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Identification of principal components in cortical evoked potentials by brief surface cooling

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Abstract

Objectives: The evoked potential recorded by a single electrode in rat's barrel cortex after whisker stimulation was shown to be composed of two main principal components shifted in time by about 3 ms. The purpose of this study was to verify the hypothesis that these components represent activity of supra- and infragranular pyramidal cell classes.

Results: Our results show that a brief cooling pulse applied to the cortical surface abolishes the shorter latency component, which may therefore be attributed to the response of supragranular pyramidal cells.

Conclusions: The longer latency principal component, which disappears only with strong cooling pulses, is proposed to represent postsynaptic activity of infragranular pyramidal neurons.

Keywords: Evoked potentials; Principal component analysis; Supra- and infragranular pyramidal cell components; Sensory cortex; Rat

1. Introduction

Cortical field potentials recorded in behaving animals after sensory stimulation are often used to evaluate the state of activation of the responding network (Mark and Hall, 1966; John et al., 1969; Basar et al., 1976; Brenner et al., 1987; Brandt et al., 1991; Cauller and Kulics, 1991;
Siegel and Sisson, 1993; Gtą¿ewski et al., 1998; Musia³ et al., 1998a; Webster et al., 1991; Wróbel et al., 1998;Frocet al., 2000). This network consists, however, of heterogeneous groups of neurons which might change their activity in quite a different manner depending on different functional contexts. Dissociation of components forming the actual evoked response should help to understand the underlying cortical processing.

Current source density (CSD) analysis attributed the main sources for cortical evoked potentials (EPs) to the large dipoles formed by synaptic excitation of apical dendritic trees of two pyramidal cell classes: supra- and infragranular (Mitzdorf, 1985; Di et al., 1990). The detailed principal component analysis (PCA) has confirmed that these sources comprise 70-90% of recorded evoked responses in the barrel cortex depending on whether the animal is anaesthetized (Di et al., 1990) or behaving (Musia³ et al., 1998b). Since both pyramidal cell classes form main outputs from the cortical column, parallel observation of their temporary activation would allow us to better understand the role of the cortical column in the dynamics of cortical processing (Wróbel et al., 1998). The difficulty in the use of the CSD and PCA methods during behavioral experiments emerged from the requirement of recording from many sites along the cortical depth. We have recently shown (Musia³ et al., 1998b) that PCA may be successfully applied in the time domain in order to reveal the principal components of the EP recorded by a single chronic electrode in behaving rats. In the current experiment, this method was applied in an attempt to identify the cellular sources of the two main principal components of the cortical EPs.

2. Methods and materials

2. 1. *Animal preparation*

The experiments were performed on 7 adult hooded rats (Chl-Ch7, all males except Ch7) weighing 250-400 g. Animals were anaesthetized with urethane (1.3-1.5 mg/kg, i.p., with 10% of the original dose added when-necessary) and placed in stereotaxic apparatus. Lignocaine (2% gel) was applied into the rats' ears and the skin over the skull was injected with Xylocaine (2%) prior to surgery. The skull was opened to expose part of the barrel field large enough to place a cooling plate. Fluid requirements were fulfilled by

s.c. injections of 0.9% NaCI and/or 5% glucose. The body temperature and electrocardiogram were monitored during the whole experiment to control the physiological condition of the animal.

2.2. *Stimulation and recordings*

The whisker stimulator consisted of a thin needle supported on a piezoelectric slab and was glued to the whisker at around 10-15 mm from the snout. Square wave pulses of 3 ms duration delivered from a PC produced a 0.1 mm vertical movement of the whisker with a frequency of 0.2 Hz. Several vibrissae were stimulated at the beginning of the experiment in order to find the one giving the best response (principal whisker, PW; typically at C2 position of the vibrissal field). An insulated 25 µm tungsten wire with a sharpened tip was used for monopolar local field potential (LFP) recording with a screw in the nasal bone used as reference. The electrode was lowered through a small hole in the cooling plate, perpendicular to the surface, to different cortical depths, but the presented data were taken at a chosen location of about layer IV. The LFP signal was amplified (1000X), filtered (0.1 Hz-5 kHz) and stored on magnetic tape of RACAL V-store recorder. The EPs evoked by PW stimulation were digitized on-line (2 kHz) with Spike-2 software for preliminary analysis. All taped data were examined for integrity and epochs with artefacts were excluded from further analysis.

2.3. Cortical cooling

In order to stop the activity in superficial and not deep cortical layers we applied brief, transient cooling pulses to the surface of the cortex by means of a small silver plate (3x3 mm) placed on the dura matter. The plate was abruptly cooled by short (about 0.5 s) puffs of aerosol Freeze75 expanding inside the plastic tube to which it was glued. A thermode fixed at the cortical side of the plate monitored the surface temperature which could drop by 35°C in 5 s (compare Fig. 2B), much faster than with the help of the originally tried Peltier device. Such surface temperature resulted in about 20°C in the deep layers of the rat's sensory cortex after a stable gradient was obtained in vivo (Diamond et al., 1992). One can expect that very short cooling pulses as applied in our experiment would produce an even steeper gradient, leaving deep layers with temperatures much closer to the physiological range (Fig. 1A).

2.4. PCA analysis

The detailed method for PCA in the time domain has been described before (Musia³ et al., 1998b). In brief, we assumed that recorded EPs resulted from several components, which were generated by separate neural populations with a unique time course with a constraint that a given function remained

EP traces were considered as variables and measurement at chosen time points as case values. To improve the interpret-ability of the results, the principal components (factors) were rotated (varimax rotation) in signal space. In all anaesthetized animals, the two first principal components (Fig. 1B) typically accounted for about 90% of the variance in the population of variables. Higher components were rejected according to Kaiser's criterion (Donchin, 1966). The PCA and correlation of principal components with single EPs were performed using the standard SPSS program.

3. Results

Typical recordings from an experiment with the cooling pulse applied to the cortical surface are presented in Fig. 1C. The consecutive EPs evoked by principal whisker stimulation during the control period are characterized by prominent N1 and P2 waves, which vary in amplitude from trial to trial. The variability of single EPs in the control series allowed for extracting two principal components, which accounted for most of the variance (Fig. 1B). The averaged course of evoked responses from this period is shown with an expanded time base in Fig. ID (dark grey line). The N1 wave in this trace becomes maximal at about 10 ms after stimulation and P2 starts to grow at about 20 ms; both values fit the average latency of excitation measured for 'single pyramidal cells in the central and neighbouring barrel columns (Armstrong-James, 1995).

The EP series in Fig. 1C show responses evoked by the same sensory stimuli after the cooling pulse was applied to the cortical surface. It is clear that the amplitudes of both waves became gradually smaller as the cooling pulse depressed more of the cortical tissue. The averaged trace obtained from potentials evoked soon after the pulse (Fig. 1 D, black line) shows that the N I wave decreased mostly at the falling phase which corresponded in time with the negative wave of the first principal component (Fig. 1B). This component in lightly anaesthetized animals also encompassed the P2 wave, which decreased accordingly after the first cooling pulse. Later, the low temperature abolished neuronal activity throughout the cortex leaving intact only the small PI response at about 4 ms latency which, therefore, may be attributed to the reflection of the thalamic volley (see insert in Fig. ID). After such deep cortical cooling, consecutive evoked responses did not usually reverse to the control level, most probably due to the damaged circulation in the recorded region of the coifex.

In order to confirm the sequence in which two main components disappear during cooling, we have performed a PCA analysis on the group of 7 animals. Fig. 2A presents results of such analysis for two representative cases. Both graphs show averaged EP traces from the control periods (thin lines) and model traces (thick lines) resulting from subtraction from the averaged EPs of the calculated values

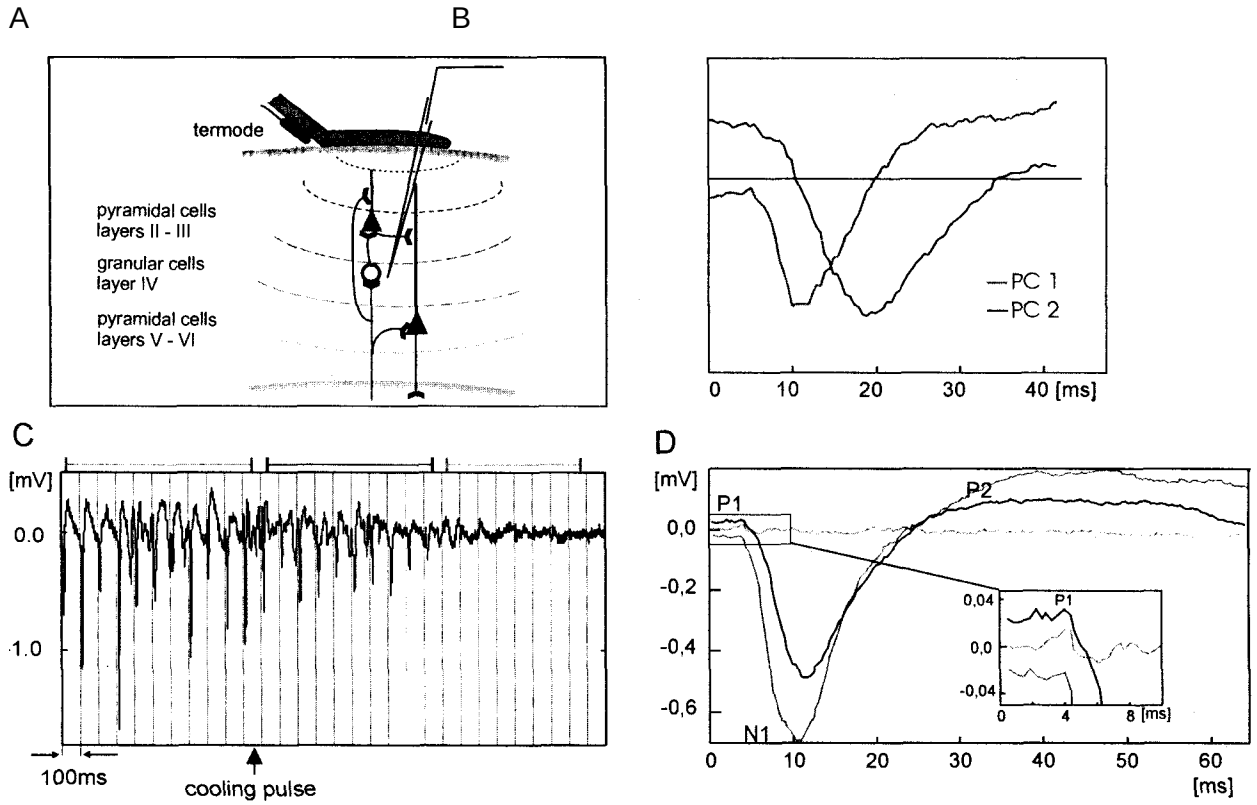

Fig. 1. (A) Cooling of the surface produces a temperature gradient along the cortical depth (symbolized by equitemperature broken lines). Evoked potentials in the cortex result from large electric dipoles formed on the apical dendrites of two pyramidal cell groups: supragranular (grey triangle) and infragranular (black triangle). If postsynaptic activity of both groups were represented by two principal components of the EP (B) then a weak cooling pulse would first abolish the superficial component (grey line, PC 1). (C) An example of a cooling experiment (Ch5) consisted of a period before and after the cooling pulse indicated by the arrowhead. The first 11 EPs show spontaneous variability, which allowed for calculation ofPCA components shown in (B). The vertical dotted lines indicate points where 5 s long LFP pieces between EPs were removed. The horizontal line over each part encompasses those EPs which were used to obtain averaged responses shown in (D). (D) Averaged EPs corresponding to 3 stages of the cooling experiment. The control potential (dark grey line) consists of a small PI followed by large N 1 and P2 waves. Immediately after the cooling pulse (black line), the falling part of N 1 and the P2 wave decreased in amplitude as expected with fading PC 1 component. After a while, the strong cooling pulse cooled most of the cortical tissue and EP consisted only of the reflection of incoming volley (PI).

experimental traces obtained after averaging the EPs immediately following the cooling pulse (grey lines) look very much like the model traces, indicating that the cooling pulse diminished the magnitude of the first PC.

A similar effect was observed with the correlation analysis presented by graphs in Fig. 2B. The black and grey lines represent correspondingly the correlation between the first and second principal components and the consecutive 40 EPs recorded before and after the cooling pulse. It is clear that in both cases, cooling of the cortical surface reduced the correlation of the next 11-12 single EPs with first principal component, leaving the correlation value between the same EPs and second component at the same level. Later on, the decreased correlation values returned to control level in the first experiment, in which a weak cooling pulse was applied (compare changes of the surface temperature). In the other experiment, the correlation between further EPs and the second principal component continuously decreased, indicating that both components were ceasing in the recorded EPs at this stage of recording.

Data from all experiments are shown in Fig. 3 and Table 1.

The changes of both principal components before and after application of the cooling pulse were estimated by measuring the amplitudes of averaged EPs at latencies corresponding to peaks of the components as evaluated independently by PCA of the control potential series (e.g. Fig. 1 B). For each rat, the averaged EP value measured in the first component range (7-11 ms after sensory stimulation. Table 1) decreased after a cooling pulse in all experiments (Fig. 3, grey bars). In contrast, the averaged potential value in the range of domination of the second component (12-17 ms) grew in 5 cases and decreased in two others. In these last cases (Chl and Ch5) the applied cooling pulses were strong and the region of the decreased temperature could also reach the deeper layers, lowering the activity of infragranular pyramidal cells. Even in these cases, however, the resulting decreases of the potential values at the second component peak were still less than when measured at the range of the corresponding first component.

The cooling was a continuous process, and accordingly we observed gradual changes of the components' amplitudes. Examples shown in Fig. 2B well illustrated the

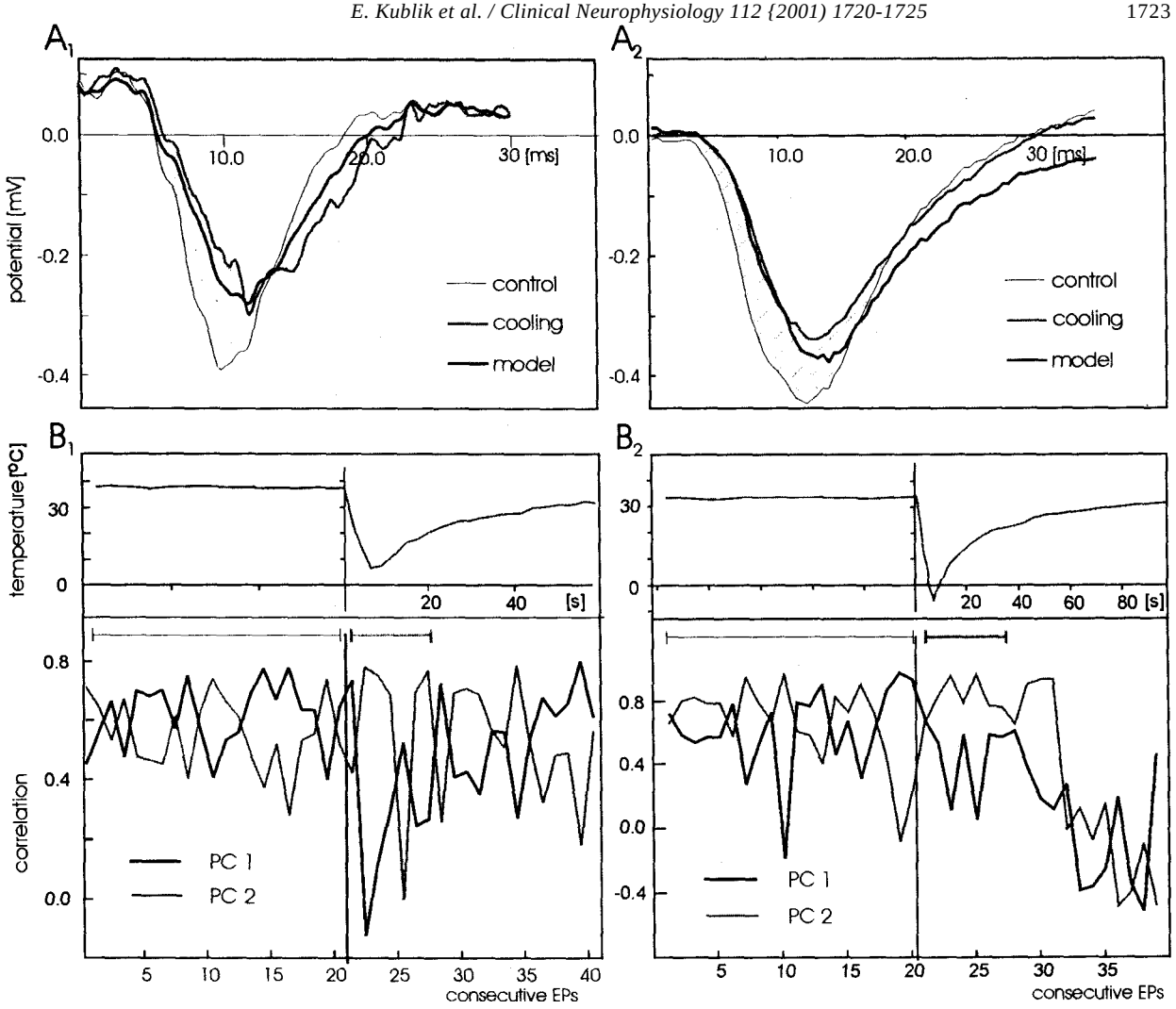

Fig. 2. (A|, A;) Averaged EPs from a control period (thin black lines, n = 20) and after cooling of the cortical surface (grey lines, n = 7) in two experiments (Ch4, Ch5). Control EPs were analyzed with PCA. Thick black lines show theoretical EPs after cooling, obtained by subtracting the first principal components from averaged control EPs. (B|, B;) Dynamics of cortical responsiveness is shown by correlation of 40 consecutive EPs with principal components: PC1 (black lines) and PC2 (grey lines). Vertical lines indicate the moment of application of the cooling pulse. Horizontal lines encompass these trials, which were averaged in (A). Correlations in (B;) are obtained from the same experiment as shown in Fig. 1C,D. Parallel changes of cortical surface temperature are inserted above.

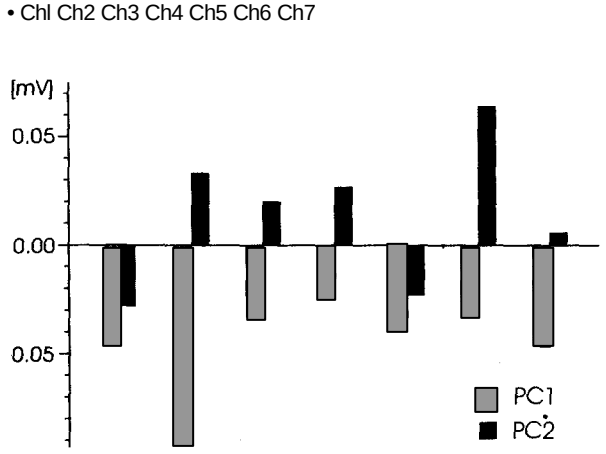

Fig. 3. Averaged differences of EP amplitudes measured at principal component maxima (as shown in Fig. 1B) before and after cooling pulses in all 7 experiments. Negative values indicate the decrease of corresponding

Table 1
Latencies measured for PC 1 and PC2 maxima, number of cooling trials, and
their effectiveness for each rat'

	Chl	Ch2	Ch3	Ch4	Ch5	Ch6	Ch7
Latency (ms)							
PC1	10	11.5	8.75	9	10.8	9,7	7.7
PC2	14	15	12	13	17.5	14.9	12.3
No. of trials							
All	2	18	12	5	1	3	4
Successful	2	13	7	5	'	3	4

' Trials in which the amplitude of the first component decreased after the cooling pulse were judged as successful. Lack of such change or smaller decrease of the first than of the second component amplitude was assumed as failure. Among all 45 trials, 35 were successful, which supports the hypothesis of superficially located source of the first PCA component

dynamics of this process. Since the thermode was positioned at the cortical surface of the cooling plate, we could not directly correlate the modification of PC amplitudes with the local temperature changes at different layers. For statistical evaluation of the direction of the observed changes, we have therefore judged as 'successes' all the decreases of the first component amplitude after the cooling pulse and the lack of such change or smaller decrease of the first than of the second component amplitude as 'failures'. Among all 45 trials (5 additional cooling cases were excluded from analysis due to recording artefacts or drastic changes in LFPs, which indicated cortical damage), 35 were successful. This favoured the hypothesis of the superficially located source of the first PCA component with significance level $P == 0.001$ in the sign test (Table 1).

4. Discussion

In the present experiment, we have shown that the principal components of the sensory EPs recorded in the barrel cortex changed in an ordered sequence after cooling of the cortical surface. Since the cooling wave encompassed first the superficial layers, we expected that the first to change would be the component generated by the supragranular pyramidal cells. All results obtained were consistent with the hypothesis that the shorter latency, first principal component represented the activity of the supragranular pyramidal cells and the second component, peaking about 3 ms later, represented the activity of infragranular neurons:

(1) the EPs recorded immediately after the cooling pulse (Fig. 2, grey lines) looked very much like the model traces deprived of the first PC component; and (2) the sequence of changes of both components following the cooling pulse was clearly shown by the correlation analysis (compare Fig. 2B). The first principal component disappeared from the evoked responses before the second one. The second component decreased only when the deeper cortical layers were inactivated by the incoming cooling wave.

We have previously shown (Musiat et al., 1998b) that PCA in the time domain may be successfully applied in order to reveal principal components of EP recorded by a single chronic electrode in behaving rats. In this paper, we demonstrate that the two first principal components obtained by this method can be specifically attributed to the postsynaptic activity in relevant cell groups: supra-and infragranular.

Similar conclusions have been reached previously by Di et al. (1990) who applied the PCA method to CSD profiles calculated from EP traces recorded at many points along the cortical depth. The present study provides, however, the necessary base for differentiation between the cortical responses recorded from behaving animals by means of monopolar, chronic electrodes (Musia³ et al., 1998b; Wróbel et al., 1998). We have previously shown that single EPs dominated by the first component characterize well habituated cortical responses and those dominated by the second component are more frequently evoked in the cortex activated independently due to the contextual (e.g. conditional) stimuli (Wróbel et al., 1998). The 'activated' EP responses are additionally characterized by the prominent P2 wave which was proposed to accompany neuronal processing at the part of the barrel field surrounding the central column (Armstrong-James, 1995; Wrobel et al., 1998) and were even found to correlate positively with sensory discrimination (Cauller and Kulics, 1991). The decreased P2 wave in the superficially cooled cortex might therefore indicate that the route for spreading specific sensory information involves the supragranular cells (Keller, 1995; Wróbel et al., 1998). Whatever the responsible gating mechanism in the barrel field, separate monitoring of the activation level of the two pyramidal cell groups can provide useful information about the momentary state of both main outputs from the cortical column and allow further investigation of the functional role played by a single columnar processing unit (Wróbel et al., 1998).

Increase of the second component amplitude after surface cooling observed in 5 out of 7 experiments (Fig. 3) could result from 3 reasons. Firstly, it could represent disinhibition of infragranular pyramidal cells resulting from decreased activity ofinterlayer inhibitory interneurons with cell bodies located superficially (Keller, 1995). Secondly, it could follow temporary increase of excitatory post-synaptic potentials (EPSPs) in infragranular pyramidal cells at the transitory temperature range (20-25°C) (Volgushev et al., 2000). This reason should, however, be of little importance in our experiments since decreased activity in superficial layers would remove a large portion of excitatory input to the infragranular cells (Armstrong-James, 1995; Wróbel et al., 1998). Moreover, PC2 amplitude was enhanced typically by about 10% of its original amplitude whereas compound EPSPs were observed to grow by at most 3% in in vitro recordings in room temperatures (Volgushev et al., 2000). Thirdly, the second component could grow because of contribution from the delayed portion of the first component. Such delay could result from slowed synaptic transmission in a cooled cortex (Volgushev et al., 2000). Comparing the cooled responses with that obtained by model calculations, we estimated that such a mechanism could explain less than 15% of the observed PC2 increase in two trials (e.g. the case shown in Fig. 2Ai) and was negligible in all other experiments (e.g. Fig. 2A,).

We have shown as well that cortical surface cooling' appears to be an excellent tool for dissociation of the activity of supra- and infragranular pyramidal cell populations. We observed that the consecutive EPs following the cooling pulse decreased monotonically in time, leaving only the specific thalamic volley reasonably stable (Fig. 1C,D). It is possible that with intact cortical microcirculation, the rapidly evoked temperature gradient is so steep that only a small fraction of cells operate in the intermediate temperature range. The hyperactivity of some cortical cells as found

in slice preparations at room temperatures (Volgushev et al., 2000) might therefore be of less importance in massive in vivo recordings.

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