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Postsynaptic potentials in cat visual cortex: dependence on polarization

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DURING the investigation of visually evoked postsynaptic potentials (PSPs) of visual cortical neurons, we recorded cell activity under different levels of membrane potential. In some cases, however, dependence of these PSPs on the level of membrane polarization appears to be inconsistent with the conventional scheme. One disagreement was the reduction, instead of an increase, of excitatory potentials during hyperpolarization of the cell. The other point was that depolarization of the cell often leads to increase of the amplitude of both excitatory and inhibitory postsynaptic potentials. This inconsistency may suggest the involvement of voltage-dependent ion channels in generating PSPs to visual stimuli. A possible way of separating the excitatory and inhibitory components of the response by polarization of the cell in spite of the presence of voltage-dependent channels and possible implications of this mechanism in the visual cortex are discussed.

Key words: Postsynaptic potentials; Polarization; Cat visual cortex

Introduction

The classical method of separation of evoked excitatory PSPs from inhibitory ones is to de- or hyperpolarize the cell. Depolarization of the cell should lead to a reduction of excitatory potentials and an increase of inhibitory ones. Hyperpolarization, on the other hand, should segregate excitatory potentials, increase their amplitude and diminish the size of inhibitory potentials. We tried to apply this method to disentangle inhibitory and excitatory components of the responses of cortical cells to visual stimuli. It appears, however, that not all cells in the primary visual cortex behave in this 'classical' way. Often the data we recorded did not fit the predictions based on classical notions of the intracellular recording. In this communication we describe the effects of membrane polarization on the visually evoked PSPs when using a low-resistance electrode normally used for patch-clamp recordings.

Materials and Methods

Experiments were done on adult cats (2.0–4.5 kg) bred in the department's animal farm. Anaesthesia was induced with Ketamine hydrochloride i.m. (Ketanest, 25 mg kg^{-1} i.m.) or with Nembutal (35–40 mg kg^{-1} i.p.), and maintained with i.v. infusion of 3–4 mg kg^{-1} h^{-1} pentobarbitone (Nembutal) without nitrous oxide or with 1–2 mg kg h^{-1} Nembutal and a gas mixture of 70% N_2O plus 29.2% O_2 and 0.8% CO_2. Muscle relaxation with gallamine triethiodide (Flaxedil) and artificial respiration was started after a stable anaesthesia with complete analgesia had been achieved. End-tidal CO_2, body temperature and EKG were continually monitored. Animals and the cortex were in good condition for 2–3 days. Whole-cell recordings from cells in the primary visual cortex of cat were done using the technique described elsewhere.[1] Visual stimuli were projected on a screen positioned 57 cm in front of the cat's eye. The eyes were focused on the screen with appropriate contact lenses. Receptive fields of the cells were localized with a hand-held projector and then investigated with computer-controlled visual stimulation. Moving and flashing stimuli were presented monocularly to the dominant eye. Responses to the visual stimulation of the cells presented here were recorded under three different levels of membrane potential: with no current applied to the cell and during application of depolarizing or hyperpolarizing currents (0.1–1.5 nA).

Results

We considered the effect of application of currents of different polarities on the excitatory and inhibitory potentials evoked by visual stimulation. Comparison of the effects produced by the current just after switching it on and after some tens of seconds revealed no significant difference. This fact, as well as the immediate restoration of PSP characteristics after switching off the current, indicates the absence of significant cumulative effects. This allowed us to pool together

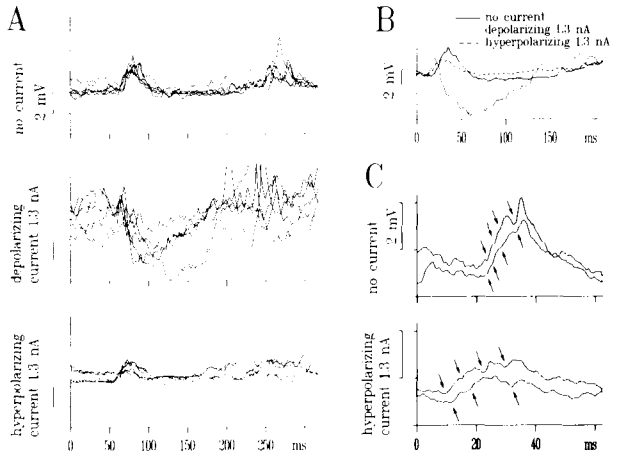

FIG. 1. Dependence of postsynaptic potentials evoked by a flashing bar on the level of membrane potential. **A**: Superimposed responses, without current application (upper traces), with depolarizing (middle) and with hyperpolarizing (lower) currents. **B**: Average of the responses shown in A. **C**: Decrease of EPSP amplitude during hyperpolarization. Only a part of the response (35–110 ms of these in A) is shown on expanded scale. The compound nature of EPSP can be seen. Onsets of single steps are indicated by arrows. Time scale begins from onset of the stimulus (A) or 35 ms later (B, C). Stimulus duration is 1 s. Mean membrane potential when no current was applied was 35 mV; calibration 2 mV.

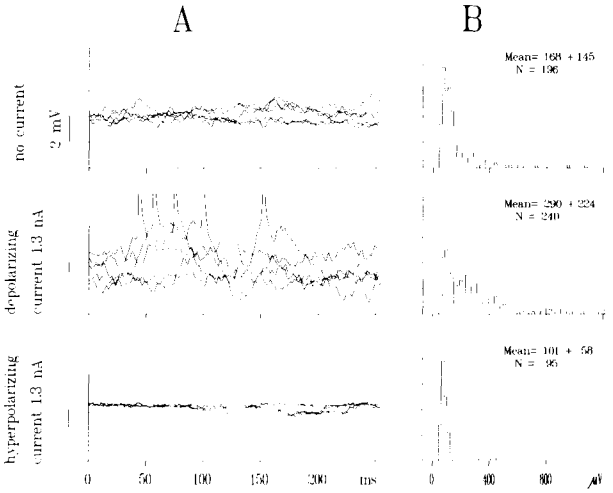

FIG. 2. Background postsynaptic activity of the cell depends on the level of membrane potential. **A**: Superimposed traces. **B**: Amplitude distributions of the positive membrane deflections. Mean membrane potential when no current was applied was 35 mV; calibration 2 mV.

results obtained during application of a constant current.

The cell shown on Figure 1 had a complex receptive field. An optimally oriented bar flashed on the receptive field of this cell evoked an excitatory potential with a latency of 63 ms and a mean amplitude of 2.2 mV (Fig. 1A, upper traces). This PSP had a compound nature, which is clearly seen on magnified records (Fig. 1C). Several steps (3 to 5), each with an amplitude of 0.5–1.5 mV (mean 0.87 mV) could be distinguished on each single sweep (arrows in Fig. 1C). After a period of silence (about 160 ms) a late excitatory component appeared (Fig. 1A). For this figure we have chosen those responses where the stimulus was displaced a bit from the receptive field center, so that spikes were not evoked.

Depolarization of the cell changed this response markedly (Fig. 1A, middle traces). First of all, a robust inhibition appeared and dominated the response. The primary excitation appeared a bit earlier (latency of 58 ms compared to 63 ms with no current), had a faster rise time (see Fig. 1B) and was attenuated by the large IPSP that hyperpolarized the cell for about hundred milliseconds. The secondary, late excitatory component of the response was enhanced, and evoked several action potentials. This enhancement of the secondary excitatory component may be at least partly due to the withdrawal of inhibition.

Depolarization also elevated the amplitude of membrane potential deflections during background activity of the cell (Fig. 2, middle traces). Eventually the firing threshold was reached and several spikes appeared. The mean amplitude of the positive deflections of the membrane potential was 0.288 mV, compared to 0.168 mV during the same activity without current. These deflections should be mainly caused by EPSPs, and an increase of their mean amplitude may reflect the increased amplitude of each incoming EPSP.

Hyperpolarization of the cell affected the response in a completely different way (Fig. 1A, lower traces). The amplitude of the compound excitatory potential was smaller (on the average 1.0 mV) Certain steps could be distinguished as before on every single sweep, but the amplitude of each of them was also smaller (0.3–0.7 mV, mean 0.48 mV; see Fig. 1C, arrows). Substantial reduction of amplitude of excitatory PSPs took place during the secondary excitatory component of the response as well. Hyperpolarization also clearly silenced the membrane when no stimulus is presented—deflections of the membrane potential are much smaller now (Fig. 2, lower traces).

The effect of current application on the inhibitory response is shown in Figure 3A, B. This cell had a simple receptive field and the figure shows response to a bar flashed on the centre of the inhibitory zone. With no current applied, an IPSP appeared with a latency of 34 ms and dominated the response (Fig. 3A, upper traces). Hardly any excitation could be recognized before it. During the course of the IPSP in spite of a variance between individual responses, positive deflections of the membrane potential always occurred around 70 and 120 ms. They appear as bumps on the averaged responses, indicating the presence of two sets of weak excitation with latencies of 70 and 120 ms. Depolarization of the cell made the initial phase of the inhibition more pronounced, but also revealed some excitation before it (Fig. 3A, middle traces, and B). This excitation was weak and did not have a constant latency, causing a small plateau in the averaged response (Fig. 3B, thin line). The secondary weak excitation around 70 ms was also enhanced and prolonged

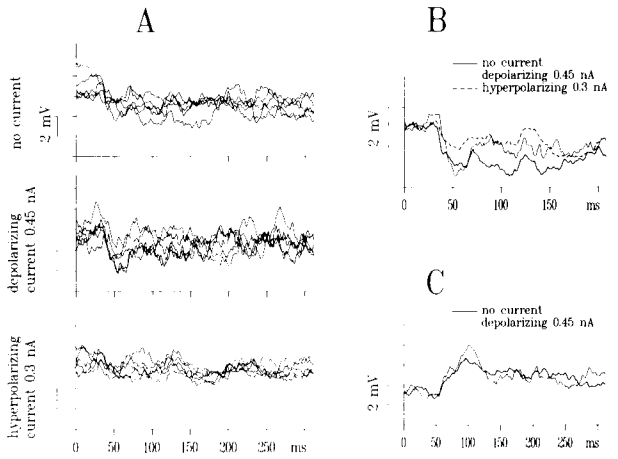

FIG. 3. Dependence of excitatory and inhibitory responses on the level of membrane potential. **A**: Superimposed responses at three levels of membrane potential. **B**: Average of responses shown in A. **C**: Enhancement of EPSP amplitude during depolarization. Averaged responses. Time scale begins from the onset of the stimulus for A, B and from offset for C (off-response); stimulus duration 1 s. Mean membrane potential when no current was applied was −30 mV; calibration 2 mV.

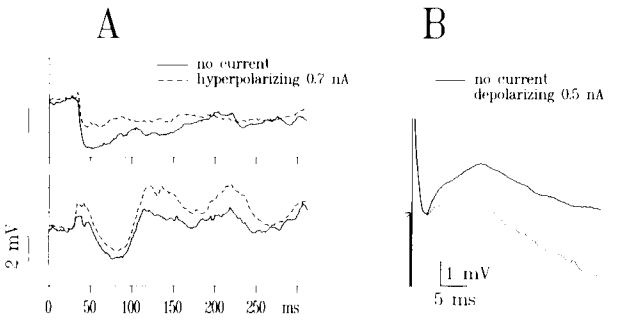

FIG. 4. Examples of conventional dependence of PSPs in visual cortex cells on the level of membrane potential. **A**: Averaged responses of a simple cell to a bar flashed in inhibitory (upper traces) and excitatory (lower traces) regions of the receptive field. **B**: Averaged responses of a cell to electrical stimulation of the LGN (0.9 mA, 0.1 ms). Artifact shows the moment of electrical stimulation. Mean membrane potential when no current was applied was −30 mV for A and −40 mV for B.

by depolarization. Hyperpolarization of the cell markedly reduced the inhibitory potential, and, as with depolarization revealed a small excitatory potential before the inhibition (Fig. 3A, bottom traces, and B). The excitation at 70 ms was reduced compared to that during depolarization or with no current; but the very late one at 120 ms was not affected (Fig. 3B). Excitatory off-response of this cell was enhanced by application of depolarizing current (Fig. 3C).

It should be added that many cells (9 out of 16 investigated with current application) do demonstrate the classical dependence of evoked PSPs on membrane potential. Hyperpolarization of the simple cell in Figure 4A increased the amplitude of EPSPs evoked by flashed bar (lower traces) and decreased the inhibitory responses (upper traces). In another cell depolarization markedly reduced the amplitudes of EPSPs evoked by electrical stimulation of the LGN (Fig. 4B).

Discussion

Our data raises several questions. First: What could cause this 'unconventional' behaviour of PSPs during de- and hyper-polarization of some cells? Second: What is it for? Third: What conclusions can be drawn from the data recorded during current application in the presence of such a mechanism?

The dependence of the recorded EPSPs on the membrane potential that we often observed was against classical notions. Such an effect can be explained, if a substantial number of voltage-sensitive channels are involved in the generation of the response. The effect may be due to the behaviour of NMDA channels in the presence of extracellular Mg^{++} ions[2,3] or in some circumstances due to inward rectification of non-inactivating Na^{+} channels.[4] Increase of EPSP amplitude during depolarization has also been observed in slices of the rat visual and cingulate cortices[5,6] (but see reference 7). The voltage-dependent magnification of the EPSPs would also explain, why the enhancement of IPSPs by depolarization was not very strong in some cases (for example, Fig. 3A, B). In such a case the enhanced EPSPs and related currents are probably strong enough to reduce substantially an on-going IPSP. This possibility is supported by the finding that the time-courses of the NMDA-component of the EPSPs and of the GABA-mediated IPSPs overlap considerably.[5]

Therefore, our data suggest that voltage-dependent channels could play a significant role in producing the response of striate cortical cells to visual stimuli. With the activation of voltage-dependent ion channels even weak inputs could evoke, under certain conditions, a postsynaptic potential of substantial amplitude. How is this mechanism used in the visual cortex? Theoretically, it could be the mechanism of involvement of the same cell in different assemblies, without the necessity to have a number of diverse sets of strong inputs. It could also serve in integrating visual information from the classical receptive field with that from remote areas.[4] It might also be involved in the refinement of some properties of cortical neurons, such as orientation selectivity. Visual stimulation usually evokes in the cell both excitatory and inhibitory potentials.[8–12] Their algebraic difference may be too small, but the depolarization of the cell may amplify this. The required depolarization could be produced, for example, by steady on-going subthreshold excitation. Another effect of the voltage-dependent channels on the properties of the cell membrane may be to restore the relative sensitivity of the cell to inhibitory and to excitatory influences. Amplification of excitatory PSPs may balance to some extent the increased driving force for inhibitory PSPs.

Our data show that in the cat visual cortex excitatory and inhibitory PSPs cannot always be readily separated by polarization of the cell. Polarization often affects

amplitudes of PSPs in unexpected ways. One piece of supporting evidence can be found in a recent paper by Ferster and Jagadeesh.[13] In their figure 10 both excitatory and inhibitory components of response were decreased by hyperpolarization. Such unconventional behaviour of some cells constrains the use of this method for separation of excitatory and inhibitory PSPs and points to a need for a more cautious interpretation of results obtained by this technique.

Conclusion

There are limitations on the extent to which depolarization and hyperpolarization can be used to separate excitatory or inhibitory post-synaptic potentials during intracellular recordings. The possible involvement of voltage sensitive channels during sustained depolarization can significantly affect the amplitudes of post-synaptic potentials recorded from the soma and cause non-linearities in the voltage-current relationship. This is especially valid when the membrane potential is reduced by depolarization. In this situation, both EPSPs and IPSPs can be enhanced, and these non-linearities preclude quantitative estimation of the strength of excitation and inhibition. The situation is somewhat better with hyperpolarization, since far fewer voltage sensitive channels are open. Even though the inhibitory potentials become smaller, the EPSPs are brought into a linear range and the strength of the excitatory input can be estimated quantitatively.

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