ORIGIN OF SLOW NEGATIVE POTENTIAL OF DIRECT CORTICAL
RESPONSE IN CATS

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To investigate mechanisms of formation of the electrocorticographic slow negative
potential (SNP) evoked by direct electrical stimulation of the cortical surface,
poststimulus single unit activity in the stimulated area was studied in anesthe-
tized cats and changes in SNP in the depth of the cortex were analyzed. The re-
sults showed that membrane hyperpolarization, accompanied by cessation of action
potential generation, develops parallel with SNP in neuron bodies in the stimulated
area. Investigation of the nature of this hyperpolarization showed that during its
development excitability of the neuron and resistance of the postsynaptic membrane
fall. It is concluded from the results that this membrane hyperpolarization is an
indicator of IPSP development in the neuron bodies. The results of laminar re-
cording showed that SNP may diminish or even disappear in the depth of the cortex
without subsequent reversal. Determination of dipoles formed along an axis per-
pendicular to the cortical surface showed that SNP has one source and two sinks,
which are located symmetrically relative to it. The presence of two symmetrical
sinks must indicate an active source, formed as a result of hyperpolarization of
the neuron membrane. On the basis of the results SNP can be regarded as a field
potential formed on the cortical surface as a result of IPSP development in the
neuron bodies.

INTRODUCTION

Despite many investigations of the mechanisms generating the response of the cortex
to its direct electrical stimulation [1, 2, 4, 5, 7, 8-12, 15, 17, 20, 23, 24] the origin
of the slow negative potential (SNP) in the direct cortical response still remains unex-
plained.

Many workers have shown that spike activity is inhibited [4, 7, 14, 15, 17, 19, 23, 24]
and membrane hyperpolarization takes place [4, 15, 17, 19, 20] in neurons of the stimulated
area of the cortex. However, several hypotheses have been put forward to explain the genesis
of SNP. It is considered [20], for instance, that SNP is a "field effect," formed as a re-
sult of hyperpolarization of the bodies of pyramidal neurons, or that SNP reflects prolonged
depolarization of presynaptic fibers [3, 23, 24]. In the opinion of other workers [8-13],
SNP formation is connected with activation of the neuroglia.

One argument against the view that SNP is a "field potential," i.e., a dipole potential
arising as a result of hyperpolarization of the neuron bodies, is the absence of sign rever-
sal in some cases in the depth of the cortex [1, 12]. However, the absence of a positive po-
tential in the depth of the cortex during laminar recording of SNP cannot be taken to indi-
cate the "nondipole" origin of this potential, for the cerebral cortex has a complex organ-
ization and it is by no means impossible that the deep positive potential is masked by inter-
action between potentials of different layers.

We know [18] that, considering the complex organization of the cortical electrical re-
sponse, in order to discover the source and sink of the dipoles the best course to adopt is
to analyze changes in the integral current density layer by layer.

The investigation described below was undertaken to continue the study of mechanisms
of SNP generation, and for that reason the nature of hyperpolarization of the neuron membrane
observed during SNP generation was studied and the gradient of the current appearing in the
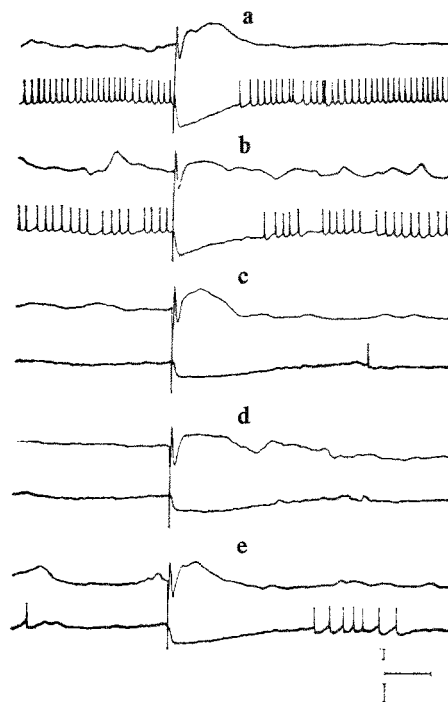thickness of the cortex during the SNP was analyzed.

Fig. 1. Intracellular recording of responses of cortical neuron to direct cortical stimulation. Top beam records global potential from cortical surface. a-e) Recording associated with different intensities of spontaneous unit activity. Calibration: 100 µV (top beam) and 10.mV (bottom beam), 100 msec.

METHOD

Acute experiments were carried out on adult cats deeply anesthetized with pentobarbital (50-80 mg/kg body weight). The sensomotor cortex or the middle part of the suprasylvian gyrus was studied. Above these areas a burrhole 2.5-3 mm in diameter was drilled in the skull for microelectrode investigations, and filled with a solution of agar-agar in physiological saline [6]. For laminar derivation of potentials, the skull was widely trephined. In every case the dura was removed. Tripolar (with one corresponding pole) Ag-AgCl electrodes were used as stimulating cortical electrodes. Square pulses 0.5 msec in duration were used for stimulation. The intracellular electrodes were glass micropipets filled with 2.5 M KCl solution, with a resistance of 20-40 MΩ. To pass an electric current through the membrane of the chosen cell a bridge circuit was used [21]. Global electrical activity was derived by Ag-AgCl wick electrodes. Derivations were monopolar and the reference electrodes were located on bone in the region of the frontal sinus. For laminar recording to global responses glass micropipets filled with 2.5 M NaCl solution and with a resistance of not more than 1 mΩ were used. To obtain accurate results, 10 to 15 responses were averaged for each depth. Data of only those experiments in which the electrode was introduced perpendicularly to the cortical surface were taken into consideration. Satisfaction of this last condition was verified morphologically. The position of the sources and sinks of the dipoles was determined by the gradient of the integral current density along an axis perpendicular to the cortical surface. Positive maxima of the integral current density gradient correspond under these circumstances to sources and negative to sinks of the dipoles [18]. Integral current density along an axis perpendicular to the cortical surface was calculated by the equation $I_z = -K(\partial V/\partial z)$, where I_z is the component of the integral current density along the z axis, V the potential relative to a distant reference electrode, K the conductance of the cortex, and z the axis perpendicular to the cortical surface. Conductance was taken to be constant for all layers of the cortex. After determination of I_z the gradient $\partial I_z/\partial z$ was determined, after which a graph was plotted to show the integral current density gradient as a function of depth of recording. The values of $\partial V/\partial z$ and $\partial I/\partial z$ were calculated by numerical differentiation.
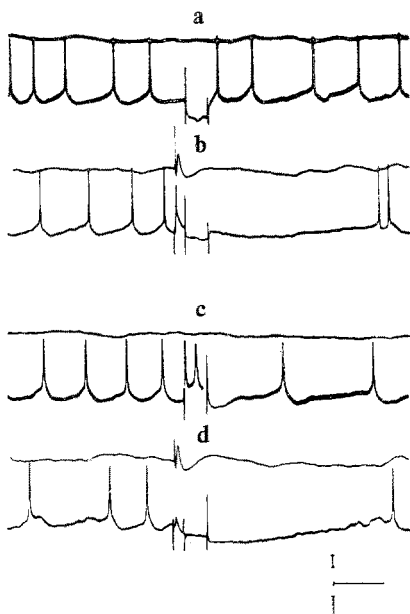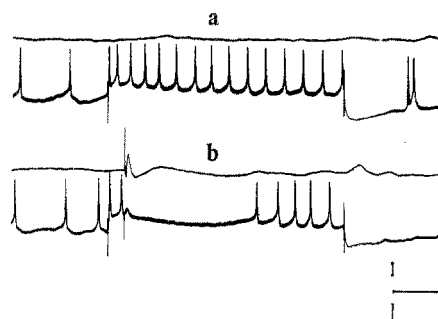
Fig. 2   Fig. 3

Fig. 2. Measurement of neuron membrane resistance by passing pulses of hyperpolarizing (a, b) and depolarizing (c, d) current during spontaneous activity (a, c) and response to direct cortical stimulation (b, d). Calibration: 100 µV (top beam) and 10 mV (bottom beam), 100 msec.

Fig. 3. Response of cortical neuron to direct cortical stimulation during intracellular injection (a, b) of depolarizing current. Calibration: 100 µV (top beam) and 10 mV (bottom beam), 100 msec.

RESULTS

Responses of Neurons to Direct Cortical Stimulation. Electrical activity of 36 cerebral cortical neurons was studied. The results showed that parallel with SNP, membrane hyperpolarization accompanied by cessation of action potential generation developed in neurons in the stimulated area of the cortex. Traces showing the development of such hyperpolarization of the neuron membrane during SNP are given in Fig. 1. They show that membrane hyperpolarization was exhibited both when the neuron discharged relatively rapidly (Fig. 1a, b) and in the absence of any discharge (Fig. 1c, d, e).

To elucidate the origin of this poststimulus hyperpolarization, changes in resistance and excitability of the membrane during development of this hyperpolarization were studied; for this purpose, a pulse of hyperpolarizing current with a strength of 10^{-9} A and duration 50 msec was passed through the intracellular microelectrode by means of a bridge circuit. It will be clear from Fig. 2a that this caused hyperpolarization of the membrane. When the current was passed during the development of poststimulus membrane hyperpolarization the degree of artificial hyperpolarization was reduced (Fig. 2b). This is an indication of the lowering of resistance. The same could also be observed when a depolarizing current was passed through the microelectrode (Fig. 2c, d). As the traces shown in this figure demonstrate, the excitatory action of the depolarizing current was suppressed against the background of poststimulus hyperpolarization.

The results of the investigation thus indicate the active inhibitory nature of this hyperpolarization.

Laminar Recording of Components of the Direct Cortical Response and Analysis of the Current Gradient in the Thickness of the Cortex. During relatively weak electrical stimulation of the cortical surface a "dendritic potential" (DP) without any subsequent SNP was recorded near the stimulating electrodes. It will be clear from Fig. 4a, which gives the results of laminar analysis of DP, that on insertion of the recording electrode deeper into the cortex the amplitude of DP gradually decreased, and at a depth of 0.9 mm its sign was
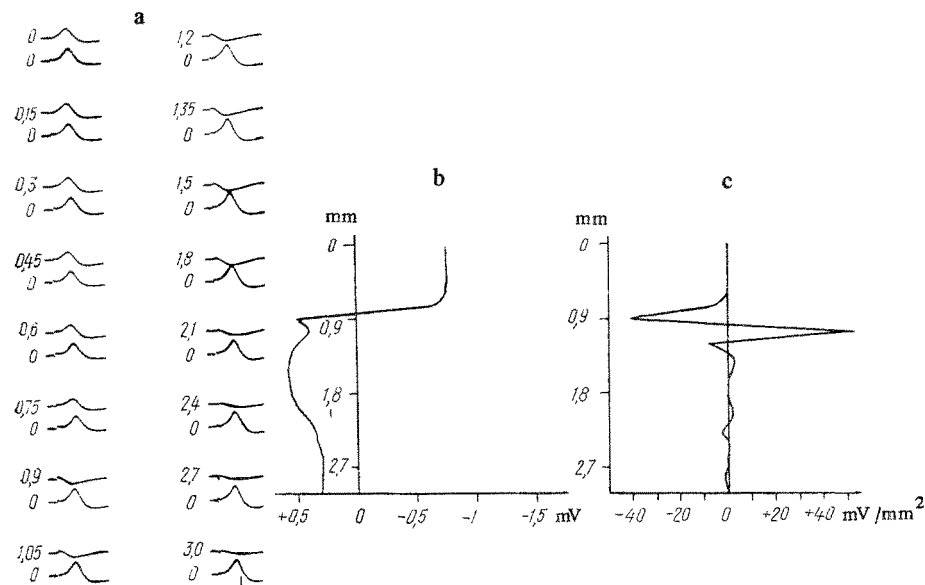
Fig. 4. Laminar recording of dendritic potentials (DP) and graphs of vertical potential distribution and corresponding current gradient. a) Dendritic responses obtained by laminar recording. Each trace contains 10 global responses. Depth of insertion of electrode tip into thickness of cortex, in mm, shown on left; for comparison in each case potential recorded from surface is shown. Calibration: 2 mV, 160 msec. b) Graph of change in DP, c) graph of current density gradient during laminar recording of DP.
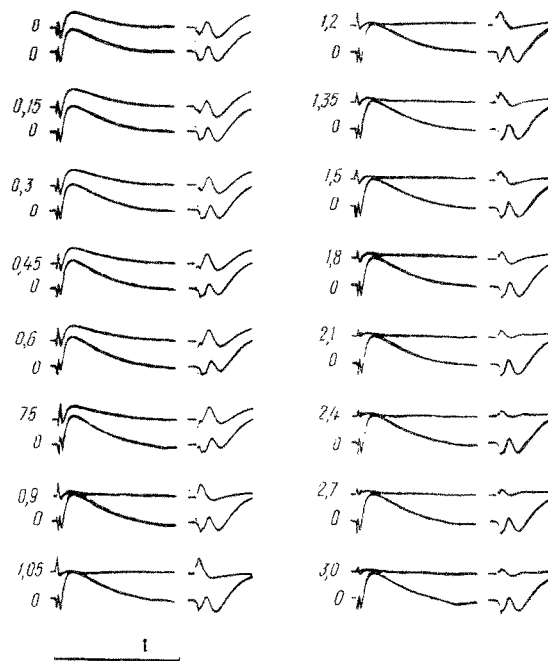

Fig. 5. Slow negative potential (SNP) and complicated SNP of dendritic responses during laminar recording. Each trace contains 10 global responses. On right — recordings made with fast sweep. On left, depth of insertion of recording electrode tip, mm. Calibration: 1 mV, 1 sec (for slow sweep) and 250 msec (for fast sweep).
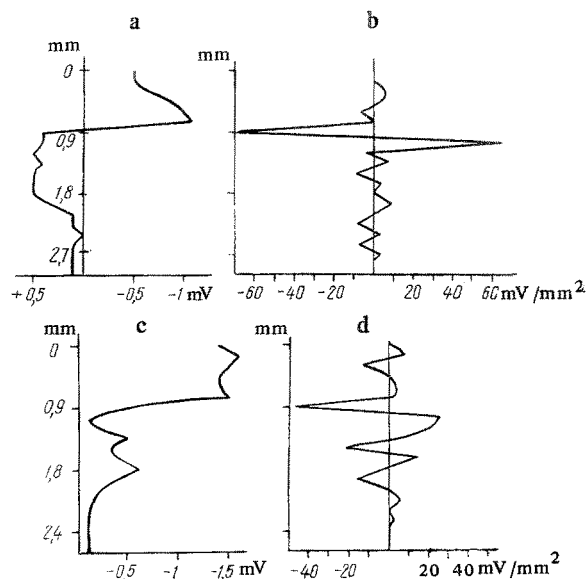
Fig. 6. Potential distributions over depth during dendritic response and slow negativity and corresponding estimates of vertical current gradients.

reversed. A graph of changes in DP along the z axis is given in Fig. 4b, and a graph of changes in the second derivative $\partial^2 V/\partial z^2$, proportional to the current density gradient along the z axis, in Fig. 4c. Analysis was carried out in this manner for 14 laminar recordings of DP and in every case the same results were obtained.

During high-intensity electrical stimulation of the cortical surface the response was complicated by the appearance of SNP immediately after DP. The results of laminar recording of DP and SNP (to facilitate analysis, DP is shown additionally with fast sweep) are shown in Fig. 5. Clearly SNP disappeared at a depth of 0.9 mm and, despite deeper insertion of the recording electrode, its sign was not reversed. So far as DP is concerned, as already mentioned, this potential was reversed at a depth of 0.9 mm. Similar results were obtained in 38 laminar recordings.

Graphs compiled on the basis of analysis of laminar recording of DP and SNP, shown in Fig. 5, are given in Fig. 6: a graph of changes in potential and a graph of current density gradient for DP in a and b, and for SNP in c and d. It will be clear from graphs a and c that at a depth of 0.8-0.9 mm both DP and SNP were sharply reduced. By contrast with DP, SNP did not change its polarity. Both SNP and DP were characterized by the presence of a sink and source (Fig. 6b, d). SNP, moreover, had one source and two sinks, arranged symmetrically relative to it (Fig. 6d).

DISCUSSION

The results of these experiments show that in response to electrical stimulation of the cortical surface, parallel with SNP of the direct cortical response membrane hyperpolarization also developed in neuron bodies of the stimulated area of cortex. This hyperpolarization of the neuron membrane is most probably an active process and not the result of a passive shift of membrane potential resulting from cessation of spike activity, for this hyperpolarization develops in the absence of such activity also (Fig. 1c, d, e). A similar view has been expressed by other workers [15, 19]. However, it is impossible to classify this hyperpolarization purely on the basis of the fact we have described as the result of development of IPSP in neuron bodies. We know [16] that during IPSP development, to correspond to hyperpolarization of the membrane its resistance decreases and excitability of the neuron falls.

The results of our experiments showed that with the development of hyperpolarization of the cortical neuron membrane its resistance falls (Fig. 2). Hyperpolarization of the neuron membrane developing in response to direct cortical stimulation is accompanied by the lowering of excitability of the neuron (Fig. 3). It can thus be concluded that the hyperpolarization observed in neurons during SNP generation is caused by IPSP.

Considering the dipole character of the electric fields generated by a cortical neuron it can be postulated that hyperpolarization of the neuron bodies due to IPSP of this kind produces an SNP on the cortical surface.

In this investigation, to determine the sink and source of the dipoles, besides laminar recording of DP and SNP in the depth of the cortex, the more accurate method of determining sinks and sources of dipoles on the basis of extrema of the total current density gradient along an axis perpendicular to the cortical surface was used. DP has one sink and one source (Figs. 4c and 6b). The remaining oscillations of the current density gradient are very small. Graphs of potential and current density gradient for SNP during laminar recording are given in Fig. 6, c and d. Comparison of graphs b and d (Fig. 6) showed with greater accuracy that the first sink of DP and SNP coincide. The sources also coincide, but SNP also has an additional sink, which lies deeper than the source. Thus DP is characterized by one source and one sink, but SNP has one source and two sinks, arranged symmetrically relative to it. The presence of two symmetrical sinks in the case of SNP is evidence of an active source formed as a result of hyperpolarization of the neuron membrane.

In the course of investigation of the distribution of global currents in the cortex the presence of dipoles during SNP generation was thus established; the position of the dipoles, moreover, is in agreement with the view that SNP is the dipole reflection of hyperpolarization of the neuron bodies.

The view that the SNP is glial in origin has met with certain objections, for despite the possibility of depolarization [22] of cortical glial cells in response to direct cortical stimulation, unless it is assumed that the glial cells are united into a certain geometrically organized network, it is impossible to imagine that the glia can affect an SNP recorded from the cortical surface.

The results of the present investigation are thus evidence in support of the view that SNP is a field potential, i.e., a dipole potential arising as a result of hyperpolarization of the neuron bodies.

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