NeuroReport 9, 2627-2631 (1998)

USING principal component analysis, we studied trial to trial, spontaneous variability of evoked potentials (EPs) recorded from rat barrel cortex after whisker stimulation. This method allowed for extraction of two distinct components of EP which overlapped in the time domain. Our results are consonant with the previously described depth distribution of current sources and the extracted components can be therefore attributed to activities of two pyramidal cell classes: supra- and infragranular. Qualitatively similar results were found in both anaesthetized and alert animals. ***NeuroReport*** **9:** ***2627—2631*** **©1998 Rapid Science Ltd.**

Key words: Evoked potentials; Principal component analysis; Sensory cortex

Spontaneous variability reveals principal components in cortical evoked potentials

Pawe³ Musia³,CA Ewa Kublik and Andrzej Wróbel

Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur Str., 02-093 Warsaw, Poland

CAcorresponding Author

Introduction

Evoked potentials for stimuli repeated under the same experimental conditions differ from trial to trial within a short time scale of tens of milliseconds.[1] The origin and character of this variability have been studied in more detail by analyzing spike trains.[2-6] Traditionally, the uncontrollable variability is removed by averaging EPs over many trials. This widely used approach is based on an assumption that EP is a linear sum of background neural activity (uncorrelated with stimulus) and a reproducible response, stable within a period of averaging. This is a poor assumption because, for example, responses of the brain might not necessarily be the same from trial to trial. Many physiological phenomena, uncontrollable by the experimenter, can cause such variability. Moreover, when a number of sources contribute to EP, each of them can be influenced by various factors, in a different manner.

The existence of a multitude of simultaneously active generators is one of the main problems in interpreting electrical field recordings from the brain. Among multivariate statistical methods for resolving sources of electrical activity, principal components analysis (PCA),[7-9] has been used successfully in many studies.[10-14] The advantage of the PCA approach is that it considers values sampled from the whole time period of a registered signal instead of only some values measured at chosen moments (e.g. locally extreme negativities and positivities of EP). We attempted to use the PCA method for resolving components of sensory EPs based on their variability over consecutive trials as recorded by one electrode, placed at a constant location within a rat's barrel cortex. Such an approach could open new possibilities for elaborating simultaneous activity of a few distinct cortical sources. It would provide an especially powerful tool for studying dynamic changes of intracortical brain activity in chronically implanted, behaving animals.

Materials and Methods

Animal preparation: The experiment consisted of two parts, in which we recorded cortical EPs from acute (A) and chronic (C) animals. For the acute condition, five hooded rats (250—320 g) were anaesthetized with urethane (1.3 mg/kg, i.p., with 10% of original dose added when necessary), and placed in stereotaxic apparatus (Kopf). Anaesthetic gel was applied into the rat's ears and the skin was injected with xylocaine prior to surgery. Fluid requirements (10 ml/100 g/24 h) were met by s.c. injections of 0.9% NaC1 and/or 5% glucose. The animal's physiological condition was monitored during the whole experiment by constant recording of temperature and electrocardiogram. The skull was opened to expose part of the barrel field, and the electrode placed into the cortex perpendicularly to its surface (typically in C2 barrel region). Monopolar recordings were obtained at different cortical depth but the presented data were taken at chosen location of about layer IV.

The chronic preparation has been thoroughly described previously.[15] Briefly, before any experimental sessions rats were accustomed for 2—3 weeks to stay still in a specially designed restraining chamber. After this, the electrodes were implanted into the barrel cortex at the depth of layer IV (500—700 p~m). The surgical procedures did not differ from those in acute experiments, except that we used chloral hydrate (1 ml of 3.5% solution/100 g body weight) for anaesthesia. The electrodes were secured to the skull with dental cement. The wound was rinsed with local antiseptic and the animals were left for a few days for recovery.

Ordinary histology was used after representative experiments in both conditions to confirm the exact electrode locations.

Stimulation: The somatosensory stimuli and recording procedure were similar in acute and chronic groups. The whisker stimulator consisted of a thin needle glued to a piezoelectric slab. The loose end of the needle was attached to the whisker —10 mm from the snout. Square wave pulses of 3 ms duration delivered from the PC computer produced a 0.1 mm vertical movement of the whisker. An average of eight evoked potentials (EPs) from all whiskers was initially registered to choose the best responding one for further experiments. During an experimental session, lasting for about 1 h, the chosen whisker received 100 stimuli with a frequency of 0.2 Hz (A) or with random interstimulus intervals (30 s on average) (C).

Recordings: Insulated 25 p~m tungsten wire with a sharpened tip was used for monopolar local field potential recordings (EEG) with a screw in the nasal bone used as a reference. The EEG signal was amplified (l000x), filtered (from 0.1 Hz to 5 kHz) and stored on magnetic tape by means of a RACAL V-store recorder. The field potentials (EPs) evoked by whisker stimulation were digitized on-line (2 kHz frequency) with Spike2 software for preliminary analysis. Stimulus markers, EGG, temperature and comments on the animal's behavior (C group) where stored on the analog tape. All taped data were examined for integrity and epochs with artifacts were excluded from further analysis. For PCA analysis, epochs of 25 ms duration (starting with the stimulus) were digitized off-line at a frequency of 10 kHz and stored on a PC hard disk.

PCA analysis: Using the PCA method we assumed that recorded EPs resulted from several components which were generated by separate neural populations. The activity of each population was thought to have a unique time course (but could occur simultaneously) with a constraint that a given function remained stable (in particular, no variability of latency was allowed) and only the magnification factor could vary.

We applied the PCA method considering EP traces as variables and measurements in chosen time points as case values. In most of previous applications the chosen time points were taken as variables and whole EPs, as cases. Thus the proposed approach interchanges the variable and case concepts in comparison to previous applications of PCA to EP analysis.[8,t2,16] The EP traces were already used as variables in some applications 10,14,17 but they were always recorded in different electrode locations. Such a method allows for better interpreting results of the analysis — factor scores (factor values in consecutive cases) correspond to time activities of extracted sources and factor loadings[9] (correlations of factors with variables) to correlations of these activities with successive EP recordings. For improving interpretability of the results the principal components (factors) have been rotated (varimax rotation) in signal space. In physiological terms, what is achieved after this operation is greater separation of extracted components over observation trials.

Results

Figure 1A shows representative EPs recorded in 16 consecutive trials from an electrode positioned at layer IV in urethane anaesthetized animal. The evolution of activity of sources extracted by PCA is shown in Fig. lB. The two principal components are shifted in time by about 1.5—4 ins, as roughly indicated by shift in their maxima (Fig. 1B). Similar results were obtained in the remaining four anaesthetized animals. As can be seen in Fig. 1A, only exceptional EPs follow exclusively the evolution of either the first or second component (thick lines). Most of the recorded EPs are, however, composed of uneven contributions from both components. These data suggest that the second extracted component is really embedded in the data and is not just a 'latency adjustment component'[13] arising from substantial latency variation of only one underlying source.

Qualitatively similar results were obtained with the group of alert animals (Fig. 2A). The difference between alert and anaesthetized animals could be better traced when two components are compared in both groups (Figs 1B,2B). In the former group both principal components seemed to be less synchronized (with broader negative peaks) and difference between peak latencies was typically bigger (up to 4 ins).

In all anaesthetized animals the two first principal components typically accounted for about 90% of the variance in population of variables. Further

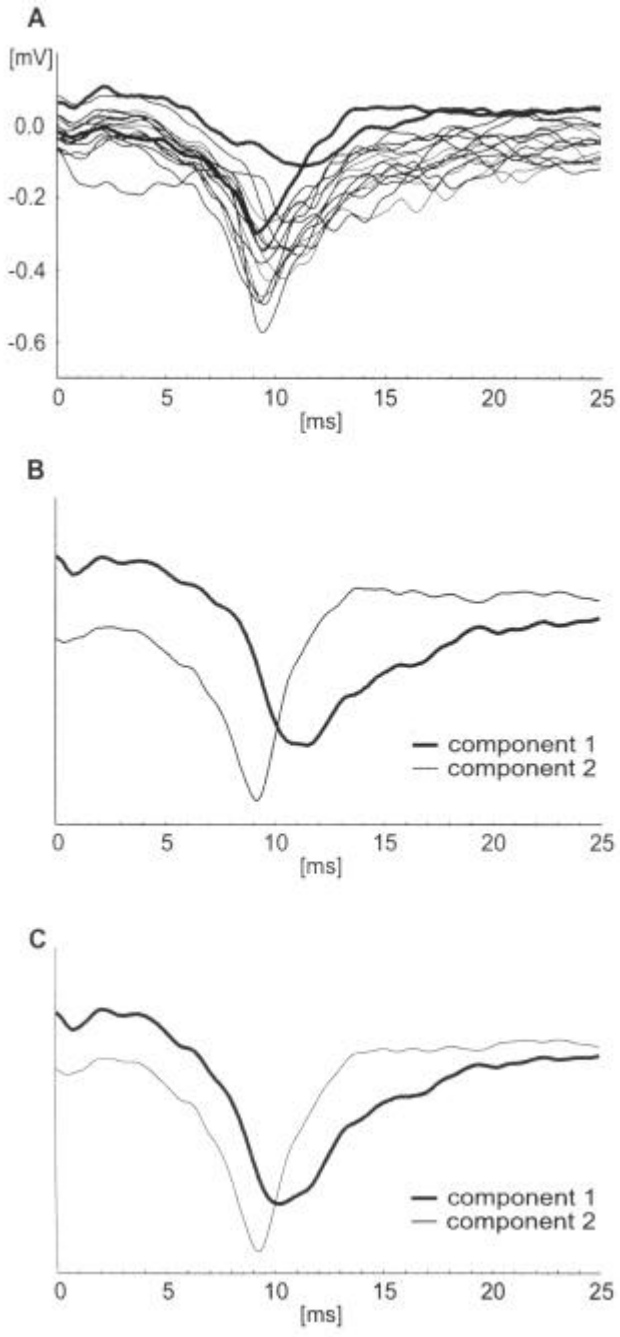

FIG. 1. (A) Evoked potentials (16 trials) recorded from the barrel cortex of anaesthetized rat after whisker stimulation. Thicker lines represent responses dominated by one of the two extracted components. (B) First two principal components extracted from the data presented in (A) (accounting for 92% of variance in population of variables) after varimax rotation. (C) As B, but after oblimin rotation. Correlation between components is 0.58.

components were rejected according to Kaiser's criterion.[12] In alert animals group, however, two first components accounted only for about 70% of variance, due to greater number of factors contributing to EP variability. In Fig. 2B we present only two first components of the PCA as calculated for the alert rat, in order to compare them directly with those obtained for the sleeping animal (Fig. 1B).

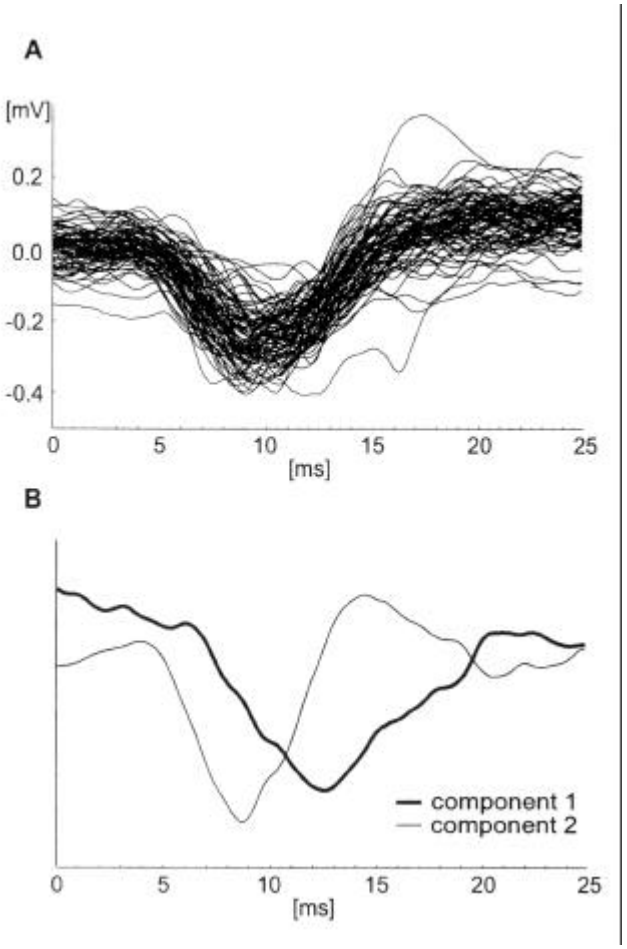

Fig. 2. (A) Evoked potentials (87 trials) recorded from the barrel cortex of alert rat after whisker stimulation. (B) First two principal components extracted from the data presented in (A) (accounting for 73 % of variance in population of variables) after varimax rotation.

Discussion

We suggest that two principal components extracted from cortical EPs reflect activation of two distinct

neuronal sources with second one lagging behind the first by about 1.5—3 ins. This proposal is based on the repeated observations that the two components change in a different manner from trial to trial. Strong support for this conclusion comes from our previous experiment in which we have found that the two components are differently influenced by a brief cooling pulse applied to the cortical surface: the short latency component ceased transiently after a pulse, while the second one remained untouched. Finally, time courses of the two components obtained in present study correspond to those shown previously on ketainine anaesthetized rats.[t0] Di *et al.*[t0] applied the PCA method to current source density profiles

calculated from detailed EP data recorded at many points along cortical depth. With a good spatial resolution they calculated the accurate depth distribution of sinks and sources of extracellular currents and concluded that the two extracted principal components originated from two separately activated pyramidal cell classes — the first component from supragranular, and the second one from infragranular cells. Our results indicate that activities of two pyramidal cells populations can be separated from recordings obtained at one electrode location but registered from trial to trial, in a longer time domain. It should be noted, however, that two components derived by the proposed PCA analysis could have their origin not only from two independent sources differently contributing to the summed responses but also from latency jitter of single EPs.[13] With a single electrode recording this possibility could not be directly ruled out. We think that such an explanation is unlikely considering the supporting results from independent experiments,[10,18] as discussed above.

The observed variability of cortical EPs seems to be of the same nature in sleeping and alert rats (Figs 1B,2B). The only difference might be a more noisy recordings from alert animals which resulted in slightly broader principal components. This is in agreement with what we know about neuronal processing in alert and sleeping brain. Similar conclusions come from more numerous studies on variability of single cell responses, e.g. recordings from visual cortex of behaving monkeys.[6,19] It is important to know whether the two phenomena measured at different levels (EP and single neurons[2-6,19]) are related to each other. Evoked potentials reflect summed postsynaptic processes arising in many neurons at the same time. Assuming that the cells are activated independently, and not in groups, then linear summation of field potentials (according to the superposition rule) should lower only the random variability. Most EPs recorded in the present experiment have two underlying components (Figs 1A,2A), suggesting the existence of organized inputs from many single neurons. Similar observation was made in visual cortex of the anaesthetized monkey.[2] By recording spike activity with multi-electrode matrix (5 x 6 wires) Bach and Kruger[2] observed highly correlated response variability within some groups of neurons and, at the same time, uncorrelated variability between these groups. They suggested that in observed neuronal populations variability of neuronal responses originated from synchronized outer inputs rather than from intraneuronal noise processes.

Application of PCA followed by varimax rotation for analysis of evoked potentials was questioned on the basis of its orthogonality, which is not physiologically justified.[16,20] In fact the varimax rotation limits possible solutions only to components that do not covary in time. EP components in our study are not necessarily uncorrelated and this is why their actual time courses may differ from those derived by the PCA analysis. To investigate this effect for the representative sample of data (set of 16 EP records) we applied rotation of oblimin type (Fig. 1C) which, like varimax, leads to maximal separation of factors (components) over trials but allows for correlation between these factors. When compared to results of oblimin rotation (Fig. 1C, correlation 0.58) the components obtained from varimax rotation (Fig. 1B) are shifted vertically which, in this case, assures lack of correlation between them. However, the time courses of the principal components obtained with both methods are nearly the same. This indicates that distortion caused by orthogonality was not prominent. The reason why we did not use oblimin rotation more widely in our analysis was caused by its higher sensitivity to small number of recordings. Each oblique rotation, as compared to orthogonal one, requires a greater number of rotation parameters to be estimated and thus it is more strongly affected by limited sample of trials. Although oblimin approach better approximates real data than varimax, the obtained results are more skewed by the acquired sample of data. For these reasons varimax rotation seems to be the reasonable option in our analysis.

In fact, neither varimax nor oblimin rotation can lead to separation of factors that evolve similarly in consecutive trials. Thus extracted components may contain phenomena that could not be further separated. Nevertheless, since qualitatively similar results are obtained with different rotational strategies (varimax and oblimin) and for different data (of Di *et al.*[10] and this study) the PCA proves its usefulness and reliability in application to EP data.

We conclude that spontaneous, trial to trial variability of EPs recorded from one location in barrel cortex can be used, with aid of PCA, to reveal activity of distinct populations of supragranular and infragranular pyramidal cells in both anaesthetized and behaving rats.

References

1. Arieli A, Sterkin A, Grinvald A and Aertsen A. *Science* 273, 1868—1871 (1996).
2. Bach M and Kruger J. *Exp Brain Res* 61, 451—456 (1986).
3. Dean AF. *Exp Brain Res* 44, 437—440 (1981).
4. Hartveit E and Heggelund P. *J Neurophysiol* 72, 1278—1289 (1994).
5. Schiller PH, Finley BL and Volman SF. *Brain Res* 105, 347—349 (1976).
6. Vogels R, Spileers W and Orban GA. *Exp Brain Res* 77, 432—436 (1989).
7. Chapman RM and McCrary JW. *Brain Cogn* 27, 288-310 (1995).
8. G)aser EM and Ruchkin DS. *Principles of Neurobiological Signal Analysis.* New York: New York Academic Press, 1976.
9. Herman HH. *Modern Factor Analysis,* 3rd edn, revised. Chicago: University of Chicago Press, 1976.
10. Di S, Baumgartner C and Barth D. *J Neurophysiol* **63,** 832—840 (1990).
11. Barth DS, Di S and Baumgartner C. *Brain Res* 484, 13—35 (1989).
12. Donchin E. *IEEE Trans Biomed Eng* 13, 131—139 (1966).
13. Donchin E and Heffley EF. Multivariate analysis of event-related potential data: a tutorial review. In: Otto D, ed. *Multidisciplinary Perspectives in Event-related Brain Potential Research.* Washington, DC: US Government Print Office, 1978: 555—572.
14. Maier J, Dagnelie G, Spekreijse H and Van Dijk BW. *Vis Res* **27,** 165—177 11987).
15. Musiaf P. Kublik E, Panecki SJ and WrObel A. *Brain Res* **786,** 1—10 11998).

16. Wood CC and McCarthy G. *Electroenceph and Clin Neurophysiol* **59,** 249—260 11984).
17. Baumgartner C, Sutherling WW, Di S and Barth D. *J Neurosci Methods* **30,** 175—184 (19891.
18. Kublik E, Musiat P and WrObel A. *Acts Neurobiol Exp* **57** (Suppl.), 65 (1997).
19. Snowden Ri, Treue S and Andersen RA. *Exp Brain Res* **88,** 389—400 11992).
20. Collet W. *Biol Psychol* **28,** 163—172 119891.

ACKNOWLEDGEMENTS: E.K. and P.M. received special doctoral funds from State Committee for Scientific Research No. 4 PO5A 053 10 and 4 PO5A 054 11. A.W. was supported by grant no. 4 PO5A 079 09 from the same source. Excellent technical help at each step of the experiment was provided by Dorota Krakowska and Wojciech Borkowski.

Received 28 April 1998;
accepted 29 May 1998