HST.583 - Lab 2: Physiology Lab Manual

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Introduction

The purpose of this lab is to familiarize you with the effects of global physiological changes on the BOLD signal. It is important to be aware of such effects in fMRI because some experimental protocols may lead to unintentional physiological changes. An example would be a cognitive experiment in which intense concentration or anxiety about performing the task correctly leads subjects to change their breathing. As you will see in this lab, even a modest shift in breathing rate can have a significant effect on the BOLD fMRI signal.

Another reason for studying BOLD responses to global physiological changes is that this is a good way to examine *regional differences in BOLD sensitivity*. The fractional change in BOLD signal for a given change in blood flow can vary in different parts of the brain, depending on the local density of blood vessels and the physiological state of the particular tissues at rest. It's difficult to study this using neuronal stimulation, because there's no easy way to ensure *equivalence* of the stimuli used to activate different regions. Respiratory perturbations like the one used in this lab provide a good way to circumvent this problem, because as *global* perturbations they have a simultaneous and similar effect on all parts of the brain.

In the exercises below you will be asked to make a number of observations and answer questions (highlighted in red). Your lab report will be the summary of these observations and answers, so be sure to make notes and printouts as you go.

Description of acquired data

In the exercises below you will examine the effects of a relatively modest but sustained change in breathing on the BOLD fMRI signal. In the data acquisition component of this lab, we acquired a number of BOLD EPI datasets including three during which the subject underwent different combinations of *breathing change* and *neuronal activation*:

- **visual stimulation and simultaneous right-hand movement task:** in this seven minute long scanning run, the subject was exposed to a visual stimulus throughout the third and fourth minutes. The subject was also instructed to perform repetitive finger tapping movements with her right hand for the two minute period that the visual stimulus was on. During the rest of the scan (the first two minutes and the fifth through seventh minutes) she viewed a uniform grey screen and did not move her hand.
- **deep breathing, no task:** in this run, also seven minutes long, the subject did not receive visual stimulation or move her hand. Instead, she deliberately increased the depth of her breathing for a four minute period during the scan (the second through fifth minutes). Deep breathing lowers the amount of carbon dioxide in the blood, which in turn lowers blood flow throughout the brain (CO_2 is a vasodilator). This scan should therefore show us the effects of such a change in blood flow on the BOLD signal in different parts of the brain.
- **visual stimulation/hand movement *plus* deep breathing** In this run we combined the events in the two previous scans. That is, the subject breathed more deeply for four minutes and in the middle of this period of *hyperventillation* she underwent visual stimulation and performed the hand movement task. The purpose of this run is to see how breathing-related effects combine with the responses to visual and motor tasks.

The timing of the conditions described above is summarized in the graph below. The elevated portions of the blue and red plots represent the periods during which the subject was breathing deeply and/or undergoing the visual-motor task.
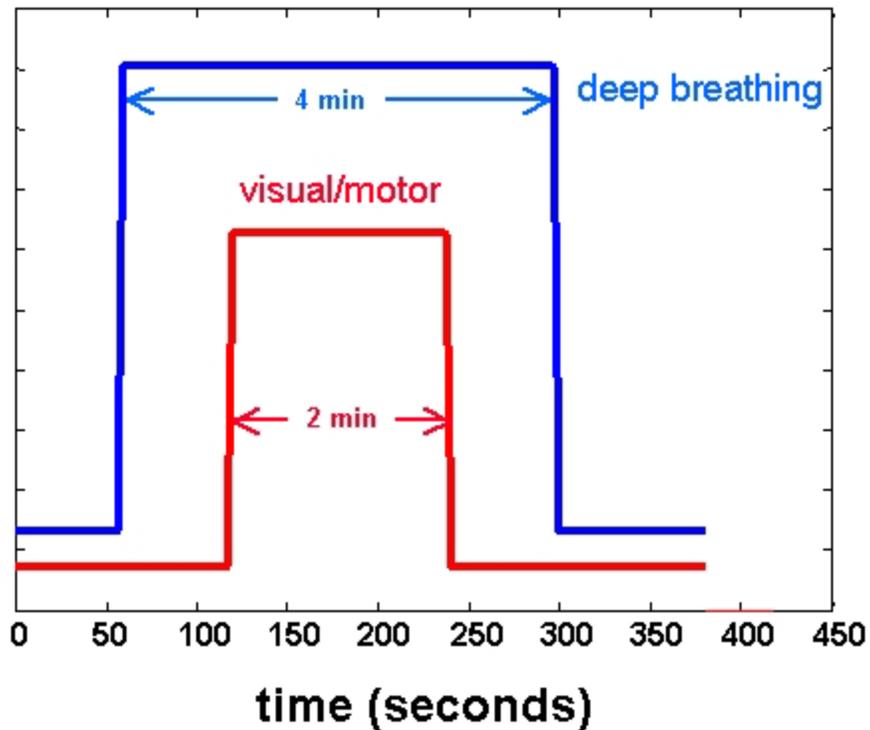
There are also two BOLD EPI series which will be used for mapping projections of the retina and right hand in the brain:

- a series with a semicircular visual stimulus rotated through three cycles of 360 degrees.
- a series in which the subject alternated between a right and left-hand motor task (3 cycles of each)

These provide a robust and specific means of identifying tissue volumes stimulated by the visual and motor inputs applied during the previous scans. We could also have tried to generate activation maps using the visual stimulation/hand movement scan without deep breathing, but there are two things that make this less than ideal: 1) the visual stimulation and hand movement periods had identical timing, complicating discrimination between hand and motor responses, and 2) the timing of the tasks was designed around the slow evolution of the breathing-related effects and is not optimal for response detection. For more information on the phase-encoding approach used to identify retinal and hand projections, see the section on retinotopy in the manual for Lab 1.

> **Pulse sequence details - functional scans:** All BOLD functional scans in this experiment were performed with the following parameters:

- acquisition type: 2D echo-planar imaging (EPI)
- spatial resolution = 4x4x4mm3
- time needed to acquire one brain volume: approx. 1 second
- repetition time (TR) = 3 seconds
- echo time (TE) = 30 milliseconds
- echo type: gradient-echo
- flip angle = 90 degrees
- image matrix size: 64x64 x 20 slices
- static magnetic field = 2.9 Tesla

Also included in the lab data are a 3D T1-weighted anatomic scan and a 3D MR *angiogram* (for visualizing vasculature).

Pulse sequence details - 3D T1-weighted scan: The anatomic scan used the following parameters:

- acquisition type: 3D Magnetization Prepared RApid Gradient Echo (MP-RAGE)
- spatial resolution = 1x1x1mm3
- time needed to acquire one brain volume: 4 minutes, 30 seconds
- repetition time (TR) = 11 milliseconds
- echo time (TE) = 4 milliseconds
- echo type: gradient echo
- flip angle = 8 degrees
- image matrix size: 256x256 x 160 slices
- static magnetic field = 2.9 Tesla

Pulse sequence details - MR angiogram: For the magnetic resonance angiogram, we used the following parameters:

- acquisition type: 3D RF-spoiled, gradient-echo scan with magnetization transfer preparation
- spatial resolution = 0.4x0.4x0.8mm3
- time needed to acquire one brain volume: 6 minutes, 44 seconds
- repetition time (TR) = 35 milliseconds
- echo time (TE) = 3.6 milliseconds
- echo type: gradient echo
- flip angle = 20 degrees
- image matrix size: 512x512 x 90 slices

> ○ static magnetic field = 2.9 Tesla
>
> The combination of short TR and relatively large flip angle result in *inflow enhancement* of the blood. A magnetization transfer prepulse is also used to selectively attenuate the tissue (but not the blood).

Now lets look at some of these scans. To get started, we need to initialize our working environment. Then we'll generate the regions of interest we will need, and apply these to the other EPI series with deep breathing and/or visual-motor activity.

Region of interest generation

Up until now, most of the signals you've looked at have been for single voxels. As the basic imaging unit, the voxel is important to think about. However, the signal-to-noise ratio of our observations can be greatly enhanced by averaging over multiple voxels. Note that this is only helpful to the extent that a set of voxels can be considered equivalent in terms of their tissue makeup and sensitivity to a particular stimulus. Such a grouping of similar voxels, averaged to enhance the signal-to-noise ratio, is often referred to as a *region of interest* or ROI.

Retinal projection ROI

First, generate an *F statistic map* for the retinal mapping scan. This is basically a map of the spectral power (relative to noise) in the time-series at the frequency of a periodically modulated input stimulus:

1. open the file **is-0-allegra-20002-20011003-163352-9-mri.mnc**
   (this contains the data needed for retinal mapping)
2. from the **Tools->Statistics** submenu, select **F statistic for periodic design**
3. click **OK**
   (the default parameters should be good - we skip the first 8 frames and have three cycles of stimulus modulation over the remaining 120)

After a short wait you should see the F-statistic map in the Dview viewports. The activated region should be at the back of the brain towards the lower (smaller z value) edge of the volume.

What do those parameters mean in the F statistic menu? This analysis actually produces *two* output volumes. Under the **Select** menu the phase volume will appear with the -fftp suffix and the F-statistic (i.e. the FFT magnitude-derived map) will have the -fftm suffix. In the case of the alternating right-hand/left-hand task, the F statistic image will highlight areas associated with *both* hands. The phase will allow us to discriminate the left and right hand representations in cortex. In order to perform the required spectral analyses, the following information is needed:

- o **Excluded initial frames: [1:8]** In the retinal mapping scan we want to exclude the first eight frames because these include bright, non-steady-state scans and also because they include the short dummy cycle of stimulus modulation.
- o **Number of periods (after excluded frames): 3** After the excluded frames, we performed three full cycles of stimulus modulation over the remaining 120 frames (achieved by rotating the semicircular mask over the radial checkerboard pattern three times). This corresponds to a modulation period of 40 frames, so we will look at the equivalent frequency in the temporal power spectrum to identify retinotopically organized cortex.
- o **Modulus threshold (for phase computation): 0.1** This parameter tells the software to only compute the phase in voxels in which the F statistic is above the entered value. The F statistic map will highlight voxels in which the temporal BOLD signal is strongly periodic with the same frequency as the stimulus modulation. It tells us nothing about the *phase* of such periodic responses, however. The phase of the temporal power spectrum at the stimulus modulation frequency allows different cortical locations to be associated specifically with different polar angles in the visual field. The problem is that the phase values in voxels that are not affected by the stimulus will be wildly random (their distribution has no central tendency or mean value), making it difficult to use thresholding for ROI delineation.
- o **Phase offset in radians: pi./2** This is a constant added to the value of each voxel in the phase image, based on arbitrary aspects of the experimental design. It can be useful to prevent phase-wrapping of values greater than 2π back to 0 in regions where there should really be a continuous gradient in the cortical mapping of visual field. This has no effect on the F-statistic image.

The next step will be to generate an ROI based on this activation map. This is done by automatically selecting all voxels in the volume in which the intensity values (in this case F-statistics) are in a prescribed range. The resultant volume of tissue will be highlighted in pink, and can be applied to other volumes acquired in the experiment to compute the average signal within the ROI.

1. from the **Tools->Region of Interest** submenu, select **Create ROI->by thresholding current volume**
2. Name the ROI **Visual**
3. enter a lower limit of **0.12**
4. click **OK**

You should now see the ROI overlaid on the F-statistic map in pink. Now we'll map the right-hand sensorimotor projection.

Right hand projection ROI

To map hand projections, we had our subject alternate between one minute intervals of left or right hand movement. Because she was always moving one hand, there should be little modulation of non-specific sensorimotor pathways. In tissue with specific projections to the right or left hand however, there will be a squarewave modulation of activity with a two-minute period.

Identifying the right-hand projection is a little more complicated than for the retina, because we have both right and left hand activation in the F-statistic map. To discriminate between the two hands, we'll make use of the *phase* of hand-related response waveforms. We can do this because the right and left hand movement periods were 180 degrees out of phase with respect to each other.

1. open the file **is-0-allegra-20002-20011003-163352-10-mri.mnc**
   (this contains the data needed for hand mapping)
2. from the **Tools->Statistics** submenu, select **F statistic for periodic design**
3. change **Modulus threshold (for phase computation)** to **0.175**
4. change **Phase offset in radians** to **0**
5. click **OK**
   (the rest of the default parameters should be good)

Again you should see an F-statistic map in Dview after a short wait. There should be two main activation foci on the left and right sides of the brain, although you will probably have to scroll through the brain to find them. The coronal view gives what is probably the best depiction of the bilateral activation foci. This time we will use the *phase* image to discriminate between left and right hand activity (note: don't confuse the phase of the response waveform with transverse magnetization phase!).

Again let's make an ROI:

1. using the **Select** menu, select the file **is-0-allegra-20002-20011003-163352-10-mri-fftp.mnc**
   (this is the phase of the response)

2. from the **Tools->Region of Interest** submenu, select **Create ROI->by thresholding current volume**
3. Name the ROI **Rhand**
4. change the *upper* limit to **-1.7**
(the lower limit should be fine)
5. click **OK**

You should now see the ROI overlaid on the F-statistic map in the usual ugly pink. Now we are ready to apply these ROI's to the other data we acquired.

ROI-based signal analysis

In the previous steps, you used functional datasets that were designed to optimize detection power for identification of 3D tissue volumes containing retinal and hand projections. These tissue volumes will necessarily undergo blood flow changes during the other scans, because we have either lowered arterial CO_2, activated neurons within the ROI, or both. In this exercise we will average signals within the retinal and right hand ROI's to look at the resultant BOLD responses.

Visually evoked response

1. open the file **is-0-allegra-20002-20011003-163352-6-mri.mnc**
(this contains the data during simultaneous visual and hand activation)
2. from the **Tools->Region of Interest** submenu, select **Apply ROI to current file** and pick **Visual** (the ROI we created). You should see the ROI overlaid on the EPI volume. The plotted signal will now be the *average* signal within the ROI (it will no longer track the yellow cursor).
3. using the selection box in the lower right corner of the Dview window, change the signal type from **Raw signal** to **Percent signal**
4. note the strong signal at the start of the signal (time = 0); this is due to fully relaxed magnetization prior to application of repeated RF excitation pulses and arrival at steady-state
5. to exclude the initial non-steady-state frames, select **Tools->Stimulation/Timing->Enter frames to exclude->as MATLAB® expression** and click **OK** to accept the default (the MATLAB® vector `[1 2 3]` tells Dview to exclude the first three frames here).
6. the visually evoked response, lasting from 120-240sec, should be clearly visible - to see a depiction of the stimulation timecourse, select **Tools->Stimulation/Timing->Enter design input matrix->as MATLAB® expression** and enter the following MATLAB® expression:

M = [zeros(1,40) ones(1,40) zeros(1,60)];

you should see a depiction of the stimulation time-course plotted in red over the signal, and the average percent change will be reported.

7. *make a note of the average percent change in the visual ROI during the visual/motor task.*

> **How does Dview compute changes during activation?** By now you should have noted that, after entering a design matrix, Dview tries to compute *percent changes* during periods of activation relative to some baseline period. Here is a brief outline of the procedure used: periods in which the design input matrix is *zero* are used as the reference baseline. Periods in which the design input matrix is *one* are deemed to reflect the non-baseline condition of interest. Design matrix values that are neither zero nor one are not used in calculating changes. Percent change is calculated as the average signal level during activation (design = 1) minus the average signal during baseline (design = 0), divided by the average baseline signal level and multiplied by 100. Points that have been *excluded* (flagged by green x's in plots) are never included in calculations. When the plot mode is set to **Raw signal** the difference value is in raw MR signal units - not percent.

Sensorimotor response

1. open a second instance of the file **is-0-allegra-20002-20011003-163352-6-mri.mnc**
   (you can do this by just opening the file again without closing the first instance)
2. from the **Tools->Region of Interest** submenu, select **Apply ROI to current file** and pick **Rhand** (the other ROI we created)
3. using the selection box in the lower right corner of the Dview window, change the signal type from **T signal (raw)** to **Percent signal**
4. once again, to exclude the initial non-steady-state frames, select **Tools->Stimulation/Timing->Enter frames to exclude->as MATLAB® expression** and click **OK** to accept the default (this excludes the first three frames)
5. the sensorimotor response, lasting from 120-240sec, should be clearly visible - to see a depiction of the stimulation timecourse, use **Tools->Stimulation/Timing->Enter design input matrix/vector->as MATLAB® expression**; the expression you entered last time should now be the default, so just click **OK**
6. *make a note of the average percent change in the right-hand ROI during the visual/motor task. Is it different from the response in the visual area?*

> **Comparing the visual and sensorimotor responses**   When comparing the visual and sensorimotor responses, it's important to remember that they were evoked by quite different stimuli. The visual stimulus, a rapidly modulated (8Hz) high-contrast radial checkerboard pattern, is quite potent as visual stimuli go. On the other hand, the motor task performed was of relatively low complexity. Simpler tasks and subtler sensations do not always lead to weaker responses, but in many situations there is a correlation (or at least a dose-response curve) between perceived intensity and amplitude of the associated BOLD response (the relationship between contrast of a visual stimulus and response in V1 is a good example).
>
> It is also worth thinking about the factors that dictate the *spatial extent* of regions activated by the visual and hand tasks. The boundaries of the visual response are determined by the area of the retina (or, equivalently, visual field) that is stimulated. The size of the area associated with the finger movement task is determined by the distribution of motor units in the hand that are involved (basically how many fingers were used).
>
> Note that we could easily arrange to have a large area of activation with small response amplitude by exposing a large extent of the retina to a very low contrast visual stimulus.

Responses to deep breathing

Now we'll look at the BOLD signal changes in both the visual and right-hand ROI's produced by deep breathing (with no stimulation/activation). Remember that deep breathing will change blood flow *everywhere* in the brain - including our ROI's.

Visual ROI:

1. open the file **is-0-allegra-20002-20011003-163352-8-mri.mnc** (this file contains the deep-breathing-only data)
2. change the signal type to **Percent signal**
3. apply the **Visual** ROI to this file
4. exclude the first three frames
5. enter the following design input matrix (note that it's slightly different from the one used above for visual and sensorimotor tasks):

   M = [zeros(1,20) ones(1,80) zeros(1,40)];

6. *make a note of the average percent change in the Visual ROI during deep breathing. Is the BOLD signal decrease caused by deep breathing larger or smaller than the increase induced by visual stimulation?*

Right-hand ROI:

1. open another instance of the file **is-0-allegra-20002-20011003-163352-8-mri.mnc**
2. change the signal type to **Percent signal**
3. apply the **Rhand** ROI to this file
4. exclude the first three frames
5. enter the same design input matrix you just used for the visual ROI
6. *make a note of the average percent change in the Right-hand ROI during deep breathing. Is the breathing-related negative change in BOLD signal the same size as the signal increase caused by the motor task? Compare the average breathing-related changes in BOLD signal in visual and motor cortices.*

Grey and white matter ROI's:

Here, you will manually paint regions of interest in grey and white matter regions that should not be affected by the visual and/or motor tasks. The purpose is to compare grey and white matter signal changes during a global blood flow change.

1. open yet another instance of the file **is-0-allegra-20002-20011003-163352-8-mri.mnc**
2. right-click on the transverse view and select **Copy this view to big window**
3. change the signal type to **Percent signal**
4. exclude the first three frames
5. enter the same design vector you used previously to depict the time course of deep breathing
6. from the **Tools->Region of Interest** submenu, select **Create ROI->by manually painting it**. Give it the name "Frontal".
7. Now you can paint an ROI on the current volume by clicking the *middle* mouse button. Note painting a voxel does *not* force the triplanar display to that location, but you can still use the left mouse button to move around in the volume. Paint a region of interest of about 10 grey-matter voxels in frontal cortex.
8. *make a note of the average percent change in the frontal grey-matter ROI during deep breathing. Repeat the above steps for a grey matter ROI in parietal cortex, and a white matter ROI anywhere in the brain (call them Parietal and WhiteMatter - or something; get about 10 voxels in each*

region). Compare the breathing-induced signal changes in the three regions of interest.

Responses to visual and sensorimotor stimulation during deep breathing

Now we'll look at the BOLD signal changes in the visual and right-hand ROI's produced neuronal activation induced during a period of deep breathing. The goal here is to see how the activation and breathing-associated responses interact. *If we cause neuronal activation against a BOLD signal baseline that has been lowered by deep breathing, will the changes summate linearly? Is the BOLD signal during activation independent of the baseline?* Discuss possible responses to these questions in your report.

Visual ROI:

1. open the file **is-0-allegra-20002-20011003-163352-7-mri.mnc** (this file contains the scan with visual and sensorimotor activation combined with deep breathing)
2. change the signal type to **Percent signal**
3. apply the **Visual** ROI to this file
4. now using **Tools->Stimulation/Timing->Enter frames to exclude->as MATLAB® expression** exclude the first 23 frames and the last 40 (enter `Exclude=[1:23 101:140]`)
5. enter the design input matrix for visual/motor stimulation to obtain the visually-evoked signal change against the decreased hypocapnic baseline. Is this change the same size as the visually-evoked response during normal breathing?

Right-hand ROI:

1. open another instance of the file **is-0-allegra-20002-20011003-163352-7-mri.mnc**
2. change the signal type to **Percent signal**
3. apply the **Rhand** ROI to this file
4. again, using **Tools->Stimulation/timing->Enter frames to exclude->as MATLAB® expression** exclude the first 23 frames and the last 40 (enter `Exclude=[1:23 101:140]`)
5. enter the design input matrix for visual/motor stimulation to obtain the motor task-induced signal change against the decreased hypocapnic baseline. Is this change the same size as the sensorimotor response during normal breathing?
6. *prepare a brief and qualitative description of the interaction between the breathing and activation-related signal changes (visual and motor)*

Examination of physiological signals

During the experimental session, we also recorded several physiological signals from the subject. These included heart rate, end-tidal CO2 (a measure of CO2 in the arterial blood), and tidal volume. Now you will examine these recordings, which are contained in Microsoft Excel files, and see how they correspond to the observed MR signals.

1. open another instance of the file **is-0-allegra-20002-20011003-163352-8-mri.mnc**
   (this run is the deep breathing-only data)
2. change the signal type to **Percent signal**
3. exclude the first three frames
4. apply the **Rhand** ROI to this file
5. from the **Tools->Stimulation/timing** menu, select **Enter design matrix/vector->from Excel file**
6. select the file **Run8-ETCO2.xls** to see the decrease in end-tidal CO2 during deep breathing; note the correspondence to the period of BOLD signal decrease
7. to see the plot of end-tidal CO2 with axis scaling, execute the following MATLAB® commands:

   ETCO2 = xlsread('Run8-ETCO2.xls');
   plot(ETCO2)
   xlabel('frame')
   ylabel('End-tidal CO2 (mmHg)')

   make a note of the initial "baseline" value, and the minimum value at the end of the period of deep breathing (around the 100th frame); does end-tidal CO2 return to baseline by the end of the final two minute recovery period?

8. now read in the design vector from the file **Run8-TidalVolume.xls** to see changes in breathing depth during the scan. You can also load **Run8-HeartRate** to see heart rate during the scan.
9. repeat the steps above for the **Visual** ROI, a frontal grey-matter ROI, and a white matter ROI.
10. *prepare a brief and qualitative description of the relationship between the different physiological parameters measured and the BOLD signal. Does this relationship vary across the brain?*

Magnetic resonance angiography

At the end of the session, we acquired an *MR angiogram*. This scan shows blood vessels as regions of higher intensity, and can be used to identify vascular responses in BOLD activation experiments. Series number 12 (is-0-allegra-20002-20011003-163352-12-mri.mnc) is the angiogram - load the file in Dview and adjust the window levels to obtain good depiction of the vasculature. You can use the vascular images to position the cursor, then select an EPI series to see the BOLD signal at the approximate location of the vessel. Note that, with a 512x512x90 scan matrix, this file is huge and will take a long time to load. It's also important to realize that not all blood vessels show up strongly on such angiograms. Veins carrying blood which has spent a lot of time in the imaged volume may be very faint since the inflow enhancement effect diminishes after repeated RF excitations.

Lab report

Carry out the above exercises, and submit a concise report consisting of the observations you were directed to make above. You can print the Dview display window under the **File** menu, so feel free to make use of annotated printouts like this to describe your findings.

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