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BOLD Signals Correlate with Ensemble Unit Activities in Rat's Somatosensory Cortex

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Abstract

Evoked neural activity (ensemble single-unit activity and evoked field potential) and functional magnetic resonance imaging (fMRI) changes of the primary somatosensory cortex in response to electrical stimulation of the hind paw were studied in rats under anesthesia. The effects of stimulation frequency (ranging from 0.3 to 10 Hz) and types of anesthetics (α-chloralose and sodium pentobarbital) on blood oxygen level dependent (BOLD) activation and neural activation were compared. Both ensemble single-unit activity and BOLD signal changes achieved maximal activation at 3 Hz of stimulation and responses were significantly stronger under α-chloralose anesthesia. The maximal activation of the integral evoked potential (ΣEP), in contrast, was the highest at 10 Hz; and the values were similar for α-chloralose and pentobarbital. These analyses revealed that fMRI image changes were better correlated with ensemble single-unit activity than with ΣEP during somatosensory stimulations.

Key Word: fMRI, evoked potential, ensemble neuronal activity, somatosensory cortex

Introduction

Functional magnetic resonance imaging (fMRI) is a powerful tool for detecting the functional responses in the brain. When a brain area is activated, deoxyhemoglobin is indirectly produced. The presence of deoxyhemoglobin in the blood changes the proton signal from water molecules surrounding a blood vessel in gradient-echo MRI, producing a blood oxygenation level-dependent (BOLD) contrast (21, 22). Compared to other functional imaging technique, such as PET, fMRI is non-invasive, does not use radioactive substances, has relatively good temporal and spatial resolutions, and produces contrast images of soft tissue. It can detect activities in different slices of the brain to locate the most active regions, and it can be used to construct a three-dimensional functional view of the entire brain.

The most fundamental function of the brain is its electrical activity. It is of utmost importance, therefore to learn how BOLD signal change relates to the underlying electrical signal change. In this aspect, it has been documented (7, 13, 14) that changes in multi-unit, synaptic activity (as represented by evoked field potential) and fMRI image intensity changes are correlated. Furthermore, in a visual study (14) Logothetis *et al*. found that fMRI changes correlate better with evoked field potential than with multi-unit activities. However, in the somatosensory system, it has been only shown that in the primary somatosensory cortex (SI), somatosensory-evoked unit activities correlate well with either local blood flow (7, 32), tissue oxygenation level (30) or local metabolic rate (27). No direct comparison, be it field potential change or unit activity change, has been made to correlate with BOLD signal change in the somatosensory system.

The unit activity studies cited above were per-

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formed using a single microelectrode (7, 14, 27, 30, 32). Recently, electrical recording techniques have become so advanced that the ensemble neuronal activity in a given brain region can be assessed with multiple single-unit recording from an array of microwires (19). Multiple-channel, multiple single-unit activity (ensemble single-unit activity) is a better index of the cortical neuronal activation than the multi-unit activity, because, in addition to multiple sampling channels, single-units are less contaminated by near-field fiber activities and far-field EMG activities. Moreover, multi-unit data or field potential data have traditionally been analyzed with peri-event histograms within a limited time frame (3, 15, 16, 20). With the ensemble single-unit data, it is possible to count the number of spikes in the on-off sequences and subject the data to analysis more similar to those usually performed on the fMRI data.

In this study, we compared fMRI activation versus two types of neuronal activations, namely, integral field potential and ensemble single-unit activities, evoked by supra-threshold intensity of electrical stimulation of the hindpaw of rats at stimulation frequencies ranging from 0.3 to 10 Hz under two types of anesthetics. With this wide range of input - output arrangement, we found a complicated non-linear correlationship pattern among the BOLD signal, ensemble unit activity and field potential changes.

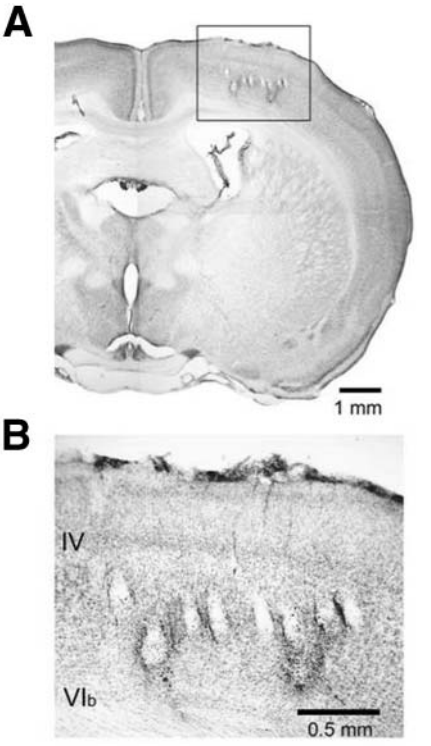

Fig. 1. An example of the recording sites of the implanted 8-channel microwire array. The tips of the implanted microwires in the infra-granular layers of the hind paw region of the primary sensorimotor cortex are shown in lower magnified (A) and higher magnified (B) photomicrographs. Photo B is an enlarged view of the boxed area in A. IV: layer 4 of the SmI; VIb: layer 6b.

Materials and Methods

Subjects

Experiments were performed on 15 Long-Evans rats weighing between 280 and 350 g. They were purchased from the National Breeding Center for Experimental Animals and group housed in the vivarium of Institute of Zoology in National Taiwan University. Surgical and recording procedures complied with NIH (USA) recommended guidelines for animal use and care, and were approved by the Animal Use and Care Committee of the National Taiwan University, Taipei, Taiwan, ROC.

Electrophysiological Experiment

Seven rats were used in the neuronal recording experiments. The microwire array electrode was chronically implanted into the hindpaw region of the primary sensorimotor cortex (SmI, (5)) on the right side. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), the rat was mounted on a stereotaxic apparatus. A midline incision was made over the skull. After retraction of the skin and cleaning of the soft tissue, small craniotomies were made to implant the intracortical microelectrode into the SmI on the right side. The microelectrode used was a linear array of eight stainless steel wires individually insulated with Teflon (50 μm o.d.), and the electrode array was placed about 1 mm deep (Fig. 1, see also (31)). The receptive fields of the individual channels were ascertained when the rat was still under anesthesia. Ketamine hydrochloride (50 mg/kg, i.m.) was administered as necessary to maintain proper anesthetic depth so that the animal was areflexic throughout the surgical period. The hole on the skull and the implanted electrode were sealed and secured with dental cement. The rat was allowed to recuperate for at least 1 week after surgery.

Each rat in this group was tested under two anesthetics, pentobarbital and α-chloralose. This was performed in two stages on separate days. Sodium pentobarbital (50 mg, i.p.) was used first. Supplementary doses of the same anesthetics were given as necessary. After the rat recovered from the pentobarbital anesthesia (24 to 48 h later), it was tested under α-chloralose anesthesia. For α-chloralose anesthesia, the rat was initially anesthetized by 4% halothane. The dose of α-chloralose was 50 mg/kg intravenously *via* a femoral vein cannula. During all experiments,

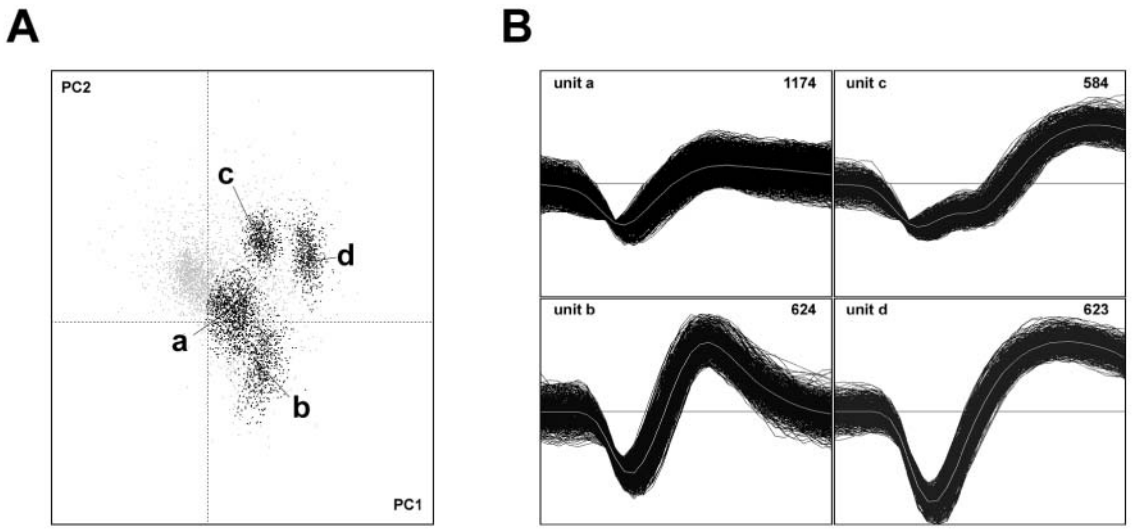

Fig. 2. A representative example of multiple single-units recording from one implanted SmI microwire. Four single-units were classified. (A) All wavelets plot on the PC1 and PC2 axes of their principal component analysis (PCA) results. Each dot represents one wavelet. (B) Superimposed waveforms of individual single-units. The numeric numbers in the top right corners of each waveform plot are the number of wavelets superimposed. The x-axis represents 1.2 ms.

the rectal temperature was monitored and maintained at 37°C by a feedback-controlled heating pad. Electrical stimulation was applied to the left hind paw with square pulses of 1~2 mA amplitude, 2-ms duration at frequencies of 0.3, 1, 3, 10, and 30 Hz. To simulate the on-off fMRI protocol, after a 50-s control recording, stimulation at the specified parameters was applied for 50 s. Changes in single-unit activities and the evoked potential were determined (see next paragraph). After the recording session under μ-chloralose anesthesia, electrical lesions were made on selected electrodes of the microelectrode array. The animal under deep anesthesia was perfused intracardially by saline followed by 10% formalin. The brain was frozen and sectioned serially at 50 μm with a sliding microtome. Sections were stained with cresyl violet. The positions of the electrode tips were confirmed by microscopic observation.

The evoked potential and multiple single-unit activity were acquired by a 32-channel Multichannel Neuronal Acquisition Processor system (MNAP, Plexon, Dallas, TX, USA). The electrical signals passed from the headsets to the amplifiers and band-passed filtered (spike preamp filter: 500 Hz ~ 3000 Hz, field potential filter: 3 Hz ~ 90 Hz). Real-time spike sorting was controlled by SortClient (Plexon), and the sampling rate of individual channels was 40 kHz. The saved wavelet data were analyzed using the principal component analysis (PCA) method and re-sorted by the software Offline Sorter (Plexon). An example is shown in Fig. 2. Spike train activity and field potential were analyzed with Neuroexplorer (Nex Technologies, Littleton, MA, USA).

fMRI Experiment

Two groups of rats, four in each, were used in this experiment. They were given either pentobarbital or α-chloralose. The anesthesia and stimulation were the same as described in the previous section. The rectal temperature was maintained at 36 ~ 37°C by a circulating water pad surrounding the animal in the scan chamber. The trachea was cannulated. Expiratory CO_2 and arterial blood O_2 and CO_2 concentrations were monitored. In the eight experimental animals, those parameters were within physiological ranges. The fMRI experiments were performed in an active shielded 900-mm bore size 3T Bruker MRI/MRS system (Bruker BIOSPEC Gradient system S116, Bruker, Karlshruhe, Germany). The magnet was equipped with a 120-mm inner diameter self-shielded gradient insert system (maximum gradient strength 200 mT/m; minimum inductive rise time 250 μs) and radio frequency pulses were transmitted by a self-developed surface coil with a diameter of 4 mm.

Functional MRI imaging was performed in the rat brain on axial slices of 2-mm thickness without gaps, employing a Bruker implementation of BLIP Echo Planer Imaging (BEPI, TR 2,500 ms (multi-slice); matrix size 128 × 128; FOV 60 × 60 mm; NEX 2). Anatomical images were obtained using an echo time TE of 14.5 ms, TR of 1869.5 ms, and a matrix size of 128 × 128 with high resolution, and these images were compared with anatomical data from the atlas by Paxinos and Watson (24). Data were analyzed by the software FACT (6). The total scanning

time of every image was 5 s. The electrical stimulation was applied 10 images off and 10 images on. There were five cycles in one scan.

Data Analysis

Spike train activity and field potential were analyzed with Neuroexplorer (Nex Technologies, Littleton, MA, USA). The units recorded from the electrodes which were certain in the receptive field were summarized as ensemble unit activities (EUA). The rate histograms of ensemble unit activities were obtained by Neuroexplorer. The ratio of average firing rates in the stimulating periods and non-stimulating periods were counted. The peak-to-peak amplitudes of averaged evoked potentials (EP) were obtained. The integral evoked potentials (ΣEP) (16) were calculated as the product of the peak-to-peak amplitude of the evoked potentials multiplied by the stimulation frequency. The integral evoked potential in 0.3 Hz stimulation under pentobarbital anesthesia was taken as 100%. The frequency dependent-responses between two anesthetics were tested by one-way repeated measure ANOVA followed by Newman-Keul pair-wised test. The correlations between fMRI signal changes and ΣEP or ensemble unit activities were analyzed by Pearson product moment correlation.

Results

Electrical stimulations of the left hindpaw activated the hindpaw region of the contralateral SmI. The threshold intensity for electrical activation was in the range of 0.2 to 0.4 mA. Supra-threshold intensity of 1 to 2 mA (five times the threshold intensity) was used in the present study. For the fMRI study, a fixed level of 2 mA was used, because a preliminary series of intensity-response experiments showed that at this stimulation intensity induced the most consistent BOLD signal of activation. The BOLD signal of fMRI followed the stimulation pattern better in rats under α-chloralose anesthesia. There were a tighter cluster of significantly correlated pixels under the α-chloralose anesthesia than those under the pentobarbital anesthesia (Fig. 3A and B). With the data pooled from four rats, the BOLD signal changes were the highest at stimulation frequency of 3 Hz under both types of anesthesia (Fig. 3C). However, only under α-chloralose did the change reach statistical significance ($P < 0.05$).

Figure 1 shows a typical coronal section bearing implanted microwire tracks from the brain of a representative rat. Unit activities were recorded by the implanted microwires. When limited to units responsive to light probing of the hindpaw, all SmI units responded to contralateral hindpaw electrical stimulation. The responses, however, were complex with brief excitation and variable long latency effects. When examined across the entire time-span, therefore, similar to data obtained in Smith *et al*. (27), we found that many of the SmI single-units recorded were not responsive. A few SmI units were even inhibited. When multiple-channel, multiple single-unit data were examined, more consistent results emerged. A representative example of activity changes in the ensemble SmI unit with different stimulation frequencies and two types of anesthesia of one rat is shown in Fig. 4. The ensemble unit activity showed significant increase at 3- and 10- Hz stimulation under the α-chloralose anesthesia. It showed a significant change to 10-Hz stimulation only under sodium pentobarbital anesthesia. Percentage-wise, increase of the firing rate was the most pronounced at 3 Hz under the α-chloralose anesthesia in this rat. Frequency-dependent unit activity changes were studied in seven rats. In each of them, the effects of both α-chloralose and sodium pentobarbital effects were examined. The results are summarized in Fig. 5. Ensemble unit activities were significantly increased in the SmI hindpaw region (the "In" region) at stimulation of 3 Hz under α-chloralose (Fig. 5A), and at stimulation of 10 Hz under sodium pentobarbital (Fig. 5B).

The responses of the units recorded from channels not responsive to light probing of the contralateral hindpaw (the "Out" region), their responses were not significant. The responses of evoked field potentials were quantitatively studied using the peak-to-peak value of the short-latency evoked potential. We defined the integral evoked potential (ΣEP) as the product of the peak-to-peak amplitude of the evoked potential multiplied by the stimulation frequency. A representative example of data obtained from one rat is shown in Fig. 6. Under both α-chloralose and pentobarbital anesthesia, amplitudes of the evoked potential of SmI decreased with an increase in the stimulation frequency (Fig. 6A, 6B). Multiplying with the frequency value yielded a bell-shaped curve (Fig. 6C). The value of ΣEP was the highest at 10 Hz stimulation under pentobarbital anesthesia but at 3 ~ 10 Hz stimulation under α-chloralose anesthesia. The value of ΣEP under α-chloralose anesthesia was significantly different at 3 Hz stimulation from that under pentobarbital anesthesia (Fig. 6C).

Functional MRI images and neural activities showed frequency- and anesthetic-dependent changes under somatosensory stimulation. Firing rate changes of ensemble unit activities showed a maximal increase at 3 Hz stimulation. The BOLD signal changes showed a pattern of activation similar to that observed in the ensemble unit activities on both the frequency and the anesthetics variables (Fig. 7A, 7B). Amplitudes of the ΣEP augmented with an increase in

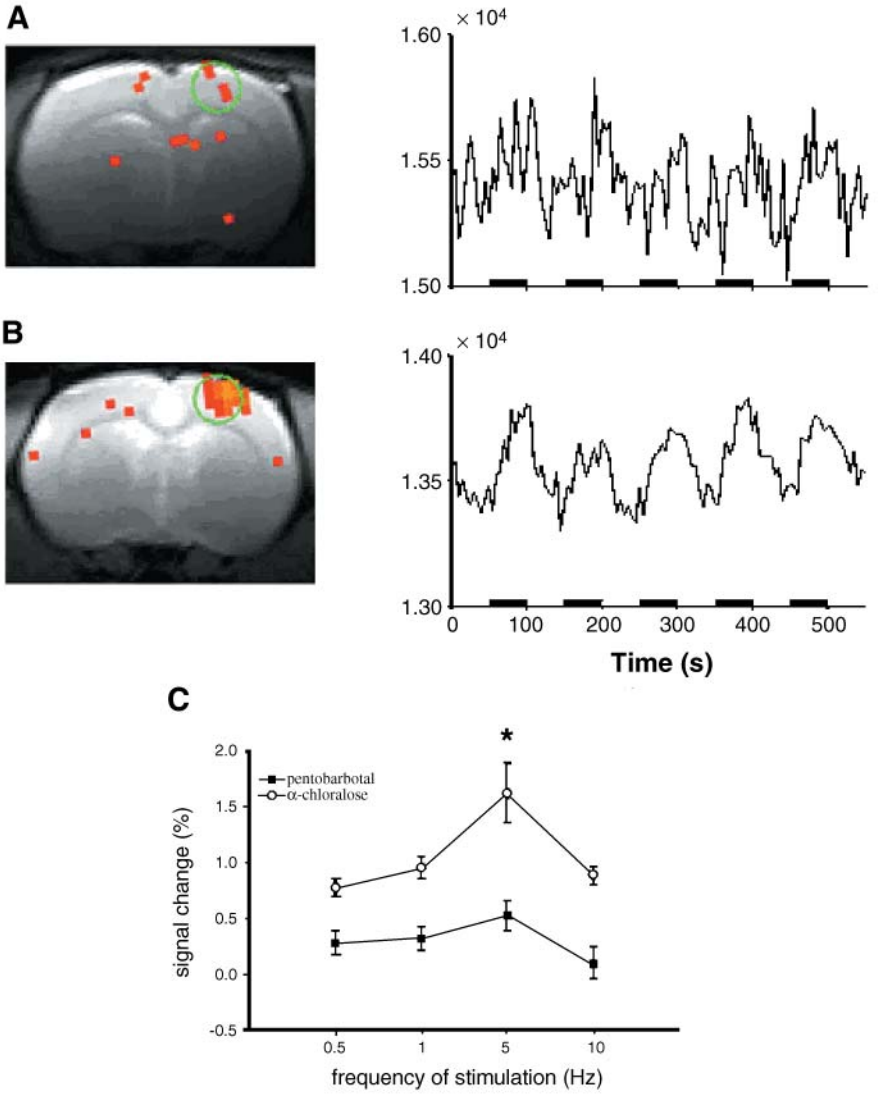

Fig. 3. Results of the BOLD fMRI activation in the hindpaw region of the SmI during 2 mA, 3-Hz left hind paw stimulation. (A) A rat under sodium pentobarbital anesthesia, and (B) another rat under α-chloralose anesthesia. The figures on the left are activation maps on which significantly correlated pixels are marked with red dots. Figures on the right are time-varying average BOLD values in the ROIs (regions of interest, as circled in the brain maps on the left) used for the calculating of the percentage changes. The lower panels in the right hand side denote when the electrical stimulation was on (solid bars) and off (no bar). (C) Frequency-dependent changes of BOLD signals change under two kinds of anesthesia. The highest BOLD signal change occurred at 3 Hz of stimulation in rats under α-chloralose anesthesia. *: $P < 0.05$, compare to different anesthetics.

stimulation frequency (Fig 7C, 7D). The correlations between BOLD signal and ensemble unit activity or ΣEP were calculated. The correlation coefficient of BOLD signal and ensemble unit activity (r = 0.71, P = 0.29) is higher than the one of BOLD signal and ΣEP (r = 0.06, P = 0.94) under α-chloralose anesthesia. Under pentobarbital anesthesia, the correlation coefficients between BOLD signal and ensemble unit activity is -0.2 (P = 0.8) and the one between BOLD and ΣEP is 0.07 (P = 0.92). We summarized the responses of BOLD signal, ensemble unit activity and ΣEP under both α-chloralose and pentobarbital anesthesia and calculate the correlation coefficient. The summary correlation coefficient between BOLD signal and ensemble unit activity (0.46, P = 0.25) is higher than the one between BOLD signal and ΣEP (-0.13, P = 0.76).

Discussion

This study compared the effects of stimulation frequency and anesthetics on cortical unit activity, field potential and fMRI BOLD changes induced by electrically stimulating the hind paw of the rat. Cor-

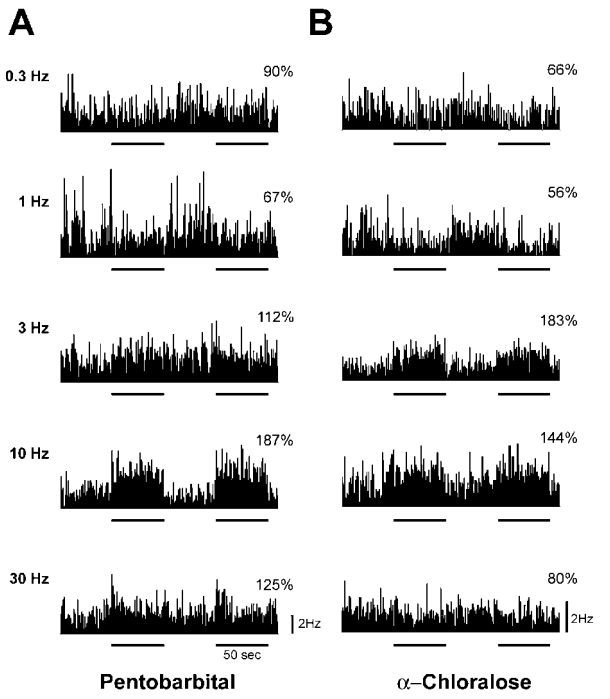

Fig. 4. Averaged ensemble single-unit activity of six hindpaw SmI units during electrical stimulation of the contralateral hind paw at 0.3 to 30 Hz and 1.5 mA. Thick bars under each graph represent the stimulation. The rat was under pentobarbital anesthesia (A) and under α-chloralose anesthesia (B). Percentage values on the top right corner of each figurelet are relative ensemble unit activities during stimulation periods compared to values during periods not stimulated. Note in this rat the highest activation occurred at 10 Hz stimulation under pentobarbital anesthesia and at 3 Hz under α-chloralose.

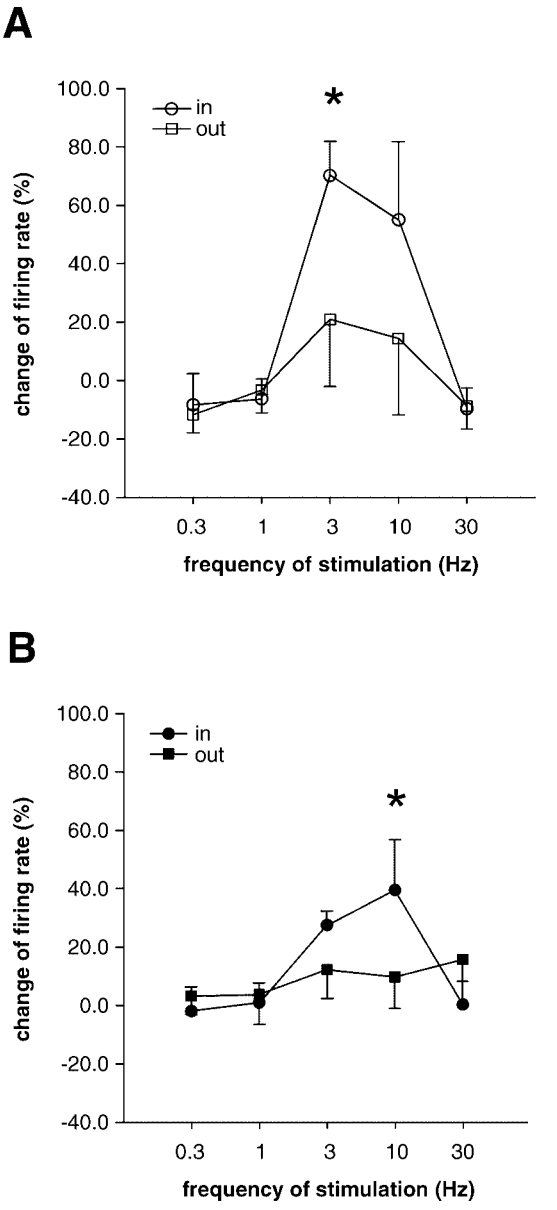

Fig. 5. Frequency-dependent changes ensemble single-unit activity of cortical regions in (In) and near (Out) the hind paw region of the primary sensorimotor cortex. Electrical stimulations were applied to the hind paw of rats under α-chloralose (A) and under sodium pentobarbital (B) anesthesia. The average firing rate in baseline period was taken as 0%. *: $P < 0.05$, compare to Out region group.

tical single-units showed responses varying from excitation to inhibition. However, with multiple-channel, multiple single-unit recording, ensemble activation was more consistent in response to the hindpaw stimulation and showed a frequency-dependent effect correlated the best with fMRI activation under α-chloralose anesthesia

Electrical Signal vs. BOLD Signal

Many fMRI studies have shown that somatosensory stimulation of limbs or electrical stimulation of peripheral somatic nerves activated BOLD signals in the homotypic SI region (2, 4, 9, 10, 11, 23, 25, 29, 33). Given that electrical activity is often viewed as the most pertinent indicator of neuronal functions, a question arises as to how the BOLD signal changes related to the electrical signals. Several studies compared functional imaging change with evoked potential (1, 3). Linear coupling was found. The range of the stimulation frequency was too narrow (1.5 to 6 Hz in (3) and 10-100 Hz in (1)), however to account for the bell shaped frequency-response curve repeatedly demonstrated for the relationship between somatosensory stimulation frequency and cerebral blood flow or functional image change (7, 15, 16, 17, 18, 20, 26).

Another often-used neural activity index is the firing rate of the local neurons. Several studies have attempted correlating evoked unit activities with the local blood flow, metabolic rate, or BOLD intensity

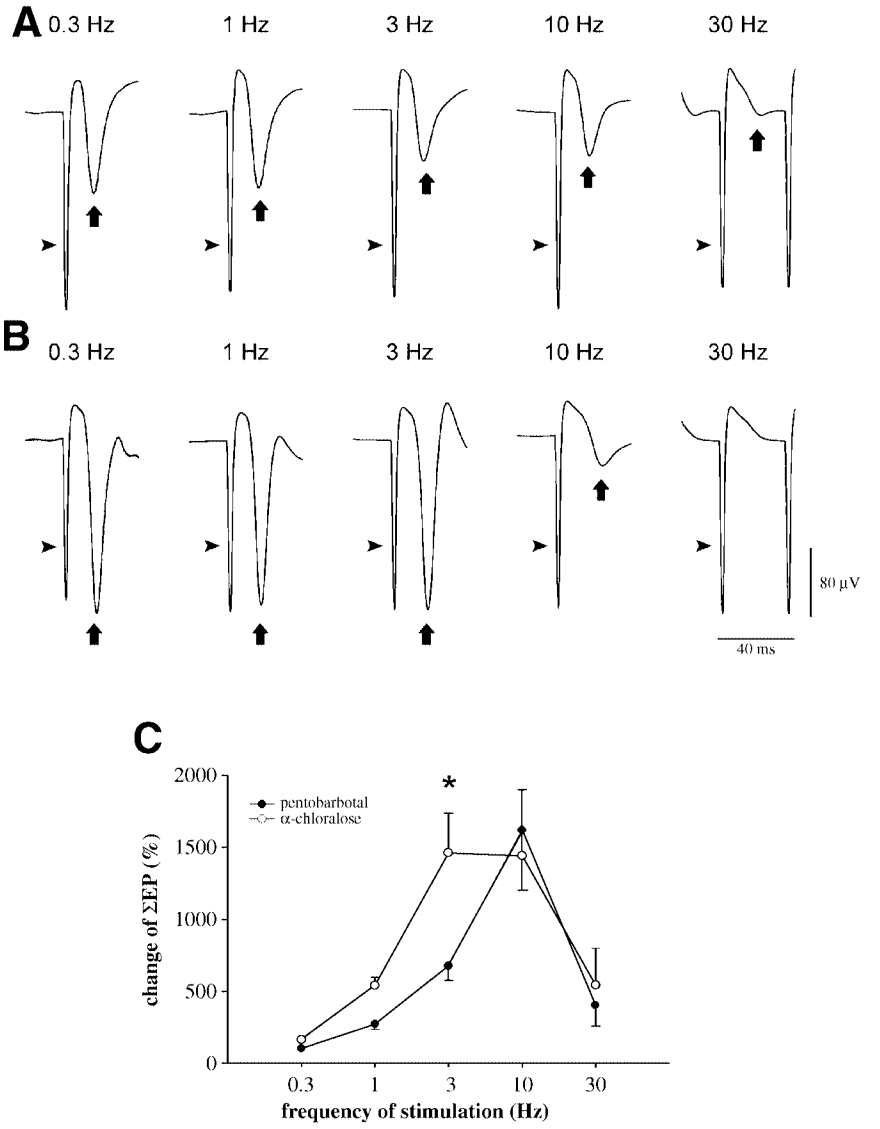

Fig. 6. Frequency dependent changes of field potential in the SmI evoked from electrical stimulation of the contralateral hind paw. Square wave pulses 2 mA in intensity and 2 ms in duration. (A) The rat was under pentobarbital anesthesia; (B) the same rat under α-chloralose anesthesia. Arrowheads point to the artifact of the electrical stimulation. Arrows point to the evoked field potential. Note evoked field potential persisted in the SmI of the rat under pentobarbital anesthesia at higher stimulation frequencies (10 and 30 Hz). (C) Summary of frequency-dependent changes of evoked field potential in SmI under the two kinds of anesthesia. Change of ΣEP calculated as the % difference in integral evoked potential compared with that at 0.3 Hz stimulation. *: $P < 0.05$, compare to different anesthetics.

in the cerebral cortex (7, 14, 27, 30, 32). In all of these studies, a significant positive correlation has been observed between the unit activities and blood flow, metabolic rate or changes in the BOLD signal. Studies by Logothetis *et al.* (13, 14) and by Devor *et al.* (7) measured the local field potential and unit activities simultaneously in the same animal. Using anesthetized and paralyzed monkey, Logothetis *et al.* (14) reported that changes in local field potential correlated notably better than unit activity with the BOLD signal changes in the visual cortex. On the other hand, Devor *et al.* (7) found a linear correlation of local field potential and multi-unit activity in the barrel cortex throughout the full range of whisker stimulation in the rat. The present study, employing a multiple-channel, multiple single-unit recording method, probed the same relationship with the hindpaw activated cortical responses. Our data indicate that in rats anesthetized with α-chloralose, in the SmI, under the condition of intermittent electrical stimulation of the skin, multiple single-unit responses correlated better with the BOLD signal changes than did the

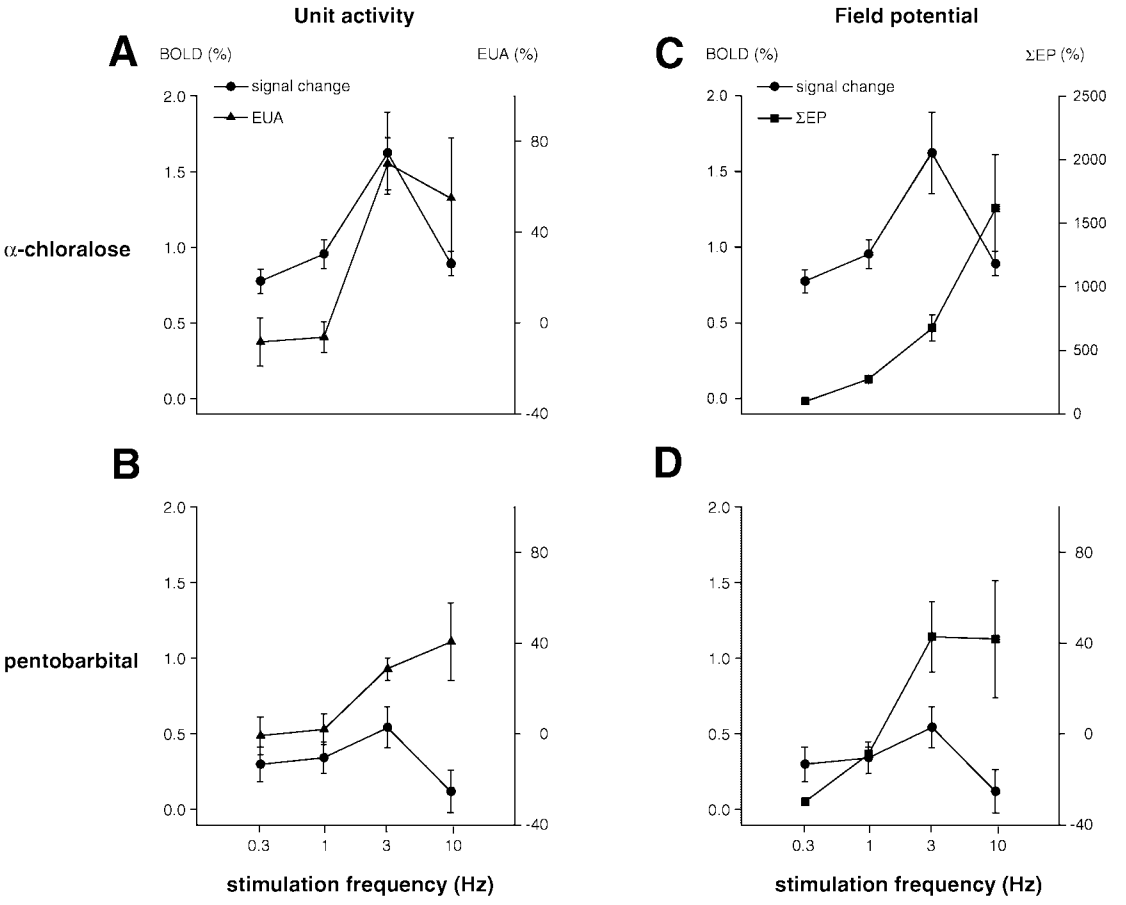

Fig. 7. Comparisons between BOLD signal versus ensemble unit activity or ΣP change. (A) and (B) are BOLD signal change (solid dots) versus ensemble unit activity (triangles). The correlation coefficients are 0.71 (A) and -0.2 (B) respectively. The summary correlation coefficient between total BOLD signal and total ensemble unit activity changes under both anesthesias is 0.59. (C) and (D) are BOLD signal change (solid dots) versus ΣEP (squares). The correlation coefficients are 0.06 (C) and 0.07 (D). The correlation coefficient between total BOLD signal and total ΣEP changes under the two anesthesias is -0.13. The upper panels (A and C) are under α-chloralose anesthesia. The lower panels (B and D) are under pentobarbital anesthesia.

evoked local field potential responses (Fig 7A). Multiple single-unit activity consistently increased at a range of stimulation frequencies within which elevation in the BOLD signal was also observed. The ensemble unit activity correlated slightly better with BOLD changes than did the integral evoked potentials in terms of their anesthetics dependency (Fig. 7). Although the correlation coefficients did not reach significance level, it implies a trend that BOLD signal correspond well with ensemble unit activity.

Many factors may underlie the discrepancy among these findings. The most important ones are the stimulation paradigm and the nature of the afferent fibers involved. In the studies of Logothetis *et al.* (14), computer-generated visual patterns were continuously presented; whereas in the whisker or the hind paw studies, stimulation pulses were given intermittently. With each stimulation pulse, both units and local field potential in the SmI show strong responses which habituate little. In contrast, to the continuous presentation of the visual pattern, while both field potential and multi-unit responses of the primary visual cortex adapt in a few seconds, the field potential nevertheless maintains a slowly adapting component. In addition, the somatosensory integral evoked potential sampled only the cortical synchronized responses of large myelinated axons (A-beta fibers), whereas the cerebral blood flow and BOLD signals might be reflecting activations from various types of afferent fibers. It might be that afferent inputs to the primary visual cortex and barrel cortex are from large myelinated axons, and hence the consistency between the responsiveness of the field potential and the BOLD signal. On the other hand, the stronger the electrical current used to stimulate the paws and peripheral nerves, the higher the cortical

blood flow or BOLD signal responded (4, 20, 26, 29). These responses do not plateau until maximal C-fiber activation is reached. It is not surprising, therefore, that the A-beta fiber evoked field potential did not correspond well with the BOLD signal as illustrated in the present study.

The significance of the present study lies in the comparison between single-unit data versus multiple channel, multiple single-unit data. While single-unit activities were elevated or depressed from the baseline in response to somatic stimulation, ensemble neuronal activity was much more consistent. We suggest that, in somatosensory studies involving A-delta or C fibers, a better field potential index should be explored which truly reflects activation from all relevant afferent fiber types.

Pentobarbital vs. α- Chloralose

The second part of the present study compared the effects of two anesthetics, α-chloralose and sodium pentobarbital, on the fMRI and neuronal activity changes. Anesthetics in the barbiturate group greatly reduce cerebral metabolism and resting CBF (8, 28, 32). α-chloralose is usually employed in fMRI experiments because of it has fewer effects on cardiovascular and respiratory functions and reflex activities (32). Ueki *et al*. (34) compared the effects of anesthesia on metabolic coupling in the rat's somatosensory cortex for α-chloralose, halothane, pentobarbital and nitrous oxide. They found that somatosensory stimulation induced a focal increase in the regional cerebral metabolic rate of glucose only under α-chloralose anesthesia. The effects of halothane, α-chloralose and thiobarbiturate on the CBF response were also compared. The result indicated that the increase in CBF induced by vibrissae stimulation was more obvious and stable under α-chloralose anesthesia (12). A non linear relationship was observed in the present study. On the one hand, a good correlation was found between fMRI and ensemble unit responses in rats under α-chloralose anesthesia (correlation coefficient 0.71). On the other hand, poorer correlation ($r = -0.2$) was found for the same correlation in rats anesthetized with pentobarbital. This illustrates a complicated relationship in which other important factors other than neuronal activity should be considered, for example, differential vascular or metabolic factors.

In summary, we examined the relationship between the fMRI signal and the underlying neural activity by recording these responses in anesthetized rats under the same constellation of conditions. The data show that a local increase in the fMRI signal correlated with an increase in neuronal activity only under restricted conditions. The fMRI signal correlated to ensemble single-unit activity better than integral evoked potential in α-chloralose anesthetized rats.

Acknowledgments

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