Comprehensive correlation between neuronal activity and spin-echo blood oxygenation level-dependent signals in the rat somatosensory cortex evoked by short electrical stimulations at various frequencies and currents

Ikuhiro Kida[1] and Toru Yamamoto[2]

[1]Integrated Neuroscience Research Team, Tokyo Institute of Psychiatry, Setagaya-ku, Tokyo 156-8585, Japan

[2]Division of Biomedical Engineering and Science, Graduate School of Health Sciences, Hokkaido University, Sapporo, Hokkaido 060-0812, Japan

Number of pages: 16

Number of tables and figures: tables, 0; figures, 3.

Corresponding author:

Ikuhiro Kida *Tel: +81-3-3304-5701*

Tokyo Institute of Psychiatry *Fax: +81-3-3329-8035*

2-1-8 Kamikitazawa, Setagaya-ku

Tokyo 156-8585, Japan *E-mail: kida-ik@igakuken.or.jp*

ABSTRACT

It is essential to elucidate the relationship between blood oxygenation level-dependent (BOLD) signals and neuronal activity for the interpretation of the functional magnetic resonance imaging (fMRI) signals; this relationship has been quantitatively investigated by animal studies measuring evoked potentials as indices of neuronal activity. Although most human fMRI studies employ the event-related task design, in which the stimulus duration is short, few studies have investigated the relationship between BOLD signals and evoked potentials at short stimulus durations. The present study investigated this relationship in the somatosensory cortex of anesthetized rats by using electrical forepaw stimulation at a short duration of 4 s and comprehensively analyzed it at different frequencies (1–10 Hz) and currents (0.5–2.0 mA). Somatosensory evoked potential (SEP) responses were measured at the scalp using silver ball electrodes. The sum of the peak-to-peak amplitude (ΣSEP) and average SEP (avg. SEP) responses were calculated. BOLD signals were obtained using a spin-echo echo-planar imaging sequence at 7 T. The relationship between the avg. SEP and BOLD signals varied with frequency, whereas that between ΣSEP and BOLD signals showed a significant correlation at varying frequencies and currents. In particular, the relationship between ΣSEP and ΣBOLD, which is the sum of the BOLD signals obtained at each time point reflecting the area under the BOLD response curves, mostly converged, irrespective of the frequency. Our results suggest that ΣBOLD obtained using a spin-echo sequence reflects the neural activity as quantified by ΣSEP, which was determined at different frequencies and currents.

Section: Regulatory Systems

Keywords: BOLD; fMRI; Forepaw; Neurovascular coupling; SEP

Abbreviations: BOLD, blood oxygenation level-dependent; CBF, cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen consumption; EPI, echo planar imagining; TE, echo time; fMRI, functional magnetic resonance imaging; LDF, laser Doppler flowmetry; SEP, somatosensory evoked potential; T_2, transverse relaxation time

1. Introduction

Functional magnetic resonance imaging (fMRI) based on blood oxygenation level-dependent (BOLD) effect has been widely used in neuroscience and psychology to study brain function. fMRI reflects cerebral hemodynamic changes, and its signals have been interpreted by assuming a neuronal hemodynamic response (Friston et al., 1995) based on the 19th century hypothesis of Roy and Sherrington that neuronal activity induces an increase in the regional cerebral blood flow (CBF) (Roy and Sherrington, 1890). Thus, fMRI is an indirect method for assessing neuronal activity. Therefore, it is essential to elucidate the relationship between BOLD signals and neuronal activity for interpreting the fMRI signals; this relationship has been investigated mainly by animal studies measuring evoked potentials as indices of the neuronal activity in the somatosensory cortex (Brinker et al., 1999; Van Camp et al., 2006; Goloshevsky et al., 2007; Huttunen et al., 2008; Sanganahalli et al., 2009). Although most of the human fMRI studies employ the event-related task design that has a short stimulus duration, animal studies generally investigate the relationship between BOLD signals and evoked potentials with a stimulus duration of several tens of seconds (Brinker et al., 1999; Van Camp et al., 2006; Goloshevsky et al., 2007; Huttunen et al., 2008; Sanganahalli et al., 2009). Unlike BOLD studies, numerous optical studies performed using laser Doppler flowmetry (LDF) (Ngai et al., 1999; Matsuura and Kanno, 2001; Ureshi et al., 2004, 2005) and optical imaging (Devor et al., 2003; Martindale et al., 2003; Sheth et al., 2003, 2004; Jones et al., 2004; Hewson-Stoate et al., 2005; Martin et al., 2006) have investigated the hemodynamic responses such as CBF and deoxygenated hemoglobin changes to neuronal activity in the rat cortex with a short stimulus duration (2–5 s) (Matsuura and Kanno, 2001; Martindale et al., 2003; Sheth et al., 2003, 2004; Jones et al., 2004; Ureshi et al., 2004, 2005; Hewson-Stoate et al., 2005; Martin et al., 2006). Thus, an animal fMRI study with short stimulus duration is expected to bridge the results of the optical studies using short stimulus durations and those of the BOLD studies using long stimulus durations.

Furthermore, most of the optical studies performed using electrical stimulation varied either in frequency (Ngai et al., 1999; Matsuura and Kanno, 2001; Martindale et al., 2003; Sheth et al.,

2003; Ureshi et al., 2004), or current (Jones et al., 2004, Ureshi et al., 2005), or both (Sheth et al., 2004; Hewson-Stoate et al., 2005). Animal fMRI studies on somatosensory activation investigated the relationship between BOLD signals and neuronal activities by changing the stimulus frequency while maintaining a fixed current strength (Brinker et al., 1999; Van Camp et al., 2006; Goloshevsky et al., 2007; Huttunen et al., 2008; Sanganahalli et al., 2009), and few fMRI studies have comprehensively investigated this relationship at different stimulus frequencies and currents.

Thus, the present study aimed to investigate the relationship between BOLD signals and neuronal activities in the somatosensory cortex of anesthetized rats for forepaw electrical stimulation at a short duration of 4 s by changing both frequencies (1–10 Hz) and currents (0.5–2.0 mA). BOLD signals with a spin-echo echo planar imagining (EPI) sequence at 7 T and scalp-based somatosensory evoked potentials (SEP) were obtained from individual rats.

2. Results

The BOLD fMRI activity during the electrical stimulation of the forepaw was observed in the somatosensory cortex contralateral to the forepaw stimulation. The mean time course of changes in BOLD signals from the 5 most activated pixels in the somatosensory cortex is shown in Fig. 1A. The BOLD signal intensity increased approximately 1.0 s after the onset of stimulation and continued to increase even after the offset of stimulation. Higher stimulation currents were associated with long-lasting post-offset changes in the BOLD signals. Neither an initial dip nor a post undershoot were observed for any stimulation frequencies or currents. Fig. 1B shows how the BOLD signals varied with changes in stimulation frequencies and currents. BOLD signal values (avg. BOLD and ΣBOLD) increased monotonically with an increase in the stimulation current for any stimulation frequency; this increase was significant (Friedman's test $\chi^2 > 8.22$, df = 2, $p < 0.05$). The peak of the avg. BOLD was observed at either 3 Hz (Wilcoxon's test $Z > 2.07$, $p < 0.05$ for 1 and 10 Hz) or 5 Hz (Wilcoxon $Z > 2.19$, $p < 0.05$ for 1 and 10 Hz) for both stimulation currents of 1.0 and 2.0 mA, although there was no significant difference between the stimulus frequencies of 3 and 5 Hz ($p > 0.3$). On the other hand, the peaks of ΣBOLD appeared at 3 Hz for 1.0 mA

(Wilcoxon Z = 2.67, $p < 0.01$ for 1, 5, and 10 Hz) and 2.0 mA (Wilcoxon Z = 2.67, $p < 0.01$ for 1 and 10 Hz and Wilcoxon Z = 1.84, $p < 0.07$ for 5 Hz) (Fig. 1B). Although not statistically significant (Friedman's test $\chi^2 < 7.13$, df = 3, $p > 0.05$), BOLD signals associated with 0.5-mA stimulation also appeared to peak at 3 Hz.

SEP amplitude during the stimulation of the forepaw is shown in Fig. 2. For a stimulation frequency of 1 Hz, the SEP amplitude remained constant throughout the 4-s stimulation period (Fig. 2A). For 3 Hz, there was a gradual decline in the amplitude from the initial SEP, and for both 5 and 10 Hz, there was almost an immediate inhibition reaching a steady-state level (Fig. 2A). For higher stimulus frequencies, the SEP amplitudes showed an oscillating pattern attributed to inhibitory mechanisms that have been observed by other researchers (Matsuura and Kannno 2001; Herman et al., 2009). The avg. SEP and ΣSEP values with changes in stimulation frequencies and currents are shown in Fig. 2B. Both avg. SEP and ΣSEP increased significantly with an increase in the stimulation current strength for any stimulation frequency (Friedman's test $\chi^2 > 14.22$, df = 2, $p < 0.01$). However, the frequency dependence differed greatly. The avg. SEP values decreased with an increase in the stimulation frequency for all stimulus currents: adjacent values along stimulus frequency differed significantly (Friedman's test $\chi^2 = 27$, df = 3, $p < 0.01$) (Fig. 2B). On the other hand, the ΣSEP values significantly peaked at 3 Hz for 1.0 and 2.0 mA (Wilcoxon Z > 2.54, $p < 0.05$) (Fig. 2B). This frequency dependence of ΣSEP is in agreement with the results for the BOLD signals (Fig. 1B).

Fig. 3 shows the relationship between the BOLD signals and SEP amplitudes for different stimulus frequencies and currents. Although there was a linear correlation between avg. SEP and the BOLD signals for each stimulation frequency (Fig. 3A and 3B), the slopes differed. On the other hand, the slopes for ΣSEP and a BOLD signal at each stimulus frequency tended to converge (Fig. 3C and 3D). Furthermore, there were significant correlations between ΣSEP and the BOLD signals for all the data points of stimulus condition: avg. BOLD (r = 0.932) and ΣBOLD (r = 0.992). The correlation coefficient of ΣBOLD was significantly better than that of avg. BOLD (comparison of 2 correlation coefficient, $p < 0.05$). Thus, there was a converged correlation between ΣSEP and ΣBOLD, irrespective of the stimulus frequency and current.

3. Discussion

We demonstrated a comprehensive correlation between the neural activity and BOLD signals in the rat somatosensory cortex during the forepaw electrical stimulation for a short duration (4 s) by changing both stimulation currents and frequencies (Fig. 3). Although there have been few reports on the electrical somatosensory stimulation for short durations like our study, several researchers have investigated the relationship between the BOLD signals and neuronal activity with long duration of electrical somatosensory stimulation (Brinker et al., 1999; Van Camp et al., 2006; Goloshevsky et al., 2007; Huttunen et al., 2008). They measured the neuronal activity using SEP (Brinker et al., 1999; Van Camp et al., 2006; Goloshevsky et al., 2007) or local field potential (LFP) (Huttunen et al., 2008). Our results support the findings of previous studies that demonstrate a linear relationship between neuronal activity and BOLD signals obtained by varying the stimulus frequency (Van Camp et al., 2006; Huttunen et al., 2008). We have elucidated the relationship between neural activity and BOLD signals for not only stimulus frequency but also stimulus current. Evidence that the convergence of the slopes improved dramatically for ΣSEP implies that ΣSEP is a better index than avg. SEP to represent the intensity of neuronal activity (Fig. 3). This is understood by the relation between avg. SEP and ΣSEP: avg. SEP = ΣSEP/(4 × frequency). ΣSEP is the sum of the neuronal responses for all events, i.e., for every 0.3-ms electrical stimulation pulse in our study, whereas avg. SEP does not explicitly reflect the number of events during the stimulation (4 × frequency). Accordingly, avg. SEP decreases from a good index (ΣSEP) with an increase in stimulus frequency, resulting in an increase in the slopes as shown in Fig. 3A and 3B (BOLD signal versus avg. SEP). On the other hand, the slopes in Fig. 3C and 3D (BOLD signal versus ΣSEP) tended to converge. In particular, ΣBOLD correlated more strongly with ΣSEP ($r = 0.992$), and this correlation was represented by a unique slope irrespective of the stimulus frequency. Therefore, the results in the present study suggest that ΣBOLD strongly reflects the neuronal activity quantified by ΣSEP.

The relationship between neuronal activity (e.g., summation of SEP or field potentials) and hemodynamic response (e.g., summation or maximum of deoxygenated hemoglobin or CBF) during short stimulus duration has been investigated previously with optical imaging and LDF. By manipulating the frequency of stimulation at a fixed current strength, these studies found a linear correlation between neuronal activity and hemodynamic response (Matsuura 2001; Martindale 2003; Sheth 2003; Ureshi 2004). On the other hand, the studies in which stimulation current was manipulated found a non-linear relationship (Sheth 2004; Ureshi 2005). The study in which both stimulus frequency and current were changed also showed a non-linear relationship (Hewson-Stoate et al., 2005). To understand the reason for the linear or non-linear relationship, we introduced the intensity of the stimulation pulse (ISP): the product of the stimulation current and stimulation pulse width. We observed a linear relationship between ΣSEP and BOLD signals for the ISP range of 0.15–0.6 μA•s. The relationship between neuronal activity and hemodynamic response tends to be non-linear in the ISP range, higher or lower than that used in our study. Sheth et al. (2004) employed the ISP range of 0.4–1.2 μA•s and found a non-linear relationship showing greater hemodynamic responses at higher ISPs than ours. However, their relationship was linear at lower ISPs (i.e., 0.4–0.6 μA•s) that were within the ISP ranges used in our study. In contrast, Hewson-Stoate et al. (2005) employed the lower ISP range of 0.06–0.48 μA•s and found a non-linear (power law) relationship between the maximal CBF and integrated field potentials. In their case, the data in our ISP range seemed to be linear. Another study with a lower ISP range of 0.10–0.25 μA•s found a non-linear relationship between integrated CBF and field potential (Ureshi et al., 2004). Thus, the type of the relationship between neuronal activity and its responses is roughly classified on the basis of ISP, and our results showing the linear relationship do not contradict those of previous studies using optical techniques.

SEP amplitude is mainly attributed to the synchronized extracellular currents induced by the total postsynaptic potentials (Nunez, 1981; Lopes da Silva, 1991), which are associated with the release of neurotransmitters (Lopes da Silva, 1991; Kida et al., 2006). Following the synaptic activity, the reestablishment of ionic concentration gradients and the uptake of glutamate in astrocytes as well as neurons occur, leading to demand majority of cerebral oxygen consumption

(Attwell and Laughlin, 2001; Hyder et al., 2006). This is supported by the evidence that the local field-evoked potential is attenuated by blocking glutamate release during forepaw stimulation (Kida et al., 2001, 2006). This decrease in field-evoked potential is linearly related with the decrease in the evoked $CMRO_2$ as well as BOLD signal and CBF (Kida et al., 2001, 2006), consistent with the result that the activation-dependent release of glutamate and the glutamate-glutamine cycles require a proportionate oxidative energy consumption (Sibson et al., 1998). Furthermore, a linear relationship between BOLD signals and oxygen consumption associated with cortical activation produced by either physiological perturbations or forepaw stimulation has been reported (Kida et al., 2000; Sanganahalli et al., 2009; Herman et al., 2009). These findings regarding cerebral oxygen consumption that correlate with both SEP and the BOLD signal support the correlation between BOLD signal and SEP amplitude obtained in the present study.

The spin-echo BOLD signals at a higher magnetic field originate largely from the capillaries in the activated cortex (Yacoub et al., 2003) because the spin-echo refocuses the dephasing of the spin around the pial draining vein, and the intravascular effect is negligible due to extremely short transverse relaxation time (T_2) of the blood (Uludağ et al., 2009). Therefore, the level of blood oxygenation in capillaries could be monitored by the spin-echo BOLD signals at a higher magnetic field. Since oxygen delivery rate to the tissue is proportional to the level of blood oxygenation in capillaries (Hyder et al., 1998), the spin-echo BOLD signal at each time point reflects the rate at which oxygen is delivered to the cortex. Thus, ΣBOLD, regarded as the integral part of the BOLD signal, represents the total oxygen delivered to the tissue. On the other hand, the SEP linearly correlates with oxidative energy consumption as aforementioned, and ΣSEP represents the total oxygen consumption by the neuronal activity. Evidence of a good correlation between ΣBOLD and ΣSEP (Fig. 3D) suggests that ΣBOLD reflects the total oxygen consumption by the neuronal activity, supporting that ΣBOLD represents the total oxygen delivered to and consumed by the tissue. Oxygen is delivered to the cortex by oxygen-concentration-dependent-cerebrovasucular control (Gordon et al., 2008) until the consumed energy is compensated (Sanganahalli et al., 2009; Herman et al., 2009), because the reestablishment of ionic concentration gradients in mainly astrocytes continues even after the offset of stimulation (Wang et al., 2006; Schummers et al.,

2008). Therefore, ΣBOLD, representing the total oxygen consumption by the neuronal event, is a better index of neuronal activity than avg. BOLD, especially for short stimulation duration. The studies at 11.7 T using the gradient-echo EPI have shown nearly the same linearity between the BOLD signals and neuronal activity measured by LFP and multiunit activity (Sanganahalli et al., 2009; Herman et al., 2009). Because they set the ROI (region of interest) in the middle cortical layers, thereby reducing the influence of the magnetic field distortion due to the pial draining vein, the gradient-echo BOLD signals in capillaries were enhanced to yield similar results as were obtained in the present study using spin-echo EPI. Since the influences of the draining vein on the gradient-echo BOLD signal at a higher magnetic field are persistent, further studies are required to elucidate the gradient-echo BOLD signal while taking these influences into consideration.

In summary, in the rat forepaw model with a short stimulus duration, there is a unique comprehensive correlation between BOLD signals (ΣBOLD) and neuronal activity (ΣSEP) for different frequencies and currents; the BOLD signals measured by a spin-echo EPI sequence at a high magnetic field. Thus, ΣBOLD may quantitatively reflect neural activity, and thereby help in the interpretation of fMRI signals in human studies.

4. Experimental procedures

4.1. Animal preparation

Male Sprague-Dawley rats weighing 244 ± 44 g were used (n = 9). These rats were anesthetized by using 4% isoflurane in a 70%/30% N_2O/O_2 mixture and tracheotomized: they were mechanically ventilated with 2% isoflurane in 70%/30% N_2O/O_2 throughout the surgical procedure. The femoral artery and vein were cannulated using a polyethylene catheter (PE-50 and PE-10) to enable blood sampling for gas analysis, blood pressure monitoring, and pancuronium bromide administration. Intraperitoneal catheters (PE-50) were placed for administering α-chloralose. Arterial blood gas levels were checked periodically, and respiratory volume was adjusted to maintain normal gas levels (pH: 7.42 ± 0.06; pCO_2: 34.1 ± 4.6 mmHg; pO_2: 194 ± 45 mmHg).

Whiskers were cut to prevent any MRI signals arising from them. Animals were placed on and covered by a heated water pad to maintain an appropriate body temperature (37 ± 0.5°C), and the head was secured with a stereotaxic frame. Anesthesia was maintained with a periodical α-chloralose supplement (40 mg/kg/h) and an i.v. injection of pancuronium bromide (1 mg/kg) administered every hour to prevent movements. Rectal temperature, arterial blood pressure, and heart rate were continuously monitored by a PC. Upon completion of the procedures, isoflurane anesthesia was discontinued and an i.p. bolus of α-chloralose (80 mg/kg) was administered. All the animal procedures were approved by the Hokkaido University Institutional Animal Care and Use Committee.

4.2. Forepaw stimulation

Electrical stimulation was achieved via 2 needle electrodes inserted beneath the skin of the right forepaw (between digits 1 and 2 and digits 3 and 4). A function generator (PowerLab, ADInstruments, USA) was used to control the stimulation frequency and current via a constant-current dense stimulus isolation device (Nihon Koden, Japan). Rectangular electrical pulses of 0.3-ms duration were applied for 4 s at fixed currents of 0.5, 1.0, and 2.0 mA. For each of these currents, stimulation was applied at 4 fixed frequencies: 1, 3, 5, and 10 Hz (yielding 12 different stimulation conditions). Currents above 2.0 mA were not used because of associated changes in blood pressure (Ureshi et al., 2005) that could influence the results. A block-design stimulus paradigm was preceded by a 16-s control condition: the 4-s stimulation period was repeated 3 times with an inter-stimulus interval of 40 s. It has been reported that a 40-s inter-stimulus interval prevents habituation of the BOLD signals (Kida and Yamamoto, 2008). The SEP and fMRI experiments with the aforementioned stimulus paradigm for each stimulus frequency and current were repeated to obtain 2 data sets (total 6 stimulations), which were free from confounding influences such as the movement of the rat head.

4.3. fMRI experiments

fMRI experiments were performed using a 7-T horizontal bore spectrometer interfaced with a Varian Unity^{INOVA} (Varian Inc., USA): this system uses a 110-mm-diameter shielded gradient coil with a maximum gradient field strength of 120 mT/m. Radiofrequency pulses were transmitted and MRI signals were received via a 10-mm surface coil placed over the somatosensory region (i.e., around the bregma). High-resolution coronal gradient-echo images with a 20-mm field of view and 1.0-mm slice thickness were acquired as scout images. After positioning the functional imaging slice, slice shimming was performed, first automatically and then manually. For the BOLD fMRI experiments, single-shot spin-echo EPI images were acquired with a data matrix of 32 × 32, field of view of 20 × 20 mm, slice thickness of 2 mm, echo time (TE) of 40 ms, and repetition time of 1 s. Since the T_2-dependent signal changes are the most sensitive mathematically when the TE is set to the resting T_2 value and the value in the rat somatosensory cortex under deep anesthetic condition like this experiment is 40 ms (Kida et al., 2000), we employed 40 ms for the TE.

4.4. Electrophysiological experiments

Electroencephalography was performed using silver/silver chloride ball electrodes placed on both the sides of the scalp above the somatosensory cortex: the reference electrode was inserted in the depilated skin of the nose. Signals were amplified 1000 times (ER-1 amplifier; Cygnus Tech. Inc., USA), band-pass filtered (0.5–500 Hz), digitized by PowerLab software (ADInstruments, USA) at a sampling rate of 1 kHz, and transferred to a PC for display and storage. An evoked potential was obtained for each 0.3-ms stimulation pulse, and P1 (the maximum voltage) and N1 (the minimum voltage) were measured to determine the SEP amplitude (P1-N1). P1 and N1 appeared 0–20 ms and 10–30 ms after the stimulation pulse, respectively. When these peaks were below the level of spontaneous activity, the SEP amplitude was considered to be zero in the subsequent analysis. The sum of the SEP amplitudes (ΣSEP) and average SEP (avg. SEP) were calculated for each 4-s stimulation period, and the SEP amplitudes were averaged for each

stimulation frequency and current (6 stimulations per condition per animal in 9 animals yielded a total of 54 stimulations at each frequency and current).

4.5. Data analysis

BOLD fMRI data were processed using MATLAB (Math Works Inc., USA) and an in-house software. Images with head-movement artifacts (identified by center-of-mass analysis) were excluded from the analyses. Functional images were created as Student's *t*-test statistical images on a pixel-by-pixel basis, by using the images obtained before and after the onset of stimulation. Activated regions were defined as pixels showing a statistically significant response ($p < 0.05$). The 5 most activated pixels appeared with a stimulation current of 2.0 mA, and the BOLD signals associated with these pixels were averaged for each stimulation frequency and current (6 stimulations per condition per animal in 9 animals yielded a total of 54 stimulations at each frequency and current). We analyzed BOLD signals in the following 2 ways: avg. BOLD indicates the average value for a 4-s stimulation period, and ΣBOLD is the summation of the BOLD signals at each time point reflecting the area under the BOLD response curves.

The BOLD signals (avg. BOLD and ΣBOLD) and SEP amplitudes (avg. SEP and ΣSEP) at each stimulus frequency and intensity were averaged in all animals. Difference in each value along frequency and current was tested by Friedman's test, and the existence of peak along frequency in the BOLD signal and the SEP amplitude was tested by Wilcoxon's test (Figs. 1B and 2B). The correlation coefficients between ΣSEP and the BOLD signals in Fig. 3C–D were statistically analyzed. Data are presented as mean ± standard error.

Acknowledgments

The authors express their gratitude to Eiji Yamada, an engineer at the Nuclear Magnetic Resonance Laboratory at Hokkaido University, for his technical support. This work was supported by grants from JSPS KAKENHI (21791803 to I.K.; 18500339 and 21613001 to T.Y.).

References

Attwell, D., Laughlin, S.B., 2001. An energy budget for signaling in the grey matter of the brain. J. Cereb. Blood Flow Metab. 21, 1133-1145.

Brinker, G., Bock, C., Busch, E., Krep, H., Hossmann, K.A., Hoehn-Berlage, M., 1999. Simultaneous recording of evoked potentials and T2*-weighted MR images during somatosensory stimulation of rat. Magn. Reson. Med. 41, 469–473.

Devor, A., Dunn, A.K., Andermann, M.L., Ulbert, I., Boas, D.A., Dale, A.M., 2003. Coupling of total hemoglobin concentration, oxygenation, and neural activity in rat somatosensory cortex. Neuron 39, 353-359.

Friston, K.J., Holmes, A.P., Poline, J.B., Grasby, P.J., Williams, S.C., Frackowiak, R.S., Turner, R., 1995. Analysis of fMRI time-series revisited. NeuroImage 2, 45-53.

Goloshevsky, A.G., Silva, A.C., Dodd, S.J., Koretsky, A.P., 2007. BOLD fMRI and somatosensory evoked potentials are well correlated over a broad range of frequency content of somatosensory stimulation of the rat forepaw. Brain Res. 1195, 67-76.

Gordon, G.R., Choi, H.B., Rungta, R.L., Ellis-Davies, G.C., MacVicar, B.A., 2008. Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature 456, 745-749.

Herman, P., Sanganahalli, B.G., Blumenfeld, H., Hyder, F., 2009. Cerebral oxygen demand for short-lived and steady-state events. J. Neurochem. 109 Suppl 1, 73-79.

Hewson-Stoate, N., Jones, M., Martindale, J., Berwick, J., Mayhew, J., 2005. Further nonlinearities in neurovascular coupling in rodent barrel cortex. NeuroImage 24, 565–574.

Huttunen, J.K., Gröhn, O., Penttonen, M., 2008. Coupling between simultaneously recorded BOLD response and neuronal activity in the rat somatosensory cortex. NeuroImage 39, 775-785.

Hyder, F., Shulman, R.G., Rothman, D.L., 1998. A model for the regulation of cerebral oxygen delivery. J. Appl. Physiol. 85, 554-564.

Hyder, F., Patel, A.B., Gjedde, A., Rothman, D.L., Behar, K.L., Shulman, R.G., 2006. Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. J. Cereb. Blood Flow Metab. 26, 865-877.

Jones, M., Hewson-Stoate, N., Martindale, J., Redgrave, P., Mayhew, J., 2004. Nonlinear coupling of neural activity and CBF in rodent barrel cortex. NeuroImage 22, 956-965.

Kida, I., Kennan, R.P., Rothman, D.L., Behar, K.L., Hyder, F., 2000. High-resolution $CMRO_2$ mapping in rat cortex: a multiparametric approach to calibration of BOLD image contrast at 7 Tesla. J. Cereb. Blood Flow Metab. 20, 847-860.

Kida, I., Hyder, F., Behar, K.L., 2001. Inhibition of voltage-dependent sodium channels suppresses the functional magnetic resonance imaging response to forepaw somatosensory activation in the rodent. J. Cereb. Blood Flow Metab. 21, 585–591.

Kida, I., Smith, A.J., Blumenfeld, H., Behar, K.L., Hyder, F., 2006. Lamotrigine suppresses neurophysiological responses to somatosensory stimulation in the rodent. NeuroImage 29, 216-224.

Kida, I., Yamamoto, T., 2008. Stimulus frequency dependence of blood oxygenation level-dependent functional magnetic resonance imaging signals in the somatosensory cortex of rats. Neurosci. Res. 62, 25-31.

Lopes da Silva F., 1991. Neural mechanisms underlying brain waves: from neural membranes to networks. Electroencephalogr. Clin. Neurophysiol. 79, 81-93.

Martin, C., Martindale, J., Berwick, J., Mayhew, J., 2006. Investigating neural-hemodynamic coupling and the hemodynamic response function in the awake rat. NeuroImage 32, 33-48.

Martindale, J., Mayhew, J., Berwick, J., Jones, M., Martin, C., Johnston, D., Redgrave, P., Zheng, Y., 2003. The hemodynamic impulse response to a single neural event. J. Cereb. Blood Flow Metab. 23, 546-555.

Matsuura, T., Kanno, I., 2001. Quantitative and temporal relationship between local cerebral blood flow and neuronal activation induced by somatosensory stimulation in rats. Neurosci. Res. 40, 281-290.

Ngai, A.C., Jolley, M.A., D'Ambrosio, R., Meno, J.R., Winn, H.R., 1999. Frequency-dependent changes in cerebral blood flow and evoked potentials during somatosensory stimulation in the rat. Brain Res. 837, 221–228.

Nunez, P.L., 1981. A study of origins of the time dependencies of scalp EEG: II--experimental support of theory. IEEE Trans Biomed. Eng. 28, 281-288.

Roy, C.S., Sherrington, C.S., 1890. On the regulation of the blood supply of the brain. J. Physiol. 11, 85-108.

Sanganahalli, B.G., Herman, P., Blumenfeld, H., Hyder, F., 2009. Oxidative neuroenergetics in event-related paradigms. J. Neurosci. 29, 1707-1718.

Schummers, J., Yu, H., Sur, M., 2008. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science 320, 1638-1643.

Sheth, S.A., Nemoto, M., Guiou, M., Walker, M., Pouratian, N., Toga, A.W., 2004. Linear and nonlinear relationships between neuronal activity, oxygen metabolism, and hemodynamic responses. Neuron 42, 347–355.

Sheth, S., Nemoto, M., Guiou, M., Walker, M., Pouratian, N., Toga, A.W., 2003. Evaluation of coupling between optical intrinsic signals and neuronal activity in rat somatosensory cortex. NeuroImage 19, 884-94.

Shulman, R.G., Rothman, D.L., 1998. Interpreting functional imaging studies in terms of neurotransmitter cycling. Proc. Natl. Acad. Sci. U.S.A. 95, 11993-11998.

Sibson, N.R., Dhankhar, A., Mason, G.F., Rothman, D.L., Behar, K.L., Shulman, R.G., 1998. Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc. Natl. Acad. Sci. U.S.A. 95, 316-321.

Van Camp, N., Verhoye, M., Van der Linden, A., 2006. Stimulation of the rat somatosensory cortex at different frequencies and pulse widths. NMR Biomed. 19, 10-17.

Uludağ, K., Müller-Bierl, B., Uğurbil, K., 2009. An integrative model for neuronal activity-induced signal changes for gradient and spin echo functional imaging. NeuroImage 48, 150-165.

Ureshi, M., Matsuura, T., Kanno, I., 2004. Stimulus frequency dependence of the linear relationship between local cerebral blood flow and field potential evoked by activation of rat somatosensory cortex. Neurosci. Res. 48, 147–153.

Ureshi, M., Kershaw, J., Kanno, I., 2005. Nonlinear correlation between field potential and local cerebral blood flow in rat somatosensory cortex evoked by changing the stimulus current. Neurosci. Res. 51, 139-145.

Wang, X., Lou, N., Xu, Q., Tian, G.F., Peng, W.G., Han, X., Kang, J., Takano, T., Nedergaard, M., 2006. Astrocytic Ca^{2+} signaling evoked by sensory stimulation in vivo. Nat. Neurosci. 9, 816-823.

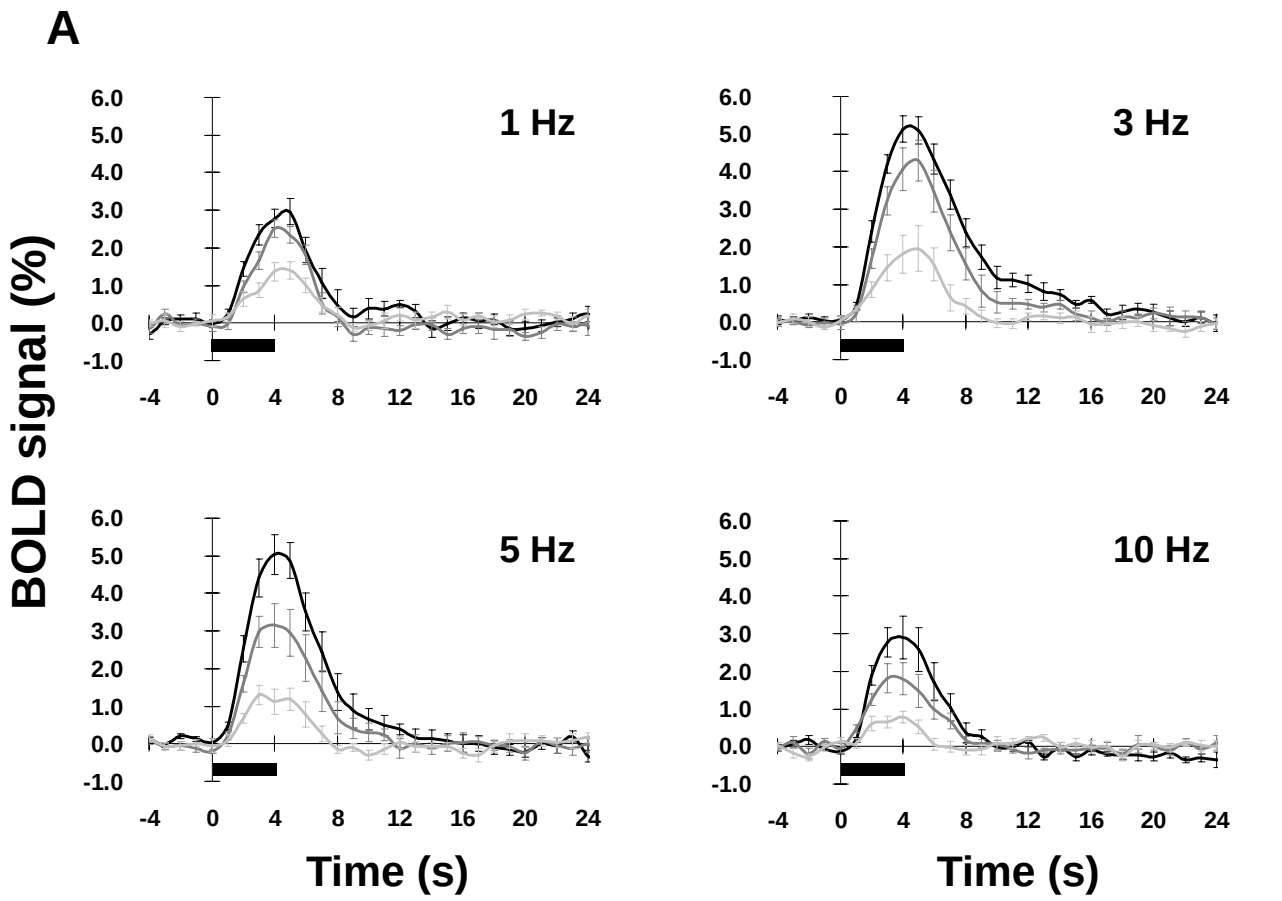
Yacoub, E., Duong, T.Q., Van De Moortele, P.F., Lindquist, M., Adriany, G., Kim, S.G., Uğurbil, K., Hu, X., (2003) Spin-echo fMRI in humans using high spatial resolutions and high magnetic fields. Magn. Reson. Med. 49, 655-664.

Figures legends

Fig. 1 (A) Time course of the blood oxygenation level-dependent (BOLD) signal from the 5 most activated pixels in the somatosensory cortex. The time course was averaged across animals for each stimulation frequency. Light gray, dark gray, and black lines represent current strengths of 0.5, 1.0, and 2.0 mA, respectively. The black bars under the time course indicate the stimulation period. (B) BOLD signals (averaged across animals) as a function of stimulation frequency and current. The peak of the avg. BOLD was observed at stimulation currents of 1.0 and 2.0 mA applied at either 3 or 5 Hz (values were significantly higher than those for 1 and 10 Hz, $p < 0.05$), whereas the peak of ΣBOLD significantly appeared at 3 Hz for 1.0 mA ($p < 0.01$ for 1, 5, and 10 Hz) and 2.0 mA ($p < 0.01$ for 1 and 10 Hz and $p < 0.07$ for 5 Hz). Although not statistically significant ($p > 0.05$), BOLD signals at stimulation currents of 0.5 mA also appeared to peak at 3 Hz. The triangles, squares, and circles show the values for stimulation currents of 0.5, 1.0, and 2.0 mA, respectively.

Fig. 2 (A) Temporal profiles of the somatosensory evoked potential (SEP) amplitude. SEP amplitude was averaged across the animals. Stimulation paradigms identical to those of the MRI experiment were performed using the stimulation frequencies and currents. Light gray, dark gray, and black lines show the SEP amplitude for stimulation currents of 0.5, 1.0, and 2.0 mA, respectively. Each abscissa axis represents a 4-s stimulation period. **(B)** SEP amplitude (averaged across animals) as a function of stimulation frequency and current. The triangles, squares, and circles show the values for stimulation currents of 0.5, 1.0, and 2.0 mA, respectively.

Fig. 3 (A and B) The relationship between blood oxygenation level-dependent (BOLD) signals and average somatosensory evoked potential (SEP) amplitudes for all stimulation conditions. The slopes at each stimulation frequency differed. (C and D) The relationship between BOLD signals and ΣSEP amplitude for all stimulation conditions. The slopes at each stimulus frequency tended to converge. The circles, squares, triangles, and diamonds show the values for stimulation frequencies of 1, 3, 5, and 10 Hz, respectively. The colored symbols indicate the values for stimulation currents of 0.5 (white), 1.0 (gray), and 2.0 mA (black).


A
BOLD signal (%)
1 Hz
3 Hz
5 Hz
10 Hz
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-4
0
4
8
12
16
20
24
Time (s)

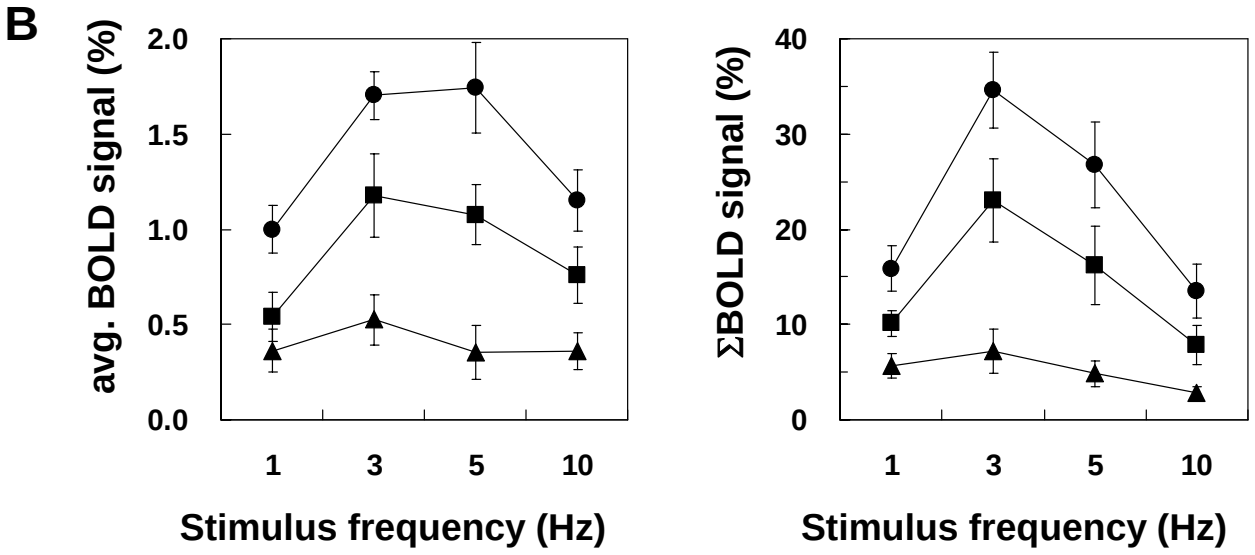
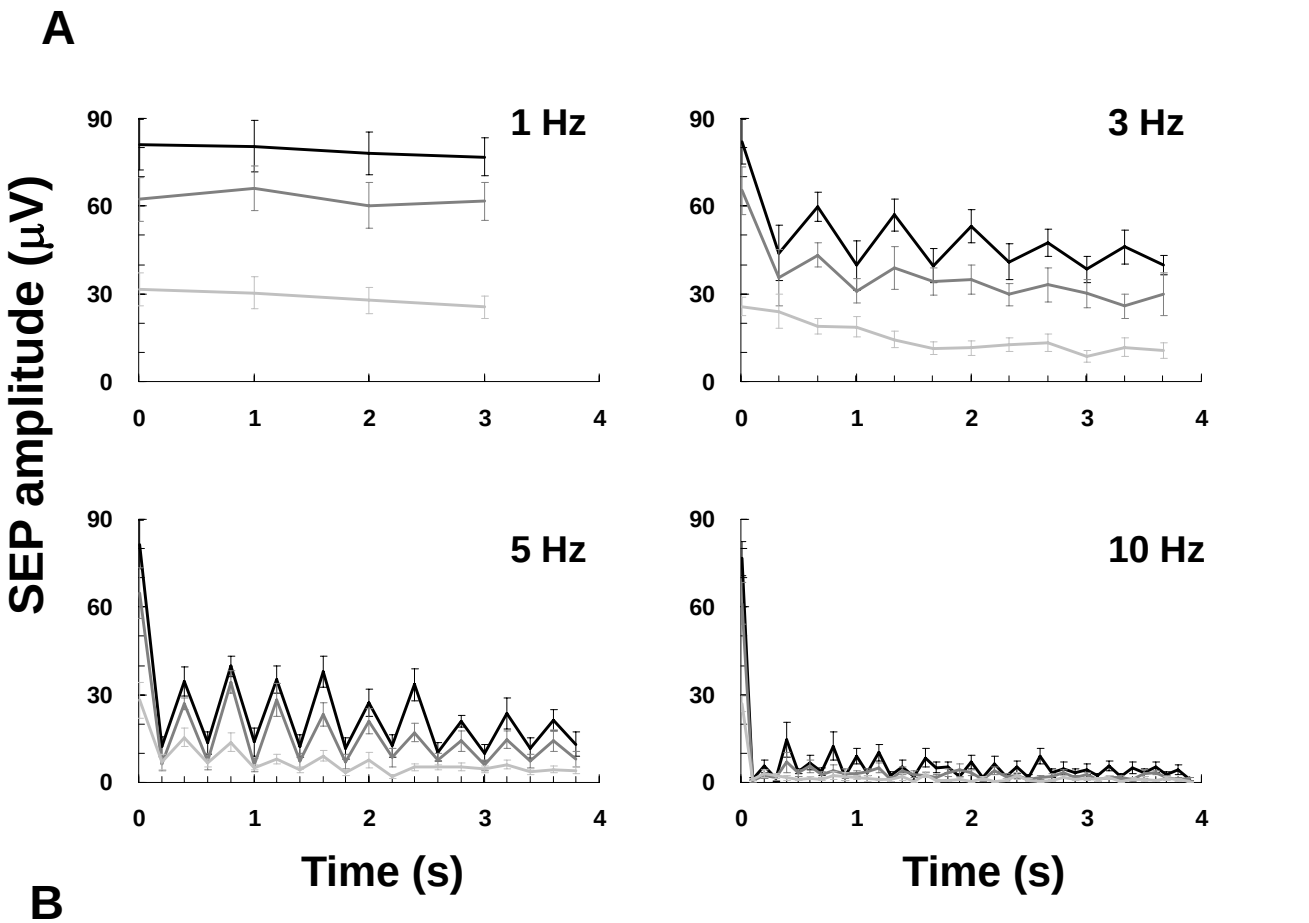

B
avg. BOLD signal (%)
2.0
1.5
1.0
0.5
0.0
ΣBOLD signal (%)
40
30
20
10
0
1
3
5
10
Stimulus frequency (Hz)

Fig. 1


A
SEP amplitude (μV)
1 Hz
3 Hz
5 Hz
10 Hz
Time (s)
Time (s)

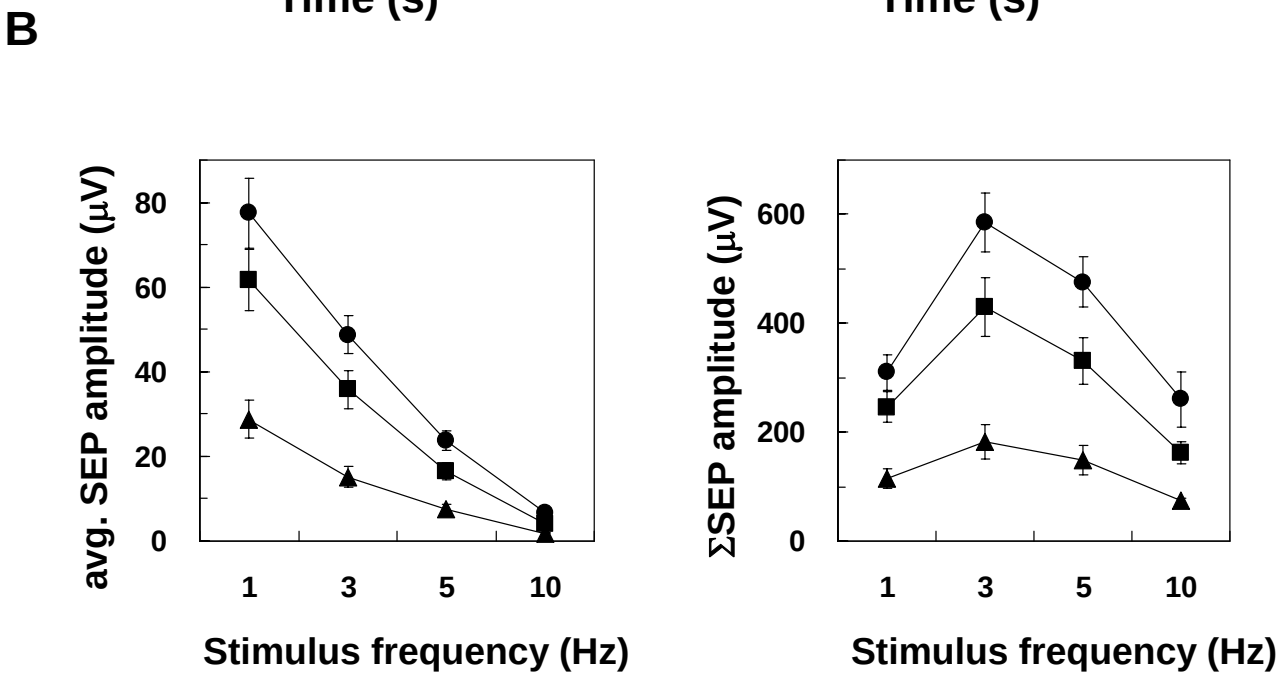

B
avg. SEP amplitude (μV)
ΣSEP amplitude (μV)
Stimulus frequency (Hz)
Stimulus frequency (Hz)

Fig. 2

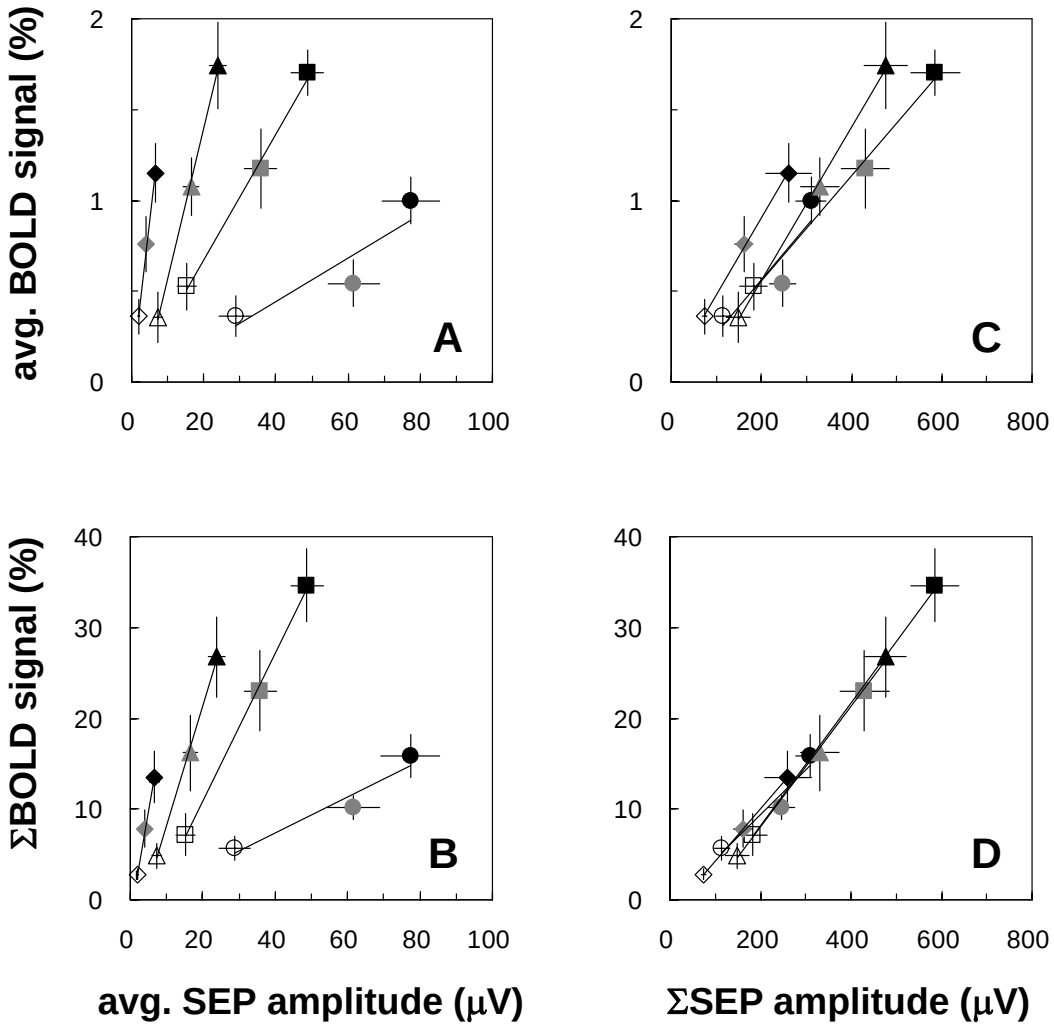

avg. BOLD signal (%)
ΣBOLD signal (%)
avg. SEP amplitude (μV)
ΣSEP amplitude (μV)
A
B
C
D
0
1
2
0
20
40
60
80
100
0
200
400
600
800
10
30
40

Fig. 3