Visualization of the spread of electrical activity in rat hippocampal slices by voltage-sensitive optical probes

A. Grinvald, A. Manker and M. Segal

*J. Physiol.* 1982;333;269-291

This information is current as of September 7, 2007

This is the final published version of this article; it is available

*The Journal of Physiology Online* is the official journal of The Physiological Society. It has been published continuously since 1878. To subscribe to *The Journal of Physiology Online* go to: [http://jp.physoc.org/subscriptions/](http://jp.physoc.org/subscriptions/). *The Journal of Physiology Online* articles are free 12 months after publication. No part of this article may be reproduced without the permission of Blackwell Publishing: [JournalsRights@oxon.blackwellpublishing.com](mailto:JournalsRights@oxon.blackwellpublishing.com)
at:
http://jp.physoc.org/cgi/content/abstract/333/1/269

This version of the article may not be posted on a public website for 12 months after publication unless article is open access.
VISUALIZATION OF THE SPREAD OF ELECTRICAL ACTIVITY IN RAT HIPPOCAMPAL SLICES BY VOLTAGE-SENSITIVE OPTICAL PROBES

BY A. GRINVALD, A. MANKER AND M. SEGAL

From the Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel

(Received 26 October 1981)

SUMMARY

1. Voltage-sensitive membrane-bound dyes and a matrix of 100 photodetectors were used to detect the spread of evoked electrical activity at the CA1 region of rat hippocampus slices. A display processor was designed in order to visualize the spread of electrical activity in slow motion.

2. The stimulation of the Schaffer collateral-commissural path in the stratum radiatum evoked short latency (2–4 msec) fast optical signals, followed by longer latency (4–15 msec) slow signals which decayed within 20–50 msec. Multiple fast signals were frequently detected at the stratum pyramidale; they propagated toward the stratum oriens with an approximate conduction velocity of 0.1 m/sec.

3. The fast signals were unaltered in a low Ca\(^{2+}\) high Mg\(^{2+}\) medium but were blocked by tetrodotoxin. These signals probably represent action potentials in the Schaffer collateral axons. Their conduction velocity was about 0.2 m/sec and their refractory period about 3–4 msec.

4. The slow signals were absent in a low Ca\(^{2+}\) medium and probably represent excitatory post-synaptic potentials (e.p.s.p.s) generated in the apical dendrites of the pyramidal cells. They were generated in the stratum radiatum, where the presynaptic signals were seen, and spread into somata and basal dendrites (the stratum pyramidale and oriens, respectively).

5. The timing of the signals with fast rise-time, which were detected at the stratum pyramidale, approximately coincided with the timing of the extracellularly recorded field potentials. These multiple discharges probably represent action potentials of the pyramidal cells. They spread back into the apical dendrites but with significant attenuation of the amplitudes of the high frequency components of the pyramidal action potentials.

6. Hyperpolarizing potentials could be detected when strong stimuli were applied to the stratum radiatum or alveus. The net hyperpolarizations were detected only in the stratum pyramidale and the border region between the stratum pyramidale and radiatum. Frequently the inhibition was masked by the large e.p.s.p.s. However, its existence could be demonstrated by treatment of the slice with picrotoxin or a low Cl\(^{-}\) medium. Under these conditions a long-lasting depolarization of the apical dendrites was evoked by the stimulation. This was associated with an increase of the multiple discharges in the stratum pyramidale and oriens.

7. These studies illustrate the usefulness of voltage-sensitive dyes in the analysis
of passive and active electrical properties, pharmacological properties and synaptic connexions in mammalian brain slices, at the level both of small neuronal elements (dendrites, axons) and of synchronously active neuronal populations.

INTRODUCTION

The hippocampus constitutes an organized cortical structure amenable to a physiological analysis of synaptic interactions among neurones. The advent of the slice preparation facilitated the study of neural interactions as it allows prolonged intracellular recording and control of the cellular environment. Thus, recent studies have characterized some properties of hippocampal neurones in vitro (Yamamoto, 1972; Schwartzkroin, 1975; Andersen, Sundberg, Sveen & Wigstrom, 1977; Segal, 1980).

Conventional electrical recording methods have some inherent limitations which may impede the analysis of the neuronal circuits: only a restricted number of electrodes can be placed in the analysed tissue and intracellular recording can be made only from reasonably large elements, e.g. cell bodies and large dendrites, but not from smaller ones (fine dendrites, axons). These limitations hamper the interpretation of data from experiments directed toward an analysis of functional properties of synaptic connexions and of neuronal circuits in the mammalian brain.

Optical methods for monitoring neuronal activity offer an alternative approach. The principle of the technique is simple. The preparation is stained with a physiological solution containing 1–400 μM voltage-sensitive dye which binds externally to the neuronal membrane. These dye molecules serve as molecular transducers which transform changes in membrane potential into optical signals. Voltage-clamp experiments on squid giant axons indicate that many optical signals are linearly related to changes in membrane potential and have a fast response time. Therefore, optical recordings are equivalent, in time course, to intracellular electrical recordings (Cohen, Salzberg, Davila, Ross, Landowne, Waggoner & Wang, 1974; Ross, Salzberg, Cohen, Grinvald, Davila, Waggoner & Wang, 1977; Gupta, Salzberg, Cohen, Grinvald, Kamino, Boyle, Waggoner & Wang, 1981). Optical methods have some advantages over the classical recording techniques; a large number of photodetectors can detect simultaneously changes in membrane potential in a large number of neurones at different locations (Salzberg, Grinvald, Cohen, Davila & Ross, 1977; Grinvald, Cohen, Lester & Boyle, 1981a). Furthermore, the use of these dyes enables recording from small neuronal elements (Grinvald, Ross & Farber, 1981b; Grinvald & Farber, 1981). The use of optical methods for the study of invertebrate central nervous systems has been described (Salzberg, Davila & Cohen, 1973; Grinvald, Salzberg & Cohen, 1977, Grinvald et al. 1981a). The implementation of the technique to the study of mammalian neurones in tissue culture was reported recently (Grinvald et al. 1981b), and optical studies of the development of excitability in cardiac muscle were described (Fujii, Hirota & Kamino, 1981a, b). A few reviews are also available (Waggoner & Grinvald, 1977; Cohen & Salzberg, 1978; Cohen, Salzberg & Grinvald, 1978).

We investigated the application of voltage-sensitive dyes to study rat hippocampus slices and report here on membrane-potential changes induced by stimulation of an
OPTICAL MONITORING OF ACTIVITY IN SLICES

excitatory afferent pathway as detected simultaneously by an array of 100 photodetectors each sampling an area of 45 x 45 µm. Since each photodetector receives light from several neuronal elements, the observed optical signal probably reflects the intracellular activity of a population. The high degree of organization of the hippocampus allows intracellular population recordings from relatively well-defined neuronal elements i.e. dendrites, somata, axons.

Preliminary results have been presented (Grinvald, Ross, Farber, Saya, Zutra, Hildesheim, Kuhnt, Segal & Kimhi, 1980).

METHODS

Adult (200–300 g) male Wistar rats were decapitated, their brains removed, and the hippocampus of one hemisphere was dissected out. Transverse slices of 250 µm were cut with a McIlwain tissue chopper and placed, for 1–2 hr, in a chamber containing fresh oxygenated Krebs solution. Slices were then transferred to the recording chamber, which was fixed on a Zeiss universal microscope stage. The plexiglass chamber had a 15 mm diameter hole in the centre. This hole was covered with a microscope cover-slip attached to the chamber with a thin layer of Vaseline. The slices were placed in the chamber and perfused continuously with physiological solutions. The perfusion did not interfere with the optical measurements provided that the solutions were filtered through 0-22 µm millipore filters. The slices transmitted 30–50 % of the illuminating light. Photometrical measurements indicated that the transmission through the 'opaque' area of the dendrites was only about 25 % less than that through the relatively transparent zone of the cell somata.

Up to three slices were kept in the optical chamber. They were covered with a silk net made of fine threads and at spacings of 1-5 mm. The net was glued to a thin platinum ring. Slices were left to recover for a period of at least 15 min. Usually the experiments were carried out 4–12 hr after the preparation of the slices. Two or three hydraulic M-103 Narashige micromanipulators mounted on the same microscope stage were used to place 1–15 mΩ, 0-5 m-NaCl filled glass micropipettes in the tissue. Such monopolar stimulating electrodes were placed in the stratum radiatum, oriens or alveus while a recording electrode was placed in the stratum pyramidale. The stimulation was controlled by a WPI constant current stimulator. Electrical signals were recorded with a M707 WPI electrometer. The stimulus duration was 0-3–0-5 msec, and the current was adjusted to yield a 1–3 mV extracellular population spike detected in the stratum pyramidale. To facilitate comparison with the optical recordings which were also filtered with a RC filter having a time constant of 0-7 msec. (The filtration and the presently low time resolution of the computerized recordings from 100 channels resulted in extracellular recordings which are somewhat distorted relative to their traditional presentation.) Intracellular recordings were also attempted, but stable recordings were not obtained, probably because of the low angle of the electrode under the water immersion objective (23°), and the type of manipulator used. Therefore, to demonstrate the performance of the optical probe and the detection system, simultaneous optical and intracellular recordings were carried out on tissue culture cells, as described in detail elsewhere (Grinvald et al. 1981 b).

The Krebs solution contained (in mm): NaCl, 124; KCl, 5; KH₂PO₄, 1-25; NaHCO₃, 26; MgSO₄, 2; CaCl₂, 2; and D-glucose, 10. The solution was oxygenated with a gas mixture of 95 % O₂ and 5 % CO₂; the pH was 7-4. In some experiments a low Ca²⁺ medium was used, containing 0-2 mm-Ca²⁺ and 4-0 mm-Mg²⁺. In a 'low Cl⁻' medium the NaCl was replaced by Na⁺-propionate. The experiments were performed at room temperature (24–28 °C).

The optical apparatus

The optical monitor was described in detail elsewhere (Grinvald et al. 1981 a, b). The apparatus is depicted in Fig. 1. A Zeiss universal microscope was rigidly mounted on a vibration-isolation table (Newport Research, CA, U.S.A.). A 12 V/100 W tungsten/halogen lamp was used. Slices were viewed with a long-working-distance 40 × water immersion objective (Zeiss 46-07-15). Transmitted light was detected by a 10 x 10 square array of photodiodes, each 1-4 x 1-4 mm (Centronix, London). Each photodiode received light from a 45 x 45 µm area of the microscope objective field, and was coupled to a current-to-voltage converter and amplifier. The amplifiers filtered the optical data with
Fig. 1. Schematic diagram of the optical monitor of neuronal activity. The microscope objective forms a real magnified image of the preparation at the microscope image plane. A 10×10 array of photodetectors, positioned at the image plane, records the changes in light intensities that are related to neuronal activity. The optical signals are amplified and fed into the computer memory by a direct memory access interface. A Vidicon camera projects the picture of the preparation on a TV monitor screen. A computer-to-video display processor plots a grid on the TV picture so that each square corresponds to a given photodetector element. The interface displays, in slow motion, flashes of light which correspond to the activity in each photodetector at a given time. The scheme on the TV monitor shows the picture of the displayed activity exclusively at the Schaffer collateral zone, 1-4 msec following the stimulus (the bright diagonal strip marks the stratum pyramidale). Optically detected electrical activity is also displayed in the conventional way on a graphic display terminal.
resistance-capacity (RC) filters having a time constant of 0.7 msec. The signals were a.c. coupled with a switch-selected time constant of 100 msec or 800 msec and amplified again (× 1000–4000). In most of the experiments the a.c. time constant was 100 msec. With a.c. coupling of 100 msec, fast signals (< 5 msec) are not distorted while slower signals (> 10 msec) are. The output of the amplifiers was multiplexed and digitized by two multiplexer 8-bit a./d. converted cards (Adac, MA, U.S.A.), and deposited in the PDP 11/34 computer memory. The recording from each channel was displayed on the screen of a Tektronix 4010 display terminal. Permanent records were made on a Tektronix 4631 hard copy unit. The object field was viewed with a Vidicon camera and stored on videotape for further localization of the photodiode array with respect to the slice. A computer-to-video display processor was designed in order to visualize the spread of electrical activity in slow motion (Manker, 1982). It projected the outlines of the array on the TV monitor superimposed with the picture of the preparation. In addition, it displays a star in each of the 100 elements of the picture. The size of each star was related to the amplitude of the detected signal at a given time interval (Fig. 1).

Procedures

In most experiments the stimulating and recording electrodes were positioned in the microscope field of view. The dye, WW401 (Grinvald et al. 1981b), (51-γ-sodium sulphopropyl-4(1H) quinolylidene)-3-propylrhodanine), a merocyanine-rhodanine derivative, was then applied at a concentration of 0.2 mM in normal medium, for 20 min. (The perfusion was stopped). The staining solution was washed away for an additional 20 min and the size of the evoked potentials, which were often reduced during the staining with the dye, was restored. Using this dye voltage-sensitive optical signals can be obtained at a wavelength range of 510–780 nm. The largest optical signals were obtained at a wavelength of 690 nm and a band width of 30 nm (Grinvald et al. 1981b). To minimize bleaching of the dye molecules and photodynamic damage (Ross et al. 1977), a shutter, transmitting the light onto the slice, was opened only for the duration needed to measure the optical responses (10–30 sec for averaging ten to thirty trials using 1 Hz stimuli).

The correction procedure for light-scattering signals

Light-scattering optical signals were detected in the slice in response to electrical stimulation. Their time course was independent of the wavelength of the light passing through the tissue, whereas the maximal voltage-sensitive dye signals were recorded with a 690 nm interference filter. Virtually no dye-related response was seen when a 810 nm filter was used. Thus, a routine subtraction of light-scattering responses measured with a 810 nm filter (multiplied by a correction factor), from responses obtained with a 690 nm filter was used to yield a net dye-related signal in most of the recordings presented below. The proper correction factor for the light-scattering signals was calculated from the measurements of the transmitted light intensities at 690 and 810 nm. (The scattering signal measured at 690 nm was ~ 1.3 times larger than the one observed at 810 nm, for equal excitation intensities.)

RESULTS

Stimulation of the stratum radiatum produced a short latency (2–4 msec), fast (3.5–6 msec) signal in a strip 90–180 μm wide corresponding to the Schaffer collateral–commissural system (two top traces of Fig. 2). The signals could be traced in adjacent detectors for a long distance but only along the collateral system (not shown). These fast signals probably reflect the action potentials in the Schaffer collateral axonal fibres.

A second wave of excitation was detected in the same region with a latency of 4–15 msec, but this wave ‘travelled’ along the dendritic tree toward the strata pyramidale and oriens. These slow signals were assumed to represent the dendritic e.p.s.p.s. (The possibility that the dendrite can fire action potentials and other contributions to the slow signals are discussed below.)
The dendritic depolarization triggered multiple optical signals at the pyramidal cell bodies. These optical signals probably represent the action potential discharges there. The optical signals propagated into the stratum oriens with an averaged conduction velocity of 0.1 m/sec. Its passive spread back into the apical dendrites was noticeable only over a short distance, under normal conditions (Fig. 2). The optical signals detected at stratum oriens probably reflect the activity of the basal dendrites and axons of the pyramidal cells as well as the activity of interneurons there.

There was an excellent correlation between the electrically recorded field potential and the optical signals from the stratum pyramidale; whenever there was a large population spike in the electrical recording, there were also large optical signals. Furthermore, a single stimulus would occasionally generate secondary and tertiary or even quaternary population spikes on both the electrical and optical recordings.
from the stratum pyramidale. The time of the peaks of the field potentials preceded the peaks of the optical spike by 0.7–1.4 msec. Such small time differences were frequently observed and they are discussed below. Both the electrical and optical responses were markedly reduced by reversing the polarity of the stimulus. Finally, various drugs (see below) did affect the two types of recording in a similar manner.

Below are given the results obtained in a series of experiments designed to clarify and establish the proposed identity of the optical signals.

Fig. 3. Light scattering signals in the slice. Stimulation was applied at stratum radiatum and responses from an unstained slice were recorded. A, the effect of stimulus strength on light-scattering signals; a, weak stimulus, top trace, shows the extracellular recordings from the pyramidal layer. Bottom traces are optical recordings from: (1) the stratum radiatum; (2) the stratum oriens; and (3) the stratum pyramidale. b, stronger stimulus, 1–3 same positions as a. The signals are larger than those in a and the differences in the latencies of the light-scattering signals in the different areas are clearer. The thick arrows show the second and third peaks of multiple discharges on the slow light-scattering signals. Thin arrows: time of stimulus onset. B, pulse-pair facilitation of the light-scattering signals. a, responses to weak twin pulses; 1 is from the stratum radiatum 50 μm away from the stimulating electrode. 2 is 300 μm away from the stimulating electrode; The electrical field potential recording is from the stratum pyramidale. b, light-scattering responses to stronger stimuli. Same as a, except that the facilitation is smaller. Twenty trials were averaged.

**Light-scattering optical signals from unstained slices**

The light-scattering response to the stimulation had characteristics that were clearly different from the dye responses. The time course of the light-scattering signal was independent of the wavelength. It was present also at a wavelength of 690 nm, and presumably added to the dye response at 690 nm. The light-scattering signals produced by the stimulation had a rather slow time course and outlasted any known changes in neuronal membrane potential (Fig. 3). The time constant for the decay of the light-scattering signal was also measured using the slower a.c. coupling of 900 msec, rather than the fast a.c. coupling (100 msec) (see Methods). A value of
250 msec was obtained for the decay of the light-scattering signals. Evidently the slow light-scattering signals are not linearly related to membrane potential changes. However, the signals had spatial and temporal characteristics which were correlated with electrical activity. The signals were generated first in the stratum radiatum, had a longer latency in the stratum pyramidale and the longest in the stratum oriens. Interestingly, the largest magnitude change was found in the stratum pyramidale (Fig. 3Ab). Both the magnitude and the latency to the onset of the light-scattering response were dependent on the stimulation intensity. With low stimulation intensity the light-scattering responses were small and had a slow rise-time whereas high stimulation intensity resulted in shorter latencies of the light-scattering responses, faster rise-times, and larger signals (compare Fig. 3Aa with Fig. 3Ab). The onset of the light-scattering responses in the st. pyramidale had the same latency as that of the population spike (Fig. 3Ab).

When twin pulse stimulation was applied the light-scattering response to the second stimulus was augmented in comparison with that of the first stimulus. This was more noticeable in the stratum radiatum far away from the stimulation site than next to the electrode and only at a moderate stimulus strength (Fig. 3B). Reversal of the stimulus polarity led to a marked decrease in the size of the light-scattering signals as well as in that of the population spike recorded electrically. Application of tetrodotoxin abolished the light-scattering signals (see below).

In summary, the light-scattering signals were triggered by the physiological activity and were not merely an artifact of stimulation. The signals were similar to those recorded previously in squid giant axons and crab nerves (Cohen, Keynes & Hille, 1968; Cohen, Keynes & Landowne, 1972; Cohen & Keynes, 1971), and may be related to those recorded from cerebral cortical slices (Lipton, 1973).

Dye-related signals

There is a striking similarity between intracellular electrical recording and optical recording from single cells. Such a comparison carried out using tissue-culture cells stained with the probe WW401 is shown in Fig. 4A. Fig. 4B shows the optical signals measured from a slice stained with the same dye. These signals were recorded from the stratum radiatum, using three different wavelengths. The two optical signals at 690 nm and at 540 nm had opposite signs as reported previously (Grinvald et al. 1981b). The slow signal measured at 810 nm, where the dye has negligible absorption, is a light-scattering signal; It was almost identical before and after the staining.

Ca\(^{2+}\) dependency of the fast and slow responses

To test if the fast signals are action potentials, and if the long-latency slow signals indeed represent e.p.s.p.s generated in the apical dendrites of CA1 pyramidal neurones, a low Ca\(^{2+}\) (0.2 mM)–high Mg\(^{2+}\) (4 mM) solution was used to block post-synaptic activity. The field potential was monitored continuously and found to be completely suppressed after 20 min, at which time optical responses were measured (Fig. 5). While the initial fast response was somewhat enhanced by the low Ca\(^{2+}\) medium, the long-latency slow response was completely blocked. The occurrence of fast signals was restricted to the region of the Schaffer collaterals (over an inspected distance larger than 900 \(\mu\)m). The increase in the spike size may reflect recruitment
Fig. 4. The dye-related optical signals. A, comparison between optical (top) and intracellular electrode recordings (middle) from a 60 μm neuroblastoma cell maintained in culture. The bottom trace shows the timing of the intracellular stimulating current. The open arrow shows the distortion of slow optical signals due to the a.c. coupling of the optical signal (see Methods). However, it is evident that for the action potential the distortion is minimal. The measurement was made using 690 nm interference filter. B, wavelength dependence of optical signals from the slice. Slices were bathed in a medium containing only 0-2 mM-Ca^{2+} and 4-0 mM-Mg^{2+}, to block synaptic activity. The wavelength was selected with 30 nm interference filters. The signals were not corrected for light-scattering the transmission changes detected at 810 nm before and after staining were almost identical. The stimulating electrode was less than 100 μm away from the recording site. The arrows on the calibration bar for the optical signals show increase in absorption (decrease in transmission). s: time of stimulus onset. Ten trials were averaged in A and B.

Fig. 5. The effect of Ca^{2+} removal on the optically detected evoked activity. A, optical and electrical recordings in normal medium from the stratum radiatum. B, optical recording in a medium containing 0-2 mM-Ca^{2+} and 4-0 mM-Mg^{2+}. Note that the size of the fast signals was increased and the slow response disappeared. C, optical recording 20 min after the low Ca^{2+} medium was replaced by the normal medium. The stimulating electrode was less than 150 μm away from the optical recording site. The field potential was recorded from the stratum pyramidale. Small arrows marked by s: time of stimuli onset.
of additional presynaptic elements in the low Ca\(^{2+}\) medium, possibly due to an increased excitability. These effects were reversible and the original response was restored by perfusion with the normal medium.

**Effects of tetrodotoxin on the fast responses**

If indeed the fast signals represent voltage-activated Na\(^+\) action potentials, they should be blocked by the Na\(^+\) channel blocker, tetrodotoxin (TTX). TTX (5 \times 10^{-6} \text{ M})

![Diagram](image)

Fig. 6. The effects of tetrodotoxin on the optical responses. A. Recordings in normal medium. a, from the stratum radiatum; the presynaptic and post-synaptic responses are evident. b, from the stratum pyramidale. Ex, extracellular recordings. B, recordings from the same locations after the application of 5 \times 10^{-6} \text{ M-TTX}. C, the position of individual photodetectors in the array that recorded the average activity presented in records D–F. D–F, optical responses as a function of the distance from the tip of the stimulating electrode in the presence of TTX. The stimulating electrode was placed in the proximal part of stratum radiatum. D, responses near the electrode in the presence of TTX. The polarity of the stimulus is positive. a, the photodetector at the tip of the micro-electrode. b, average response of the four photodetectors monitoring an area 45 \mu m away from the stimulating electrode. c, same as b but 80 \mu m away. d, same as b but 135 \mu m away from the electrode tip. E, Same as D, but the polarity was reversed. The slow component of the signals remains similar, but the fast component reverses its direction. F, Same as E, but the measurement was carried out at a wavelength of 810 nm, where voltage-sensitive responses should disappear. The arrows show the time of the stimuli onset.

was applied to the slice and the disappearance of the field potential was monitored. The optical responses diminished simultaneously with the disappearance of the electrical responses (Fig. 6A and B). These experiments illustrate that the fast signals indeed reflect Na\(^+\) action potentials and not locally evoked dendritic Ca\(^{2+}\) action potentials.
Optical ‘artifacts’ near the stimulating electrode

A small local response was often detected in the presence of TTX. It decayed rapidly in space and was not seen 150 µm away from the stimulating electrode (Fig. 6D and F). These small signals may partially represent the local passive polarizations, the sign of which depends on the stimulus polarity (Fig. 6D and E). Such local and direct depolarizations are present near the stimulating electrode and are probably responsible for the fact that, in a few cases the latency for the fast spike was zero (e.g. Figs. 4 and 5). The same type of local response is also observed using intracellular electrical recording if the stimulating electrode was close and a high stimulus intensity was employed. However, some local signals persisted also at a wavelength of 810 nm (Fig. 6F), where voltage changes cannot be detected, and hence may be due to a mechanical effect of the stimulation on the slice (movement artifact) or to electrophoretic effects of the electric field on the bound dye molecules, which may unbind and change their colour, thus giving rise to an optical signal.

In summary, it seems preferable to record optically far away from the stimulating electrode. However, when a moderate stimulus is used and a correction for light scattering is made, optical recordings near the stimulating electrode are only slightly distorted.

Properties of the unmyelinated axons in the hippocampus slice

Effects of tetraethylammonium (TEA)

TEA blocks K\(^+\) channels when applied to hippocampal neurones intracellularly (Schwartzkroin & Prince, 1980). TEA (2 mM) was applied in the incubation medium and its effect on the evoked optical responses was assessed (Fig. 7A). A marked broadening of the fast responses was observed; there was no change in latency or magnitude of the evoked optical response but it decayed more slowly than under control conditions. The control response could be restored after 20–30 min of washing with normal medium (Fig. 7A).

Conduction velocity

The conduction velocity along the Schaffer collateral system was estimated by measuring the delay between the peaks of the fast responses, which were measured at different locations along the Schaffer collateral system. Due to the limited time resolution of the present apparatus (0.7 msec), an accurate estimate could be obtained only across fairly long distances. The microscope field of view was shifted and an adjacent field was sampled. In one such case, three adjacent fields were sampled yielding a total length of over 1 mm of the fibres (Fig. 7B); a conduction velocity of 0.2 m/sec was determined. Similar conduction velocities were found in other slices over shorter distances. The present results agree with the value of 0.2–0.35 m/sec which was reported for the conduction velocity of these fibres in guinea-pig hippocampal slices measured at 37 °C (Andersen, Silfvenius, Sundberg, Sveen & Wigstrom, 1978). The magnitude of the fast optical response diminished gradually in size the farther the recording was from the stimulation site and the signal broadened. These results are essentially identical to earlier observations which were made using extracellular recordings (Andersen et al. 1978). This can be attributed to
the possible reduction in the number of intact fibres that are activated away from the stimulating electrodes (due to the slice dissection angle, and to non-uniformity in velocity. Obviously near the stimulating electrode a small contribution of activity from other neuronal elements is added to the fibres' signal. However, in a few experiments the size of the presynaptic action potential was fairly constant over one field of view (450 μm).

![Figure 7](image)

**Fig. 7.** Properties of unmyelinated axons. **A**, The effect of TEA on action potentials in the stratum radiatum. The stimulating electrode was placed 150 μm away from the recording point. *a*, the average response in a medium containing low Ca²⁺. *b*, 10 min after application of 2 mM-TEA. *c*, 20 min after the TEA solution was washed out. **B**, measurement of conduction velocity in the Schaffer collateral system. The Schaffer collaterals were stimulated 100 μm away from the first shortest latency recording point. The experiment was done in a low Ca²⁺ high Mg²⁺ medium. The distance from one to the next monitored location is 180 μm. The calculated conduction velocity is 0.2 m/sec. **C**, measurements of the refractory period of action potentials in the Schaffer collaterals. The Schaffer collaterals were stimulated 120 μm away from the recording point. Recording was carried out in normal medium. Traces from locations with minimal post-synaptic responses were selected for this figure. *a*, interpulse interval of 3 msec; *b*, 4 msec; *c*, 7 msec.

**Refractory period**

The refractory period of the activated axons was estimated by applying two pulses at various interpulse-intervals (Fig. 7C). There was almost no response to the second stimulus when it was applied 4 msec or less after the first one, and a partial response when it was applied 4 msec after the first one; a full response was obtained with a 7 msec delay between the pulses. It appears that the refractory period is in the range of 3–4 msec. Our results were close to the value of 2 msec, which was determined for the refractory period of similar fibres in guinea-pig hippocampal slices (Andersen et al. 1978). The lack of a second response in Fig. 7Cb also indicates that the contribution of local direct polarization to the fast and short latency signals was minimal.
OPTICAL MONITORING OF ACTIVITY IN SLICES

Post-synaptic responses

Excitatory post-synaptic responses

It was already suggested that the longer latency slow responses probably represent excitatory post-synaptic potentials (e.p.s.p.s), generated at the apical dendrites of CA1 pyramidal neurones, because such a response was absent in a low Ca²⁺ medium (see Fig. 5). Fig. 8 provides another example of the responses observed along the axis of a CA1 pyramidal cell. In this experiment the stimulating electrode was 500 μm away from the recording site, and therefore (e.g. Fig. 7B) the presynaptic action potentials were small (open arrows on top traces). No delay could be observed between the peaks of the slow responses at stratum radiatum and the peaks of the fast responses detected at the stratum pyramidale. In fact, the gradual diminution of the 'dip' between the two spikes (from the pyramidale toward apical dendrites) may suggest that the fast spikes were initiated at the stratum pyramidale and their passive deflexion is superimposed on the slow e.p.s.p. It is possible that presynaptic and post-synaptic activity of other interneurones also contribute to the slow signals detected at the stratum radiatum. The e.p.s.p. observed at stratum pyramidale was

Fig. 8. Intracellular population response along the axis of CA1 pyramidal cells. The simplified scheme at the right shows the long axis of CA1 pyramidal cells. The experiment was carried out with the same slice as that shown in Fig. 2, but 500 μm further away from the stimulating electrode. See Fig. 2 for more details. Thick arrow, the time of the stimulus onset. Open arrow, the peak of the presynaptic action potentials. Note the slow conduction velocity of the population activity at the stratum oriens.
often much smaller than expected (not shown). This cannot be solely attributed to the attenuation caused by passive spread from the point of origin and the e.p.s.p. was probably masked by hyperpolarizing synapses localized there (see below).

**Hyperpolarizing inhibitory potentials**

Hyperpolarizing potentials could be detected when strong stimuli were applied. Under such conditions (Fig. 9A) the e.p.s.p. was followed by a long lasting hyperpolarization of a short (1–3 msec) latency. This was pronounced in the stratum pyramidale and also in the transition area between the strata radiatum and pyramidale. Inhibitory GABA synapses are indeed found on the somata of the pyramidal cells, and thus these hyperpolarizing signals probably reflect i.p.s.p.s.

Antidromic stimulation of CA1 pyramidal cells by stimulating the alveus is known to activate a recurrent inhibitory pathway (Andersen, Eccles & Loyning, 1964). Fig. 9B demonstrates that upon antidromic stimulation i.p.s.p.s were indeed recorded at some loci, whereas in response to orthodromic stimulation only depola-

---

**Fig. 9.** Inhibitory post-synaptic potentials in the stratum pyramidale. A, activity was evoked by stimulation of the stratum radiatum 100 μm away from the recording site. Four optical traces are shown (top to bottom): from the stratum radiatum 300 μm away from the layer of cell somata; from the stratum radiatum 150 μm away from the stratum pyramidale; from the stratum pyramidale; from the stratum oriens. The extracellular recording electrode was placed 200 μm away from the site of the optical recording of pyramidale activity. B, a simplified diagram of a recurrent inhibitory circuit in the CA1 area. The dark neurone is the inhibitory interneurone; it accepts excitatory input from axonal collaterals of CA1 pyramidal cells and its inhibitory synapses are located at the stratum pyramidale. s, stimulating electrode; Ortho, orthodromic stimulation at the stratum radiatum; Anti, antidromic stimulation at the alveus. C, optical responses at the stratum pyramidale to stimuli at different loci. Top trace, the response to a strong antidromic stimulus of the alveus. Thick arrows, time of stimulus onset. Thin arrows show the time of the stratum pyramidale field potentials (500 μm away from the optical recording site). Middle trace, the response to a three-fold weaker stimulus, with inhibition no longer evident. Bottom trace, the response, at the same location, to orthodromic stimulation.
rizations were detected there. Because of the nature of intracellular population recordings, it is clear that a lack of a net hyperpolarization does not imply an absence of hyperpolarizing inhibitory responses in the slice (masked by the depolarization). Pharmacological manipulations are helpful in demonstrating the various types of synapses (see below).

The cellular discharges

Large signals having a fast rise-time were observed in the strata pyramidale and oriens. The optical response at its rise-time appeared to coincide with the extracellularly recorded population spikes from the same area (e.g. Fig. 2). These action potential-like fast signals were not as evident in the stratum radiatum but were first seen in the stratum pyramidale and were even more evident in the transition zone between the strata pyramidale and oriens, where they had the fastest rise-time. They were associated with a high but not a low stimulation intensity and were occasionally followed by secondary and tertiary spikes. These, too, were not as prominent in the stratum radiatum. It appears that these fast signals represent action potentials generated in the axon hillocks and spreading into the strata oriens, pyramidale and radiatum. Interneuronal activity is probably also contributing to the optical signals detected at the stratum oriens.

Effects of picrotoxin

Picrotoxin, a GABA antagonist, was applied in the superfusion medium at a concentration of 10 \( \mu \)M. In the presence of picrotoxin, the stimulation of the stratum radiatum produced a greater depolarization than under control conditions. In addition, the stimulation produced an oscillatory response consisting of three to four successive peaks (Fig. 10). This oscillation was most pronounced in the strata oriens and pyramidale but was also seen in the stratum radiatum. The timing of the optical signals at the stratum pyramidale was similar to that of the field potentials. If indeed the oscillatory response represents repetitive synchronous discharges, which originate in the axonal hillock region, then their appearance in the stratum radiatum indicates their passive or active spread from the border zone of the strata pyramidale and oriens. Their effective spread back into the dendrites is in line with the theoretical electrotonic length of about 1–2 for the apical dendrites (Turner & Schwartzkroin, 1980; Brown, Fricke & Perkel, 1981). Similar results were obtained when the experiment was carried out in a low chloride medium (data not shown).

Spatial spread of the excitation of the Schaffer collaterals

Fig. 11 demonstrates the main advantage to optical recording, i.e. the feasibility of simultaneous recording from many (hundreds of) neighbouring loci. It illustrates the spread of a focal excitation along the Schaffer collateral commissural axons and post-synaptically along the apical dendrites of the CA1 neurones up to the axonal hillock region of the pyramidal cells, where the multiple discharge was initiated and spread toward the oriens. Occasionally the size of the dendritic depolarization was not the largest at the location of the synapses in the stratum radiatum (e.g. top of columns 5–7). One possible explanation is that \( \text{Ca}^{2+} \) action potentials were evoked by the e.p.s.p.s. The slow after-hyperpolarization and the shape of the dendritic responses
Fig. 10. The effect of picrotoxin on the spread of excitation along pyramidal cells. The scheme at the right shows the locations from which recordings were made. Thick traces, responses in normal medium; thin traces, 15 min after application of $10^{-5}$ M-picrotoxin; see Fig. 2 for more details. Bottom right: The slow lateral spread at the oriens 45 μm away from stratum pyramidale. Each recording is 90 μm away from its neighbour.

Fig. 11. Simultaneous optical recordings of the electrical activity at multiple loci. Top left, a scheme of the slice showing the area monitored by the array. Top, the optical traces from 9 x 10 detectors are shown at their appropriate locations in the field of view. Activity was evoked by stimulation of the stratum radiatum. The fast-rising short-latency signals (left side) are the optically detected presynaptic action potentials. The slow waves represent mostly the post-synaptic responses in the apical dendrites of the pyramidal cells. The e.p.s.p.s spread along the dendrites, resulting in action potential discharges of the pyramidal cells. The last two columns on the right show the activity of neuronal elements in the stratum oriens, i.e. the basal dendrites, axons of CA1 pyramids and other interneurones. The results of twenty trials were averaged. The ordinate bar represents the fractional change in light intensity. Each detector samples a square area of 45 x 45 μm. The optical signals were corrected for light-scattering. (Traces from four bad photodetectors were replaced by their neighbours, and a few traces with excessive noise were retouched.) Bottom, visualization of the activity presented in the top Figure. The four TV frames show the pictures formed by the display processor. They show the spread of activity from the stratum radiatum to the stratum oriens. The appropriate time interval is marked below each frame. (The superimposed TV picture of the preparation was omitted for clarity, see Fig. 1.)
OPTICAL MONITORING OF ACTIVITY IN SLICES
support this interpretation, in line with other recent observations (Schwartzkroin & Slawsky 1977; Wong, Prince & Basbaum, 1979; Llinás & Sugimori, 1980).

The main message obtained from Fig. 11 is that optical recording facilitates the detection of dynamic patterns of electrical activities of cell populations, and that these patterns are heterogeneous even in a relatively small area in the highly ordered CA1 region. Further experiments are required in order to shed light on the synaptic networks and morphological correlates underlying the observed patterns of activity. Analysis of the data presented in Fig. 11 is not a trivial job. There are \( \sim 10^6 \) bits of information there, which can be collected in less than 1 sec. Detailed analysis is feasible by using conventional approaches and multiple data presentation similar to Figs. 2, 8 and 10. Such an approach is obviously time consuming and inefficient. Therefore we have designed the display processor which affords an on-line slow motion visualization of the spread of electrical activity. The display of electrical activity at four different time intervals is demonstrated in the bottom of Fig. 11.

**DISCUSSION**

**The origin of the optical signals**

The present series of experiments demonstrate the usefulness of optical methods in the study of electrical responses and pharmacological properties of neurones, employing the slice preparation. These studies demonstrate, for the first time in mammalian brain, that certain dyes bind to neuronal membranes and change their optical properties in response to electrical activity.

A series of experiments was designed to verify that the optical signals do reflect changes in membrane potential. Firstly, a correlation could be established between the optical recording and the extracellular recording from the stratum pyramidale in terms of size and pattern of responses. Secondly, the results deduced from the optical signals in response to various pharmacological treatments (TTX, TEA, picrotoxin, low Cl\(^-\), low Ca\(^{2+}\)) were similar to those obtained with conventional methods. Thirdly, the conduction velocity, the refractory period, the facilitation and the particular loci of a given optical signal support our identification of the various signals. It should be noted that, in principle, some of the slow optical signals may come from glia, rather than neurones, and their possible contribution remains to be evaluated.

While the optical and electrical signals share many characteristics, certain differences exist between the two types of signals. The presynaptic volleys recorded by Andersen et al. (1978) are shorter in duration than the present optical signals. This is due to the following: (1) Extracellular spikes are usually narrower than intracellular ones. (2) The spatial resolution of the extracellular recordings is smaller than that of the optical recordings, which pick up the activity from all the elements in a cube of \( 45 \times 45 \times 45 \) \( \mu \)m. These widely spread elements are probably active with a smaller degree of synchronousness than that observed from fibres adjacent to the extracellular electrode. (3) The temperature in the present experiment was lower. (4) The present time resolution of 0.7 msec may also ‘increase’ the width of the signals. (5) Averaged responses may be wider than those detected in a single sweep.

The patterns of optical and extracellular field potential were identical. However,
the electrical signals usually precede the optical signal by 0.7–2.1 msec. This is approximately the expected timing relationship for extracellular and intracellular recordings (we verified it by simultaneous intracellular and extracellular recordings of action potentials from single cells in culture). Small differences in the timing of the two types of recording may also arise from the fact that the two recordings cannot be done from precisely the same elements. (It is also difficult to visualize the exact position of the electrode tip, and the differences in timing between neighbouring areas can be quite large, e.g. bottom right of Fig. 10.)

Initiation and spread of action potentials

Previous studies have produced inconclusive findings with respect to the localization of the action potential generating zone in CA1 neurones. Spencer & Kandel (1961) and Andersen & Lømo (1966), suggested that dendrites generate spikes which travel into the soma to trigger a somatic spike. Using a depth profile analysis Andersen and Lømo (1966) suggested that the dendritic spike is generated about midway between the Schaffer collateral zone and the stratum pyramidale. Direct intracellular recordings from dendrites in CA1 region also suggest that fast action potentials in the dendrites can be evoked by stimulation of the stratum radiatum (Wong et al. 1979). On the other hand, MacVicar & Dudek (1981) reported that the small fast prepotentials detected at the somata of CA3 pyramidal cells are a reflexion of electrical coupling rather than of dendritic spikes. Knowles & Schwartzkroin (1981) recently suggested that the fast prepotentials are a passive reflection of action potentials in axon collaterals of the pyramidal cells. Jeffereys (1979) found that in dentate granular cells, the action potential is generated in the soma and is only triggered by the dendritic e.p.s.p. Not being able to record directly from remote dendrites or axons simultaneously with somatic recording, these results are only indirect and tentative and they emphasize the need for new techniques. The ability to measure potential changes with voltage-sensitive dyes may permit localization of the initiation site of action potentials. The results of the present experiments (e.g. Figs. 2, 8 and 10) suggest that the secondary and tertiary action potentials are probably generated in the stratum pyramidale or the axon hillock region and not in the dendrites. Once these action potentials are generated, they travel into the apical dendrites of the recorded cells. This observation confirms Jeffereys’ (1979) in the dentate granular cells. However, the present time resolution of our measurements makes it impossible to conclude whether the first population spike is generated in the soma or already in the apical dendrites. Thus, the possibility of a somatopetal conduction of a dendritic spike can not be excluded.

Advantages and limitations of optical recording

Advantages

Compared with electrical recording, optical methods have several inherent advantages. Firstly, there is no lower limit to the size of the neuronal element recorded, provided a voltage-sensitive dye can attach to its membrane. (However, a large number of such elements have to be synchronously active or, alternatively a large number of responses have to be averaged, in order to detect responses from small elements.) Intracellular-optical recordings can thus be obtained from unmyelinated
axons as well as from very thin dendrites. These have never been recorded intracellularly from mammalian preparations. Secondly, this method is a non-invasive one: there is no need to impale the membrane and risk injuring it to be able to record. For this reason, recording can be obtained from the same elements for a considerable length of time. Thirdly, the detection and localization of synchronously active populations of neurones are feasible. Finally, the major advantage is that a large number of detectors can be placed side by side to monitor simultaneously the electrical activity from hundreds of loci.

Difficulties exist with the present state of optical recording in brain slices, and they were exemplified in the present study.

**Instrumental difficulties**

The present instrument has a time resolution of 0.7 msec. The duration of each trial was limited to 350 msec, and in averaging experiments the light shutter had to be opened at least 1.5 sec prior to each of the recording periods, to allow for the 'settling' of the a.c. coupled signals. These limitations are now being improved with modified hardware and software.

**Present limitations of optical probes**

(A) The size of the optical signals is small. Therefore, spontaneous activity of single cells could not be unequivocally identified. The signal-to-noise ratio for the optical detection of an action potential in single mammalian neurones maintained in culture, or in invertebrate neurones, was in the range of 10–20 under optimal conditions (Grinvald et al. 1981a, b). The signal-to-noise ratio for an action potential from a population of pyramidal cells was only 2–3 in the present work. It was reported that the sensitivity of an optical probe may depend on the preparation (Ross & Reichardt, 1979; Gupta et al. 1981). Because only two dyes were tested in these experiments it is likely that more sensitive optical probes already exist for the present preparation. Thus, extrapolating from the results obtained in other preparations (Grinvald et al. 1981a, b), it is expected that also in a brain slice experiment, clearly detectable spike activity from single cells will be recordable.

(B) Because the signals are presently small, the activity-dependent light scattering signals from the slice distort the voltage-sensitive optical signals. (These light scattering signals were subtracted from the optical response.)

(C) Pharmacological side effects can be expected when extrinsic probe molecules are bound to the neuronal membrane. We did not observe significant pharmacological side effects, in the present studies, using field potential recordings from stratum pyramidale. However, more stringent tests using intracellular recording are required to assess the extent of pharmacological side effects. Different voltage-sensitive probes have very different chemical structures and different charges. It is therefore unlikely that all of them (more than 200) will cause similar pharmacological side effects. An example has been reported (Grinvald et al. 1981a), where the proper choice of the probe eliminated pharmacological side effects on a behavioural reflex mediated by a polysynaptic pathway.

(D) Bleaching: during the course of an experiment part of the dye bleaches, resulting in deterioration of the optical signals. To minimize bleaching, the exposure time of the slice to light should be reduced to a minimum.
(E) Photodynamic damage: the dye molecules sensitize the formation of reactive singlet oxygen, in the presence of intense illumination. These reactive radicals attack membrane components and damage the cells (Cohen et al. 1974; Ross et al. 1977). The photodynamic damage limits the duration of the experiments. Using the present probe, continuous measurements can be made for at least 1 min without marked damage. If the duration of each trial is 50 msec, then more than 1000 trials can be carried out before significant damage occurs.

(F) The resting potential can not be readily calculated and certainly not manipulated. The inability to evaluate reversal potentials for various e.p.s.p.s and i.p.s.p.s limits the interpretation of some of the observed responses.

In addition to these difficulties there are some limitations to the optical recording techniques that are specific to brain slices.

(G) The size of optical signals is related to the membrane area and the extent of binding. There are differences in ‘concentrations’ of membrane elements across the slice. In distal dendrites there are many processes and therefore a larger membrane area. There is much less membrane in the somata layer and the size of the optical signals from the stratum pyramidale is roughly estimated to be three to four times smaller than those from the stratum radiatum. Furthermore, a lower density of bound dye molecules in some parts of the tissue will result in a smaller signal size. Thus, a direct comparison of the amplitudes of optical signals in different regions may not be straightforward and the interpretation has to rely mostly on the time course of the signals.

(H) The recording is made from the entire depth of the slice (250–300 μm) which consists of different neural elements. (The depth of field resolution (Salzberg et al. 1977) could be improved if an objective with a higher numerical aperture was employed.) Although the hippocampus was selected because of its clear stratification, neurones of various types co-exist and signals from these neurones reaching the same detectors can undoubtedly obscure signals from a single population of neuronal elements, i.e. axons, dendrites, pyramidal somata, etc. Thus, the activity detected at the stratum oriens reflects the activity of the CA1 pyramidal axons as well as that of the interneurones there. The activity detected at the stratum radiatum reflects the action potentials of presynaptic elements, the post-synaptic responses of apical dendrites, the passive spread from pyramidal cells’ somata as well as the activity of other interneurones. Proper manipulation of stimulus size, location, frequency and pharmacological treatments should permit a separation of the various components.

Development of higher resolution dyes and the possible ionophoretic injection of a suitable fluorescent dye into single cells, could overcome some of the above difficulties. Successful experiments along these lines were performed recently. (Agmon, Hildesheim, Anglister & Grinvald, 1982).

Note added in proof. Recently we have found other optical probes (e.g. RH–155, RH–27, WW375, available upon request), which were five to eight times more sensitive than WW401. Thus, averaging was no longer required. (U. Kuhnt and A. Grinvald, unpublished result.)
We thank Drs L. B. Cohen, M. Gutnick, Y. Yarom and U. Kuhnt for their constructive comments. Special thanks to U. Kuhnt who participated in preliminary experiments. Supported in part by grants from the Israel Centre for Psychobiology, the Charles E. Smith Family Foundation, the USPHS NS-14716, and a grant from the Paralysed Veterans of America’s Technological Research Foundation to A.G.

REFERENCES


OPTICAL MONITORING OF ACTIVITY IN SLICES


Visualization of the spread of electrical activity in rat hippocampal slices by voltage-sensitive optical probes
A. Grinvald, A. Manker and M. Segal

*J. Physiol.* 1982;333;269-291

This information is current as of September 7, 2007

<table>
<thead>
<tr>
<th>Updated Information &amp; Services</th>
<th>including high-resolution figures, can be found at: <a href="http://jp.physoc.org">http://jp.physoc.org</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Permissions &amp; Licensing</td>
<td>Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://jp.physoc.org/misc/Permissions.shtml">http://jp.physoc.org/misc/Permissions.shtml</a></td>
</tr>
<tr>
<td>Reprints</td>
<td>Information about ordering reprints can be found online: <a href="http://jp.physoc.org/misc/reprints.shtml">http://jp.physoc.org/misc/reprints.shtml</a></td>
</tr>
</tbody>
</table>