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The Effects of Aging upon the Hemodynamic Response Measured by Functional MRI[1]

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We compared the characteristics of the visually evoked hemodynamic response (HDR) in groups of young and elderly adults. Checkerboard stimuli were presented for 500 ms either singly or in pairs separated by a 2-s intrapair interval while gradient-echo echoplanar fMRI images were acquired concurrently every 1 s. Activated voxels, identified by correlation with an empirically derived reference waveform, were found for both groups in cortex along the calcarine sulcus and in the fusiform gyrus, with the mean HDR latency in calcarine cortex peaking approximately 300 ms earlier than the HDR evoked in the fusiform gyrus. On average, younger subjects had twice as many activated voxels as older subjects. The mean HDR had a similar onset time, rate of rise, and peak amplitude in both groups. However, the HDRs of older subjects reached their peak earlier and were more variable across subjects. Despite having average HDR amplitudes similar to those of younger subjects, older subjects had higher noise levels in activated voxels, resulting in lower signal-to-noise ratios. Distribution analyses of voxel statistics (*t* value, peak amplitude, peak latency) revealed that older subjects had proportionally fewer small-effect-size voxels, due to their increased voxelwise noise. This finding was consistent with the smaller spatial extent of activation in older subjects. To investigate age differences in the refractory period of the visual HDR, the HDR evoked by the second stimulus of each pair was isolated by subtracting the HDR evoked by a single stimulus from the composite HDR evoked by a pair. Recovery measures were similar across the age groups.

INTRODUCTION

Functional magnetic resonance imaging (fMRI) has proved a useful tool for the noninvasive investigation of sensory, motor, and cognitive processing in the human brain. The increased use of fMRI in the study of brain dysfunction associated with dementia and other neurological and psychiatric diseases has led to the increased use of fMRI in elderly subjects. For this reason, it is critical to understand how normal aging may influence the fMRI-measured hemodynamic response (HDR).

Anatomical, physiological, and metabolic changes in the human brain have been reported to be associated with aging. Reported anatomical changes have included sulcal widening, increased ventricular size, and loss of synapses (Goldstein and Reivich, 1991; Raz, 2000). However, these structural changes may not be concomitant with changes in neuronal density over the course of normal aging. While early studies advanced the idea that neuron loss accompanies the normal aging process, even in the absence of pathological states (Brody, 1955), recent studies using advanced sampling and stereological techniques have shown negligible neuronal loss in normal aging (Long *et al.*, 1999). These studies have suggested that the functional decline observed in normal aging may not be due to changes in neuronal density (Morrison and Hof, 1997). Furthermore, differences in dendritic growth or regression across different brain regions make simple conclusions about age-related declines in function problematic (see Coleman and Flood, 1987, and Flood, 1993, for reviews). Finally, the issue of whether age-related anatomical changes differ for men and women is not settled, as studies support both increased male and increased female vulnerability to age-related changes (Coffey *et al.*, 1998; Murphy *et al.*, 1996; Raz *et al.*, 1993).

Age-related changes have also been reported in the cerebral vasculature, including thickening of the vascular basement membrane and thinning of the endothelium (see Kalaria, 1996, for review). Less well established are the effects of aging upon cerebral blood flow and metabolism: some authors report age-related declines, while others do not (see Goldstein and Reivich, 1991, for review). More recent studies suggest that age-related changes in resting brain metabolism may

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differ across brain regions with significant changes occurring in frontal cortex and little change reported for visual cortex (Loessner *et al.*, 1995).

Physiological studies have revealed age-related changes in the latencies of sensory evoked potentials, with increased latencies observed in elderly subjects (see, for example, Celesia and Daly, 1977). Age-related declines in evoked potential amplitudes have also been reported by some investigators (e.g., Allison *et al.*, 1984). Positron emission tomography (PET) studies of cerebral blood flow have also demonstrated widespread age-related changes in the activation patterns evoked by cognitive challenges (Grady *et al.*, 1999; Madden *et al.*, 1996, 1997, 1999).

Blood oxygen level-dependent (BOLD) contrast reflects both cerebral blood flow and oxygen extraction evoked by neuronal stimulation (Buxton *et al.*, 1998). We would presume, therefore, that any age-related change in brain anatomy, neuronal density, vasculature, metabolism, or neuronal responsivity would influence the fMRI-measured HDR. Few studies, however, have investigated the effects of aging upon fMRI-derived measurements. Taoka and colleagues (1998) measured the temporal characteristics of fMRI activation in motor cortex during an extended-duration (10 s) hand-squeezing task. Using a regression technique across a wide range of subject ages (20–76 years), they found that there were age-related increases in the rise-time of the fMRI signal, but not in the fall-time. This slowing of signal rise was attributed to vascular effects, including stiffening of the arterial wall. Age-related differences have also been reported for a photic stimulation task using fMRI, such that signal amplitude was decreased in elderly subjects, but spatial extent of activation did not differ from that of young subjects (Ross *et al.*, 1997). D'Esposito and colleagues (1999) investigated the characteristics of the fMRI-derived HDR evoked in a motor task in groups of young and elderly subjects. Subjects performed a periodic reaction time task, pressing a button when a fixation point changed to a circle (every 16 s). Young subjects exhibited both more suprathreshold voxels and higher signal to noise ratios than did elderly subjects. However, there were no significant between group differences in either the shape or within-group variability of the HDR. These results suggest that fMRI imaging analyses can be profitably conducted on elderly subjects, although the authors caution that differential spatial extents of activation may lead to intensity differences after spatial smoothing used in typical preprocessing of data.

In this study, we investigated the amplitude, latency, refractoriness, and spatial extent of the HDR evoked by brief visual stimuli in young and elderly subjects. On each trial, subjects were presented with either a single full-field checkerboard stimulus or a pair of checkerboards separated by 2 s (onset-to-onset). Each checkerboard's duration was 500 ms regardless of whether it was presented singly or in a pair. Subjects passively viewed the checkerboards with eyes at fixation. This design is adapted from recent studies by our group showing that the HDR evoked by the second of two closely spaced stimuli is attenuated in amplitude and increased in latency compared to a single stimulus (Huettel and McCarthy, 2000a, 2000b). At an intrapair interval (IPI) of 1 s, the response evoked by the second stimulus was approximately one-half the amplitude evoked by a single stimulus, but at a 6-s IPI, the response evoked by the second stimulus was approximately the same as evoked by the first stimulus (Huettel and McCarthy, 2000). In designing the present study, we reasoned that age-related vascular changes might alter the recovery of the HDR to the second of a pair of close-spaced stimuli—i.e., there might be age effects upon the refractory period of the HDR. We investigated HDR changes in regions of interest in the cortex surrounding the calcarine sulcus and in the fusiform gyrus.

METHODS

Subjects

Two subject groups were tested under identical conditions: 11 young subjects (mean age = 23 years, range 18–32 years) and 11 elderly subjects (mean age = 66 years, range 57–76 years); each group consisted of 7 males and 4 females. Young subjects were recruited through advertisements at Duke University. Elderly subjects were drawn from a population of healthy adults who had previously participated in a MR spectroscopy study. All elderly adults had been screened for dementia and other cognitive deficits and had tested in normal ranges on cognitive testing. All subjects had normal vision or were corrected to normal vision using MR-compatible lenses.

Stimuli and Experimental Design

Throughout each run, a small white fixation cross ($< 1°$) was visible against a neutral gray background. The experimental stimulus was a black and white, high-contrast, radial checkerboard subtending 20 by 15° of visual angle. The stimulus was projected into the scanner bore onto a screen behind the subject's head, by using a LCD projector with a custom throw lens. The subject viewed the display using mirrored goggles.

Checkerboards appeared singly or in pairs. On single-stimulus trials, one checkerboard was presented for 500 ms, followed by a 16.5-s intertrial interval (ITI). Paired-stimulus trials consisted of two checkerboards, each presented for 500 ms, separated by a 2-s intrapair interval (IPI; measured onset to onset) followed by a 16.5-s ITI. A run consisted of 20 trials (10 of each type) and each subject participated in either six or seven

runs (elderly mean = 6.63 runs, young mean = 7.00 runs).

Imaging Parameters

MR scanning was conducted on a 1.5T GE SIGNA scanner with an NVi high-performance system for fast echo-planar imaging and 41 mT/m gradients. Image transfer and reconstruction was conducted using a GE Advanced Development Workstation. A vacuum-pack system restricted head motion without compromising patient comfort. Twelve axial slices (5 mm thick, no skip) parallel to the line connecting the anterior and posterior commissures were selected in each subject following initial sagittal structural imaging (2-D SPGR; nine slices around midline). These axial slices were selected to encompass inferior temporal through occipital cortex. High-resolution spin-echo structural images were acquired for each slice (in-plane resolution: 0.94 mm^2) for later anatomical identification of the calcarine sulcus. Functional images were acquired using gradient-echo echoplanar imaging (TR: 1000 ms, TE: 40 ms, Flip Angle: 81°, in-plane resolution: 3.75 mm^2). This procedure enabled us to acquire images of occipital and temporal cortex with temporal resolution of 1 s.

Data Analysis Techniques

Identification of active voxels. The MR signal for each voxel was temporally aligned to correct for the interleaving of slice acquisition within each TR. This was accomplished by fitting a cubic spline function to the MR signal time series for each voxel and then resampling the series aligned to the onset of each TR. Epochs time-locked to the onset of each stimulus event were then extracted from the continuous time series and averaged according to trial type (single stimulus or paired stimulus). These averaged epochs consisted of the 5 vol preceding and 13 vol (19 s total) following stimulus onset (i.e., the onset of the single checkerboard or the onset of the first checkerboard of the pair). An empirically derived reference waveform obtained from our prior study (Huettel and McCarthy, 2000a) was cross-correlated with the averaged time series of every brain voxel to determine which voxels were significantly activated in the single-stimulus condition. This reference waveform represented the group activation to an identical 500 ms duration single checkerboard stimulus. T-statistics were derived from the resulting correlation coefficients, with threshold for activation set at $t > 3.5$ ($P < 0.001$, uncorrected). The result of this procedure was to identify the set of voxels in each subject that showed significant activation to the single checkerboard stimulus.

Regions of interest. We identified two anatomical areas using the structural MR images: calcarine cortex and fusiform gyrus. Calcarine cortex was identified by mapping the path of the calcarine sulcus in successive sagittal views. For all subjects, the region of interest (ROI) subsequently labeled as "Calcarine Cortex" contained the mapped calcarine sulcus, so that it included both V1 and portions of other visual areas. The fusiform gyrus (FFG) was circumscribed anatomically in axial structural images. All subjects showed significant activation in calcarine cortex; one elderly subject showed no activation in fusiform gyrus. The time course of activation within each ROI was determined by averaging the time courses of its constituent activated voxels as defined by cross-correlation above.

In addition, we selected the single voxel with the largest *t* value (e.g., best correlation) from calcarine cortex so that our results could be directly compared to those of D'Esposito and colleagues, who used a single-voxel analysis (cf. D'Esposito *et al.*, 1999). Thus, for each subject, we obtained three HDR waveforms—from activated voxels within the calcarine cortex ROI, from activated voxels within the fusiform gyrus ROI, and from the maximally activated voxel in calcarine cortex. The peak amplitude and latency to peak of each HDR was measured for each subject. In addition, secondary measures of peak amplitude and latency to peak were obtained by fitting a cubic spline to the empirical HDR. The resulting function was then sampled with a temporal resolution of 250 ms.

Signal–noise ratios. We calculated the signal-to-noise ratios (SNRs) of the HDR evoked at each voxel as the base-to-peak amplitude of the average HDR waveform divided by the standard deviation of the signal variation in that same voxel over the duration of each of the several runs. The density of stimulation over time was very small (1 stimulus event per 17–19 s); nevertheless, the contribution of the evoked signal to the noise estimation was removed prior to calculation. This was accomplished by subtracting the averaged single or paired HDR from the appropriate time points that followed each stimulus presentation before calculating the standard deviation of the run (in practice, this precaution had virtually no effect upon the SNR estimate). The SNR measure is identical to the *z* score deviation of the HDR amplitude from the mean noise variation in the voxel's time series.

Analysis of refractory effects. We investigated the characteristics of the HDR to the second stimulus in a pair in the same ROIs described above. We subtracted the HDR evoked by a single checkerboard from the composite HDR evoked by a pair of checkerboards on a voxel-by-voxel basis. Since the HDRs evoked by the single checkerboard and by the first checkerboard of each pair should be identical, the residual response following this subtraction is the HDR evoked by the second stimulus of the pair. This amplitude and latency of the second of the pair HDR

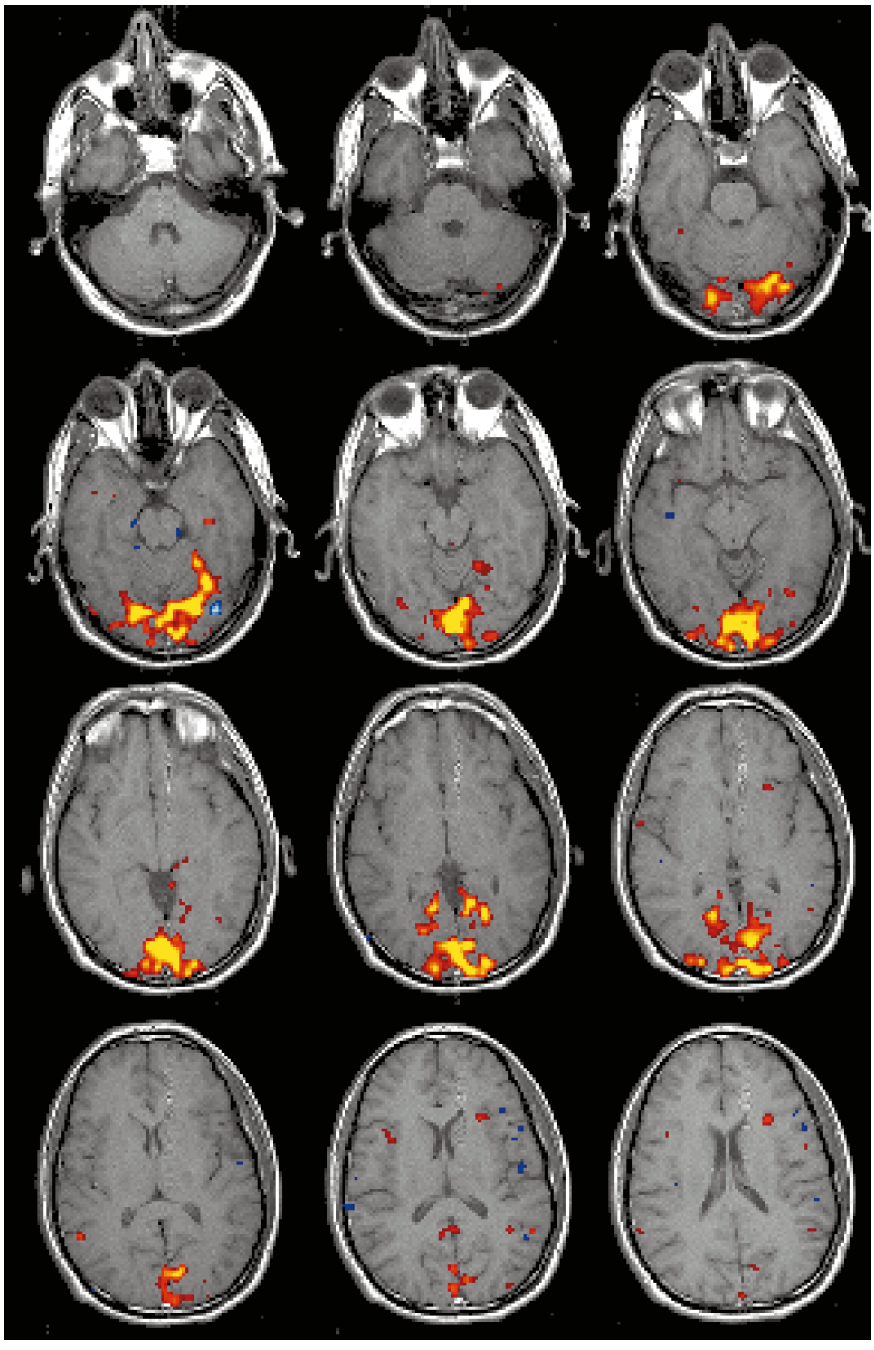

FIG. 1. The pattern of activation observed in a single randomly chosen subject. Overlaid upon a structural T1-weighted image is a statistical map generated by comparing control-trial activation to a predicted HDR function. Voxels active at a t value of greater than 3.5 ($P < 0.001$) are indicated on the colored activation overlay (red-yellow, for t values from 3.5 to 8.0+).

could then be directly compared to that evoked by a single stimulus.

RESULTS

The spatial pattern of activation evoked by a single checkerboard stimulus is shown in Fig. 1. The pseudocolored activation map represents the strength of the correlation between the reference waveform and the averaged time course of each voxels (correlations were converted to t statistics and thresholded above 3.5). This activation map has been overlaid upon the coregistered structural images. The typical pattern of activation is evident: bilateral calcarine cortex extending into bilateral fusiform cortex. This pattern was similar for the two subject groups. However, the spatial extent

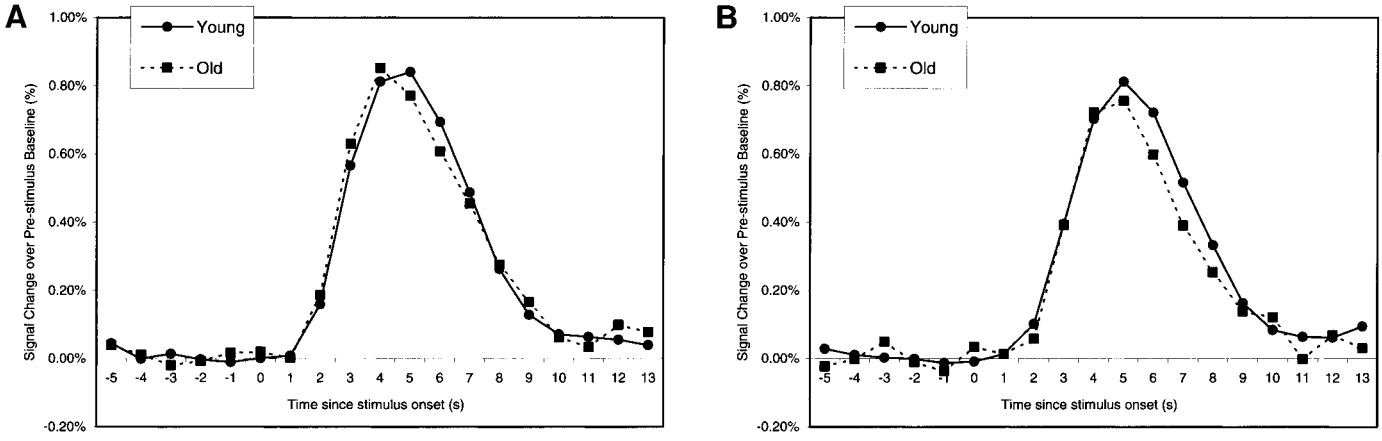

FIG. 2. The HDR, to a brief visual stimulus, in calcarine (A) and fusiform (B) cortices. A single checkerboard stimulus was presented, for 500 ms, beginning at time 0 s (x-axis). For our subject groups of young and old subjects, the average HDR is represented as signal change as a percentage of the prestimulus baseline period (y-axis). Notably, young and old subjects showed similar forms of the HDR, although the old subjects showed an earlier fall from peak than did the young subjects. In calcarine cortex, the latency to peak was significantly earlier in elderly subjects [$t(20) = 2.16$; $P < 0.05$]. In the fusiform gyrus, the difference between the groups was numerically similar and approached significance [$t(19) = 1.92$; $P = 0.069$].

of activation was greater in young subjects than in elderly subjects. In the sections below, we consider first the general spatial and temporal properties of the HDR, identifying similarities and differences between the groups. Then, we systematically evaluate intragroup variability in our two subject populations, both in form of the HDR in regions and voxels of interest and in distribution of voxel statistics across the brain.

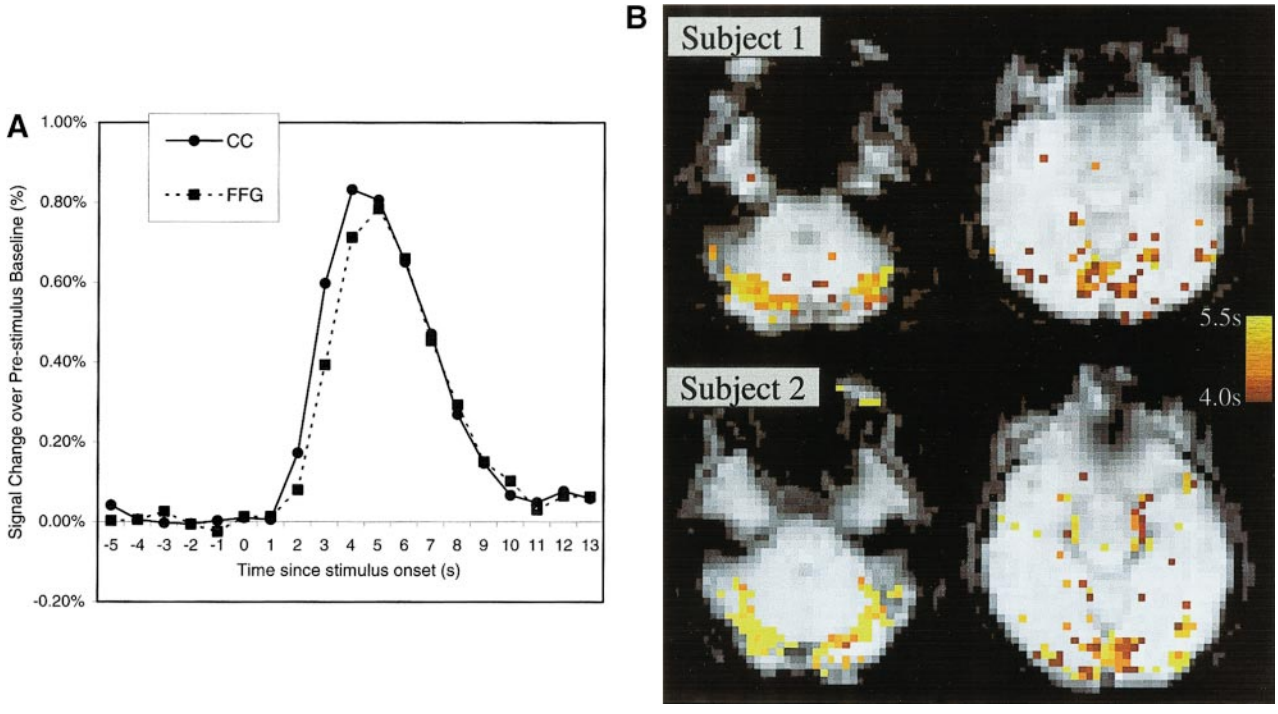

FIG. 3. Evidence for latency differences in the HDR in calcarine cortex and fusiform cortex. In (A), signal change averaged across both subject groups (y-axis) is presented as a function of the time since the presentation of the checkerboard stimulus (x-axis). Although the HDRs are generally of similar amplitude, there is a significant latency shift such that the response in calcarine cortex (CC), compared to that in fusiform gyrus (FFG), both is larger at its onset [at 2 s; $t(19) = 2.28$, $P < 0.05$] and peaks earlier [$t(19) = 2.97$, $P < 0.01$]. (B) shows data from two representative subjects, with latency to peak activation (red-yellow colormap indicating 4.0–5.5 s) in significantly active voxels overlaid upon echoplanar images. As can be readily identified in the images, the activation in fusiform cortex (left images) peaks later than that in medial calcarine cortex (right image), although there is significant variability across voxels. Similar results were found across all subjects.

TABLE 1

Summary Statistics for ROIs Tested

Age Group	Type	Peak amplitude		Latency to peak		Maximum SNR	Number of active voxels	
		Mean	SD	Mean	SD		Mean	SD
Young	CC	0.88%	0.20%	4.75	0.42	0.52	53.09	15.62
Old	CC	0.92%	0.27%	4.23	0.68	0.35	25.82	12.33
Young	FFG	0.85%	0.15%	5.05	0.56	0.41	79.00	24.93
Old	FFG	0.83%	0.20%	4.55	0.62	0.30	35.60	15.56
Young	Max	1.67%	0.67%	4.91	0.59	0.86	—	—
Old	Max	1.61%	0.95%	4.89	0.73	0.47	—	—

Note. Presented are peak amplitude of the HDR (percentage signal change over baseline; mean and standard deviation), latency to peak (seconds; mean and standard deviation), maximum signal–noise ratio, and number of active voxels in regions of interest (mean and standard deviation). Each measure is indicated for both subject groups (young and old) and for the three analyses: calcarine cortex ROI (CC), fusiform cortex ROI (FFG), and maximum-significance voxel (Max).

Finally, we evaluate whether differences in recovery of the HDR are present in our subject groups.

The Form of the Hemodynamic Response

The averaged HDR functions for the young and elderly groups are presented in Figs. 2A (calcarine cortex) and 2B (fusiform gyrus). Young and elderly subjects show similar mean amplitudes. A significant difference in latency to peak between groups can be observed in the calcarine cortex ROI with older subjects reaching their peak value about 500 ms earlier than younger subjects [$t(20) = 2.16$; $P < 0.05$]. For the fusiform gyrus, there was a similar numerical difference between the groups that approached significance [$t(19) = 1.92$; $P = 0.069$]; one elderly subject had no fusiform activation. Close examination of the response functions reveals that this latency difference begins approximately 5 s after stimulus onset. To assess the statistical significance of this numerical difference, we performed a Mann–Whitney *U* test on the normalized (to a peak amplitude of 1.0) subject data. We normalized each subject's data due to the presence of significant intersubject variability (see Fig. 4 below), which dominates the relatively small difference between the subject groups. We chose the nonparametric Mann–Whitney *U* test, because changes in the relative size of effects introduced by normalization would affect a parametric test (e.g., *t*). For calcarine cortex, there was a significant difference between the groups 5 s after stimulus onset [*Z*-adjusted (10) = 2.04; $P < 0.05$], and a marginally significant difference 6 s after onset [*Z*-adjusted (10) = 1.67; $P < 0.10$]. For the fusiform gyrus, there were no significant differences between the groups, although there were trends toward significance at 6 s [*Z*-adjusted (10) = 1.48; $P = 0.14$] and at 7 s following onset [*Z*-adjusted (10) = 1.62; $P = 0.10$]. There were no other significant differences between the groups in amplitude at any time point. Given the difference between the groups in latency to peak, the similar rise patterns, and the tendency for the elderly subjects to have reduced amplitude on the HDR fall, we conclude that the HDR shows a more rapid return to baseline in elderly subjects. No trends toward sex differences were found in peak amplitude, peak latency, or number of active voxels between the groups ($P > 0.10$). Summary values for amplitude, latency, signal-noise ratio, and number of active voxels are presented in Table 1.

Figure 3A presents the across-groups HDRs for calcarine cortex (CC) and fusiform cortex (FFG). The amplitude of the curves are similar, with peak amplitude of 0.91% signal change in calcarine cortex and 0.85% signal change in fusiform cortex. There is a latency shift between the curves, such that the HDR peaks about 300 ms earlier in calcarine cortex [paired *t* test (one subject had no fusiform activation), $t(19) = 2.97$, $P < 0.01$]. This latency difference was consistent in each group: in young subjects, the average difference was 300 ms; in elderly subjects, 320 ms. Additionally, the difference in latency to peak was mirrored by a difference in the onset of the HDR. Although no difference between the ROI functions is present at 1 s, by 2 s the response is larger in calcarine cortex [paired *t* test, $t(19) = 2.28$, $P < 0.05$].

Analyses of Within-Group Variability

The analyses of variability reported below are restricted to activated voxels within the calcarine cortex ROI.

Head motion. To investigate whether measures of variability were related to subtle differences in head motion, we conducted separate within-groups analyses of the correlation between amplitude of activation and subject head movement (D'Esposito *et al.*, 1999). Head motion was defined as the change in the position of the center of mass of the brain during each TR. The values

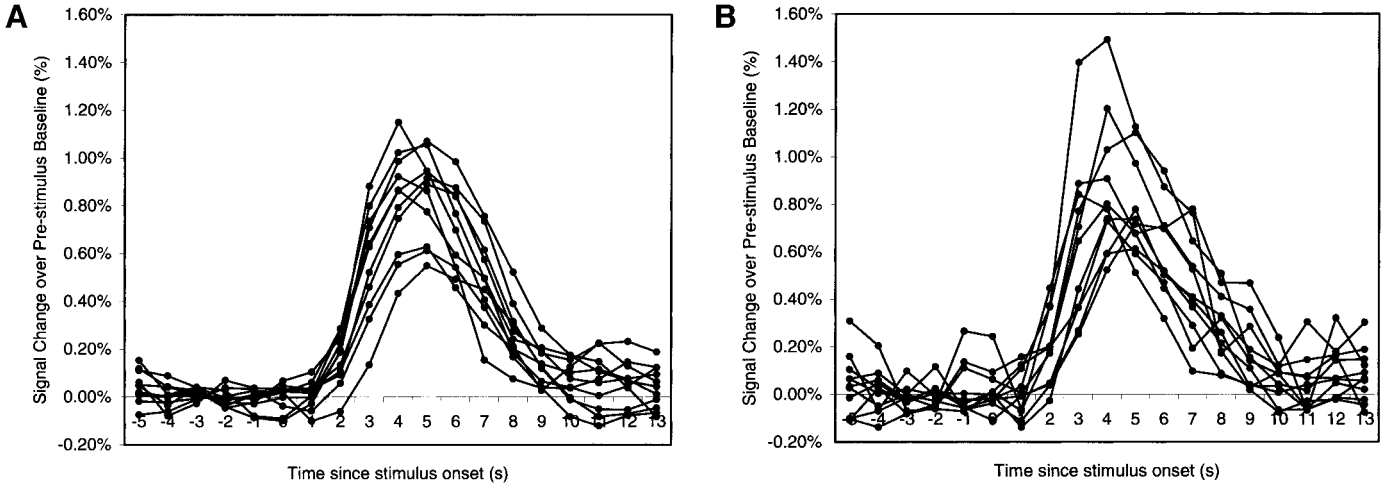

FIG. 4. The individual HDRs in the calcarine cortex ROI (see text) for all subjects in the young (A) and old (B) subject groups. Signal change over a prestimulus baseline (y-axis) is presented as a function of the time since the presentation of the checkerboard stimulus (x-axis). As evident in the averaged data, the general forms and amplitude of the HDR are similar across the groups, but the older subjects show more intersubject variability and intrasubject noise (see text for analyses).

for change in each of the three orthogonal directions were combined into a single vector, the length of which was measured for each TR and summed across all TRs. The movement vector was approximately 40% longer in elderly subjects. However, the correlation between path length and response amplitude was not significant in either young or elderly subjects [young, $r = -0.34$, $P > 0.10$; elderly, $r = 0.34$, $P > 0.10$]. We tested whether these correlation values were significantly different from one another using Fisher's Z test, finding no significant difference between them [$Z = 1.42$, $P > 0.10$]. Given the small magnitude of these nonsignificant correlations, we conclude that head motion did not differentially influence the intersubject variability results presented below.

Region of interest analysis. Figure 4 presents the calcarine cortex ROI HDRs for young (Fig. 4A) and elderly (Fig. 4B) subjects. As evident in Fig. 4A, all young subjects showed flat prestimulus (−5 to 0 s) baseline and smooth HDRs. In contrast, elderly subjects (Fig. 4B) appear to have more variability both in the prestimulus period and in the HDR following stimulus onset. As a first test for group differences in variability, we conducted F' tests on the summary values for peak amplitude and latency, and nonparametric tests on the epoch time courses. The two groups did not significantly differ in their standard deviations (SD) of peak amplitude (elderly SD: 0.27%; young SD: 0.20%) or of peak latencies (elderly SD: 0.68 s; young SD: 0.42 s), as revealed by F' testing of variance differences [amplitude $F' = 1.35$; latency $F' = 1.62$; P > 0.10 for both].

The standard deviation, for each subject group, at each time point in the peristimulus epoch investigated is provided in Fig. 5A, for the calcarine ROI. As expected, the variability values are largest from 3 to 7 s following stimulus onset, at which times the HDR is at its largest amplitude. To determine whether there were group differences in variability when the entire epoch time course is considered, we conducted a nonparametric statistical comparison of the standard deviations at all epoch time points (simple sign test). For the calcarine ROI, there was a higher within-group standard deviation in the elderly subject sample at 18 of the 19 epoch time points [$P < 0.001$]. Similar results were found for the fusiform ROI [not shown; 18 of 19 time points higher in elderly, $p < 0.001$]. We conclude that, although no differences between groups were found when only peak latency or peak amplitude values were considered, the elderly subjects show more amplitude variability across the time course of the HDR.

Best voxel analysis. We investigated variability in the HDR obtained from the single "best" voxel, defined as the voxel in calcarine cortex with the highest t value (i.e., the strongest correlation with the reference waveform) in both subject groups. Figure 6A presents the HDRs for the young subjects and Fig. 6B presents the HDRs for the elderly subjects. All voxels show flat prestimulus baselines and well-defined HDRs. The mean peak amplitude is similar across groups (1.67% in young, 1.61% in elderly), with a larger standard deviation within the elderly group (0.67% in young, 0.95% in elderly). Mean latency to peak and its standard deviation are also similar across the groups. The plot of intersubject standard deviations (Fig. 5B) mirrors the form of the HDR itself, with greatest variability 4−8 s following stimulus onset. As in the ROI comparisons above, there were no significant difference between groups in the variability of peak amplitude

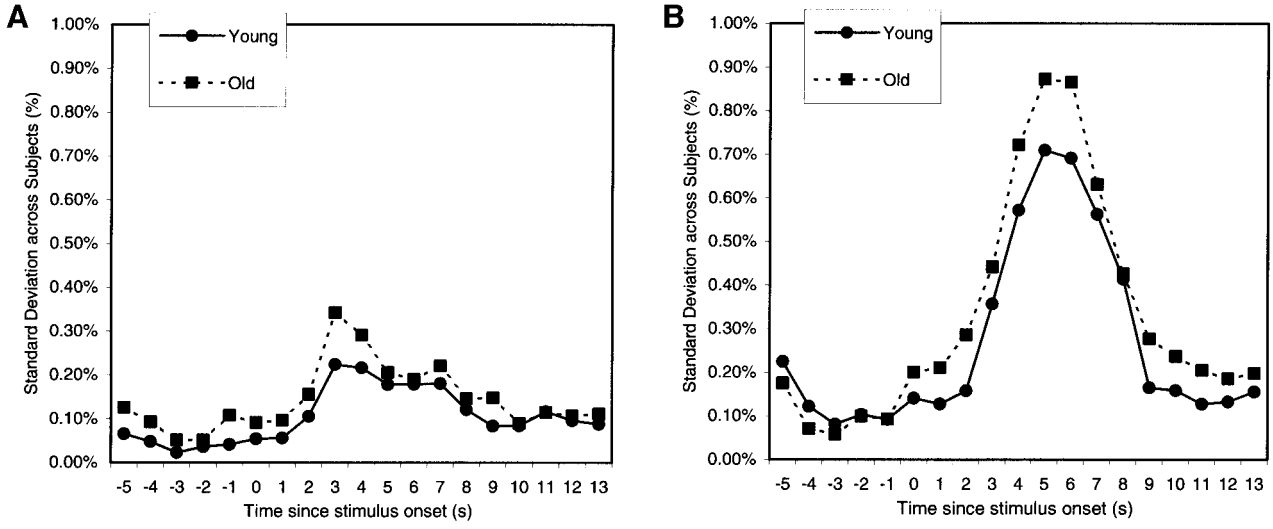

FIG. 5. Intersubject variability within the time course of the HDR in calcarine cortex (see text), for the ROI as a whole (A) and for the single best voxel within calcarine cortex (B). The standard deviation across subjects within each age group (y-axis) is plotted as a function of time since stimulus presentation (x-axis). For the ROI, the older subjects have higher intersubject variability throughout the stimulus epoch [18 of 19 time points, $p < 0.001$], with the greatest intergroup differences occurring 3–4 s following stimulus onset. For the single best voxel, the older subjects have slightly higher intersubject variability following stimulus onset, but prestimulus baseline variability levels are similar between the groups [15 of 19 time points higher in elderly, $P < 0.01$].

(elderly SD, 0.95%; young SD, 0.67%) or of peak latencies (elderly SD, 0.73 s; young SD, 0.59 s), as revealed by F' testing of variance differences [amplitude $F' = 1.42$; latency $F' = 1.24$; $P > 0.10$ for both]. Nonparametric comparison of relative standard deviation across all epoch time points revealed that the group of elderly subjects had a higher standard deviation than had the young in 15 of the 19 time points ($P < 0.01$). Notably, the young had slightly greater standard deviations over the first four time points (prestimulus baseline), while the elderly were more variable over the remainder of the epoch.

Analyses of Signal–Noise Ratio

The SNRs were markedly different between groups. Figure 7 presents the mean SNR across epoch time points for a region of interest in calcarine cortex and for the single voxel with the highest t value in calcarine cortex. The greatest signal noise ratio, observed 5 s after stimulus onset, for the young subjects was about 0.52 for the calcarine cortex ROI, while the greatest for the elderly subjects was about 0.35. The young had significantly higher SNR than the elderly at all time points in the epoch from 2 to 7 s following stimulus

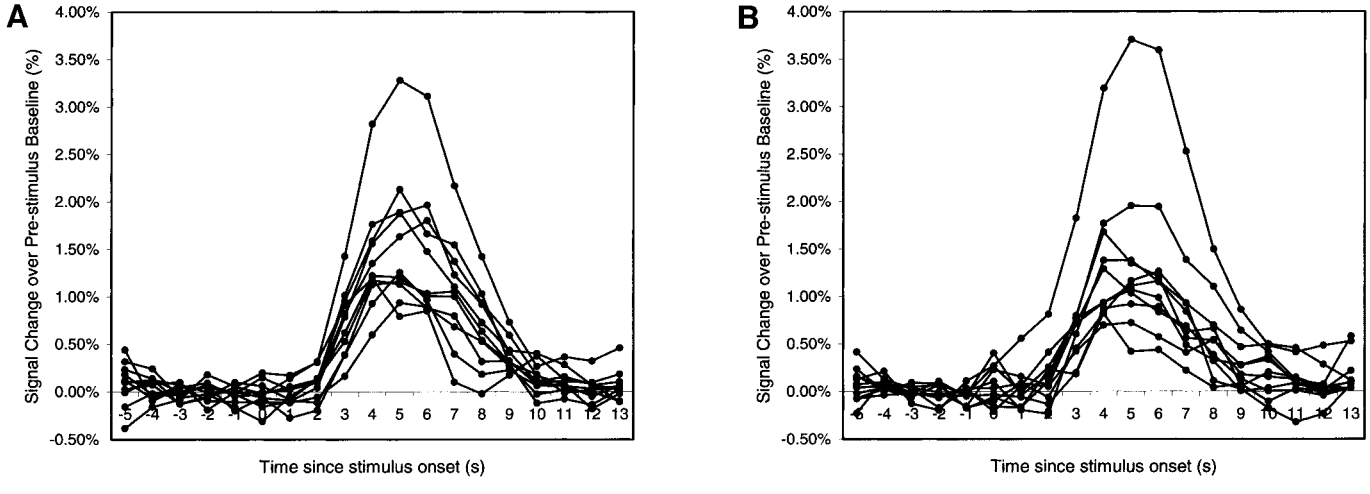

FIG. 6. The individual HDRs in the single most-significant voxel (see text) for all subjects in the young (6a) and old (6b) subject groups. Signal change over a prestimulus baseline (y-axis) is presented as a function of the time since the presentation of the checkerboard stimulus (x-axis). The general forms and amplitude of the HDR are similar across the groups (see text for analyses).

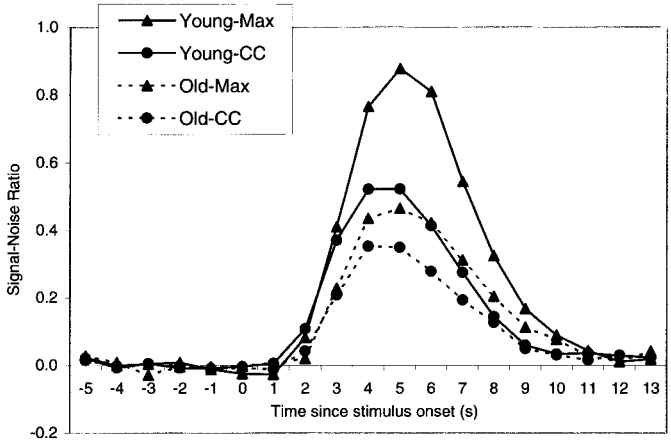

FIG. 7. Signal to noise ratios (SNRs). Analyses were conducted that examined SNR in young and old subjects, both for the calcarine cortex ROI (CC) and for the single most-significant voxel (Max). Plotted is SNR (y-axis; see text for calculation) as a function of the time since the presentation of the checkerboard stimulus (x-axis). Significantly higher SNR values were found for the young subjects at all time points in the epoch from 2 s to 7 s following stimulus onset [*t* comparison; all $P < 0.05$]. Combined with the finding of similar effect sizes between the groups (e.g., Fig. 2), this indicates that the older subjects had higher voxelwise noise levels.

onset [*t* comparison; all $P < 0.05$]. For both groups, SNR was larger when only the single best voxel was considered (young = 0.88, elderly = 0.46): in the young at epoch time points from 4 to 9 s following stimulus onset [paired *t* comparison, all $P < 0.05$], and in the old at points from 5 to 10 s [all $P \leq 0.0$]. Although both groups showed SNR improvements when considering only a single voxel compared to the mean of a ROI, the increase was proportionally larger in the young subjects. Since the mean HDR amplitudes did not differ across groups, the decreased SNR for the elderly reflects increased noise. This noise is evident in the variable prestimulus baselines for the elderly shown in Fig. 4B.

The higher SNR in the young subjects compared to the elderly was not due to less head motion. We compared the path length of head motion to the SNR of each subject 5 s following stimulus onset (for methods, see *Analyses of Within-Group Variability* above). Head motion was not significantly correlated with SNR within either group, nor were the two groups significantly different from one another [young, $r = -0.20$, $P > 0.10$; elderly, $r = 0.06$, $P > 0.10$; Fisher's $Z = 0.52$, $P > 0.10$]. Thus, differences in SNR across groups may reflect physiological variability, not head motion.

Distributions of Voxel Statistics

The analyses described heretofore in this section investigate whether young and elderly subjects differ in the temporal properties of the HDR. To complement these analyses, we investigated whether the two subject groups differ in their distributions of active voxels. We hypothesized that two sorts of intergroup differences might occur. First, older and younger subjects may differ in the number of voxels that pass the criterion threshold. We investigated this possibility in the regions of interest analyzed above and in the brain as a whole. Second, there could be intergroup differences in the distributions of voxel characteristics, whether in *t* values, peak amplitudes, or latencies to peak. For each of these summary statistics, we compared the distributions of all suprathreshold (i.e., statistically active) voxels in the brain.

Spatial extent of activation. Younger subjects had significantly more suprathreshold voxels than older subjects for ROIs tested in both calcarine cortex [$t(20) = 4.55$, $P < 0.001$] and fusiform cortex [$t(19) = 4.39$, $P < 0.001$; see Table 1]. This was also true when all activated voxels within the brain were measured—young subjects had about twice as many active voxels (mean, 1078 voxels) as did elderly subjects (mean, 495 voxels). This group difference was significant [$t(20) = 3.68$, $P < 0.01$].

Voxel characteristics. The histogram of voxel *t* values, for all voxels in the brain, is presented in Fig. 8. Each bin label indicates the midpoint *t* value (e.g., the bin labeled "0" contains all voxels with $-0.5 < t < 0.5$. The y-axis uses a logarithmic scale to accommodate all *t* values. As evident in Fig. 8, the young subjects diverge from the older subjects at *t* values greater than about 2.5. This analysis rules out the possibility that the specific *t* value chosen (i.e., $t > 3.5$) biases the differences between groups in spatial extent of activation. For any *t* threshold greater than about 2.5, the younger subjects will show a higher proportion of active voxels than the older subjects.

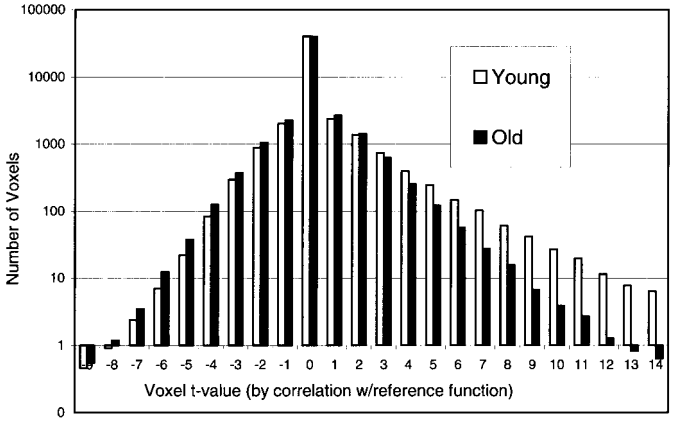

FIG. 8. Histogram of voxel *t* values for young and old subjects. Plotted is the total number of voxels in the brain (y-axis; logarithmic scale) for each *t* value bin (e.g., −0.5 to +0.5). The distributions of young and old subjects diverge at *t* values greater than about +2.5, indicating that threshold selection greatly determines the resulting relative spatial extent of activation, although young subjects have greater spatial extents for all positive thresholds.

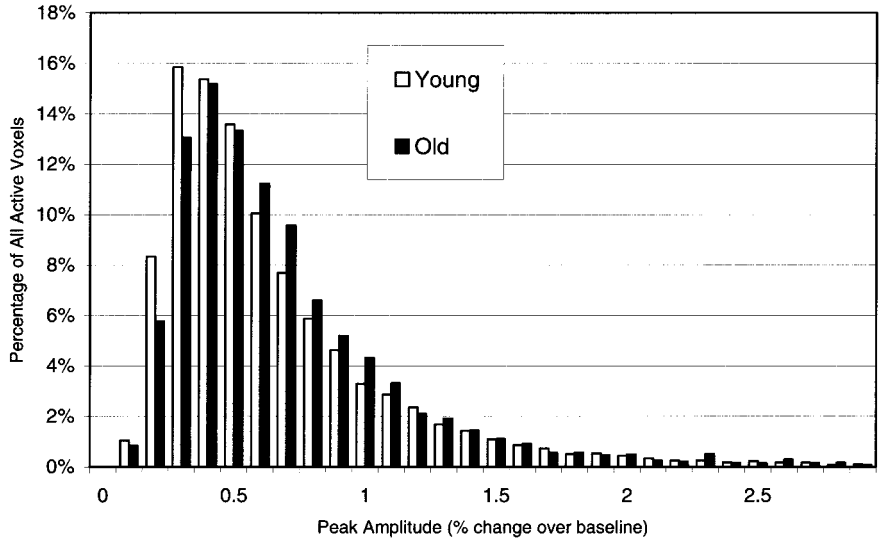

FIG. 9. Histogram of peak amplitude values for young and old subjects. Plotted is the percentage of significantly active voxels (y-axis; see text for methods) at each value of peak amplitude (x-axis). The proportion of high-amplitude voxels (e.g., >1.0% signal change) is similar between the groups, but the young subjects have proportionally more low-amplitude voxels (e.g., < 0.3% signal change). We suggest that this difference reflects the increased noise level in the old subjects, which reduces the likelihood that a low-effect-size voxel will pass significance thresholds.

Given this difference in spatial extent, it is critical to compare peak amplitude and peak latency for both groups. Figure 9 presents the distribution of peak amplitude values for all suprathreshold voxels in the brain, normalized within each group by the total number of active voxels. This normalization corrects for the approximately 2:1 difference in the number of active voxels in young and old subjects. Both distributions are heavily skewed, with the majority of voxels having peak amplitudes between 0.3 and 0.7%, and with a positive tail extending to amplitude values greater than 2.0. However, the distributions have similar forms, with that of the elderly subjects slightly less skewed. There was a near-significant trend for the young subjects to have more low-amplitude (peak amplitude $\leq 0.3\%$) active voxels than had the elderly [$t(20) = 1.96$, $P = 0.06$].

The distribution of peak latency values for all suprathreshold voxels is presented in Fig. 10. The vast majority of voxels have peak responses between 4.25 and 6.25 s. There are no significant differences between the two groups in their latency distributions.

Recovery of the HDR Amplitude and Latency in Paired Stimuli

The mean HDRs in calcarine cortex to a single stimulus and to the second of two stimuli are plotted in Fig.

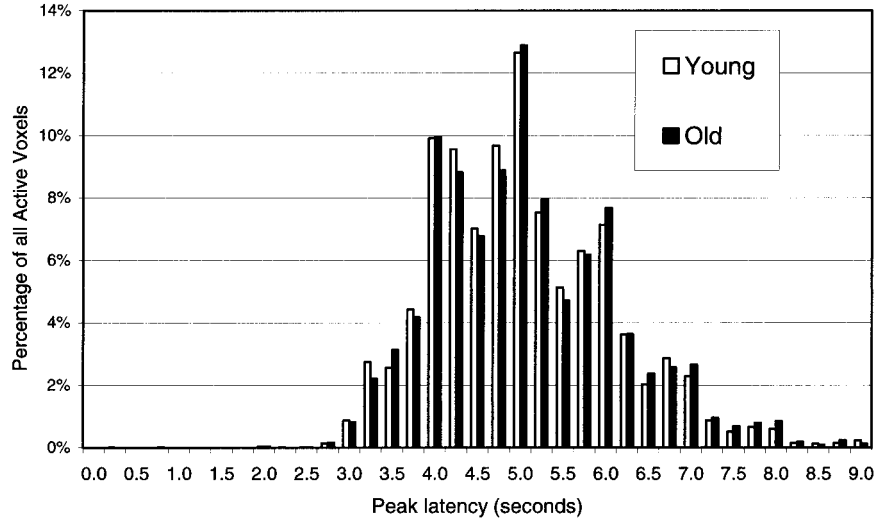

FIG. 10. Histogram of peak latency values for young and old subjects. Plotted is the percentage of significantly active voxels (y-axis; see text for methods) at each value of peak latency (x-axis). No systematic differences exist between the groups.

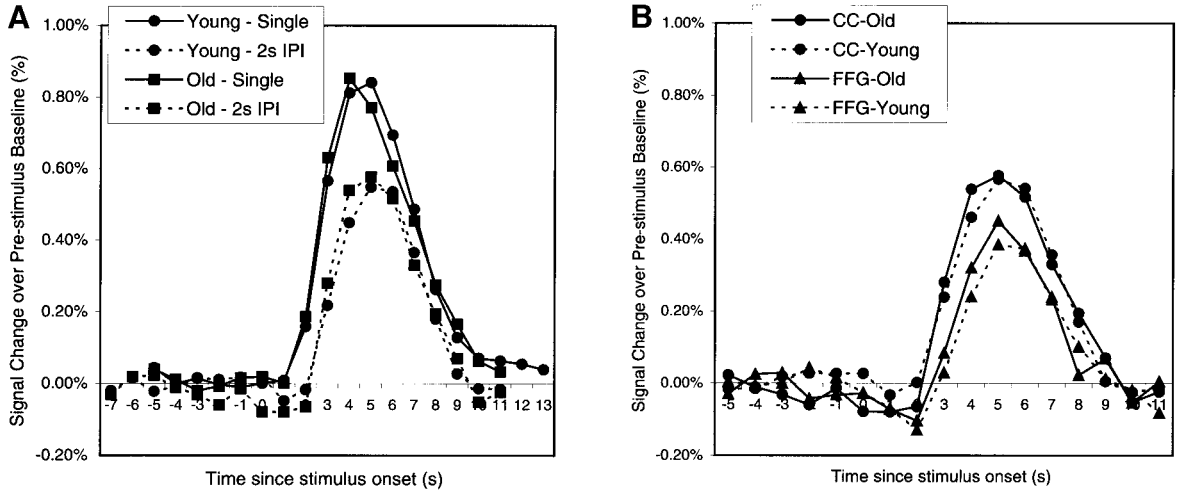

FIG. 11. Refractory effects for young and old subjects. The HDR is plotted (A) in percent change over a prestimulus baseline (y-axis), for single-stimulus trials (solid lines), and for the second stimulus in a pair (dashed lines; see text for methods). For both younger (circles) and older (squares) subjects, there were significant attenuations in amplitude and increases in latency in the HDR to the second stimulus in a pair. These refractory effects were similar between the subject groups; furthermore, both were similar to earlier published reports (Huettel and McCarthy, 2000a). At right (B), the HDR to the second stimulus in a pair is plotted for calcarine cortex (circles) and fusiform cortex (triangles), for both young and old subjects. As the amplitude of the HDR to a single stimulus was not significantly different across these conditions, differences in the response to the second stimulus in a pair reflect differences in proportional recovery (not initial amplitude). There was significantly greater recovery in calcarine cortex than in fusiform cortex, consistent with earlier experiments (Huettel and McCarthy, 2000b).

11. The HDRs evoked by the second stimulus of a pair are smaller and longer in latency than HDRs evoked by single stimuli in both young and elderly subjects (Fig. 11A). Peak amplitude of the HDR to the second stimulus was significantly attenuated in younger subjects [$t(10) = 2.67$; $P < 0.05$], but did not differ from the first to second stimuli in the elderly subjects [$t(10) = 0.73$; $P > 0.10$]. However, when the poststimulus HDRs to the first and second stimuli are compared, both groups of subjects show a numerical reduction in amplitude at all 11 time points [$P < 0.001$]. The amplitude of the HDR to the second stimulus was reduced by 34% in the younger subjects and by 32% in the older subjects. The latency of the HDR to the second stimulus was significantly increased in both groups, by 0.75 s in the younger subjects [$t(10) = 4.82$; $P < 0.001$], and by 0.50 s in the older subjects [$t(10) = 2.43$; $P < 0.05$]. Thus, the two-subject groups showed similar refractory period characteristics.

As can be seen in Fig. 11B, amplitude of the HDR to the second stimulus was greater in calcarine cortex than in fusiform cortex, even though the two regions showed similar activation to a single stimulus. This was true for both groups at every time point from 2 to 9 s following stimulus onset [$P < 0.001$]. The latency to peak of the second stimulus was similar (5.0 s) for all four response curves.

DISCUSSION

There were two primary findings in the current study. First, for visual cortex, the amplitude, general form, and refractory properties of the hemodynamic response (HDR) were similar in young and elderly adults. Second, there was a change in voxel noise levels over the lifespan, such that elderly adults had significantly higher noise levels than younger adults. Corollaries of the higher noise levels in the elderly were reduced spatial extent of activation, increased intersubject variability, and changes in the distributions of voxel statistics. A secondary finding was a systematic change in HDR latency from calcarine to fusiform cortex, such that the HDR peaked about 300 ms earlier in calcarine cortex. Below, we consider the implications of these findings in turn.

The Form of the Hemodynamic Response

Our conclusion from systematic analysis of the fMRI HDR in visual cortex is that it is fundamentally similar in healthy younger and older subjects. HDR amplitude, measured in percent signal change over a prestimulus baseline, was not significantly different between the groups, under both regions of interest and single-voxel testing. This indicates that functional MRI studies are feasible in populations composed of older subjects. The overall similarity between the groups is consistent with the results of D'Esposito and colleagues (1999), who found no age differences in the fMRI HDR in motor cortex. However, there was one consistently observed difference in the form of the HDR between our young and elderly subjects: namely, the HDRs of the elderly peaked earlier than those of the younger subjects and consequently returned to baseline earlier.

This effect occurred despite a similar onset latency and rate of rise in both groups. For visual cortex, therefore, vascular changes accompanying aging do not appear to influence the processes controlling the onset of the HDR, but instead slightly affect the form of the response function.

These results differ from those of Taoka and colleagues (1998), who identified an age-related increase in rise time of the HDR in a blocked-design motor-squeeze task (2 Hz right-hand grasping for 10 s). Although there was considerable intersubject variability at all ages tested, there was a significant positive correlation between age and the time required for the HDR to reach one-half of its maximum value (*T*-inc, which was time locked to the onset of the motor response). In contrast, no difference between groups was found on the time for the signal to return to baseline following task cessation (*T*-dec). We applied the analysis from Taoka and colleagues (1998) to our single-stimulus data from calcarine cortex (Fig. 5), in which the older and younger subjects had similar averaged response functions, but the older subjects showed more intra-group variability. No significant differences in *T*-inc were found ($P > 0.10$).

Refractory Effects in the Hemodynamic Response

The recovery in amplitude to the second of paired stimuli was similar in both groups. Both young and elderly subjects showed significant refractory effects to a second stimulus presented at 2 s IPI, with marked amplitude attenuation [young: −34%; elderly: −32%] and latency increase [young: +0.75 s; elderly: +0.50 s). These values replicate closely those previously reported for similar stimuli at 2 s IPI (Huettel and McCarthy, 2000a). In this earlier work, which parametrically tested IPI effects on the refractory period in the HDR in a population of younger subjects (mean: 27 years; range: 19–41 years), the change in peak amplitude at 2 s IPI was −33%, and the change in latency at 2 s IPI was +0.70 s. In addition, both groups showed similar patterns of recovery across brain regions, such that greater recovery was found in calcarine cortex as compared to fusiform cortex. This regional difference in recovery confirms our previous study using paired face stimuli (Huettel and McCarthy, 2000b).

Changes in Noise Level with Aging

Younger subjects had higher SNR values than older subjects had, despite the similarity in the mean amplitude (signal) of the HDR across the groups. The difference between the groups must therefore be due to a difference in noise levels. While we cannot isolate a single source for this noise difference within these data, we are able to rule out head motion as a primary contributor. As described under Results, head motion was not correlated with SNR within each group, reducing its explanatory power across groups. Real-time physiological monitoring may enable extraction of additional physiological differences between the groups, such as heart or respiration rates, allowing additional improvements in SNR for both. Even with such correction, it is empirically possible that the noise differences between the groups result from physiological changes accompanying aging, which would necessarily contribute to the measurement of HDRs using fMRI.

In practical terms, the difference in SNR, but not form of the HDR, between age groups indicates that different numbers of trials may need to be signal-averaged in each group to achieve equivalent SNR. Consider the measured SNR values for calcarine ROIs of 0.52 for young subjects and 0.35 for elderly subjects, which give a ratio between the groups of about 1.5. Improvements in SNR for trial-averaged data increase approximately with the square root of the number of trials averaged (for uncorrelated noise). The elderly subjects will require about 2.25 times as many trials as the younger subjects to ensure similar SNR values in the averaged HDR (see simulation below). In experimental designs without this compensation, such as found in the present study, the elderly subjects may show reduced spatial extent of activation, increased intrasubject variability, and reduced number of voxels with small effect sizes.

Spatial extent of activation. We found similar patterns of activation in both age groups: bilateral activation of calcarine and fusiform cortices, with little activation elsewhere in the brain. However, the groups differed in the spatial extent of activation, such that the young subjects showed significantly larger regions of activation, measured by the number of suprathreshold voxels within our regions of interest. This result is consistent with previous observations using both fMRI and PET of age-related reductions in the spatial extent of activation for motor cortex (D'Esposito *et al.,* 1999), for parietal and frontal cortices (Grady *et al.,* 1999), and visual cortex (Madden *et al.,* 1996, 1997, 1999b).

The differences between groups in the spatial extent of activation could be attributed to a higher proportion of active voxels with large response amplitudes in the young subjects. However, this possibility was not supported by the data. As evident from the response amplitude histogram (Fig. 9), the elderly subjects had a proportion of large-amplitude voxels (> 1%) that was similar to that of the young subjects. Furthermore, the averaged response amplitudes in the regions of interest tested were similar across the groups (Fig. 2). Instead, we believe that the spatial extent differences were due to the higher voxelwise noise levels in elderly subjects. As indicated by the SNR data discussed above, low SNR reduces the likelihood of identifying significant activations in voxels with small HDR am-

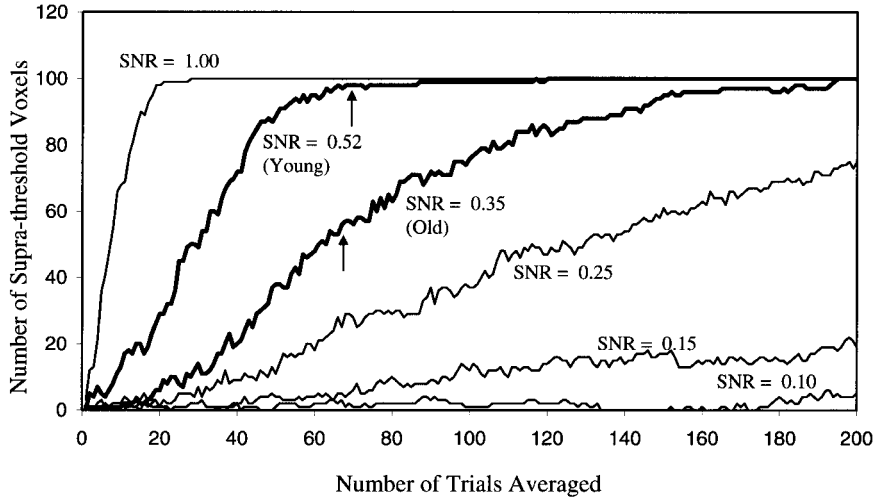

FIG. 12. Effects of signal averaging upon the detection of active voxels. We simulated how the signal–noise ratios (SNRs) measured in our experiments affect the identification of active voxels, for different numbers of averaged trials (see text for details). The form of the hemodynamic response and the noise distribution were empirically derived from our experimental data. Plotted are the two SNR values measured in our young and elderly subjects (bold lines) and four other SNR values for comparison. The two arrows indicate the average number of trials we recorded for the two subject groups. For the number of trials we averaged (about 70), the lower SNR in the elderly subjects results in a much smaller spatial extent of activation, even if the underlying distributions of neural activity are similar.

plitudes (see Fig. 9) when a limited number of trials are averaged.

To test this hypothesis, we used our empirical data to simulate the effects of noise level upon detection of active voxels. We constructed a "brain volume" of 1000 voxels, of which 100 were defined to be active, with signal amplitudes drawn from a gamma distribution modeled on our empirical distribution of amplitudes (alpha = 3.31, beta = 0.26; see Fig. 9 for distribution). On a simulated trial, the response in the active voxels was defined as signal + noise, where signal waveform was the empirical HDR measured in these experiments (see Fig. 2A), and the response in the inactive voxels was defined as noise alone. Six different SNR values were used for calculation of the noise: two values derived from our subject groups (young = 0.52, elderly = 0.35), and four other reference values (0.10, 0.15, 0.25, and 1.00). Noise on each trial, for each of the six SNR conditions, was drawn from a Gaussian distribution with mean zero and standard deviation of effect amplitude/SNR. We evaluated the number of statistically significant voxels ($t > 3.5$) following signal averaging of N trials ($N = 1$ to 200); analysis techniques were identical to those described in the methods section above. Note that all parameters in this simulation were derived directly from our empirical data and that the underlying voxel activation was similar for all SNR values. While this simulation technique simplified the structure of fMRI data by assuming that the voxels had no spatial or temporal correlation, it nevertheless provided an empirically grounded method for estimating the effects of SNR upon spatial extent of activation.

Figure 12 provides the results of this simulation. Notably, for smaller numbers of trial averages (e.g., 30–35 trials), the predicted difference in spatial extent of activation due to SNR differences between young and old subjects was as large as 5:1. Following averaging of 66 or more of the young subjects' trials, 97 or more active voxels were detected as statistically significant. However, only 53 of the active voxels passed the criterion threshold for the older subjects when 66 trials were averaged. As indicated in the methods section, the young subjects in our experiment participated in 70 trials each, while the elderly participated in an average of 66 trials. Given our number of trials (arrows on Fig. 12), our simulation predicts that the SNR differences between the groups will lead to the young subjects having about 1.83 times as many suprathreshold voxels as the elderly subjects. For the calcarine cortex ROI that formed the basis for our simulation, there were approximately 2.05 times as many active voxels in the young subjects. The similarity between the simulation results and observed data suggests that differences in the spatial extent of activation between young and old subjects may be primarily related to group differences in SNR.

Variability differences between age groups. In the present study, the older subjects showed significantly more intragroup variability in the form and amplitude of the HDR than was found in the young subjects. As seen on Fig. 4a, all younger subjects had similarly shaped response functions, although amplitude (and latency, to a lesser degree) differed across subjects. The elderly subjects differed more in form of their response functions, with large variability in shape across sub-

jects (Fig. 4b). In addition to these qualitative differences, there were quantitative differences in intragroup variability as well.

The younger subjects showed significantly less intragroup variability in amplitude of the HDR than was found for the older subjects, both for region of interest and best-voxel analyses (Fig. 5). For the region of interest analysis, which represents a typical technique used in event-related fMRI, the younger subjects had a more stable prestimulus baseline, in addition to less variability following stimulus onset. The implication of this difference for analysis of event-related fMRI is straightforward. The greater variability present in the elderly reduces the effective power of experimental designs when compared to those with young subjects. That is, experimental analyses are less likely to identify real effects in the older, more-variable population than in young subjects, independent of any spatial differences between the groups (see previous section).

In their study of the variability of the HDR in motor cortex, D'Esposito and colleagues (1999) found no differences in intragroup variability between young and old subjects. This results conflicts with our positive finding of increased variability in the elderly subjects. Although our study differed from that earlier work in several design aspects, most notably in investigating visual cortex rather than motor cortex, we suggest that methodological differences made our design more sensitive to detection of intragroup variability. We tested each subject on a larger number of trials (about 70 single-stimulus trials), whereas D'Esposito and colleagues estimated the HDR from only 20 trials. Reducing the number of trials in which each subject participated decreases the accuracy of each subject's HDR estimate. In turn, this would necessarily lead to a reduced chance of identifying group differences in variability. Furthermore, we sampled the HDR at a higher temporal resolution (TR: 1 s, compared to TR: 2 s in D'Esposito *et al.*, 1999), again improving our HDR estimates. In their earlier study, fully one-quarter of the elderly sample (5/20 subjects) failed to show suprathreshold activation in one or more voxels. The exclusion of data from those subjects may have had the effect of reducing the variability in the elderly sample by excluding the worst-performing subjects. In contrast, all of our subjects had significant activation in the region of interest in calcarine cortex, although some showed less activation than others.

We also suggest that the use of a random-voxel selection technique, while possibly appropriate for subjects with only a few active voxels, may mask population differences in variability. To test this, we replicated the analysis of D'Esposito and colleagues (1998) by choosing randomly a single active voxel, for each subject, within calcarine cortex. The peak amplitudes of the HDR were similar across subject populations (0.77% in young, 0.69% in elderly). However, the intragroup variability for both age ranges increased considerably as compared to region-of-interest analyses, with the young subjects actually showing more amplitude variability than the elderly did. The random voxel technique thus provided variability results that were at odds with the results from our region of interest and best voxel analyses. We conclude that the use of a single random voxel to describe the hemodynamic characteristics of a region is potentially misleading, in that differences in the distributions of voxel characteristics make small-sample-size analyses problematic.

Temporal Characteristics of the fMRI HDR

One interesting finding is that of a significant latency shift between calcarine and fusiform cortex. In both young and elderly subjects, we found that the HDR in calcarine cortex anticipates that of fusiform cortex by about 300 ms (Fig. 3). Direct analysis of latency to peak response within all active voxels reveals that significant increases in latency can be observed in extrastriate visual areas in single-subject analyses. These results obtained using fMRI are consistent with information obtained from electrophysiological techniques indicating a latency difference in neuronal activation between calcarine and fusiform cortices (Allison *et al.*, 1999).

We suggest that similar latency analyses may be used to identify the relative timing of neural events using fMRI. For discrete neural events, temporal ordering is straightforward, given the assumption of similar HDRs. However, when the form of the HDR to a stimulus varies across brain regions, the relative ordering of events is a more complex problem. If the underlying neural response to a visual stimulus presented in a response-time task is much longer in motor cortex than in visual cortex, for example, then latency to peak amplitude cannot, in itself, provide relative timing information. Nevertheless, we believe that these results encourage future development of fMRI analysis techniques that will allow direct investigation of both patterns of activation and their relative timing.

Summary

The fMRI hemodynamic response to a brief visual stimulus was similar in amplitude and form across groups of young and elderly adults. Therefore, similar experimental designs and analysis techniques may be used across age groups. However, the elderly adults showed greater voxelwise noise, which contributed to differences between the age groups in spatial extent of activation and intergroup variability. We suggest that additional signal averaging through increased number of trials may ameliorate, but not eliminate, these differences, allowing better comparison between subject populations.

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