Post-stimulus fMRI Changes in Cerebral Blood Flow, Volume and Oxygenation Following Visual Stimulation and Breath-hold Provide Evidence for the Hemodynamic Response Being Neurotransmitter-mediated

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Background and Objective. Functional MRI (fMRI) is commonly performed using the blood-oxygenation-level-dependent (BOLD) approach [1], which is sensitive to ensemble changes in cerebral blood flow (CBF), cerebral blood volume (CBV), and the cerebral metabolic rate of oxygen ($CMRO_2$). While such *hemodynamic* changes are generally consequential to neuronal activity, the fundamental mechanism governing neuro-vascular coupling (NVC) remains unknown. Specifically, it is not clear whether NVC is driven by local tissue energy demands or more directly by neurotransmitter-mediated vascular effects. An understanding of this relationship would be important for identifying the neuro-physiological mechanisms fueling fMRI contrast and may have important implications in clinical scenarios where NVC relationships are impaired. To investigate this relationship, BOLD fMRI was performed concurrently with arterial spin labeling (ASL) fMRI, which is sensitive to CBF changes [2], and vascular-space-occupancy (VASO) fMRI, which is sensitive to CBV changes [3]. Earlier work using short TR VASO showed that following visual stimulation, VASO and ASL signal returned to baseline when the BOLD signal continued to change (undershoot), providing the first experimental evidence of an uncoupling of $CMRO_2$ from CBF and CBV changes [4]. However, VASO signal has recently been shown to have a CBF contribution at short TR [5], which has rendered earlier conclusions on CBV changes ambiguous. Here, BOLD, long-TR VASO, and ASL experiments are performed on human volunteers during both visual stimulation and breath-holding. During visual stimulation, $CMRO_2$, CBF and CBV all increase [6], while during brief breath-hold CBF and CBV increase, yet $CMRO_2$ remains unchanged [6]. Therefore, comparison of hemodynamic responses during breath-hold and visual activation may help elucidate the relationship between CBF/CBV and $CMRO_2$. The hypothesis to be investigated is that the BOLD undershoot observed after visual stimulation will reduce or disappear in BOLD breath-hold experiments if this is due to persisting $CMRO_2$ [4,7], which is unchanged in breath-hold.

Methods. *Experiment.* Ten healthy volunteers were scanned at 3.0T (Philips Medical Systems); Y_a, $EtCO_2$, and *heart rate* were recorded throughout the scan. BOLD, VASO and ASL data were acquired on each volunteer, separately for visual and breath-hold tasks (six scans per subject). Visual paradigm: 56s/14s cross-hair fixation/flashing (f=8 Hz) checkerboard stimulation, repeated four times. Breath-hold paradigm: 52s/4s/14s normal breathing/exhale/breath-holding, repeated four times. Following long breath-hold (20s+), subjects responded with mild hyperventilation. To prevent this compensatory response, the breath-hold task was kept reasonably short (14s). Common scan parameters: single-slice through calcarine fissure, FOV=240x240 mm^2, voxel size=3x3x3 mm^3, single-shot gradient echo EPI, SENSE=2.5. Technique-specific parameters: BOLD: TR/TE=3000/45 ms, 112 image acquisitions; VASO: TR/TI/TE=5000/1054/13 ms, 68 image acquisitions; ASL [8]: TR/TI/TE=2000/1500/13 ms, 84 $\Delta M/M_0$acquisitions. In VASO, TR=5000 ms was used for specific CBV sensitivity [5]. *Analysis.* All images were motion-corrected and co-registered using FLIRT [9]; artifactual BOLD contributions were removed from ASL images [10]. For fMRI analysis, a *z*-test was performed on voxels within the visual cortex. Although gray matter CBV and CBF change globally in breath-hold, only occipital parenchyma was analyzed so that comparison could be made more directly with the visual data. Activation criteria: z≥2.5 (BOLD, ASL), z-score≤-2.5 (VASO), cluster size≥4 (BOLD, VASO and ASL). Separate BOLD, VASO, and ASL time courses were calculated for voxels meeting activation criteria in *both* visual and breath-hold experiments.

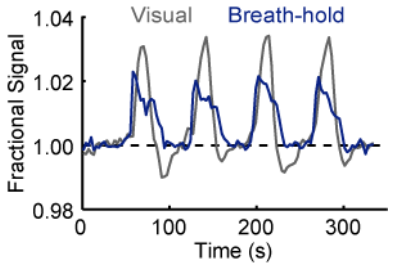

Fig. 1. BOLD visual (gray) and breath-hold (blue) time courses; note the absence of an undershoot in breath-hold data.

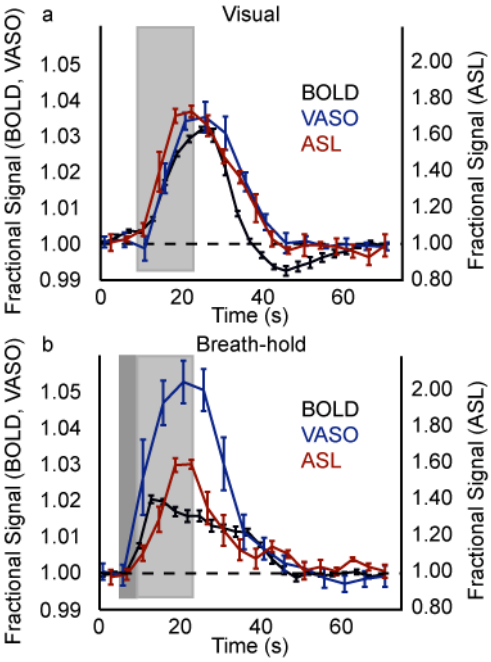

Fig. 2. Subject- and task-averaged time courses for visual (a) and breath-hold (b). Light gray=task period; dark gray=exhale period.

Results and Discussion. One of the ten volunteers could not complete the study and was excluded. Of the remaining nine volunteers, aside from $EtCO_2$ which dropped to zero during breath-hold, vital signs did not deviate beyond error either between visual and breath-hold experiments or within task periods. Fig. 1 shows the subject-averaged BOLD time courses for visual (gray) and breath-hold (blue) tasks. Note the absence of the undershoot in the breath-hold experiment, which is reproducible over all task periods. Fig. 2 shows the average response for different image methods and tasks; error bars are standard error (n=9). Average values are shown in Table 1. First, note that only the BOLD visual experiment contains a post-stimulus undershoot. Second, for visual experiments, less time was required for VASO (22±3s) and ASL (19±4 s) signal to return to baseline than for BOLD (34±4s) signal to do so; Table 1. In breath-hold, no such return discrepancy was apparent as the BOLD time course returned to baseline at the same time (23±6s) as VASO and ASL. An unexpected observation was that the BOLD breath-hold response was smaller than the BOLD visual response, whereas the VASO breath-hold response was much larger than the VASO visual response. Since the ASL response did not vary beyond error between functional tasks, it is possible that the Grubb relationship may differ between visual stimulation and breath-hold, which has been previously suggested [11]. The observation that the VASO and ASL visual signal returns to baseline during the BOLD visual post-stimulus undershoot, combined with the observation that no BOLD undershoot is detectable following breath-holding supports the conclusion that the normoxic BOLD undershoot is due to persisting $CMRO_2$ elevation unmatched by CBV and CBF. These observations are consistent with the hypothesis that NVC may be elicited independently of $CMRO_2$, by neurotransmitters, such as glutamate, which induce vasodilation through the synthesis of vasodilatory intermediates [12]. In this so-called neurotransmitter pathway, the vasodilatory response is not coupled to $CMRO_2$. Persisting $CMRO_2$ following stimulation may be due to increased energy demand required to restore Na^+/K^+ ion gradients or vesicle recycling of neurotransmitters, both ATP-dependent processes. We expand on previous multi-modal fMRI work and show that at 3.0T and with CBV-specific VASO parameters, evidence exists for an uncoupling of $CMRO_2$ from CBV and CBF.

	Signal change %		Time (s) to cross baseline		Time (s) to return to baseline	
	Visual	B-h	Visual	B-h	Visual	B-h
BOLD	3.4±0.8	2.3±0.3	14±1	23±6	34±4	23±6
VASO	3.8±1.1	5.6±1.0	21±4	22±6	22±3	22±6
ASL	67±10	60±12.2	19±4	19±5	19±4	19±5

Table 1. Signal changes and baseline return times for Visual and Breath-hold (B-h) experiments.

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