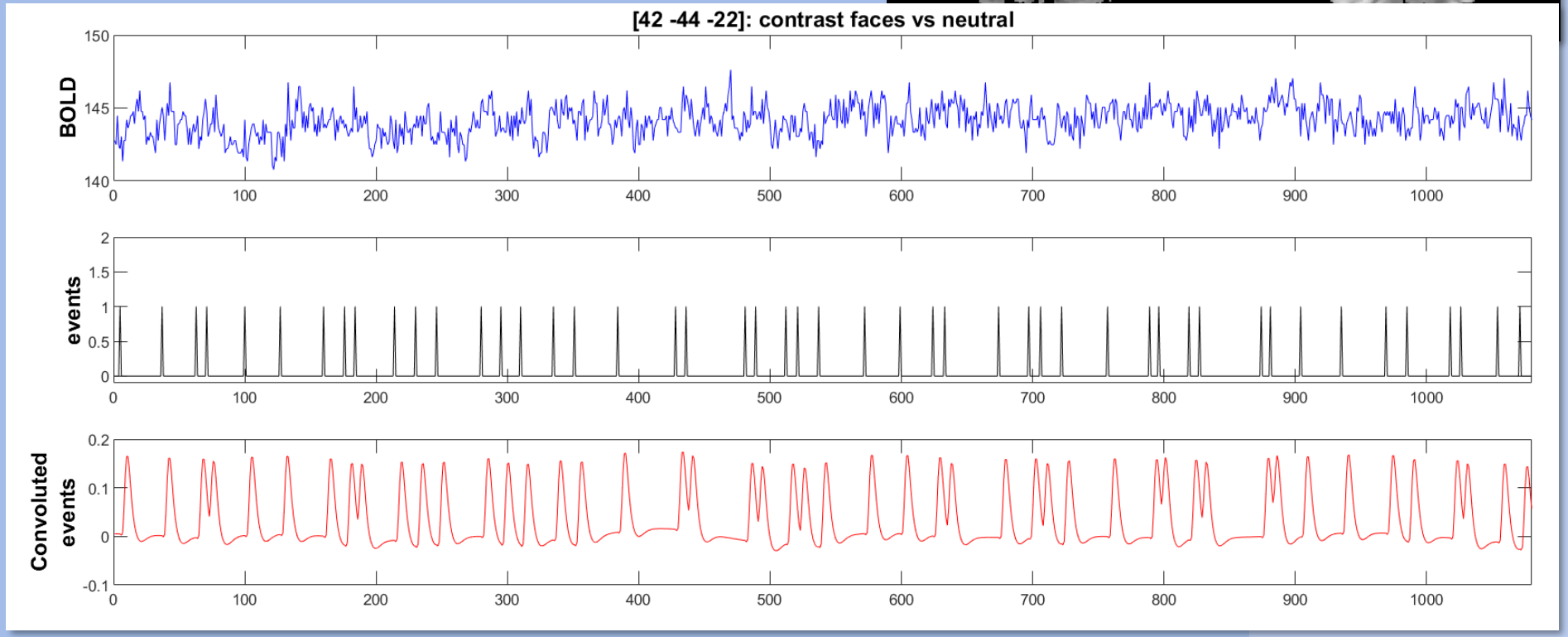
$$BOLD_{Signal} = b_0 + b_1 * X_{\substack{Stimulus \\ events}} + e$$



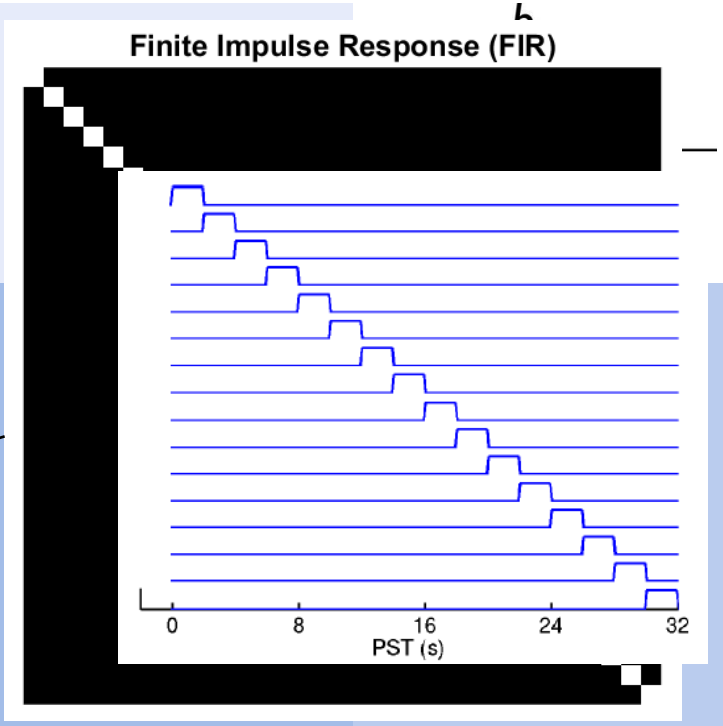
```
[betas,dev,stats] = glmfit(Bold_model , Bold_signal);
```

Event related fMRI



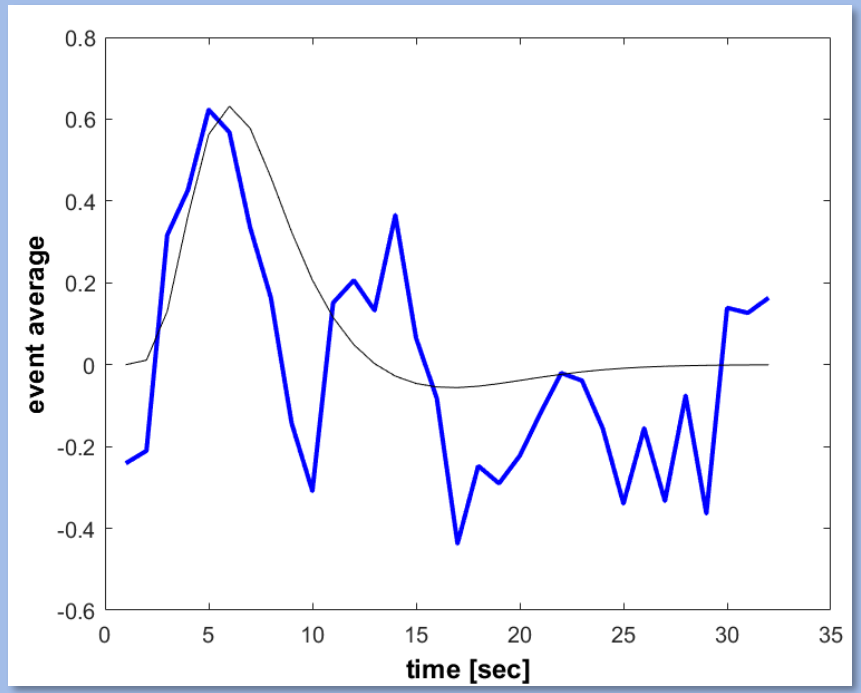


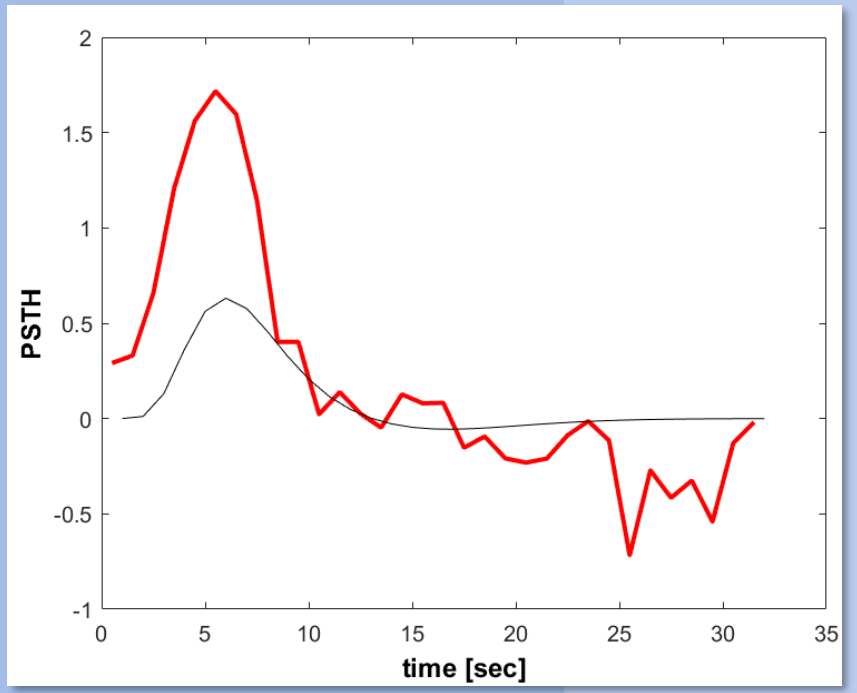
Event related fMRI




```
N=size(Events,1)
FIR=eye(32);
estimated_HRF=zeros(N,32);
for n=1:N
    Twindow=single(yBOLD(Events(n)+2:Events(n)+33));
    for time=1:32
        [b,de,st]=glmfit(FIR(time,:),Twindow);
        estimated_HRF(n,time)=b(2);
    end
end
estimated_HRF=mean(estimaed_HRF,1);
```

How sure can you be ?

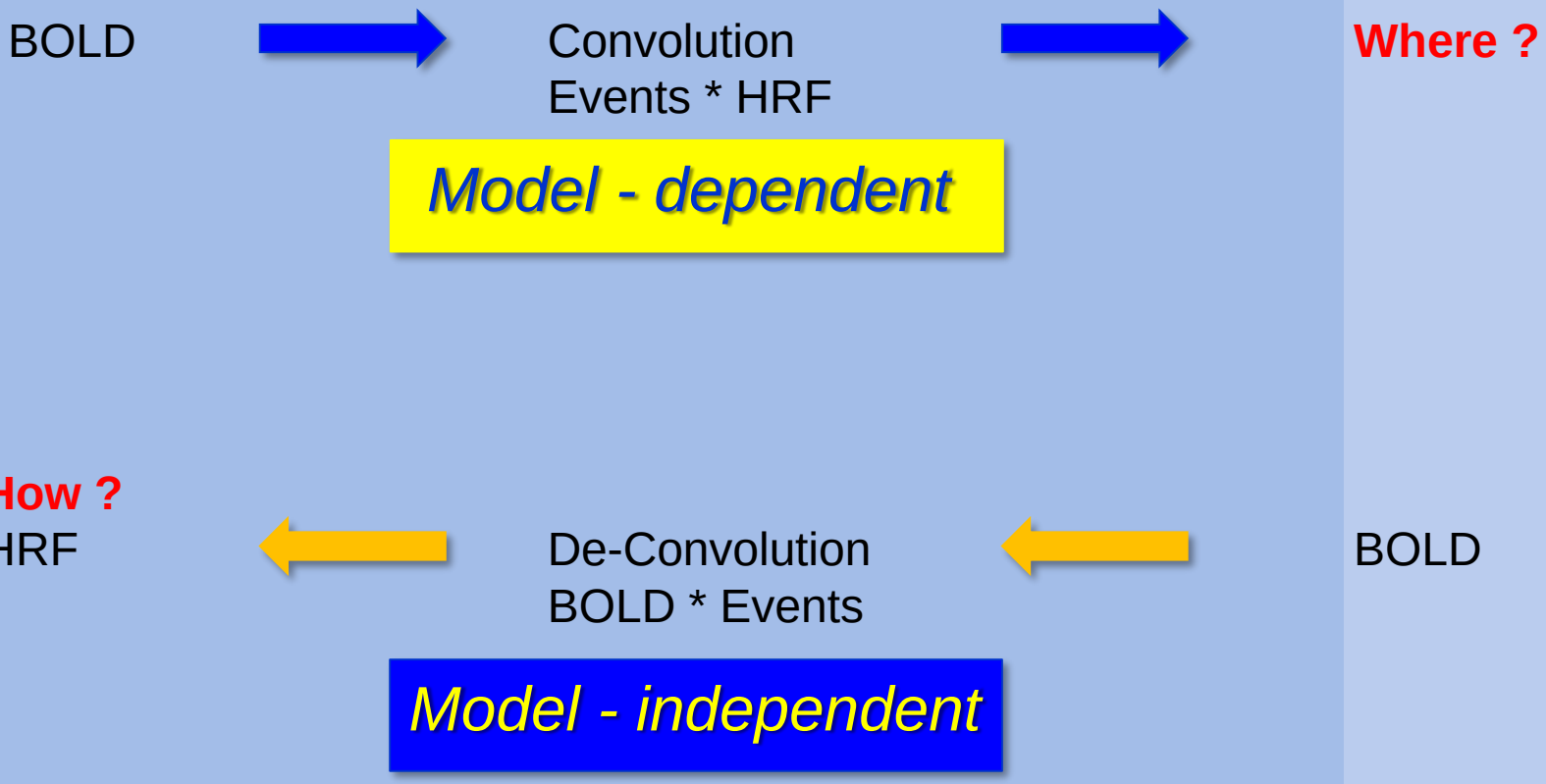




`estimated_HRF`

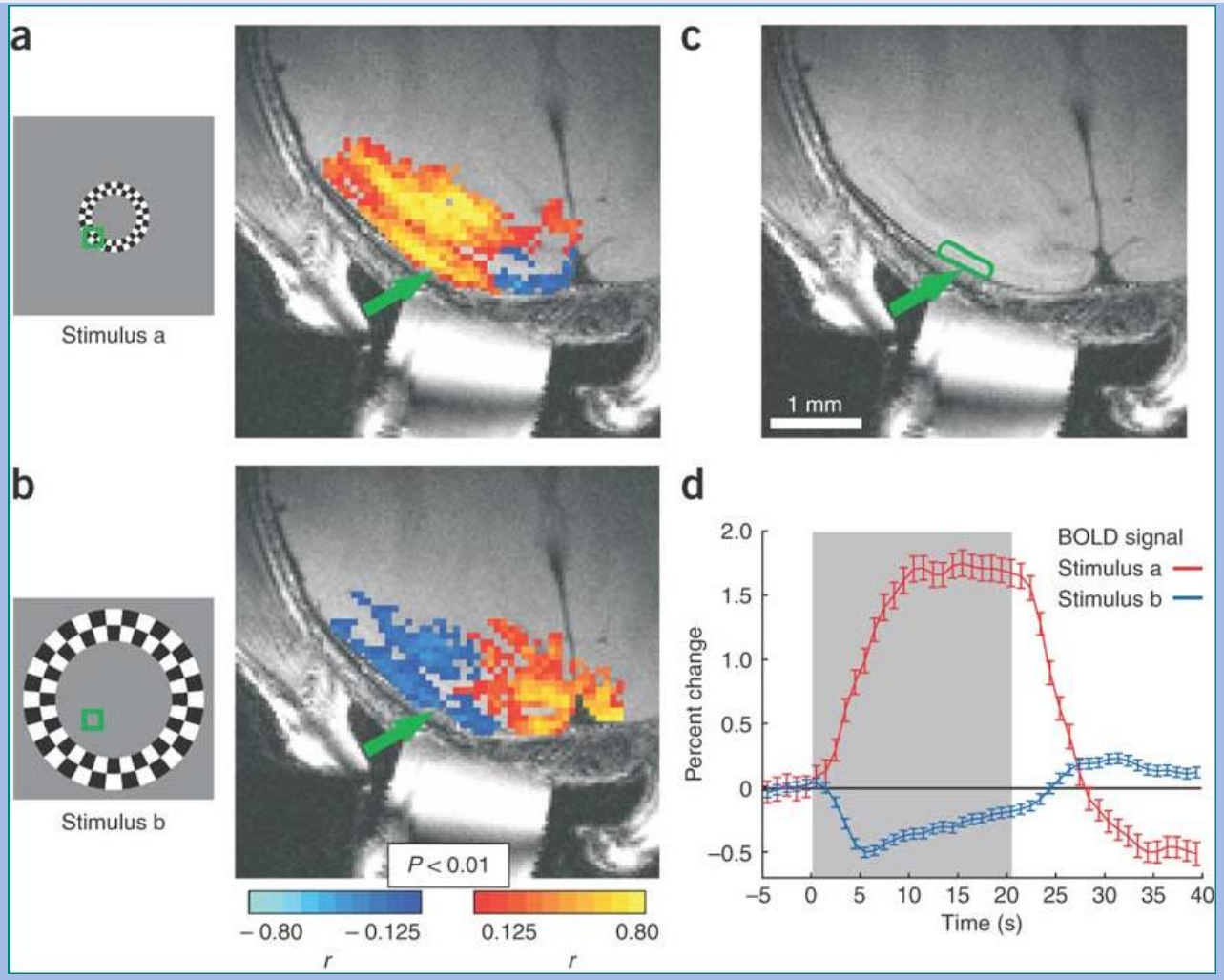
Event related fMRI: check results

UNIVERSITÄT BERN



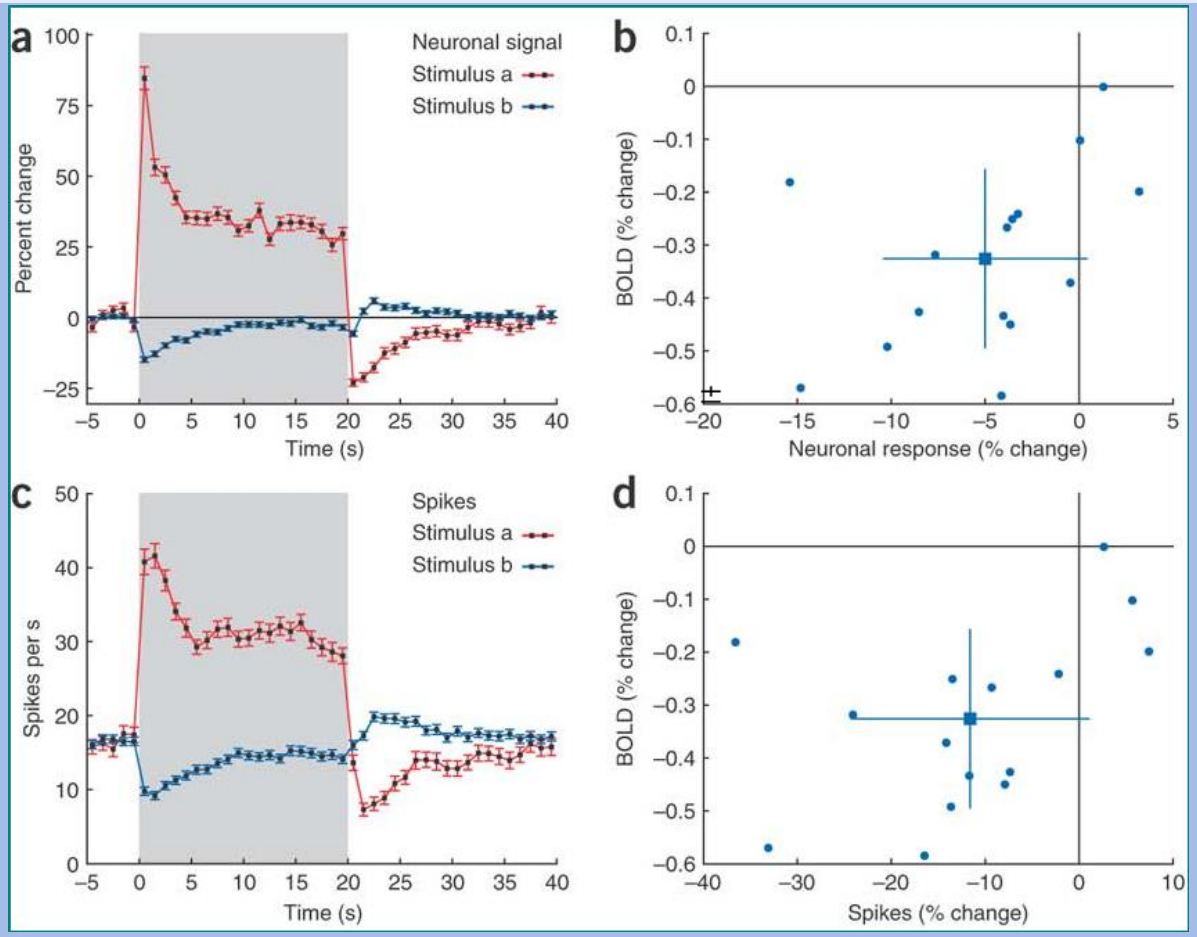
HRF: Hemodynamic Response Function

Negative BOLD: challenging



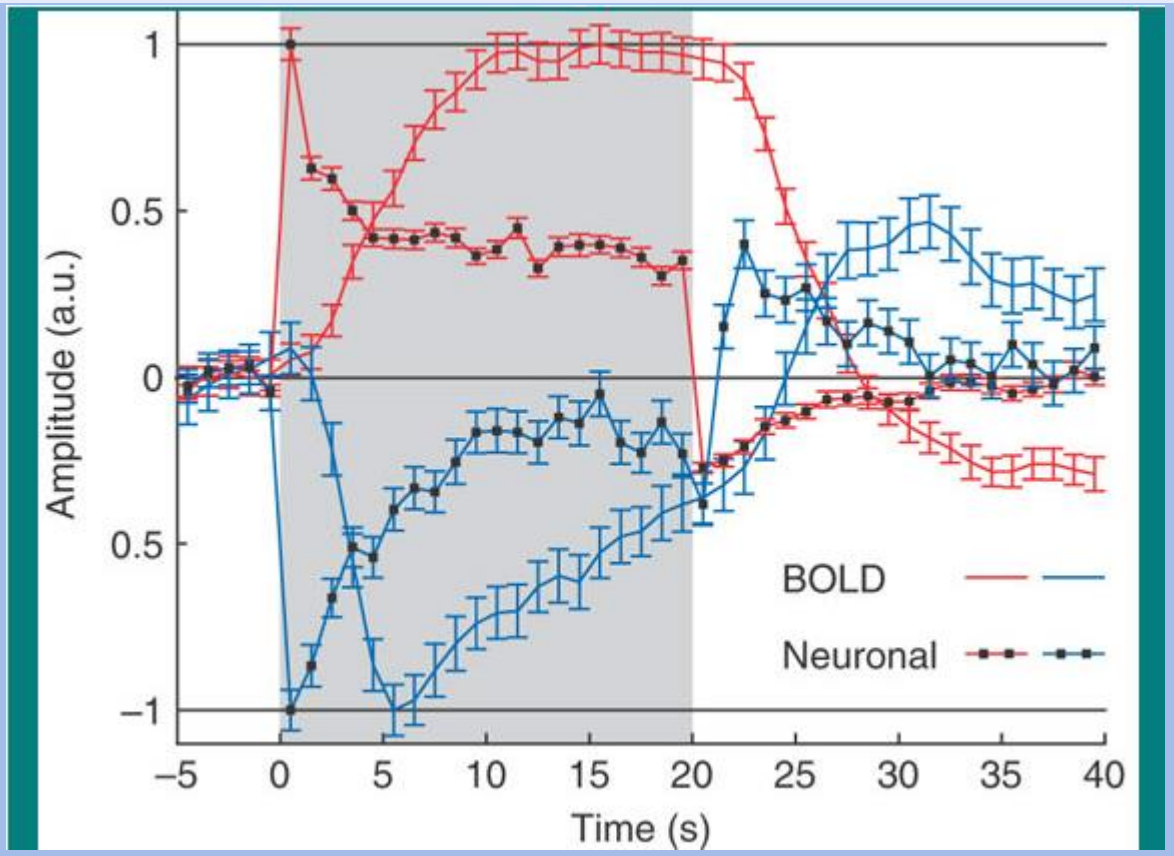
Shmuel A. et al., 2006 *Nat Neurosc* 9(4):569-577.

Negative BOLD: challenging



Shmuel A. et al., 2006 *Nat Neurosc* 9(4):569-577.

Negative BOLD: challenging

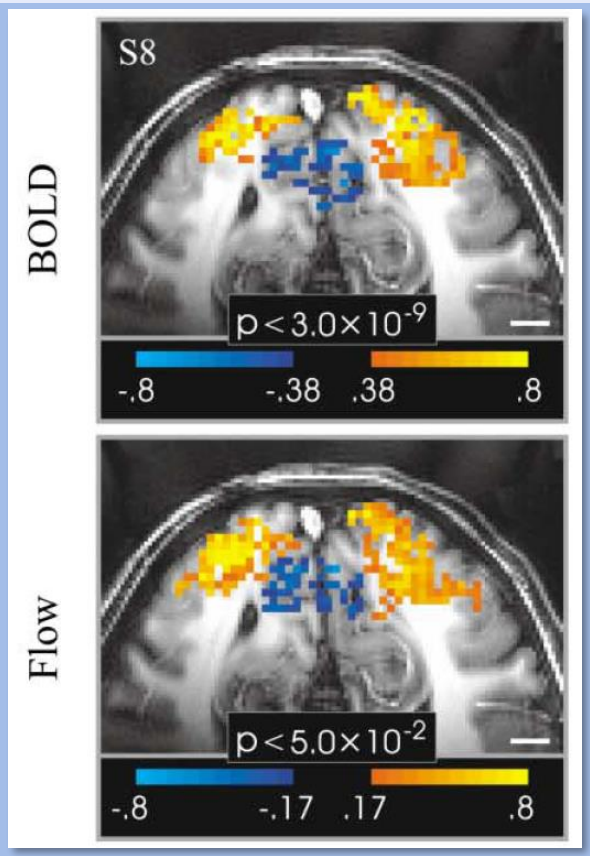


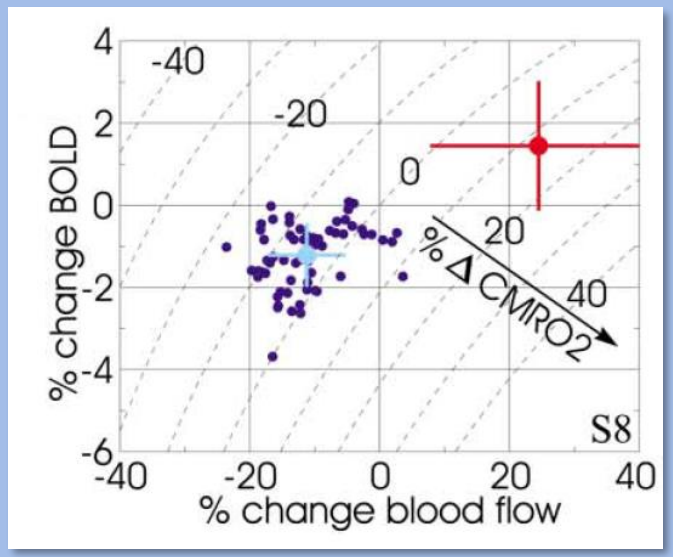
Could NBR be originated by ↓ CBF ? Caused by hypoxia
Could NBR be originated by «vascular steel» ?

Shmuel A. et al., 2006 *Nat Neurosc* 9(4):569-577.

NBR: BOLD and CBF* measure

* Cerebral Blood Flow (CBF)

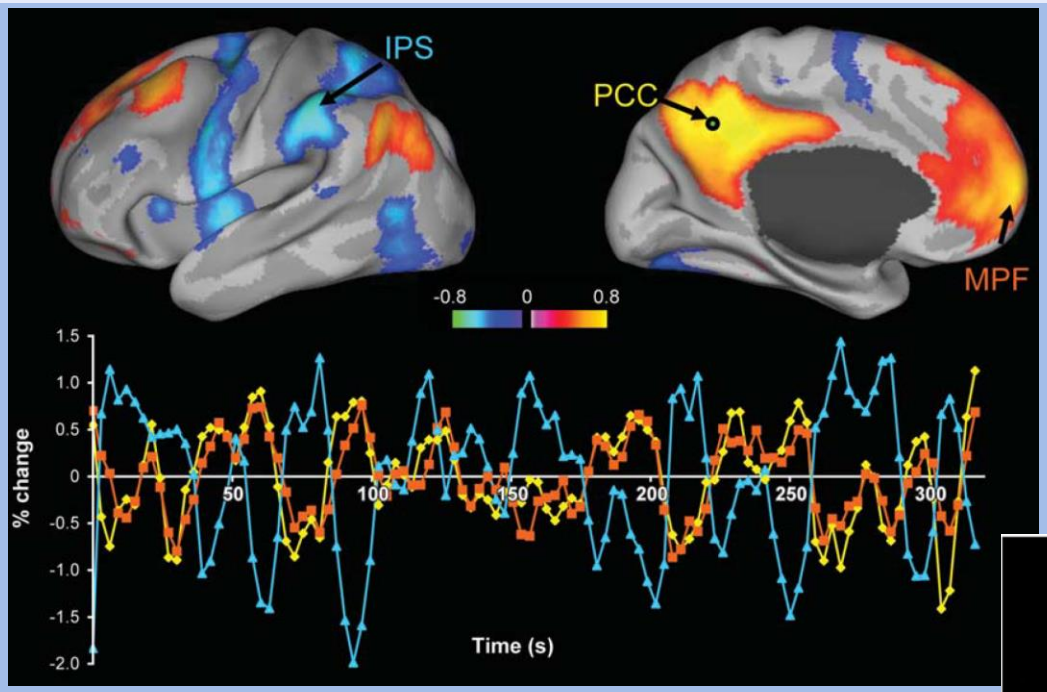


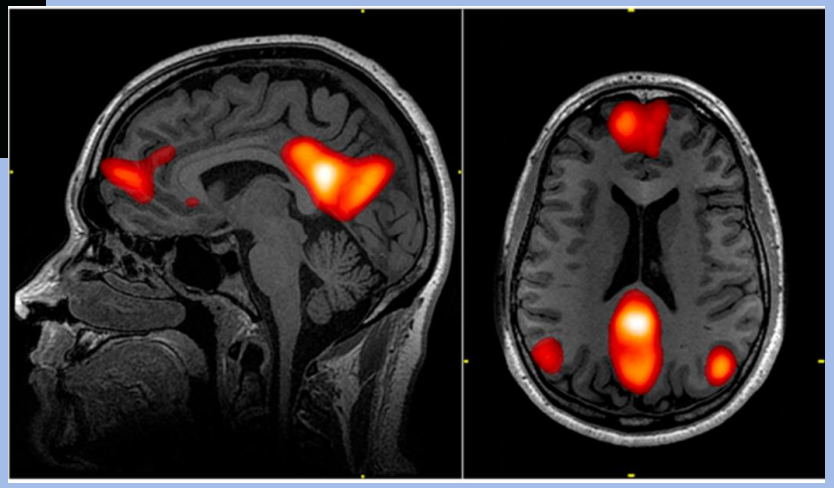


Conclusion: NBR is associated with a decreased $CMRO_2$

Shmuel A. et al., 2002 *Neuron* 36: 1195-1210.

Resting state/functional connectivity





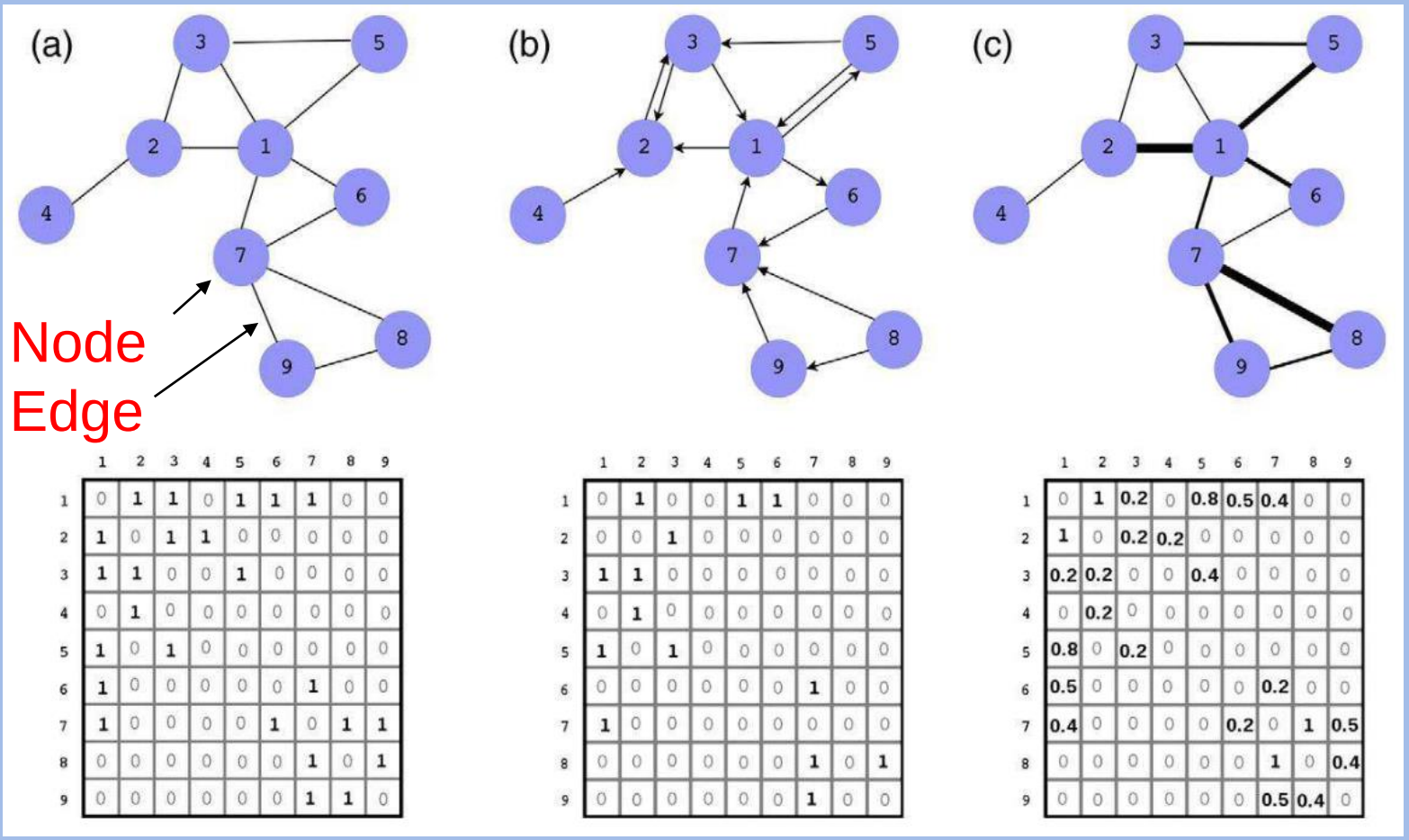
Fox MD. et al 2005 *PNAS* 102: 9673-78

Raichle ME. et al 2001 *PNAS 98*:676-82

Network analysis

UNIVERSITÄT BERN

undirected directed weighted



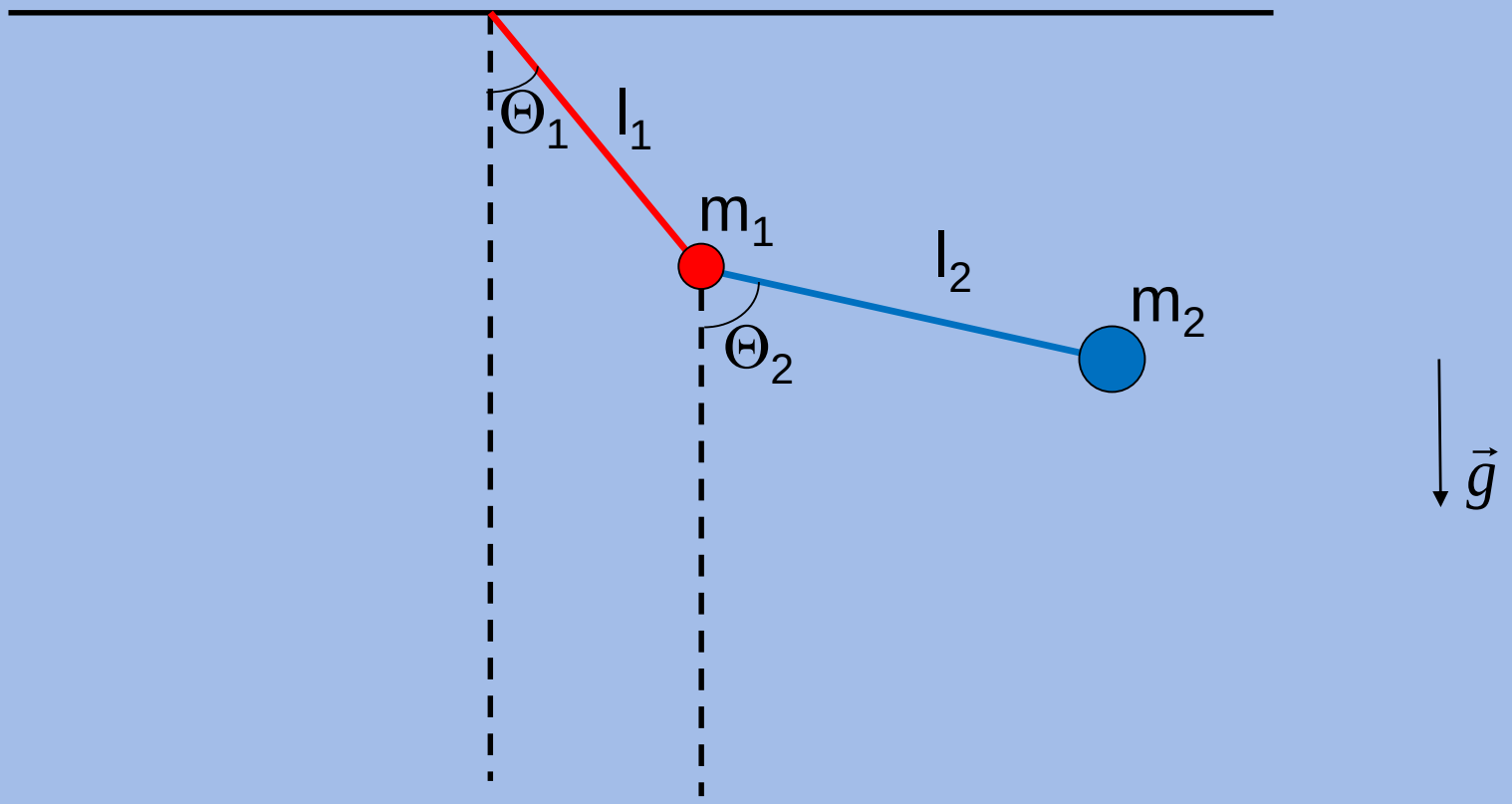
	1	2	3	4	5	6	7	8	9
1	0	1	1	0	1	1	1	0	0
2	1	0	1	1	0	0	0	0	0
3	1	1	0	0	1	0	0	0	0
4	0	1	0	0	0	0	0	0	0
5	1	0	1	0	0	0	0	0	0
6	1	0	0	0	0	0	1	0	0
7	1	0	0	0	0	1	0	1	1
8	0	0	0	0	0	0	1	0	1
9	0	0	0	0	0	0	1	1	0

	1	2	3	4	5	6	7	8	9
1	0	1	0	0	1	1	0	0	0
2	0	0	1	0	0	0	0	0	0
3	1	1	0	0	0	0	0	0	0
4	0	1	0	0	0	0	0	0	0
5	1	0	1	0	0	0	0	0	0
6	0	0	0	0	0	0	1	0	0
7	1	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	1	0	1
9	0	0	0	0	0	0	1	0	0

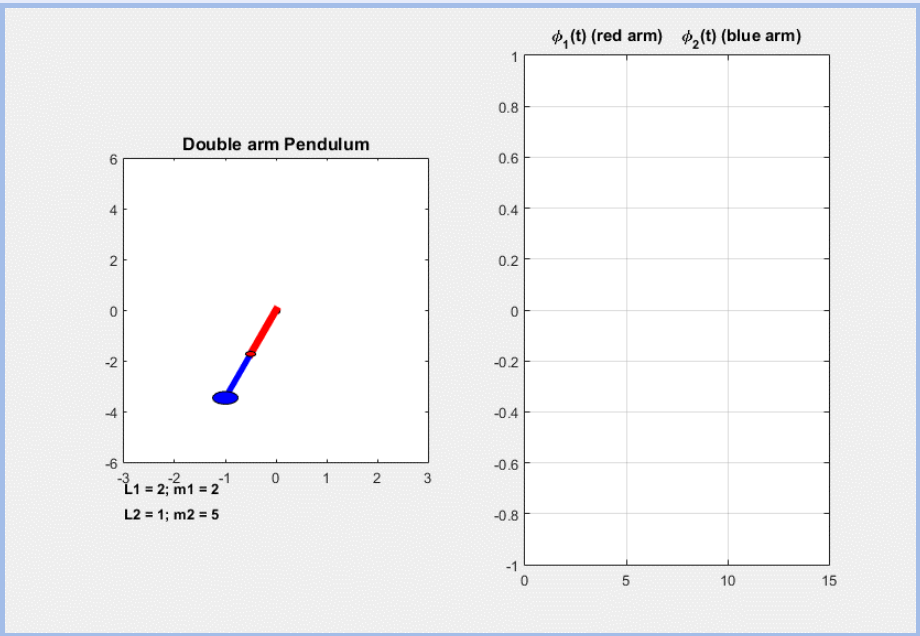
	1	2	3	4	5	6	7	8	9
1	0	1	0.2	0	0.8	0.5	0.4	0	0
2	1	0	0.2	0.2	0	0	0	0	0
3	0.2	0.2	0	0	0.4	0	0	0	0
4	0	0.2	0	0	0	0	0	0	0
5	0.8	0	0.2	0	0	0	0	0	0
6	0.5	0	0	0	0	0	0.2	0	0
7	0.4	0	0	0	0	0.2	0	1	0.5
8	0	0	0	0	0	0	1	0	0.4
9	0	0	0	0	0	0	0.5	0.4	0

Islam R., et al Am J of Appl Scien 2017, 14 (12): 1186.1208

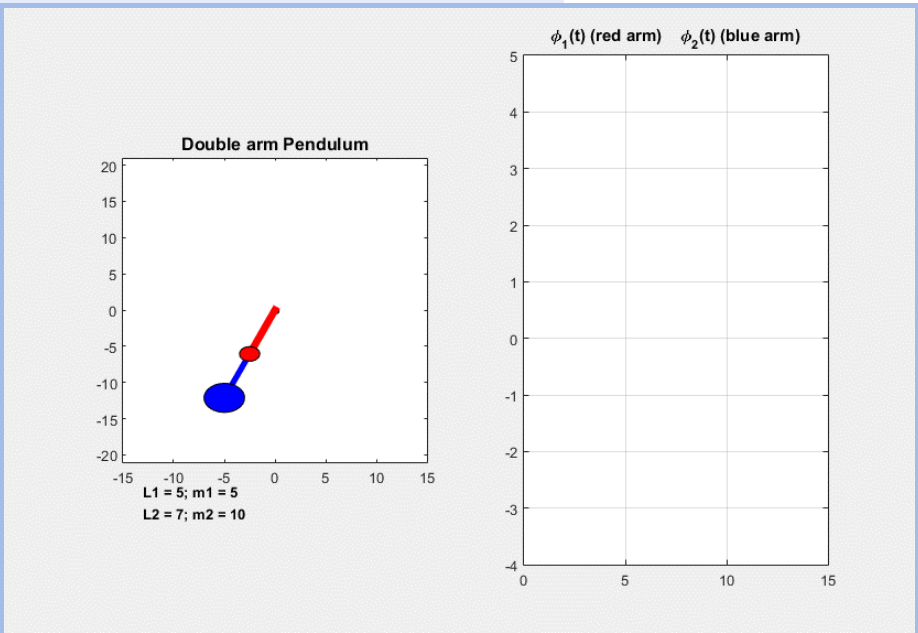
Double Pendulum: approaching connectivity



Functional coupling



r=0.6166; p<0.001



r=-0.0106; p=n.s.

> coupling present

> connectivity is visible in the angle of both arms

> interaction of red-to-blue arm

Functional connectivity: assumptions

> ≈ homogeneous medium in GM

> ≈ homogeneous medium in WM

> ≈ micro vasculature

> ≈ nerve conduction velocity

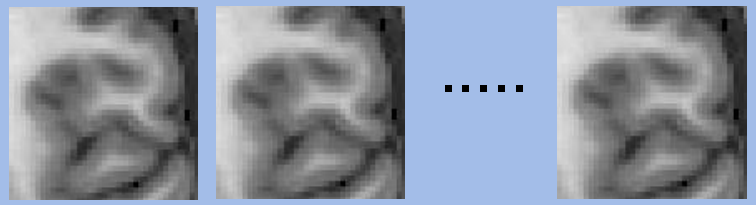
> ≈ oxygen extraction fraction

> ≈ neuro vascular coupling

> ≈ *k*[ATP]

> .....

roi_1 roi_2 ..... roi_n



Functional connectivity: features

Transport: energy, information

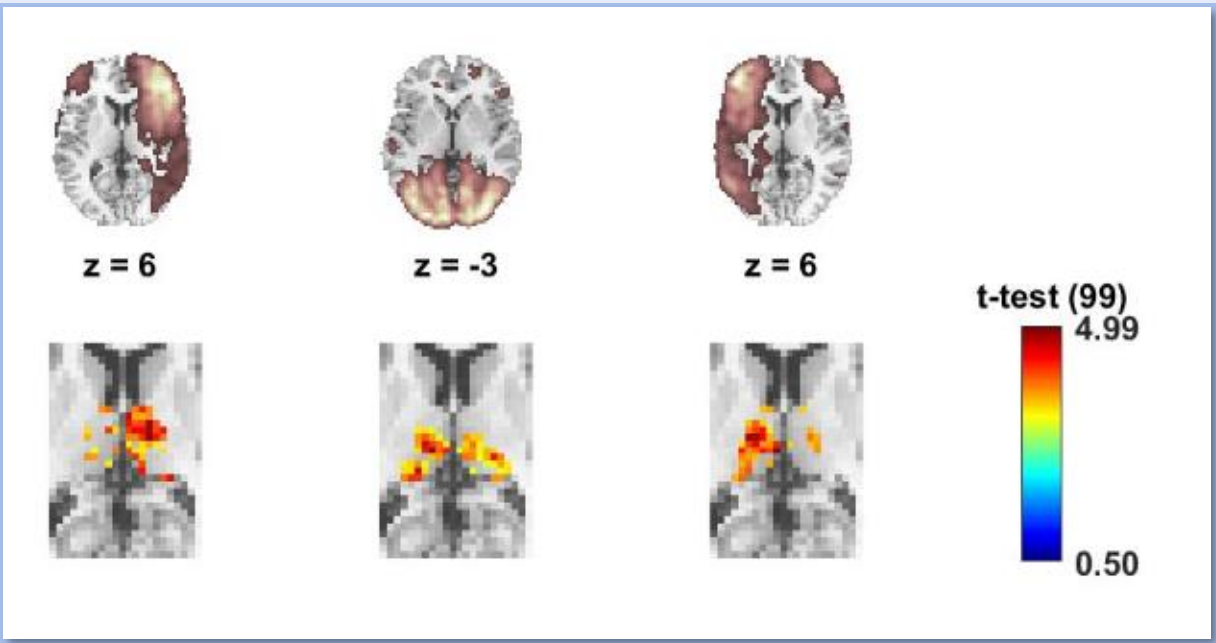
fast

> large diameter axons
> high NCV
> extracellular
> U-shape fibers

slow

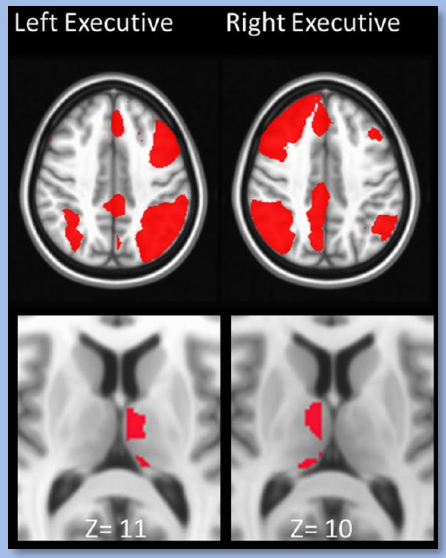
> small diameter axons
> low NCV
> intracellular
> frontal regions

Thalamo-cortical Network



Thalamus as „seed“ ROI

Network analysis (independet components):
i.e. each IC corresponds to a specific Network



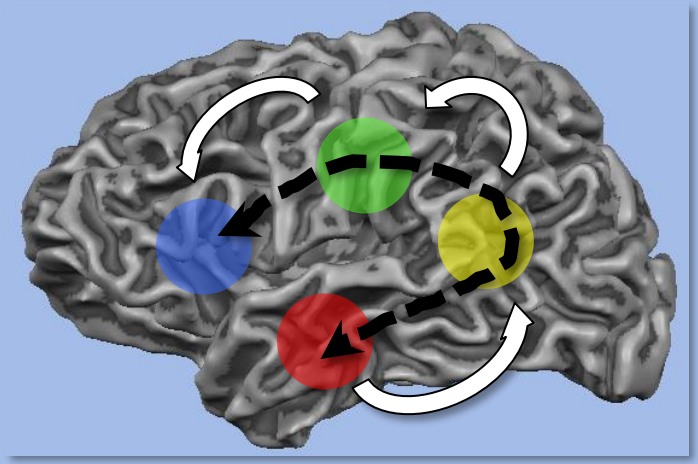
Yuan R. et al. Brain Struc Func Scien 2016, 221(4): 1971-1984

Ideas behind RSN

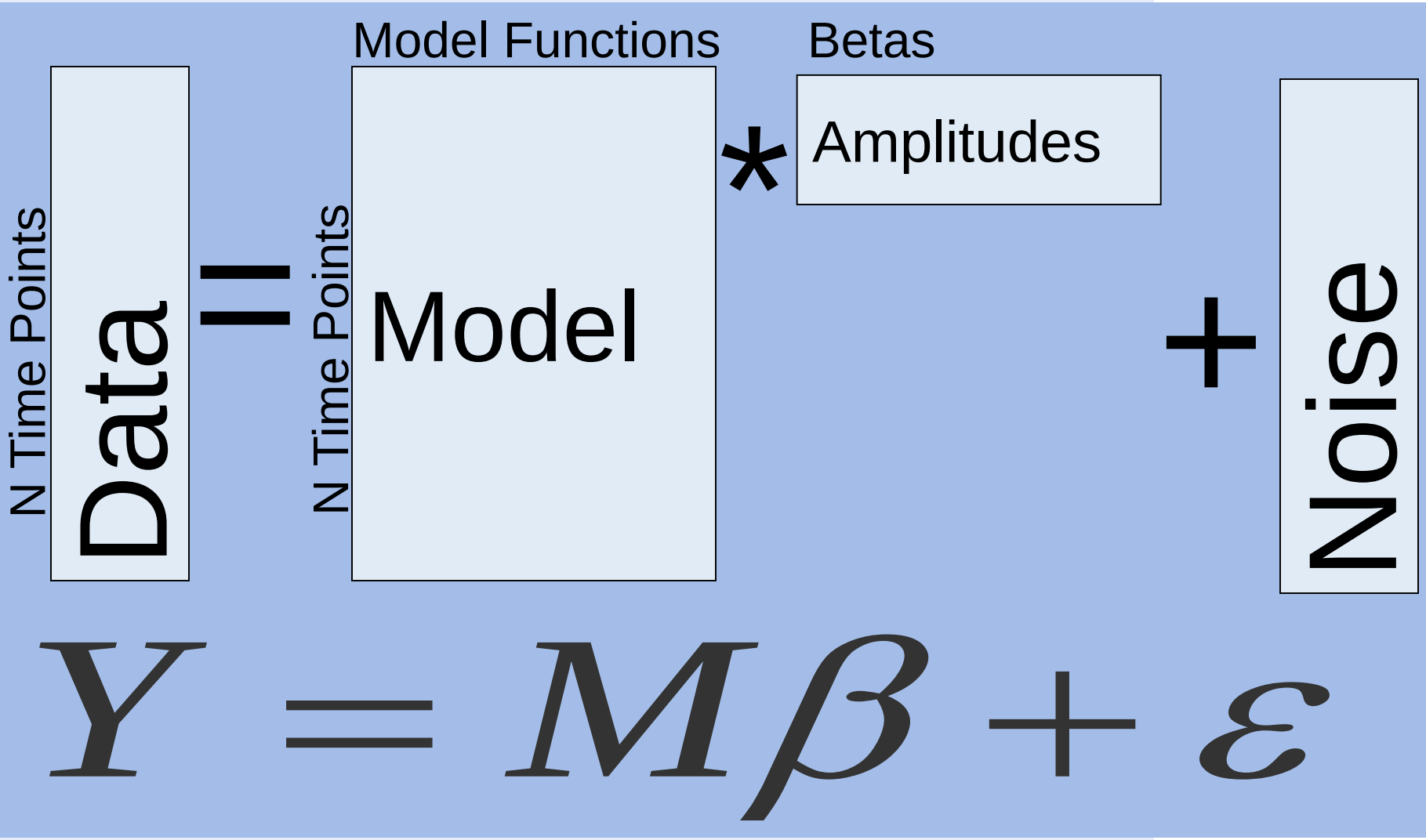
UNIVERSITÄT BERN

Localization <> Causality

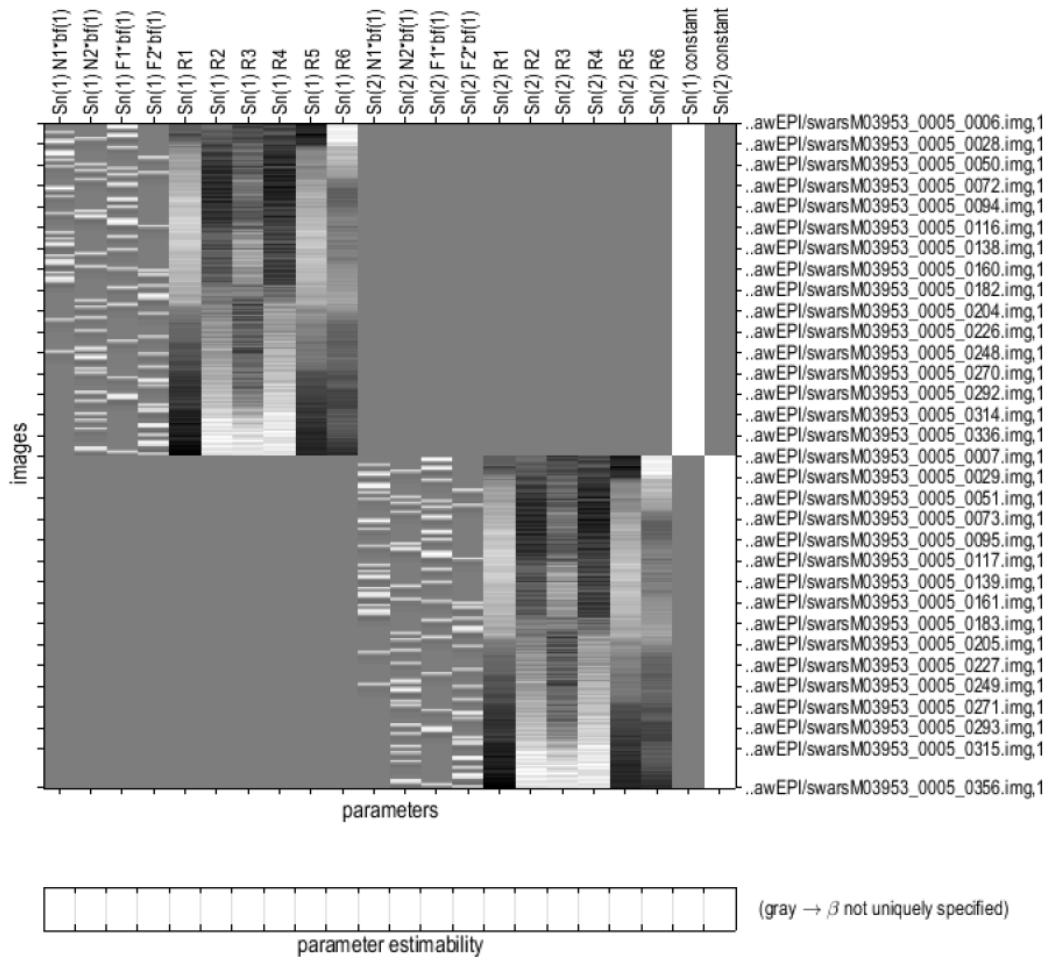
functional integration



Statistical steps: GLM



Design matrix



Design description...

Basis functions :	hrf
Number of sessions :	2
Trials per session :	4 4
Interscan interval :	2.00 {s}
High pass Filter :	[min] Cutoff: 128 {s}
Global calculation :	mean voxel value
Grand mean scaling :	session specific
Global normalisation :	None

Level of statistics

1. Level: subject‘s level

task performance, motion, etc.

2. Level: between subjects and within subjects

Group comparison

result-generating statistics

Different pathes of analysis in fMRI

Fixed Effects Analysis – (FFX)

concatenating all the subjects runs

Random Effects Analysis – (RFX)

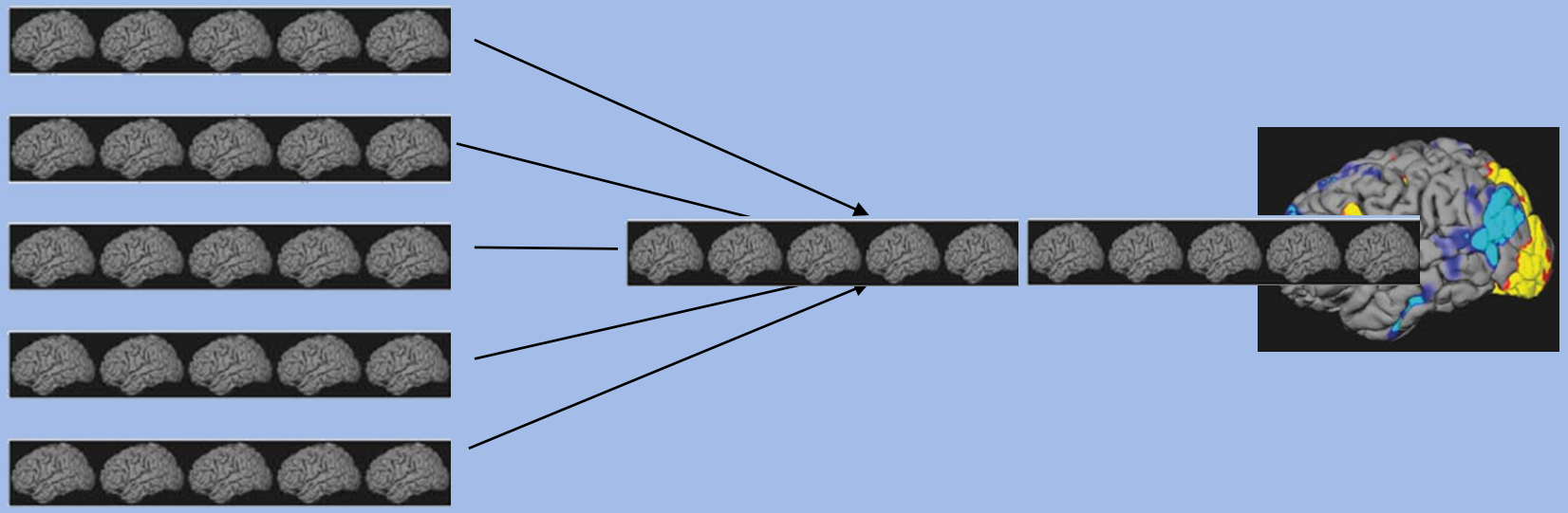
generalization to the population level

Fixed Effects Analysis FFX

concatenate subjects

degree of freedom „big“

Allows inference to subject‘s sample

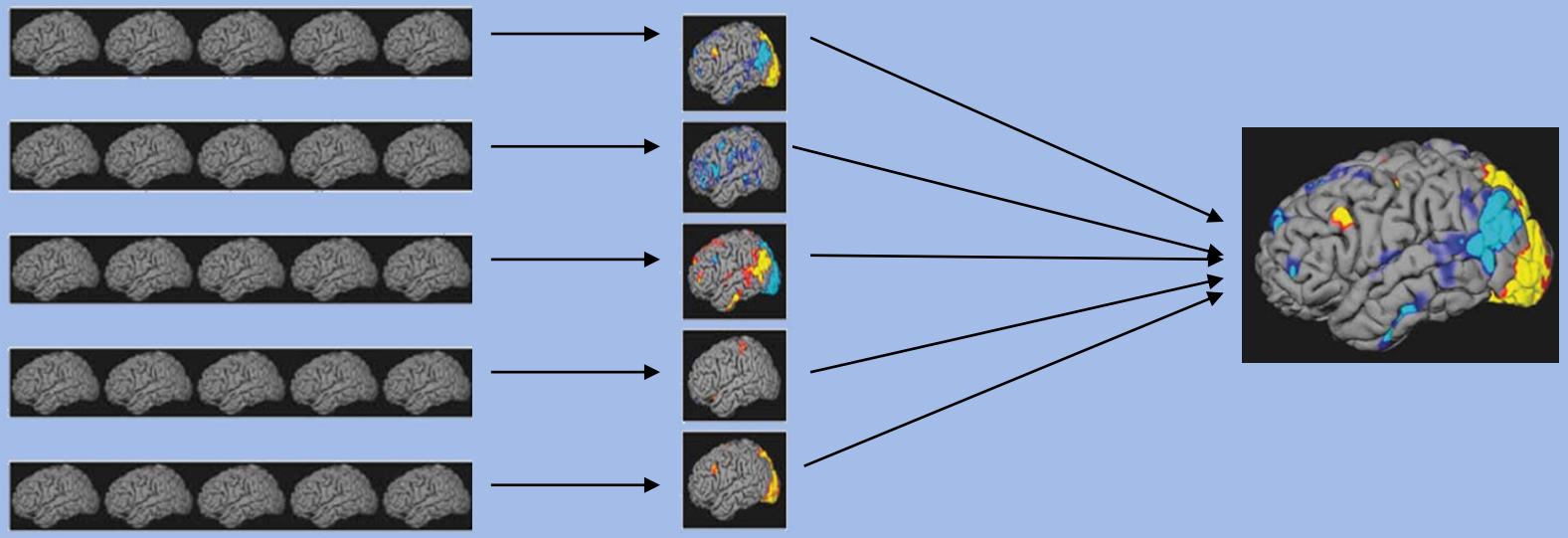


Random Effects Analysis RFX

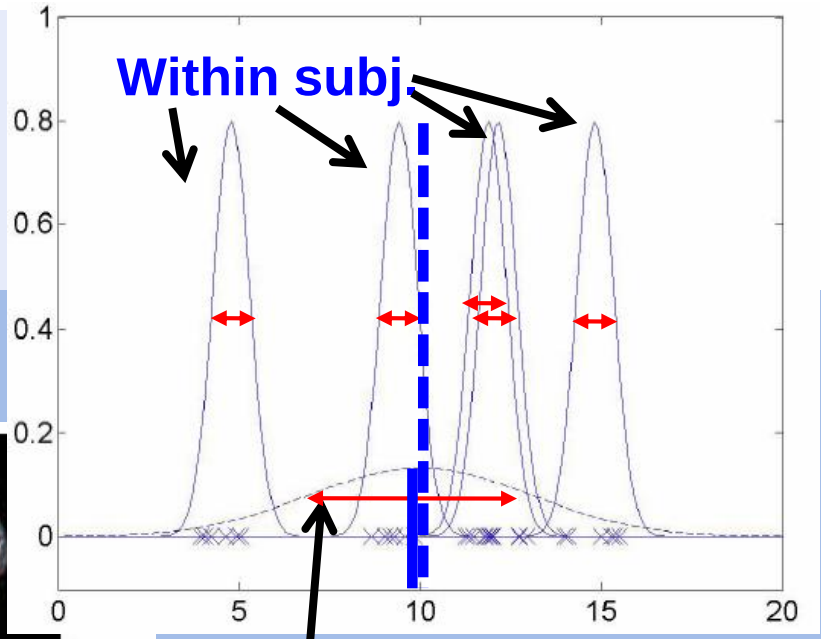
concatenate subjects

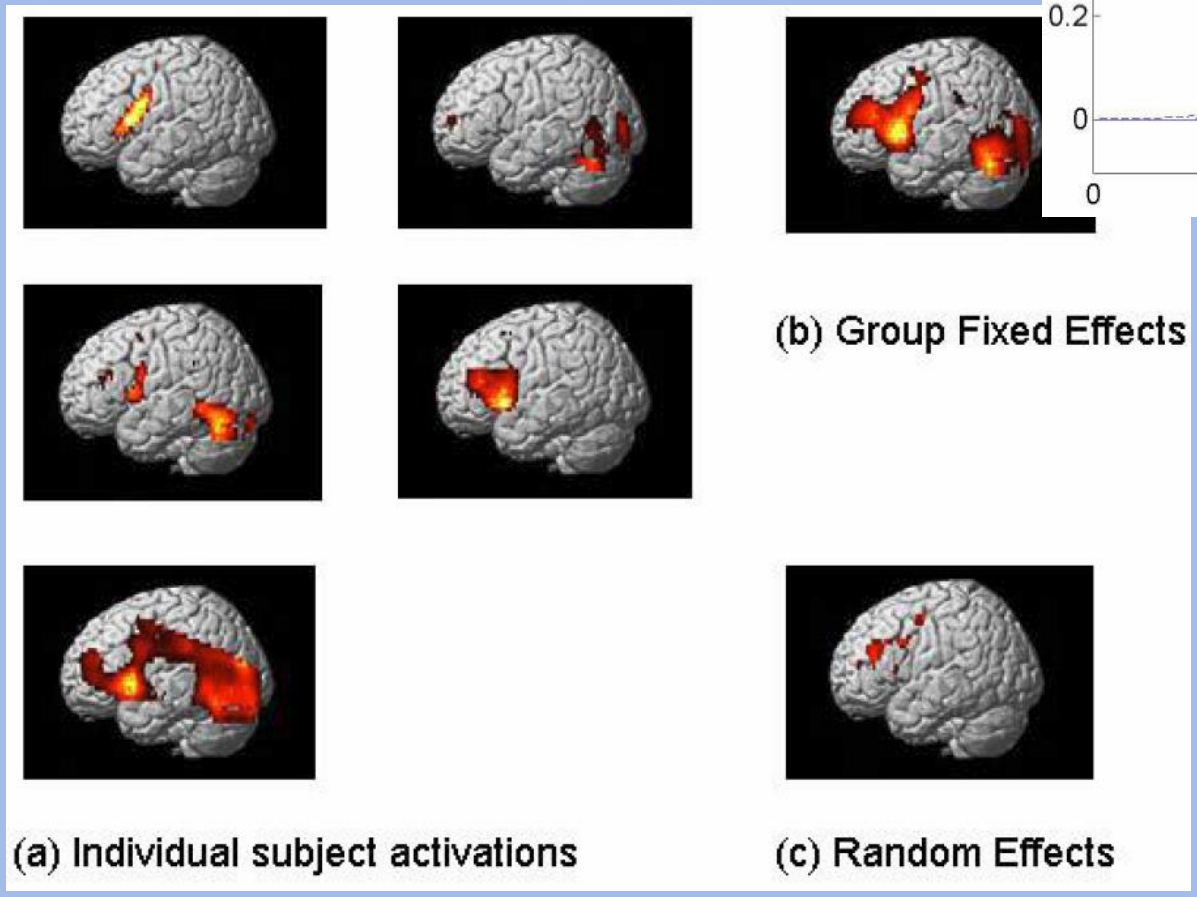
degree of freedom „smal“

Allows inference to population from the sample cohort



FFX vs RFX





↔ *variance*

average

That‘s science: it‘s all about assumptions

UNIVERSITÄT BERN

Procedure	Assumptions
Design	Previous literature
Record data	Patient/Control; Drugs; Cyrcadian rhytm; age; gender; social status; etc... MR scanner; Resolution ($\vec{x}, t$); Temperature; Pressure; etc...
Prepocess data	Gaussian distribution; serial correlation; Coregistration; Normalize Template; Smooth; etc...
1-level statistics	Gaussian distribution; linear trend; GLM residuals; etc...
2-level statistics	Gaussian distribution; variance; independent data; GLM residuals; etc.
Inference statistics	Correct p for multiple comparison; Random Field Theory; Smooth Field; Spatial autocorrealtion; etc...

List NOT complete !

Multiple testing

UNIVERSITÄT BERN

Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates

Anders Eklund[a,b,c,1], Thomas E. Nichols[d,e], and Hans Knutsson[a,c]

PNAS 2016 Jul 12;113(28):7900-5

What's all about ?

- H_a: alternative hypothesis — There is activation!
- H_0: null hypothesis — No effect
- α: false positive rate
  probability to *reject* H_0
  when H_0 is TRUE — type I error
- β: false negative rate
  probability to accept H_0
  when H_a is TRUE

The probability to make at least one type I error [Family Wise Error Rate (FWER)]

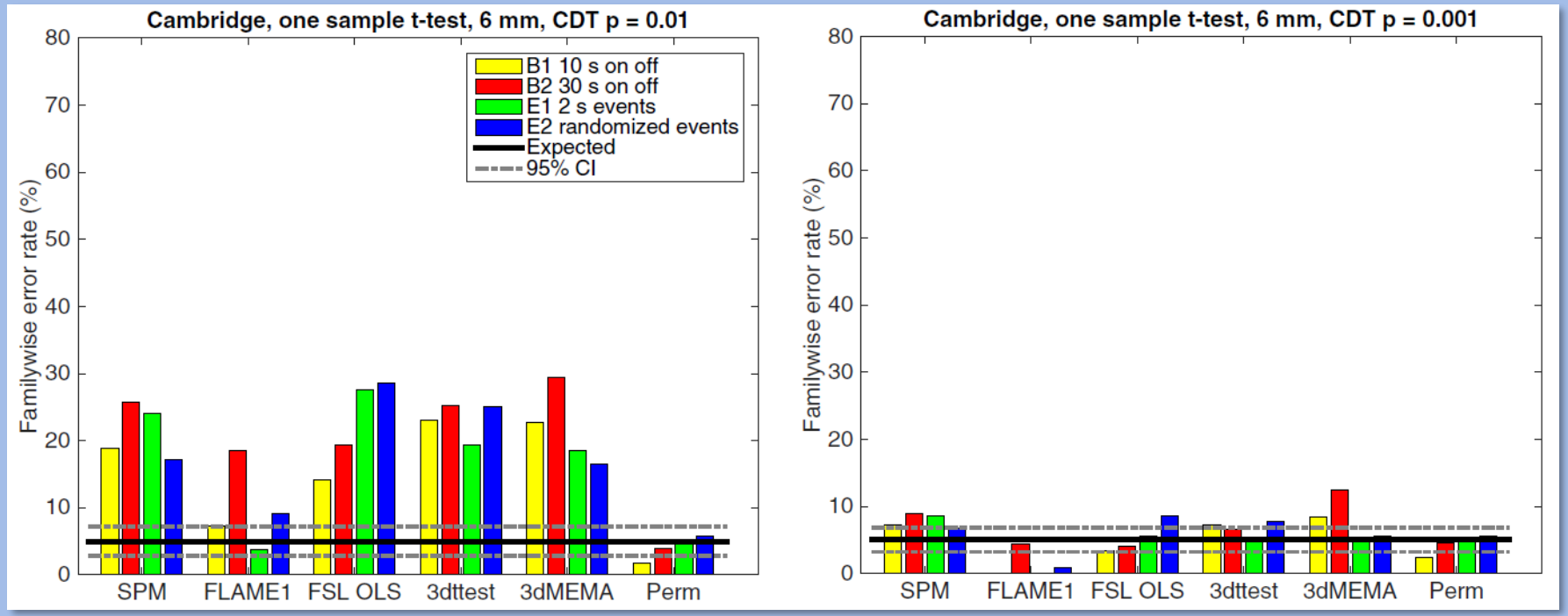
Mutiple testing

UNIVERSITÄT BERN

FWER (voxels): find p_α =0.05 so that there is a 5 % chance to find at least 1 activated voxel

FWER (cluster): with p_α =0.05 find the # voxels in a cluster so that there is a 5 % chance in the cluster to find at least 1 activated voxel

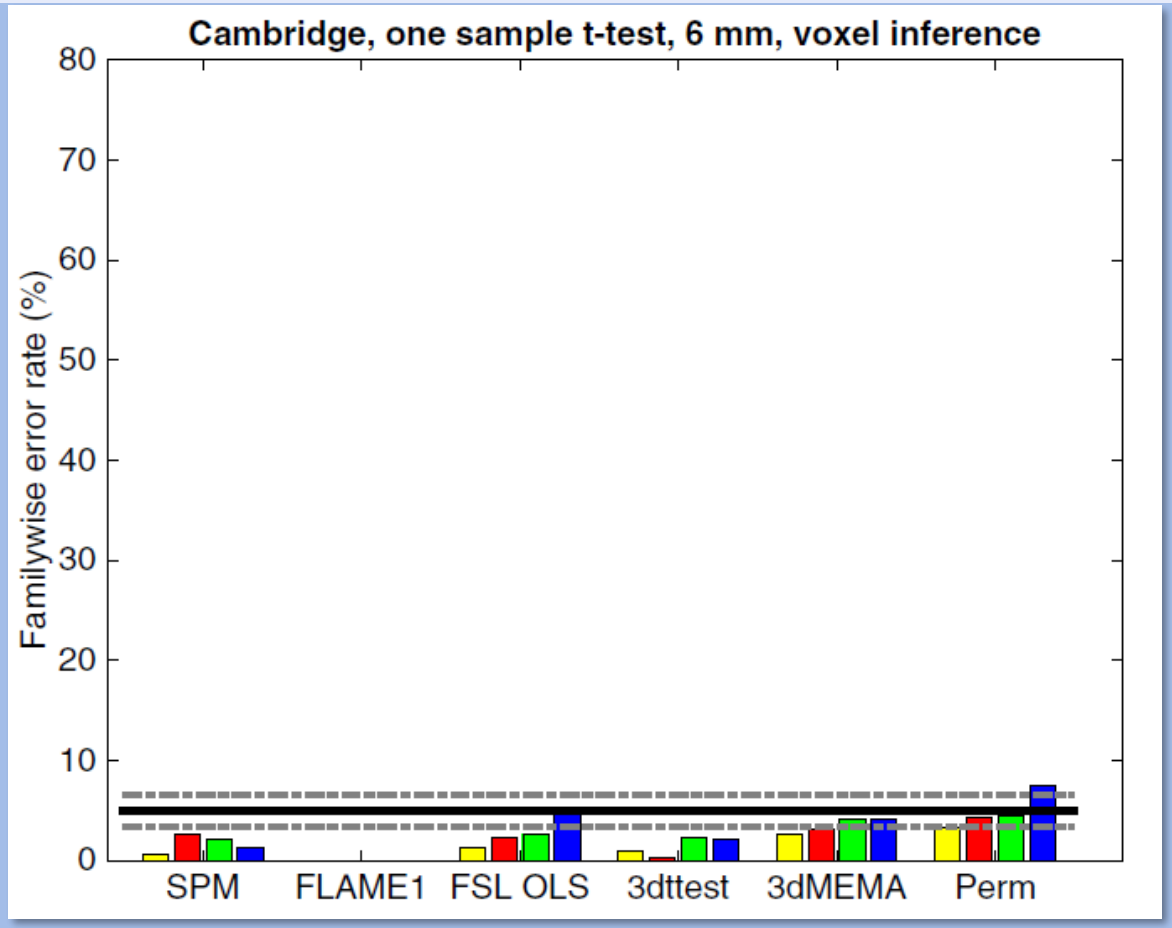
SPM, FSL, AFNI, non-parametr.



Cluster wise threshold: Not OK for p<0.01

~OK for p<0.001

Voxel wise threshold OK



Multiple testing (Bonferroni)

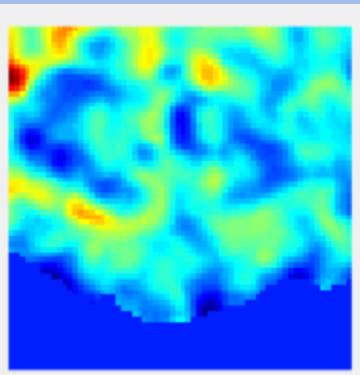
If we have 64*64 voxles we do 4096 test:

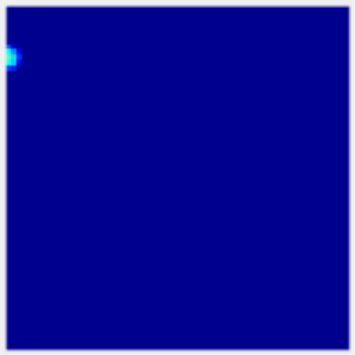
$$p \leq {}^{0.05}\!/_{4096} = 0.0000122$$

Example:

df=30;
p=0.0000122
t = 1-tinv(0.05/4096,30);

= 5.9834
too conservative !





no correcction t>5.98

Random Field Theory

mathematical model:

-estimate the # RESEL in your search volume

-estimate the # cluster (thresholded at some level)

-and correct the thresholded level

Random Field Theory

UNIVERSITÄT BERN

Independent data: data of one voxel should be independent of its neighbourhood

in fMRI spatial correlation is present !

Smoothness: should be constant over the brain

how to check this ?

Problems when:

- Small sample size
- errors/residuals not normaly distributed and not smooth

Take home

UNIVERSITÄT BERN

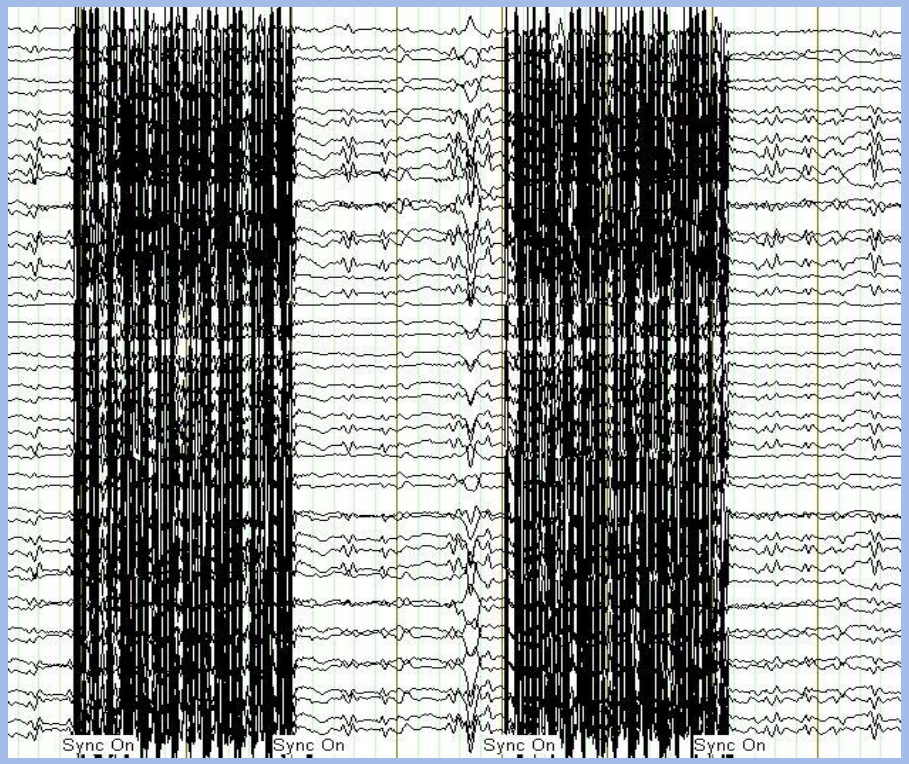
- Check your data carefully (assumptions Y/N ?)
- Investigate into Signal and Noise in your data !
- Careful interpretation of results;
  especially when dealing with (large) clusters
- Non-parametric SnPM may be an optimal choice
- COBIDAS* White paper with guidelines «best-practices»

Committe on Best Practice in Data Analysis: http://dx.doi.org/10.1101/054262

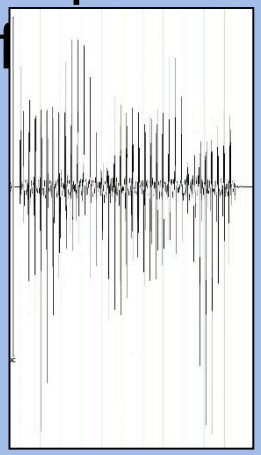
DATA PRE-PROCESSING

UNIVERSITÄT BERN

Scan-Puls artifact correction



Subtraction of a template-artif

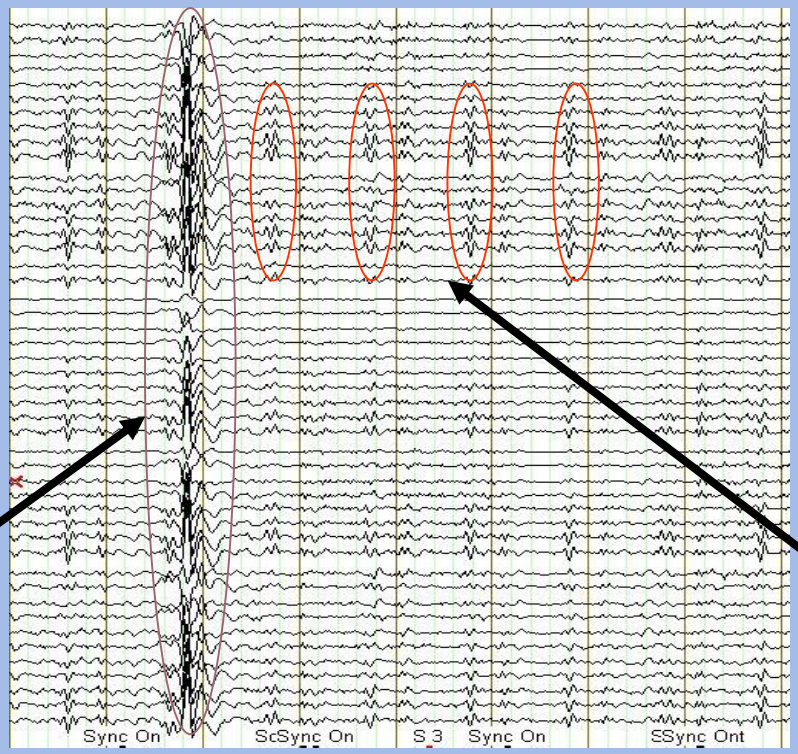


EEG in 3T MRT

DATA PRE-PROCESSING

UNIVERSITÄT BERN

After Scan-Puls artifact correction



movemen
t- artifacts

cardioballistic
artifacts

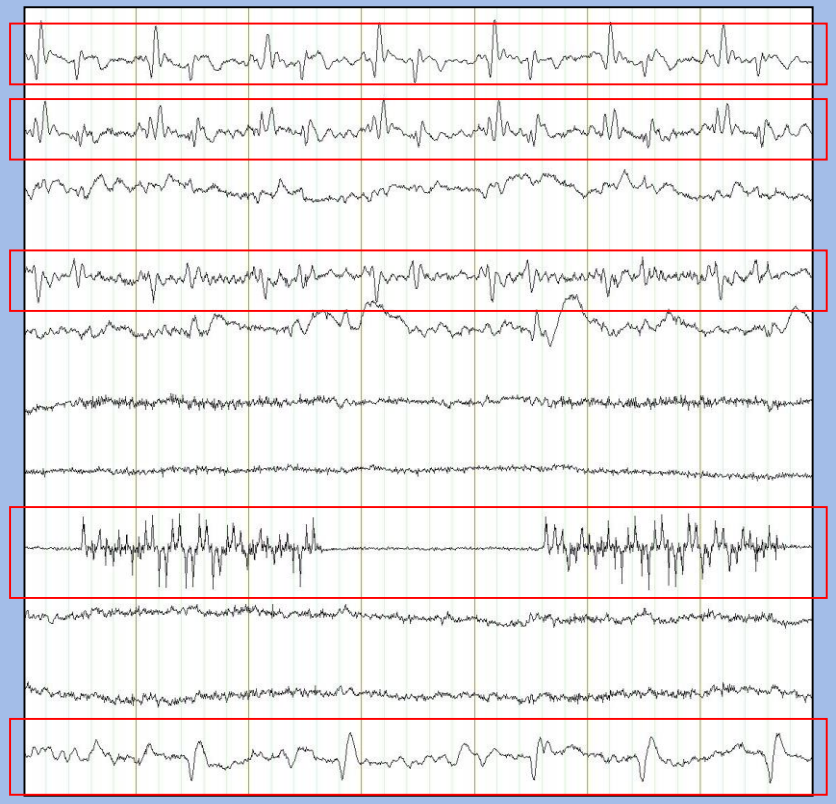
DATA PRE-PROCESSING

UNIVERSITÄT BERN

Independent Component Analysis

Decomposition of EEG data into independent factors.

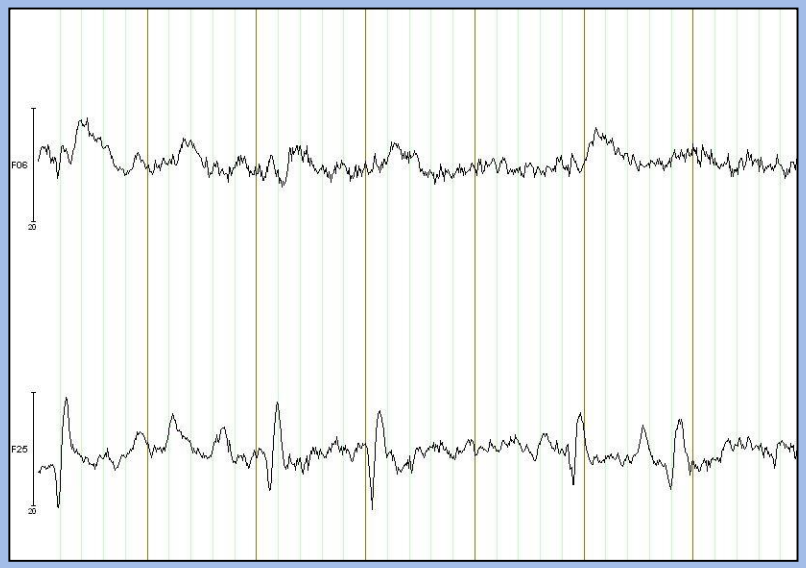
- Scan-Puls artifact
- Cardioballistic artifact
- Eye blinks
- Epileptiform activity
- Other



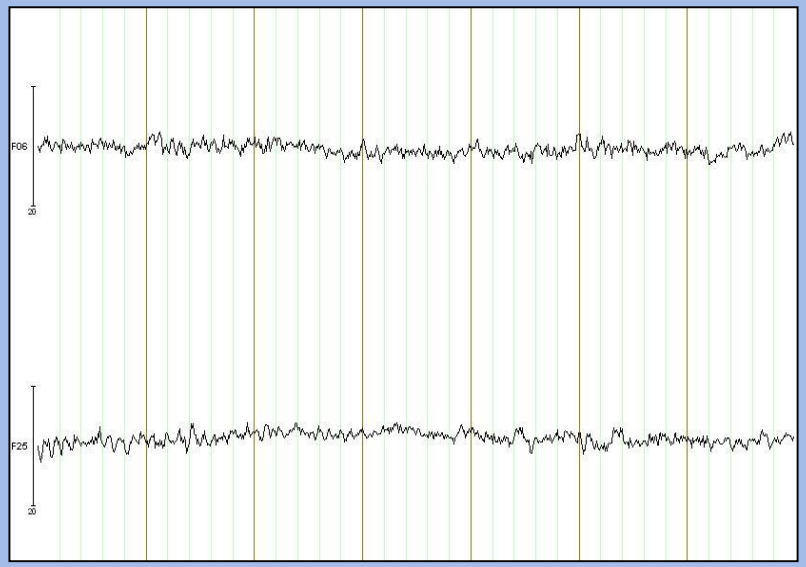
ICA FACTORS (EXAMPLE)

UNIVERSITÄT BERN

Example factors coding for epileptiform activity



Epoch **WITH** interictal spikes.

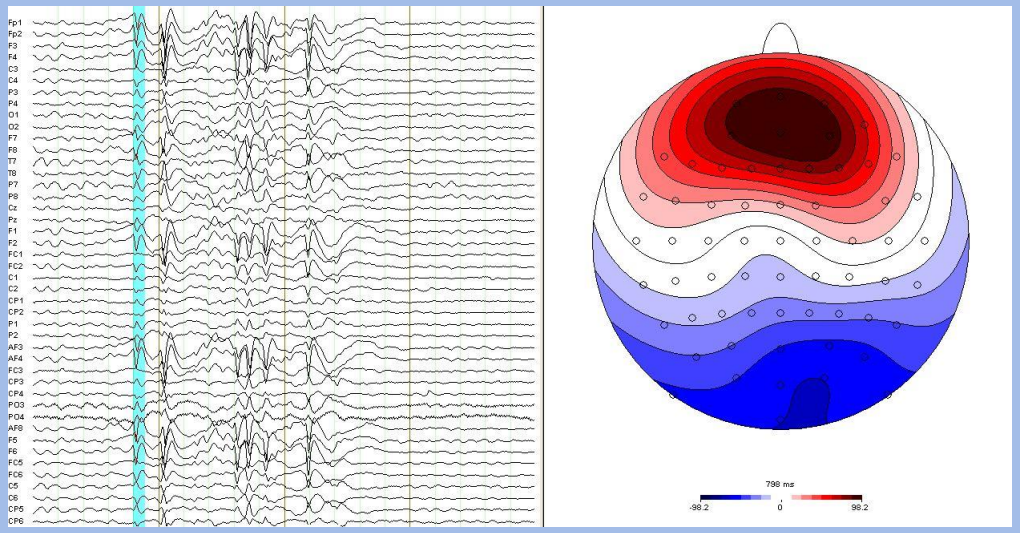


Epoch **WITHOUT** discharges.

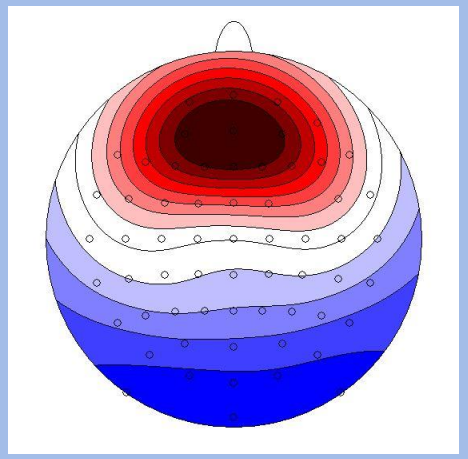
ICA FACTORS (EXAMPLE)

UNIVERSITÄT BERN

Selection of factor further dependent on topography



EEG topography.

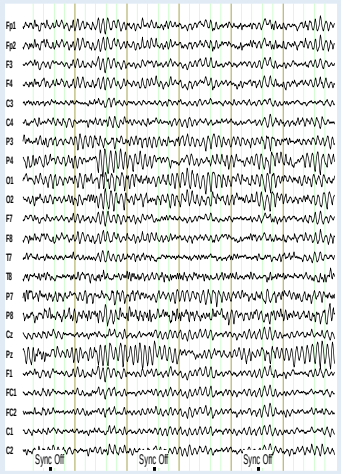


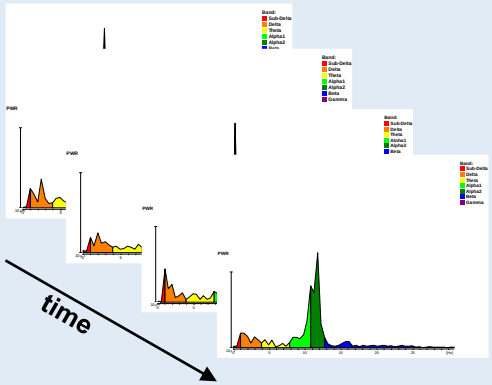
ICA factor topography.

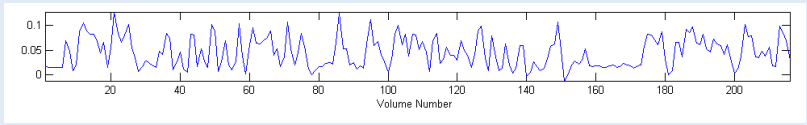
Features extracted from EEG

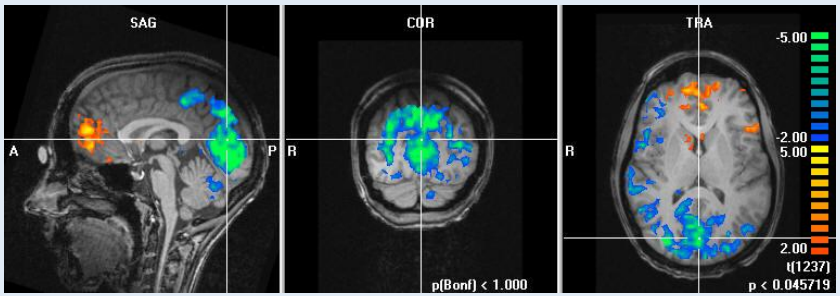
UNIVERSITÄT BERN

Spontaneous Activity - Frequency domain









Jann et al. (unpublished)

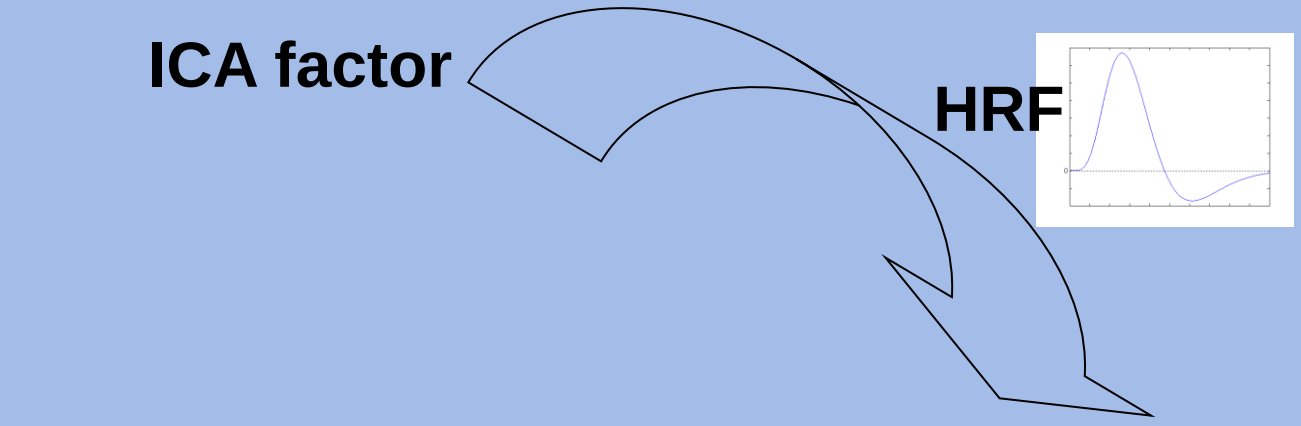
DATA PROCESSING

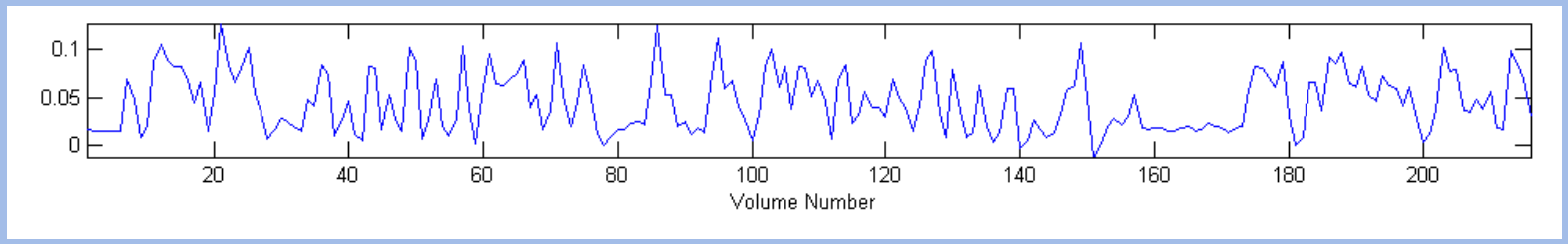
UNIVERSITÄT BERN

Convolution with a ‘Hemodynamic Response Function’ (HRF)

ICA factor

HRF



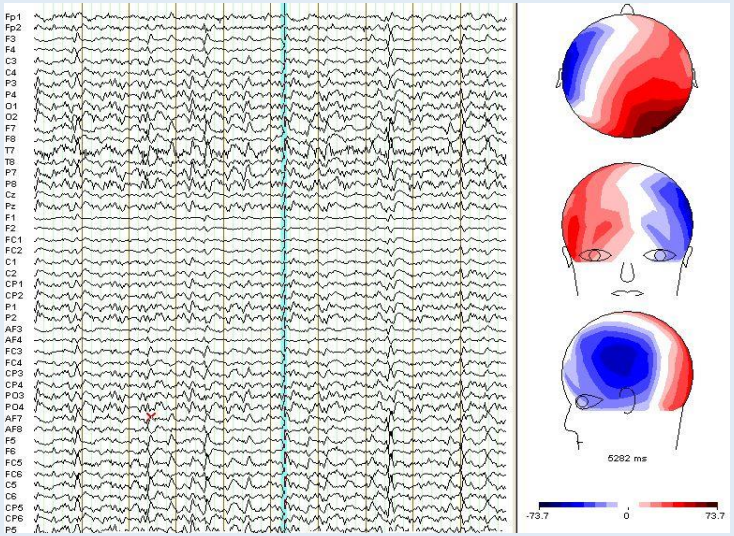


Predictor for fMRI BOLD signal

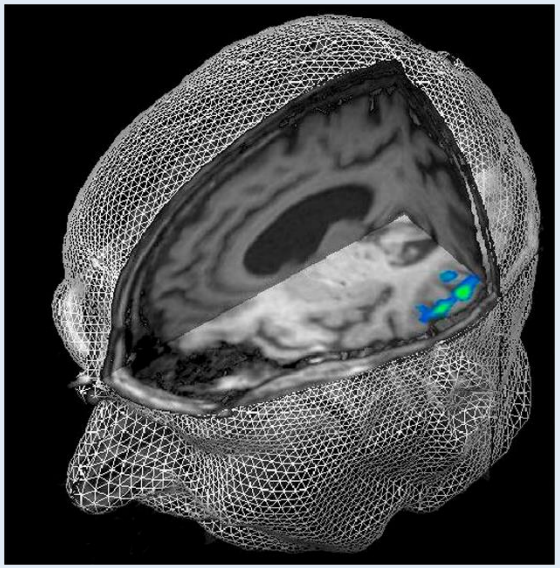
Features extracted from EEG

Spontaneous Activity - Single events

e.g. Epilepsy: unpredictable events



Occurence of IEDs as predictor for BOLD

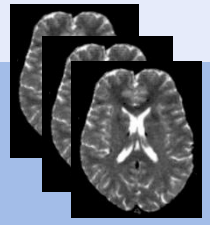


Adapted from Jann et al., Neuroimage, 42 (2008), p635-648

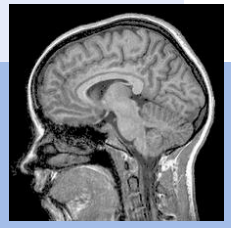
fMRI study

Experimental setup

Recorded time series (EPI)



Coregistration of functional and anatomical data



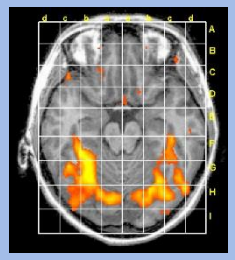
Anatomical images

Preprocessing

Spatial normalisation

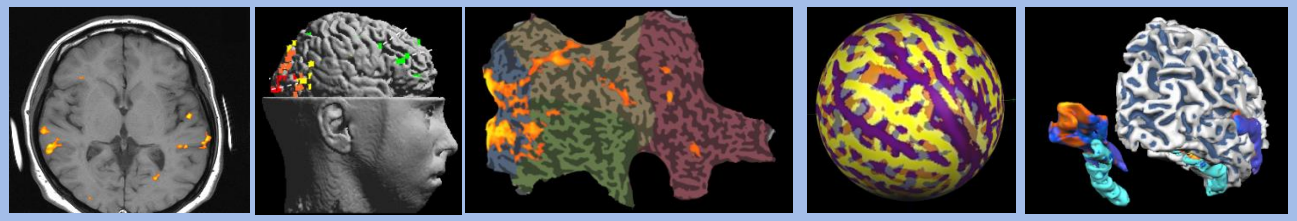
Segmentation
Cortex reconstruction

Statistical localization of brain activation, functional maps



Group analysis I
Talairach space

Single subject analysis



Group analysis II – Surface-based alignment