The Study of Brain Activity at “Rest”
Activity Baseline in Brain Functional Studies

Experimental Paradigm – Block Design

Baseline

Task

BOLD

Rest Task
Resting State Brain Activity and the Default Mode Network
“Resting State” is an operational definition referring to a constant condition without imposed stimuli or other behaviorally salient events.

The eyes may be closed or open, with or without visual fixation.

The objective of resting state experiments is to capture the statistical properties of endogenously generated (i.e., spontaneous) neural activity. In contrast, the objective of event-related studies is to measure evoked or induced responses.
History: How active is a «resting» brain?

In 1929 Hans Berger, inventor of the electroencephalogram, deduced from the ceaseless electrical oscillations detected by the device that “we have to assume that the central nervous system is always, and not only during wakefulness, in a state of considerable activity.” But his ideas about how the brain functions were largely ignored, even after noninvasive imaging methods became available in neuroscience laboratories.

The brain maintains a high level of activity even “at rest.” Behavioral tasks require minimal extra energy (~5% increment) over what is already consumed in the baseline state.
Quantitative maps of blood flow (Upper) and oxygen consumption (Lower) during quiet rest with eyes closed. Note the large variation in blood flow and oxygen consumption across regions of the brain. These vary most widely between gray and white matter. Despite this variation, blood flow and oxygen consumption are closely matched.
Regions of the brain regularly observed to decrease their activity during attention demanding cognitive tasks. These data represent a meta-analysis of nine functional brain imaging studies performed with PET. In each of the studies included, the subjects processed a particular visual image in the task state and viewed it passively in the control state. One hundred thirty-two individuals contributed to the data in these images. These decreases appear to be largely task independent. The images are oriented with the anterior at the top and the left side to the reader’s left.
The DMN includes part of the medial temporal lobe, part of the medial prefrontal cortex, and the posterior cingulate cortex, along with the adjacent ventral precuneus and the medial, lateral and inferior parietal cortex.
The **Default Mode Network (DMN)** is a network of brain areas that are active when the individual is not focused on specific tasks requiring attention to external stimuli and the brain is at wakeful rest.

The DMN is characterized by **coherent functional oscillations** at a rate lower than **0.1 Hz** (one every ten seconds).

The DMN preferentially activates when individuals focus on internal tasks such as **daydreaming, envisioning the future, retrieving memories, and gauging others' perspectives**.
During goal-oriented activity, the DMN is deactivated and another network, the **Task-Positive Network** (TPN) is activated.

The DMN is negatively correlated (anti-correlated) with brain systems that focus on external signals.
### TABLE 1 | Characterizations of experiences when the default mode is active.

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 “unconstrained verbally mediated thoughts”</td>
<td>Shulman et al., 1997, p. 648</td>
</tr>
<tr>
<td>2 “semantic knowledge retrieval, representation in awareness, and directed manipulation of represented knowledge for organization, problem-solving, and planning”</td>
<td>Binder et al., 1999, p. 80</td>
</tr>
<tr>
<td>3 “active retrieval of past experiences and planning of future experiences”</td>
<td>Andreasen et al., 1995, p. 1576</td>
</tr>
<tr>
<td>4 “retrieval and manipulation of past events, both personal and general, in an effort to solve problems and develop future plans”</td>
<td>Grecius et al., 2003, p. 257</td>
</tr>
<tr>
<td>5 “enhanced watchfulness toward the external environment (e.g., waiting for upcoming task-relevant stimuli or attending to scanner noise and incidental light)”</td>
<td>Gilbert et al., 2007, p. 43</td>
</tr>
<tr>
<td>6 “inner thought, self-reflective thinking in terms of planning for the future, or simulation of behavior... interrupted... into a... extrospective... state of mind... characterized... as increased attention and readiness... sensorimotor planning for future routes of action in response to potential changes in the inner and outer environment”</td>
<td>Fransson, 2005, p. 26</td>
</tr>
<tr>
<td>7 “not focused on the external environment... internally focused tasks including autobiographical memory retrieval, envisioning the future, and conceiving the perspectives of others”</td>
<td>Buckner et al., 2008, p. 1</td>
</tr>
<tr>
<td>8 “spontaneous, internally directed cognitive processes”</td>
<td>Andrews-Hanna et al., 2010, p. 322</td>
</tr>
<tr>
<td>9 “spontaneous mental contents which are unrelated to perception and coordinate[c]... so that they are maintained in the face of competing sensory information”</td>
<td>Smallwood et al., 2012, p. 67</td>
</tr>
<tr>
<td>10 “an ultimate state of inspection of the self”</td>
<td>Wicker et al., 2003, p. 229</td>
</tr>
<tr>
<td>11 “stable, unified perspective of the organism relative to its environment (a ‘self’)”</td>
<td>Gusnard and Raichle, 2001, p. 692</td>
</tr>
</tbody>
</table>
Jack: Jack is rubbing with his thumb the fabric that holds the writing board, and he feels the snagging of the fabric on his thumb. This is more a sense in his thumb than of the fabric. He is involved with determining whether this is wool or synthetic, but that is a part of the sensation, not a cognitive act.

Sensory awareness
Otto: Otto is saying in inner speech “I just turned 30.” He doesn’t know why he is saying that or to whom, but he is clear that that is what he is experiencing and that he is emphasizing the word “turned.”

**Inner speaking**
Susan: Susan innerly sees the actress Sigourney Weaver in a cryogenic tank from the movie Alien. She sees Sigourney’s face from above, below the glass window of the tank – the rest of Sigourney’s body is vaguely or blurrily present. Mostly Susan is searching for the word used in the movie: cryogenic chamber, cryogenic tank, etc., waiting for the right word to appear. This is primarily a state of suspended animation, waiting for the word – she does not see or hear pieces of words, etc.

Inner seeing
Neural activation patterns largely consistent across time

Large individual differences in inner phenomena

The resting state itself may differ substantially from one participant to the next
The function of the DMN is still *unclear*. In humans, the DMN has been hypothesized to be involved in *introspection* or *self-referential thoughts*. In particular, it may be related to generation of *spontaneous thoughts, mind wandering* and *creativity*.

Alternatively, the DMN may represent *underlying physiological processes going on in the brain* that are *unrelated to any particular thought or thoughts*.

The DMN properties are potentially modulated by a variety of interventions and processes.
Rats and Monkeys possess a DMN that is broadly similar to the DMNs of humans. A well-organized, intrinsically coherent DMN appears to be a fundamental feature in the mammalian brain.

Lu et al., PNAS 2012
The similarity between functional and structural connectivity is highest in regions of the default mode network.
Prominent locations for network hubs in adults include precuneus/posterior cingulate cortex, medial PFC, anterior cingulate cortex, bilateral parietal lobule, and bilateral insula.

In infants, the majority of cortical hubs are located in the homomodal cortex, foremost in the auditory, visual and sensorimotor areas, and to a lesser extent in the PFC.

Fransson et al., Cereb. Cortex 2011
The level of connectivity in the DMN in children is a subject of debate and intense investigation. The main finding so far is that children tend to show **diffuse correlations near seed locations** and **few long-distance correlations**. Long-distance correlations begin to emerge during adolescence.
EEG correlates of the Default Mode Network
**Attention to external stimuli:** A shift of attention toward specific stimuli or tasks is associated with a reduced connectivity within the DMN.

**Meditation:** Long-term meditation practitioners may show reduced activation and reduced functional connectivity in the DMN.

**Psychedelic drugs:** Psilocybin has been shown to be associated with a reduction of the blood flow to areas of the DMN (posterior cingulate cortex and medial prefrontal cortex).

**Sleep:** Sleep deepening is associated with a decrease in connectivity between the posterior cingulate cortex and medial prefrontal cortex.

**Sleep deprivation:** Sleep deprivation results in a decrease in connectivity within the DMN. A decreased anti-correlation between the DMN and the TPN can also be observed.
The DMN overlaps areas involved in major brain disorders. In fact, alterations of the DMN have been observed in several pathological conditions. Discerning precisely which aspects of the network are affected by different disorders may provide new diagnostic tools.

Some examples...

**Alzheimer’s Disease**: Brain areas that atrophy in Alzheimer’s overlap very closely with major centers of the DMN.

**Depression**: Hyperconnectivity in the DMN seems associated with rumination in Depression.

**Autism**: Patients may fail to reduce DMN activity during cognitively demanding tasks.
The AD group showed decreased resting-state activity in the posterior cingulate and hippocampus. Of note a posterior cingulate hypometabolism is commonly detected in positron emission tomography (PET) studies of early AD.
Patients and controls were asked to examine negative pictures passively and also to reappraise them actively. In widely distributed elements of the DMN, depressed, but not control subjects, exhibited a failure to reduce activity while both looking at negative pictures and reappraising them.

Moreover, looking at negative pictures elicited a significantly greater increase in activity in other DMN regions (amygdala, parahippocampus, and hippocampus) in depressed than in control subjects.
Abnormal Functional Connectivity in Autism Spectrum Disorder Patients

Compared to controls, ASD patients showed decreased FC between areas of the DMN.

FC in these regions was inversely correlated with the severity of patients' social and communication deficits (Autism Diagnostic Observational Schedule).

Association between ASD deficits and DMN under-connectivity?

Assaf et al., NeuroImage 2010
Methods for Exploration of the Resting State Brain Activity
Three main approaches are commonly used to study resting state functional MRI data (rs-fMRI):

- **Map-Based Methods**
  - Seed-based Functional Connectivity (FC) Analysis
  - Independent Component Analysis (ICA)

- **Graph-Based Methods**
  - Connectivity Matrices and Graph Analysis
**Seed-based correlation mapping** requires the a priori identification of a target region of interest and requires extensive preprocessing to minimize the influence of non-neuronal sources of variance.

In contrast, **ICA** provides a direct means of separating artifact from neural signals but is less suited to investigating targeted regions of interest.

The topography of the **DMN** appears more or less the same regardless of which method is used.
Signal from a certain voxel or cluster of voxels (seed region of interest, seed-ROI) is used to calculate the temporal correlation (usually Pearson’s correlation coefficient) with other voxels of the brain. Usually the seed-ROI is placed on precuneus / posterior cingulate cortex (PCC) or on anterior cingulate / medial prefrontal cortex for the study of DMN.
Correlation is NOT Causation

**Global Average Temperature vs. Number of Pirates**

- **Global Average Temperature, °C**
  - 13.0
  - 13.5
  - 14.0
  - 14.5
  - 15.0
  - 15.5
  - 16.0
  - 16.5

- **Number of Pirates (Approximate)**
  - 35000
  - 45000
  - 20000
  - 15000
  - 5000
  - 400
  - 17

- **Data Points**
  - 1820
  - 1860
  - 1880
  - 1920
  - 1940
  - 1980
  - 2000

- **Legend**
  - STOP GLOBAL WARMING: BECOME A PIRATE!

- **Cartoon**
  - Panel 1: I used to think correlation implied causation.
  - Panel 2: Then I took a statistics class. Now I don’t.
  - Panel 3: Sounds like the class helped. Well, maybe.
The term “Functional Connectivity” (FC) indicates analyses of temporal covariance. Differently, the term “Effective Connectivity” (EC) commonly indicates analyses of causal relationships between different brain areas, and typically implies a temporal influence of an area over another region.

The most common approach for FC analyses is based on the calculation of the Pearson correlation coefficient. The Pearson coefficient is a measure of the linear correlation between two variables X and Y, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation.

\[
\rho(A, B) = \frac{\text{cov}(A,B)}{\sigma_A \sigma_B}
\]

\[
\rho(A, B) = \frac{1}{N-1} \sum_{i=1}^{N} \left( \frac{A_i - \mu_A}{\sigma_A} \right) \left( \frac{B_i - \mu_B}{\sigma_B} \right)
\]

\[\text{cov} = \text{covariance}; \ \sigma_A = \text{standard deviation of } A; \ \mu_A = \text{mean of } A\]
Independent component analysis (ICA) is a computational method for separating a multivariate signal into additive subcomponents. The ICA separation is based on two assumptions: (1) The source signals are independent of each other; (2) The values in each source signal have non-gaussian distributions. ICA is a special case of blind source separation (BSS).
ICA separates a signal into non-overlapping spatial and time components and for this reason can be used in the detection of resting state networks. It is highly data-driven and allows for better removal of noisy components of the signal (motion, scanner drift, etc). It also has been shown to reliably extract default mode network as well as many other networks with very high consistency.
Resting State Networks

- Default Mode
- Salience
- Primary Visual
- Auditory
- Sensory-Motor
- Right Fronto-Parietal
- Etc...
Resting-state networks consist of **anatomically separated**, but **functionally connected** regions displaying a high level of correlated BOLD signal activity. These networks are found to be quite consistent across studies, despite differences in the data acquisition and analysis techniques. Most of these resting-state components represent functional networks of regions that are known to share and support specific cognitive functions.
**Graph-Analysis – Typical Data Processing**

### Data Acquisition
- **T1 weighted Structural Image**
- **Continuous fMRI**
- **Resting State**

### Brain Parcellation
Can be based on individual anatomical or functional data, group-level data or standard atlases.

### Correlation Matrix
For most analyses, a threshold is applied (e.g., $r^2 > 0.3$) to obtain a binary matrix (connection present/absent).

### Signal Extraction and Connectivity Computation
Simplest Case: Pearson’s correlation coefficient between each pair of ROIs.
Graphs are mathematical structures used to model pairwise relations between objects. A graph is made up of vertices or nodes and edges or lines that connect them.

**Global efficiency:** Is a measure of how efficiently a network exchanges information. It is the average inverse shortest path length in the network, and is inversely related to the characteristic path length.

**Clustering coefficient:** Is a measure of the degree to which nodes in a graph tend to cluster together. It is the fraction of triangles around a node and is equivalent to the fraction of node’s neighbors that are neighbors of each other.

**Characteristic path length:** is the average shortest path length in the network (lengths of shortest paths between all pairs of nodes).

Etc...
Potential confounds arises from:

- Head/Body movements
- Cardiac and respiratory cycles,
- Variations in arterial CO2 concentration
- Blood pressure/cerebral autoregulation
- Vasomotion
Movement of the head causes the content of each voxel to change, and this will determine a change in the BOLD signal! This is particularly problematic at tissue interfaces and around large vessels.
Functional connectivity conclusions may be erroneous when motion artifacts have a differential effect on resting BOLD signals for between group comparisons!

For Instance...

Children and patient populations are usually more uncomfortable in the MR environment than young healthy controls, resulting in a greater amount of head motion!
Cardiac and respiratory cycles are relatively high frequency (~1 Hz and ~0.3 Hz, respectively) compared to the low-frequency (< 0.1 Hz) BOLD fluctuations.

Bulk motion related to the cardiac and respiration cycles will lead to similar confounds as motion of the head itself.

Pulsations of the vessels caused by cardiac-related pressure changes will generate small movements in and around large blood vessels.
Changes in **arterial CO₂ concentration** and **blood pressure** (both under sympathetic and parasympathetic nervous system control) can determine low-frequency BOLD fluctuations.

Regional low frequency oscillations may artificially inflate functional connectivity measures or cause spurious connectivity patterns where BOLD connectivity is not present or low.
Hypercapnia arising from breath-holds increases BOLD signal whilst hypocapnia arising from hyperventilation reduces it.

An increase in CO$_2$ concentration determines an increase in CBF as well as in the BOLD signal: the increased CBF reduces deoxyhaemoglobin concentration!

The response of the vasculature to CO$_2$ is very sensitive, thus any changes in respiration rate and resulting CO$_2$ variations will have consequences for resting BOLD signal fluctuations.
Cerebral autoregulation is the intrinsic dynamic ability of cerebral vessels to maintain steady-state CBF despite fluctuations in arterial blood pressure.

The arterial and arteriolar systems allow to maintain stable CBF by changing vascular tone through vasoconstriction and vasodilation processes.

Blood pressure variations are a key source of CBF fluctuations due to the delay in dynamic autoregulation.

Fluctuations in arterial blood pressure are likely to have a great influence on resting-state BOLD signal variations.
Implicit to the use of connectivity estimates over extended resting-state sessions is the assumption that the brain state of subjects will be homogeneous.

Brain states associated with considerable neurophysiological changes should be monitored and avoided.

Vigilance shifts indeed occur during rest, therefore it is in the interest of researchers to maintain a steady brain state during fMRI acquisition (e.g., wakefulness).
Simultaneous EEG-fMRI recordings

Sleep Scoring

SVM Classifier

Based on fMRI connectivity matrices
Accuracy corresponding to 75%
Sleep classification was conservative
Fluid Boundaries Between Wake and Sleep: Implications

- EEG-fMRI data collection
- Classifier trained to detect sleep
- 1147 fMRI datasets Analyzed
- Transition to sleep in $\frac{1}{3}$ within 3’

Tagliazucchi and Laufs, Neuron, 2014
Fluid Boundaries Between Wake and Sleep: Implications

Tagliazucchi and Laufs, Neuron, 2014

- Wake < N2 sleep
- Wake > N2 sleep

social anxiety disorder VS healthy controls
Exemplificative Pipeline for the Analysis Of Resting-State fMRI Data
3T scanner, Discovery MR750
GE Medical Systems

- 5 min eyes-open EPI resting-state scan: repetition time: 2000 ms, echo time: 25 ms, flip angle: 60, voxel size: 1.8 x 1.8 x 3.5 mm, plane: 128 x 128, number of slices: 40, total number of volumes: 150.

- high-resolution FSPGR T1 anatomical scan: repetition time: 8.2 ms, echo time: 3.2 ms, inversion time: 450 ms, flip angle: 12, voxel size: 1 x 1 x 1 mm, plane: 256 x 256, number of slices: 156.
Example: Data Preparation

Elimination of Initial Dummy Volumes (fMRI signal stabilization)

\[ 3dTcat -prefix GB_{resting\_cut} GB_{resting+orig}'[5..149]' \]

Slice temporal alignment

\[ 3dTshift -prefix GB_{resting\_tshft} GB_{resting\_cut+orig} \]

Spatial alignment (Head Motion Correction)

\[ 3dvolreg -verbose -maxite 50 -dfile GB_{resting\_out\_base} GB_{resting\_cut+orig}'[0]' -prefix GB_{resting\_vr} GB_{resting\_tshft+orig} \]

Elimination of Artifactual Spikes

\[ 3dDespike -prefix GB_{resting\_despike} GB_{resting\_vr+orig} \]
Example: Basic Preprocessing

Spatial Smoothing (6mm FWHM)

```shell
3dmerge -doall -1blur_fwhm 6 -prefix GB_resting_fwhm6
GB_resting_despike+orig
```

Spatial Transformation in Common Space (Talairach Space)

```shell
adwarp -apar GB_anat+tlrc -dpar GB_resting_fwhm6+orig -dxyz 2 -prefix
GB_resting_fwhm6
```

Generation of a Brain Mask (Exclusion of voxels outside brain parenchima)

```shell
3dAutomask -SI 130 -dilate 4 -prefix GB_resting_mask GB_anat+tlrc
```

Calculation of Signal Average for each voxel (baseline)

```shell
3dTstat -prefix GB_resting_mean GB_resting_fwhm6+tlrc
```

Signal normalization using the mean (baseline correction)

```shell
3dcalc -fscale -a GB_resting_fwhm6+tlrc -b GB_resting_mean+tlrc -c
GB_resting_mask_res+tlrc -prefix GB_resting_norm -expr '(a/b*100)*c'
```
**Example: Preparation for Data Cleaning**

Extraction of Signal from a White Matter ROI

```
3dmaskave -quiet -mrange 1 1 -mask ROI_CLEAN_fr+tlrc
GB_resting_norm+tlrc > ROI_WM.1D
```

Extraction of Signal from a Ventricular ROI

```
3dmaskave -quiet -mrange 2 2 -mask ROI_CLEAN_fr+tlrc
GB_resting_norm+tlrc > ROI_LV.1D
```

Deconvolution including stimuli of no interest

```
3dDeconvolve -jobs 4 -input GB_resting_norm+tlrc \
-polort 2 \
-num_stimts 8 \
-stim_file 1 'GB_resting.out[1]' -stim_file 2 'GB_resting.out[2]' -stim_file 3 \
 'GB_resting.out[3]' \
-stim_file 4 'GB_resting.out[4]' -stim_file 5 'GB_resting.out[5]' -stim_file 6 \
 'GB_resting.out[6]' \
-stim_file 7 'ROI_WM.1D' -stim_file 8 'ROI_LV.1D' \
 -cbucket out_cbucket -x1D out_x1D
```

Timeseries extracted from one WM region and one CSF region (vs Global Signal Regression used in the past)

6 rigid body head motion parameters (derived by retrospective motion correction)
Example: Data Cleaning

Extraction of waveforms representing signal of no-interest

3dSynthesize -cbucket out_cbucket+tlrc -matrix out_x1D -select all -prefix out_effectsofnointerest

Removal of signal of no-interest from data

3dcalc -datum float -a GB_resting_norm+tlrc -b out_effectsofnointerest+tlrc -expr 'a-b' -prefix GB_resting_norm_clean

Low-pass filter at 0.1 Hz

3dFourier -prefix GB_resting_norm_clean_bp -lowpass 0.1 GB_resting_norm_clean+tlrc

BOLD modulations of neural origin are absent above this frequency, whereas artifacts of various origin, including cardio-pulmonary pulsations, are not
Seed-Based Functional Connectivity Analysis: FC Calculation
Seed-Based Functional Connectivity Analysis: Results
Conclusions
• Resting-State paradigms can be used to study the brain functional organization: several networks can be identified!

• The Default Mode Network is the main functional network at rest, and its activity is reduced during active tasks

• Changes in the DMN and in other resting-state networks seem be associated with specific clinical conditions.

• Resting-State networks are investigated using seed-based functional connectivity or independent component analysis

• Several factors can modulate Resting-State connectivity: physiological noise may lead to spurious correlations!


