Signal Analysis Techniques for Multi-site Recordings of Somatosensory Evoked Potentials in Free Moving Rats

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Abstract

Multi-site recording techniques have been used for characterizing the activities of large populations of neurons involved in brain processing. The aim of this study is to evaluate the multi-site recording technique for brain activities and utilize signal analysis for decomposing the underlying information in a study of free moving rats. In our study, male Wistar rats were first anesthetized and then transferred to a stereotaxic apparatus for the implantation of a multi-wire electrode into the primary somatosensory cortex (SI). During the experiment, sessions of somatosensory evoked potential (SEP), induced by electrical stimulus at rat's tail base, were recorded. Using multi-site recordings, independent component analysis (ICA) was used to remove the electrical stimulus artifacts. The decomposed signals, reconstructed from selected components based on cumulative power spectra, were represented in a topographic form in order to observe the spatiotemporal distribution of the rat's brain. Our results indicated that the application of an ICA can extract the dominant components of SEPs related to drowsy and awake states of somatosensory stimuli on the subject animal. The techniques developed in this study would benefit neuroscience studies of awake, free moving rats while performing neuropsychological task.

***Keywords*:** Multi-site recording, Somatosensory evoked potentials (SEPs), Independent component analysis (ICA), Free moving rat

Introduction

Recent advances in neuroscience have made it possible to utilize microelectrode array assemblies, surgically implanted in the subject's brain, to simultaneously record the extracellular neuronal activities of the brain. Varied types of recording electrodes, including multi-wire and silicon-based multi-electrode, have been developed for obtaining concurrent recordings of multiple neurons *in vivo* [10]. Developments in the yield, performance and durability of multi-site recordings, along with the growing power of computers, have allowed neurophysiologists to execute new experimental models and to observe the subject animal's behavior during a wide variety of brain activities. It is to be noted that even the simplest behaviors will significantly depend on the synchronous activation of great populations of neurons [12]. As a consequence of these developments, some researchers have been able to monitor the extracellular activity of neurons in various species of rodents and primates [11].

For multi-site recording of neural activities, the Identification of independent specific neural responses is essential in analyzing the interaction of multiple neurons. Ideally, the information that is distributed across a neuronal ensemble should be represented as patterns of correlated activity among the neurons. Unfortunately, it is extremely complicated to resolve the original structure of information contained among concurrently recorded neurons. An important goal is to understand multivariate statistical methods for dimension reduction that might be able to reconstruct functional interactions between multiple neurons. Typically, dimension reduction techniques have been used to transform a large set of observed variables into a smaller set of arbitrary variables. Principle component analysis (PCA), also known as single value decomposition, has been commonly used for separating varied types of biological signals [2, 3, 5]. Although PCA has been utilized for data dimension reduction, it mainly maximizes the variances of the principle components of signals without specifying the individual characteristics of each signal [2, 3]. A Recent development in independent component analysis (ICA) is aimed at identifying independent characteristics of ensemble biological signals which are straightforwardly related to the analyses of higher-order correlations. With these special features, ICA has been shown to be useful in analyzing the relationship amongst multiple,

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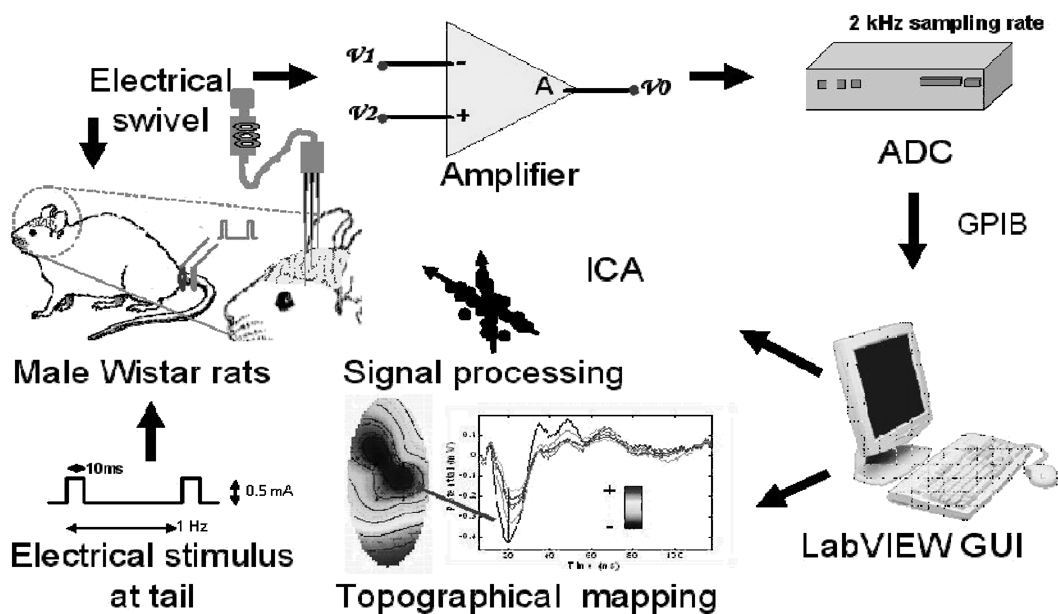

Figure 1. Schematic representation of the somatosensory stimulation, data acquisition system, and signal analysis method.

simultaneously recorded ensemble neural activates [4, 5, 6].

Among the various brain activity recordings, somatosensory evoked potential (SEP), an electrophysiological signal extracted from electroencephalogram (EEG), provides a highly effective method for the understanding and monitoring of somatosensory information collected from animals. When elicited by high intensity electrical stimulation, this response reflects the level of activation within the nociceptive system next to the activation of the tactile system after stimulation of peripheral nerves [1]. By using the multi-site recording technique, one can acquire data about brain activity which is distributed across multiple brain structures of the animals receiving the stimulus. However, when recording and eliciting these SEPs in rats, the rats used have generally been anaesthetized [8, 9, 15]. It is well known that anesthetic drugs could strongly modify the responses to noxious stimulation, thus modifying the SEP signal. As a consequence, these specific SEP waveforms do not necessarily represent the physiological functioning in conscious, rats and could not be used as the baseline data for investigating numerous neuroscience applications, e.g, studies on the anti-nociceptive efficacy of analgesic drugs. To utilize the SEP as a monitoring tool for the adequacy of analgesia, a more stringent and better standard definition in both stimulus/ recording procedures for alert, ambulatory animals is required [11].

The aim of this study is to design a multi-site brain recording system and employ these in animal experiments in order to measure the SEPs in active, mobile rats as well as to evaluate the signal processing method for identifying independent characteristics of recorded SEPs. The experimental protocols and signal analysis on SEP developed in this study can serve as a validation process for the implementation of multi-set recording techniques as well as the foundation for future animal behavior studies.

Material and Methods

Experimental Protocols

The animals used in these experiments are male Wistar (250-350 gm at surgery). All research protocols were approved by the National Cheng Kung University Medical Collage Animal Use Committee. All requirements of the “Guide for the Care and Use of Laboratory Animals” were conducted according to the standards set by the research council.

Before performing the SEP experiment, the rats received chronic implantation of 10 microelectrode arrays, which were arranged in two rows (spaced 0.5 mm apart) of five microelectrodes each, with an intermicroelectrode distance of approximately 200 μm. All animals were anesthetized to a surgical level with intraperoneal injections of pentobarbital sodium (50 mg/kg), and then placed into a stereotaxic apparatus (Model 902, David Kopf Instruments, Tujunga, CA). Supplementary injections were administered as required to keep the animals surgically anesthetized at the proper level using pentobarbital sodium (0.1-0.5 mg/kg, s.c.). A topical antibiotic was also applied. A bilateral craniotomy located in layer V of both SIs (-1.3~-2.3 mm caudal from bregma, 2.5 mm mediolateral, and ~1.5 mm depth from skull surface) [8, 9, 15] was performed. Three weeks following the recovery from surgery, each rat was placed in a Faraday cage five times (2 h/day) to habituate it to the experimental environment. On the day of the experiment, the animal was placed in the recording cage for 30 minutes prior to the recording session. The experiment was performed in a well-controlled, sound attenuated room. After completion of the experiment, the animal was sacrificed with an overdose of sodium pentobarbital (150-200 mg/kg).

To elicit the SEP, somatosensory electrical stimulation was applied using multistrand stainless steel wires which were

clamped around the tail base. These wires were held in place with an adhesive clamp, and electrode gel was applied to enhance the contact. A constant stream of pulses electrical current pulses of 10-ms duration were delivered to the tail. The intensity of the electrical current was set at 0.5 mA. This current level was chosen because it activates the low threshold cutaneous receptors. The interstimulus interval of electrical stimulation pulses was kept at 1 s. Simultaneous recordings of neural activity in free moving rats were carried out using an electrical swivel system and a series of amplifiers. The electrical swivel system has sliding gold wire contacts and gold slip rings to reduce noise. There are 8 electrical leads which are used in connection with head mounted electrodes for the long-term monitoring of brain activities. The head stage contains junction field effect transistor (JFET) input operational amplifiers (Motorola TL064) that feature high input impedance, low input bias current and low input offset current. The JFET input operational amplifiers were set as voltage followers with unit gain in all recording procedures. The preamplifiers are instrumentation amplifiers (BURR-BROWN INA118) and are used for very low offset voltage, high common-mode rejection and have a wide bandwidth, even at high gain (70 kHz at gain=100). Their output signals are transmitted to the second OP-Amps (gain=100) for a total gain of 10,000. The data acquisition system (ADC488/8SA, IO tech.) has simultaneous sample-and-hold function and 16-bit resolution and was used to sample the SEP at 2 kHz. A graphics user interface was programmed in LabVIEW for data acquisition (National Instrumentation, Austin, TX). The recorded raw data was analyzed through numerical computation software (MATLAB, The MathWorks, Natick, Massachusetts) for further off-line analysis. The entire setup for the electrical stimulation, SEP recording and signal processing procedures is illustrated in Fig. 1.

Independent Component Analysis for Somatosensory Evoked Potential

Independent Component Analysis (ICA) is a technique that maximizes the entropy of each independent component and minimizes the mutual information among independent components. As in many other linear transformations, ICA assumes that at a given instant in time the observed n-dimensional data vector, $x(k)=[x_1(k),........x_n(k)]^T$ is given by the model

$$x(k)=\sum_{i=1}^{m} a_i s_i(k) = As(k) \tag{1}$$

The source signals, $s_1(k),.........,s_m(k)$, are supposed to be stationary, independent and, together with the unknown coefficients of the mixing matrix, $A=[a_1,......,a_m]$. The goal is to estimate both unknowns from $x(k)$, with appropriate assumptions on the statistical properties of the source distributions. The solution is sought in the form

$$\hat{s}(k) = Bx(k) \tag{2}$$

where B is called the “separating matrix”.

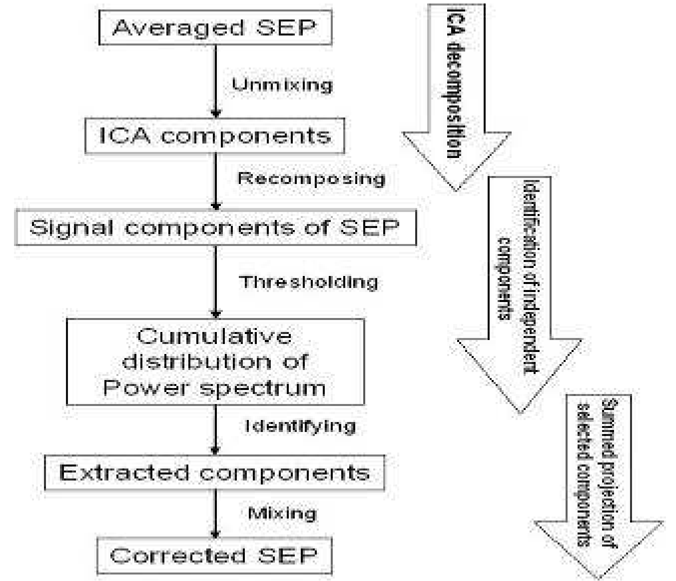

Figure 2. Procedures for selecting dominant components of independent components using cumulative distribution. The decomposed independent components were reconstructed from the selected components with no or minimal stimulation artifact or interference.

A FastICA algorithm is adopted in this study [3, 16]. FastICA uses a fourth-order cumulant, also called kurtosis. Kurtosis is negative for source signals whose amplitude has sub-Gaussian probability densities (distributions flatter than Gaussian), positive for super-Gaussian (sharper than Gaussian), and zero for Gaussian densities. Maximizing the norm of kurtosis leads to the identification of non-Gaussian sources. The FastICA is a fixed point algorithm which, maximizing the absolute value of the kurtosis, finds each of the columns of the separating matrix and so identifies one independent source at a time. The corresponding independent source signal can be found and the algorithm may be run repeatedly. It is, nevertheless, necessary to remove the information contained in the solutions already found, to estimate a different independent component each time [7, 16].

In our study, 8 channels of SEP signals were recorded. At most, 8 independent components can be derived using ICA. Although ICA is effective in extracting SEP from the stimulus artifact, there are some drawbacks in using an ICA algorithm. The assignment of independent components is arbitrary and no weighting is given to each component. We usually correlate the decomposed components with the original signal. In this study, we compute the cumulative signal power in the decomposed components in order to determine which component should be considered to be correlated to the SEP. In addition, the numbers of statistically independent signals contributing to the electrodes are unknown. Our approach to the physiological interpretation aspects is to observe from the spatio-temporal distribution of SEP. The entire signal analysis process for decomposing the components, removing the artifact, and spatio-temporal observation is depicted in Fig. 2.

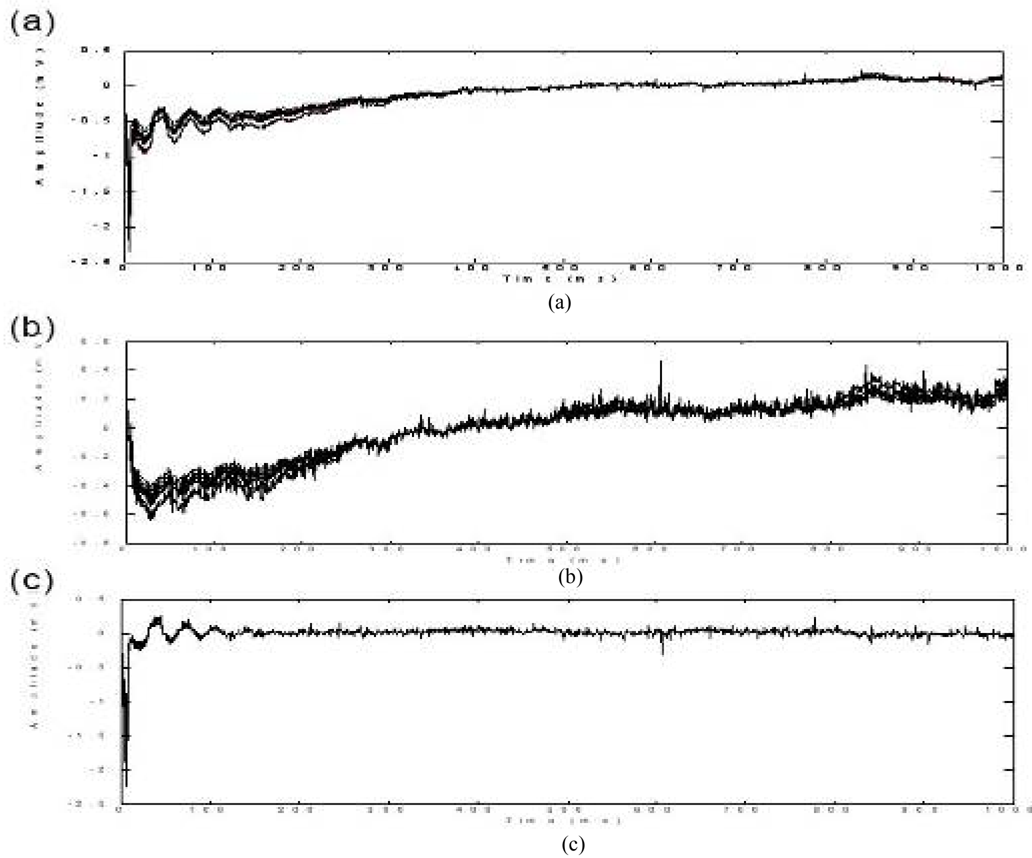

Figure. 3. The removal of stimulus artifacts in SEP signals after ICA. The ensemble average of SEP signals before ICA, (a), exhibits clearly stimulus artifact. (b) The stimulus artifact components can be extracted as an independent component with negative amplitude. (c) The coherent average of SEP signals after removing stimulus artifact shows major features in dominant peaks occurred at reasonable time latencies.

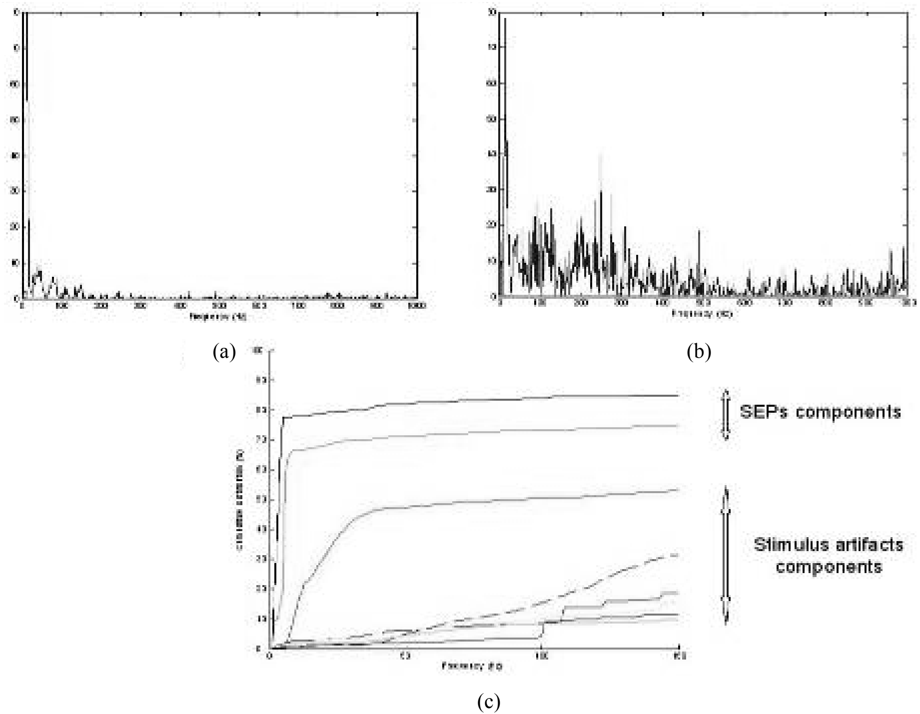

Figure. 4. Power spectra of the components extracted from SEP raw data, including (a) SEP and (b) stimulus artifact. The cumulative frequency distribution for these two major components (c).

Results

ICA for artifact suppression in an anesthetized rat

In our data analysis, ICA was first applied to decompose the SEP data collected from a Wistar rat which underwent electrical stimulation at the base of its tail while anesthetized. Figure 3(a) shows a portion of eight-channel of SEP signals from the implanted electrodes. Although the isolation circuitry of the amplifier was used, the predominantly negative activity pattern induced by the stimulus artifacts is still observed. After subtracting the stimulus artifact (Fig. 3(b)), the coherent average of SEPs exhibits clear, dominant peaks, as shown in Fig. 3(c). The criterion for determining spectrum to determine which independents components to be included is based on the cumulative spectral of the decomposed components.

Figure 4 (a) and (b) show the power spectra of the SEPs and the stimulus artifact, respectively. We can observe that the spectra of the major component of SEP are distributed across the low frequency band usually below 150 Hz. This characteristic was used to determine the components be considered as the SEP. In this study, we utilized the cumulative distribution of the SEP spectrum, as shown in Fig. 4(c), as a criterion in determining the numbers of included SEP components. There are two components which have 70 % of the in signal power below 150 Hz, and so were identified as SEP. The others 6 components were assumed to be either the stimulus artifact or white noise.

Signal Interpretation of SEPs in Rat

Two sessions of SEPs recorded in drowsy and alert states were compared for the interpretation of ICA analysis of the SEPs. The drowsy state is the transition stage, with a lower arousal level. Usually it is brought on by injecting pentobarbital sodium (0.1-0.5 mg/kg, i.p.) when the rats are ready to be connected to the recording modules for free moving activity. The SEP signals were recorded when the rat started moving its whisker but without any visible smooth limb movement. The rat is considered when it begins to perform voluntary free movement. Researchers have indicated that there are significant differences in the SEP properties recorded during these two distinct states [13-15].

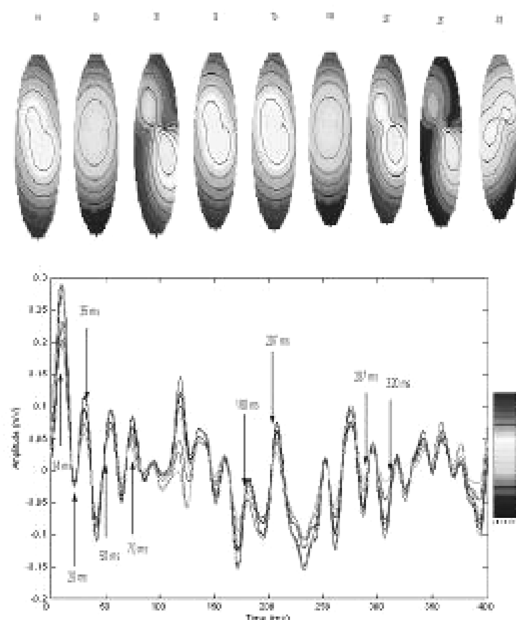

Figure. 5. The topographic brain mapping of SEP in drowsy state demonstrated that peaks occurred at the targeted time latencies, i.e., P14, N20, P35, and P50.

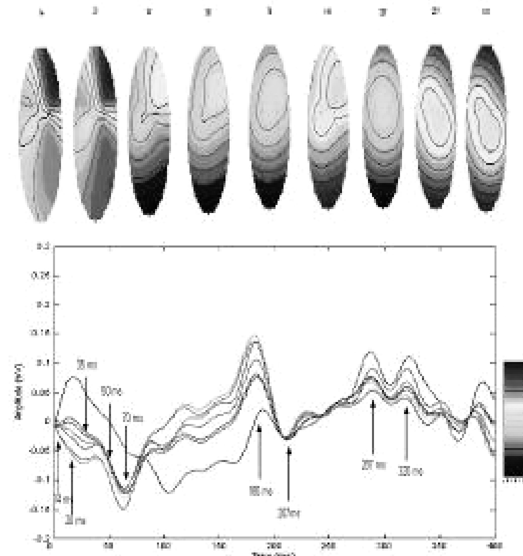

Figure. 6. The topographic brain mapping of awake state SEP. Dominant peaks distribution occurred at the reasonable time latencies, i.e., N70, N180, N207, P289 and P320.

Fig. 5 demonstrates the SEPs recorded in the drowsy state and the corresponding anatomical locations of the electrodes. The time latencies appeared around P14, N20, P35 and N50, respectively. The peak amplitude distribution of the individual SEPs exhibits characteristics similar to the SEP signal during the anesthetized state [8, 9]. Observing the topographical brain mapping, the spatio-temporal brain activity patterns during drowsy state SEP are very similar to those of anesthetized state. After averaging, the spatial mappings of SEP at different times are shown in Fig. 5. We can clearly observe that the potential in primary somatosensory area is nearly zero at both the initial (at 14ms) and final (at 100ms) stages. During the peak of SEP (at 20ms), the foci are located in the enter of the primary somatosensory. Similar foci distributions can be observed in the SEP measured at 35 ms. Moreover, a contour with two obvious foci can be found in SEPs measured at 14 and 35 ms. These might be considered as two independent sources in the brain.

Fig. 6 shows the SEPs of rat recorded in the awakened state. The first obvious peak appeared at about N70. The other predominant peaks appeared at P180, N207, P287 and P320. Compared to those in the drowsy state, the topographic brain mapping exhibited significantly different patterns, having longer time latencies compared with those found in the awakened stage. After averaging, the spatial mappings of SEPs at different time are shown in Fig.6. We can clearly observe that the potential in primary somatosensory area is nearly zero at the initial (at 14ms) and final (at 100ms) stages. During the

peak of SEP (at 20ms), major foci can be found at the center of the primary somatosensory. However, the foci observed around 35 ms of drowsy SEPs were not present in the SEPs of the awakened state. Moreover, a contour with two obvious foci can be found at SEPs measured at 180, 287 and 320 ms that might be considered as two independent sources in the brain. The corresponding anatomical locations of the electrodes are shown in Figure 6.

Discussion and Conclusions

The present study demonstrated the signal analysis technique which can extract the dominant components of SEPs recorded in response to electrical stimulation of the base of a rats' tail. The stimulus artifacts were extracted and removed based on the cumulative power spectra of reconstructed independent components. After removing of artifact signals, the key issue becomes how to verify that the reconstructed signals, after the ICA-based method contained no or minimal cerebral activity other than the artifacts we are examining. Our approach observed the physiological interpretation from the dominant peaks of averaged SEPs, and from the spatio-temporal distribution of SEPs.

From the averaged SEP, the peak amplitudes and latencies were pointed at the designated time as having originated from the recorded site, the primary somatosensory cortex (SI). From the frequency distribution of an SEP ranging from 0-150 Hz [16], we utilized the cumulative power spectrum as a criterion in determining the ICA components. Thus, the stimulus artifact was able to be easily identified and removed from the raw signal. After removing the stimulus artifact, the features of the SEPs are able to be observed from their averaged profile and from the interpolated mappings of the multi-site recordings. Our results showed significant prominent phenomena with peak amplitudes and latencies which coincided with previous studies (N20; P40 and N50) [10, 11].

Observing from the topographic mapping of the averaged SEP, distinct patterns can be differentiated for drowsy and awakened rats. From temporal brain mapping analysis, a localized concentric source of field potential was observed in the SI only after activating of the stimulus. Larger responsive cortical areas were found in response to more fiber activation.

Only a limited number of studies have reported SEPs recorded in awake, free moving rats [13,14,15,18]. The present study not only can successfully record SEPs evoked by electrical stimulation in awake, freely moving rats but also verify the noxious components from the SEPs recorded in both the drowsy and awakened states. It is known that the approach of using free-moving rats is less stressful when the altering the neural modulation of the anesthetized subjects. In this study, SEPs of both the drowsy and the awakened state are recorded to compare the characteristics of SPE at different arousal levels. In the drowsy state, the SEPs indicated that the peaks occurred in a similar way to those in the anesthetized state, even when the rats had began to move their whiskers and interact with the environment. In the same, the similar topographic brain mapping can be observed both in drowsy and anesthetized states. In contrast, SEPs measured in the awakened state are totally different from those of drowsy state. From the averaged SEPs, a large negative 100 ms (N100, in 70 ms) peak can be found, which might be linked to the motor-evoked potential. Although the SEPs of the rat tail have been triggered, the nociceptive stimuli of electrical stimulation might trigger movement of rat's tail in order to prevent the sensation of pain. The peaks occurring at N100 and P200 (positive peak at about 200 ms) are considered to be the processing of attention and appeared in the free moving rats as a result of the tail stimuli. Other peaks at P300a and P300b (double positive peaks in about 300 ms) might be related to the cognitive procedures due to its prolonged and delayed response.

In summary, the application of ICA is feasible for observing the multi-site recordings of both anesthetized and alert rats. With the removal of the stimulus artifact, determined from the cumulative spectral distribution, results in a better brain mapping of somatosensory stimuli which could facilitate a better understanding of various part of cortical brain functions involved in the processing of stimuli. Further investigation of the features of SEPs and the firing patterns of the multi-site cortex recordings would benefit neuroscience studies of awake, free moving rats performing various neuropsychological tasks.

Acknowledgements

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自由移動大鼠多頻道紀錄體感覺誘發電位之訊號分析技術

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摘　　要

多頻道紀錄的技術已經被使用在取得影響資訊處理的廣大叢集神經元的特徵。本研究的目的是評估多頻道紀錄腦部神經元活性的系統，以及利用訊號處理的方法應用於自由移動大鼠之研究。我們將以完成一系列的動物實驗，來印證所提出的紀錄系統與分析方法爲適用。首先，將麻醉 Wistar 公鼠，再將其放在立體定位儀器上，以將電極植入主要感覺皮質區。在實驗過程中，紀錄由刺激老鼠尾巴根部所產生的體感覺誘發電位。多頻道的電訊號則可利用多變數的統計方法，稱爲獨立成分分析法，在神經元同時被記錄時，重新探尋訊號來源，而且也能除去在電極和獨立的訊號來源間的部分同時發生之資訊。將被分解出來的訊號，利用腦部電位拓譜圖來表示，以觀察訊號在腦部時空的分布。我們的結果指出獨立成分分析法的演算法，在同時紀錄體感覺誘發電位的實驗中，能夠將電刺激的雜訊與體感覺誘發電位分離出來，而且在這同時紀錄的體感覺誘發電位中，能再分離出明顯的獨立成分。本研究可完成多頻道紀錄腦部細胞外神經科學研究，以探討自由活動的動物行爲實驗。

關鍵詞：多頻道紀錄、體感覺誘發電位、獨立成分分析法、自由移動大鼠

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