Observation and correction of a vascular time-lag effect, disruptive to functional connectivity analysis.

O. Grigg[1], G. Goelman[1]

[1]Hadassah Hospital Medical Center, Jerusalem, Israel

Synopsis:

To investigate the sensitivity of the functional connectivity approach, we applied connectivity histogram analysis on the layers of the rat's cortex. We discovered a time lag effect, which disrupts connectivity measures. We hypothesize that this effect is hemodynamic in nature, caused by propagating hemodynamic changes. Using several analysis tools, we aimed to understand the physiological basis of this effect and to correct it.

We describe our analysis methods, findings, the theorized physiological basis of them, and significance to functional connectivity analyses.

Introduction:

Functionally connected regions of the brain exhibit a high degree of temporal coherence of blood flow fluctuations. This synchrony implies the existence of neural connections that facilitate coordinated neural activity. In this work, we aimed to investigate the sensitivity of the functional connectivity approach by applying it on the layers of the rat's cortex. We chose to use Correlation-Coefficient (CC) histogram analysis in order to retain all the information, by applying no cutoff and no averaging on the data.

We hypothesized that layers more rich in synapses (such as layer 2 and 4) will exhibit higher intra-connectivity, and layers, which are known to be functionally connected to each other (such as layers 6➔1 and 2➔5) will exhibit higher inter-connectivity.

Unexpectedly, we discovered two phenomena: **1.** 89% of all histograms contained negative flanks as big as the positive flanks, i.e., contained large proportions of negative CC's. **2.** A significant depth dependent decrease in the overall connectivity measures. We used several analysis tools to investigate the physiological basis of these phenomena and to correct them, and created a vascular lag effect theory to explicate them.

Here, we describe our analysis methods, findings, the theorized physiological basis of them, and significance to functional connectivity analyses.

Methods:

Experiment protocol: 12 SD rats were anesthetized with Isoflurane, and held at a sub-awake state. fMRI measurements were performed using a 4.7 T BioSpec system and a GE-EPI sequence. A single transverse slice at Bregma 0.48 mm was selected. Slice thickness=1 mm, FOV=3 cm, acquisition matrix=192 x 128, zero filled to 256 x 256; producing effective voxel dimensions of 117 x 117 x 1000 µ. Each fMRI set consisted of 400 scans at a TR=1 sec. (x 2 averages) and TE=25 msec.

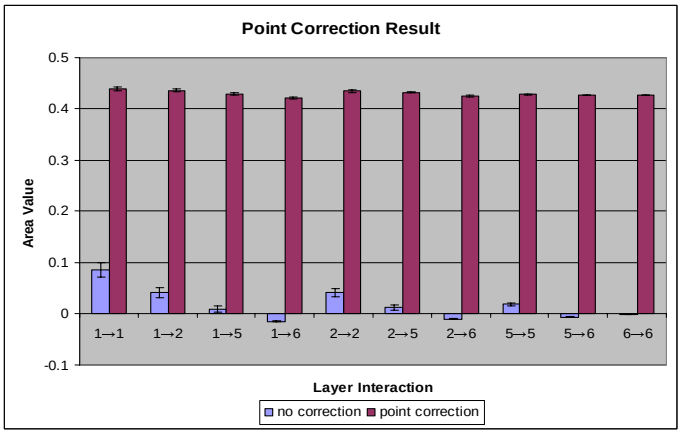
Defining regions of interest: The cortical layers of the motor and sensory strips of each hemisphere were defined as regions of interest (ROIs), using a rat brain atlas and IDL software written by us. Since layer 4 is of varying thickness along the cortex (in some areas absent altogether), it was included in the layer 2+3 ROI. Thus, we obtained 16 ROIs: 4 cortical areas (motor R, motor L, sensory R, and sensory L) X 4 layers (layer 1, layers 2+3+4, layer 5, and layer 6).

Preparing and segmenting the data sets: All data sets were low-pass filtered (0.15Hz) to remove the cardiac and respiratory frequencies. The first 10 scans from each dataset were deleted, and the remainder was divided into 12 segments of 30 scans each, in order to examine connectivity changes in time.

Connectivity analysis: Correlation analysis within and between the ROIs for all segments was performed, resulting in 4 (motor and sensory, L and R) X 10 (all interactions) X 12 (segments) = 480 CC distribution histograms. Every such connectivity analysis between ROIs computes the interaction between all the pixels contained in the first ROI and all pixels in the second ROI and, accordingly, was termed "an interaction". The weighted area under each histogram was computed.

Results and Discussion:

We chose to use CC histogram analysis in order to retain all the information by applying no cutoff and no averaging on the data. The weighted area of the histograms provides an overall assessment of the connectivity value; the *closer* it is *to* zero, the more symmetrical around zero the histogram is, and the *farther* it is *from* zero (both higher/positive or lower/negative), the histogram as a whole has more positive/negative CC bulk. We encountered two unexpected phenomena: **1.** 89% of all CC distribution histograms were centered around zero (had an area of between ±0.15), i.e. had negative flanks as big as the positive flanks. **2.** Connectivity measures were much lower than expected, with further decreases as deeper layers were examined. A possible and plausible source for the *negative CC histogram flanks*, and for the *depth phenomenon* we have observed, could be a time shift in the fluctuations of the hemodynamic activity, between pixels. Meaning, assuming that fluctuations are pseudo-periodic, when a time-shift between two pixels exhibiting the same periodicity, is such that one time-course is in a half-phase delay relative to the second time-course, a negative CC will result. We found typical delays between pixels to be in the time order of seconds. The hemodynamic delay, as seen in BOLD MRI is known to be in the time order of seconds also. Therefore, we assume the time lag we found to be hemodynamic in origin. A physiological source for such a time lag can be a propagating hemodynamic change; a change in one site that propagates, through the vasculature, to sites in the vicinity. With the existence of such propagation, the correlation of any two pixels will decrease respectively to their distance, as, upon examining our data, we indeed found. We also found that the lag found could be partially corrected by shifting the "lagged" time-course forward to eliminate the lag. In order to determine the lag, "correction curves" were computed, based on the degree of shift necessary to maximize pixels' correlations. The correction curves showed correspondence between pixels' distance and the shift needed. Two correction methods were examined: Point correction – assumes the source of the hemodynamic propagation for a pixel is the pixel itself, thus, the correction curve is computed radially, from the pixel outwards. Vertical correction – assumes that the source of the hemodynamic propagation is the apex of the cortex, which is known to be dense with vasculature. Thus, the correction curve is computed vertically, from top to bottom. Only the point correction eliminated all negative CC's, with the overall outcome of raising all interactions towards much higher connectivity measures. **To conclude**: we hypothesize the existence of propagating hemodynamic changes in the brain's vasculature, which disrupt correlation analysis between pixels/regions in the brain, by creating a lag between pixels' time-courses, resulting in negative CC's. By nature, functional connectivity analysis is over-sensitive to this phenomenon, and steps to correct this purely vascular component should be taken. We propose a preliminary correction method.



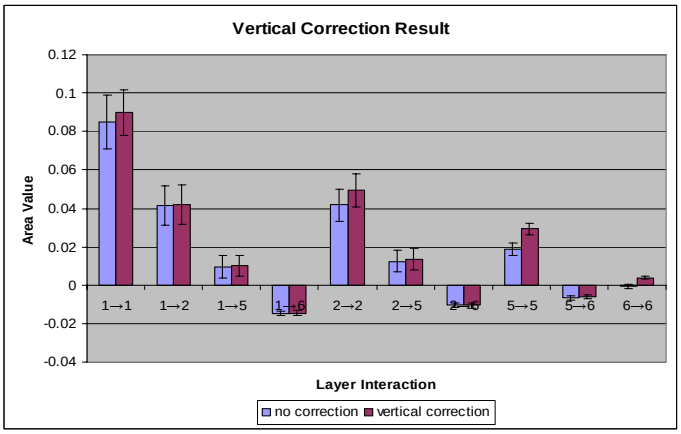


Fig 1. The outcome of the two correction methods used, versus the original results, averaged for all animals. For simplification, we show results averaged for the entire cortex, as results for the motor and sensory areas were similar. **Right**: Vertical correction managed to eliminate some of the negative CCs in the histograms. **Left:** Point correction managed to eliminate all negative CCs, and increased the overall measures toward more reasonable values.