Stimulus frequency dependence of blood oxygenation level-dependent functional magnetic resonance imaging signals in the somatosensory cortex of rats

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Abstract

Understanding the mechanism of coupling between neuronal events and hemodynamic responses is important in non-invasive functional imaging of the brain. The stimulus frequency dependence of hemodynamic responses has been studied using a rat somatosensory cortex model; most results for short stimulus durations reveal peak frequencies at which the hemodynamic response is maximized. However, such peak frequencies have not been observed in studies using blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signals with long stimulus durations. To clarify whether the stimulus frequency dependence of BOLD signals depends on the stimulus duration, we measured BOLD signals at 7 T with short and long stimulus durations for stimulating rat forepaw at 1–10 Hz using spin-echo echo-planar imaging to enhance changes in activation focus. For both these durations, BOLD signals were significantly higher at stimulus frequencies of 3 or 5 Hz in agreement with the results of previous studies using optical techniques. Our results show that stimulus duration has little influence on the stimulus frequency dependence of BOLD signals in the rat somatosensory model. The discrepant results of most previous fMRI studies using gradient-echo sequence may be ascribed to the difference of imaging to enhance activation focus or draining vein.

Keywords: blood oxygenation level-dependent; cerebral blood flow; forepaw stimulation; functional magnetic resonance imaging; metabolism; optical imaging

1. Introduction

In order to map the functional activation of the human brain, neuroimaging techniques such as functional magnetic resonance imaging (fMRI), intrinsic optical imaging, and positron emission tomography are used to measure the local variations in hemodynamics and metabolism that accompany neuronal activity. Since neuroimaging techniques cannot directly assess neural events, it is important to understand the mechanism of the coupling between neuronal events and hemodynamic responses for the interpretation of neuroimaging signals. In order to investigate this relationship, several neuroimaging studies have been conducted by using animal models because such methods of study are more flexible in that (1) pharmacological perturbations can be used, (2) the animals can be genetically controlled, and (3) less variations are observed between individual animals.

A large number of studies have been conducted by using electrical stimulations on the rat somatosensory model by using the blood oxygenation level-dependent (BOLD) fMRI (Brinker et al., 1999; Gyngell et al., 1996; Huttunen et al., 2008; Keilholz et al., 2004; Kida et al., 2001; Ogawa et al., 2000; Van Camp et al., 2006), laser Doppler flowmetry (LDF) (Ances et al., 1999; Detre et al., 1998; Matsuura and Kanno, 2001; Ngai et al., 1999; Ureshi et al., 2004), and optical imaging (Sheth et al., 2004; 2005). Since BOLD signals correlate with the deoxygenated hemoglobin content (Ogawa et al., 1990), the stimulus frequency dependence of BOLD signals should correspond to the deoxygenated hemoglobin content assessed by optical imaging, and a similar dependence is expected with regard to changes in the cerebral blood flow (CBF) (Hoge et al., 1999; Kida et al., 2000; Silva et al., 2000). However, conflicting results were obtained with regard to the stimulus frequency dependence of the BOLD signals measured by MRI and the changes in CBF measured using LDF. The intensity of BOLD signals was observed to decrease with increase in the stimulus frequency applied to rat forepaw from 1.5 Hz (Brinker et al., 1999; Gyngell et al., 1996), whereas the peak CBF was recorded by using LDF at a stimulus frequency of 5 Hz during the stimulation of the forepaw (Ances et al., 1999; Detre et al., 1998) and hindpaw (Matsuura and Kanno, 2001; Ureshi et al., 2004). This discrepancy in the results of stimulation is probably because of the difference in the stimulus durations. In the studies that employed rat somatosensory models, the stimulus duration in LDF techniques was relatively short

(2–5 s) (Ances et al., 1999; Detre et al., 1998; Matsuura and Kanno, 2001; Ureshi et al., 2004), whereas that in BOLD fMRI techniques was relatively long (several tens of seconds) (Brinker et al., 1999; Gyngell et al., 1996; Huttunen et al., 2008; Keilholz et al., 2004; Kida et al., 2001; Van Camp et al., 2006). However, no fMRI studies have investigated the stimulus frequency dependence for short stimulus durations in order to compare the results obtained using the fMRI and LDF techniques in the rat somatosensory model. Therefore, whether the stimulus frequency dependence of BOLD signals is dependent on the stimulus duration remains unknown.

In this study, we first investigated the stimulus frequency dependence of the BOLD signal at 7 T in an α-chloralose-anesthetized rat; the rat forepaw was electrically stimulated at frequencies of 1, 3, 5, and 10 Hz for 4 s, which is the same duration as that used in LDF. In order to enhance the BOLD signals in the activation focus, we employed spin-echo echo-planar imaging (EPI) instead of gradient-echo EPI, which was employed in the previous fMRI studies. Next, to clarify the effect of the stimulus duration on stimulus frequency dependence, we recorded BOLD fMRI signals at the same stimulus frequencies applied for a longer duration (32 s).

2. Materials and Methods

2.1. Animal preparation

The fMRI experiments were conducted using 18 male Sprague-Dawley rats weighing 220–280 g. These rats were initially anesthetized with 4% isoflurane in 70%:30% N_2O:O_2 and then tracheotomized. Anesthesia was maintained by administering 1.8%–2.0% isoflurane in 70%:30% N_2O:O_2 through an artificial ventilation system throughout the surgical procedures. The femoral artery was cannulated with a polyethylene catheter (PE-50) to withdraw blood samples for blood gas analysis and to monitor the blood pressure. The femoral vein was cannulated by using another polyethylene catheter (PE-10) for injecting pancuronium bromide, a muscle relaxant. PE-50 catheters were inserted for intraperitoneal administration of α-chloralose. In all the animals, the ventilation parameters were adjusted such that the arterial blood gas tension was

maintained within the normal range throughout the experiment (pH, 7.39 ± 0.06; pCO_2, 37.4 ± 4.9 mmHg; and pO_2, 143 ± 32 mmHg). A pair of copper needle electrodes was inserted underneath the skin of the rat forepaw. All the whiskers were cut to prevent signal contamination from the whisker region in the somatosensory cortex. The rat was placed in a home-built holder, and its head was tightly secured in a stereotaxic manner by using a bite plate and ear bars to minimize head movements. A temperature-controlled water blanket was placed under the rat to maintain its body temperature (37 ± 0.5°C) throughout the experiments. After the completion of all these procedures, the administration of isoflurane anesthesia was discontinued, and anesthesia was then maintained by the intraperitoneal administration of α-chloralose (40 $mg \cdot kg^{-1} \cdot h^{-1}$). Pancuronium bromide (1 $mg \cdot kg^{-1} \cdot h^{-1}$) was administered intravenously as a muscle relaxant throughout the experiment.

2.2 Forepaw stimulation

The forepaw was electrically stimulated using a pair of copper needle electrodes. A signal with a rectangular pulse width of 0.3 ms and an intensity of 2 mA was applied using a constant current power supply (Nihon Kohden, Tokyo, Japan). The stimulus frequency dependence was investigated at frequencies of 1, 3, 5, and 10 Hz. Two stimulation paradigms were employed. The paradigm for the short stimulation was a block design initiated under a 16-s control condition, followed by 3 alternating cycles of 4-s stimulation with a stimulus interval of 40 s (n = 12). The paradigm for the long stimulation was a single-stimulation design initiated under a 16-s control condition, followed by stimulation for 32 s (n = 6). Both stimulation paradigms were repeated twice at each stimulus frequency. The stimulations at different frequencies were randomly performed. A total of 72 and 12 BOLD signals were obtained in response to the 4-s and 32-s stimulations, respectively, from all the animals at each stimulus frequency. Under all the stimulus conditions, no variations were observed in the mean arterial blood pressure during stimulation (Ureshi et al., 2005).

2.3. fMRI experiments

All the fMRI data were obtained using a 7-T horizontal bore spectrometer (Varian Inc., CA). This instrument consists of a shielded gradient coil (diameter: 11 cm) with a maximum gradient field strength of 120 mT/m. Radiofrequency pulses were transmitted, and the MRI signals were received using a 10-mm surface coil placed over the somatosensory area (i.e., around the bregma). To determine the positions of the functional images of the somatosensory cortex, eight high-resolution coronal gradient-echo images with a 20-mm field of view and 0.6-mm slice thickness were acquired as scout images. After positioning, slice shimming was first performed automatically and then manually.

Single-shot spin-echo EPI images for the BOLD fMRI experiments were acquired under the following conditions: data matrix, 32 × 32; field of view, 20 × 20 mm; slice thickness, 2 mm; echo time, 40 ms; and repetition time, 1000 ms.

2.4. Data analysis

The BOLD fMRI data were processed using the MATLAB software (MathWorks, Inc., MA) and an in-house written software. For each BOLD fMRI experiment, the functional images were obtained as Student's *t*-test statistical images on a pixel-by-pixel basis by using the 4 images obtained before and after the stimulus onset. The activated regions were defined as pixels that revealed the statistically significant responses ($p < 0.05$). The time courses of the BOLD signals in the forepaw-related region of the contralateral somatosensory cortex of the rat were obtained by determining the activated area based on the Student's *t*-test values that were averaged for each stimulus frequency. Images with head-movement artifacts, which were identified by a center-of-mass analysis, were not considered for further analyses.

The BOLD signals for all frequencies were averaged during stimulus duration and analyzed with the Kruskall–Wallis test, a non-parametric test for several independent samples. A comparison of the BOLD signals for each stimulus frequency was performed with the Mann–Whitney U-test, a nonparametric test for two independent samples. All the data have been represented as mean ± standard deviation (S.D.).

3. Results

Figure 1 shows the Student's *t*-test statistical images obtained at each stimulus frequency averaged for 3 repeated 4-s stimulations of the right forepaw. During the forepaw stimulation, significant variations were detected in the left somatosensory area contralateral to the stimulated side. The same area was activated at all the frequencies; however, the increase in the signal intensity in the activated area differed. For each stimulus frequency, repeated stimulation-induced BOLD signals were obtained from 5 voxels with the largest significant differences in the somatosensory area (Figure 1). Since similar signal changes were observed thrice with a 40-s stimulus interval for each stimulus frequency (Figure 1), the time courses of the signals were first averaged over 3 repeated stimulations and then across all the animals, as shown in Figure 2a. The BOLD signals obtained for all the stimulus frequencies revealed similar initial responses after the stimulus onset (Figure 2). The signal intensity began increasing 1.5 s after the stimulus onset. On the other hand, after the stimulus offset (4 s), the signal intensity at stimulus frequencies of 1, 3, and 5 Hz increased until 5–6 s, whereas that at the stimulus frequency of 10 Hz immediately returned to the baseline value. For all the stimulus frequencies, no initial dip and post-undershoot were observed in the rat somatosensory model. Figure 2b shows the time course of the BOLD signals averaged over all the animals for the 32-s stimulation. At each stimulus frequency, the initial increases in the BOLD response to the 32-s stimulation are identical to those to the 4-s stimulation; these responses begin to increase at 1.5 s after the stimulus onset. However, the responses after attaining the peak value differ across different stimulus frequencies. For the 1-Hz stimulation, the increased signal intensity remained almost stable at 82 ± 13% of the peak intensity during the period from 10 s till the end of the stimulation. However, for the 10-Hz stimulation, the signal intensity peaked at 4.2 ± 1.0 s and decreased to 36 ± 25% of the peak intensity during the period from 10 s till the end of the stimulation. During stimulation, high stimulus frequencies exhibited a greater rate of reduction in the BOLD signal intensity.

To compare the BOLD signals of the 4- and 32-s stimulations, the BOLD signals were averaged during stimulus duration (Figure 3). The averaged BOLD signals peaked at intermediate frequencies, i.e., 3 and 5 Hz, for the 4-s stimulations; the BOLD signals at the intermediate frequencies were significantly greater than those at the lowest (1 Hz) and highest (10 Hz) frequencies ($p < 0.05$) (Figure 3a). Although the BOLD

signals that were averaged for the 32-s stimulation exhibited a similar stimulus frequency dependence (Figure 3b), the peak frequency tended to shift lower; the significant difference was not detected between the lowest (1Hz) and 5 Hz frequencies ($p > 0.2$) and observed between the lowest (1 Hz) and highest (10 Hz) frequencies ($p < 0.05$).

4. Discussion

In this study, by using the rat somatosensory stimulation model, we investigated the stimulus frequency dependence of the BOLD signals obtained in response to the short (4 s) and long (32 s) stimulus durations. We observed that the BOLD signals peaked at the stimulus frequency of 3 or 5 Hz during both 4- and 32-s stimulations (Figure 3). The stimulus frequency dependence has been reported in previous studies that employed different techniques such as LDF (Ances et al., 1999; Detre et al., 1998; Matsuura and Kanno, 2001; Ureshi et al., 2004) for short stimulus durations such as the 4-s stimulus duration in our study. The CBF responses measured by LDF showed a peak at 5 Hz during the stimulation of the forepaw (Ances et al., 1999; Detre et al., 1998) and hindpaw (Matsuura and Kanno, 2001; Ureshi et al., 2004). This stimulus frequency dependence of the CBF response does not vary with stimulus currents, i.e., 1.0 mA (Ances et al., 1999; Detre et al., 1998), and 1.5 mA (Matsuura and Kanno, 2001; Ureshi et al., 2004). On the other hand, an optical imaging study conducted by performing hindpaw stimulations reported that no frequency peak was observed in the deoxygenated hemoglobin response; a monotonic decrease in the response from the lowest frequency (2 Hz) was observed when a current of 0.8 mA was applied during the 2-s stimulation (Sheth et al., 2004). Another similar study reported no differences between the 2–10 Hz stimuli when a current of 1.2 mA was applied during the 2-s stimulation (Sheth et al., 2005). These conflicting results, which reveal the absence of the peak stimulus frequency reported by optical imaging studies conducted by hindpaw stimulation, could be attributed to the differences in the stimulus pulse width (Van Camp et al., 2006) because the previous optical imaging used stimulus pulse width of 1.0 ms (Sheth et al., 2004; 2005) instead of 0.3 ms in the present study; however, the precise reason remains unknown.

Few studies have been conducted on the stimulus frequency dependence of BOLD signals in the rat somatosensory model. Ogawa et al. (2000) investigated the BOLD signals at 7 T during the forepaw stimulation at 2 stimulus frequencies—approximately 1.6 Hz (stimulus interval, 620 ms) and 3.2 Hz (stimulus interval, 310 ms); their results demonstrated that the BOLD signals at low stimulus frequencies (1.6 Hz) were larger that those at higher frequencies (3.2 Hz) for the same number of stimulus pulses. However, the stimulus duration of the 1.6-Hz stimulus is twice of the 3.2-Hz stimulus for the same number of stimulus pulses. By re-plotting the data of Ogawa et al. (2000) for the same stimulus duration, the BOLD signal was observed to be larger at 3.2 Hz. This frequency dependence of the BOLD signal is consistent with the results of the 4-s stimulation experiment.

With regard to the longer forepaw stimulation, previous fMRI studies on stimulus frequency dependence conducted by Gyngell et al. (1996) (40-s duration) and Brinker et al. (1999) (50-s duration) indicated that BOLD signals measured using the gradient-echo sequence at 4.7 T decreased with increase in the stimulus frequency from 1.5 Hz. On the other hand, studies conducted by using the spin-echo EPI sequence (Huttunen et al., 2008; Keilholz et al., 2004) showed that the BOLD signal measured at 4.7 and 11.7 T peaked at 3 Hz during the forepaw stimulation (30 to 45-s duration). These results and ours using the spin-echo sequence contradict those using the gradient-echo sequence (Gyngell et al., 1996; Brinker et al., 1999). Several studies have reported that spin-echo sequences with high magnetic field strengths have an advantage over gradient-echo sequences, that is, they allow the observation of local activation (Duong et al., 2003; Kida et al., 2000; Lee et al., 2002). The BOLD signals obtained using spin-echo sequences represent the following 3 components: (1) extravascular effect of the capillary bed, (2) intravascular effect of both the large and small blood vessels, and (3) absence of the transverse relaxation time (T_2) effect such as the inflow effect (Duong et al., 2003; Lee et al., 2002). At high magnetic field strengths, the T_2 value of blood is extremely low (e.g., 7 ms at 7 T, but 180 ms at 1.5 T). Hence, the intravascular effect is negligible for long echo time and at high magnetic field strengths. Studies on the echo time dependence of BOLD signals and the utilization of a diffusion gradient have revealed that the non-T_2 effect is very low at high magnetic field strengths (Duong et al., 2003; Lee et al., 2002). Therefore, BOLD signals obtained using spin-echo sequences at high magnetic field strengths mainly originate from the extravascular effect of the capillary bed, which provides a highly

accurate estimate of the activation area. On the other hand, the signals obtained using the gradient-echo sequences include the intravascular and extravascular effects of large draining veins or downstream venules at low magnetic field strengths such as 1.5 T or even at 4 T (Duong et al., 2003; Yacoub et al., 2005). Since the extravascular effect cannot be suppressed by gradient-echo sequences because of the absence of a refocus radiofrequency pulse, large venous vessels induce larger BOLD signals as compared to smaller vessels (Duong et al., 2003; Lee et al., 2002). The vascular responses in the activated capillary bed are regulated rapidly by smooth muscle bands at the branching points in the capillaries (Harrison et al., 2002; Vanzetta et al., 2005). However, the responses in the downstream venules and veins are slow (Vanzetta et al., 2005) since they are contaminated by the flow from the surrounding non-activated area; this flow is referred to as the watering-the-garden effect (Turner and Ordidge, 2000). Therefore, the stimulus frequency dependence of the vascular response in the capillary bed may differ from that in the downstream venules and veins. Since we obtained the BOLD signals by using spin-echo sequences at high magnetic field strengths, our results reflect the stimulus frequency dependence in the activation focus in the capillary bed. On the other hand, the extravascular effects of draining veins is dominant in gradient-echo EPI experiments at low magnetic field strengths; moreover, with a decrease in the signal-to-noise ratio, it becomes difficult to observe the effects of small vessels that reflect the activation focus. Therefore, the stimulus frequency dependence of the BOLD signals may vary with the magnetic field strength, particularly in gradient-echo EPI experiments. However, the BOLD signal peaked at an intermediate stimulus frequency during forepaw stimulation even in the gradient-echo EPI experiments at 7 T with a longer stimulus pulse width (i.e., 10 ms) (Van Camp et al., 2006). This stimulus frequency dependence may be explained on the basis of evidence suggesting that at high magnetic field strengths (>7 T), the contribution of large blood vessels decreases relative to the effect of small vessels (Duong et al., 2003; Yacoub et al. 2003); another explanation may be that neuronal responses vary according to the stimulus pulse width (Ahissar et al., 2001), which affects BOLD signals (Logothetis et al., 2001; Smith et al., 2002).

The effect of stimulus duration on the frequency dependence of the BOLD signals could be attributed to neuronal responses because responses in CBF and BOLD signal are correlated with the local field potential (Logothetis et al., 2001; Ngai et al., 1999, Ureshi et al., 2004). We postulate that "total neuronal response" is represented by the product of the averaged amplitude and the number of the neuronal responses during

stimulus duration. The averaged amplitude of neuronal response decreases with an increase in stimulus frequency due to the influence of adaptation (Sheth et al., 2003; 2004; Ureshi et al., 2004) (left chart in Fig. 4). On the other hand, the number of neuronal responses during stimulus duration proportionally increases with the stimulus frequency and duration (middle chart in Fig. 4). Therefore, the "total neuronal responses" have a peak in frequencies (right chart in Fig. 4). When the stimulus duration becomes longer, the number of neuronal responses increases and the averaged amplitude of neuronal responses during the stimulus duration rapidly decreases with stimulus frequency because the influence of adaptation becomes larger for longer stimulus duration. Consequently, a peak frequency of the "total neuronal responses" slightly shifts to a lower frequency for longer stimulus duration. Actually in the present study, the BOLD signals had a peak at intermediate frequencies, i.e., 3 and 5 Hz, for the 4-s stimulations, whereas the peak of the stimulus frequency for 32-s stimulation slightly shifted to the lower frequency (Fig. 3). It is noted that the frequency dependence of the "total neuronal response" and BOLD signal could be attributed to the variations in the resting baseline condition because the neuronal adaptation during stimulation is depended on the variations in anesthetic agents (Masamoto et al., 2007; Sheth et al., 2003; 2004).

In conclusion, BOLD signals obtained by spin-echo EPI at a magnetic field strength of 7 T peaked at an intermediate stimulus frequency. The stimulus duration showed little influence on the stimulus frequency dependence of the BOLD signals.

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FIGURE LEGENDS

Figure 1

Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) maps (left column) and time courses (right column) of the BOLD signals detected in the activation area of the rat somatosensory cortex during the short stimulus duration (4-s stimulation). The BOLD fMRI maps are obtained by Student's *t*-test performed on a pixel-by-pixel basis with the threshold ($p < 0.05$) by using the 4 images obtained before and after the stimulus onset; the maps are overlaid on the corresponding anatomical image. The time courses of the BOLD signals detected in the activation area of each rat brain at each stimulus frequency are averaged across 3 trials. The black bars in the time course indicate the stimulation period.

Figure 2

Time course of the blood oxygenation level-dependent (BOLD) signals at frequencies of 1, 3, 5, and 10 Hz during (a) 4-s and (b) 32-s stimulation. The time courses of the averaged BOLD signal in the somatosensory cortex at different stimulus frequencies during 4-s stimulation are obtained for a total of 72 responses from all the animals (n = 12). The time courses of the averaged BOLD signal during 32-s stimulation are obtained for a total of 12 responses from all the animals (n = 6). The stimulation period is marked in gray.

Figure 3

Stimulus frequency dependence of the blood oxygenation level-dependent (BOLD) signals. BOLD signals for (a) 4-s and (b) 32-s stimulations are averaged for the entire stimulation period. The BOLD signals for the 4- and 32-s stimulations were statistically different for all frequencies (Kruskal–Wallis, $p<0.01$). The error bars indicate the standard deviation (S.D.). Significant differences ($p < 0.05$) performed with the Mann–Whitney U-test are indicated by the asterisk symbol.

Figure 4

Model of stimulus frequency dependence of neuronal response. The averaged amplitude of neuronal response is adapted for repetitive stimuli to result in a decrease of neuronal response with an increase in stimulus

frequency (left chart). This tendency becomes large for longer stimulus duration, e.g. 32-sec stimulus duration (blue line) compared to shorter stimulus duration, e.g. 4-sec stimulus duration (red line). The number of neuronal response proportionally increases with increasing stimulus frequency and duration (middle chart). "Total neuronal response" during stimulus duration is represented by the product of the averaged amplitude and the number of the neuronal responses (right chart).

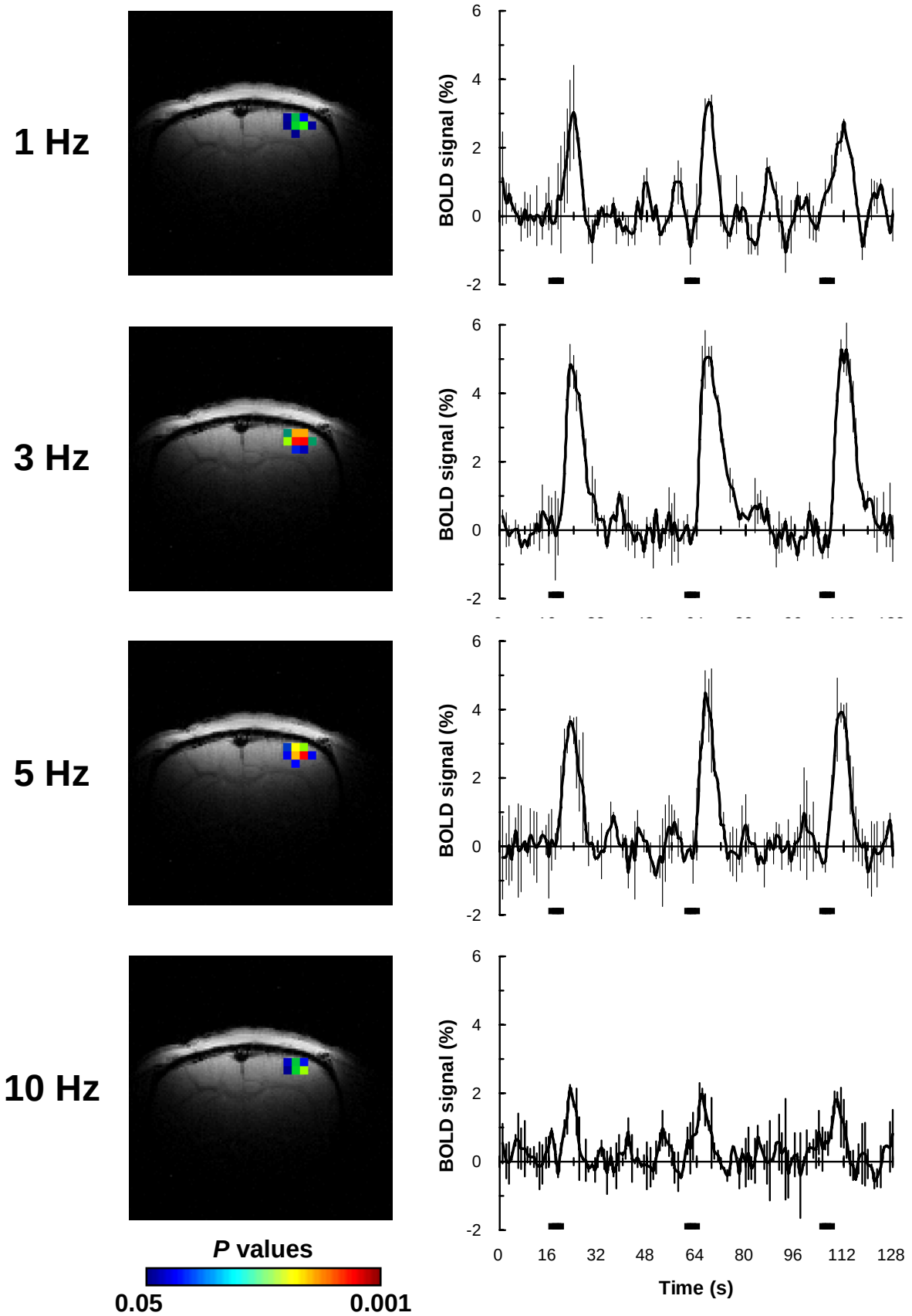

1 Hz
3 Hz
5 Hz
10 Hz
BOLD signal (%)
6
4
2
0
-2
0
16
32
48
64
80
96
112
128
Time (s)
P values
0.05
0.001

Figure 1

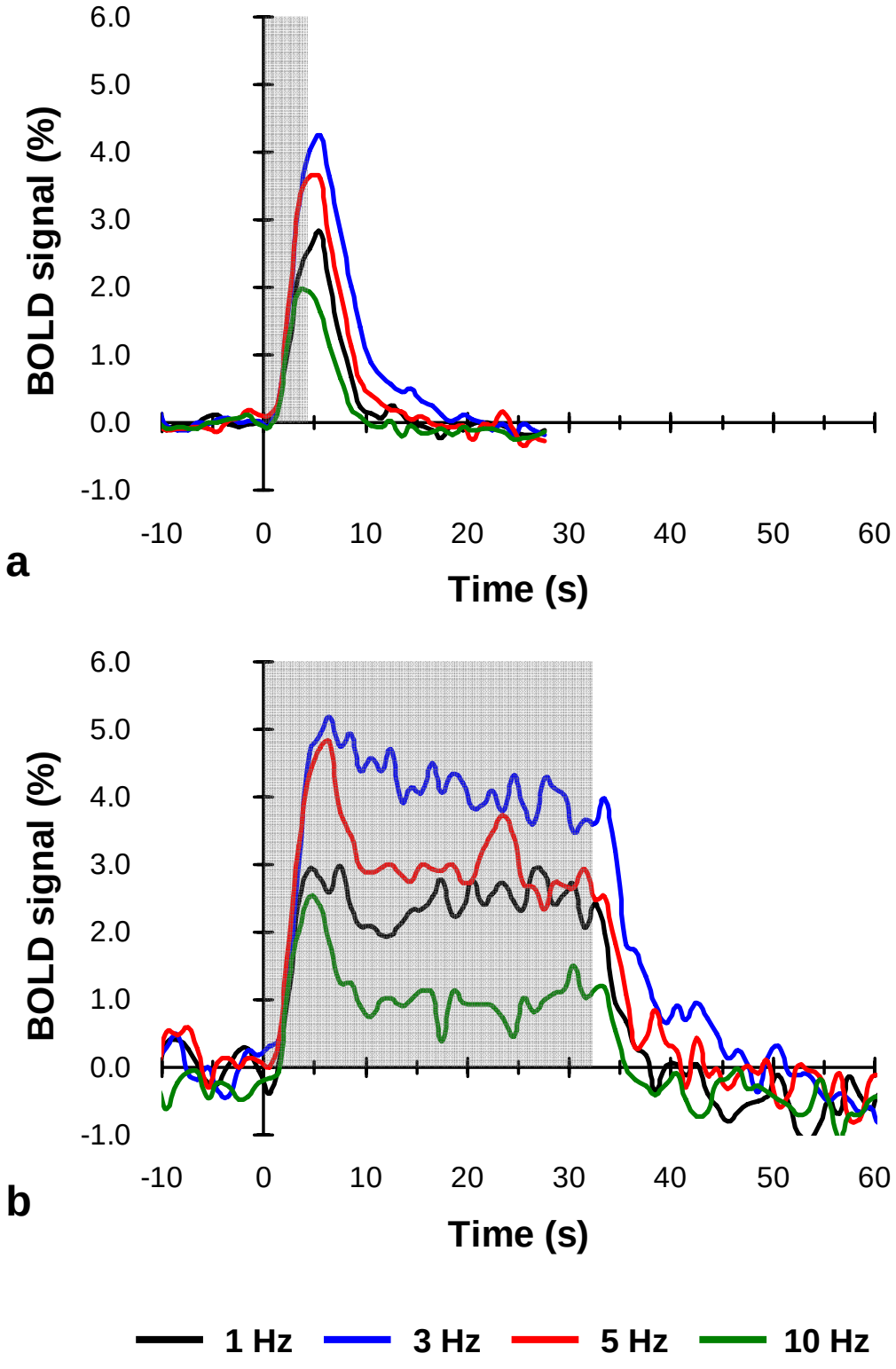
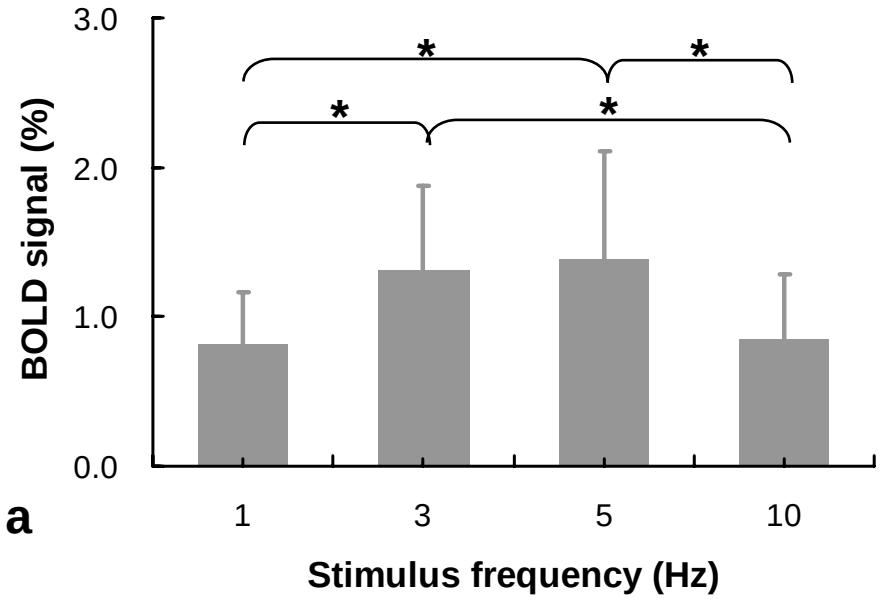
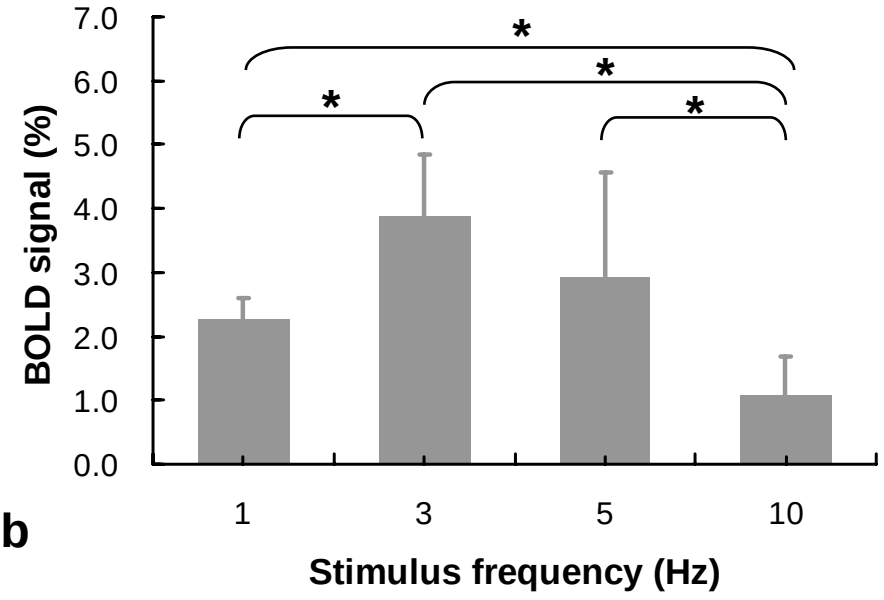

6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
BOLD signal (%)
-10
0
10
20
30
40
50
60
Time (s)
a
b
1 Hz
3 Hz
5 Hz
10 Hz

Figure 2


3.0
2.0
1.0
0.0
BOLD signal (%)
*
*
*
*
1
3
5
10
Stimulus frequency (Hz)

a


7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
BOLD signal (%)
*
*
*
*
1
3
5
10
Stimulus frequency (Hz)

b

Figure 3

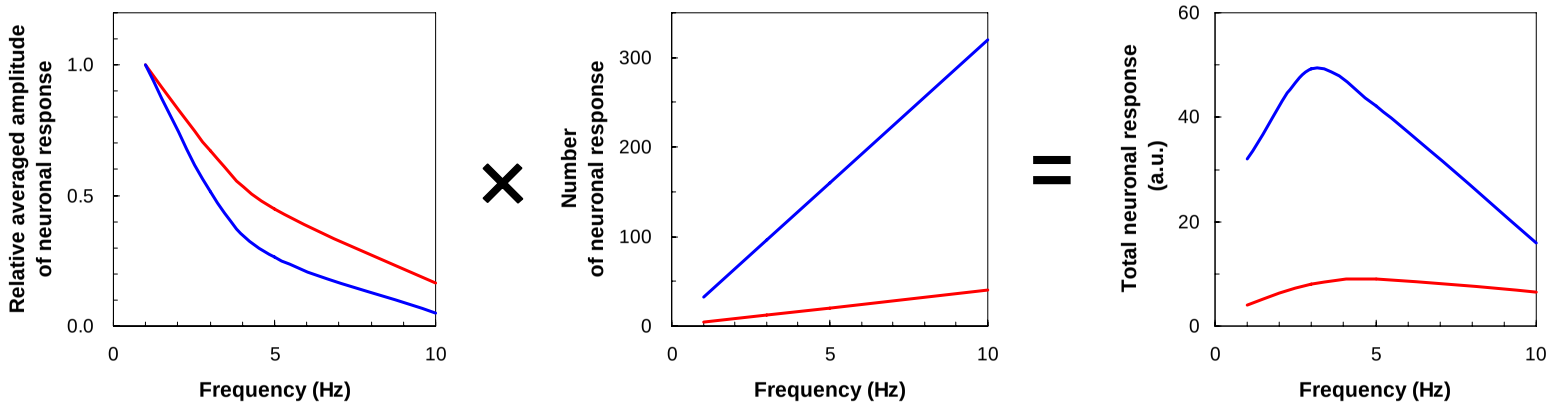

Relative averaged amplitude of neuronal response
1.0
0.5
0.0
0
5
10
Frequency (Hz)
×
Number of neuronal response
300
200
100
0
0
5
10
Frequency (Hz)
=
Total neuronal response (a.u.)
60
40
20
0
0
5
10
Frequency (Hz)

Figure 4