

# Propagation of Voltage Transients in Arborized Neurites of Retzius Cells of the Leech in Culture

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Propagation of electrical signals is studied in Retzius cells of the leech in culture using voltage-sensitive fluorescent dyes at a spatial resolution of  $8 \times 8 \mu\text{m}^2$  and  $14 \times 14 \mu\text{m}^2$  and at a sampling interval of 0.12 ms. The neurons are stimulated by a microelectrode impaled in the soma. Action potentials of a halfwidth of 2–3 ms are triggered close to the end of the primary neurite dissociated from the leech. They propagate back to the soma at invariant halfwidth at a velocity of 50–230  $\mu\text{m}/\text{ms}$ . They pervade extended arborized secondary neurites which are grown on extracellular matrix protein. Their width is enhanced up to a factor two. The velocity is around 100–150  $\mu\text{m}/\text{ms}$  such that delays up to 3.5 ms are observed. Accordingly the neuritic trees are not isopotential. The features of propagation are found to be incompatible with passive spread.

## Introduction

The morphology of many neurons in brain tissue is dominated by extended dendrites. The model of branched cables with ohmic conductances is applied frequently to describe signal propagation in arborizations (Rall [1], Koch *et al.* [2]). A more involved signal processing may be attained if voltage-gated conductances are distributed in a compartmentalized tree (Hounsgaard and Mitgaard [3]). In fact *e.g.* voltage gated calcium channels have been located in dendrites (Llinas [4]). The elucidation of dendritic signal processing must be based, of course, on direct observation of the membrane potential at sufficient resolution in space and time.

As a model to study voltage transients in arborized neurons at high resolution we use neurons of the leech in culture. “An advantage of the leech is that identified neurons of known function can be used and that the regenerative response of every neuron type in culture can be compared with the performance of the same cell *in situ*” (Chiquet and Nicholls [5]). Cultivation of leech neurons was pioneered by Nicholls and coworkers (Dietzel *et al.* [6], Chiquet and Acklin [7], Chiquet and Nicholls [5], Chiquet *et al.* [8], Stewart *et al.* [9]). Recordings of voltage transients in small arborizations were

reported using an optical absorption technique at low resolution with extensive signal averaging (Ross *et al.* [10, 11]). We describe in the present paper the propagation of voltage transients in Retzius cells as detected by a single-sweep fluorescence technique at a high spatio-temporal resolution. We study soma and primary neurite as dissociated from the ganglia and extended arborizations of secondary neurites as grown in culture. A preliminary report of the work has appeared (Fromherz and Vetter [12]).

## Materials and Methods

### Neurons

Leeches (*Hirudo medicinalis*, Biopharm/Swansea) were maintained at 4 °C in water. The segmental ganglia were dissected and pinned on a Sylgard coated dish in Leibowitz-15 medium (Gibco/Eggenstein) as supplemented with 50  $\mu\text{g}/\text{ml}$  gentamycinsulfate (Sigma), with 6 mg/ml glucose and with 2% foetal calf serum (Gibco) (Dietzel *et al.* [6]). The capsules around the ganglia were opened with forceps. The ganglia were incubated in dispase/collagenase (Boehringer/Mannheim) (2 mg/ml L-15 medium) for 1 h. The Retzius cells (soma with 50–100  $\mu\text{m}$  of axon) were removed by aspiration into a fire-polished pipette and washed by transfer through several drops of medium.

Ganglia with opened capsules were incubated for several hours in 2% (vol/vol) triton X-100 in 10 mM Tris/HCl, pH 7.4 with 2 mM phenylmethyl-

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sulfonylfluoride, 5 mM N-ethylmaleimide and 2 mM EDTA (all from Sigma/München). The insoluble material was washed extensively with water and incubated overnight in 4 M urea in the same medium as above (Chiquet and Acklin [7]). The supernatant was dialysed against 10 mM Tris/HCl, pH 7.4 overnight. (All operations at 4 °C.) The effective component of this extract of the extracellular matrix (ECM) – with respect to growth – is a laminin-like protein (Chiquet *et al.* [8]).

Cover slips (0.15 mm thick) were attached to silicone chambers (Flexiperm-mikro 12, Heraeus, Hanau). ECM-extract was air-dried on the cover slips. Retzius cells were plated on this substrate in supplemented L-15 medium (Chiquet *et al.* [8]). Usually the cells were cultivated for 2–5 days at room temperature. The morphology of the cells was checked by phase contrast microscopy and – after fixation and dehydration – by scanning electron microscopy.

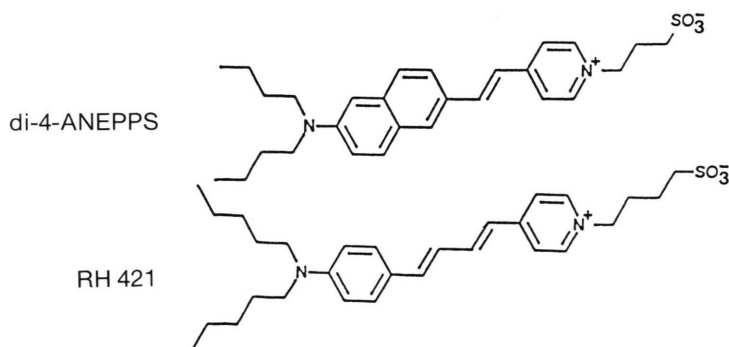
#### Optical recording

We used the method of voltage sensitive dyes (Cohen and Salzberg [13], Cohen and Leshner [14]). We applied the amino-stilbazolium dyes RH-421 (Grinvald *et al.* [15]) and di-4-ANEPPS (Fluhler *et al.* [16]) (Molecular Probes, Junction City, Oregon. The dyes were used without purification.) We used di-4-ANEPPS for studies of primary neurites and RH-421 for studies of secondary neurites.

We stained the cells by a vesicle technique: Dye (1 mM) was dispersed with egg lecithin (20 mM) in leech Ringer (115 mM NaCl, 1.8 mM CaCl<sub>2</sub>, 4 mM KCl, 10 mM Tris-maleate, pH 7.4) by sonication (Branson sonifier, level 3, at 0 °C, three times for 5 min with intervals of 5 min). After centrifugation the stained vesicles (diameter 90 nm as

checked by dynamic light scattering) were added to the growth medium (final dye concentration 4–8 μM). The cells were stained presumably by release of vesicle-bound dye to the medium. The method is superior to the application of ethanolic solutions as it avoids precipitation of the dye with ill-defined concentration as well as toxic effects of alcohol. The wavelengths of the maxima of excitation and emission of the dyes as bound to Retzius cells are 501 nm and 634 nm for RH-421 and 472 nm and 616 nm for di-4-ANEPPS respectively. Depolarization of the cell lowers the fluorescence yield, shifts the excitation and emission spectra to the blue and broadens the fluorescence spectrum due to an unknown mechanism (Grinvald *et al.* [15], Loew *et al.* [17], Ephardt and Fromherz [18], Fromherz and Lambacher [19]). The response is linear. An optimal signal-to-noise ratio was obtained by illumination at 541 nm taking advantage of the blue shift of absorption and by detection at >590 nm taking advantage of the drop of yield.

The chambers with the Retzius cells were mounted on the stage of an inverted microscope (Axiomat, Zeiss/Oberkochen) as fixed on an air-damped table. The central part of the arc of a mercury high pressure lamp (HBO 100 W/2, Osram) was imaged onto the cell through an interference filter (maximum 541 nm, width 20 nm, Schott/Mainz) and a dichroitic beam splitter (Ft 570, Zeiss). Illumination could be blocked by a shutter. The cell was projected onto the first image plane through a cut-off filter (RG 590, Schott). We used two objectives with different magnifications: (i) Planapo 100×/1,3 Oel Ph3 (Zeiss). (ii) Neofluar 100×/1,3 Oel (Zeiss) which was adapted to the Axiomat by an additional lens such that its actual magnification was 175×.



An array of 100 photodiodes each of a size  $1.4 \text{ mm} \times 1.4 \text{ mm}$  (MD 100-2, Centronics/Bristol) was mounted in the image plane. Thus the spatial resolution was  $14 \mu\text{m}$  with the Planapo ( $100\times$ ) and  $8 \mu\text{m}$  with the Neofluar ( $175\times$ ). In each measurement cell and array were carefully adjusted to attain optimal match for three selected diodes by superposing a video picture with a computer diagram of the array. The diagram was calibrated by moving a light spot across the object plane which was observed simultaneously on the monitor and at the output of selected diodes. At a resolution of  $8 \mu\text{m}$  the precision was better than  $1 \mu\text{m}$ . The photocurrents of the central 64 diodes of the array were fed into 64 current-voltage converters as mounted in direct contact to the array (operational amplifiers OPA 121 (Burr Brown/Filderstadt),  $100 \text{ M}\Omega$  resistors (electronic GmbH/Munich),  $2 \text{ pF}$  capacitors (time constant  $0.6 \text{ ms}$ )). It was the noise of these current-voltage converters which governed the overall signal-to-noise ratio of the measurements. The shot-noise of the photons and the fluctuations of the arc were negligible. The outputs were fed directly into 64 linear amplifiers or fed first into 64 sample-and-hold amplifiers where the offset of stationary fluorescence was subtracted from the transients. The amplifiers were adjusted such that no significant differences of gain and no significant time lags were introduced. 3 diodes could be selected by a multiplexer. The signals of these 3 channels were digitized in parallel (resolution 8 bit, sample interval  $120 \mu\text{s}$ , sample time  $20 \mu\text{s}$ ) and read simultaneously into a microcomputer, such that no time delays were introduced by multiplexing.

The soma was impaled by a microelectrode filled with  $3 \text{ M KCl}$  (resistance  $10\text{--}20 \text{ M}\Omega$ ) as used in *in vivo* experiments (Nicholls and Purves [20], Lent [21]). The electrode was attached to a micro-manipulator (Leitz/Wetzlar) on the stage of the microscope. It was used for stimulation by current injection ( $1\text{--}4 \text{ nA}$ ) and for recording of the membrane potential. The resting potential of the Retzius cells was around  $-40$  to  $-55 \text{ mV}$ . The potential was held at  $-50$  to  $-55 \text{ mV}$  to prevent spontaneous firing. Action potentials of an amplitude of  $55\text{--}80 \text{ mV}$  were stimulated by current injection. The signal of the electrode was digitized and read into the computer in parallel to the three optical channels.

Each neuron was tested 1 h after the application of the dye by stimulation. Experiments were started with cells with action potentials of an amplitude above  $60 \text{ mV}$ . The cells were adjusted with respect of the diode array. Three diodes were selected. The protocol of a measurement was: (1) Opening of the light shutter. (2) Recording of fluorescence after  $30 \text{ ms}$ . (This long delay was chosen because of fast initial photobleaching of the dye.) (3) Start of sample and hold amplifier to record transients after  $0.5 \text{ ms}$ . (4) Stimulation after  $4.5 \text{ ms}$ . (5) End of stimulation after  $20 \text{ ms}$ . (6) End of record after  $5 \text{ ms}$ . (7) Closing of shutter. During the measurement the three optical records and the electrical record were read simultaneously into the computer. After a delay of  $30$  seconds the cycle was repeated without stimulation. The signal obtained without stimulation was fitted by a function  $f(t) = a + b \exp(c \cdot t)$  ( $t$  time,  $a$ ,  $b$ ,  $c$  free parameters) using a Marquardt algorithm. The function was subtracted from the stimulated signal to eliminate bleaching effects. The change of fluorescence was divided by the absolute fluorescence intensity to eliminate effects of inhomogeneous illumination. Series of measurements with displaced array were aligned in time by using the time-to-peak of the action potential as a marker as recorded by the microelectrode. The error introduced by this procedure was about  $0.1 \text{ ms}$ .

The dyes were phototoxic. Illumination of stained cells lead first to a drop of amplitude and later also to an increase of width of the action potential. For both dyes the amplitude dropped typically from  $70 \text{ mV}$  to about  $50 \text{ mV}$  within  $3 \text{ sec}$  of illumination. The time of illumination for a single measurement was  $120 \text{ ms}$ . In most experiments we restricted the number of measurements such that the drop of amplitude was negligible.

## Results

We studied Retzius cells in two states: (i) Neurons which consisted of the soma with an appendix of a primary neurite dissociated from the ganglia. (ii) Neurons which consisted of soma, primary neurite and a secondary arborized neurite sprouted on ECM-extract in culture.

### Primary neurites

An example of a dissociated Retzius cell with superimposed diagram of the diode array is shown

in Fig. 1A. The resolution was  $14\ \mu\text{m}$  per diode. An action potential was triggered by an impaled microelectrode. The fluorescence transients (di-4-ANEPPS) were recorded at three sites: (1) At the end of the neurite, (2) near the root of the neurite and (3) at the soma. The changes of fluorescence – normalized by their amplitude – are compared in Fig. 1B for site 1 and 2 and in Fig. 1C for site 2 and 3. No significant noise is visible at site 2. The noise in trace 1 and trace 3 is due to digitization: The amplitudes of the transients (diode currents) were  $-110\ \text{pA}$ ,  $-190\ \text{pA}$  and  $-40\ \text{pA}$  for the sites 1, 2 and 3 on a level of total fluorescence of  $1100\ \text{pA}$ ,  $1900\ \text{pA}$  and  $700\ \text{pA}$ . The digitization (8 bit) was matched to the largest transient at site 2.

The earliest peak of the action potential was observed near the terminal of the primary neurite (site 1). It arrived at the root of the neurite (site 2) with a delay of  $0.6\ \text{ms}$ . From there the action potential jumped across the soma without visible delay. The shape of the pulse was invariant. The amplitudes were similar ( $\sim 10\%$ ) for site 1 and 2, but lower ( $\sim 5.7\%$ ) at site 3.

We studied a series of cells using several positions of a triplet of diodes. From the time course of the action potentials we evaluated the delay of the time-to-peak and the amplitude. The width of  $2.0\text{--}3.0\ \text{ms}$  was almost invariant. The results for two cells sketched in Fig. 2A are plotted in Fig. 2B and 2C versus the distance along the neurite as measured from the location of the earliest time-to-peak.

A constant velocity of pulse propagation,  $105\ \mu\text{m}/\text{ms}$  and  $230\ \mu\text{m}/\text{ms}$  respectively, was observed along the primary neurites. As the pulse reached the root it jumped across the soma at a velocity too fast to be resolved. This behaviour was found in all cells studied. The range of velocities was  $50\text{--}230\ \mu\text{m}/\text{ms}$ .

The correlation of the amplitudes of relative fluorescence with distance was less definite (Fig. 2C). There was a tendency of a drop by about  $50\%$  from the site of the earliest time-to-peak to the most distant part of the soma. Whether the drop of amplitude is a continuous process along neurite and soma – as suggested by the right cell in Fig. 2 – or whether there is a step at the boundary of neurite and soma – as may be suggested by Fig. 1 and the left cell in Fig. 2 – cannot be decided.

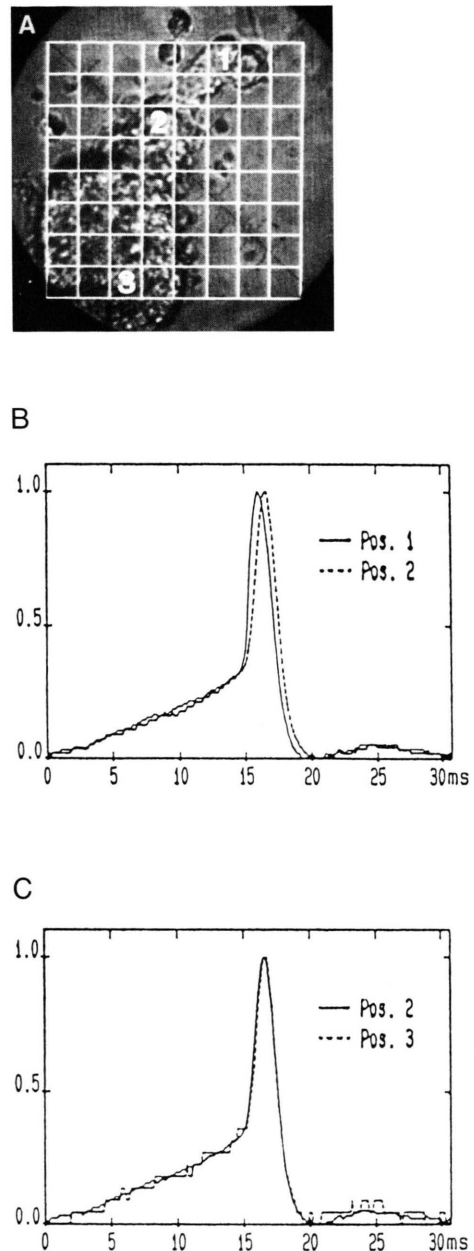


Fig. 1. Voltage transients in Retzius cell. (A) Photograph taken from the monitor superposed by a diagram of 64 diodes which are matched in scale such that one diode detects an area of  $14\ \mu\text{m} \times 14\ \mu\text{m}$ . The cell was stimulated and three diode signals (site 1, 2, 3) were recorded. (B and C) Relative negative change of fluorescence (normalized amplitude) versus time for sites 1, 2 and 3 (single sweep). The actual amplitudes of change of photodiode current are  $-110\ \text{pA}$ ,  $-190\ \text{pA}$  and  $-40\ \text{pA}$  at total signals of  $1100\ \text{pA}$ ,  $1900\ \text{pA}$  and  $700\ \text{pA}$ . *I.e.* the relative changes are  $-10\%$ ,  $-10\%$  and  $-5.7\%$ . The window of digitization is matched to the largest change (site 2).



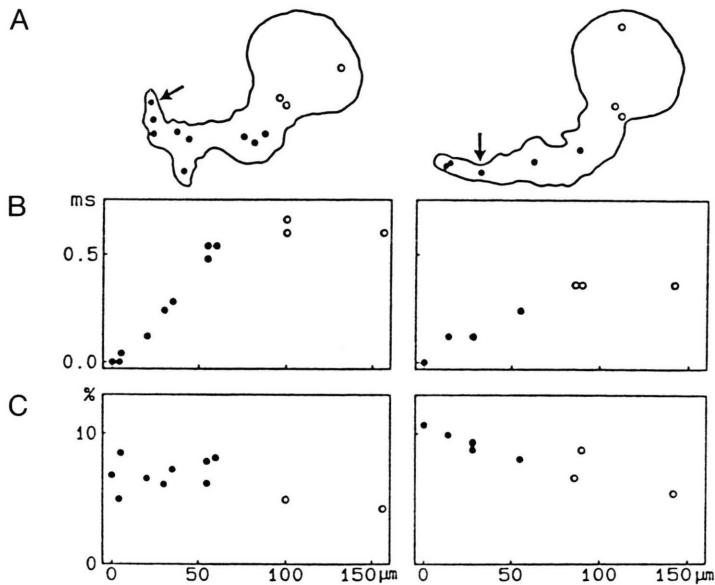


Fig. 2. Signal spread in primary neurite and soma of dissociated Retzius cell. (A) Shape of two cells. The location of measurements is indicated. Four (three) different adjustments of three diodes (spatial resolution 14  $\mu\text{m}$ ) are used in the left (right) cell. The arrows mark the location of the earliest action potential. (B) Delay of time-to-peak versus distance from the arrow along neurites and somata. (C) Maximal negative change of relative fluorescence intensity along neurites and somata. Each measurement is repeated 2–4 times. Delay and amplitude are averaged.

#### Secondary neurites

A typical cultivated Retzius cell with arborized neurite is shown in Fig. 3 A. We adjusted the diode array to various parts of the tree at a resolution per diode of 8  $\mu\text{m}$ . The cell was stimulated and optical

records (RH-421) were taken at four sites: On the soma (site 1), on the primary neurite (2) and at two sites in the arborized secondary neurite (3, 4). The normalized relative changes of fluorescence are plotted in Fig. 3 B. An abrupt change of amplitude

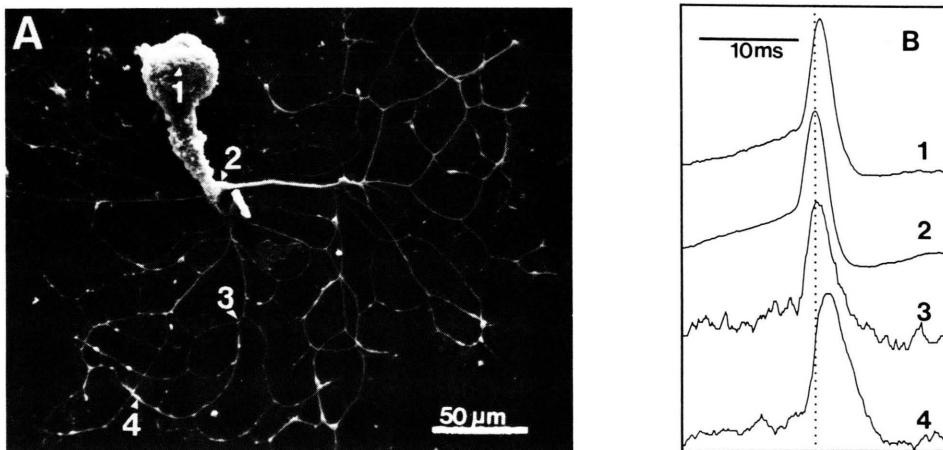


Fig. 3. Signal propagation in cultivated Retzius cell. (A) Scanning electronmicrograph. The dissociated cell is cultivated for 3 days on ECM-extract. Voltage transients as triggered by stimulation of the soma are recorded at the positions 1–4. (B) Negative relative change of fluorescence (arbitrary scale of normalized amplitude) in the soma (1), in the primary neurite (2) and at two locations in the secondary neurite (3, 4). The dotted line marks the earliest time-to-peak (primary neurite). The amplitudes at positions 1, 2, 3 and 4 are –5.0%, –8.3%, –1.3% and –1.8%. Spatial resolution 8  $\mu\text{m}$ .

was observed in all cells at the boundary of primary and secondary neurite. We assign this effect to a change of sensitivity of the probe which may be caused by a different environment or to a higher background. The signals in soma and primary neurite (amplitudes  $-5\%$  and  $-8.3\%$  at site 1 and 2 respectively) did not show significant noise. In the secondary neurite (amplitudes  $-1.3\%$  and  $-1.8\%$  at site 3 and 4 respectively) the signal-to-noise ratio was lower.

The earliest peak of the action potential appeared near the end of the primary neurite (Fig. 3B). From there it spread into the soma and into the neuritic tree. The shape of the voltage transient changed as it propagated along the neuritic tree. The halfwidth was enhanced from 2.9 ms to 4.2 ms. In particular the decaying phase was affected.

The action potential pervaded all parts of neuritic trees. 24 sites of measurement are marked in Fig. 4 as investigated in a single tree with 8 different adjustments of a triple of diodes. The numbers indicate the sequence of measurements. In order to obtain this extended set of data we had to study the cell for a rather long time (8 adjustments, on average 4 measurements per adjustment) such that the photodynamic damage could not be avoided. The action potential dropped from 80 to 40 mV. Nonetheless we may conclude: (i) The action potential – triggered close to the soma – penetrates all parts of an extended arborization. (ii) There exists a general correlation of time-to-peak and distance from the soma as approximated by a velocity of 120  $\mu\text{m}/\text{ms}$ .

A set of voltage transients along a single path was measured in a neuritic tree of simple morphology as shown in Fig. 5. The traces 1–6 along the path were obtained with six adjustments of the diode array. For control purpose trace 7 was taken simultaneously with trace 6. The time-to-peak was delayed up to 2.4 ms: In the terminal of the arborization (trace 6) the depolarization reached its maximum at a time when it had decayed almost completely in the soma (trace 1). Thus the neuron was far from being isopotential. From the distance of 360  $\mu\text{m}$  between position 1 and 6 we evaluated a velocity of peak propagation of 150  $\mu\text{m}/\text{ms}$ . The pulse width was enhanced from 2.9 ms to 4.8 ms. The amplitude of relative fluorescence was rather invariant along the secondary neurite as  $-1.1\%$ ,

$-1.0\%$ ,  $-1.5\%$ ,  $-1.1\%$ ,  $-1.1\%$  at sites 2–6. In the soma it was higher ( $-8.2\%$  at site 1) as usual. In all cells studied we found a similar deformation of the action potential. The maximal delay was 3.5 ms, the maximal factor of broadening was 2.1.

An important phenomenon of inhomogeneous pulse propagation was observed in the neuron

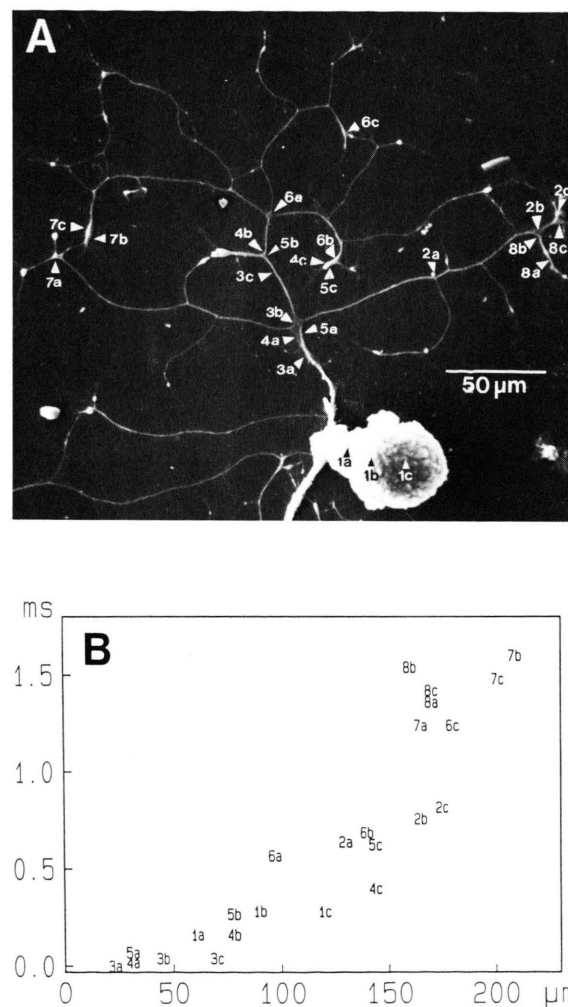


Fig. 4. Time-to-peak of action potential in arborized Retzius cell. (A) Scanning electronmicrograph. The dissociated cell is cultivated for 3 days on ECM-extract. Voltage transients are observed at 24 locations with 8 adjustments of the diode array (spatial resolution 8  $\mu\text{m}$ ). The numbers indicate the sequence of measurements. The letters refer to different diodes. (B) Delay of time-to-peak versus distance as evaluated from the fluorescence transients. Each point is an average of 3–6 time delays as evaluated from single sweep records.

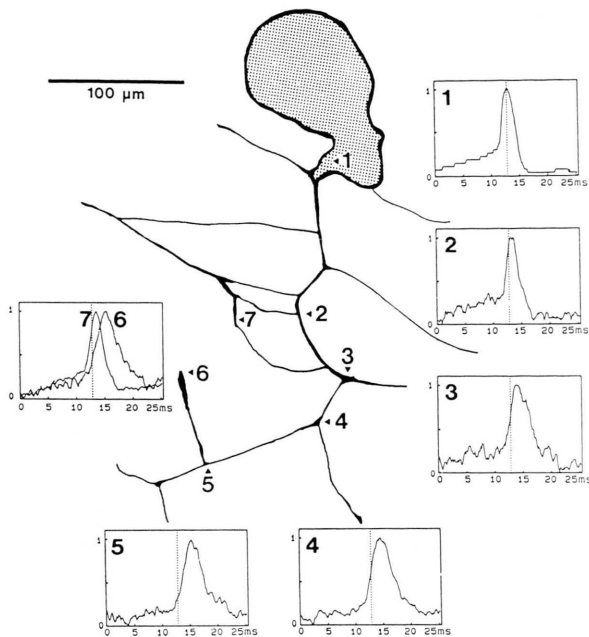


Fig. 5. Signal propagation in arborized Retzius cell. The negative relative changes of fluorescence (normalized) are plotted for seven locations. Site 1 is on the primary neurite, sites 2–6 are along a path in the secondary neurite. The signals of site 1–6 are recorded with sequential stimulations. The signal of site 7 is obtained simultaneously with that of site 6. The dotted line marks the earliest time-to-peak (site 1). Spatial resolution 8  $\mu\text{m}$ .

drawn in Fig. 6. After a series of measurements at various positions of the arborized neurite – the action potential was still 50 mV – the triplet of signals shown in Fig. 6 was recorded simultaneously. These transients were detected reproducibly at a given position of the array. From position 1 to 2 – within 22  $\mu\text{m}$  – the halfwidth broadened from 4.1 ms to 7.0 ms. From position 2 to 3 – within 44  $\mu\text{m}$  – the halfwidth dropped to 5.0 ms.

## Discussion

Stimulation of the soma of cultivated Retzius cells triggers fluorescence transients near the end of primary neurites dissociated from the ganglia. (i) The signals propagate towards the soma at a velocity of 50–230  $\mu\text{m}/\text{ms}$  at invariant halfwidth of 2–3 ms. They spread instantaneously across the soma. The amplitude of relative fluorescence change is several percents, dropping from primary

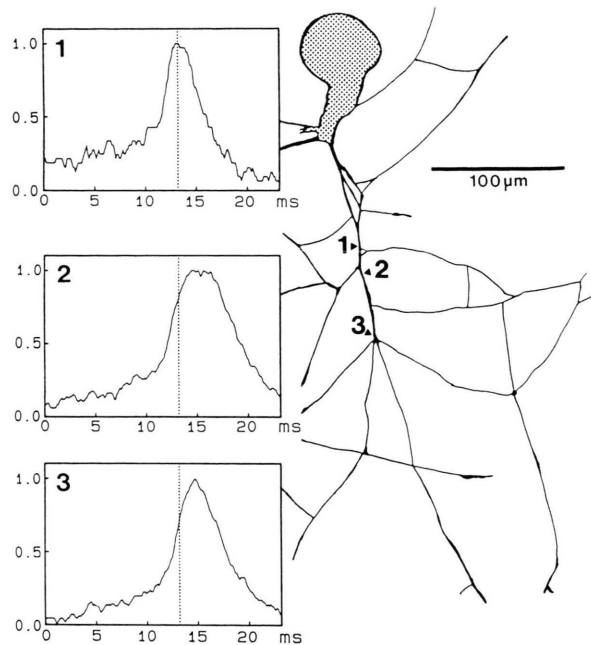


Fig. 6. Inhomogeneous signal propagation in cultivated Retzius cell. The negative relative change of fluorescence (normalized) as detected simultaneously at the sites 1, 2 and 3 is shown. Spatial resolution 8  $\mu\text{m}$ . The dotted line marks the time-to-peak at site 1.

neurite towards the soma. (ii) The transients pervade the finest processes of secondary neurites as grown in culture. The velocity of propagation is 100–150  $\mu\text{m}/\text{ms}$ . Delay times up to 3.5 ms are observed. The halfwidth broadens up to a factor 2.1. The amplitude of relative fluorescence change is around –1% with no systematic change along the arborization.

Are we allowed to interpret the fluorescence transients in terms of intrinsic transients of the membrane potential? We consider two questions: (i) Do the dyes affect the electrical properties of the neuron? (ii) Do the dyes reflect the change of the membrane potential?

With respect to the first issue we may note that considerable changes of amplitude and width of electrical transients are induced by the dyes in case of illumination. However, in all measurements – where we compared times-to-peak and pulse-widths – we restricted the duration of illumination

such that no significant changes of the action potential were recorded by the microelectrode in the soma (with the exception of a few points in Fig. 4 as mentioned). A direct check of damage is not possible in the arborizations. However, we found that the time delays and the changes of width were independent from the sequence of measurements *i.e.* whether we measured first near the primary neurite and later near the periphery of *vice versa*. So we exclude that the features of pulse propagations are caused by photodynamic damage.

With respect to the second issue we may note that an assignment of fluorescence change to voltage change is valid certainly with respect to the time-to-peak (velocity) and with respect to the pulse width as in that case only linearity of the response is to be assumed. This linearity was checked by direct comparison of fluorescence and voltage on the soma. The amplitude, however, has to be considered with more caution. The effective sensitivity of the stain may vary along the neuron due to a changed dye/membrane interaction or to a changed background. An example for such an effect is the sudden drop of fluorescence amplitude at the boundary of primary and secondary neurite.

To rationalize the features of signal propagation we consider two elementary models: (i) "Passive" spread in a homogeneous cable with ohmic conductances (Rall [22]). (ii) "Active" propagation in a homogeneous cable with voltage-gated conductances (Hodgkin and Huxley [23]).

We evaluate a rough model of passive spread: We apply a Gaussian pulse (halfwidth 2.8 ms) of voltage to one end of a homogeneous cable (length 400  $\mu\text{m}$ , sealed ends) and integrate the Kelvin equation,  $\tau \partial_t V = \lambda^2 \partial_{xx} V - V$  for the difference  $V$  between membrane potential and resting potential (Rall [22], Jack *et al.* [24], Clement and Redman [25]) with time constant  $\tau$  and length constant  $\lambda$ . We express  $\tau$  and  $\lambda$  by the specific capacitance  $c$  and conductance  $g$  of the membrane, by the specific resistance  $\varrho$  of the core and by an effective radius  $a$  as  $\tau = c/g$  and  $\lambda^2 = a/2 g \varrho$ . It is not possible to fit the observed velocities in the range of 50–230  $\mu\text{m}/\text{ms}$  with standard values  $c = 1 \mu\text{F}/\text{cm}^2$  and  $\varrho = 50 \Omega \text{ cm}$  of membrane capacitance and plasma resistance using the geometric radius of about 5  $\mu\text{m}$ . In order to describe the delays we have to assume radii of  $a = 0.01$ – $0.1 \mu\text{m}$  at conductances in the range of  $g = 5 \text{ pS}/\mu\text{m}^2$ . Those fits

imply a significant broadening of the pulses. On the basis of these simple computations we exclude a mechanism of passive spread in the primary neurite as (i) significant broadening is not observed and as (ii) the value of the fit parameter  $a$  is by orders of magnitudes smaller than the geometric radius.

A comparison of the simple model with arborized neurites is appropriate for certain geometric conditions (Rall [22]). Even if those conditions are not valid an equivalent cable may be taken as an approximation as simulations of arbitrary trees lead to similar transients (Koch *et al.* [2], Segev *et al.* [26]). A satisfactory fit of the normalized fluorescence transients – of the delayed times-to-peak and of the broadened widths – is obtained with  $a = 0.06 \mu\text{m}$  and  $g = 4 \text{ pS}/\mu\text{m}^2$  as shown in Fig. 7. The fit parameter  $a$  is by an order of magnitude smaller than the geometric radius of secondary neurites of 0.5–1  $\mu\text{m}$ . The difference makes

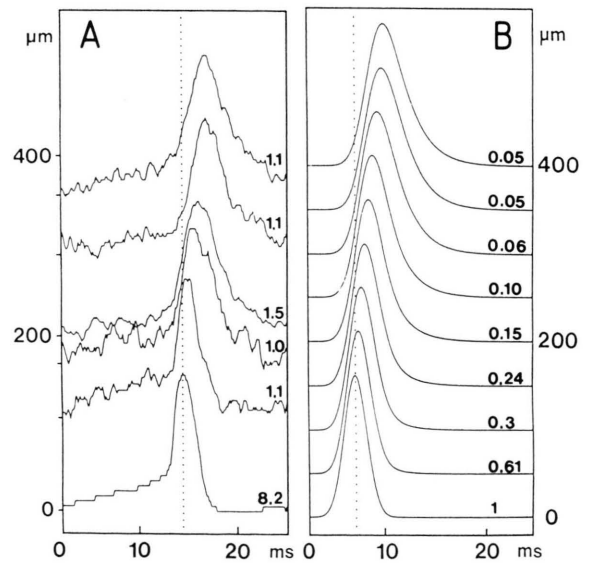


Fig. 7. Experimental and theoretical transients. (A) Normalized transients of negative relative fluorescence as taken from Fig. 5 versus distance from soma. The zero level of the transients is marked on the left distance scale. The actual amplitudes (percent of negative fluorescence change) are indicated. (B) Normalized voltage transients in a linear cable. Propagation of Gaussian pulse (half width 2.8 ms) in cable of length 400  $\mu\text{m}$  with sealed ends (membrane capacitance  $c = 1 \mu\text{F}/\text{cm}^2$ , membrane conductance  $g = 4 \text{ pS}/\mu\text{m}^2$ , electrolyte resistance  $\varrho = 50 \Omega \text{ cm}$ , effective radius  $a = 0.06 \mu\text{m}$ ). The values of the amplitudes (fraction of initial amplitude) are indicated.



passive spread unlikely. As a further argument we take into account here the amplitudes: The amplitude of the theoretical voltage pulse drops from the start (100%) to a level of 5% at a distance of 360  $\mu\text{m}$  (Fig. 7). No such trend is observed in the transients of fluorescence with amplitudes of  $-1.1\%$ ,  $-1.0\%$ ,  $-1.5\%$ ,  $-1.1\%$ ,  $-1.1\%$ . (The value of  $-8.2\%$  of trace 1 cannot be taken as a start value as it is measured on the soma, where the sensitivity is generally higher.) To identify the experiments with a mechanism of passive spread we would have to postulate a systematic increase of dye sensitivity by a factor of 20 towards the periphery to compensate for the decaying voltage. We do not see any basis for such an effect. The discrepancy of the amplitudes of the voltage transient and of the fluorescent transient – which is observed in all cells studied – is so drastic that we take it as a conclusive argument against a mechanism of passive spread. We have to point out, however, that propagation of a pulse at invariant amplitude and enhanced width is not compatible with a Hodgkin-Huxley-type mechanism in an homogeneous neurite either. An inhomogeneous cable, however, may give rise to deformations of a Hodgkin-Huxley pulse (Manor and Segev [27]). The result of Fig. 6 shows that inhomogeneities exist actually in the secondary neurites.

The cultivated cells are similar to cells *in vivo* in certain aspects: (i) The action potential originates far from the soma and spreads towards soma and periphery (West and Lent [28], Lent [29]). (ii) The velocity of propagation of the action potential is

similar (Lent [29]). These similarities suggest that cultivated neurons are intact not only with respect to their local electrical properties (Fuchs *et al.* [30]) but also with respect to signal propagation.

It may appear that our observations are not compatible with the results of Ross *et al.* [10, 11] who studied the correlation of voltage transients and calcium influx in cultivated neurons of the leech. These authors did not report delays or deformations of pulses. However, their set-up was not designed to detect such effects as they used small arborizations and large areas of detection ( $40 \times 40 \mu\text{m}^2$ ).

Our set-up of optical recording in cell culture allows the observation of details of voltage transients which were not resolved hitherto. A detailed rationalization of signal propagation in neuritic trees must be based on a study of arborizations of defined geometry (Fromherz and Schaden [31]) using stimulating pulses of various shape and sign (Fromherz and Vetter [32]).

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