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# Separation of signal and noise from in vivo optical recording in Guinea pigs using independent component analysis

Shinichi Maeda[a], Shinji Inagaki[a], Hideo Kawaguchi[b], Wen-Jie Song[a,*]

[a]Department of Electronic Engineering, Graduate School of Engineering, Osaka University, Suita 565-0821, Japan
[b]Advanced Research Laboratory, Hitachi, Ltd., Saitama 350-0395, Japan

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**Abstract**

Optical recording in vivo severely suffers from the interference of heartbeat noise. So far, heartbeat noise has been minimized by subtracting from each experimental trial an average of interlaced control recordings. This method, however, is time-consuming and increases tissue damage due to phototoxicity. Here we applied independent component analysis (ICA) to in vivo optical recordings, for separation of auditory signals and noises. Our results show that ICA can be successfully used to separate sound-evoked signals and heartbeat noises. Compared with the previous method, ICA has a comparable power of separation and does not require background recordings. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords*: Optical recording; Independent component analysis; Noise reduction; Guinea pig; Auditory cortex

Information is represented in a distributed manner in the nervous system. Study of information transformation in the brain thus requires knowledge of spatio-temporal neuronal activity. Multi-channel optical recording in vivo is potentially a powerful technique for studying spatio-temporal neural dynamics [4,8]. By using voltage-sensitive dyes, optical recording systems can be made to have high spatial and temporal resolutions [4]. One drawback of the technique is that the fluorescence signal is much smaller than background artifact, with a ratio around $10^{-3}$ [2,15]. The two major sources of the noise are generated by the animal's heartbeat and respiration [2,6,8]. Respiration noises can be removed by temporarily stopping artificial respiration [2,6]. The heartbeat noise however, cannot be removed in the same way, and thus has typically been removed by subtracting averaged recordings obtained without stimulus from a stimulus-evoked recording (referred to as sAVR method in the following) [2,6]. This sAVR method is thus time-consuming and does not remove noises that survive averaging. The requirement of control recordings also increases cellular photodamage. In recent years, the method of independent component analysis (ICA) has been developed for blind source separation, and has been successfully applied to electroencephalogram, functional magnetic resonance imaging, magnetoencephalography, and optical imaging of intrinsic signals for signal/noise separation [11,13,14,16]. Because ICA does not require pre-stimulus recordings, we tested if ICA might be applicable to in vivo optical recordings using a voltage-sensitive dye in the auditory cortex. Our results showed that ICA was a useful alternative for signal/noise separation for in vivo optical recordings.

Optical recordings with the voltage-sensitive dye RH795 [9] was done from the primary auditory cortex of four anesthetized guinea pigs. All animals were treated in accordance with the principles approved by the Council of the Physiological Society of Japan. The recording system has been described previously [6,15] and is schematically depicted in Fig. 1A. With our optics, the $12 \times 12$ photodiode array covered a cortical area of $3 \times 3$ mm, corresponding to the size of the primary auditory cortex [17]. Signals from all channels were amplified and fed in parallel into a computer via a holding and sampling circuit. The sampling frequency was 1 or 2 kHz and the recording time was 1 s. For each stimulus, recordings were repeated for ten or 16 trials. Respiration was controlled with a respirator and was stopped for 1.5 s during each recording to eliminate respiration artifact. Recordings were synchronized to heartbeat by using the R wave of electrocardiogram as a trigger, for the execution of the sAVR method. By simultaneously recording local field potential (LFP) using a glass microelectrode

* Corresponding author. Tel.: +81-6-6879-7786; fax: +81-6-6879-7784.
*E-mail address:* song@ele.eng.osaka-u.ac.jp (W.-J. Song).


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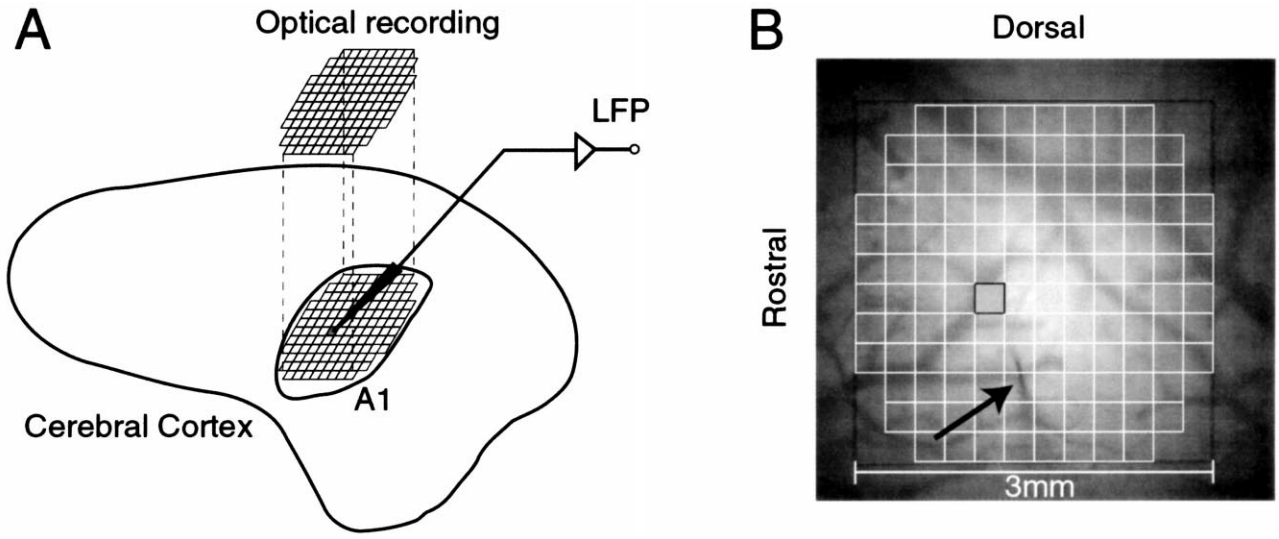


Fig. 1. Arrangement of the experiment. (A) A schematic diagram for the arrangement of optical recording and simultaneous LFP recording. The photodiode array is represented by the grating, which picks up optical signal from the whole area of the primary auditory cortex. Upper is dorsal and anterior is to the left. (B) A photograph of the recorded cortical area. The grid marks the position of the photodiode array. Arrow points to the electrode for LFP recording. The tip of the electrode, not visible in this photo, was located in the shaded pixel.

filled with 4 M NaCl (6–8 MΩ Fig. 1), we were able to evaluate and compare both sAVR and ICA methods in signal/noise separation [3,8,15].

A total of 206 trials were recorded during presentation of a sound stimulus, alternating with an equal number of control (no sound) recordings. The stimulus was either pure tones (1~7 kHz) or guinea pig vocal calls. In 45 of the 206 trials, LFPs were simultaneously recorded. Shown in Fig. 2A is a raw optical recording of the response to a 1 kHz tone. It is clear from the figure that the recording is heavily contaminated by a large sinusoidal-like component, caused by the animal's heartbeat. Our goal was to test if

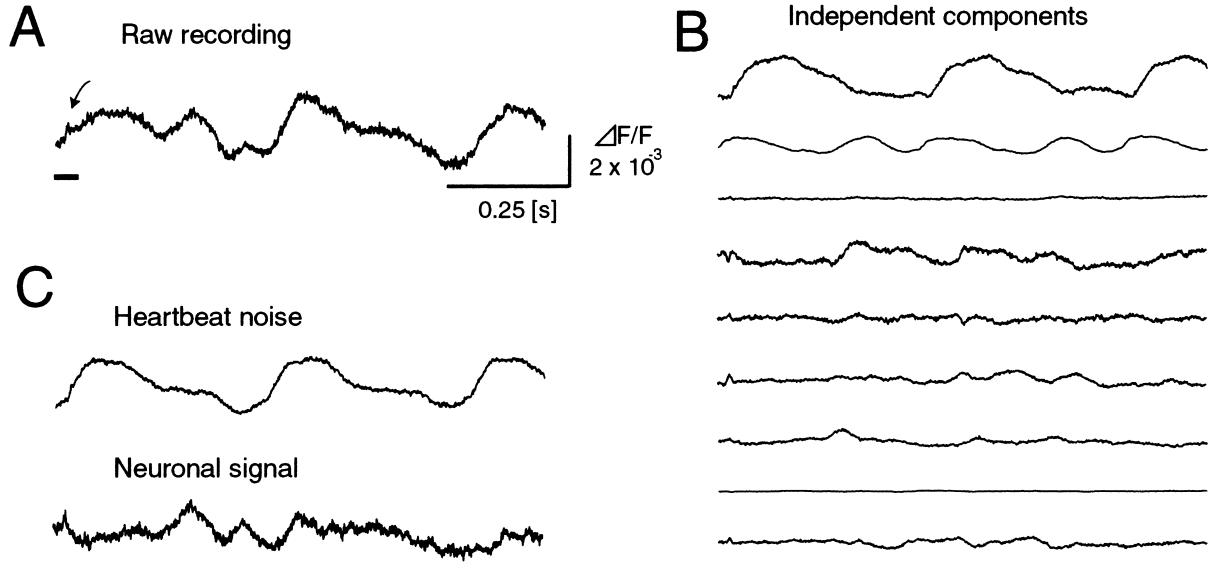


Fig. 2. Signal/noise separation with ICA. (A) An optical recording in a single trial, recorded from the shaded site shown in Fig. 1. The stimulus was a 1 kHz pure tone, whose timing is marked by the bar. The evoked response is indicated by the arrow. (B) Independent components for the recording in (A). The first two components are considered as heartbeat components from their periodicity. (C) The neural signal and the heartbeat noise of the recording in (A). Top trace: sum of the first two traces in (B), representing the heartbeat noise. Bottom trace: subtraction of the top trace from the raw recording in (A), representing the neural signal. This trace is virtually identical to the sum of the last seven traces in (B), with a minor difference caused by dimension reduction in principal component analysis. Scale bars in (A) apply to all traces.

heartbeat noise could be removed by using ICA. ICA has been developed as a signal processing technique to recover independent signal sources, **s**, from their mixtures, $\boldsymbol{x} = \mathbf{As}$, where **A** is a mixing matrix [5]. To recover the independent sources, ICA adaptively finds a matrix **W** to make components of $\boldsymbol{u} = \mathbf{Wx}$ mutually as independent as possible. Such components of $\boldsymbol{u}$ are then regarded as estimated independent signal sources. Our initial attempts to isolate heartbeat noises using ICA for recordings of single trials (1 s long data) always failed. We then treated the ten or 16 repeated trials as one recording to make the signal appear more stationary. In practice, ICA algorithms isolate the same number of sources as the number of recording sites [10,12]. Here we have recordings of 128 channels, but it was not necessary to assume the number of sources to be 128, considering the purpose here was only to separate signal from noise. The number of sources had to be assigned, because the true number was not known in advance. Assuming too large a number of independent sources would cause difficulty in computation and too small a number would not adequately represent the signals. Here we compressed the 128 channel recordings into nine major components using principal component analysis, before application of ICA. Changing the number of principal components from eight to 11 did not affect ICA analysis. We used both the Fast ICA algorithm [10] and the Extended ICA [12] algorithm, and obtained the same results (also see Ref. [7]).

Returning to Fig. 2, one can see an example of independent components decomposed from a raw recording. The raw recording in Fig. 2A was separated into nine independent components as shown in Fig. 2B. Whether an independent component represents noise or signal is not known, but to be determined based on knowledge of the signal or noise. Here we calculated the power spectrum of each independent component and assigned a component to heartbeat noise, if its power at the heartbeat frequency and twice the frequency exceeds a quarter of the total. Based on this criteria, two to five independent components were judged as heartbeat noise components, in each of a total of 206 cases studied. In the case of Fig. 2B, the first two traces were judged as heartbeat components. Heartbeat artifacts were then composed from the two components (Fig. 2C, top trace), and subtracted from the raw recording to obtain neural activity (Fig. 2C, bottom trace). To evaluate how well ICA separates neural signals from heartbeat noises, we calculated the correlation coefficient between neural signals separated with ICA and that separated with sAVR. Because in vivo optical signals resembled LFP [3,8,15], we further calculated the correlation between neural signals separated with ICA and LFP, and compared the correlation to that between signals separated with sAVR and the LFP. The three traces shown in Fig. 3A is the signal separated with ICA (top trace, the same as the bottom trace in Fig. 2C), the signal separated with sAVR (middle trace), and the LFP (bottom trace), all from the same recording site. It is clear from Fig. 3A that the three traces have similar waveforms. Shown in Fig. 3B is the correlation between the signal sepa-

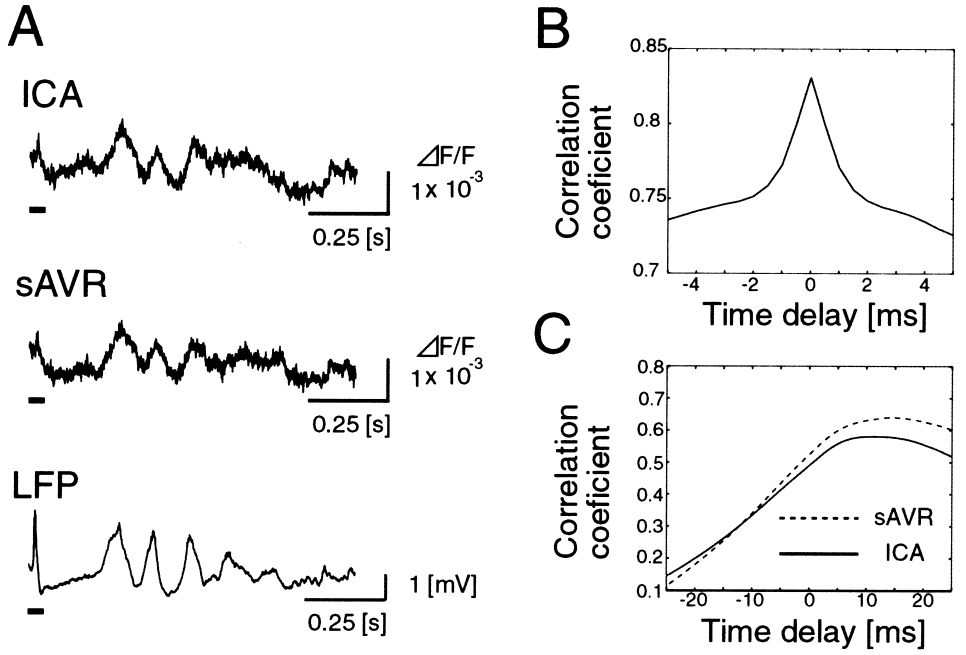


Fig. 3. Comparison of ICA method with the sAVR method. (A) Top trace: the neural signal, separated with ICA, as shown in Fig. 2C; middle trace: a neural signal separated with the sAVR method; bottom trace: an LFP recording. All traces are derived from the same recording site shown as the shaded site in Fig. 1. Bars mark the timing of stimulation. (B) Correlation coefficients between the signal separated with ICA and that separated with the sAVR method. C) Correlation coefficients between the LFP and the signal separated either with ICA (solid line) or with the sAVR method (dotted line).

rated with ICA and that separated with sAVR. The correlation coefficient curve has a peak value of 0.8309 at zero ms lag between the two signals. In all 206 cases studied, the averaged peak correlation coefficient was 0.8071 (standard deviation (SD) = 0.1207), suggesting that ICA effectively separated neural signals from heartbeat noises. This notion was further supported by the observation that the signal separated with ICA also correlated with the LFP, as did the signal separated with sAVR (Fig. 3C). The peak value of the correlation coefficient was shifted about 14 ms, because the time course of LFP reflected that of the extracellular current, while the optical signal reflected membrane potential. The averaged coefficient between LFP and the signal separated with sAVR was 0.4714 ($n = 45$, SD = 0.1859) at a 14 ms time shift. The averaged correlation coefficient between LFP and the signal separated with ICA was 0.3801 ($n = 45$, SD = 0.2120). Assuming that these values of correlation coefficient follow the normal distribution, the Akaike information criteria (AIC) [1] favors a model of equal variance with different mean (the AIC values were −28.85 for equal variance with different mean, −27.63 for different mean and different variance, −26.25 for equal mean and equal variance, and −24.47 for equal mean with different variance). The small difference in AIC values of different models, however, suggests that ICA-based signal/noise separation is closely as effective as the sAVR method.

We thus have shown that ICA can be used to successfully remove heartbeat noise from optical recordings obtained in vivo. Because ICA does not require pre-stimulation recordings, an obvious advantage of using this method is the shortening of the time required for experiments. This will in turn lead to a reduction of photodamage of neurons, and thus better data.

Compared with the sAVR method, however, ICA gave rise to a smaller value of the averaged correlation coefficient between LFP and the separated neural signal. We speculated that this was caused by the synchronization between sound stimulus and the heartbeat, because the synchronization tended to make the neural response depend on the heartbeat noise. Nevertheless, ICA was effective in the separation, probably because of the fluctuation of neural responses during repeated trials. The synchronization was done for the execution of the sAVR method, but it was not required for ICA. It is anticipated that by removing the synchronization, ICA would be even better in separating neural responses from the heartbeat noises.

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