

LONG and stable recordings of post-synaptic, action and membrane potentials from visual cortical neurons *in-vivo*, are possible with the patch-clamp technique. These are comparable to the whole-cell configuration, but with an incomplete seal. EPSPs and IPSPs of normal time course and up to several mV can be recorded. DC potentials ranged from -30 to -60 mV and input resistances from 50 to 150 M Ω . Injected currents have the same effect as if applied intracellularly. Membrane conductance after electrical stimulation of the lateral geniculate nucleus is increased during the first 20 ms, but decreases from 60 to about 130 ms, during return of the membrane potential to its resting level. The recording method is compared to other intracellular recording techniques *in-vivo* and *in-vitro*.

Key words: *In-vivo* whole cell recording, Patch-clamp technique, Cat visual cortex

Whole cell recording and conductance measurements in cat visual cortex *in-vivo*

Xing Pei,¹ M. Volgushev,^{1,2}
T.R. Vidyasagar^{1,3} and O.D. Creutzfeldt^{1,CA}

¹Dept. of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, P.O.B. 2841, W-3400 Göttingen-Nikolausberg, Germany;

²Institute of Higher Nervous Activity and Neurophysiology, USSR Academy of Sciences, Moscow, USSR;

³Visual Neurosciences Group, John Curtin School of Medical Research, Australian National University, G.P.O. Box 334, Canberra, A.C.T. 2601, Australia

^{CA}Corresponding Author

Introduction

Intracellular recordings from cortical neurons are rarely sufficiently stable for prolonged investigation. In the visual cortex, additional complications arise because of the small neuron size and brain pulsations. Hence, only a few extensive reports on intracellular records from visual cortical neurons during visual stimulation have been published¹⁻⁴ and many questions are not yet clarified.^{1,5-8}

Application of techniques used for patch clamp recordings in isolated cells^{9,10} and brain slices¹¹ helped us achieve stable whole cell recordings of membrane and post-synaptic potentials (PSPs) over several hours in an *in-vivo* preparation, but with an incomplete seal of the microelectrode on to the neuronal membrane. We also measured membrane conductance changes during stimulus evoked PSPs.

Method and Results

General preparation: Adult cats (2.0–4.5 kg) bred in the department's animal farm were anaesthetized with ketamine hydrochloride i.m. (Ketanest[®]) (25 mg kg⁻¹ i.m.) or with Nembutal[®] (35–40 mg kg⁻¹ i.p.), and maintained on i.v. infusion of 3–4 mg kg⁻¹ h⁻¹ pentobarbital (Nembutal[®]) without nitrous oxide or on 1–2 mg kg⁻¹ h⁻¹ Nembutal[®] and with a gas mixture of N₂O: O₂: CO₂ (70:29.2:0.8). Muscle relaxation with gallamine triethiodide (Flaxedil[®]) and artificial respiration were started after a stable anaesthesia with complete analgesia had been achieved. End-tidal CO₂ was adjusted to around 3.6% and body temperature was maintained around 37–38°C. The ECG was

continually monitored and the absence of heart rate alteration during squeezing of the pinna was tested every few hours to ensure adequate anaesthesia. For improving mechanical stability, a hydraulic microelectrode holder (David Kopf) was mounted directly on the skull using screws and dental cement. Brain pulsations were kept to a minimum by producing a bilateral pneumothorax and by suspending the animal on the second thoracic vertebra. A brass-cylinder (diameter 20 mm) was cemented over a craniotomy (5 mm diameter) done over area 17 and centered at P₄/L₁ (Horsley-Clark). A small hole was cut into the dura just large enough to let in the electrode. After positioning the electrode, 2–3% agar was poured into the chamber.

Electrodes and solutions: The electrodes were similar to those used for single channel recording,¹⁰ pulled from hard Borosilicate glass (o.d. 1.5 mm; i.d. 1.05 mm) with a two step puller (Patch-Clamp Pipette Puller L/M-3P-A) and the tips were usually additionally heat-polished. The tip diameter was 1–3 μ m and the resistance 2–7 M Ω with the filling solution we used:¹¹ 130 mM K-gluconate, 5 mM NaCl, 10 mM EGTA, 10 mM HEPES, 1 mM ATP, 1 mM CaCl₂, 2 mM MgCl₂, pH 7.4 (KOH). The electrode was fixed to an electrode-holder that allows positive and negative pressures to be applied.⁹

Recordings: While advancing the electrode into the cortex, we applied a continuous pressure of 200–400 cm H₂O to prevent blockage of the electrode tip. The electrode resistance was continually checked with current pulses 0.05–0.1 nA, 50 ms, 0.5–1.0 Hz. Without pressure application, the electrode resistance increases quickly to ≥ 100 M Ω , indicating clotting

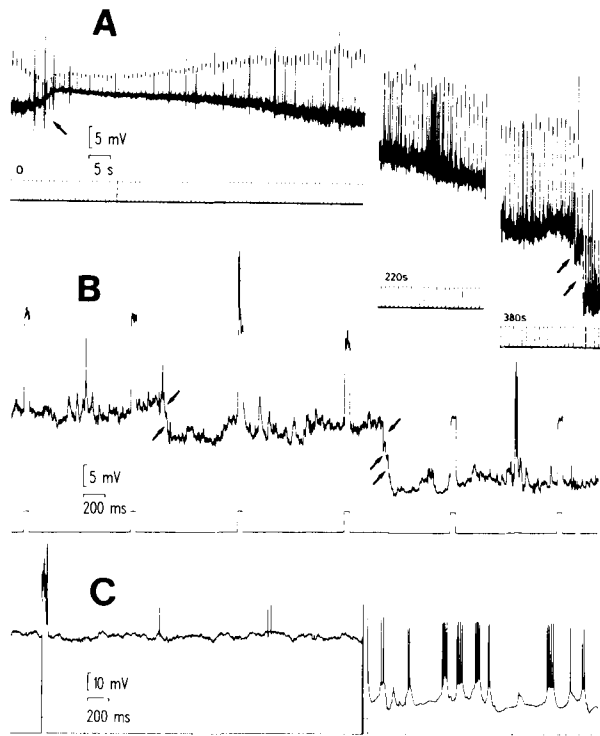


FIG. 1. Slow approach to the final recording situation. A: Top: Voltage recording, bottom: positive current pluses of 0.05 nA, 50 ms, 1 Hz. First section: The electrode is close to the membrane. Increasing the pressure inside the electrode (arrow) decreases the input resistance (smaller amplitude of voltage changes caused by the constant current pulses), the DC-potential at the tip and the synaptic noise. Slight suction and forward movement of the electrode slowly increases all three parameters (right part of first section and middle section). When membrane potential and input resistance have reached a stable level (right section), additional suction is applied (arrows at right), leading to sudden increase of the negative DC-potential and decrease of input resistance. This is shown at higher time resolution in B: Arrows point to stepwise increase of negative potential during suction. CC: Record from another cell, in which opening of the membrane was achieved by application of a positive 3 nA pulse leading to a negative voltage drop and a slight increase of discharge rate. The amplitude variations of action potentials are due to digital conversion of data.

with tissue débris. At a depth of 400–1000 μm the pressure in the electrode is reduced to 30–50 cm H_2O , and the electrode is further advanced during the search for visually responsive cells. Changes of electrode resistance often appear without signs of neuronal activity, indicating possible contact with various membrane elements. Close contact with a neuron is clearly recognized by the appearance of large action potentials and an increase of electrode noise up to several 100 μV . Then the positive pressure is again reduced to 1–10 cm H_2O . As the electrode is further advanced, the serial resistance increases and the tip potential becomes more negative. Increasing the pressure reduces the resistance (see Fig. 1A, arrow in section 1); reducing the pressure increases the resistance again and may be accompanied by a slight negative shift of the DC-potential (Fig. 1A, end of section 1).

At this point, a small negative pressure (1–20 cm H_2O) is applied to the electrode while it is further

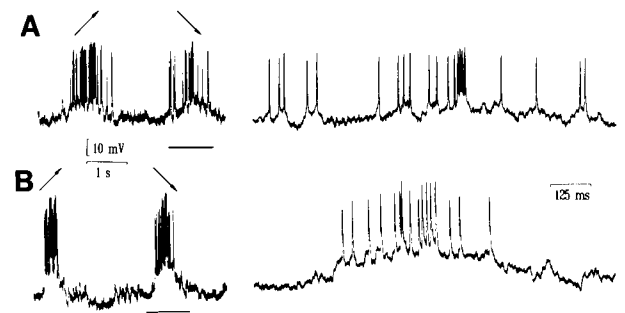


FIG. 2. Responses of a complex cell to an optimally oriented light bar moving across its receptive field in the directions indicated by the arrows. The recordings at the top (A) and bottom (B) are separated by 2 h, but the speed of bar movement is higher in B. Left: Low time resolution. Right: Higher time resolution of the underlined portions of the left records. Differences in action potential amplitude are caused by digital conversion.

advanced (Fig. 1A, sections 1 and 2), sometimes with a continuous negative current (0.05–0.5 nA). During this procedure, the electrode noise increases significantly, the DC-potential gradually becomes more negative, and the action potentials become essentially monophasic with only a small negative afterpotential. At this stage, sudden negative increments of the DC-potential by a few mV are only occasionally seen. When the DC-potential and the resistance have reached a stable level (usually -10 to -20 mV and 100–300 $\text{M}\Omega$, respectively) (Fig. 1A, beginning of section 3), we apply a strong negative pressure of -30 to -100 cm H_2O for a few seconds (Fig. 1A, arrows in section 3) and/or single positive current pulses (20–50 ms, 0.1–10 nA). During these procedures an additional negative increment of the membrane potential by 10–20 mV may appear (see Fig. 1B and C). Any negative pressure may then be released. The drop of membrane potential during these negative pressure pulses may consist of several steps of a few mV each (arrows in Fig. 1B) indicating that the membranes between the electrode and the cell interior are broken in successive steps. The breakage through the membrane is also often accompanied by a sudden drop of the resistance of the pipette-cell assembly to values around 100 $\text{m}\Omega$ (typically 50–150 $\text{M}\Omega$). In the example of Fig. 1A and B, the resistance dropped from 225 to 130 $\text{M}\Omega$. The recording situation is now stable and may thus be maintained for several hours (see Fig. 2). During one day of recording about 2–3 cells (usually one per electrode) could be thoroughly investigated, i.e. up to 6–8 cells per experiment.

The records indicate an intracellular recording situation as judged from the monophasic action potentials with a duration of 1.5 to 5 ms, from the amplitude, form and stimulus dependence of EPSPs and IPSPs, from the relatively constant firing threshold for action potentials and, especially, from the effect of intracellular current pulses. Positive pulses depolarize the cell and above threshold, induce cell firing without signs of

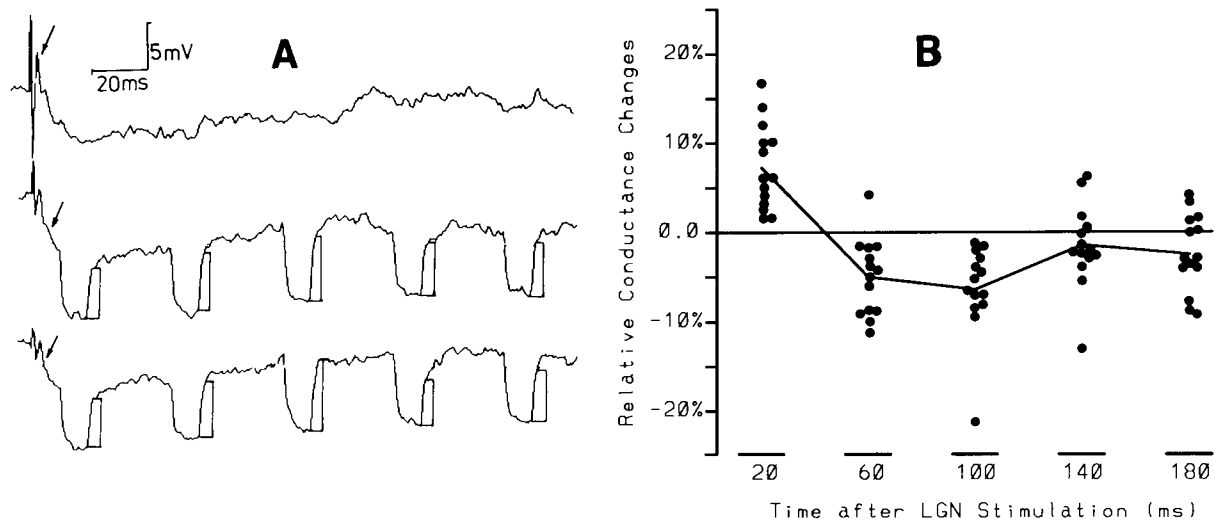


FIG. 3. A: Conductance changes following electrical stimuli in the LGN. Top trace: Responses of the cell (simple, resting potential: -40 mV, action potentials: 30 mV, input resistance: 130 M Ω) with short-latency EPSP (arrow) followed by strong and long lasting IPSP. Lower traces: Five negative current pulses (10 ms, 0.05 nA) are given after the LGN-stimulus, the first one 10 ms after the LGN-stimulus and the following ones at 40 ms intervals. The voltage change is measured from the end of the pulse until 4 ms later (see vertical lines after the pulses). B: Relative conductance changes of 14 visual cortical neurons following LGN-stimulation. Values are given as percentage (ordinate) of control measurements without LGN-stimulation; each point is the mean of 8 – 10 measurements on one cell. Note conductance increase at 20 ms and decrease at 60 and 100 ms following LGN-stimulus.

adaptation, while negative pulses polarize the cells. The amplitude of EPSPs increases during negative and decreases during positive current injection and, *vice versa*, for IPSPs.

There are however indications that the electrode may not be in direct contact with the cytoplasm of the recorded cell as in whole cell recordings in cortical slices.^{9,12} This is indicated by the relatively low membrane potential which ranged between -30 and -60 mV, and small amplitude action potentials, ranging from 30 to 50 mV. Threshold positive currents to excite a cell were in the range of, or only slightly higher than, those in classical intracellular recordings. In rare instances, whole cell recordings with large membrane and action potentials (60 – 80 mV) were achieved, but their incidence and stability were as capricious as those of classical intracellular recordings.

Conductance measurements: Even though it was difficult to estimate true cell resistance, it was possible to measure changes of input resistance during evoked post-synaptic activity. Figure 3 shows conductance changes after electrical stimulation of LGN (lateral geniculate nucleus). The LGN-stimulus led to an initial excitation with an EPSP latency of 2 ms and followed by an IPSP of 100 ms duration (Fig. 3A, top trace). The lower records show the effect of applying a sequence of five current pulses. The amplitude of voltage change caused by the pulse is proportional to the input resistance and inversely related to conductance. The first pulse is slightly smaller than the following ones, indicating a higher conductance during the initial part of the IPSP, but decreased conductance during its decay phase.

Conductance changes following LGN stimulation were measured in 14 cells and Fig. 3B shows the relative conductance changes at different intervals after the LGN stimulus. Conductance increased in these cells 10 – 20 ms after the LGN stimulus, i.e. during the peak of the IPSP, by 7.2% on average. At 60 and 100 ms, conductance was decreased (average 5.0%) and was then close to the baseline level 140 – 180 ms after the LGN stimulus. The differences between the mean values of the first and those of the second and third groups were significant (*t*-test, $p < 0.001$). We have also measured conductance during visual stimulation in the optimum and non-optimum orientations as well as during strong intracellular hyperpolarizing or depolarizing current injections, but have rarely seen significant conductance changes under any of those conditions.

Discussion

Applying methodology adopted from patch-clamp techniques clearly improved the yield and stability of intracellular recordings in the visual cortex *in-vivo*. Yet the recording was not as clear as one would expect if the electrode were in direct contact with the intracellular compartment such as in classical intracellular recordings or in tight-seal whole cell recordings.¹⁰ Sizes of membrane and action potentials were only about one to two thirds, while the amplitude of PSPs and their time course were in the same range and current injections had the typical polarity-dependent effects. In these respects, our recordings are comparable to the 'quasi-intracellular' recordings from thalamic cells.¹³ Gradual increase of

the resting potential during penetration is also similar in both recording methods.

One reason for the small size of the potentials in our recordings may be that the electrode is in direct contact with the intracellular compartment, but that the seal between the electrode and the membrane is not sufficiently tight, causing current leakage. However such leakage can lead to injury discharges, which were not usually observed. Another possibility is that the electrode tip is either not in direct contact with the intracellular compartment or only through a small hole because the tip is still covered with some membrane remains of cells overlying the recorded neuron. Such overlying membranes, sandwiched between the electrode tip and the neuronal membrane and possibly partly broken over the electrode opening, are compressed at the electrode edge which is therefore not directly sealed to the neural membrane. In such a situation, the resistance for recording intracellular events should be high, but the total input resistance would be reduced by the parallel shunting resistance across the incomplete seal.¹⁴ The cell is then not or only a little damaged, but the measured membrane potential should be lower than the true membrane potential. The fact that PSPs are still well recognized while action potentials are attenuated suggests that this arrangement has a low pass filtering property. Large current pulses may break the membrane completely yielding true intracellular records, though usually injuring the cell and often limiting recording time. Our recordings may incorporate both the above possibilities to different degrees.

The conductance changes during post-synaptic excitation and inhibition measured with this technique are in the range of those reported by others in *in-vivo* recordings from cortical cells in cats and rats^{15,16} but they are clearly smaller than those measured in cortical slices.¹⁵ The reason for this may be partly the relatively large shunt, but it should also be realized that *in-vivo* cells are under a continuous barrage of EPSPs and IPSPs² keeping total cell resistance low. Electrical stimulation of the LGN or, for that matter, visual stimulation only adds to this ongoing postsynaptic activity. In fact, total cell resistances of *in-vivo* recorded cells using fine microelectrodes range between 10 and 50 M Ω ,¹⁷⁻¹⁹ which are lower than those measured in slices.¹⁷ However, we cannot explain the enormous difference between cell resistances reported for whole cell recordings in slices^{12,20} and those from *in-vivo* measurements.

The conductance increase following the LGN-stimulus was restricted to the initial 20 ms. This is the time during which EPSPs appear and includes the rise time of the post-excitatory IPSP.²¹ From 50

to about 130 ms, conductance was decreased, slowly returning to its control level. This corresponds approximately to the time course of cortical IPSPs after electrical stimulation of cortical afferents. Since this inhibition involves all cortical neurons¹⁹ many intrinsic synapses should also be silent. Furthermore, the electrical stimulus in the LGN also activates the inhibitory circuits in the LGN itself and thus silences LGN-neurons for about 100–150 ms,^{22,23} so that the synapses of thalamo-cortical afferents are also not active. As a consequence, postsynaptic ion channels are closed and conductance should indeed decrease during the later part of the IPSP. Our data thus indicate that earlier estimates of synaptic currents during IPSPs^{16,19,24} are too long, but they are in agreement with recent measurements of post-synaptic inhibitory currents in whole cell recordings in slices.²⁰

Conclusion

Whole cell recording from cortical cells *in-vivo* even with an incomplete seal is a considerable methodological improvement which allows one to analyse spontaneous and evoked postsynaptic events in visual cortical neurons over long time periods.

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