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BOLD fMRI and Somatosensory Evoked Potentials Are Well Correlated Over a Broad Range of Frequency Content of Somatosensory Stimulation of the Rat Forepaw

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Abstract

Electrical stimulation of the rat paw is commonly used to study the hemodynamic, metabolic, and neuronal mechanisms of functional MRI (fMRI) responses in somatosensory cortex. Several groups have reported good correlation between the Blood Oxygenation Level Dependent (BOLD) fMRI signal and somatosensory evoked potentials (SEPs) using short, typically 300 μs, square stimulation pulses. The spectral power of these short pulses is evenly distributed over a wide range of frequencies and thus the effects of the frequency content of the stimulation pulse on fMRI responses have not been previously described. Here, the effects that different stimulation pulse waveforms with a range of frequency content have on neuronal activity, as measured by SEPs, and on the amplitude of the BOLD fMRI signal in rat somatosensory cortex are investigated. The peak-to-peak SEP amplitudes increased as the power in the high frequency harmonics of the different pulse waveforms increased, using either triangular or sinusoidal stimuli waveforms from 9 Hz to 180 Hz. Similarly, BOLD fMRI response increased with increased high frequency content of the stimulation pulse. There was a linear correlation between SEPs and BOLD fMRI over the full range of frequency content in the stimulations.

Keywords

fMRI; BOLD; somatosensory evoked potential; forepaw

1. Introduction

The blood oxygenation level-dependent (BOLD) contrast, routinely used for functional MRI (fMRI) of the brain, arises from changes in cerebral blood flow (CBF), oxygen consumption rate ($CMRO_2$) and cerebral blood volume (CBV) that accompany modulations in neuronal activity (Huettel et al., 2004, Friston, 2005). The contrast in fMRI measurements does not

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directly reflect neuronal activity, but rather arises from changes in these hemodynamic properties (Mandeville et al., 1999). The knowledge of the mechanisms of the neurovascular coupling is thus of major importance for the applications of functional neuroimaging (Villringer and Dirnagl, 1995), and several models describing dynamics and features of the hemodynamic responses originating from brain activity have been recently proposed (Buxton et al., 2004). Therefore, there has been increasing interest in correlating results of BOLD fMRI with direct electrophysiological measurements such as recordings of somatosensory evoked potentials (SEPs) (Brinker et al., 1999; Ogawa et al., 2000; Logothetis, 2002; Smith et al, 2002; Arthurs and Boniface, 2003; Shmuel et al., 2006; Van Camp et al., 2006). Studies have also been performed reporting on the relationship between neuronal activity and cerebral blood flow, measured with MRI and other techniques such as laser Doppler (Leniger-Follert and Hossman, 1979; Gerrits et al., 2000; Nielsen et al., 2001; Caesar K et al., 2003; Jones et al., 2004; Ureshi et al., 2004; Sheth et al., 2004; Devor A et al., 2005). Generally speaking, a tight coupling (often a linear relationship) between the neuronal activity and the fMRI and cerebral blood flow have been demonstrated in these works unless there have been pharmacological manipulation of neural vascular coupling (Stefanovic et al., 2007).

The scope of the fMRI studies investigating the neurovascular coupling can be further expanded by the broadening of the range of experimental conditions, which would selectively target different aspects and mechanisms of the coupling. For example, a number of studies have optimized stimulation protocols for the model of electrical somatosensory stimulation of a rat paw. Typically, the amplitude, repetition rate of short current pulses and the duration of the train of pulses have been varied (Leniger-Follert and Hossman, 1979; Gyndell et al., 1996; Brinker et al., 1999; Silva et al., 1999; Ogawa et al., 2000; Keilholz et al., 2004). Using this paradigm, short pulses of a few hundred microseconds of a few mAmp current and repetition rates of 3-5 Hz elicit the maximum evoked SEP and fMRI responses. The low repetition rates that maximize the response of the paw somatosensory cortex are assumed to be due to refractory phenomena, when a stimulus reduces the response to the subsequent stimulus, and a train of activations occurring at too high a repetition rate cannot sustain maximum effects (Brinker et al., 1999; Ogawa et al., 2000; Keilholz et al., 2004).

It is possible to separate the frequency content of the specific stimulation pulses from the repetition rate of these pulses. The influence of the frequency content of the stimulation pulse shapes or pulse durations on neuronal activity have not been well investigated. The spectral power of the short, 0.3 ms, pulses is evenly distributed over a wide range of frequencies. Therefore, in this work we studied the effects of changes in pulse shapes and pulse durations which altered the frequency content of the stimulation on the neuronal activity measured by SEPs and BOLD fMRI in the rat paw somatosensory cortex. The peak-to-peak SEP amplitudes increased as the power in the high frequency harmonics of the different waveforms increased. Times to peak of the SEP responses were longer for stimuli with more power at low frequencies that were longer to reach peak stimulation current. BOLD fMRI studies demonstrated a similar trend to the SEPs. Stimuli with more power at higher frequencies led to increased amplitudes of the BOLD responses in SI compared to stimuli with power at lower frequencies. Therefore, a good correlation between SEPs and BOLD fMRI held over a broad range of frequency content in the stimulations. This work also establishes conditions where strong fMRI activation of somatosensory cortex can be achieved with relatively long stimulation pulses which should be useful for broadening the scope of the rat forepaw stimulation model for fMRI studies.

2. Results

2.1. Somatosensory Evoked Potentials

Fig. 1 shows the utilized stimulation pulses and their Fourier transforms. The short rectangular pulses have power over a broad range of frequencies and the sinusoidal pulses have power at

the frequency that was applied. As can be seen in Fig. 2, the representative entire time series of the SEPs were not susceptible to time-dependent effects, and therefore the amplitudes of the mean waveforms could be used to quantify the SEP responses. Representative mean profiles of SEP recordings from one of the rats are shown in Fig. 3. Each profile consisted of an early positive deflection (P1), followed by a larger negative wave (N1) and a second positive wave (P2). The SEPs, shown in Fig. 3A, correspond to the triangular-shaped stimulation current pulses of different base-widths. The figure demonstrates that the amplitudes of the negative N1 waves of SEPs increased as the base-widths (and the rise times) of the pulses decreased. It is interesting that the amplitudes of the positive P1 waves did not always follow the same trend and for some animals a smaller N1 wave was preceded by a larger P1 deflection (as shown in Fig.3A). The onset times of the evoked neural responses followed the stimulation maxima, and the times to peak of the SEPs were longer for the longer times to peak of the stimuli.

The representative SEP waveforms, shown in Fig. 3B, correspond to the 111.1 ms long, sinusoidally-shaped stimulation current pulses at 6 different frequencies (9, 18, 27, 45, 90, and 180 Hz). The amplitudes of the negative N1 waves of SEPs depended on the waveforms of stimuli and increased as the frequency of stimulus increased.

Fig. 4 summarizes the SEP data averaged over all the animals. The SEP amplitudes were calculated as the peak-to-peak of the P1 and N1 waves and were normalized by the amplitude of the response obtained with the 330 μs rectangular stimulation pulse obtained from each individual animal. The average SEP amplitudes for all stimuli are summarized in Table 1. For the triangular waves and the sinusoidal waves increasing the frequency content of the stimulation increased the SEP amplitude. The largest amplitude was obtained with the 330 μs rectangular pulse that has the power evenly distributed over a broad range of frequencies. About 75% of the maximal SEP is obtained with sinusoidal pulses of 45 Hz. Below this, SEPs decrease and there are small increases in SEP amplitude for higher frequency pulses.

2.2. Functional Magnetic Resonance Imaging

The intensities of the cortical activation maps obtained with BOLD fMRI showed the same trend as SEPs. Figure 5 shows typical fMRI activation maps overlaid on an EPI image used to obtain the time course data. The activation maps were calculated by cross-correlating the fMRI time course with the stimulation paradigm for each pixel, and for the maps shown the threshold on the values of cross-correlation coefficient (CCC) was set at 0.3. These maps agree with previous work and in addition to strong activation in the proper somatosensory area there is activation along the surface of the brain, which represents a draining vein (Keilholz et al., 2006). Figure 5A shows fMRI maps obtained from one rat using the short, square wave pulse and Figure 5B shows maps from the different triangular wave pulses. Figure 6A and B show representative time courses from a rat with the different stimulation protocols. The scale in Figure 6 was calculated as percent signal change from the pre-stimulation baseline signal. As can be readily seen from the fMRI maps and time courses, there was increased fMRI activation as the high frequency content of the stimulation pulses increased. The longest base-width of the triangular stimuli and the 9 Hz sinusoidal pulse gave the smallest activation.

Fig. 7 shows fMRI data averaged from all the rats and normalized to the fMRI response obtained from the short, 330 μs square pulse stimulation. The value of the BOLD signal was averaged over the entire stimulation period as a measure of the intensity of activation. Similar to the SEPs, the higher the frequency content of the pulses the higher the BOLD fMRI signal. 80% of the peak fMRI response was achieved with the sinusoidal stimulation at 45 Hz. All of the SEP and BOLD data is summarized in Table 1.

The relationship between the normalized SEP amplitudes and BOLD contrast intensities is shown in Fig. 8 for the triangular waveforms (Fig 8A) and the sinusoidal waveforms (Fig 8B).

In both cases a linear dependence fits the data well with very similar slope of response for the triangular and sinusoidal waveforms.

3. Discussion

The results obtained in SEP recordings and fMRI for the triangular-shaped stimuli showed that the evoked neuronal responses and fMRI signal amplitude depend on the frequency content of the stimuli. This frequency dependence was further investigated by application of stimuli with a well-defined frequency content. Sinusoidally-shaped current pulses of 111.1 ms duration were applied at different frequencies to further investigate the impact of different frequency ranges of the stimuli on SEP and fMRI. It was demonstrated that for both triangular and sinusoidal stimulation pulses the amplitudes of the SEP N1-waves and the BOLD fMRI amplitude increased when the power in the high frequency harmonics increased. The responses evoked by the stimulation protocols with a substantial power of the high frequency content were very similar, despite the differences in stimulus shapes and durations (for instance, the triangular-shaped 3 ms pulse and sinusoidally-shaped 111.1 ms pulses above 40 Hz). The typically used 330 μs square wave stimulation gave the strongest SEP and BOLD fMRI response and was used to normalize the data. Differences in absolute values from animal to animal in both SEPs, for example, due to electrode placement, and fMRI signal changes, for example due to baseline MRI signal, makes this normalization important enabling each animal to have an internal control.

That high frequency stimuli elicit strong responses may be explained within what is known about the physiological properties of the afferent sensory fibers and the neuronal mechanisms of perception (i.e. the ability to distinguish between different types of sensations). During the electrical stimulation with needle electrodes the peripheral sensory receptors of the forepaw are bypassed and the afferent axons of the sensory nerves are stimulated directly (Desmedt, 1988). Electrophysiological stimuli in SEP studies have been traditionally limited to the short pulses of electrical current (with very fast rise times and broad frequency content) due to the advantage of minimized temporal dispersion in the resulting afferent volleys of neuronal impulses arriving at the cortex and correspondingly large SEP signals. Very slow increases of the stimulus may lead to inactivation of sodium channels (Nicholls et al., 2001) and the absence of responses. However, it has been demonstrated that the afferent sensory fibers are highly receptor-specific in their physiological properties (Koerber and Mendell, 1998; Koerber et al., 1988) and thus lead to effective discrimination by the brain (Vallbo, 1981; Kelly et al., 1997). The utilization of very short stimulation pulses leads to a lack of specificity with regard to the type of activated fibers (because all fibers are stimulated) and therefore, a loss of natural discrimination. Electrical stimuli of various ramp rates have been previously utilized in voltage-clamp studies of inactivation kinetics of neurons (and single sodium channels) (Magistretti et al, 1999). Recently, it was shown that some isoforms of sodium channels are resistant to inactivation (Rush et al., 2007). Therefore, altering the rise times of the stimuli leads to a degree of selectivity unattainable with the electrical stimulation by short, fast rising pulses. Here we show that there is a strong correlation between SEP amplitude and fMRI over pulses with a wide range of frequency content and varying rise times.

Fast adapting and slowly adapting types of sensory fibers in the forepaw respond differently when activated by the same stimuli. When slow changing (low frequency) stimuli are applied, only the slow-adapting neurons are expected to sustain a neuronal response. At high frequencies of stimulation, however, both fast adapting and slow adapting types of neurons should be activated. Therefore, considering the whole forepaw (and the entire region of the cortex, corresponding to the forepaw), the slow changing stimuli produce less neuronal activity, probably due to the fact that primarily the slow adapting afferents are activated. On the other hand, when the higher frequency, fast-rising stimuli are applied, both types of the afferents

sustain their neuronal activity leading to an overall larger response. This warrants further study because the possibility of quantification of the functioning of fast adapting somatosensory vs. the slow adapting somatosensory pathway may be useful for studying neurological disorders of the peripheral and central nervous system.

It is well known in paw and whisker somatosensory cortex that neuronal responses can be affected by the repetition time of stimuli with different systems having maximal responses at different repetition times. There has been little work studying how the frequency content or rise time of the stimulation effects neuronal responses. Recently it has been shown that in the rodent whisker barrel cortex the deflections of whiskers with very high frequency content can elicit strong somatosensory responses (Andermann et al., 2004; Moore, 2004). Similarly, we find that the paw somatosensory system also responds to high frequency content in the stimulation.

The correlation between BOLD and SEPs was linear over the broad range of frequency content of the stimulation (Fig 8). This agrees with a number of studies that have shown strong correlation between BOLD and electrical measures of activity (Brinker et al., 1999; Van Camp et al., 2006) and indicates that the correlation holds over a broad range of stimulation conditions. There has been much discussion of whether the vascular responses due to neuronal activity in the cortex are due to local field potentials or spiking (Nielsen and Lauritzen, 2001;Logothetis, 2002;Kayser et al., 2004;Thomsen et al., 2004;Lauritzen, 2005;Niessing et al., 2005). Similar to local field potentials, SEP signals represent a summation of the coherent electrical activity evoked throughout the area of recording at the brain surface. In terms of the representative frequency range, SEPs have higher power at lower frequencies and thus are thought to primarily indicate the input synaptic activity (Logothetis, 2001;Logothetis and Wandell, 2004). However, it should also be noted that the frequency domains of local field potentials and spikes are not clearly separated (for example, a synchronization of spikes may result in a low frequency component), and therefore it is difficult to absolutely distinguish the input and output signals in a neuronal ensemble. Also, it is worth noting that the positive and negative SEP deflections may overlap when the long sinusoidally-shaped stimuli are applied and thus there is a possibility of errors in estimation of neuronal activity by SEP recordings. The bulk of the evidence in this model of rat somatosensory cortical activation is that the vasodilators responsible for the fMRI signal are produced by NO synthetase (NOS) and cyclooxygenase 2 (COX2) (Kaufmann et al., 1996;Cholet et al., 1996;Iadecola, 2004;Lauritzen, 2005). Both of these enzymes are predominantly localized in dendrites of pyramidal neurons, astrocytes and interneurons (Kaufmann et al., 1996;Sancesario et al., 2000;Burette et al., 2002;Zonta et al., 2003). This indicates that it is dendritic activity, astrocyte or interneuron activity that couples electrical activity to a BOLD response. Overall, however, care should be exercised in attributing too much significance to the magnitudes of linear correlations observed between BOLD and SEPs in this and previous studies, considering the known complexity of activation of the cortical neuronal network, the known complexity of neural vascular control and the known complexity of the BOLD signal response (Friston, 2005). Recent data further indicates the complexity of the fMRI/SEPs relation by demonstrating that it can be dramatically altered with pharmacological intervention (Stefanovic et al., 2007).

The times to peak of the evoked neural responses were longer for the stimuli that were longer to reach peak stimulation current. For instance, SEP onset for the 9 Hz pulse was delayed by about 15 ms relative to the 330 μs stimulus. This effect could be used in future imaging studies in rats with enhanced temporal and spatial resolution to further investigate the laminar specificity of fMRI signal onset times (Silva et al., 2002).

Finally, this work demonstrates that it is possible to give electrical stimulation pulses of relatively long duration (at least 100 ms) that result in robust fMRI activation of somatosensory cortex as long as there is high frequency information within the envelope of these pulses. Most work on rodent behavior, such as fear conditioning with electrical shock, utilizes long DC pulses (Otto et al., 2000; Rosen et al., 2006). These DC pulses do not give significant fMRI activation (data not shown), which may limit their use for fMRI studies of behavior in rodents. From the work reported here it should be possible to utilize long pulses with high frequency content that would result in strong somatosensory activation and also induce strong behavioral effects.

In conclusion, the dependence of BOLD fMRI and SEPs from rat somatosensory cortex was measured using electrical stimulation of the forepaw with pulses of different shapes and frequency content. Largest SEP and BOLD fMRI responses were obtained with pulses that had frequency content above 40 Hz, which corresponded to pulses with fast rise times. There was a linear correlation between BOLD and SEPs over the entire frequency range and pulse shapes studied. To the best of our knowledge, this is the first study of this kind in rodent somatosensory cortex. It should be possible to use stimulation pulses of varying frequency content to correlate the fMRI responses to the facilitation of sensory processing through the somatosensory pathways, as well as to elicit strong cortical fMRI responses in the context of behavioral studies that use electrical stimulation.

4. Experimental Procedure

4.1. Animal preparation

All experiments were performed in compliance with guidelines set by the National Institutes of Neurological Disorders and Stroke ACUC. Male Sprague-Dawley rats (200-300 g) were anesthetized with halothane (5% induction, 1.5% maintenance) and orally intubated for artificial ventilation. Catheters were placed into the femoral artery and vein for arterial blood pressure monitoring, sampling of blood gases and drug delivery. Halothane was discontinued and anesthesia was switched to α-chloralose (80 mg/kg initially, followed by a continuous infusion of 26.7 mg/kg/hr). The animals were placed into a stereotaxic head holder with ear and bite bars. Expired CO_2, rectal temperature and blood pressure were continuously monitored. Blood gases were measured and maintained at normal levels. An intravenous injection of pancuronium bromide (4 mg/kg) was given once per hour to prevent motion.

4.2. Forepaw stimulation

Needle electrodes were inserted into a forepaw. A forepaw was stimulated by application of trains of pulses from a constant current source at a repetition rate of 3 Hz. Waveforms of the pulses were varied and consisted of rectangular pulses of 330 μs duration, triangular pulses of different base-widths and sinusoidally-shaped pulses of 111.1 ms duration at 9, 18, 27, 45, 90 and 180 Hz. Figure 1 shows the different pulses used and the Fourier transform of these pulses to show the power content of these pulses at different frequencies. The sinusoidal pulses have power at the frequency that was applied, and the power of the short rectangular pulses is distributed over a broad range of frequencies with little variation. Maximum current amplitude was 2 mA in all stimulations. Voltage waveforms were created and output using a MP150 Biopac system (Biopac Systems, Inc, Goleta, CA). An A395 linear stimulus isolator (World Precision Instruments, Sarasota, FL) was used for voltage-to-current conversion/isolation.

4.3. Recordings of Somatosensory Evoked Potentials

Four separate animals were used for the SEP recordings. The SEPs were acquired on the bench outside the magnet. The rat skull was exposed and 700 μm burr-holes were drilled at 3.5 mm on both sides lateral to the bregma for the signal electrodes. One additional burr-hole 10 mm

caudal to the bregma was drilled for the ground electrode. The electrodes (Plastics One Inc, Roanoke, VA) were gently inserted until the dura was touched. SEPs were recorded using the EEG module of the MP150 Biopac system (differential input, gain of 5000, the passing range of the filter was from 1 to 35 Hz).

The stimulation paradigm employed for the recordings consisted of 2 epochs of 45 s during rest, 30 s during stimulation of a series of pulses applied at 3 Hz, and 45 s during rest. To illustrate the procedure, the entire series of SEPs recorded over a 30 s long stimulation are shown in Figure 2 for both slow and fast changing triangular stimulation pulses. Mean profiles of SEP responses were subsequently obtained by averaging over the entire series with a time-window step of 333.3 ms (synchronized to the start of a stimulation pulse). AcqKnowledge software package (Biopac Systems) was used to calculate the averaged profiles of SEP responses, as well as the Fourier transforms of the SEPs and the stimulation pulses.

4.3. Functional Magnetic Resonance Imaging experiments

Five separate animals were used for the fMRI experiments. Functional MRI experiments were performed in an 11.7/31 cm magnet (Magnex Scientific, Ltd., Abington, UK) interfaced to a Biospec-Avance console (Bruker-Biospin, Corp, Billerica, MA) and equipped with a 9 cm ID gradient set (Resonance Research, Billerica, MA). A laboratory-built, 2 cm receive-only surface coil was used inside a 7 cm ID transmit-only coil.

A spin-echo EPI sequence was employed for BOLD fMRI studies. FOV was 1.92×1.92 cm^2 with a 64×64 matrix (in-plane resolution of 300 μm). The bandwidth was 200 kHz, TE was 30 ms and TR was 1.5 s. Five 1.5 mm thick slices were acquired, covering the forebrain and middle brain. The stimulation paradigm to generate fMRI maps was 60 images (90 s) during rest, 30 images (45 s) during stimulation, and 60 images (45 s) during rest.

Functional maps were calculated by cross-correlation of the fMRI time courses with the stimulation paradigm. STIMULATE software (Strupp, 1996) was used for the calculation. The cross-correlation threshold was set at a value (≥ 0.2 for permitting to observe activation in the case of the least responsive stimulation paradigm (e.g. the 30 ms base-width triangular pulse and the 9 Hz harmonic pulse). Only groups of at least 4 activated pixels were taken into consideration. The selected ROIs corresponded to the forepaw representation as determined by the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1998). All time courses shown in this work were averaged over the pixels in ROI.

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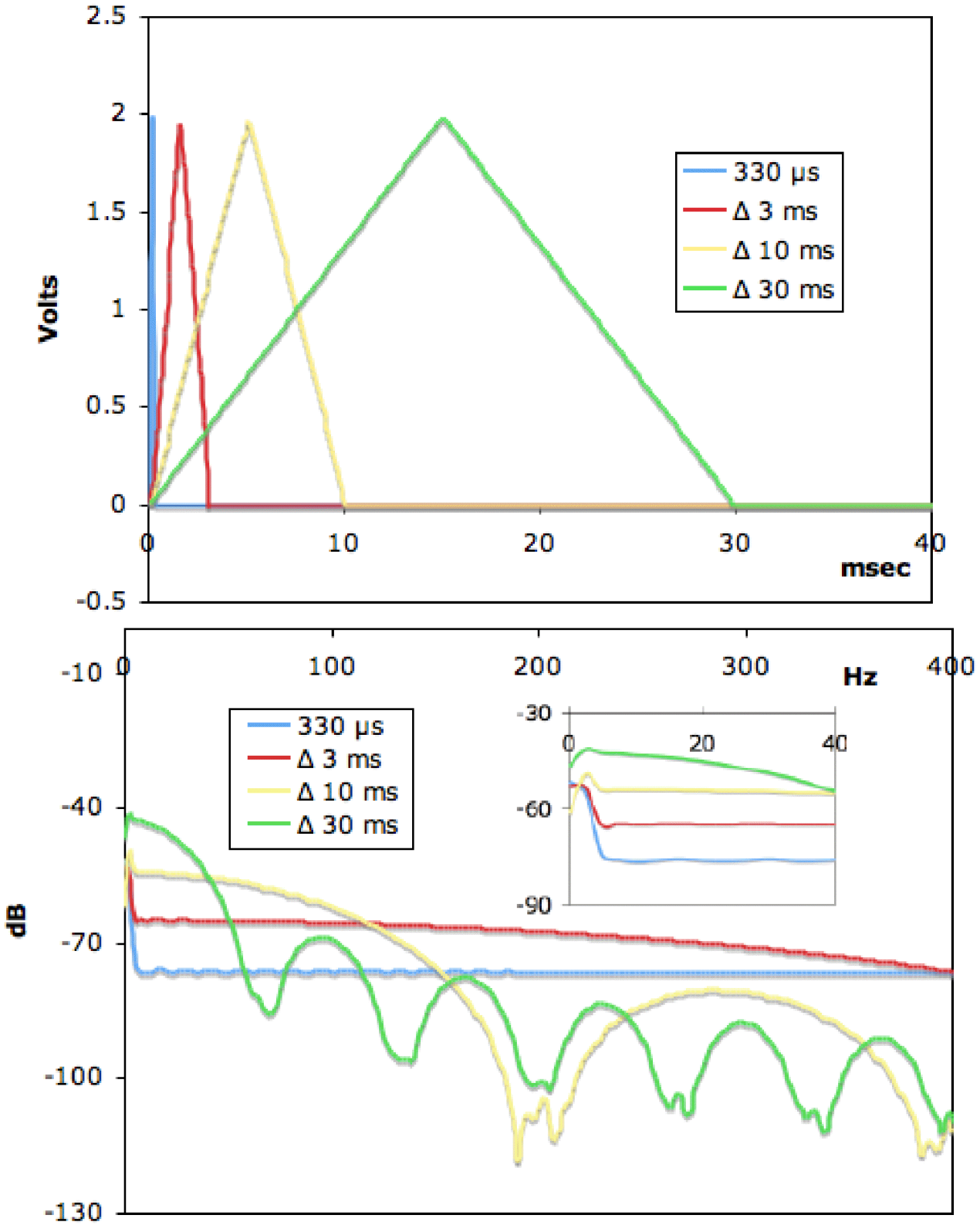


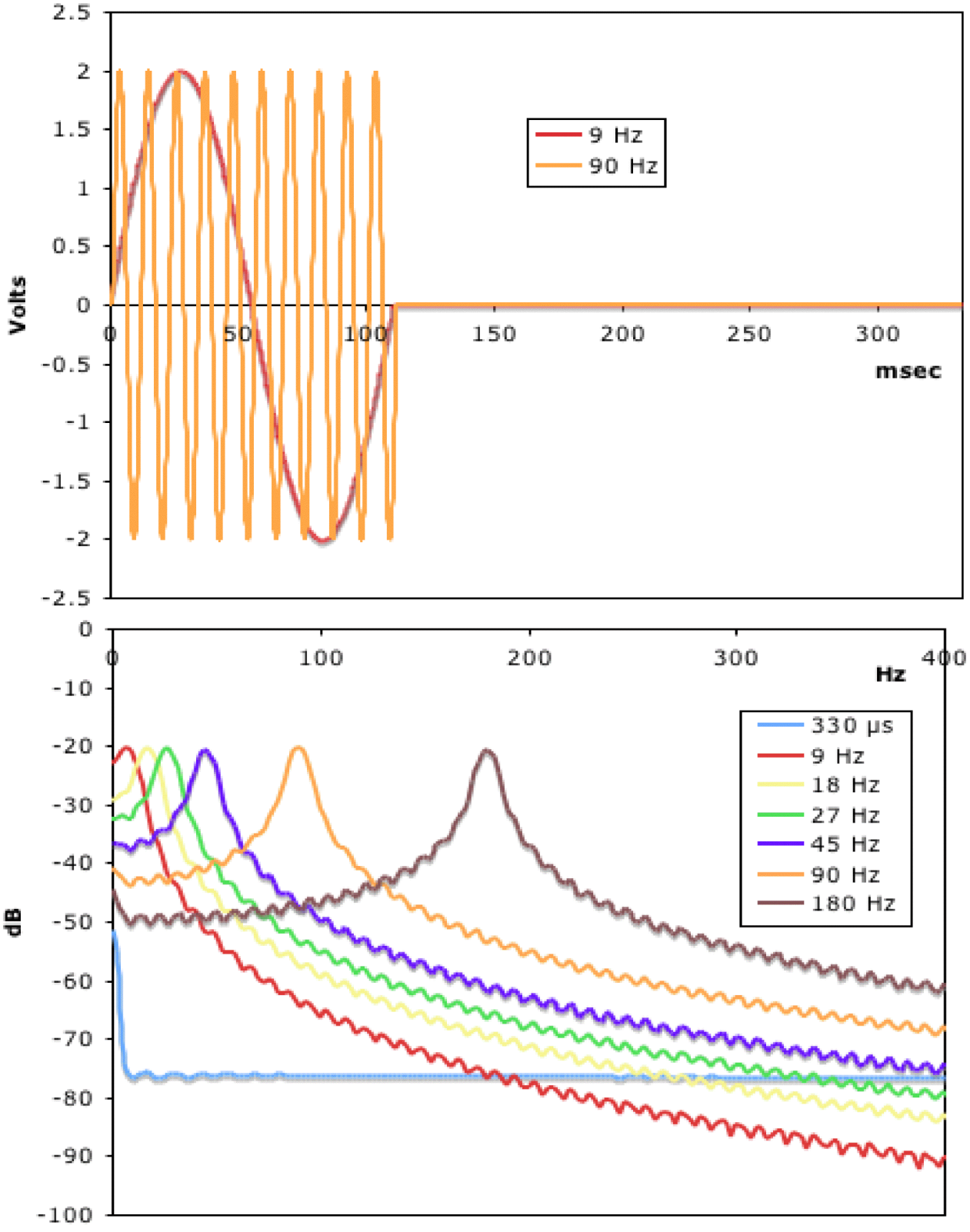


Fig. 1.

Waveforms and power spectra (Fourier transforms) of stimuli used in this work: (A) triangular pulses with base-widths of 3, 10 and 30 ms and (B) their power spectra; (C) sinusoidally-shaped pulses of 111.1 ms duration (frequencies of 9 and 90 Hz are shown) and (D) their power spectra. Each plot contains a rectangular 330 μs pulse for comparison; 2 Volts corresponded to 2 mA.

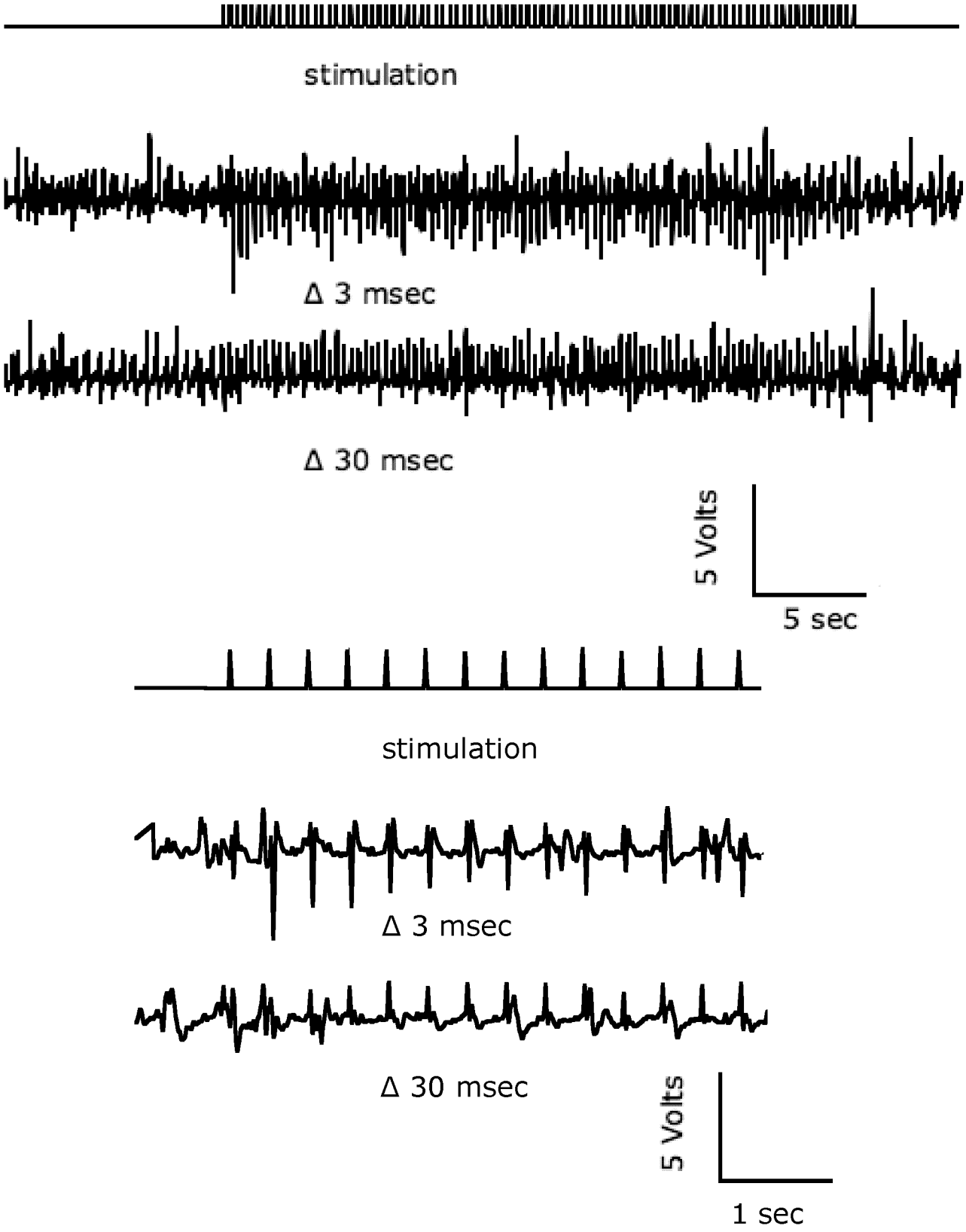


Fig. 2.

SEP response: (A) entire series recorded over a 30 s long stimulation with triangular pulses of 3 ms and 30 ms base-widths; (B) first 5 s of the series.

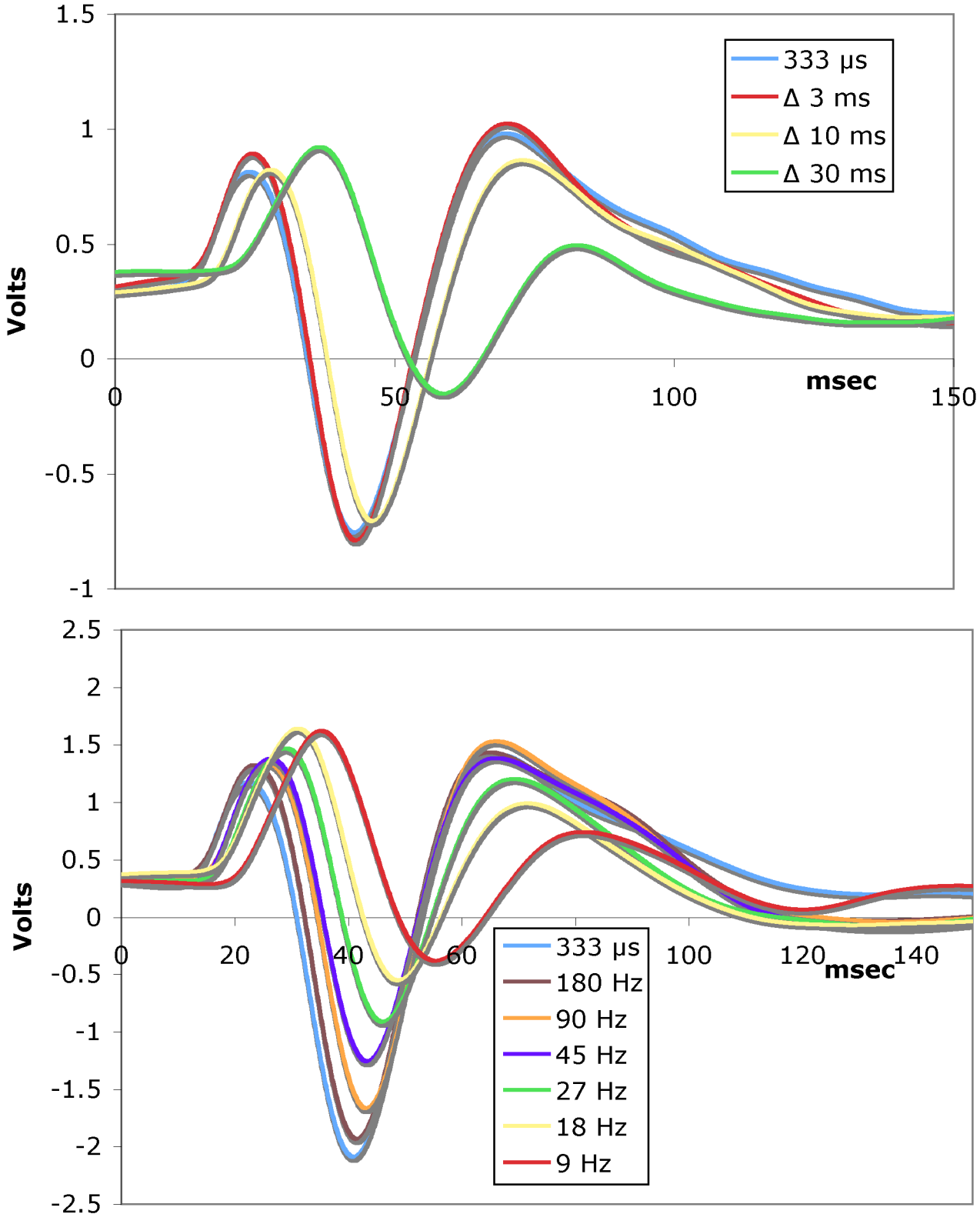


Fig. 3.

Somatosensory evoked potentials (SEPs) from one rat for various stimuli waveforms: (A) triangular pulses and (B) sinusoidally-shaped pulses. Shown SEPs are representative of all the animals used. SEP recorded with a rectangular 330 μs stimulation pulse is shown for comparison.

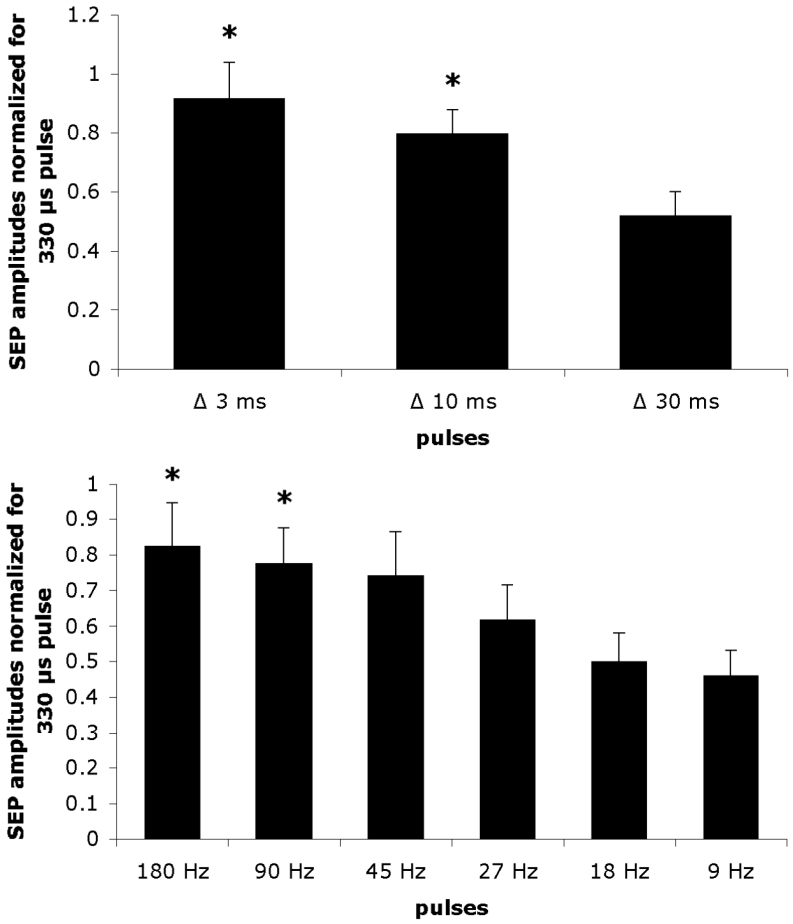


Fig. 4.

Peak-to-peak SEP amplitudes obtained with (A) triangular pulses and (B) sinusoidally-shaped pulses and averaged over all the animals (statistically significant difference from the minimal response: *, $P < 0.05$). Prior to averaging, SEPs were normalized for each individual animal with the response from the 330 µs rectangular stimulation pulse.

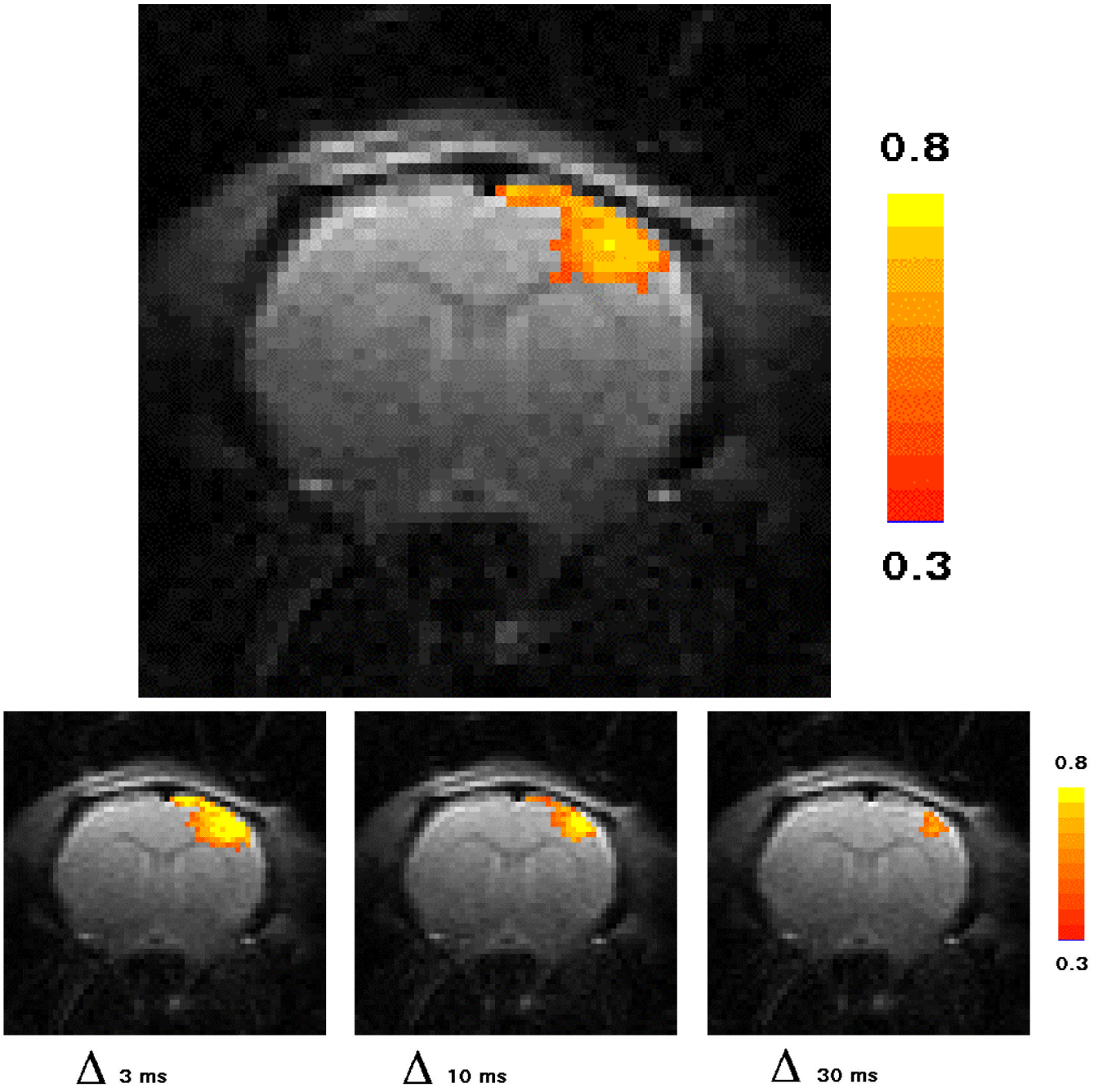


Fig. 5.

Typical activation maps from one of the rats, obtained with (A) rectangular 330 μs stimulation pulse and (B) triangular stimuli, overlaid onto the corresponding anatomical EPI images. The activation maps were generated from pixels representing a cross-correlation coefficient (CCC) larger or equal to the value of 0.3. The color bar indicates the scale of CCCs.

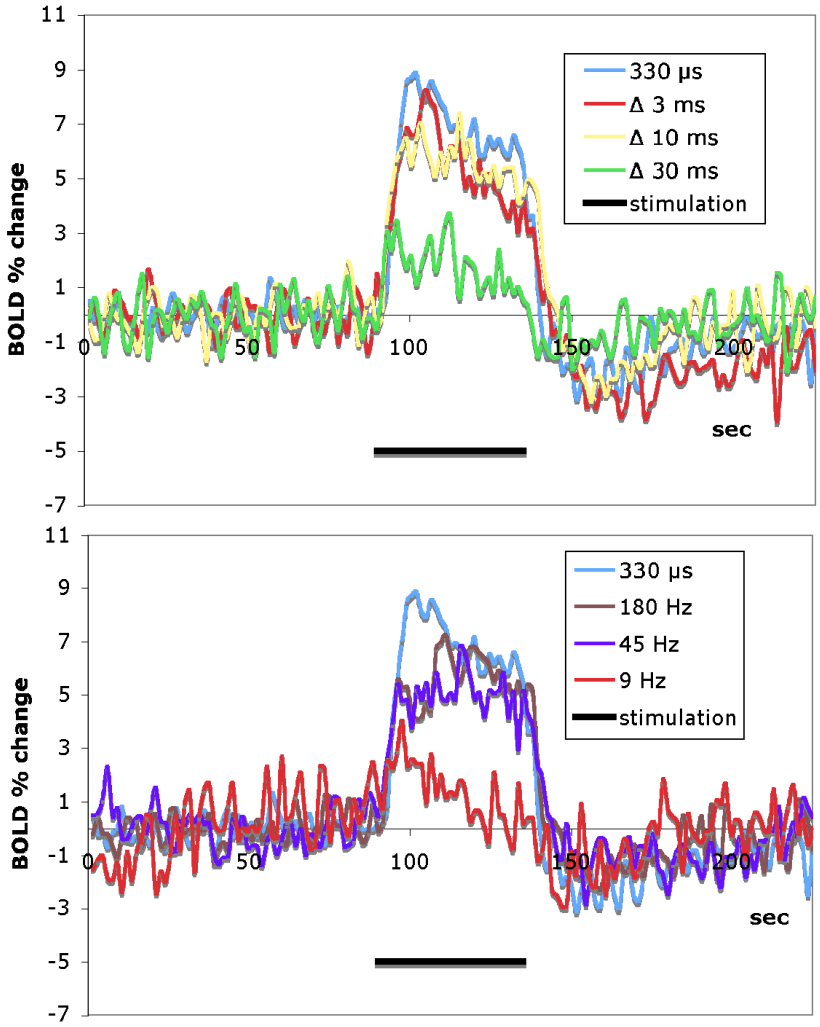


Fig. 6.

Time courses of BOLD contrast percent change from one of the rats for (A) triangular pulses and (B) sinusoidally-shaped pulses (for clarity, the time courses for pulses at only selected frequencies of 9, 45 and 180 Hz are shown). The time course obtained with a rectangular 330 μs stimulation pulse is shown for comparison.

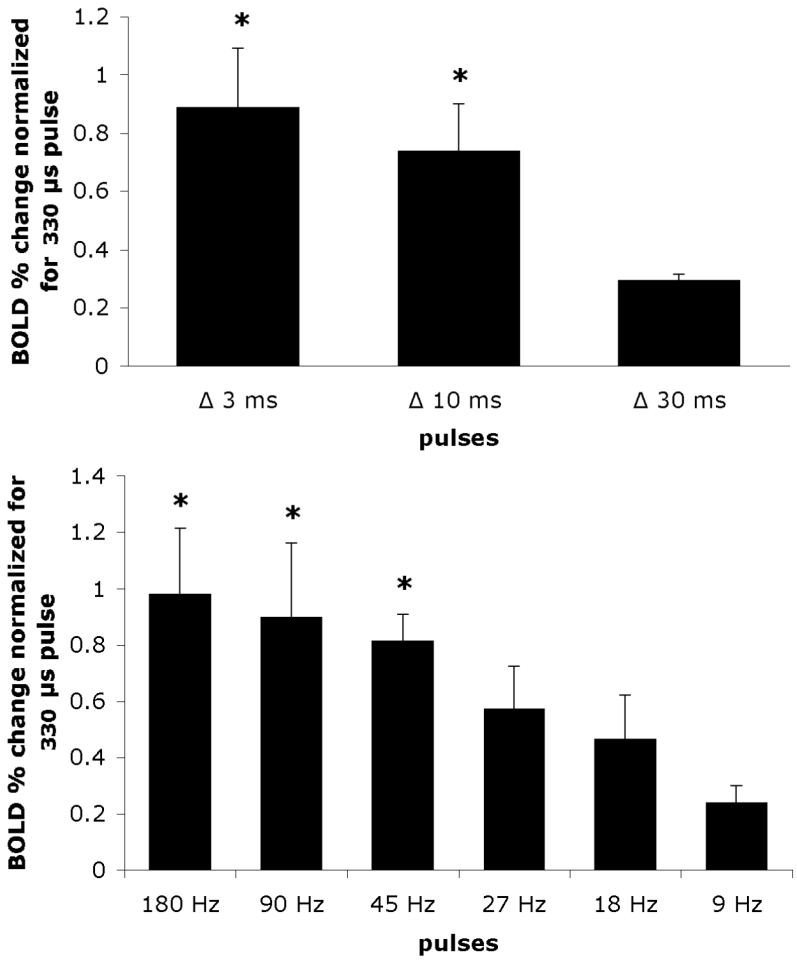
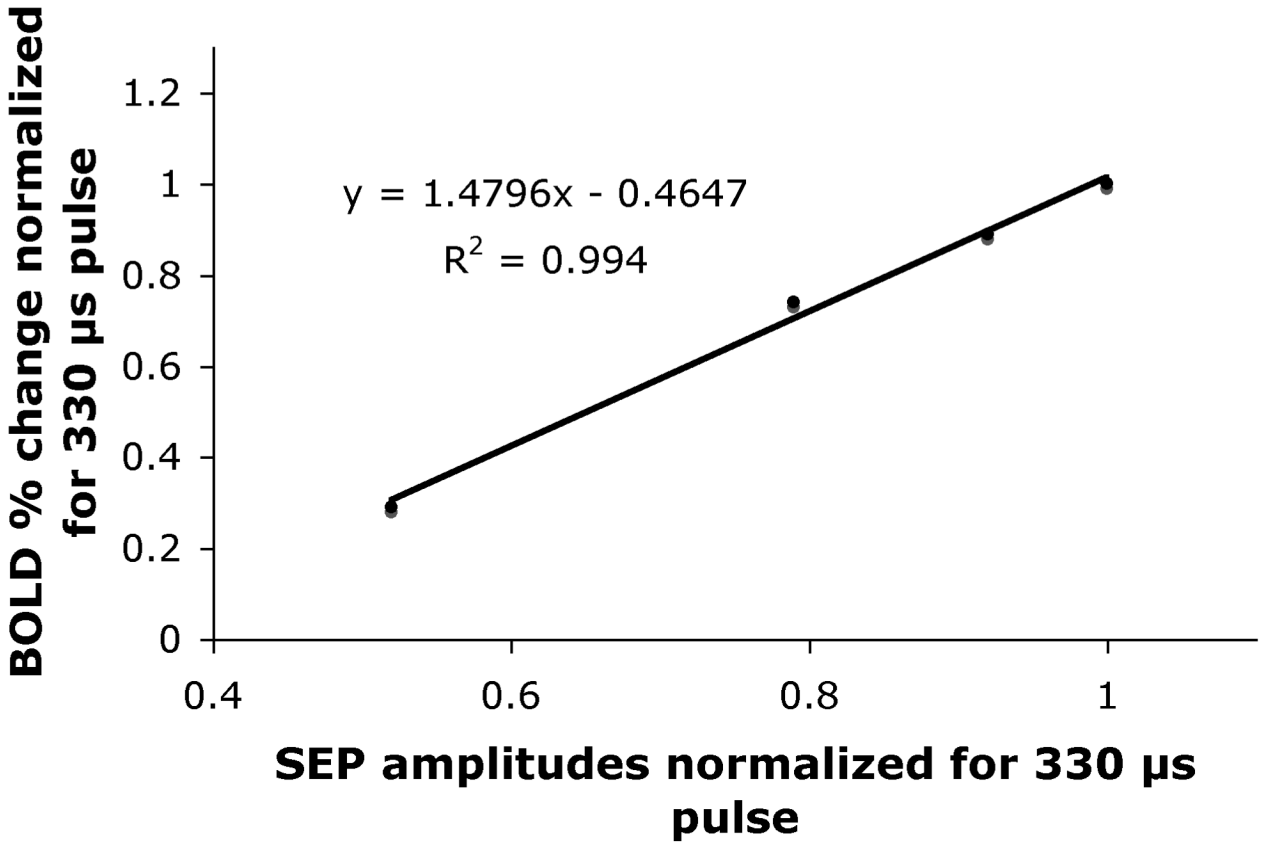


Fig. 7.

Intensities of BOLD changes obtained with (A) triangular pulses and (B) sinusoidally-shaped pulses and averaged over all the animals (statistically significant difference from the minimal response: *, $P < 0.05$). A mean value of the BOLD time course over the stimulation period was taken as a measure of the intensity of activation. Prior to the averaging across the animals, the activation intensities of individual animals were normalized to the value obtained with the 330 µs stimulation pulse.



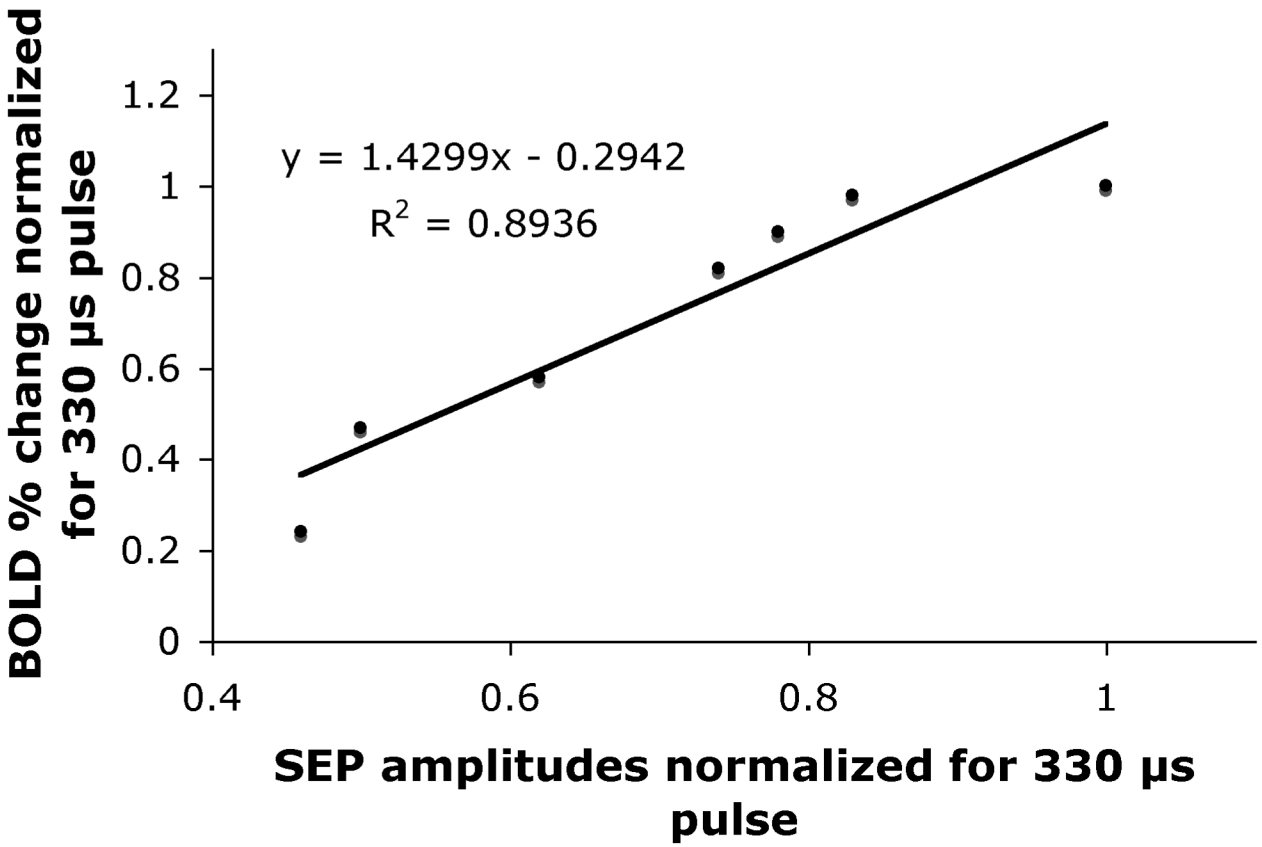


Fig. 8.

Relationship between BOLD contrast intensities and SEP amplitudes for (A) triangular pulses and (B) sinusoidally-shaped pulses. Regression lines are plotted to demonstrate a linear correlation. SEP amplitudes and BOLD intensities were normalized to 330 μs pulse responses and then averaged across all the animals.

Table 1

SEP amplitudes and BOLD signal percent changes obtained with triangular stimulation pulses of different base-widths and 111.1 ms long, sinusoidally-shaped stimulation pulses at different frequencies. The values were normalized for 330 μs stimulation pulses (mean ± SD, * - statistically significant difference from the minimal response at Δ 3 ms pulse and ** - from the minimal response at 9 Hz pulse, $P < 0.05$)

	SEPs	BOLD
Δ 3 ms	0.92 ± 0.12	0.89 ± 0.20
Δ 10 ms	0.79 ± 0.08 *	0.74 ± 0.16 *
Δ 30 ms	0.52 ± 0.08 *	0.29 ± 0.02 *
9 Hz	0.46 ± 0.07	0.24 ± 0.06
18 Hz	0.50 ± 0.08	0.47 ± 0.16
27 Hz	0.62 ± 0.10	0.58 ± 0.15
45 Hz	0.74 ± 0.12	0.82 ± 0.09 **
90 Hz	0.78 ± 0.10 **	0.90 ± 0.26 **
180 Hz	0.83 ± 0.12 **	0.98 ± 0.23 **