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Multi-phase passband balanced SSFP fMRI with 50 ms sampling rate at 7 Tesla enables high precision in resolving 100 ms neuronal events

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ABSTRACT

Passband balanced steady state free precession (b-SSFP) fMRI employs the flat portion of the SSFP off-resonance response to obtain microscopic susceptibility changes elicited by changes in blood oxygenation following enhancement in neuronal activity. This technique can reduce geometric distortion and signal dropout while maintaining rapid acquisition and high signal-to-noise ratio (SNR) compared with traditional fMRI techniques. In the study, we developed a novel multi-phase passband b-SSFP fMRI technique that can achieve a spatial resolution of a few mm³ and a high temporal sampling rate of 50 ms per slice at 7 Tesla. This technique was further applied for an event-related (ER) fMRI paradigm. As a comparison, gradient-echo echo-planar imaging (GE-EPI) with similar spatial resolution and temporal sampling rate was carried out for the same ER-fMRI experiment. Experiments with visual cortex stimulation were carried out at 7 Tesla to demonstrate whether the multi-phase b-SSFP technique and GE-EPI are able to differentiate temporal delays in hemodynamic response function (HRF) separated by 100 ms in stimulus onset. Consistent with ERP results, the upslope of the HRF of both techniques can differentiate 100 ms delay in stimulus onset, with the former showing a lower level of intersubject variability. The present study demonstrated that the multi-phase passband b-SSFP fMRI technique can be applied for resolving neuronal events on the order of 100 ms at ultrahigh magnetic fields.

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1. Introduction

Since its inception in the early 1990s, blood oxygenation level dependent (BOLD) fMRI has been the predominant tool for functional neuroimaging during task activation and resting state [1–4]. During the past two decades, considerable progresses have been made to improve the spatial and temporal resolutions of BOLD fMRI. With the recent development of simultaneous multi-slice (SMS) or multiband imaging [5,6], whole brain BOLD fMRI using gradient-echo echo-planar imaging (GE-EPI) can be performed with an isotropic spatial resolution of a few

mm³ and a sampling rate of a few hundred milliseconds per volume, as demonstrated by the Human Connectome Project (HCP) [7,8]. The availability of ultrahigh magnetic fields (e.g. 7 Tesla and beyond) also improved the sensitivity and fidelity of fMRI as the BOLD signal at ultrahigh fields arises primarily from capillaries instead of veins compared to lower field strengths of 1.5 and 3 T [9]. As a result, fMRI with a sub-millimeter spatial resolution has been applied for studying human brain function at the level of cortical columns and layers at 7 Tesla [10–15].

One of the limitations of BOLD fMRI is that the hemodynamic response function (HRF) is relatively slow in time (delay by a few seconds from neuronal event), limiting the capability of fMRI for resolving temporally adjacent neuronal events. Although the recently developed multiband fMRI allowed sub-second temporal resolution, it has been mainly used for filtering out cardiac and respiratory pulsation effects as well as for dynamic functional connectivity analysis [16]. For

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standard GE-EPI, the sensitivity of fMRI also suffers from the T1 saturation effect with shorter TR, even with the use of Ernst angle for signal excitation. Nevertheless, in 1998 Menon et al. [17] found in their study that GE-EPI with a TR of 100 ms at 4 T is able to differentiate temporal events separated by 125 ms in stimulus onset, by measuring the latency of the inflection point from the resting baseline of the HRF. This study suggests that the initial portion (upslope) of the HRF may have adequate sensitivity and specificity to trace the temporal onset of neuronal responses – a technique dubbed mental chronometry. More recent studies have applied a novel magnetic inverse imaging technique to differentiate a relative timing of 100 ms in visual cortex activation [18,19]. By combining graph-based visualization of self-organizing maps (SOM) with Granger causality analysis, Katwal et al. [20,21] showed that a timing difference as short as 28 ms could be detected in the HRF curves from visual fMRI experiments.

The purpose of the present study was to present a novel multi-phase segmented passband balanced steady-state free precession (b-SSFP) technique for event-related fMRI (ER-fMRI) with a temporal sampling rate of 50 ms. Passband b-SSFP fMRI utilizes the flat portion of its magnetization profile to detect microscopic susceptibility changes induced by regional hemodynamic response to neuronal activation. Compared to GE-EPI, passband b-SSFP has the advantages of high SNR efficiency that does not suffer from T1 saturation effects as EPI for short TRs. Given the relatively wide and flat portion of its off-resonance profile, passband b-SSFP is able to offer wide brain coverage, high spatial resolution and high temporal sampling rate for fMRI applications with minimal distortion [22–26]. In the present study, standard GE-EPI based ER-fMRI was carried out with matched temporal sampling rate and spatial resolution as multi-phase b-SSFP, and the results of both fMRI techniques were compared with electrophysiological recordings using event-related potential (ERP). Our hypothesis was that both b-SSFP and GE-EPI based ER-fMRI with 50 ms sampling rate will be able to resolve a latency of 100 ms in neuronal events, approaching the capability of ERP recordings.

2. Materials and methods

2.1. Simulation

Simulations were carried out based on analytical solutions to steady state conditions using custom Matlab programs to understand the behavior of b-SSFP signals compared to a standard gradient-echo (GRE) sequence, as well as GE-EPI [27]. The parameters used for simulation of GRE and SSFP were $T1 = 2000$ ms, $T2 = 50$ ms, $T2^* = 31$ ms (GRE), $TE = TR/2$ (SSFP) [17], and the magnitude was calculated and plotted with a range of TRs or flip angles (FA) as a function of off-resonance frequency. A fixed TR of 4 ms was used in simulations with varied FA, and the FA was set to 20 degrees in simulations with multiple TRs. For GE-EPI, TR was varied between 50 and 2000 ms based on in vivo experiments, TE was 21 ms and FA was set as the Ernst angle. The simulation results were used for determining the optimal parameters for in vivo fMRI experiments.

2.2. Multi-phase b-SSFP pulse sequence

The multi-phase passband b-SSFP sequence was modified from the cine b-SSFP imaging technique with segmented acquisitions that was originally developed for cardiovascular imaging [28–32]. The original cine b-SSFP technique was designed to acquire a series of time-resolved cardiac images between two heartbeats. Each cine b-SSFP acquisition is triggered by ECG gating. Following a linear ramp of RF pulses to stabilize the b-SSFP signal, the signal is then continuously acquired by segmented multi-phase b-SSFP readout with phase encoding advancing in a centric order. At the end of the b-SSFP readout, the magnetization is restored to the positive z-axis

by using an $\alpha/2$ pulse [33]. Each individual time frame of cine b-SSFP acquisition is generated from multiple segmented acquisitions synchronized to the ECG trigger (i.e., only one segment of k-space data is acquired for each ECG triggered b-SSFP acquisition).

The pulse sequence diagram of the multi-phase b-SSFP fMRI technique is shown in Fig. 1a. Instead of using ECG, the multi-phase b-SSFP acquisition was synchronized with the stimulus presentation through a TTL (transistor–transistor logic) pulse generated by the scanner. Following the linear ramp of 20 RF pulses to stabilize the b-SSFP signal, the visual stimulation was presented immediately or at 100, 200 and 300 ms delays respectively (see below) while the signal was continuously acquired by the segmented multi-phase b-SSFP readout. Each TTL-triggered multi-phase b-SSFP acquisition lasted 16 s to acquire the full HRF for ER-fMRI, which consisted of 303 time frames or phases resulting in a temporal sampling rate of 52.65 ms (16 s/303 phases excluding linear RF ramp) per image frame. Six segments (first segment was used as dummy scan to reach the steady state, while the rest were used for data acquisition) with fifteen k-space lines per segment were acquired for a complete dataset of 303 time-resolved image series of the HRF synchronized to the stimulus presentation.

2.3. MR experiment

Eighteen healthy volunteers (age 20–29 years, 11 males) participated in the fMRI study while 5 healthy (age 19–26 years, 3 males) volunteers participated in the ERP experiments. Two subjects participated in both b-SSFP fMRI and ERP experiments. All subjects have given written informed consent prior to the study in accordance with the guidelines and approval of the Institutional Review Board of the Beijing MRI Center for Brain Research.

ER-fMRI experiments using multi-phase passband b-SSFP and GE-EPI were performed on a 7 Tesla Siemens Magnetom whole-body system (Erlangen, Germany) with a volume excitation and 32-channel receive head coil (Nova Medical, Cambridge, MA, USA). Nine subjects (5 M/4 F) participated in the b-SSFP fMRI experiment (Exp. 1) and 9 subjects (6 M/3 F) in the GE-EPI fMRI experiment (Exp. 2). ER-fMRI was performed with a full screen 16 Hz-flashing counter-phased checkerboard (flashing duration 62.5 ms) as the visual stimulus. In Exp. 1, following the linear ramp of 20 RF pulses to stabilize the b-SSFP signal, a TTL trigger was sent to the stimulus computer and the checkerboard was presented with a time delay of 0, 100, 200 or 300 ms (d0, d100, d200, or d300 respectively) relative to the TTL trigger pulse (Fig. 1b). The order of stimulus delay was randomized during each scan. The subjects were required to focus on a cross-hair fixation located in the center of the visual field throughout the experiments. All stimulus paradigms were presented with Psychtoolbox in Matlab. The imaging parameters of Exp. 1 were: data acquisition window = 16 s with a temporal sampling of 52.65 ms per frame, $FOV = 19.2 \times 19.2 \text{ cm}^2$, $TE = 1.76$ ms, $TR = 3.52$ ms, single slice of 5 mm thickness, spatial resolution = $1.4 \times 1.7 \times 5 \text{ mm}^3$, $FA = 19^\circ$, GRAPPA with an acceleration rate of 2, and each HRF cycle = 96 s (6 segments, 16 s per segment). In Exp. 2, a standard single shot GE-EPI sequence was applied for ER-fMRI and the TTL trigger was sent at the beginning of each acquisition window or trial. The imaging parameters were: $FOV = 19.2 \times 19.2 \text{ cm}^2$, $TE = 21$ ms, $TR = 50$ ms, single slice of 3 mm thickness, spatial resolution = $1.6 \times 1.6 \times 3 \text{ mm}^3$, $FA = 20^\circ$, GRAPPA with an acceleration rate of 3, data acquisition window = 16 s consisting of 320 TRs. These imaging parameters were determined by simulations as well as data from a pilot study [34]. Previous studies also demonstrated that an acquisition window of 16 s was sufficient for the ER-fMRI signal to return to the baseline [35,36].

In both experiments, each subject underwent a three-plane localizer scan, three dimensional T1-weighted scan with the MP-RAGE sequence. From the T1-weighted images, the calcarine sulcus was identified to locate the two-dimensional slice for the ER-fMRI scans using the

Fig. 1. Pulse sequence of multi-phase b-SSFP and stimulus for ER-fMRI designs. (a) Pulse sequence diagram of multi-phase b-SSFP. The arrow in the left with a checkerboard in the figure implies the TTL pulses to trigger the stimulus presentation. (b) Stimulus for event-related fMRI of Exp. 1 and Exp. 2. The 16 Hz-checkerboard is presented with full screen. There are four conditions (0 ms, 100 ms, 200 ms, 300 ms) of delays relative to the TTL trigger.

multi-phase b-SSFP and GE-EPI sequence. Furthermore, the location of the calcarine sulcus was confirmed with block design fMRI using a GE-EPI sequence with full brain coverage. The stimulus was 20s flashing checkerboard followed by 20s resting state.

2.4. Electrophysiological experiment

Electrophysiological signals using ERP recording were acquired on 5 subjects (3 M/2 F) using the Compumedics full-montage standard Electroencephalograph SynAmp2 NeuroScan system (Charlotte, NC, USA), for comparison with the fMRI experiments. The sampling rate was 1000 Hz with a full brain acquisition of 64 channels. The identical stimulus paradigm of ER-fMRI was applied in the ERP experiment. Compared to the ER-fMRI HRF, the ERP signal directly records electrical activities of neural circuits and is much faster than the HRF. Therefore a randomized delay of 1–2 s was applied after the stimulus presentation in ERP experiments to allow the signal to return to the baseline. There are 3 main peaks in the ERP responses in our experiments. The P300 is an endogenous potential that has nothing to do with the physical attributes of the stimulus. The P1 and N1 components are attributed to the use of different visual stimuli, and reflect the robust latency originating from the visual stimulus [37].

2.5. Data analysis and statistical analysis

The fMRI data was processed using SPM8. In-plane motion correction was performed on the series of b-SSFP and GE-EPI images. Physiological noise related to cardiac and respiratory pulsation effects was temporally filtered (low pass filter, 0.05 Hz) based on fast Fourier transform (FFT). The general linear model (GLM) was then employed to detect the spatial localizations of the initial dip and subsequent positive response. The reference function of the positive response was generated by convolving the stimulus paradigm with a canonical HRF

according to Friston et al. [38], while the reference function of the initial dip was modeled by the time course of previous published data [39–41]. The HRF curves were temporally smoothed using the LOWESS (locally weighted scatterplot smoothing) [42] function with a span of 20% of the data.

The ERP signal was recorded using 8 channels of the occipital lobe electrodes (left hemisphere: O1, PO3, PO5, PO7, right hemisphere: O2, PO4, PO6, PO8). The ERP signal was processed with EEGLAB and plotted as averaged ERP contour maps. One thousand voltage points before the beginning of each trial were averaged as the baseline, and the time series of electrical voltage was calculated relative to the baseline. The temporal window from -250 ms to 1250 ms was used to show the ERP response. The ERP response curve was smoothed with the same LOWESS function as fMRI data. There were several peaks in the ERP response (P1, N1, P300). P1 was chosen to estimate the latency and delay of each experimental condition, since P1 is a robust peak known to originate from the visual cortex in ERP literature using visual stimulation [37].

Since the sampling rate of HRF curves acquired using b-SSFP fMRI and GE-EPI was ~50 ms, in order to accurately estimate the delay between HRF curves (e.g., 100 ms, 200 ms, 300 ms in this study), linear interpolation was applied on the fMRI data to virtually increase the sampling rate of fMRI to 1000 Hz. The upslope (3–5 s post TTL trigger) was chosen as the representative range for the calculation of the delay. Take the d0 and d100 HRF curves as an example, after linear interpolation, cross-correlation coefficient was calculated between the original d0 curve and a temporally shifted d100 curve every 1 ms shift. The temporal shift yielding the largest correlation coefficient was determined as the delay between the HRF curves. The same methods were applied on d200 and d300 HRF curves to determine their delays relative to d0 respectively. In the ERP experiments, the latency of the P1 peak was calculated for each experimental condition (d0, d100, d200 and d300) respectively.

Using the methods above, the delay of each subject in all fMRI and ERP conditions was calculated and subject to repeated measures

ANOVA (analysis of variance). Based on results of the Mauchly's test of sphericity, the multivariate test method (Pillai's Trace) or within subjects effects method (sphericity assumed) was used for detecting the statistical difference within and between groups in each condition. The post-hoc tests were carried out between each pair of two conditions to determine the statistically significant difference. The F test was carried out to detect the difference of variances between each condition of Exp. 1 and Exp. 2 respectively. After the comparison of homogeneity of the variances, T test was applied for comparison of the mean value of each condition in Exp. 1 and Exp. 2 respectively.

3. Results

3.1. Simulation results

The b-SSFP signals were calculated with different TRs as a function of off-resonance frequency which is plotted in Fig. 2a. For comparison, the mean signal of standard GRE sequence was calculated at each TR across the frequency range as well (Fig. 2a). The magnitude of b-SSFP with different FAs as a function of off-resonance frequency is plotted in Fig. 2b, and the period is $1/TR$ (250 Hz for $TR = 4$ ms). As an example, only two periods of the simulation results are displayed. Passband and transition band of b-SSFP can be distinguished in the profiles clearly. The FA range of 15° – 20° yielded the highest passband b-SSFP signal, therefore $FA = 19^\circ$ (take SAR into consideration) was chosen as the optimal FA for our fMRI experiments at 7 Tesla. Furthermore, the mean signal intensity for GE-EPI sequence was calculated and displayed in Fig. 2c for the ranged of TR (50 ms to 2000 ms) for in vivo experiments. The signal of GE-EPI decreased with shorter TR due to saturation effects. In contrast, the magnitude of the passband b-SSFP signal was very stable across TRs and only dropped slightly over TR due to T2-relaxation. The results show that the magnitude of passband b-SSFP is able to maintain a steady state with varied TRs resulting in a high SNR

even with a short TR, while the magnitude of the GRE or GE-EPI sequences decreased with reduced TR.

3.2. The fMRI results and characteristics of HRF curves

The representative functional images from Exp. 1 and Exp. 2 are displayed in Fig. 3. The image quality of b-SSFP was better than GE-EPI, with minimal distortions. The activation maps of the positive and initial dip negative HRF activation from a representative subject in Exp. 1 are plotted in Fig. 4a (Family Wise Error (FWE) corrected t-test, $p < 0.05$). A significant initial dip was detected in each subject in Exp. 1. For comparison, the activation maps from a representative subject in Exp. 2 are plotted in Fig. 4b (FWE corrected t-test, $p < 0.05$). The initial dip was not obvious compared with that of Exp. 1. The SPM-t magnitude was obviously larger in the positive response than that of the negative response. Both the initial dip and positive activation occurred mostly in gray matter, with the former being more focal. Fig. 4 also shows difference in spatial smoothness between activation maps of b-SSFP and GE-EPI which may be attributed to the blurrier image of GE-EPI as shown in Fig. 3.

The multi-phase b-SSFP and GE-EPI signals were extracted from regions of interests (ROIs) of the activation clusters in the primary visual cortex. The baseline was determined from 20 images before the TTL trigger. The averaged HRF curves (with standard deviation) of Exp. 1 and Exp. 2 are displayed as percentage signal changes of the subsequent time series of b-SSFP and GE-EPI functional images, as shown in Fig. 4a and b respectively for the stimulus without delay relative to the TTL trigger (d0 condition). In Exp. 1, one sample t-test on the values of the time window (0–4 s) from each subject shows that the HRF signal during the initial dip was significantly below zero ($p = 0.015$), and the positive HRF response was significantly above zero ($p = 0.0001$). The initial dip in Exp. 2 was not as obvious as that of Exp. 1 from the HRF curves. The HRF signal during the initial dip

Fig. 2. Simulations of b-SSFP, GRE and GE-EPI. (a) The magnitude of b-SSFP (left) and GRE (right) sequences with different TRs in simulations. (b) The simulated magnitude of b-SSFP with different FAs. The passband and transition band profiles are distinguished clearly in the figure, and the FA range of 15° – 20° yields the highest passband b-SSFP signal at 7 T. (c) The magnitude of GE-EPI in simulations with TRs ranging from 50 ms to 2000 ms, and FA is calculated as the Ernst angle in each TR. The magnitude of passband b-SSFP can maintain a steady state with varied TRs resulting in a high SNR even with a short TR, while the magnitude of the GRE or GE-EPI sequences decrease with reduced TR.

Fig. 3. Functional images from a representative subject in Exp. 1. The functional images of multi-phase b-SSFP (a) and GE-EPI (b) with a sampling rate of 50 ms were shown. The GE-EPI image was blurred and distorted, while the quality of b-SSFP is much better with minimal distortions.

was barely below zero ($p < 0.05$), while the positive HRF response was significantly above zero ($p < 0.0001$).

3.3. fMRI signals capturing the onset timing of visual stimulus

In Exp. 1, the HRF curves elicited by visual stimulation with four different delays (d0, d100, d200, d300) relative to the TTL trigger pulse are displayed in Fig. 5a respectively. The rising slopes (3–5 s) of the HRF curves were chosen to calculate the fitted delay. The initial dip and post-stimulus undershoot can be detected in all conditions, and the HRF curves of the four delay conditions can be clearly distinguished from each other based on the rising slopes of the HRFs. However, the initial dip of the HRF curves is not able to differentiate the physical delay of 4 conditions. After the HRF signal reaches peak, the remaining

portions of the HRF do not have sufficient precision to distinguish the 4 delay conditions. The HRFs return to the baseline in 16 s.

The ER-fMRI results of Exp. 2 are shown in Fig. 5b, in which the HRFs of 4 stimulus conditions (d0, d100, d200, d300) are presented. The HRF curves of the four delay conditions can be clearly distinguished from each other based on the rising slopes of the HRFs as well. Compared with the HRF curves in Exp. 1, the HRF curves of Exp. 2 show a greater level of intersubject variability (see below for F test statistics).

3.4. Visual stimulus delay results of ERP

The average ERP curves of four delay conditions from 5 subjects are plotted in Fig. 6. The ERP curves of each experimental condition

Fig. 4. Activation maps of both b-SSFP and GE-EPI. (a) Positive activations of the SPM-t map overlain onto the MP-RAGE T1 structural image in d0 condition (as an example) of Exp. 1 (left). The initial dip in the SPM-t map is overlain onto the same structural image (middle). The mean HRF curve from the selected ROIs averaged across 9 subjects with error bars (standard deviation) is plotted in the right panel. The black bar is the time period for detecting the initial dip, while the 16 s duration is for the HRF positive activation detecting. (b) GE-EPI shows similar activation patterns yet smaller activation regions than b-SSFP, especially in the initial dip. However, the signal change percentage is larger than that of b-SSFP. Both the positive and negative activations (initial dip) in fMRI with b-SSFP and GE-EPI sequences occur most in gray matter at 7 Tesla.

Fig. 5. The BOLD response function curves. The HRF curves averaged across 9 subjects in Exp. 1 (a) and Exp. 2 (b) are shown with four delay conditions (d0, d100, d200, d300). The insets indicate the upslope (3–5 s) that reflect the delays between different HRFs.

with marked main peaks are plotted as well. It can be clearly seen that the ERP curves for different stimulus conditions are shifted in time, corresponding to the onset timing of the stimulus presentation. The latency of the P1 peak of ERP curves was calculated as the delay of four conditions (d0, d100, d200, d300). The measured temporal delay was close to the physical stimulus delay in each condition.

3.5. Statistical analysis

Fig. 7 shows the average estimated fMRI delays with error bars of standard deviation for different experimental conditions in Exp. 1 and 2, respectively. Repeated measures ANOVA of estimated HRF delays of fMRI revealed a significant statistical difference across four delay conditions in Exp. 1 ($p = 6.01 \times 10^{-6}$) and Exp. 2 ($p = 1.73 \times 10^{-5}$). The post-hoc test showed that there were significant differences between each pair of two conditions ($p = 8.56 \times 10^{-8}$ in Exp. 1, $p = 6.84 \times 10^{-5}$ in Exp. 2). From the one-way ANOVA (F test) results, the standard deviations (error bars) of the d100 and d200 conditions were significantly ($p = 0.011$, $p = 0.041$) greater than those in Exp. 2, indicating a higher level of intersubject variability in Exp. 2. The standard deviations (error bars) of the d300 condition, however, did not

show statistical significance between b-SSFP and EPI ($p = 0.401$). In addition, the mean fitted delay of each condition did not show significant difference between Exp. 1 and Exp. 2. For comparison with the fMRI results, the latency to P1 of the ERP curves of the corresponding stimulus conditions are displayed in Fig. 7. Repeated measures ANOVA revealed significant differences across four delay conditions ($p = 0.00007$). The post-hoc t-test showed that there were significant differences between each pair of two conditions ($p < 0.001$).

4. Discussion and conclusions

In this study, a new fMRI technique was presented by adapting the cine multi-phase b-SSFP originally developed for cardiovascular MRI to achieve a temporal sampling rate of a few tens milliseconds and a spatial resolution of a few cubic millimeters for ER-fMRI. As comparison, the standard single shot GE-EPI was carried out for the same ER-fMRI experiment with matched spatial resolution and temporal sampling rate. The HRF curves of both b-SSFP (Exp. 1) and GE-EPI (Exp. 2) are able to discern a stimulus onset delay as short as 100 ms, as validated with electrophysiological recordings using ERP. GE-EPI shows higher percentage signal changes compared to that of

Fig. 6. The averaged ERP responses of 4 conditions and main representative peaks. The ERP responses in Exp. 1 averaged across 5 subjects and in 4 conditions respectively (left). The ERP curves of every experimental condition with main peaks marked are plotted individually as well (right). Three representative peaks are indicated with arrows, and the delay to each P1 peak is estimated, which shows that the estimated delay to the P1 peak is close to the real stimulus delay.

Fig. 7. Statistical analysis for the fitted delay of both fMRI and ERP. The mean estimated fMRI delay in Exp. 1 and Exp. 2 with error bars (standard deviation) based on HRFs of every condition. For comparison with fMRI results, the latency to P1 in the ERP curves of the corresponding stimulus conditions is displayed as well (blue line).

b-SSFP. However, the intersubject variability of the fitted delay of HRF curves was lower in multi-phase passband b-SSFP than that of GE-EPI (for 100 and 200 ms stimulus delay). In addition, the HRF curve of b-SSFP in Exp. 1 provides more reliable estimate of the initial dip, compared to that of GE-EPI in Exp. 2.

4.1. Comparison of multi-phase b-SSFP and GE-EPI fMRI

While BOLD fMRI has been widely applied for functional neuroimaging studies in both health and disease, one of its limitations is that the BOLD contrast arises from the hemodynamic response secondary to neuronal activation. The HRF of BOLD fMRI is considered both delayed in time (on the order of seconds) and blurred in space (arising from venules and veins instead of capillaries and tissue), imposing a physiological limit on the spatial and temporal resolutions that can be achieved by fMRI. Nevertheless, recent advances in optical imaging and microscope provide new evidence that cerebral blood flow can be precisely and rapidly regulated on an extremely fine scale at the level of capillaries [43–46]. Therefore, there has been renewed interest in pursuing fMRI with high temporal sampling rate and spatial resolution especially at ultrahigh magnetic fields [47].

The purpose of the present study was to evaluate the capability of b-SSFP and GE-EPI based fMRI with a high sampling rate for discerning a temporal delay of 100 ms in neuronal responses. The multi-phase passband b-SSFP technique inherits the advantages of passband b-SSFP, such as high SNR efficiency and relatively low magnetic susceptibility artifacts, and further capitalizes on the unique advantage of b-SSFP for steady state imaging to allow for a high temporal sampling rate. Another appealing property of b-SSFP based fMRI is that its contrast mechanism is similar to T2 based fMRI rather than T2* or GRE based fMRI. Past studies have shown that T2 based fMRI (e.g., spin-echo EPI) arises from water diffusion around microvasculature instead of larger veins, thereby is closer to neuronal activation sites in terms of spatial localization [48]. While the limitation of T2 based fMRI has been the lower signal strength, performing fMRI studies at ultrahigh magnetic fields (e.g. 7 T and beyond) can overcome this limitation and further improve the spatial resolution of fMRI. Based on our pilot studies, translating multi-phase b-SSFP fMRI to 3 T appears to be challenging due to reduced SNR and functional sensitivity. Therefore, performing multi-phase b-SSFP fMRI at ultrahigh magnetic fields offers an appealing approach to achieve high fidelity fMRI with millisecond temporal sampling rate and (sub-)millimeter spatial resolution.

GE-EPI can also be performed with 50 ms sampling rate, and is able to discern 100 ms delay in temporal events. As expected, the

percentage signal change of the HRF of GE-EPI is greater than that of b-SSFP based HRF. However, the intersubject variability of the fitted delay of HRF curves was lower in multi-phase passband b-SSFP than that of GE-EPI (for short stimulus delays of 100 and 200 ms). The reason for increased variability of fitted HRF delay in GE-EPI is not well understood, and may be related to the different sensitivity of GE-EPI and b-SSFP to physiological noise. In our pilot study [49,50], the power spectrum of GE-EPI time series shows greater power in the frequency range of cardiac and respiratory pulsation compared to that of passband b-SSFP. As mentioned, passband b-SSFP utilizes the relatively wide and flat portion of its off-resonance profile, resulting in relatively low sensitivity to frequency shifts caused by physiological fluctuations. This may be an advantage of the proposed multi-phase passband b-SSFP technique for resolving temporally adjacent neuronal events.

However, the multi-phase b-SSFP method requires segmented acquisition resulting in increased scan time compared to that of GE-EPI. The segmented acquisition may also make the b-SSFP technique susceptible to head motion and physiological noise between segments. Due to the relatively long scan time for b-SSFP or GE-EPI ER-fMRI studies, we were not able to perform comparison of the two techniques in the identical cohort of 9 subjects. Therefore, the reported differences in terms of initial dip and intersubject variability between b-SSFP and GE-EPI need to be interpreted with caution and replicated in future studies.

4.2. Visual response latencies measured by fMRI and ERP

In both Exp. 1 and 2, the full field checkerboard with 100 ms difference in stimulus onsets produced the exact delays in the HRF of fMRI signals (calculated from the rising slope, Fig. 5a), as well as in the early ERP components (calculated from the peaks of P1, Fig. 6). Our results are consistent with previous research [17,19], showing that GE-EPI with a TR of 100 ms is able to differentiate temporal events separated by 100–125 ms in stimulus onset, by measuring the latency of the inflection point from the resting baseline of the HRF. These findings suggest that the early portion of the HRF is sensitive to delays of stimulus onset and is able to trace early neuronal responses in the visual cortex as indicated by ERP. However, the initial dip and later portion of the HRF (once the signal reaches its peak) fails to differentiate delays in stimulus onset. This finding suggests that a typical HRF of BOLD fMRI may consist of multiple temporal components corresponding to different underlying physiological processes.

Friston et al. [51,52] proposed a hemodynamic response model that consists of a linear dynamic model of changes in regional cerebral blood flow (CBF) caused by neuronal activities and the Balloon model that accounts for the relationship between CBF and BOLD signal. The neurogenic component of CBF response is fast in time and focal in space, and may be mediated by nitric oxide (NO) that has a time constant of 100–1000 ms. In contrast, the later BOLD response arises primarily from venous blood volume changes driven by CBF increases, as well explained by the Balloon model. Our observations that the upslope of the HRF of both b-SSFP and GE-EPI is able to differentiate stimulus delay as short as 100 ms (while the later portion of HRF fails) suggests that the early portion of the HRF may primarily reflect initial CBF increases to neuronal activation, likely mediated by dilation of arterioles.

Past studies also showed that the initial dip may present in the HRF of BOLD fMRI, depending on the stimulus and imaging parameters. The initial dip is thought to reflect the temporal mismatch between initial oxygen metabolism induced by neuronal activation and increased CBF. In the present study, the HRF of b-SSFP appears to show more apparent initial dip compared to the HRF of GE-EPI. This finding is consistent with the contrast mechanism of passband b-SSFP which is similar to T2 based fMRI and arises primarily from microvasculature wherein the oxygen extraction takes place. However, since b-SSFP and GE-EPI

experiments were not performed on the identical subjects, this finding needs to be interpreted with caution and replicated in future studies.

4.3. Alternative ultrafast fMRI techniques

During recent years, a variety of ultra-fast fMRI techniques have been developed, such as MR encephalography (MREG) [53,54] and its combination with generalized inverse imaging (GIN) [55] to achieve a temporal sampling of a few tens or even a few milliseconds. MREG and its variants have shown promise for filtering out cardiac and other physiological noise during resting state fMRI [56]. Alternative ultra-fast fMRI techniques include inverse imaging based methods [18] that are analogous to source localization of MEG and EEG. Inverse imaging, however, is challenging given highly undersampled data that require regularized reconstruction. In addition, most of existing ultra-fast fMRI techniques are based on gradient-echo EPI that suffers from the T1 saturation effect with high temporal sampling rate (short TR).

Compared to MREG and its variants, the multi-phase b-SSFP sequence can obtain functional images with high spatial resolution and minimal distortion, while the limitation of the proposed multi-phase b-SSFP technique is that the total scan time is relatively long especially for achieving both high temporal sampling rate and high spatial resolution as well as adequate imaging coverage. In order to circumvent this limitation, 3D imaging as well as multi-band b-SSFP techniques in combination with parallel imaging can be applied in future studies. Other accelerated 4D imaging techniques such as view sharing and keyhole can also be applied with constrained image reconstruction. In addition, the inverse imaging methods that have been developed for MREG can be further applied to achieve whole-brain multi-phase b-SSFP based ER-fMRI. Recent studies have shown that multi-band GE-EPI can be performed at 7 T with an acceleration factors up to 12 [57]. It will be interesting to test the feasibility of accelerated multi-band GE-EPI with a high temporal sampling rate of 50 ms in future studies.

4.4. Novel methods for measuring relative timings in HRF

Multi-phase passband b-SSFP, GE-EPI as well as MREG are all emerging fMRI techniques with a high sampling rate or a very short TR that can all be applied for detecting short timing differences from the early portion of HRF. In addition, recent progress has been made in the post-processing methods [21,58] by utilizing Granger causality analysis in conjunction with data-driven self-organizing maps (SOM) for detecting the activated voxels while resolving short temporal delays in brain activities. In a recent study [21], a visual ER-fMRI experiment was carried out using GE-EPI with a TR of 250 ms at 7 T, and the time-to-peak estimated from the HRF curves was able to detect a stimulus onset difference of 112 ms. With Granger causality analysis, sub-100 ms differences (as short as 28 ms) from HRFs can be further resolved. In the future, Granger causality analysis can be combined with the multiphase passband b-SSFP technique for resolving sub-100 ms latencies in neural activities across brain regions.

In conclusion, the multi-phase passband b-SSFP fMRI offers a promising approach for high fidelity functional neuroimaging with millisecond temporal sampling rate and millimeter spatial resolution at ultrahigh magnetic fields. These features are ideal for detecting neural activities with precision and high spatiotemporal resolution, such as the initial dip in HRF. With accelerated imaging speed and innovative post-processing algorithms, the technique may find a variety of applications in cognitive neuroscience and clinical medicine in the future.

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