

**HEMODYNAMIC CORRELATES OF STIMULUS REPETITION
IN VISUAL AND AUDITORY CORTICES:
An fMRI STUDY**

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ABSTRACT

We examined the effects of stimulus repetition upon the evoked hemodynamic response (HDR) in auditory and visual cortices measured by magnetic resonance imaging in two experiments. Experiment 1 focused on the effects of the interval duration between two identical stimuli on HDR. Pure auditory tones (1000 Hz) of 100 ms duration were presented singly or in pairs with intrapair intervals (IPIs: onset-to-onset) of 1, 4 and 6 s. In Experiment 2, using a within-subject design, we aimed to compare the HDR refractory period in both sensory cortices, as well as the HDRs to auditory and visual stimuli. Identical auditory tone as described above and visual stimuli of 500-ms high-contrast checkerboard patterns were presented singly or in identical pairs with an IPI of 1 s. Images were acquired at 1.5 Tesla using a gradient-echo echoplanar imaging sequence sensitive to blood-oxygenation-level dependent contrast. Experiment 1 revealed that the HDR evoked by an auditory stimulus is followed by a refractory period of 4-6 s. in auditory cortex, as indicated by smaller HDR amplitudes to the second of each pair of stimuli. Furthermore, peak latency was dependent upon IPI, with longer latencies observed for shorter IPIs. Experiment 2 revealed that the HDR evoked in both sensory cortices by paired stimulus presentations is suppressed and delayed similarly by the refractory effects imposed by the preceding stimulus, suggesting similar refractory properties of the HDR at this specific IPI. We also provide evidence for additional neural resource allocation in response to repeated stimuli.

INTRODUCTION

Early studies have demonstrated the feasibility of event-related fMRI acquisitions (Akaishi and Sakuma 1985; Buckner, Bandettini et al. 1996; McCarthy, Puce et al. 1996; Zarahn, Aguirre et al. 1997; Friston, Josephs et al. 1998) to detect the hemodynamic responses (HDR) associated with isolated trials of a task. Although event-related fMRI procures the advantage of identifying vascular response patterns to single stimuli, it often requires a large number of events per task condition to achieve sufficient signal to noise. Furthermore, these events need to be separated by long inter-stimulus intervals (ISIs: onset-to-onset) in order to allow the BOLD (Blood Oxygen Level Dependent) responses to recover back to baseline. Increasing the number of trials and the ISI leads to significant increases in imaging time, while decreasing the ISI results in complex HDRs that require analysis methods to decompose the HDR into its subcomponents. In an effort to develop more efficient stimulus presentation protocols during event-related fMRI studies and improved procedures for decomposing the resulting complex HDRs, investigators have examined among other factors the effects of stimulus duration and contrast (Vazquez and Noll 1998; Liu and Gao 2000), stimulus presentation rate (Binder, Rao et al. 1994; Friston, Josephs et al. 1998), and the sampling procedure of the HDR function upon the characteristic profile or characteristic profile or time course of HDR curve (Miezin, Maccotta et al. 2000).

It is also well established that the characteristics of the HDR vary across cortical regions as a function of stimulus properties and experimental parameters. For example, frontal HDRs show late onset and sustained duration of the HDR peak while motor and visual sensory cortices show earlier onset and shorter duration in the HDR to single

stimuli (Schacter, Buckner et al. 1997). Furthermore, the amplitude and latency of the HDR appear to change from cortical to subcortical regions as a function of ISI as well, indicating that the HDR recovers earlier in subcortical regions as compared to cortical areas (Pollmann, Wiggins et al. 1998). Evidence of variation between cortical regions in HDR properties and sensitivities to stimulus presentation characteristics also comes from reports indicating that stimulus presentation rate does not alter HDR in bilateral frontal regions for instance, which shows a categorical response to the presence of words irrespective of rate, while activation in bilateral occipito-temporal regions increase linearly and posterior auditory association cortex exhibits a nonlinear (inverted U) relationship with increasing word rate (Buchel, Holmes et al. 1998; Pollmann, Wiggins et al. 1998). Thus, the latency and amplitude of the HDR waveform varies across brain regions (Buckner, Bandettini et al. 1996; Kim, Richter et al. 1997; Aguirre, Zarahn et al. 1998; Buckner 1998; Robson, Dorosz et al. 1998; Miezin, Maccotta et al. 2000; Huettel and McCarthy 2001).

Previous studies suggested that the amplitude of the HDR increases 'roughly' linearly with increased stimulus duration (Boynton, Engel et al. 1996; Dale and Buckner 1997). For example, ISI durations between 4 to 12 s evoked roughly linear increases in the amplitude of the BOLD response (Friston, Josephs et al. 1998; Pollmann, Wiggins et al. 1998). At shorter ISIs however, the HDR does not appear to add linearly as a function of increased stimulus presentation rates, thus creating the need for methods of overlap correction (Dale and Buckner 1997) in order to recover the full HDR. Significant non-linear changes in HDR amplitude with increases in stimulation rates have also been demonstrated through simulation studies of the peri-auditory cortical regions and the

posterior superior temporal gyrus (STG) regions (Friston, 1998). The finding of nonlinear additivity has also been demonstrated in the visual cortex using ISIs of 1, 2, 4 and 6 second durations between two consecutive stimuli (Huettel and McCarthy 2000; Huettel and McCarthy 2001). The nonlinear additivity in that study was attributed to the presence of a refractory period following stimulus presentation, which in turn modulated the amplitude of the HDR to subsequent stimuli. Subsequently, numerous studies (Huettel and McCarthy 2000; Grill-Spector K 2001; Huettel and McCarthy 2001; Kourtzi Z 2001; Soon CS 2003) have described similar phenomena, such as repetition suppression, repetition priming, or FMR-adaptation. Many investigators have used this phenomenon to examine the role of particular cortical regions in processing specific stimulus attributes, with the hypothesis being that if stimulus attributes are processed in overlapping neural spaces, the HDR elicited in those regions by repeated stimulation would show suppression effects consistent with refractory properties. (Grill-Spector K 2001; Kourtzi Z 2001). Furthermore, previous studies have also demonstrated that the characteristics (such as amplitude and latency), as well as refractory properties of the HDR vary across different cortical regions (Huettel and McCarthy 2001). Despite the numerous studies describing refractory or adaptive properties of the HDR, the exact mechanism and nature of this complex phenomenon remains unclear. Since a combination of multiple physiological changes associated with neuronal activity, such as CBF, CMRO₂, and CBV contributes to the BOLD signal change, it is difficult at this point to distinguish the differential role of these physiological contributors upon changes (linear or not) in the BOLD signal associated with stimulus/task attribute changes. Nevertheless, the “HDR refractory period” (or “repetition priming” or “FMR-

Adaptation”) bears significant resemblance to the refractoriness exhibited by neurons in other cortical regions, such as shape adaptation in macaque IT neurons (Sobotka 1993), and hence may reflect a hemodynamic counterpart associated with such a phenomenon.

In the present study, we sought to further evaluate the refractoriness of the HDR in the auditory and visual cortices at different interstimulus intervals. In Experiment 1, we examined the effect of stimulus onset asynchrony (SOA: onset-to-onset) upon the refractory properties of the HDR elicited in the auditory cortex to successive identical auditory tones using a similar paradigm previously used to assess the refractoriness of the HDR in visual cortex (Huettel and McCarthy 2000). In Experiment 2, using a within-subject design, we compared the degree of suppression of the HDR evoked by paired stimuli in the visual and auditory cortices during a fixed SOA.

MATERIALS AND METHODS

Subjects:

Twenty-four healthy subjects participated in 2 fMRI experiments. In Experiment 1, 10 healthy subjects (5 female, 5 male) performed an auditory monitoring task (described below). The average age of the subjects was 25.2, ranging from 19 to 32. In Experiment 2, 14 healthy subjects (9 female, 5 male) performed a multimodal sensory monitoring task. The average age of the volunteers was 24.2, ranging from 19 to 57. Each subject was interviewed prior to the scan and provided informed consent in accordance with the UNC and Duke University Medical Center Institutional Review Boards. The subjects had no history of or current medical illness, or clinically diagnosed neurological deficits. They also had no history of or current diagnosis of substance dependence or

current substance abuse, nor were female subjects pregnant. All of the subjects had normal or corrected to normal vision.

Experimental Design:

Experiment 1:

Subjects were presented with auditory tone, delivered as single tones or in pairs at intrapair intervals (IPIs: onset-to-onset) of 1, 4, 6 sec. Successive single or paired stimulus presentations were separated by a stimulus onset asynchrony (SOA) that varied between 17-20 s (Figure 1). This design yielded 4 trial types, i.e. 1 s IPI paired, 4 s IPI paired, 6 s IPI paired, and single tone trials. Auditory pure tones of 100 ms duration and 1000Hz frequency were delivered at 85 dB SPL using headphones (Resonance Technologies). Subjects were asked to maintain fixation on a central crosshair back projected on a screen mounted at the head of the gurney. Each trial type was repeated 40 times and randomly intermixed throughout 10 runs (~3.5 min each). Subjects were instructed to passively listen to the tones.

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Insert Figure 1 around here
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Experiment 2:

Subjects were presented with paired and single visual and auditory stimuli. Visual stimuli consisted of a black and white high-contrast, radial checkerboard pattern displayed for a duration of 500 ms. The auditory stimuli were identical to the ones utilized in Experiment 1. Visual and auditory paired stimuli were presented with a 1 s inter-stimulus interval. Visual and auditory trials were presented in an interleaved pattern

alternating between modalities in order to avoid overlapping activations in the same sensory cortex. As a consequence, trials of the same modality were separated by 16-20 s while consecutive trials (always of different modality) followed each other by 8-10s (Figure 2).

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Overall, subjects were presented 60 paired visual stimuli, 60 paired auditory stimuli, 60 single tones, and 60 single checkerboards. Each trial type was randomly intermixed throughout 10 runs (~ 4 min each). Subjects were again instructed to passively view the task.

Imaging Protocol

Scans for both experiments were performed on a General Electric 1.5T scanner equipped with 41mT/m gradients for high-speed echo-planar imaging. Sagittal T1-weighted localizer images were first collected. The functional images were acquired from 12 contiguous axial oblique slices (5mm thick with no gap) parallel to anterior-commissure and posterior commissure (AC-PC) plane, beginning at around 15mm below the AC, and extended to approximately 40mm above. 4th slice is aligned with AC-PC plane for each subject. This volume allowed us to image the occipital and the superior temporal cortical regions associated with visual and auditory processing, respectively. The functional images were acquired using a T2*-weighted gradient-echo, echo-planar imaging sequence (Echo Time (TE)=40msec; Repetition Time (TR)= 1 sec; in-plane resolution= 3.75x3.75mm; Field of View (FOV) = 24cm). The first 5 image acquisitions

in each run were discarded to allow stabilization of longitudinal magnetization. Twelve T1-weighted anatomical images (2D spin echo; TE = min; TR = 450 ms; FOV = 24; 5 mm slice thick, no gap) were acquired using the same slice prescription to aid in identifying landmarks of functional activation and delineating regions of interest (ROIs).

ANALYSIS

For both Experiments 1 and 2, segments consisting of 19 time-point images including 5 timepoints (5 s) prior to stimulus onset, and 13 timepoints following stimulus onset (13 s) were excised from the continuous functional time series and analyzed as the epochs of interest. For the paired stimulus conditions epochs were identified around the onset of the first stimulus in a pair. Epochs were averaged for each trial type. ROIs were defined on each subject's high-resolution T₁-weighted axial images slice by slice. Image analyses were performed using a region of interest (ROI) approach. Regions of interest were manually traced for each subject based on anatomical landmarks on individual co-planar high-resolution anatomical images, which were acquired parallel to AC-PC axial plane in a manner to coincide the 4th slice to the AC-PC plane for all subjects. Subsequent comparisons of total and percent voxels counts for each region across subjects revealed no significant differences in ROI size. The following brain regions and slice levels were traced for ROI analyses based on anatomical sulcal landmarks and with the guidance of brain atlases (e.g. Duvernoy, 1991): For Experiment 1: The auditory cortex (STG) was traced to include Brodman areas 41, 42, and 22 extending from AC-PC plane to 20 mm superior to AC-PC plane. For Experiment 2, two ROIs were defined. STG (as described above) and the visual cortex (VC) including

Broadman areas 17,18,and 19 extending from 15 mm below the AC_PC plane to 15 mm above the AC_PC plane. The activated voxels within these ROIs for each subject did not extend beyond these limits. The ROIs were not restricted to primary sensory cortices due to the extension of the clustered activation of the voxels beyond these regions. Changes in the BOLD response to repeated stimulation was investigated by selecting voxels activated during the single stimulus presentation, and using them as a mask to examine suppression effects at those voxels in response to the repeated stimulus presentation, voxels activated by the single stimulus condition served as a mask to map/select those voxels. Voxels activated by a single stimulus were identified by correlating the time course of every voxel in the averaged single stimulus epoch with an empirically determined HDR obtained from Huettel and McCarthy (2000). The t-value used to determine active voxels ($t > 3.6$) was chosen *a priori* as a conservative threshold for active voxels, with p-value of approximately 0.001 (one-tailed; 0.002 two-tailed). Masked ROIs were created by selecting only those voxels within each ROI that were activated beyond this threshold by single stimulus presentations. These masked ROIs were further interrogated for their activation in response to paired stimulus presentations (Figures 3 and 4).

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Extracting the response to the second stimulus in a pair:

The response of each voxel to the paired stimulus conditions resulted in a composite HDR that contained a response elicited by both the first and second stimulus in

the pair. To isolate the contribution of the second stimulus in a pair to the composite HDR, the HDR within the voxels that responded to the single stimulus condition was subtracted from the HDR within the same set of voxels (masked ROI) evoked by the paired condition and the resulting HDR was shifted by the corresponding IPI of that trial, which was 1 sec, 4 s or 6 s in Experiment 1 and 1 s in the Experiment 2. This shift aligned the zero timepoints of the subtracted HDR and the single stimulus HDR. The amplitude measurements of the subtracted HDR were compared with the amplitude of the response to the single stimulus condition.

Suppression Index Analysis:

To compare the degree of suppression in BOLD activation across the visual and auditory cortices (Experiment 2), a suppression index was calculated for each subject for each modality within the corresponding region. This index was calculated as:

Suppression index (SI) = $[(S2/S1)*100]$. A higher index was expected to represent less suppression of the HDR. For example; an index of '100' would show no suppression whereas '0' would represent total suppression of the HDR.

Analysis of the spatial extent of the activation:

The spatial extent of activation for each individual was calculated as the percentage of the active voxels to the total number of voxels within each anatomically defined ROI (STG, VC) and referred to as 'Percent Active Voxels'. The group average of 'Percent Active Voxels' was computed for each trial type (single visual, paired visual, single auditory, paired auditory).

Modality/Domain effect in visual and auditory cortices:

In Experiment 2, since auditory and visual trials were presented in an interleaved fashion, we evaluated the cross-activation effects between auditory or visual cortices. Based on previous evidence showing visual cortex deactivation during auditory stimulation, we expected to observe cross-inhibition effects between visual and auditory cortices. This analysis was performed by averaging the epochs of BOLD signal within each anatomically defined ROI during the single stimulus presentation of the opposite modality (i.e. average BOLD response within the VC during single auditory tone presentation and vice versa).

RESULTS

Experiment 1

Figure 3 depicts activation of the STG in response to unpaired auditory tone. A one-way ANOVA with the factor of trial type (single, paired 1sec IPI, paired 4sec. IPI, and paired 6sec IPI) was performed on the dependent measures of peak amplitude (percent signal change) and peak latency (seconds) of the HDR in the STG. This analysis revealed that the peak percent signal change evoked by the second auditory tone in the paired-stimulus condition was significantly suppressed in all paired conditions ($F(3,36) = 2.87, p < 0.0001$) (Figure 5). Paired comparison analyses performed between the peak percent signal change in the single condition and each of the paired conditions (Table 1) revealed that the suppression effect of the first stimulus of a pair on the HDR to the second stimulus in the pair persisted even 6 s following the onset of the first stimulus (paired t-tests, two-tailed: (single vs. IPI=1 sec, $t(9) = 5.7, p < 0.001$); (single vs. IPI=4 sec, $t(9) = 7.4, p < 0.001$); (single vs. IPI=6 sec, $t(9) = 4.5, p < 0.001$)). Analysis of the peak

latencies of the HDR demonstrated that the latency of the HDR to the second stimulus in a pair was delayed in the 1 s IPI condition. ($t(9) = -4, p < 0.004$, two-tailed).

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Experiment 2

Hemodynamic responses in Visual Cortex:

T-tests for paired samples were performed between the peak percent signal change in response to the single stimulus condition and the subtracted HDR (as described above) elicited by the paired stimuli condition. A similar analysis was also done on the peak latency measurements. The peak response to the second stimulus in a pair was significantly smaller (suppressed) ($t(13) = 7.35, p < 0.001$, two-tailed) and occurred later (delayed) ($t(13) = -4.50, p < 0.001$, two-tailed) than the peak response to the single stimulus (Table 1, Figure 6).

Hemodynamic responses in Superior Temporal Gyrus:

T-tests for paired samples were performed on the peak percent signal change and peak latency in response to single and the second stimulus in the pair condition. The peak amplitude of the HDR to the second stimulus in a pair was significantly smaller (suppressed) ($t(13) = 8.27, p < 0.001$, two-tailed) and occurred later (delayed) ($t(13) = -3.37, p < 0.005$, two-tailed) than the peak response to the single stimulus (Table 1 and Figure 7).

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Insert Figure 6 and 7 and Table 1 around here

Suppression index:

T-tests for paired samples were performed on the suppression indices of auditory and visual modalities. The results showed that there was no significant difference in the degree of suppression across sensory cortices ($t(13) = -1.23, p < 0.2$, two-tailed).

Spatial Extent of Activation:

T-tests for paired samples were computed on the ‘Percent Active Voxels’ in response to single and paired stimuli presentation for each ROI separately. In both cortices, the paired condition activated more voxels than the single condition. This increase was significant in the auditory modality ($t(13) = 1.77, p = 0.001$, two-tailed).

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Modality specificity of the activation in the sensory cortices

As the visual and auditory stimuli were separated only by 8-10secs, we performed an analysis to examine the cross-activation effect of the visual and auditory modalities upon each other. The HDR time course analysis of each condition in each ROI revealed that sensory cortices showed significant increase in the BOLD signal in response to the corresponding stimulus domain and a slight decrease during non-corresponding stimulus presentation. This cross-inhibition was more prominent in visual cortex during auditory stimulation as consistent with the previous studies showing visual deactivations during auditory stimulation (Laurienti PJ 2002).

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Qualitative measures:

In addition to these quantitative results, our data also revealed a qualitative difference in the HDR characteristics within the VC and STG to single (unpaired) modality-specific stimulus presentations. As such, the HDR to single stimulus presentation appears to peak later and has a slightly higher peak in the VC as compared to the HDR in STG (Table 2), a finding that could be partially attributable to the different properties of the visual and auditory stimuli, as discussed below.

DISCUSSION

The present study revealed three significant findings. First, Experiment 1 indicated that properties of the HDR to stimulus repetition in the STG are similar to those demonstrated in earlier studies in the visual cortex. Specifically, they are nonlinearly additive, indicative of a hemodynamic refractory period in this region, which extends beyond 6 sec following stimulus onset in the auditory cortex. Secondly, Experiment 2 revealed that the HDR elicited by the second of a pair of stimuli was reduced and delayed significantly across both visual and auditory modalities, replicating the prior refractory period observations reported for the visual cortex, and extending these findings to the auditory cortex. Thirdly, Experiment 2 also revealed that the paired condition activated more voxels than the single condition in both cortices, possibly reflecting compensatory neural/vascular recruitment in the presence of localized HDR refractoriness.

Our first experiment examined the modulation of the HDR in auditory cortex under repetitive stimulation conditions. We identified significant and differential changes in the peak latency and amplitude of the HDR elicited by repetitive stimulation. Accordingly, the peak of the HDR to the second tone in a pair of identical tones was delayed and suppressed relative to the peak of the first tone. Furthermore, while the delay of the peak latency recovered by 6 seconds, the suppression of the peak amplitude of the second tone did not resolve by 6 seconds, leading to a nonlinear increase in the HDR amplitude in auditory cortex. The findings of non-linear changes in HDR at short IPIs (under 6 sec) are consistent with previous studies showing non-linear increases during stimulation periods below 4 sec, while stimulation durations of about 6 s or longer yielded a more linear increase in the HDR (Dale and Buckner 1997; Vazquez and Noll 1998; Liu and Gao 2000). Similar non-linear changes have been observed during increased stimulation rates (Binder, Rao et al. 1994), further suggesting that the dynamics guiding the HDR properties change as a function of stimulation and experimental protocol parameters. The non-linear changes described in previous studies for short stimulus durations as well as fast stimulus presentation rates have been interpreted as indicative of the refractory properties of the hemodynamic response as described in this and previous papers (Huettel and McCarthy 2000). Using a similar experimental design in the visual modality, Huettel et al. (Huettel and McCarthy 2000) showed parallel refractory effects on peak latency and amplitude in visual cortex, where both resolved by 6 seconds. The differential measures of refractoriness obtained for the peak latency and amplitude of the HDR in auditory cortex is interesting, and the neurophysiological

mechanisms underlying such an unparallel refractory effect in auditory domain bear further exploration.

Experiment 2 directly compared the refractory properties of the visual and auditory cortical regions, and revealed comparable quantitative measures of HDR suppression in the sensory regions at 1 second intrapair intervals, with some qualitative differences in the characteristics of the HDR curves elicited in the visual and auditory cortices by single stimulus presentations. The comparable degree of suppression observed in these sensory regions at 1 second IPI indicates that when designing fMRI experiments the timecourse of the activation to single stimuli should be modeled based on information about the time since preceding stimulus presentation. The present study extends previous findings by indicating that the peak-latency and peak-amplitude of the HDR in the auditory cortex is also altered as a function of the temporal distance between consecutive auditory stimuli.

While the present study found comparable refractory properties in the visual and auditory cortical regions, the duration of the refractoriness has previously been reported to extend longer in non-primary sensory cortices relative to primary sensory regions, as revealed by the refractory period extending up to 8 seconds in the fusiform gyrus for visual stimuli (Huettel and McCarthy, 2001), suggesting that the duration of refractory period varies between cortical subregions within the sensory modalities. Such differential refractory properties across cortical regions may be due to differences in the neural response latencies between different cortical fields (Liegeois-Chauvel, Musolino et al. 1991; Rosen, Buckner et al. 1998) or in neural vasculature, capillary density (Kuschinsky and Paulson 1992) and differential sampling of vessels across regions (Lee, Glover et al.

1995). In fact, neighboring voxels have been shown to vary in onset time (up to 2 s) and amplitude (1-5% in range), suggesting different effects of micro and macro-vasculature rather than being due to neural activity (Binder, Rao et al. 1995); (Buckner, Bandettini et al. 1996). Furthermore, stimulus properties and experimental parameters, such as stimulus duration (Vazquez and Noll 1998), stimulation train duration (Robson, Dorosz et al. 1998) and stimulus rate (Friston, Josephs et al. 1998) also affect the length of the refractory period during which HDR to subsequent stimuli are suppressed. Such differences in the characteristics of the HDR function elicited in different cortical regions extend beyond sensory areas, as they have been demonstrated in the prefrontal cortex as well (Schacter, Buckner et al. 1997).

The qualitative differences in the curves of the HDR elicited by the single visual and auditory stimuli in Experiment 2 consisted of earlier HDR peaks in STG as compared to the HDR in the visual cortex. However, given the differences in stimulus properties (e.g. auditory tones sustained a shorter duration), and evidence that increasing stimulus duration up to 3 seconds leads to higher and delayed activation peaks (Vazquez and Noll 1998; Liu and Gao 2000). Hence the interpretation of the differential activations in the visual and auditory cortices should be interpreted conservatively. Nevertheless, the finding that the HDR in auditory cortex peaks around 4 seconds after the stimulus onset is consistent with the previous studies which have demonstrated even earlier peaks (3-4 sec), with different subregions of the auditory cortex giving rise to different peak latencies, such as 3 sec. in primary auditory cortex and 4 sec. for more anterior and lateral regions of auditory cortex (Belin, Zatorre et al. 1999), perhaps reflecting variability in peak latency across Heschl's gyrus and other secondary auditory cortical subregions. The

tracing of finer definitions for our ROIs, possibly guided by independent retinotopic/tonopic maps (Sereno MI 1994) would enhance our understanding of both differences of the characteristics of the HDR in primary and secondary cortical regions, as well as differences in refractory properties of eth HDR across cortical regions.

Although the suppression of the HDR is a complex phenomenon, it has been reported under many experimental conditions, and has been interpreted as a reflection of adaptive properties of sensory processing regions that respond to selective attributes of stimuli. For example, Grill-Spector and Kourtzi demonstrated that FMR-A, a phenomenon similar to repetition priming, is primarily driven by the neurons' sensitivity to different attributes of the presented stimuli. As such, this phenomenon has served to investigate the differential and selective sensitivity of particular sensory regions to specific stimulus attributes (Grill-Spector K 2001; Kourtzi Z 2001). Similarly, Soon et al examined the HDR refractoriness by presenting identical and non-identical faces, and demonstrated that the signal recovers less in response to identical faces compared to different faces in mid-fusiform and right prefrontal regions (Soon CS 2003). In addition, suppression of the HDR within cortical subregions to in response to repeated attributes of stimuli has also been described in studies of "repetition priming" (Grill-Spector K 2001; Kourtzi Z 2001), further supporting neural adaptation or refractoriness in visual cortical regions. Our results extend these findings by demonstrating comparable adaptive properties in both visual and auditory cortical regions.

Despite these relatively robust and replicated observations of adaptive properties of the HDR under repeated stimulation conditions across studies, the nature and mechanism of this complex phenomenon remain unclear. It is known that a combination

of multiple physiological changes associated with neuronal activity, such as CBF, CMRO₂, and CBV contributes to the BOLD signal change. It is difficult at this point, and certainly not possible from this experiment, to distinguish the differential role of these physiological contributors upon changes (linear or not) in the BOLD signal associated with stimulus/task attribute changes. Nevertheless, we recognize the “refractory period” or “repetition priming”, or “fMRI-Adaptation” bears significant resemblance to the refractoriness exhibited by neurons in other cortical regions. While the neurophysiological mechanisms of the refractory properties of the HDR are not clear, the refractory period has been associated with ‘neural refractoriness’ (Binder, Rao et al. 1994; Friston, Josephs et al. 1998; Huettel and McCarthy 2000), a period during which neural responsivity and/or hemodynamic coupling is reduced. This refractoriness has further been shown to depend on the ISI of the paired stimuli (Friston, Josephs et al. 1998). It has been suggested that non-linear characteristics of HDR may result from neuronal refractory periods or from non-linearities in the mechanism of blood flow regulation (Binder, Rao et al. 1994; Friston, Josephs et al. 1998; Huettel and McCarthy 2000). Consistently, previous studies have suggested a decoupling of vascular refractoriness and an underlying neural refractoriness. For instance, the HDR in rat cortex is delayed despite the absence of a delay in accompanying electrophysiological measures, suggesting a decoupling primarily induced by vascular responses (Cannestra, Pouratian et al. 1998b). The observation of a nonlinear BOLD response change during very fast stimulation frequencies has also been demonstrated to be limited to the BOLD signal, and to not affect CBF, where transduction of neural activity to rCBF has been found to be linear (Mechelli, Friston et al. 2000; Mechelli, Price et al. 2001), suggesting a nonlinear

transduction of rCBF to BOLD signal. Thus, the findings of a linear relationship between the transduction of neural activity and rCBF, combined with optical imaging (Cannestra, Black et al. 1998a) and single cell response recordings (Tolhurst, Walker et al. 1980) strongly link the refractoriness of the HDR to neural refractoriness.

The results also revealed that the extent of activation, as reflected by the proportion of activated voxels within an ROI, was greater under the paired condition relative to the unpaired of each modality. The larger area recruited during the paired stimulation conditions in our study as compared to single stimulus presentation may reflect the recruitment of novel or additional neural pools. Alternatively, more likely, given the stimuli timing on the order of seconds, the greater extent of activation may also be a consequence of slow vascular recovery in these regions, yielding to additional vascular recruitment from adjacent areas to perfuse local neural space. These results may also reflect a change in contrast to noise ratio related to greater stimulation and an increase in deoxy-hemoglobin. This in turn may lead to a compound effect at short ISI and a stronger and consequently greater extent of activation, reflecting an increased contrast to noise ration, associated with improved signal detection capability. In summary, the greater extent of activation to repeated stimuli may reflect recruitment of additional neuro-vascular resources.

An outstanding question is the potential effect of cross-modality interactions upon the hemodynamic refractoriness of sensory regions. For instance, in the present study, BOLD activation in the auditory cortex in response to the tones was accompanied by a simultaneous negative shift in the BOLD response measured at the visual cortex (Figure 9B). In contrast, activation in the auditory cortex did not appear to show a similar

“below-baseline” decrease in the BOLD response elicited during the visual stimulation. This relatively reduced BOLD signal in the visual cortex during the auditory stimulation may simply reflect the lingering of the visual cortex activation elicited by the preceding visual stimulus. Figure 9 is consistent with the latter explanation, as it demonstrates that the activity in the visual cortex indeed lingers for a longer period than that in the auditory cortex, hence resulting in a longer sustained activation period in visual cortical regions following stimulus offset, overlapping with the presentation of the auditory stimulation period.

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REFERENCES

Aguirre, G. K., E. Zarahn, et al. (1998). "The variability of human, BOLD hemodynamic responses." *Neuroimage* **8**(4): 360-9.

- Akaishi, T. and Y. Sakuma (1985). "Estrogen excites oxytocinergic, but not vasopressinergic cells in the paraventricular nucleus of female rat hypothalamus." Brain Research **335**(2): 302-5.
- Belin, P., R. J. Zatorre, et al. (1999). "Event-related fMRI of the auditory cortex." Neuroimage **10**(4): 417-29.
- Binder, J. R., S. M. Rao, et al. (1994). "Effects of stimulus rate on signal response during functional magnetic resonance imaging of auditory cortex." Brain Res Cogn Brain Res **2**(1): 31-8.
- Binder, J. R., S. M. Rao, et al. (1995). "Lateralized human brain language systems demonstrated by task subtraction functional magnetic resonance imaging." Arch Neurol **52**(6): 593-601.
- Boynton, G. M., S. A. Engel, et al. (1996). "Linear systems analysis of functional magnetic resonance imaging in human V1." J Neurosci **16**(13): 4207-21.
- Buchel, C., A. P. Holmes, et al. (1998). "Characterizing stimulus-response functions using nonlinear regressors in parametric fMRI experiments." Neuroimage **8**(2): 140-8.
- Buckner, R. L. (1998). "Event-related fMRI and the hemodynamic response." Hum Brain Mapp **6**(5-6): 373-7.
- Buckner, R. L., P. A. Bandettini, et al. (1996). "Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging." Proc Natl Acad Sci U S A **93**(25): 14878-83.
- Cannestra, A. F., K. L. Black, et al. (1998a). "Topographical and temporal specificity of human intraoperative optical intrinsic signals." Neuroreport **9**(11): 2557-63.
- Cannestra, A. F., N. Pouratian, et al. (1998b). "Refractory periods observed by intrinsic signal and fluorescent dye imaging." J Neurophysiol **80**(3): 1522-32.
- Dale, A. M. and R. L. Buckner (1997). "Selective averaging of rapidly presented individual trials using fMRI." Human Brain Mapping **5**: 329-340.
- Friston, K. J., O. Josephs, et al. (1998). "Nonlinear event-related responses in fMRI." Magn Reson Med **39**(1): 41-52.
- Grill-Spector K, M. R. (2001). "fMR-adaptation: a tool for studying the functional properties of human cortical neurons." Acta Psychol (Amst). **107**(1-3): 293-321.
- Huettel, S. A. and G. McCarthy (2000). "Evidence for a refractory period in the hemodynamic response to visual stimuli as measured by MRI." Neuroimage **11**(5 Pt 1): 547-53.
- Huettel, S. A. and G. McCarthy (2001). "Regional differences in the refractory period of the hemodynamic response: an event-related fMRI study." Neuroimage. **14**(5): 967-76.
- Kim, S. G., W. Richter, et al. (1997). "Limitations of temporal resolution in functional MRI." Magn Reson Med **37**(4): 631-6.
- Kourtzi Z, K. N. (2001). "Representation of perceived object shape by the human lateral occipital complex." Science **293**(5534): 1506-9.
- Kuschinsky, W. and O. B. Paulson (1992). "Capillary circulation in the brain." Cerebrovasc Brain Metab Rev **4**(3): 261-86.
- Laurienti PJ, W. M., Maldjian JA, Susi CM, Stein BE, Burdette JH. (2002). "Deactivation of Sensory-Specific Cortex by Cross-Modal Stimuli." Journal of Cognitive Neuroscience **14**: 420-429.

- Lee, A. T., G. H. Glover, et al. (1995). "Discrimination of large venous vessels in time-course spiral blood-oxygen-level-dependent magnetic-resonance functional neuroimaging." Magn Reson Med **33**(6): 745-54.
- Liegeois-Chauvel, C., A. Musolino, et al. (1991). "Localization of the primary auditory area in man." Brain **114 (Pt 1A)**: 139-51.
- Liu, H. and J. Gao (2000). "An investigation of the impulse functions for the nonlinear BOLD response in functional MRI." Magn Reson Imaging **18**(8): 931-8.
- McCarthy, G., A. Puce, et al. (1996). "Magnetic resonance imaging studies of functional brain activation: analysis and interpretation." Electroencephalogr Clin Neurophysiol Suppl **47**: 15-31.
- Mechelli, A., K. J. Friston, et al. (2000). "The effects of presentation rate during word and pseudoword reading: a comparison of PET and fMRI." J Cogn Neurosci **12 Suppl 2**: 145-56.
- Mechelli, A., C. J. Price, et al. (2001). "Nonlinear coupling between evoked rCBF and BOLD signals: a simulation study of hemodynamic responses." Neuroimage **14**(4): 862-72.
- Miezin, F. M., L. Maccotta, et al. (2000). "Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing." Neuroimage **11**(6 Pt 1): 735-59.
- Pollmann, S., C. J. Wiggins, et al. (1998). "Use of short intertrial intervals in single-trial experiments: a 3T fMRI-study." Neuroimage **8**(4): 327-39.
- Robson, M. D., J. L. Dorosz, et al. (1998). "Measurements of the temporal fMRI response of the human auditory cortex to trains of tones." Neuroimage **7**(3): 185-98.
- Rosen, B. R., R. L. Buckner, et al. (1998). "Event-related functional MRI: past, present, and future." Proc Natl Acad Sci U S A **95**(3): 773-80.
- Schacter, D. L., R. L. Buckner, et al. (1997). "Late onset of anterior prefrontal activity during true and false recognition: an event-related fMRI study." Neuroimage **6**(4): 259-69.
- Sereno MI, M. C., Allman JM (1994). "Analysis of retinotopic maps in extrastriate cortex." Cereb Cortex **4**(6): 601-20.
- Sobotka, S., & Ringo, J.L. (1993). "Investigation of long-term recognition and association memory in unit responses from inferotemporal cortex." Experimental Brain Research **96**: 28-38.
- Soon CS, V. V., Chee MW. (2003). "Stimulus repetition and hemodynamic response refractoriness in event-related fMRI." Hum Brain Mapp **20**(1): 1-12.
- Soon CS, V. V., Chee MW. (2003). "Stimulus repetition and hemodynamic response refractoriness in event-related fMRI." Hum Brain Mapp **20**(1): 1-12.
- Tolhurst, D. J., N. S. Walker, et al. (1980). "Non-linearities of temporal summation in neurones in area 17 of the cat." Exp Brain Res **38**(4): 431-5.
- Vazquez, A. L. and D. C. Noll (1998). "Nonlinear aspects of the BOLD response in functional MRI." Neuroimage **7**(2): 108-18.
- Zarahn, E., G. Aguirre, et al. (1997). "A trial-based experimental design for fMRI." Neuroimage **6**(2): 122-38.

FIGURE LEGENDS

Figure 1.

Exp 1: Auditory Task Design: A brief tone of 1000 Hz presented for 100ms either singly or in a pair. The two stimuli in a pair were separated by a variable intra-pair interval of 1, 4 or 6 seconds. Three types of pair conditions and the single stimulus condition were intermixed randomly during a presentation of 10 runs (3.5min each). Forty instances of each condition were presented during each session.

Figure 2.

Exp 2: Interleaved Auditory and Visual Task Design: Single and paired auditory and visual stimuli were presented in an alternating pattern to avoid the overlapping response of the same domain. The time between the two conditions of the same domain varied between 16-20 seconds while two consecutive trials of different modality are separated by 8-10 seconds. Visual stimuli were checkerboards displayed with a duration of 500ms, whereas the auditory stimuli were pure tones of 1000 Hz with a duration of 100ms.

Figure 3.

Exp1: The activation map (t-map) in response to a single auditory tone overlaid on a structural T_1 -weighted image in a single subject. The voxels that respond to single stimulus presentation at a t-value of 3.6 or greater were selected to evaluate the refractoriness when a second stimulus was presented with close succession to the first one.

Figure 4.

Exp 2: The activation maps in response to single and paired stimulus conditions in both modalities. The response to paired conditions was calculated by averaging the epochs of BOLD signal within the voxels activated by single stimulus presentations. Note that the t-

maps in response to single and paired conditions mostly overlap, but do not have the same spatial extent. In order to eliminate the confounding effects of newly recruited areas, the BOLD signal analysis for both trial types were restricted to the voxels that responded to single stimulus presentations.

Figure 5.

Exp1: The composite HDRs evoked by the paired conditions - aligned to the onset of the first stimulus. Black arrow indicates the onset of first stimulus in all the conditions. Colored arrows indicate the onsets of second stimuli in different IPI conditions (Blue: 1 sec IPI; Green: 4 sec IPI, Red: 6 sec IPI). The HDRs corresponds to the matching arrow condition. Legends indicate the time interval between the two stimuli in a pair. This figure demonstrates the separation of two peaks as the time between two stimuli in a pair increases. At the 6 sec IPI condition, even though the peaks are separated and the latency to peak from the onset of second stimulus recovers, the peak amplitude still remains suppressed as compared to the first peak.

Figure 6.

Exp 2: Visual Cortex. Average of hemodynamic responses in visual cortex. A) HDRs evoked by single and paired stimulus presentation. Composite HDR to the paired condition shows higher amplitude than the response evoked by single stimulus. B) To isolate the contribution of the second stimulus in a pair to the composite response, the response to the single stimulus presentation was subtracted from the composite response to the paired condition and shifted by 1 second to realign to the same time onset.

Figure 7.

Exp 2: Auditory Cortex. Average of hemodynamic responses in auditory cortex. A) HDRs evoked by single and paired stimuli presentation. B) To isolate the contribution of the second stimulus in a pair to the composite response, the response to the single

stimulus presentation was subtracted from the composite response to the paired condition and shifted by 1 sec. to realign to the same time onset.

Figure 8.

Exp 2: Percent of activated voxels in response to repeated stimuli in visual and auditory cortices (*: $p < 0.002$)

Figure 9.

A) Percent signal change evoked by single and paired visual stimulus presentations in visual and auditory cortical regions. B) The HDRs evoked by auditory stimuli in VC and STG. (**S-VC**: Single stimulus condition in VC, **P-VC**: Paired stimuli condition in VC, **S-STG**: Single stimulus condition in STG, **P-STG**: Paired stimulus condition in STG).

Table 1

Mean values of the peak amplitude (percent signal change), suppression index based on peak values ($SI = [(S2/S1) * 100]$) and latency (second) measures of the average HDR evoked in VC and STG.