Correlation Between BOLD fMRI and Theta-Band Local Field Potentials in the Human Hippocampal Area

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Ekstrom A, Suthana N, Millett D, Fried I, Bookheimer S. Correlation between BOLD fMRI and theta-band local field potentials in the human hippocampal area. J Neurophysiol 101: 2668–2678, 2009. First published February 25, 2009; doi:10.1152/jn.91252.2008. The relation between the blood-oxygen-level-dependent (BOLD) signal, which forms the basis of functional magnetic resonance imaging (fMRI), and underlying neural activity is not well understood. We performed high-resolution fMRI in patients scheduled for implantation with depth electrodes for seizure monitoring while they navigated a virtual environment. We then recorded local field potentials (LFPs) and neural firing rate directly from the hippocampal area of the same subjects during the same task. Comparing BOLD signal changes with 396 LFP and 185 neuron recordings in the hippocampal area, we found that BOLD signal changes correlated positively with LFP power changes in the theta-band (4–8 Hz). This correlation, however, was largely present for parahippocampal BOLD signal changes; BOLD changes in the hippocampus correlated weakly or not at all with LFP power changes. We did not find a significant relationship between BOLD activity and neural firing rate in either region, which could not be accounted for by a lesser tendency for neurons to respond or a greater tendency for neurons to habituate to the task. Strengthening the idea of a dissociation between LFP power and neural firing rate in their relation to the BOLD signal, simultaneously recorded LFP power and neural firing rate changes were uncorrelated across electrodes. Together, our results suggest that the BOLD signal in the human hippocampal area has a more heterogenous relationship with underlying neural activity than has been described previously in other brain regions.

INTRODUCTION

Functional magnetic resonance imaging (fMRI) is currently the dominant technique in neuroscience for measuring human brain activity during cognition. fMRI and other techniques that depend on measurement of blood flow in the brain are frequently interpreted as a proxy for “neural activity.” Thus a critical question is how changes in the blood-oxygenated-level-dependent (BOLD) signal, which forms the basis of fMRI, relate to underlying neural activity. Recordings from primary sensory neocortex suggest that both local field potentials (LFPs) (Mitzdorf 1985) and local changes in neural firing rate correlate significantly with the BOLD signal (Logothetis et al. 2001; Mukamel et al. 2005). It remains unclear, though, whether and to what extent these BOLD-electrophysiology correlations might also hold true in other brain regions (Heeger and Ress 2002; Raichle and Mintun 2006).

In this study, we compare fMRI and electrophysiological changes in the human hippocampal area, composed of the hippocampus (HC) and the parahippocampal region (PHR) (Witter 2002) the latter of which includes entorhinal cortex (ERC), perirhinal cortex (PRC), and parahippocampal cortex (PHC). The hippocampal area is critically involved in declarative and spatial memory (Spiers et al. 2001; Squire et al. 2004). Spatial navigation involves activity in several different hippocampal area subregions, which in turn may play roles in the formation of direction-independent spatial representations, or “cognitive maps” (O’Keefe and Nadel 1978). PHC activations have been reported during both virtual and imagined navigation (Aguirre et al. 1996; Jahn et al. 2004). HC activations have also been observed in tasks requiring retrieval of spatial locations (Hartley et al. 2003; Rekkas et al. 2005; Shipman and Astur 2008). Spatial navigation also induces significant hippocampal LFP changes (primarily in the theta band) in both rodents (Czurko et al. 1999; Vanderwolf 1969) and humans (Ekstrom et al. 2005) as well as significant changes in neural firing rate in rodents (McNaughton et al. 1983; O’Keefe and Dostrovsky 1971), humans (Ekstrom et al. 2003) and non-human primates (Hori et al. 2003). We thus first sought to replicate PHC and HC fMRI-based changes during navigation to validate comparisons with previous fMRI studies.

Simultaneous electrophysiological recordings and MRI are currently not possible in human brain structures deeper than superficial neocortex due to clinical safety issues associated with the use of depth electrodes in the MR environment. We therefore compared fMRI and electrophysiological activity by obtaining high-resolution structural and fMRI images of the hippocampus prior to implantation of depth electrodes and then recording electrophysiological activity in the same subjects following implantation with depth electrodes. Because movement-related physiological changes have been recorded both inside and outside the scanner in comparable brain regions (see Jahn et al. 2004; Miyai et al. 2001) and can be thought of as contextually independent, we contrasted changes in fMRI and electrophysiological activity during navigation of a virtual city. By recording wide band neural activity, we were able to simultaneously record action potentials from nearby neurons and LFPs on the same microelectrodes. Following localization of depth electrodes to areas of the brain with changes in BOLD...
activity during the task, we could directly compare BOLD and electrophysiological activity from the human hippocampal area.

**METHODS**

**fMRI/MRI**

Patients performed three runs in a blocked-design experiment as they were scanned in a 3 Tesla Siemens Allegra scanner using high-resolution echo-planar imaging (EPI) sequences (TR = 2 s, TE = 39 ms, slices = 19, voxel size = 1.6 x 1.6 x 3 mm, matrix size = 128, FOV = 200). In each navigation block of 120 s, subjects freely explored a virtual environment by searching for passengers and delivering them to designated locations (e.g., stores). In each control block, subjects followed arrows on a blank screen with the joystick for 25 s, and this sequence was repeated eight times over the three runs. Patients were required to deliver passengers to eight different destination stores with four separate deliveries to each destination interspersed over the course of the experiment. Our control task was based on previous findings (Stark and Squire 2001) showing that low-level perceptual tasks, such as determining the direction of an arrow, provided minimal hippocampal activation compared with other conditions. Fixation-based control tasks (commonly used in fMRI experiments), in contrast, actually induced hippocampal activity (Stark and Squire 2001), and thus we deemed them inappropriate for our experiment.

Prior to undertaking the navigation task during fMRI, patients received 15 min of practice on the task, navigating a city unrelated to the ones they would later experience. A matched-bandwidth sequence (TR = 5 s, TE = 66 ms, voxel size = 1.6 x 1.6 x 3 mm) permitted registration of EPI sequences to the hippocampal structural scan (TR = 5.2 s, TE = 105 ms, voxel size: 0.4 x 0.4 x 3 mm, matrix size = 512, FOV = 200). All patients participated following informed consent and all procedures conformed with the UCLA Institutional Review Board (IRB).

Functional activations were obtained in FSL FEAT (http://www.fmrib.ox.ac.uk/fsl/feat5/); we applied motion correction, high-pass filtering (100 s), spatial smoothing (5 mm), cluster-corrected thresholding (P < 0.05) (Woolrich et al. 2001), and a threshold of Z > 1.7. Activations thresholds were relevant to activation maps (e.g., Figs. 2 and 5) and did not play a role in calculating correlations between BOLD and electrophysiological activity, in which all activation data were used (see RESULTS). Spatial smoothing reduces noise and increases detection of signal changes; we used a smoothing window about three times our voxel resolution (see Worsley and Friston 1995).

Activations were modeled using a blocked design analysis with the general linear model (Beckmann et al. 2003) with equivalent amounts of navigation and control tasks (25 s of navigation compared with 25 s of the control); subsequent navigational epochs were factored out as a third explained variable. When the entire block of navigation (120 s) was included in the analysis, we noted deactivation of the PHC; therefore we restricted our analysis of navigation to the first 25 s to be consistent with previous results (Aguirre et al. 1996) although analyzing the full 120 s of navigation (see RESULTS) did not affect the correlations between BOLD and electrophysiological activity. We excluded runs on which patient absolute motion in the scanner exceeded 1 mm. We thus excluded one run of a patient (patient 6) who moved 1.2 mm (in absolute motion).

To visualize depth electrodes on two-dimensional (2D) hippocampal maps, we performed a three-way registration of the patient computed tomography (CT), whole brain MRI, and high-resolution MRI to maximize our localization accuracy using Brainlab stereotactic and localization software. The CT was first registered to the whole brain MRI, followed by registration of the whole brain MRI to the high-resolution MRI (Fig. 1B). Electrode regions of interest (ROIs) were based on registration between MR and CT images and typically encompassed about 8 mm², or ~4 voxels in-plane, with ~1 mm in-plane accuracy, which was based on the registration algorithms employed (see Ekstrom et al. 2008 for details). Because our functional images were 1.6 x 1.6 x 3 mm (i.e., larger than our anatomical ROIs), our electrode ROIs were constrained to be typically 1.6 mm in-plane.

To computationally unfold the HC from three to two dimensions, we performed the following steps. First, we segmented hippocampal gray matter by outlining white matter and CSF along HC proper and extending through the fusiform cortex. To improve the quality of the segmentation, hippocampal slices were computationally and then manually interpolated by a factor of 7 along the long-axis of the HC, producing a final voxel size of 0.391 x 0.391 x 0.429 mm (Zeineh et al. 2001, 2003). The resulting 3-D gray matter strip was then computationally flattened. Using landmarks on the 3-D hippocampal scan, we defined anatomical boundaries of CA1–CA3/CA2/dentate gyrus (CA23DG), CA1-subiculum (Sub), subentorhinal cortex (ERC), ERC-perirhinal cortex (PRC), sub-parahippocampal (PHC) cortex, and PHC-fusiform cortex. We also demarcated the beginning of hippocampal head (Anterior CADG) and the ERC-PRC-PHC boundary (Amaral and Insausti 1990; Duvernoy 1998). These boundaries were then projected into 2-D hippocampal space, thus outlining the anatomical locations defined by these boundaries in 3-D space. A more complete explanation of the methods involved in computational unfolding, including validation and precision of the method, can be found in Zeineh et al. (2001).

Group activation maps were produced by averaging the subregional boundaries of each subject and then transforming each subject’s flat map (and depth electrodes) to this template (Thompson et al. 2000). The degree of fit between each individual subject’s behavior and the BOLD signal (i.e., beta values) were then compared across subjects for each voxel using a mixed-effects t-test. Significance thresholds for activations were set above a threshold of 1.7 and P < 0.05.

**Electrophysiology**

During electrophysiological recordings, subjects performed the same navigation experiment as they did during the fMRI scan while wide-band activity was recorded on a Neuralynx 64-channel recording system (sampling = 28 kHz) with the one difference being that they explored a different spatial environment, including encountering different stores and passengers and exploring an environment with a different spatial geometry. This was done to ensure that responses would not habituate due to repetition and enhanced knowledge of the spatial environment. We return to this issue in RESULTS and DISCUSSION. Each depth electrode contained eight microelectrodes from which we recorded electrophysiological signals. Recordings were referenced to a ninth distally placed micro-wire; off-line analysis isolated local field potentials (LFPs; 1–200 Hz) and activity from nearby neurons. Although microelectrodes did not have a strict geometry, they were designed to “splay” and thus cover disparate areas of the brain (Fried et al. 1999). In the few cases in which microelectrodes adhered to each other and thus showed identical or highly similar signal, only one contact was used. Action potentials were isolated using the cluster cutting software package wave_clus (Quiroga et al. 2004). Neural events were first thresholded using the raw filtered data and then clustered based on separation of wavelet coefficients (Quiroga et al. 2004, 2005). All clusters were re-sorted manually to ensure maximum separation of different waveforms; clusters that showed contamination from 60-Hz activity were discarded. Clusters were then separated into multiunit activity and putative single-unit activity based on waveform shape, distributions of interspike intervals, and spike autocorrelation. We note that in subsequent analyses, we did not see a difference in the correlation between BOLD and multunit or single units (analysis
included in supplemental text and discussed in RESULTS), and did not find any difference in the correlation with BOLD when we averaged single and multiunits across microwires in our comparison with the BOLD signal (analysis included in supplemental text and discussed in RESULTS). Furthermore, the distinction between multiunits and putative single units during extra-cellular recordings is necessarily "post hoc" (e.g., Harris et al. 2000). Thus we generally refer to "neural firing rate" in this paper when discussing action potential recordings, unless we refer specifically to analyses preformed separately on multiunits and putative single units, which nonetheless did not differ in their outcome.

We analyzed LFPs by downsampling to 1,000 Hz, filtering between 1 and 200 Hz and applying a 60-Hz notch filter (59.9 – 60.9 Hz) to remove noise contamination. LFP channels that showed significant 60-Hz noise or evidence of significant saturation were not considered; we further excluded epochs that exceeded six times the SD of the mean of the signal (typically representing 0.1% of the signal). We then extracted wavelet power at logarithmically-spaced frequencies by convolving the raw signal with Morlet kernels (Percival and Walden 1993); wavelets provide an excellent representation of signal amplitude for isolated bouts of oscillations while still providing frequency band specificity compared with multi-taper or Fourier methods (van Vugt et al. 2007). We averaged LFP power over six different frequency bands (Canolty et al. 2006; Caplan et al. 2003), including delta (δ: 1–4 Hz), theta (θ: 4–8 Hz), alpha (α: 8–16 Hz), beta (β: 16–30 Hz), low gamma (γL: 30–100 Hz), and high gamma (γH: 100–180 Hz).

Analyses were conducted by comparing the firing rate or mean wavelet-transformed power averaged in 2-s bins during the control task using a t-test. We used 2-s bins to be consistent with our BOLD data, which were based on a TR of 2 s. Using smaller bin sizes (1 s) did not change our overall results. We note that we could not directly compare time courses among BOLD activity, LFP, and neural firing rate because patients navigated different environments between fMRI and electrophysiological recordings (a constraint imposed to control for hippocampus-based learning). We return to this issue in RESULTS and DISCUSSION. In some cases, patients participated in more than one electrophysiological recording session (e.g., Table 1). In these cases, LFP and neural firing rate data included all recording sessions for that patient on that given microelectrode.

For the analysis in which we directly correlated neural firing rate and LFP responses, significantly and nonsignificantly firing neurons were compared with the simultaneously recorded LFP on that microelectrode. Neural firing rate, averaged over epochs of 2 s of the navigation and control tasks, was correlated with the mean wavelet power during these same behavioral epochs. For a more detailed description of analysis methods, see Ekstrom et al. (2007).

The online version of this article contains supplemental data.

FIG. 1. Behavioral methods and depth electrode localization. A: patients navigated as a “taxi driver” through a virtual environment while undergoing functional magnetic resonance imaging (fMRI) scanning; the same patients then performed the identical task in a novel environment while implanted with depth electrodes. During the task, the patients were instructed to pick up passengers placed in the center of the city (in the same location every trial); they were told to bring the passengers to a specific and well-marked “store” located on the periphery. B: depth electrodes labeled by location [e.g., LMH (left medial hippocampus), LAH (left anterior hippocampus), LPC (left parahippocampal cortex), and LEC (left entorhinal cortex)] were localized in 3-dimensional (3-D) space (see METHODS) by co-registering the CT, high-resolution MRI, and whole brain MRI and then identifying the tips of microelectrode recording sites. Bottom middle panel: CT registered to patient high-resolution hippocampal MRI; top middle panel: zoomed CT to demonstrate microelectrode tips. Patient depth electrode locations were then projected into 2-D space (far right plot). This registration step allowed us to determine where fMRI activations and depth electrode recording sites overlapped.
neurons from the HC, ERC, and PHC; we simultaneously recorded and analyzed 396 LFP signals from these same microelectrodes (based on exclusion of bad channels, see METHODS). Of these neurons and LFPs, 92 neurons were isolated from PHR and 93 from HC while 191 LFP channels were recorded from PHR and 205 from HC (the discrepancy between the number of LFP channels and neurons was because neurons could not always be recorded from all microelectrodes). The total number of significant LFPs in all frequency bands we examined [delta (δ), theta (θ), alpha (α), beta (β), low gamma (γL), and high gamma, (γH)] as well as neural firing rate exceeded the type I error rate (5%) across all microelectrodes (Fig. 3, B and C).

Comparing BOLD signal changes with LFP and neural firing rate changes

To measure how the BOLD signal related to changes in LFPs and neural firing rate at specific depth electrodes, we compared the BOLD t-statistic against the LFP and neural firing rate t-statistic separately at each depth electrode. We then determined how well the variance in the BOLD signal across depth electrodes could be explained by changes in LFPs versus neural firing rate (Fig. 4). These comparisons were performed over all 396 microelectrodes recorded using a multivariate robust regression analysis to deal with the possible presence of outliers and heteroskedasticity of variance (Huber 1981). We tested our hypothesis by comparing the maximally responding voxel from an electrode ROI against the maximally responding LFP or neuron at that depth electrode (e.g., Fig. 5 and Supplementary Fig. S1). Our multivariate model included the BOLD signal as the dependent measure and different frequency LFP bands (δ, θ, α, β, γL, γH), neural firing rate, region (HC vs. PHR), and tissue normalcy (interictal vs. tissue showing no interictal discharge) as independent measures. Because of the potentially limited signal-to-noise ratio with using the maximal response, we additionally compared the mean BOLD signal at an electrode ROI against the mean LFP or neural firing rate at that depth electrode (i.e., averaged over all microelectrodes). We also compared the mean BOLD signal from an electrode ROI against LFP and neuron changes at each individual microelectrode. These analyses yielded comparable results to those reported below and are included in the supplementary text and Supplementary Fig. S2.

### RESULTS

#### BOLD activations during navigation

We found significant increases (navigation > control) in the PHC BOLD signal in all six subjects (Fig. 2), which were also reflected in the group-averaged map (Fig. 3A). Significant BOLD signal decreases (control > navigation) were also present in the HC in the group map. In individual subjects, the ERC and HC showed clusters of significant BOLD signal increases (Fig. 2; e.g., patients 1, 2, 5, and 6).

#### Depth electrode locations and electrophysiological activity

We recorded from a total of 27 different hippocampal area depth electrodes across six patients from whom we also obtained fMR/MR imaging. Depth electrodes, in many cases, were placed in areas of significant BOLD changes. In the PHR, 3/12 depth electrodes showed significant BOLD changes (all of which were increases), whereas in HC, 9/15 depth electrodes showed significant BOLD changes (1/15 depth electrodes in HC showed significant BOLD signal increases while 8/15 showed significant BOLD decreases). The mean activation (measured with the t-statistic) across PHR depth electrodes was $0.48 \pm 0.5$ ($P = 0.31 \pm 0.30$; means $\pm$ SE) with a range of $-1.4$ to $-4.0$ ($P = 0.08 / 0.0001$) and the mean activation across HC depth electrodes was $-1.1 \pm 0.5$ ($P = 0.31 \pm 0.30$) with a range of $-3.7$ to $-2.8$ ($P = 0.0001 / 0.0026$).

Each depth electrode contained 8 recording microelectrodes from which we recorded and isolated a total of 185
Regressing the maximum BOLD signal against the maximally responding LFP and neuron on that depth electrode, the overall model was significant ($t = -2.1, P < 0.05, df = 17$), with a significant effect of the maximum theta-band t-statistic on the BOLD signal ($t = 3.6, P < 0.005$; Pearson-rho correlation, $r = 0.40$; Fig. 4B). Other LFP frequency bands ($\delta, \alpha, \beta, \gamma_L, \gamma_H$), neural firing rate, region, and tissue normalcy were not significant predictors (Fig. 4, C and D). There was a difference in the BOLD signal versus theta-band correlation by region (PHR: $r = 0.73; P < 0.01, n = 12$, Fig. 4, F and G; HC: $r = 0.33; P = NS, n = 15$; Fig. 4, F and G). In the supplementary text, we show that the lack of correlation between BOLD and neural firing rate could not be accounted for by a lack of tendency for neurons to respond to the task (see also Supplementary Fig. S1), by a tendency to habituate over repetitions of the task, by a differential relation between putative multiunit and single unit to BOLD, or by any effects of spatial smoothing of the BOLD signal. These supplementary analyses suggest that our lack of correlation between BOLD and neural firing rate could not be readily explained by these four factors we additionally tested for.

To ensure that our findings regarding a correlation between BOLD and theta-band LFP activity were not a result of our selecting smaller epochs of navigation (we chose 25 s of navigation to be consistent with previous fMRI studies, see METHODS), we also analyzed BOLD and theta-band activity...
during the entire 120 s of navigation. We again found a significant correlation between BOLD signal changes and theta-band activity in the PHR but not for activations in the HC (see Supplementary Fig. S3). Because longer epochs of navigation resulted in significant PHR increases and decreases (while the 25-s analysis only resulted in significant PHR increases), it is noteworthy that we obtained strong correlations between BOLD and theta-band activity when both significant BOLD

FIG. 4. BOLD signal changes correlate significantly with theta-band LFPs. A: placement of depth electrodes across patients on the group map, same underlay as shown in Fig. 3. Note the fairly extensive coverage obtained across the medial/lateral and rostral/causal aspects of the HC. See Table 1 for labeling scheme. B: maximally responding BOLD t-statistic values [for electrode region of interest (ROI) in navigation vs. control comparison] plotted against maximally responding theta-band t-statistic values for each depth electrode site; the regression was significant (P < 0.05). HC depth electrodes are plotted in blue, parahippocampal region (PHR) depth electrodes are plotted in red. C and D: same plots for BOLD vs. neural firing (C) and BOLD vs. low gamma-band activity (D). These correlations were not significant. E: bar graph showing slope values for B–D derived from the multivariate, robust regression reported in the results, which includes all positive and negative BOLD changes in HC and PHR. The slope for theta-band activity exceeded all other slope values. F: maximal BOLD t-statistic vs. maximal theta-band t-statistic for PHR *only*; the correlation coefficient was 0.73. G: correlation coefficients for each frequency band and neural firing rate separately for HC (blue bars) and PHR (red bars).
signal changes in both directions (increasing and decreasing) were present in PHR.

**Dissociations between PHR and HC BOLD increases and decreases**

Previous studies that measured metabolic and electrophysiological activity in the rodent suggest that HC BOLD changes do not consistently correspond with changes in electrophysiological activity (Schridde et al. 2008; Uecker et al. 1997). Because we found lower (and nonsignificant) correlations between BOLD and theta-band activity in HC compared with PHR, we investigated this issue further by looking separately at BOLD increases and decreases, relative to the baseline we selected, in PHR and HC. We found that on microelectrodes showing significant increases in the BOLD signal, PHR microelectrodes had theta-band t-statistic values significantly greater than zero [t-test, t(71) = 3.5, P < 0.0008; Fig. 6A]. We could not assess significant decreases in the PHR BOLD signal because no depth electrodes in the analysis of 25 s of navigation showed significant negative BOLD activations. Looking at PHR BOLD signal decreases over 120 s of navigation (see METHODS), we found that microelectrodes with significant PHR BOLD decreases were indeed accompanied by significant decreases in theta-band activity [t-test, t(65) = 3.8, P < 0.0005; Fig. 6B]. Microelectrodes with significant PHR BOLD increases during 120 s of navigation similarly showed significant overall increases in theta-band activity [t(63) = 3.8, P < 0.001].
0.0005; Fig. 6B], consistent with our findings from 25 s of navigation.

While hippocampal microelectrodes with significant BOLD increases had theta-band t-statistics significantly greater than zero [t-test, t(24) = 2.6, P < 0.01; Fig. 6A], HC microelectrodes with significant BOLD decreases did not correspond with an overall negative theta-band t-statistic [t-test, t(104) = −0.12, P = NS; Fig. 6A]. This was also true when we looked at HC BOLD decreases during 120 s of navigation [t(158) = 0.12, P = 0.9; Fig. 6B; we could not analyze HC BOLD increases during 120 s of navigation because there were no significant HC BOLD changes at depth electrodes sites]. Excluding all HC BOLD decreases from our previous multivariate regression, resulted in significant positive correlations between BOLD and theta-band activity (see Supplementary Figs. S1 and S5). This was also true when we looked at PHR decrease during delivery (patient 1, e.g., Fig. 5, Supplementary Figs. S1 and S5), activation clusters did not change in terms of the spatial extent or magnitude following retesting in the navigation task (Supplementary Fig. S5). We found an 81% overlap in number of significantly active voxels between test and retest. These data suggest that the activation patterns we observed would not be expected to change substantially as a result of repeating the task with different spatial environments (see METHODS).

D I S C U S S I O N

The purpose of this study was to examine how changes in the human hippocampal BOLD signal related to electrophysiological changes. To investigate this question, we chose a well-studied task shown to induce changes in BOLD, LFPs, and neural firing rate in the hippocampal area: spatial navigation. We first replicated the finding of significant PHC activations during navigation (Aguirre et al. 1996; Jahn et al. 2004; Zeineh et al. 2003; Harrington et al. 2006), we compared activation patterns prior to deep implant with activations following implant to ensure that our patient activation clusters did not differ in any significant way. In one subject who returned for an fMRI following deplant (patient 1, e.g., Fig. 5, Supplementary Figs. S1 and S5), activation clusters did not change in terms of the spatial extent or magnitude following retesting in the navigation task (Supplementary Fig. S5). We found an 81% overlap in number of significantly active voxels between test and retest. These data suggest that the activation patterns we observed would not be expected to change substantially as a result of repeating the task with different spatial environments (see METHODS).

Another possibility we explored was whether there were different degrees of learning between fMRI and electrophysiology tasks, thus leading to different patterns of responses during navigation. To investigate this possibility, we compared subject’s paths from passengers to stores as they navigated the town (Newman et al. 2006). To determine subjects’ path accuracy, we first determined the ideal path from a passenger to a store and then computed the deviation of the actual path from this ideal path (in pixel units). More accurate paths thus had lower excess path values. Subjects showed significant learning of store locations over trials (based on lower excess path length) that did not differ in slope between different environments (Fig. 7, A and B), indicating that subjects learned comparable amounts of novel information between different spatial environments.

### Did repetitions of the task affect hippocampal fMRI activity?

HC BOLD activations are sensitive to the extent of learning that occurs within a given repetition of a stimulus (Zeineh et al. 2003) and within a virtual environment (Hartley et al. 2003). Therefore we sought to determine whether repetition of the task (first using fMRI, then using electrophysiological recording with a different spatial environment) could affect the hippocampal responses measured in each session. While previous reports suggest within subject activations are robust across repetitions of a task within subject (Aguirre et al. 1998; Harrington et al. 2006), we compared activation patterns prior to deep implant with activations following implant to ensure that our patient activation clusters did not differ in any significant way. In one subject who returned for an fMRI following deplant (patient 1, e.g., Fig. 5, Supplementary Figs. S1 and S5), activation clusters did not change in terms of the spatial extent or magnitude following retesting in the navigation task (Supplementary Fig. S5). We found an 81% overlap in number of significantly active voxels between test and retest. These data suggest that the activation patterns we observed would not be expected to change substantially as a result of repeating the task with different spatial environments (see METHODS).

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### DISCUSSION

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Janzen and van Turennout (2004) in our group activation maps. We note that individual subjects in our study often showed strong degrees of activations due to the task in PHC (maximum subject activation, $t = 15.5$; maximum activation at a depth electrode, $t = 4.0$; see Fig. 2). We also found significant HC BOLD changes, consistent with previous studies (Hartley et al. 2003; Rekkas et al. 2005; Shipman and Astur 2008; Uecker et al. 1997). We note that individual subjects, though, often showed activation patterns not in correspondence with the group, including several regions that did not show activations on the group map such as ERC and subiculum. These findings are relevant because although our depth electrodes were placed solely based on clinical determinations, their presence in areas of activation allowed us to determine how consistent individual activations were with electrophysiological changes there.

Consistent with previous electrophysiological findings, we found significant increases in all LFP frequency bands and in neural firing rate during navigation (Czurko et al. 1999; Ekstrom et al. 2005; McNaughton et al. 1983). Of particular relevance, the BOLD signal and neural activity during our control task. Previous human electrophysiology studies employed shorter behavioral time windows (<$2 s$) when comparing movement with control periods (Caplan et al. 2003; Ekstrom et al. 2005). Because we wished first to replicate previous cognitive task-related BOLD studies on the HC and PHR, we chose longer behavioral epochs to be consistent with previous studies on spatial navigation and fMRI. Because all three measures were modulated by our task, our findings regarding correlations (and the absence of correlations) between the BOLD signal and electrophysiological activity cannot be explained therefore by a lack of a response of any of our three measures.

We found a correlation between the BOLD signal and theta-band activity in the hippocampal area using three different correlation analyses across depth electrodes (see RESULTS). Further, we found that the correlation between BOLD and theta-band activity was significant in PHR although not in HC, a finding replicated in all three correlation analyses. This lack of a significant correlation between the BOLD signal and LFPs in HC, though, likely arose because HC BOLD decreases did not correspond consistently with significant decreases in theta-band activity. In fact, although we had a limited number of sites showing positive activations in the hippocampus, excluding BOLD decreases from the HC led to a significant relation between BOLD and theta-band LFPs there. Some caveats are important here, however. Increases and decreases in BOLD during a task are defined relative to the control task selected. We based the section of our control task, which involved subjects actively following arrows on a blank screen, on a previous study showing that nonactive control tasks (such as fixation) induce significant amounts of HC activity (Stark and Squire 2001). Thus while our data support the idea that HC BOLD decreases may have a different relation with LFPs than BOLD increases, our limited number of HC BOLD increases, and the fact that we did not rigorously test several different baseline conditions suggests that additional experiments and data are required before this conclusion can be made firmly.

Two previous studies in the HC also found no correspondence between BOLD changes and electrophysiological activity. Uecker et al. (1997) found that theta-band activity in the dentate gyrus of the rodent increased when the animal went from a resting state to active exploration while a separate group of rats showed decreases in glycogen activity from navigation to rest in the same brain region. Although we did not find a consistent increase in theta-band activity coupled with decreases in BOLD (Uecker et al. 1997), we instead found no relation between HC BOLD decreases and electrophysiological recordings, a difference that may be explained by the fact that our study examined multiple sites across the HC (e.g., Fig. 4A) while Uecker et al. focused on dentate gyrus alone. A second study by Schridde et al. (2008) found that during pharmacologically induced seizures, marked increases in LFP activity and neural firing rate were often accompanied by BOLD decreases in the HC while increases in LFP activity and neural firing rate in neocortex correlated with increases in the BOLD signal there. The Schridde et al. study provides some possible insight into why the observed correlation between BOLD and theta-band activity was nonetheless weaker in the HC than PHR. Based on their study (in which they also measured cerebral blood flow and cerebral oxygen metabolism), Schridde et al. suggest that in HC, oxygen metabolism may occasionally exceed fresh supplies of oxygenated hemoglobin, leading to a net increase in de-oxygenated hemoglobin and a net decrease in BOLD during periods of neural activation. Because the anatomy and vasculature of PHR more closely resembles that of neocortex than hippocampus (Duvernoy 1998; Van Hoesen 2002; Witter 2002), it is possible then that blood supply via the vasculature of PHR is not as comparably susceptible to fluctuations in neural activity, explaining why BOLD might correlate with neural activity better in PHR than HC under some testing conditions. It is important to note, though, that the Schridde et al. study examined abnormal seizure activity while our study looked at BOLD activations during behavior. Thus it is not clear from their study to what extent BOLD-neural activity decouplings might be expected during normal activations. At this point, we are limited to reporting our results and await more detailed experiments in awake, behaving animals to elucidate the underlying mechanisms for the BOLD-neural activity decoupling we observed in the HC.

In contrast to our BOLD-LFP correlations, we did not find a correlation between the BOLD signal and neural firing rate in either HC or PHR despite the fact that neurons in our study in many cases robustly changed firing from the control task to navigation (see RESULTS). In visual cortex, where previous BOLD signal and neural firing rate correlation have been reported (e.g., Logothetis et al. 2001; Shmuel et al. 2006), attenuation of neural firing does not impact BOLD signal changes (Viswanathan and Freeman 2007), a finding also demonstrated in the cerebellum (Caesar et al. 2003). One explanation for the correlation between the BOLD signal and neural firing rate is the partial drive of neural synchrony by the gamma-band LFP. In support of this idea, neural firing rate and LFPs are often correlated in these areas during perceptual tasks (Henrie and Shapley 2005; Mukamel et al. 2005). In contrast, we found no correlation between neural firing rate and LFPs in our study (see RESULTS) or in a previous navigational study.

IN ADDITION TO THE DECOUPLING BETWEEN NEURAL FIRING RATE AND LFPS, A SECOND REASON WHY BOLD AND NEURAL FIRING RATE MAY NOT HAVE CORRELATED IN OUR STUDY COULD RELATE TO THE HETEROGENEITY OF NEURAL RESPONSES IN THE HIPPOCAMPAL AREA. WITHIN OUR 1.6-MM VOXELS, WE ESTIMATE THE PRESENCE OF ~3,400 NEURONS (BUZSAKI 2004). AS WE NOTE THOUGH IN RESULTS AND HAS BEEN NOTED IN PREVIOUS STUDIES (REDISH ET AL. 2001), NEARBY HIPPOCAMPAL NEURONS OFTEN SHOW LOW CORRELATION IN TERMS OF BEHAVIORAL RESPONSES. THIS HETEROGENEITY, TO OUR KNOWLEDGE, STANDS IN CONTRAST TO PREVIOUS DEMONSTRATIONS IN THE NEOCORTEX WHERE NEIGHBORING NEURONS OFTEN SHARE SIMILAR BEHAVIORAL PROPERTIES (MOUNTCASTLE 1997). THE HETEROGENEITY OF RESPONSES IN THE HIPPOCampus, IN CONTRAST TO SENSORY NEO-CORTEX, ARGUES FOR A REGIONAL EXPLANATION FOR WHY BOLD AND NEURAL FIRING RATE (AND LFPS AND NEURAL FIRING RATE) ARE DECOUPLING IN OUR STUDY RATHER THAN A BEHAVIORAL ONE.

While simultaneous BOLD-LFP-single neuron recordings are currently possible in primate visual cortex (LOGOTHETIS ET AL. 2001), these recordings have not been performed in the HC. Because we focused on the HC, a structure shown to be sensitive to the degree of learning that occurs in a given stimulus repetition (ZEINEH ET AL. 2003) and within a virtual environment (HARTLEY ET AL. 2003), this placed constraints on the analyses we could conduct when comparing our fMRI and electrophysiological recordings between testing sessions. We were required to use different spatial environments between testing conditions to control for the degree of learning from the first to the next session (SEE METHODS), showing that the degree of learning did not differ between fMRI and electrophysiological recording sessions in our study (Fig. 7) nor did responses of the same recording modality habituate significantly from one session to the next. Because the spatial environment subjects explored were different in the two sessions, though, we could not directly compare time courses between sessions because subjects experienced different events during navigation, which could have in turn influenced signals at specific time points. We thus restricted our analysis to comparing t-statistic values, a commonly used statistic in the fMRI literature that captures both the mean and variance of a signal over an entire block of an experimental contrast. Of note, the amount of explained variance in the correlation between BOLD activity and the LFP for the maximum voxel analysis (when we excluded HC BOLD decreases) was consistent with LOGOTHETIS ET AL. (2001) in which BOLD activity, LFPS, and neural firing rate were simultaneously recorded in visual cortex (~53%).

A final reason, then, why BOLD did not correlate with neural firing rate in our study could relate to the selectivity of neural responses in the hippocampal area. Although we looked at neural responses to virtual movement (and showed that these responses overall were not affected by changes in spatial environments), it is still possible that while neurons responded generally to movement, they also showed selective changes to stimuli observed during navigation. Indeed we have shown previously that neurons do indeed fire selectively for specific spatial landmarks and spatial locations during navigation (EKSTRÖM ET AL. 2003). Neither multi- nor single units correlated with BOLD changes in our current study. Furthermore, averaging over many different neurons by comparing the mean neural firing rate across microelectrodes with BOLD changes there did not improve the overall correlation with BOLD compared with correlating the maximally responsive neuron at each site. These findings weaken the selectivity argument as an account for our low correlation between BOLD and neural firing rate in the hippocampal area because neural selectivity should largely be a property of single and not multiunits.

In summary, our finding that BOLD activity in the human hippocampal region during blocks of spatial navigation did not correlate with neural firing rate has important consequences for our understanding of BOLD signal changes in the human hippocampal area, given that BOLD was correlated with theta-band activity in our study under the exact same testing conditions. Overall, our results suggest that direct comparisons between BOLD activity and neural firing rate carried out in sensory neocortical regions (HEEGER AND RESS 2002; TSAO ET AL. 2006) may not apply in the hippocampal area, particularly over longer recording epochs or when selective firing of neurons compared with BOLD or LFPS might be invoked. Our results therefore have important implications for considering what information BOLD, LFPS, and neural firing rate can provide in the human hippocampal area.

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