Attenuation of Brain BOLD Response Following Lipid Ingestion

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Abstract: A great deal of heterogeneity exists in fMRI data. Even within the same subject, results on successive days or scan sessions often differ in the number of significantly activated pixels and/or the intensity of activation. We sought to assess whether controllable physiologic modulators, such as dietary factors, could influence the outcome of fMRI data. A high fat diet, for example, prior to a fMRI scan could change microvascular blood rheologic factors and potentially alter brain blood oxygen-level dependent (BOLD) signal patterns. In healthy adult volunteers, we measured brain BOLD signal during bilateral finger tapping (2 Hz) in the fasted state, and at 40 and 100 minutes post-ingestion of a 235 mL can of Ensure Plus (Ross Labs), alone or supplemented with either 25cc or 50cc of canola oil. Both the 25cc and 50cc Canola oil treatments produced a significant bilateral decrease in BOLD signal 40 and 100 minutes postprandial. No significant effect was observed with Ensure in the absence of oil. Therefore, to decrease fMRI within and between subject heterogeneity, and thereby increase fMRI statistical power, it is suggested that scanning within 2 hours post high fat ingestion should be avoided. As a corollary, a thorough understanding of a subject's physiological state, prior to an fMRI exam, may reduce the impact of other confounding variables. *Hum. Brain Mapp.* 20:116–121, 2003. © 2003 Wiley-Liss, Inc.

Key words: fMRI; brain; signal heterogeneity; fat; diet; motor cortex

INTRODUCTION

Functional magnetic resonance imaging (fMRI) is a well-accepted method to non-invasively study brain activation. However, there exists a great deal of heterogeneity in the published literature concerning the intensity and distribution of brain enhancement in fMRI studies, even during the

same activation tasks (Chen et al., 1998; Davis et al., 1997; McGonigle et al., 2000). Oxygen content, blood volume, perfusion, and/or tissue metabolism are collective physiologic factors that can potentially vary the oxy-to-deoxyhemoglobin ratio, and hence result in variation in the blood oxygen level dependent (BOLD) signal. Complex physiologic factors are, without a doubt, important contributors to between-subject variability but these may also represent an important source of within-subject heterogeneity.

Heterogeneity in fMRI BOLD signal is well known to occur as a result of physical factors such as probe quality, signal-to-noise ratio (SNR), magnetic field homogeneity, field strength, and others. It is understood that handedness (Solodkin et al., 2001) and sex (Karama et al., 2002) may produce different fMRI results with the same task. In addition, age (D'Esposito et al., 1999) and cardiac rhythm (Dagli et al., 1999) contribute to variability. Many other biological sources of heterogeneity are far less studied and thus more difficult to recognize. For example, complex biological factors including circadian and circannual rhythms, overall

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health and fitness level, diet, medications, ingestion or inhalation of bioactive compounds, use of herbal medicines, and so on, are factors that may or may not be important when considering subjects for an fMRI study. Recently, caffeine was shown to increase BOLD contrast in specific tasks (Mulderink et al., 2002). Thus, it is theoretically possible that fMRI signal heterogeneity may arise from something as simple as ingestion of vasoactive compounds, or other substances that may affect the microvascular environment. As postprandial lipemia has a known hemodynamic effect (Vogel et al., 1997), we sought to assess whether ingestion of a controlled fat meal would alter brain BOLD hemodynamic response and thus contribute to within and between subject fMRI data heterogeneity.

MATERIALS AND METHODS

Experimental Design

The research performed here on human subjects was acceptable by our local research ethics board and in compliance with the code of ethics as stated in the Declaration of Helsinki. Eight healthy right-handed adult volunteers (average age 23.1 years, range 19–34) were screened and approved for MRI compatibility. Subjects were asked to fast overnight (8–10 hours) prior to the study. Measurements were obtained prior to ingestion (baseline), and 40 and 100 minutes following ingestion of a high fat drink. The high fat drink consisted of 235 mL of Ensure Plus (Ross labs) supplemented with either 25 or 50 cc of Canola oil. Control sessions contained only Ensure. The fat content in Ensure is 11 g, which provides 17% of the drink's total caloric value. Each of the eight volunteers consumed all three drinks on separate days, separated by at least 1 week, over the course of the experiment.

A block design motor paradigm, consisting of bilateral finger tapping (2 Hz) with "on" and "off" intervals lasting 30 sec each, was tested. Twelve blocks, or six on-off cycles, were collected per run. Subjects were instructed to switch between "on" and "off," or "off" and "on" states by an optical cue; a light in the MRI suite was dimmed and brightened over a 1-sec period. A rapid optical cue was used rather than a sensory cue (i.e., someone tapping the subject on the foot) to avoid B_0 distortions caused by experimenters in the MRI suite. All subjects were able to respond to this cue with perfect accuracy. Measurements were repeated twice per time point per subject.

MRI Acquisition

Data was acquired using a General Electric (GE, Milwaukee, WI) 1.5T CV/i MRI, with maximum gradient strength of 4G/cm. A T1-weighted 3D-SPGR (SPoiled Gradient Recalled echo) was used for anatomical localization of the BOLD data (15° flip angle, 256×192 matrix, 2 NEX, 22 cm FOV, 1.5 mm thick, 110–120 contiguous slices). The resultant resolution was interpolated to 1 mm³ voxels. A fast gradient echo imaging sequence with a

spiral readout (Glover and Lai, 1998) was employed for functional scans (25 contiguous slices, 22 cm field of view, 5 mm thick, TE = 40 msec, TR = 2 sec, 1 NEX, 100 kHz bandwidth, 1 spiral interleaf containing 4,096 points). Prior to Fourier transformation, spiral k-space data was regridded to Cartesian based coordinates (Glover, 1999) giving a final voxel size of $3.75 \times 3.75 \times 5$ mm thick. Spiral k-space acquisition was used rather than a rectilinear trajectory because it is less sensitive to subtle motion and as it acquires the low spatial frequency information of k-space first, it results in greater BOLD contrast, relative to rectilinear (e.g., EPI) methods (Noll et al., 1995).

Data Analysis

Functional analysis was done using AFNI (Medical College of Wisconsin) (Cox 1996). Anatomical scans from each subject's session were registered and aligned to the initial scan (i.e., all post-ingestion scans were rotated and translated to the pre-ingestion brain orientation). Anatomical and functional images were warped into a standard brain space template (Talairach coordinates) prior to merging for group analysis (Cox and Hyde, 1997). The functional response was correlated with the motor task stimulus waveform to determine activation. Data was convolved with a Gaussian blur function (4s at FWHM). Group analysis was performed using a 3 factor 3D-ANOVA where the factors were paradigm repetition (rep1, rep2), time post ingestion (pre, post1, post2), and fat dose (0, 25, and 50 cc). The number of significantly activated voxels in each subject, at each time point, for each treatment were analysed using a 2-way ANOVA design with SAS (Cary, NC).

RESULTS

The BOLD signal and number of significantly activated voxels in the primary motor area (MI), supplementary motor area (SMA), and cerebellum, for both fat treatments (25 and 50 cc), decreased with time, post-ingestion ($P < 0.05$). The greatest effect was observed when subjects had consumed 50 cc of oil (Fig. 1). Both fat treatments resulted in a negative linear relationship with time (0, 40, and 100 min) (Fig. 2). Oil content showed a significant effect ($P < 0.023$) on the number of activated voxels over the entire brain (Table I). Evaluation of the linear contrasts $\bar{X}_1 - \bar{X}_2$ and $\bar{X}_1 - \bar{X}_3$, where $\bar{X}_1$ = pre, $\bar{X}_2$ = 40 minutes post ingestion, and $\bar{X}_3$ = 100 minutes post ingestion, in the absence of oil supplementation, demonstrated signal intensity changes due to ingestion of Ensure alone did not appear significant.

Results from each subject correlated well with the hemodynamic (ideal) wave function before and after ingestion of the fatty meal, with the greatest correlation before ingestion. The greatest BOLD contrast (i.e., difference between "on" and "off" states) was observed in the pre-ingestion state for all subjects. There were no significant within subject differences detected with any treatment.

Figure 1.

Example fMRI data from one subject showing the effect of 50 cc canola oil on the number of significantly activated pixels. Pre-treatment motor cortex (**A**) showed a greater number of significantly activated pixels than 2 hours post-ingestion of a lipid rich

drink (**B**). Stimulus waveform and data from motor cortex of the same individual pre (**C**) and post lipid (**D**) are shown. The BOLD contrast was reduced following the high fat drink.

DISCUSSION

In this report, we have addressed the potential for dietary factors to influence the fMRI signal. Nutrient influences on the brain has been studied using fMRI and human brain regions that respond to oral glucose consumption have been identified (Liu et al., 2000; Matsuda et al. 1999). In these studies hypothalamic regions showed a decrease in BOLD signal during glucose challenge. The diet has many biochemical components, and vasoactive substances, with theoretical potential for BOLD signal modulation. For example, caffeine, an adrenergic agonist, has been shown to increase BOLD signal (Mulderink et al., 2002). Alcohol (ethanol) is a vasodilator, and has been shown to decrease brain BOLD response (Levin et al., 1998). Antihistamines are vasocon-

strictors, which may alter BOLD signal. Various chemicals within food also have the potential for BOLD modulation. For example, pressor amines in foods such as strong cheeses increase blood pressure and flow (Plenker et al., 1997). Nitrates in many preserved meats have the potential to convert haemoglobin (Hb) to methaemoglobin (methHb). This redox state of Hb contains 5 unpaired electrons and is, therefore, more paramagnetic than deoxyHb, which has 4 unpaired electrons (Farahani et al., 1999). Depending on the activity of methemoglobin reductase in a person's blood, which converts methHb back to Hb, there could theoretically be a decrease in BOLD signal with nitrate consumption. We have shown here that a high fat drink also has the potential to affect BOLD signal during motor cortex stimulation.

Figure 2.

Pre (A), 40 minutes post (B), and 100 minutes post Ensure + 50 cc canola oil (C). Activation areas represent data averaged over all brains, using a 3 factor (oil volume, time, and replicate) 3d-

ANOVA, and are presented as a normalized t^* statistic. Significantly activated voxels are thresholded such that only those with a normalized t^* greater than 0.5 are displayed.

An increase in cerebral blood flow of 91% has been observed during motor cortex activation, most dramatically in MI, SMA, and cerebellum (McLaughlin et al., 1997). In our study, grouped 3-way ANOVA showed a drop in activation following Ensure + 25 cc oil or Ensure + 50 cc oil. However, Ensure alone appeared to have no significant affect on motor cortex activation. The high fat meal clearly demonstrated a negative linear effect with time. The ideal waveform (i.e., boxcar) appeared to convolve with the BOLD signal for all treatment combinations. However, the decrease in the number of significantly activated voxels, following the lipid rich drink, appeared related to decreased BOLD contrast (Fig. 1).

Studies have shown that dietary fatty acids induce changes in neurophysiological, cognitive, and behavioural variables. For instance, pure fat (triolein) and natural fat (vegetable oil) increase neuron firing in the orbitofrontal cortex (Rolls et al., 1999). Furthermore, membrane lipids and fatty acids are able to change the index of ion fluidity based on diet, which affects the types of nutrients that can be exchanged between cells, specifically nerve cells and astrocytes (Yehuda et al., 1999). Monocarboxylic fatty acids with

two cis-double bonds, such as linoleic acid, can pass through the blood brain barrier (BBB) while oleic acid with one cis-double bond cannot (Edmond, 2001). Canola oil contains 61.9% monounsaturated fatty acid, but 60% is the relatively inert oleic acid. On the other hand, Canola oil contains 29.7% essential polyunsaturated fatty acid (EPUFA), 20.1% linolenic acid and 9.6% α -linolate (Dupont et al., 1989). Astrocytes have been shown to convert linolenic acid and α -linolate into eicosanoids such as arachidonic and docosahexanoic acids and help transport these EPUFA through the BBB (Bernoud et al., 1998). These eicosanoids are more unsaturated than their precursors. Due to the multiple bonds in the chemical structure, the more unsaturated the PUFA is the easier it is oxidized. Therefore, increased eicosanoids and hence neuron firing could increase oxygen consumption and possibly result in a reduction in local oxygen. This metabolic effect would correspond to a decrease in BOLD contrast, which we observed (Fig. 1).

Human studies of high dietary fat intake have shown serum triglycerides increase 2 to 4 hours after high fat ingestion. Also, a decrease in flow mediated vasoactivity, proportional to increased triglycerides, was observed with Doppler ultrasound (Vogel et al., 1997). The changes in the triglyceride levels induced vasodilatation (Raitakari et al., 2000). If local blood flow increases occur without any metabolic changes, the net effect would be a local decrease in deoxyHb concentration and increase in the resting state BOLD signal. This leads to a decrease in BOLD contrast due to the smaller difference between rest and active states (Mulderink et al., 2002). From our results, the Canola oil may be acting as a vasodilator resulting in a decrease in deoxyhemoglobin thus decreasing BOLD contrast between rested and active states. The opposite effect was observed with caffeine (Mulderink et al., 2002). Caffeine causes an increase

TABLE I. Ratio of significantly activated voxels, relative to total brain volume, normalized to baseline (fasted state) at 40 and 100 minutes post ingestion*

	Fasted (pre-drink)	40 minutes	100 minutes
Ensure only	1.00	0.77 ± 0.10	1.02 ± 0.20
Ensure + 25 cc oil	1.00	0.90 ± 0.27	0.89 ± 0.16
Ensure + 50 cc oil	1.00	0.62 ± 0.12	0.44 ± 0.09

* A significant effect of fat content ($P < 0.023$), but not time ($P < 0.22$) was observed.

in local deoxyhemoglobin thus decreasing the baseline BOLD signal. It was concluded, in this study (Mulderink et al., 2002) that caffeine could be used as an agent to decrease resting CBF and increase deoxyhemoglobin concentration in baseline conditions giving an overall increase in BOLD contrast during activation (Mulderink et al., 2002). Although an increase in BOLD contrast was observed, for caffeine to be used as an enhancement agent one would have to assume a homogenous distribution of receptors throughout the brain. If this were true, then a homogenous increase in BOLD contrast throughout activated voxels would result. This is likely not the case. Instead, caffeine should be considered a source of fMRI between-subject heterogeneity. In our study of postprandial lipemia, the effect appears to be a reduction in BOLD contrast leading to fewer significantly activated pixels during motor cortex stimulation. Even though caffeine and lipemia are predictable in their affect on BOLD contrast, they are difficult to control in any experiment due to variability in individual subject pharmacokinetics and pharmacodynamics.

The Ensure fat content was 11 g, which provided 17% of the total caloric value of the drink. The recommended daily Intake (RDI) for fat is 30%. Yet often dietary fat content may reach much higher levels. For example, in one study to evaluate the typical North American diet 100 g of fat were ingested (Hozumi et al., 2002). The amount of fat used in our study was similar to that seen in some typical fast food type meals. Although there was no significant effect on BOLD signal observed with Ensure alone, there appeared to be a trend towards decreased activity. It is likely the fat content was too low (11 g, or approximately 11 mL) to produce a significant BOLD alteration.

This pilot study has shown how a fatty meal prior to a block design fMRI study can result in attenuation of BOLD contrast. This could be due to increased oxygen consumption, possibly via increased fatty acid oxidation, or mediated through reduced perfusion, or a combination of these factors. We suggest ingestion of high fat foods potentially decreases statistical power in a fMRI experiment. Further studies of brain physiologic and vasoactive compounds should be undertaken in order to characterize other potential sources of brain fMRI BOLD signal heterogeneity. Based on this study, we suggest pre-fMRI scan knowledge of a subject's physiological state should be carefully considered.

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