Concomitant physiologic changes as potential confounds for BOLD-based fMRI: a checklist

practiCal fMRI[1]

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Abstract

A recent conversation on Twitter led to the suggestion that someone compile a list of physiological effects of concern for BOLD. That is, a list of potentially confounding physiological changes that could arise sympathetically in an fMRI experiment, such as altered heart rate due to the stress of a task, or that could exist as a systematic difference between groups. What follows is the result of a PubMed literature search (mostly just the abstracts) where I have tried to identify either recent review articles or original research that can be used as starting points for learning more about candidate effects. Hopefully you can then determine whether a particular factor might be of concern for your experiment. This is definitely not a comprehensive list of all literature pertaining to all potential physiological confounds in fMRI, and I apologize if your very important contribution didn't make it into the post. Also, please note that I am not a physiologist so if I go seriously off piste in interpreting the literature, please forgive me and then correct my course. I would like to hear from you (comments below, or via Twitter) if I have omitted critical references or effects from the list, or if I have misinterpreted something. As far as possible I've tried to restrict the review to work in humans unless there was nothing appropriate, in which case I've included some animal studies if I think they are directly relevant. A final caution before we begin. It occurs to me that some people will take this list as (further) proof that all fMRI experiments are hopelessly flawed and will use it as ammunition. At the other extreme there will be people who see this list as baseless scare mongering. How you use the list is entirely up to you, but my intent is to provide cautious fMRI scientists with a mechanism to (re)consider potential physiologic confounds in their experiments, and perhaps stimulate the collection of parallel data that might add power to those experiments. (This article first appeared as a blog post on practicalfmri.blogspot.com.)

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BIOLOGICAL SCIENCES

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PRACTICAL FMRI [1]

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A recent conversation on Twitter led to the suggestion that someone compile a list of physiological effects of concern for BOLD. That is, a list of potentially confounding physiological changes that could arise sympathetically in an fMRI experiment, such as altered heart rate due to the stress of a task, or that could exist as a systematic difference between groups. What follows is the result of a PubMed literature search (mostly just the abstracts) where I have tried to identify either recent review articles or original research that can be used as starting points for learning more about candidate effects. Hopefully you can then determine whether a particular factor might be of concern for your experiment.

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ABBREVIATIONS

BP – blood pressure

CBF – cerebral blood flow (usually reported as ml blood per 100 g tissue per min)

CBV – cerebral blood volume

CMRO2 – cerebral metabolic rate of oxygen utilization

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BOLD – blood oxygenation level-dependent (contrast)

EEG – electroencephalography

fMRI – functional magnetic resonance imaging

ICA – independent components analysis

LFO – low frequency oscillation

MAP – mean arterial (blood) pressure

MEG - magnetoencephalography

rs-fMRI – resting state fMRI

PET – positron emission tomography

rCBF – regional cerebral blood flow

RSN – resting state network

SCA – sickle cell anemia

T1 – longitudinal relaxation time

T2 – transverse relaxation time

TR – repetition time

GETTING INTO BOLD PHYSIOLOGY

There are some good recent articles that introduce the physiological artifacts of prime concern. Tom Liu has reviewed neurovascular factors in resting-state functional MRIand shows how detectable BOLD signals arise from physiological changes in the first place. Kevin Murphy *et al.* then review some of the most commonconfounds in resting-state fMRI and cover a few ways these spurious signal changes can be characterized and even removed from data. Finally, Dan Handwerker *et al.* consider some of thefactors causing hemodynamic variations within and, in particular, between subjects.

BREATHING AND HEART RATES

These are undoubtedly the two big issues and will be top-of-mind for most people. Murphy*et al.* do a good job introducing the topic of cleaning raw data, *e.g.* using independent respiration and heart rate data. There are numerous correction methods available today (see the section, "Time series cleanup with physiological recordings" in the Murphy paper) and these references are perhaps the best places to start if you want to dig into the origins of the problems. In reverse chronological order we have:

PHYCAA+ by Churchill & Strother
GLMdenoise by Kay, Rokem, Winawer, Dougherty & Wandell.
RIPTiDe by Frederick, Nickerson & Tong (using near-infrared spectroscopy, NIRS)
DRIFTER by Särkkä, Solin, Nummenmaa, Vehtari, Auranen, Vanni & Lin.
RVT-HR by Chang, Cunningham & Glover
RVT by Birn, Diamond, Smith & Bandettini

RETROICOR by Glover, Li & Reese

Then there are also numerous ICA-based methods, including multi-echo acquisition with a model fit. I won't comment on the relative or absolute performance of any of these schemes, just let you know you have options. But one important lesson seems to be this: measuring heart rate and respiration independently isn't likely to hurt.
I'll also mention the inter-relatedness of HR and breathing, through **vagal tone**. This has implications for fMRI studies employing emotional stimuli.

CO2: HYPERCAPNIA AND HYPOCAPNIA

Inspired CO2 is a well-known and powerful **vasodilator**. Similarly, **breath holding** is known to lead to **hypercapnia** (increases in arterial CO2), hence vasodilation. Thus, under normal conditions (no external CO2 source) the arterial concentration of CO2 will be closely linked to the breathing rate, as demonstrated in this 2004 study. The quantitative relationship between breathing rate and end-tidal CO2 is further investigated in this 2009 study.

As for dealing with the variation, in a 2012 study it was demonstrated that clamping arterial CO2 to a narrow range improved retrospective physiological artifact \nremoval from resting-state and task-based data. I like this idea because I've often wondered whether not having the magnet bore properly ventilated could cause hypercapnia. I can't find any papers that have looked at this issue, however. The closest I've found is a study that used CO2 supplied artificially, clearly demonstrating the potential interaction of inspired CO2 with stimulus-driven BOLD. (There was an earlier study in rats that reached essentially the same conclusion.) It's probably best to keep the **ventilation** constant in the magnet bore for all subjects, just in case.

It turns out there may be **gender bias**, too. A 2010 study found that men have higher cerebrovascular reactivity to a CO2 challenge. So, not only do men tend to exhibit more head movement than women on average, men also appear to be more sensitive to CO2. (They also have different hematocrit on average, a subject that is dealt with below.)

O2: HYPEROXIA AND HYPOXIA

While refluxed CO2 may be a legitimate concern, incidental **hyperoxia** seems unlikely unless your MRI facility has a very strange design indeed. But, if you happen to be doing fMRI of divers, high-altitude pilots or astronauts in a simulated environment, do bear in mind the possibility of arterial BOLD from dissolved O2 if your subjects are breathing 100% oxygen. I suppose this ought to be a concern in **anesthetized** fMRI subjects, too. (I'll consider the physiologic consequences of anesthetics below.) If you're comparing normal volunteers (awake) to anesthetized patients then you might want to consider giving the former group the same gas mixture (sans anesthetic) as the latter.

What about **hypoxia**? If you have a well-designed MRI facility then ordinarily acute hypoxia shouldn't be an issue. But if your scanner is in a poorly ventilated basement you might want to consider an oxygen sensor, assuming you don't already have one, or improving your air conditioning. Otherwise you may be contributing to the variability in your subjects' BOLD responses.

Chronic hypoxia may be an important issue to track. Yan *et al.* studied immigrants who had

grown up at **high altitude** (HA, 2616–4200 m) and relocated to **sea level** (SL, < 400 m) to a group of sea-level controls, and found both anatomical and resting state fMRI differences associated with "chronic high altitude hypoxia." These changes were in spite of *"...no significant differences in hemoglobin levels, circulating red blood cell count, blood pressure, and pulse rate between HA residents and SL controls."* In a separate study, Yan *et al.* found persistent, correlated alterations in verbal working memory and BOLD responses for subjects who had relocated from HA (2,982.8 ± 478.7 m) to SL (< 400 m), even though they had been resident at sea level for at least one year and in spite of consistent hemoglobin levels.

VASOMOTION

According to Wikipedia, *"vasomotion is the spontaneous oscillation in tone of blood vessels, independent of heart beat, innervation or respiration."* Fair enough. Our concern is whether vasomotion might differ across groups, or perhaps might even vary for an individual under different conditions. Again according to Wikipedia, *"vasomotion has been shown to be altered in a variety of pathological situations, with vessels from both* **hypertensive** *and* **diabetic patients** *displaying altered flow patterns as compared to normotensive vessels."*

Murphy *et al.* say this: *"If vasomotion is independent of cardiac, respiratory, arterial CO_2 concentration and blood pressure fluctuations, its low-frequency characteristics will present another confound for resting-state fMRI BOLD oscillations."*

How serious a concern might spontaneous low-frequency variations be for your average BOLD study? A recent paper by Tong & Frederick claims that low frequency oscillations (**LFO**s) are a larger component of physiologic noise than respiration or heart rate, even when respiration and heart rate frequencies are aliased because of the relatively long TRs that are typical of most fMRI studies. There is also evidence from intraoperative recordings that vasomotion (at ~0.1 Hz) is *"spatially localized to distinct regions of the cortex, exhibited wave-like propagation, and involved oscillations in the diameter of specific pial arterioles, indicating that the effect was not the result of systemic blood pressure oscillations."* Not small potatoes, then.

What do these LFOs tell us, and (how) do they relate to the ongoing intrinsic neural activity that we assume is driving resting-state fMRI? Methinks this might become the next fashionable study area, if it's not already.

BLOOD PRESSURE

A good introduction to blood pressure (BP) and its relationship to CBF is given in the Murphy, Birn & Bandettini review. We might be interested in the subject's baseline (pre-scan) BP, but we might also be interested in the BP during a time series acquisition given that BP changes when laying supine versus standing, during stress, *etc*.

I've been unable to locate any papers showing whether TR-to-TR (real-time) BP is of any use in explaining variance in BOLD time series data. Baseline BP (before MRI) was found to offer only a small normalizing effect on visual-evoked BOLD signals when tested across two conditions; baseline venous T2 was considerably better. A study by Lui *et al.* in cocaine-dependent (human) subjects found that dobutamine infusion raised mean arterial BP (MAP) but produced only localized BOLD signal changes (in anterior cingulate) that correlated with the BP rise. Gianaros *et al.* observed a correlation between mean arterial BP and BOLD activity in several brain

regions of participants conducting a stressful Stroop task, with BP measured once for each block of sixteen 90-second task blocks.

This is Murphy *et al.*'s take:
"Evidence of the influence of **blood pressure oscillations** *on resting-state fMRI fluctuations in humans is sparse. Blood pressure levels in rats have been shown to affect evoked fMRI responses, with transient* **hypertension** *increasing BOLD (Wang et al., 2006) and CBF (Qiao et al., 2007) signals. Under* **hypotension**, *neural activity-evoked CBV increases in visual cortex are negligible compared to ~ 10% at normal blood pressure levels (Nagaoka et al., 2006). Increases in the amplitude of low-frequency BOLD fluctuations have been demonstrated with a drop in mean arterial pressure (Biswal and Kannurpatti, 2009). As supporting evidence in humans, BOLD signal correlates of heart rate and pulse height in the low frequency range have been discovered with fluctuations in cardiac rate explaining up to 11% of the variance in the resting-state BOLD signal (Chang et al., 2009, de Munck et al., 2008 and Shmueli et al., 2007)."*

What about chronic **hypertension**? Another study from Gianaros *et al.* used the Stroop test to stress healthy young volunteers as a model for determining whether reactivity to psychological stressors might be used to assess risk from hypertension. They found: *"Individuals exhibiting greater stressor-evoked MAP reactivity showed (1) greater amygdala activation, (2) lower amygdala gray matter volume, and (3) stronger positive functional connectivity between the amygdala and perigenual anterior cingulate cortex and brainstem pons."*

Hypotension may be a factor in cases of major **blood loss**, perhaps including recent **blood donation**. While I wasn't able to find any papers looking at recent blood loss (or donation) on BOLD fMRI, Kalisch *et al.* used a hemorrhage model in rats and observed heterogeneous correlations between BOLD and BP. They suggest that *"...a BOLD decrease during a decrease in BP may result from an increase in mostly venous cerebral blood volume (CBV) as an autoregulatory response to maintain cerebral blood flow (CBF) during decreased perfusion pressure."*

HEMATOCRIT LEVEL

Hematocrit level is the percentage of **red blood cells** in the blood. And since red blood cells carry **hemoglobin**, which itself transports the usable oxygen in blood, a person's hematocrit level is an important parameter when considering BOLD signal variations. Hematocrit levels could be unusually high in **endurance athletes** or folks who have just returned from an extended time living at **altitude**, for example. At the other extreme, we would be concerned about fMRI subjects experiencing **anemia**.

Levin *et al.* found a positive linear dependence of BOLD percent activation (BPA) on hematocrit level, the relationship being stronger in men than in women. Furthermore, *"...9 men were studied before and following isotonic saline hemodilution, resulting in an average 6% reduction in hematocrit and an 8-31% reduction in BPA."* This suggests checking whether your subject has just come off an intravenous **saline drip** because of food poisoning or flu.

There are also well-known **sex differences** for hematocrit level, with men having an average value several percent (5% seems to be the consensus figure) higher than women. A poster by Yang *et al.* at this year's HBM conference showed how variation in hematocrit across individuals

produced a systematic difference in several measures derived from resting-state fMRI.

Most causes of **anemia** are chronic and presumably you would be aware of them, but there are forms of anemia that might be of issue for BOLD studies in so-called normal volunteers. Recent utilization of intravenous fluids has just been mentioned, while recent blood loss, perhaps from major surgery or giving blood, was considered above. Are these important factors? Knowing about them might permit you to interpret an outlier. Low hematocrit could also be relevant to studies of certain patient groups, *e.g.* acute traumatic brain injury, if polytrauma could have resulted in recent loss of blood.

Possible variations in hemoglobin suggest a role for normalization when comparing across groups (or even within subjects over an extended period of time). Lu, Yezhuvath & Xiao investigated the utility of **baseline venous oxygenation** as a normalizing parameter, finding that *"...visual-evoked BOLD signal is significantly correlated with baseline venous T2 (P = 0.0003) and inclusion of physiologic modulator in the regression analysis can substantially reduce P values of group-level statistical tests."*

I'll include here a special case. **Sickle cell anemia** (SCA) has profound effects on the BOLD response because resting CBF is increased. Remarkably, Bob Ogg's group was able to get fMRI responses in SCA children but the BOLD signals were diminished in amplitude. What is especially intriguing is that *"...blood hemoglobin concentration and resting CBF were not predictive of BOLD signal amplitude in the SCA patients."* No attempt was made to measure venous blood T2 in this particular study. Definitely an area ripe for more research.

EXERCISE

The long-term effects of exercise, particularly in endurance athletes, should be reflected in hematocrit level and on resting heart and breathing rates. What about the short-term effects of recent exercise on BOLD? If your subjects arrive at the scanner having just come from a boot camp class at the gym, or they've ridden a bike twenty miles from home, do you need to know about it?

A study by MacIntosh *et al.* showed that twenty minutes of **aerobic exercise** less than an hour before scanning decreased CBF in grey matter for up to 40 minutes. BOLD results from a go/no-go attention task were mostly consistent with the pre-exercise baseline. However, the same group used a similar exercise regimen prior to acquiring resting-state fMRI and *"...observed a change in the resting-state BOLD functional connectivity of young healthy adults in three [resting state networks] RSNs, predominantly localized to cortical areas involved in sensorimotor activity."* The latter study was limited in that *"...at the time of the repeat rs-fMRI scan, the heart rate decreased after exercise but was nonetheless still significantly greater than the pre-exercise heart rate. Using heart rate as a covariate in the paired design group analysis did influence the session-related findings for two of the three significant RSNs. Blood pressure was not measured continuously so we cannot rule out the possibility that it too was elevated at the time of the repeat rs-fMRI scan."*

There are clear opportunities for more research in this direction, but it would appear that we should insist on baseline measures of hematocrit (or venous T2) and blood pressure in addition to heart rate and respiration, or we risk misinterpreting BOLD changes.

(DE)HYDRATION

Using thermal exercise so that subjects **dehydrated** via sweating, versus a non-thermal exercise control, Kempton *et al.* found an increased fronto-parietal BOLD response during an executive function task while cognitive performance and CBF were unchanged. The authors suggest: *"This pattern indicates that participants exerted a higher level of neuronal activity in order to achieve the same performance level."*

CAFFEINE

The effect of this **vasoconstrictor** on BOLD seem to have been studied more than any other drug (or foodstuff), probably because it's the most widely used **stimulant** in the world. There are many references to choose from, and what emerges is a picture best described as "it's complicated."

For a start it turns out that a subject's normal caffeine usage makes a difference if they're given a caffeine challenge. Laurienti *et al.* found "*...that the BOLD signal change in visual cortex was significantly greater in high users than in low users in the presence of caffeine."* Are your subjects high or low users? Would they even know? Here's a searchable website containing many of the common **dietary sources of caffeine**. Many sources are well known and obvious, *e.g.* dark chocolate. Some, though, may surprise you.

Next there is the **dose response**. The amount of caffeine alters BOLD magnitude non-linearly. According to Chen & Parrish, the greatest effects on BOLD are associated with an intermediate caffeine dose of 2.5 mg/kg. In the early 2000s it was even suggested to use caffeine as a BOLD signal booster, but the non-linear dose response seems to have nixed that idea.Laurienti *et al.* puts it this way: *"It is not possible to consistently enhance BOLD signal intensity magnitude by decreasing resting perfusion with caffeine."*

In case the picture isn't sufficiently complicated already, it has been found thatcaffeine alters the temporal dynamics of visual BOLD responses. Intriguingly, administration of caffeine has also been found to enhance the linearity of the BOLD response to rapid visual stimuli. One assumes there must be a dose response that hasn't yet been investigated, however. What about **resting-state fMRI and caffeine**? For starters, caffeine changes resting-state connectivity for motor cortex. It also increases the variability of motor cortical connectivity.

So it appears that we may have an additional, potentially large, source of inter- (and intra?) subject variability based on the pattern of normal caffeine use, right? Curiously, the answer is ambiguous. Addicott, Peiffer & Laurienti investigated the effects of a caffeine dose (or placebo) on subjects representing a range of usage levels (including abstinence). Acute caffeine administration did produce measurable effects in BOLD, consistent with prior reports. But the changes weren't moderated either by normal use or by abstinence in regular users. The authors conclude *"...that dietary caffeine use does not produce a significant effect on task-related BOLD activation."*

Why might this be? Griffeth, Perthen & Buxton used a quantitative fMRI experiment to investigate caffeine's effects on BOLD and CBF simultaneously. They observed offsetting changes in baseline blood flow and oxygen metabolism with subsequent responses to visual

stimuli, such that: *"The combined effect was that BOLD responses pre- and post-caffeine were similar despite large underlying physiological changes, indicating that the magnitude of the BOLD response alone should not be interpreted as a direct measure of underlying neurophysiological changes."*

Finally, let's assume you're interested in the neural consequences of caffeine use. How to differentiate from physiology? A good place to start is this 2010 review of caffeine's effects on cognition as well as BOLD. Then check out these more recent studies using simultaneous EEG-fMRI, comparing MEG to fMRI in the same subjects across separate sessions, or comparing fMRI to PET.

So, what's the bottom line regarding dietary caffeine as a potential BOLD confound? I don't know! But it sure looks like something that ought to be tracked, even if it's just self-reported accounts.

ALCOHOL

Ahhh, the world's favorite depressant. Let's deal with what we do know first. Acute administration of alcohol reduces BOLD activation in response to a visual stimulus, suppresses BOLD activity during a goal-directed visuomotor task, changes CBF in a dose-dependent manner, and modulates neurovascular coupling. Furthermore, acute alcohol consumption changes significantly several cognitive and visual networks mapped with a resting fMRI paradigm. (Also this study, on resting networks.) Finally, there are studies assessing the chronic effects of alcohol abuse, but I don't think chronic alcohol consumption can be considered as a potential physiologic confound because the neural effects will likely dominate.

Very surprisingly, nobody seems to have looked at the consequences of **hangovers** on fMRI. If acute alcohol administration causes changes to physiology and these changes are dose-dependent then at what point may we consider them negated? An hour? Eight hours? A night's sleep? Surely - *surely?* - someone must have looked at this. If not, it's a race to see who can get the first study reported.

NICOTINE

The literature on nicotine, and **cigarette smoking** in particular, is a mixed bag as far as potential confounds go. In a study on nicotine-dependent smokers, Jacobsen *et al.* found no effect of intravenous nicotine on BOLD signals produced by visual stimulation. However, citing *the "Considerable variability across individuals... in both the behavioral and fMRI blood oxygen level-dependent (BOLD) response to nicotine"* that had been found in prior studies, Warbrick *et al.* observed: *"...some participants showed an increase in activation while others showed a decrease in BOLD activation from the placebo to the [nasal] nicotine condition."* Nineteen of the 41 total subjects were smokers.

So much for the controlled administration of nicotine in an experiment. What about the effects of smoking itself? Smoking may be a much larger concern because cigarettes produce **carbon monoxide** (CO), and hemoglobin has a high affinity for CO. If hemoglobin is carrying CO then it can't be carrying O2. How much of a concern is this displacement for BOLD? The literature doesn't say. Inhaling cigarette smoke is associated with a high number of health issues, including reduced pulmonary function, but I didn't find any literature addressing these concerns

for BOLD-based fMRI.

One study from 2008 looked at heavy smoking as a potential confound in BOLD studies of sch\nizophrenia, but found no difference with non-smokers using a sensorimotor task. However, another study from 2008 found significant differences in breath hold and visual activation tasks for heavy smokers versus controls. Why the contradiction? No idea I'm afraid.

Given the well-documented respiratory and cardiac issues associated with chronic cigarette smoking, it seems to me that there is a very strong likelihood of systematic bias in the physiology of any group of smokers versus non-smokers. Would we compare marathon runners to couch potatoes and not expect significant differences in physiology? Surely, then, we should insist on some baseline measures of physiology, including perhaps pulmonary function tests (*e.g.* spirometry), or we should expect high inter-subject variability as well as smoker versus non-smoker group differences.

The physiologic effects produced by other nicotine delivery methods such as **chewing tobacco**, **e-cigarettes** (vaporizers) or **patches** may differ from the effects produced by cigarette smoking. Certainly that is my intuition but I wasn't able to locate any literature dealing with these issues. I think it suffices to say that we would want to uses caution when interpreting BOLD signals from smokers, and to predict systematic differences with non-smokers. That would just leave the dose effect (number of cigarettes/day, and perhaps depth and duration of smoke inhalation) to add a few more complications to the picture.

ILLICIT DRUGS

With the possible exception of cannabis (marijuana), we can probably discount most illegal (or partially legal) drugs as serious confounds for routine fMRI studies because they aren't in widespread use in the general population. (See Note 1.) But just in case, I did searches for several drugs, including **ecstasy** (MDMA), **LSD** and **psychedelics** in general, **heroin**, and **methamphetamine**, looking for direct investigations into possible confounding physiologic changes. Pattinson *et al.* studied the mu-**opioid** agonist remifentanil, finding only some small, regional modulations of the BOLD response to a hypercapnic challenge. Gollub *et al.* found that **cocaine** decreased CBF but BOLD responses to visual stimuli were normal. This doesn't imply that the underlying physiology is normal, however. As with caffeine, simultaneous changes in baseline CBF and oxygen metabolism could yield normal-looking responses in spite of different physiologic mechanisms.

Cannabis is now in widespread use in most western populations. There are numerous studies of the (neural and behavioral) effects of this compound, but only a small handful of investigations of the physiologic effects relevant to BOLD.

There are a couple of literature reviews that have looked at neuroimaging and cannabis use. In a 2010 review the authors noted the general finding *"...that resting global and prefrontal blood flow are lower in cannabis users than in controls."* An updated review, in 2013, assessed the findings as suggesting *"...different patterns of resting global and brain activity during the performance of several cognitive tasks both in adolescents and adults, which may indicate compensatory effects in response to chronic cannabis exposure."* Only neuroimaging studies involving chronic cannabis users were considered. Both reviews highlighted the methodological

limitations of the work conducted to date and the considerable heterogeneity of results.

What sort of physiologic confounds might be of concern in cannabis users, anyway? Let's look at the acute effects of smoking marijuana. O'Leary *et al.* used O-15 PET and found: *"Smoking marijuana resulted in intoxication, as assessed by a behavioral rating scale, but did not significantly alter mean behavioral performance on the attention task. Heart rate and blood pressure increased dramatically following smoking of marijuana but not placebo cigarettes. However, mean global CBF did not change significantly. Increased rCBF was observed in orbital and mesial frontal lobes, insula, temporal poles, anterior cingulate, as well as in the cerebellum. The increases in rCBF in anterior brain regions were predominantly in "paralimbic" regions and may be related to marijuana's mood-related effects. Reduced rCBF was observed in temporal lobe auditory regions, in visual cortex, and in brain regions that may be part of an attentional network (parietal lobe, frontal lobe and thalamus)."*

That there are immediate effects on physiology following cannabis administration isn't all that surprising. And, as with acute alcohol administration, subjects smoking a joint immediately before doing an fMRI experiment probably aren't the prime concern. (If they are, you might want to check the effects of carbon monoxide, as for regular cigarettes.) What about the hangover effects or the chronic effects on pulmonary function?

In a 2006 study, Sneider *et al.* obtained dynamic susceptibility contrast (DSC) MRI (*i.e.* gadolinium bolus) from twelve *"current, long-term daily cannabis users between 6 and 36 hr after the subjects' last reported cannabis use. Cannabis users demonstrated significantly increased blood volumes in the right frontal area ($p < .05$), in the left temporal area ($p < .005$), and in the cerebellum ($p < .005$) relative to comparison subjects."* They followed that study with a longer duration of abstinence, scanning subjects with DSC MRI at 7 and 28 days after last cannabis use: *"The present findings demonstrate that at Day 7, cannabis users continued to display increased blood volumes in the right frontal region, the left and right temporal regions, and the cerebellum. However, after 28 days of abstinence, only the left temporal area and cerebellum showed significantly increased CBV values in cannabis users. These findings suggest that while CBV levels begin to normalize with continued abstinence from cannabis, specifically in frontal areas, other temporal and cerebellar brain regions show slower CBV decreases."*

Acute differences in regional CBF were also found in adolescent marijuana users, but the differences had resolved after four weeks of monitored abstinence. CBF wasn't measured at intermediate times however, so we can't tell from this study how long any physiologic hangover effects might last. It also isn't clear whether the persistent effects are neural, physiologic or both, but you'd think that anything lasting more than a few days would have a strong neural basis. (This 2010 study observed BOLD signal changes in a spatial working memory task dependent on recency of use in adolescents.)

But do changes in baseline CBV and CBF imply different neurovascular coupling or altered BOLD responses? These might be more problematic for interpretation of fMRI data than persistent (neural) effects. According to Murphy *et al.*, perhaps not. They used a finger-tapping task and determined no differences between groups of users of cocaine, nicotine or cannabis and control subjects.

MEDICATIONS

Many legal (prescription or over-the-counter) **analgesic** and **anti-inflammatory** drugs don't seem to have been studied for their effects on neurovascular coupling. I couldn't find anything on **aspirin** (acetylsalicylic acid), **paracetamol** (acetaminophen), **ibuprofen**, **codeine** or **oxycodone** (oxycontin). I also couldn't find any references investigating the undesirable effects of **Viagra** (or its competitors) or **antihistamines** on BOLD physiology. (One group has even suggested using **diphenhydramine**, active ingredient in the US/Canadian version of the antihistamine drug, Benadryl as a way to combat nausea and vertigo in 7 T MRI)

Sedatives have been used in several fMRI studies, but to date there have been no investigations into concomitant physiologic effects for the popular **benzodiazepines** such as diazepam (**Valium**), lorazepam (**Ativan**) or midazolam. The **non-benzodiazepine** sedative zolpidem (**Ambien**) has demonstrated clear effects on visual BOLD signals with no concomitant change in heart rate or oxygen saturation. The same drug was shown to alter resting connectivity but the accompanying heart rate data were deemed unreliable due to equipment failures. I was unable to find any studies using another common non-benzodiazepine, eszopiclone (**Lunesta**).

Of those medications that have been considered for possible physiologic confounds, the non-steroidal **anti-inflammatory** indomethacin was studied a decade ago and found to change BOLD and CBF but not CMRO2 for subjects conducting a simple motor task.

Medicines aimed specifically at increasing a patient's blood flow are of obvious concern for fMRI. **Acetazolamide** (**Diamox**) was shown to increase resting cortical perfusion by 20% and decrease primary motor cortical BOLD activation by 35%, whereas the CBF response in primary motor cortex was unchanged in normal volunteers. A similar pattern was observed in patients with steno-occlusive coronary disease. However, another common vasodilator, **glyceryl trinitrate**, which increases resting cerebral blood volume (CBV), had no demonstrable effect on the BOLD response. The authors suggest that the glyceryl trinitrate affects large arteries only and has negligible effect on the microvascular system driving BOLD.

Blood pressure medications such as **angiotensin**, or **anticoagulants** such as **warfarin**, are presumably not something your subjects will use without telling you; your screening should catch these. Likewise, **asthma medications**. I couldn't find any studies looking at potential physiologic confounds for these medications in any case. But Grichisch *et al.* investigated the potential effects of nasal **insulin** on CBF and BOLD and found no difference to a drug-free baseline. (Insulin and blood glucose level is considered again below, under Food.)

Statins (**Lipitor**, **Zocor**, **Pravachol**, *etc.)* appear to be in widespread and increasing use in western populations. These medicines are designed to reduce cholesterol synthesis in the liver, but there appear to be secondary outcomes with implications for cerebral hemodynamics, such as "...upregulation of endothelial nitric oxide synthase (eNOS) with a subsequent increase\n in nitric oxide (NO) bioavailability." Xu *et al.* investigated the hemodynamic consequences of taking **atorvastatin** (**Lipitor**) over four months for asymptomatic middle-aged adults. They observed numerous regional changes in BOLD, including both greater and faster regional responses, compared to a group receiving placebo. They observed no change in baseline CBF nor any change in mean transit time (MTT) of cerebral perfusion, suggesting that the regional BOLD changes came about through altered (improved) small vessel vasoreactivity.

Antidepressants, including several selective serotonin re-uptake inhibitors (**SSRIs**), serotonin-norepinephrine re-uptake inhibitors (**SNRIs**), and a host of compounds belonging to other four-letter acronyms have been given to subjects in dozens of fMRI studies. But I was unable to find any systematic investigation of concomitant physiology, such as measuring CBF before and after drug administration.

And then there are the so-called "**smart drugs**" and other brain enhancers such as **methylphenidate** (**Ritalin**), **amphetamine** (**Adderall**) and their ilk. Rao *et al.* assessed BOLD and perfusion changes for subjects performing a finger-tapping task before and after oral methylphenidate and found no changes in neurovascular coupling. Otherwise, I wasn't able to find much literature addressing the potential for physiologic confounds. Marquand *et al.* looked at CBF changes due to methylphenidate while Nordin *et al.* assessed CBF changes due to amphetamine, both groups finding regional differences compared to placebo. But any implications for BOLD fMRI are unclear.

Literature coming out of the so-called pharmacological MRI (phMRI) field may provide the best clues for potential confounds to routine fMRI studies, should you have specific reason to be concerned about the use of medicines in your population of interest. Wang *et al.* reviewed the potential and challenges of using arterial spin labeling (ASL) in phMRI, *"...with an emphasis on the methodologies used to control for potentially confounding vascular and systemic effects."*

ANESTHETICS

It's unlikely you'll be surprised by use of anesthetics among your subjects. But there is certainly interest in scanning people who may have had recent surgery, *e.g.* traumatic brain-injured patients who may have required surgery to treat other injuries or even the TBI itself. You would presumably want to discount any residual neural and physiologic effects of anesthesia. For example, Qui *et al.* found *"Low-dose **sevoflurane** significantly altered the task-induced CBF-BOLD coupling."* Post surgery, one expects altered blood pressure, heart rate and breathing rate, so there are plenty of options to measure and control for concomitant physiologic effects.

FOODS

It seems that the biggest potential physiological confounds lie with those dietary substances known to alter neural activity, as already reviewed. In this section I'll try to deal with latent effects from things your subjects ingest for other reasons. For sustenance, say. Before that, though, what about the possible effects of restricted nutrition?

There are a couple of fMRI studies on **fasting**, one that found an effect in a motor task and another that found altered functional connectivity of visual cortex. There are also several reports of altered BOLD signal in response to experimental **hypoglycemia** (induced via **insulin** administration), including cognitive, visual stimulation, and sentence comprehension fMRI tasks. All three studies observed regional differences when compared to normal **glucose** levels. (See Note 2.) Patients with type I diabetes also showed different BOLD activity compared to controls. Additionally, there is one study using CBF and measures of oxidative metabolism ($CMRO_2$) to assess the effects of hypoglycemia. They found that *"...metabolism and flow remained coupled. Elementary motor task activation was not associated with any consistent larger activated flows. Thus it remains that although mild hypoglycemia induced an increase in basal flow and metabolism, a similar increase was not seen in task activation."* Finally, **glucose** and **fructose**

infusions were compared to saline and found to alter BOLD signal magnitude in opposite directions.

These are interesting studies, but you're probably more interested in any possible effect of blood glucose level on normal BOLD variability when your subjects aren't fasting intentionally or having their blood sugar levels manipulated artificially. I couldn't find any literature addressing this specific concern, however there is a very recent paper that compared an overnight fast with normal and **hyperglycemic** conditions. While the paper's abstract makes it sound like there were big changes in BOLD, in the Discussion they write: *"These effects are comparatively small, yet may interfere with design sensitivity, when fasting status or blood glucose is not controlled in fMRI experiments."* I only skimmed the paper but was left with the impression that the jury is still out on this one. Hyperglycemia, specifically, doesn't seem to be a concern for BOLD. Gruetter *et al.* observed no significant effects on BOLD for blood glucose of up to 300% of control levels.

Now let's move to what your subjects might be eating before getting a scan. In 2003 it was reported that ingesting **lipid** - 50 ml of canola oil -decreased BOLD response in a finger-tapping task. But the potential effect of**triglyceride levels in blood** was measured again in 2009 by another group, whereupon no significant effects on BOLD were observed. Why the contradiction? We can only speculate. The authors of the 2009 study offer several plausible explanations in their discussion, and I am inclined to go with the more recent result pending further experimental data.

Then there is the antithesis to the high fat diet: salad.Aamand *et al.* used a **nitrate** challenge, "corresponding to the nitrate content of a large plate of salad," to trigger changes in BOLD reactivity without altering the baseline CBF. CBF (by arterial spin labeling) response to dietary nitrate was also measured by Presley *et al.* They found no global CBF changes but did observe some regional changes, in frontal lobe. The apparent contradiction with the first report was addressed by Aamand *et al.* as perhaps due to differences in sensitivity. In Aamand*et al.* they used only the inferior half of a 32-channel head coil which reduced the SNR in frontal lobe for that study.

Dietary supplements may deserve their own entire section at some point but for now I'll include them as food because they are generally unregulated, unlike FDA-controlled medications. **Creatine**, usually as the monohydrate, is a popular sports supplement that has beenshown to decrease the magnitude of a visual BOLD response by 16%. I didn't find any literature assessing the effects of **carnitine**, **calcium**, **glycerol**, **vitamin A** (including retinol and beta-carotene), **vitamin B** (including folic acid and thiamine),**vitamin C** (ascorbic acid), **vitamin D** or **vitamin E** (including tocopherol) on fMRI signals or on cerebral blood flow.

There was also no literature dealing with**amino acids** generally, but **tryptophan** has been investigated because of its role as a serotonin precursor. Acute **tryptophan** depletion, as a way to manipulate serotonin levels, was found to modify regional BOLD responses to a cognitive task, but global effects weren't assessed. I was unable to find any literature investigating dietary **glutamate** (including as monosodium glutamate) or **arginine**.

Chronic ingestion of **omega-3 fatty acids** has been investigated using near-infrared spectroscopy, but only in frontal lobe where changes in both oxyhemoglobin and total hemoglobin were observed. Global measures of CBF weren't feasible. Taken together with prior

fMRI results, which reported greater prefrontal BOLD signals in a sustained attention task without a change in task performance, it seems that some sort of vascular changes may be occurring. As with lipid ingestion generally, more work will be needed before we know whether there are general effects of concern for BOLD.

DIURNAL FACTORS AND SLEEP

Those of you studying **anxiety** or the effects of sleep or **sleep deprivation** are presumably acutely aware of and controlling for potentially confounding physiologic changes in your study designs. The effects of intentional manipulation of **cortisol** and sleep deficiency on fMRI are well documented. What about the effects in "normal volunteers?" **Stress** (level of blood cortisol) and amount of sleep (drowsiness) may be factors for the time of day your study is conducted, and they may vary with the populations you sample from. (Ditto for caffeine use!) But I can't find any references specifically addressing these possibilities. There is a now classic study that showed how inter-subject variability is much larger than intra-subject variability, so perhaps it's all baked in. Still, you would want to avoid biasing groups or longitudinal studies by the time of day you scan.

HORMONES

I was unable to find any papers dealing with concomitant physiological changes accompanying the **menstrual cycle**. It seems reasonable to expect that significant effects might be reflected in the heart rate, respiration rate/depth and blood pressure. Direct vasodilatory or vasoconstrictive effects seem unlikely to me, but I'm no endocrinologist. There are papers dealing with performance differences across menstrual cycle, but if these effects repeat they may have a neural basis rather than being physiological artifacts.

Women (and soon men?) going on or off hormonal **contraceptives** during a study might be a bigger deal. A recent study found a marked change in BOLD responses for women using oral contraception. The effects could be neural or concomitant physiological changes, or a mixture of both.

I couldn't leave this section without mentioning **oxytocin**. It's quite in vogue. I wasn't able to find any studies that had have investigated systemic physiological effects of this compound as they pertain to BOLD. But giving subjects oxytocin - or any other pharmacological agent come to that - without checking for concomitant physiological changes is taking a leap of faith that all effects are neural. At bare minimum I would expect blood pressure to be recorded with and without the challenge, and then I would want to see heart rate and respiration recorded during fMRI.

AGE AND DISEASE

These variables should come as no major surprise if you are screening your subjects in any way at all. But in order to be complete and to give you a place to start if you're doing group studies across the lifespan, or comparing disease states to normal volunteers, I offer these papers:

Alterations in the BOLD fMRI signal with aging and disease: a challenge for neuroimagi\nng.

Age-related differences in memory-encoding fMRI responses after accounting for decline\n in vascular reactivity.

Evidence that neurovascular coupling underlying the BOLD effect increases with age dur\ning childhood.

Age-related differences in cerebral blood flow underlie the BOLD fMRI signal in childh\nood.

Age dependence of hemodynamic response characteristics in human functional magnetic re\nsonance imaging.

Neural mechanisms of age-related slowing: the ΔCBF/ΔCMRO2 ratio mediates age-differenc\nes in BOLD signal and human performance.

Calibrated fMRI during a cognitive Stroop task reveals reduced metabolic response with\n increasing age.

Visualization of altered neurovascular coupling in chronic stroke patients using multi\nmodal functional MRI.

ACCELERATION

If your subjects have just returned from a six-month stint on the International Space Station and you want to compare fMRI before and after the flight, you are no doubt fully aware of the many physiological factors to take into account where extended **microgravity** is concerned. Short duration microgravity - say, the few minutes to be experienced by lucky punters taking Virgin Galactic flights in the not-too-distant future - is probably not much of a concern, unless the flight happens the morning of their fMRI scan.

At the other end of the scale are those people exposing themselves to **high acceleration**; much greater than 1 g. Presumably these exposures are seconds or at most minutes in duration, but if the exposure occurs frequently or happens in the hours before participating in an fMRI experiment, we should be sure to exclude delayed physiologic changes. To date, though, I can find no reports detailing possible confounding effects of aerobatic plane flights, gymnastics, fairground rides, trampolining and the like. I am assuming these aren't going to be common issues for the vast majority of fMRI studies, but concomitant physiologic changes would be worth consideration if your research happens to involve fighter pilots, race car drivers, *etc.* Presumably measuring blood pressure, respiration and heart rates would capture the main effects.

FOOTNOTES

1. The other day someone told me about a subject who had answered negative to a drug screen but who then got very concerned when, on the morning of the scan, she was asked to give a urine sample. (I don't recall why the urine sample was required, and presumably it was stated on the recruitment flier which I can only assume the subject didn't read fully before applying to do the experiment. In any event, it was a vital part of the experiment and not a law-enforcement action!) She apparently returned from the bathroom with a sample of tap water in her specimen tube. The point? People will lie on screening forms if they are trying to make the $20 an hour you're offering. So if your experiment is highly sensitive to illicit drug use then you may want to include the option (or the threat?) of a urine test to help filter those people out.

2. Hypoglycemia was also one of the conditions used (in rats) byOgawa *et al.* in their

preliminary demonstration of the BOLD effect. From the abstract of their 1990 paper: *"This blood oxygenation level-dependent (BOLD) contrast follows blood oxygen changes induced by anesthetics, by insulin-induced hypoglycemia, and by inhaled gas mixtures that alter metabolic demand or blood flow."* So we're not exactly covering new ground here, are we?