Characterization of Impulse Propagation at the Microscopic Level across Geometrically Defined Expansions of Excitable Tissue: Multiple Site Optical Recording of Transmembrane Voltage (MSORTV) in Patterned Growth Heart Cell Cultures

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ABSTRACT Impulse propagation across sudden expansions of excitable tissue has been shown to exhibit various forms of conduction disturbance on a macroscopic scale, ranging from small delays to unidirectional or complete conduction block. With the present study, we attempted to characterize systematically the dependence of impulse propagation on the geometry of the underlying excitable tissue on a microscopic scale by investigating the spatio-temporal pattern of transmembrane voltage changes associated with impulse propagation from a narrow cell strand to a large cell area using multiple site optical recording of transmembrane voltage (MSORTV) in conjunction with patterned growth of neonatal rat heart cells in culture. While action potential propagation was smooth in the case of funneled expansions, delays of variable size occurred during propagation into rectangular or incised expansions. Close to the abrupt expansion, which functionally represented an increased electrical load to the narrow cell strand, the delays were accompanied by marked distortions of the action potential upstroke, exhibiting, in extreme cases, an initial depolarization to 50% followed by a delayed secondary depolarization to 100% of the full-signal amplitude. These distortions, which were based on bidirectional electrotonic interactions across the transition, were maximal immediately downstream from the expansion. The maximal slowing of impulse conduction across abrupt expansions was, in agreement with recently published results obtained from two-dimensional computer simulations, always situated in the expanded region. At high stimulation rates, the delays sometimes turned into intermittent unidirectional blocks, as revealed by reverse stimulation. These blocks were always
characterized by a marked abbreviation of the action potentials upstream from the region causing the block which might, in an appropriate network, facilitate reentry because of the associated shortening of the refractory period. Because the patterns were composed of cells having identical membrane properties, the results show that the local action potential shape can be modulated profoundly by the two-dimensional architecture of the underlying cell ensemble alone.

**INTRODUCTION**

Normal and abnormal excitation sequences of the heart are determined by the characteristics of the spread of the action potential, which depend on the spatial distribution of active and passive membrane properties. In general, impulse propagation is successful as long as the inward current generated at the wavefront of a propagating action potential (current source) is large enough to discharge the capacitance downstream (current sink) and to depolarize the membrane beyond threshold. An obvious region in the heart where this balance might be disturbed is the Purkinje fiber-ventricle junction (PVJ). In this example, the abrupt increase in the total cell membrane area to be activated behaves as a sudden elevation of the electrical load that has the potential for impairing successful conduction from the Purkinje fibers to the ventricles (current-to-load mismatch). Similar examples of geometrical current-to-load mismatch situations are to be found in the spread of the impulse from the small SA node to the surrounding atrial tissue (Joyner and van Capelle, 1986), or, in pathophysiological settings, impulse propagation across thin tissue bridges to larger cell islands forming the thin surviving epicardial tissue layer after an ischemic event ("mottled" myocardium; UrseU, Gardner, Albala, Fenoglio, and Wit, 1985) or impulse propagation through an accessory atrio-ventricular pathway.

The mechanisms by which an action potential successfully propagates through a region of sudden expansion of the excitable tissue has attracted the attention of many investigators during the past 25 years. At a macroscopic level, the situation of an abrupt expansion of a core conductor has mostly been investigated using the PVJ model (Mendez, Mueller, Merideth, and Moe, 1969; Mendez, Mueller, and Urbiguaga, 1970; Sasyniuk and Mendez, 1971; Overholt, Joyner, Veenstra, Rawling, and Wiedmann, 1984), but there have also been attempts to investigate the situation of a current-to-load mismatch in a tissue with defined or controlled geometry by shaping thin atrial walls into preparations consisting of two tissue flaps connected by means of a thin cellular bridge (De La Fuente, Sasyniuk, and Moe, 1971) or by asymmetrically crushing/cooling Purkinje fibers (Waxman, Downar, and Wald, 1980). These studies revealed the existence of a variety of conduction disturbances occurring at the sites of expansion including conduction delays, asymmetrical conduction, and unidirectional and complete conduction block. Because of the lack of a suitable system for microscopic mapping, uncertainties have persisted concerning the exact mode of propagation across these junctions, and it was shown, e.g., by Mendez and colleagues (1970), that the long delays at the PVJ described earlier (Matsuda, Kamiyama, and Hoshi, 1967) were probably not attributable to a delay generated at the junction under investigation, but were the result of a delayed activation of the postjunctional {\ldots}
site via alternate conduction pathways. This interpretation was later supported by the finding that the Purkinje fibers formed a complex superficial layer at the endocardial surface of papillary muscle that supported activation of the myocardium only at specific sites (Bukauskas, Sakson, and Kukushkin, 1976; Overholt et al., 1984).

A more detailed understanding of the characteristics of impulse propagation across these abrupt expansion of excitable tissue would obviously have benefited from a higher resolution mapping leading ultimately to the characterization of the spread of excitation on the microscopic scale. Because of the lack of appropriate experimental systems, efforts to understand the microscopic aspects of conduction in the PVJ region have, until now, been restricted to computer simulations of impulse propagation across abrupt expansions using one- or two-dimensional models (Joyner, 1981; Joyner, Veenstra, Rawling, and Chorro, 1984; Leon and Roberge, 1991). While these models have successfully mimicked propagation delays and unidirectional block, they have produced variable estimates of the critical size of the expansion causing unidirectional block, and they have arrived at different conclusions regarding the spatial evolution of conduction velocity and upstroke velocity with respect to the site of abrupt expansion.

With the present work, we have attempted to characterize experimentally the spatio-temporal pattern of transmembrane voltage changes associated with impulse propagation, on a cellular scale, in monolayer cultures of neonatal rat heart cells having defined geometries. The studies only became feasible by combining two recent methodological developments, viz., the patterning of the growth of ventricular myocytes in culture (Rohr, Schöll, and Kléber, 1991) and multiple site optical recording of transmembrane voltage (Salzberg, Grinvald, Cohen, Davila, and Ross, 1977; Grinvald, Cohen, Lesher, and Boyle, 1981; Salzberg, 1983; Rohr and Salzberg, 1994). The results show that abruptly expanding geometries induce localized delays of activation, asymmetric conduction, and unidirectional block, and they demonstrate the feasibility of designing cellular ensembles in culture that reproduce disturbances of action potential propagation similar to those observed in intact tissues.

Part of this work has previously been published in abstract form (Rohr and Salzberg, 1992a).

METHODS

Patterned growth was obtained following a protocol described in detail in a previous publication (Rohr et al., 1991). The original masks of the main patterns used in the present experiments are illustrated in Fig. 1. Each coverslip bore several different cellular patterns that were all connected by thin cell strands to a large monolayer ring of cells at the periphery of the coverslips. Before the experiments, each of the patterns was uncoupled from the peripheral cell ring using a scalpel or a fine needle. This procedure not only resulted in quiescent cellular patterns suitable for undisturbed extracellular stimulation, but it also assured that impulses elicited with an extracellular electrode reached the site under investigation in a controlled manner and not via tortuous pathways involving other regions of the culture. The preparations chosen were 5–10 mm long, and the recording site was always situated ≥2 mm from both the extracellular stimulation electrode and the end of the strand to allow only propagated action potentials to invade the region under investigation and to exclude effects from the sealed end. For the case of expanding cell structures, the width of the expansion was chosen to be
FIGURE 1. Examples of masks for the creation of patterned growth. The black regions correspond to the bare glass surfaces supporting adhesion and growth of myocytes. The calibration bar refers to all patterns. (A) Pattern for the production of linear strands. (B) Funnel patterns with variable expansion ratios and angles between the funnel sides. (C) Rectangular expansion patterns with variable expansion ratios. (D) The two patterns on top represent incisure patterns with varying angles. The pattern on the bottom is another variation of the funnel pattern.

≥ 1.5 mm, thereby amounting to four times the space constant (360 μm) as determined earlier in monolayer cultures of rat heart cells (Jongsma and van Rijn, 1972).

Selection of a Voltage-sensitive Dye

Of all the fluorescent dyes examined (di-4-ANEPPS and di-8-ANEPPS, kindly supplied by Dr. L. Loew [University of Connecticut Health Center, Farmington, CT]; RH 160, RH 237, RH 246, RH 421, RH 423, RH 1385, RH 1405, RH 1413, and RH 1419, kindly supplied by A. Grinvald and R. Hildesheim [The Weizmann Institute of Science, Rehovoth, Israel]), di-8-
ANEPPS proved to be the most suitable for our application. This dye was characterized by a high sensitivity in terms of fractional fluorescence change per unit change in membrane potential and by a very low tendency to harm the preparation as the result of phototoxic side effects during the required illumination intervals.

**Experimental Protocol**

The coverslips, with cell cultures adhering, were transferred to a temperature-controlled superfusion chamber (Rohr, 1986) and mounted on the microscope stage. The preparations were superfused by means of a Holter peristaltic pump (Critekon Corp., Tampa, FL) at 5–10 ml/min with Hanks' balanced salt solution (GIBCO BRL, Gaithersburg, MD) containing (mM) NaCl 137, KCl 5.4, CaCl2 1.3, MgSO4 0.8, NaHCO3 4.2, KH2PO4 0.5, Na2HPO4 0.5, and Na–Hepes 10, which was titrated to pH 7.35 with HCl. All experiments were carried out at 35°C. The cultures were stimulated with extracellular glass electrodes (tip diameter 3–5 µm; Corning Glass), which were filled with a 3 M KCl-agar solution, and they were attached to micromanipulators (Narishiga Scientific Laboratory, Tokyo, Japan, and Zeiss MR-3 Mot; Carl Zeiss Inc., Thornwood, NY). Impulses were generated by a stimulator (Grass Instrument Co., Quincy, MA) delivering square pulses of 1 ms duration at twice threshold intensity. After placement of the stimulation electrode(s), superfusion was stopped, and the preparations were incubated for 3–5 min with fresh staining solution containing 40–80 µg/ml of di-8-ANEPPS. The staining solution was prepared by diluting a dye stock solution (8 mg/ml of di-8-ANEPPS in 75% wt/wt DMSO and 25% wt/wt Pluronic F127 [BASF, Wyandotte, MI]) in the superfusion solution. After staining, the preparations were washed, superfusion was resumed, and experiments were begun after an equilibration period of 5–10 min.

**Multiple Site Optical Recording of Transmembrane Voltage (MSORTV)**

Optical recordings of electrical activity were carried out with an inverted microscope (IM35; Zeiss, Oberkochen, Germany) equipped for epifluorescence. The light from a xenon short-arc lamp (150 W; Osram AG, Munich, Germany), powered by a low-ripple power supply (Optiquip Corp., Highland Mills, NY), was band-limited with an interference filter (530 ± 25 nm; Omega Optics, Brattleboro, VT). The beam was deflected towards the objective by means of a 560-nm dichroic mirror (Omega Optics). Longer wavelength fluorescence emission from the preparation passed through the dichroic mirror and a barrier filter (OG 570; Schott Glass Co., Duryea, PA) that blocked the scattered excitation light. The image of the preparation was projected onto a 12 × 12-array of silicon photodiodes (MD-144-0; Centronics, Newbury Park, CA). The photocurrents generated by the central 124 detectors were converted to voltages by first-stage amplifiers and were further amplified by an AC-coupled second stage (time constant of 3 s).

The signals from an arbitrarily selectable subset of 16 out of the 124 diodes were routed to a fast PC-based data acquisition system (Northgate 486i; Northgate Computer Systems Inc., Minneapolis, MN) equipped with a Flash-12 model 1 data acquisition card from Strawberry Tree Inc. (Sunnyvale, CA). Data acquisition rates were chosen according to the objectives of the experiments and the magnifications used, and the rates varied between 5 and 62.5 kHz per individual channel.

The analysis of the acquired data was performed on a second PC (Macintosh IIci; Apple Inc., Cupertino, CA) using routines developed in a spreadsheet program (Excel; Microsoft Corp., Redmond, WA). (a) Normalization: optically recorded action potentials were compensated for their offset and normalized for comparison purposes. This normalization probably does not distort the result significantly because it may be assumed that the tissue along the recording region (720 µm total length or less, depending on the optical magnification used) was roughly isopotential, based on the space constant of 560 µm measured previously in an identical culture.
(b) Filtering: to remove occasional spikes (most probably caused by glitches occurring in the analogue-to-digital conversion process occurring as single events in ~1% of the recordings), data were passed through a median filter encompassing five consecutive values. This filter substituted outlying values by their closest neighbors without affecting the rest of the data, and, in particular, had negligible impact on the rapidly rising upstroke of the optically recorded action potential. (c) Determination of dV/dt: the maximal slope of the fast positive deflection of the optical signal was determined from the slope between adjacent measurement points. Because the presence of noise sometimes introduced false high slope values characterized as sudden jumps in the slope vs time graph, we also filtered the data with a running average over three values. This procedure tended to lower slightly the slope of data acquired at low conversion rates without, however, affecting calculated activation delay times between individual detectors. To express slope values in familiar units, we assumed, based on previous measurements (Rohr et al., 1991), the action potential amplitude to be 100 mV, and we expressed the slope in V/s accordingly.

The precise location of the individual photodetector receptive fields within a preparation was determined by recording the superimposed images of the photodetector array and the preparation reflected by the array using a CCD camera (Sony XC-77; Sony Corp., Paramus, NJ), which was connected to a computer-based image acquisition system (Apple Macintosh IIci equipped with a framegrabber, Quickcapture; Data Translation Inc., Marlboro, MA and image analysis software Image; National Institutes of Health, Bethesda, MD).

Assessing Activation at High Spatial and Temporal Resolution

Given an average conduction velocity of 0.3 m/s, an activation front requires 50 μs to traverse the distance between two adjacent photodetectors at a magnification of 100 (15 μm), i.e., the upstrokes recorded by two adjacent photodetectors occur 50 μs apart. To measure such small temporal differences, the following two conditions have to be met: (a) The exact sampling time of each value acquired by the recording system has to be known, while the overall sampling rate should be high enough to adequately resolve the upstroke of an action potential. We met this condition by using a data acquisition system that was capable of acquiring 1 million samples (12-bit) at variable conversion rates ≤1 MHz. During the simultaneous data acquisition from 16 channels, the maximal conversion rate per channel was 62.5 kHz. Because the switching rate among the channels was fixed at 1 μs, independent of the overall sampling rate, the delay between the first and the last scanned channel was always exactly 16 μs, even at slower than maximal conversion rates (burst sampling). (b) The bandwidths of all detector-amplifier combinations have to be identical and, consistent with signal-to-noise considerations, should be broad enough to preserve the original shape of the action potential. We satisfied these criteria by fine tuning the RC time constant of each amplifier to 300 ±15 μs (−3-dB point at 530 Hz). To increase the homogeneity of response times even further, the temporal deviations among the detectors were assessed at the beginning and at the end of each experimental series by means of a square light pulse from a light-emitting diode (LED). The mean temporal differences between 40 and 60% increase in light current among all amplifiers during this LED pulse were determined in increments of 2%, and the average was used to correct the timing of the original signals. This procedure, which also corrected for the scanning delays originating with the data acquisition system (see above), reduced the variability in response times among the different photodetectors to <±5 μs, i.e., to a range that permitted the determination of activation delays at the maximum optical magnification used. A more detailed account of our optical methods, together with probe evaluation, is published elsewhere (Rohr and Salzberg, 1994).
The description of the characteristics of impulse propagation in this work uses the following nomenclature:

**Activation time.** This term defines the time at which the upstroke of an action potential, as recorded by an individual detector, reaches a certain percentage of the full signal amplitude.

**Activation delay.** This term defines the difference between activation times obtained from two adjacent detectors. Specifically, this value was determined as follows: using linear interpolation between consecutively acquired values, activation times were determined at 40–60% of the full signal amplitude in evenly spaced intervals of 2%, and the activation delay was equated to the average of the differences in activation times between the two sites determined at these 11 amplitude levels. This procedure tended to decrease the influence of noise in cases of signals with smaller signal-to-noise ratios and yielded exact and reproducible values for activation delays between detectors exhibiting monotonically rising action potentials. However, in the cases of action potentials exhibiting large distortions of the upstroke in the 40–60% depolarization interval (see Results), it must be understood that an absolutely precise definition of individual local activation delays, and, therefore, of local conduction velocity, was not feasible. Nevertheless, it was possible, even in these cases, to follow, at least qualitatively, the evolution of conduction disturbances.

**Delayed propagation.** This term refers to the occurrence of disproportionately large activation delays (see Results).

**Total delay.** In most instances of locally delayed propagation, the region giving rise to this delay extended over a distance corresponding to several detectors. In these cases, the total delay was determined by graphing the cumulative activation delays vs distance. In these plots, an initial linear portion (uniform activation delays upstream from the region producing delayed propagation) changed over in a sigmoidal fashion (region of delay) into a second linear portion (uniform activation delays downstream from the region producing delayed propagation). The total delay was then determined as the vertical difference between two lines fitted to the initial linear portion and the second linear portion, respectively.

**Downstream.** This term refers to the direction of propagation of the action potential.

**Upstream.** This term refers to the direction opposite to that of the propagation of the action potential.

**RESULTS**

**Impulse Propagation in Two-dimensional Linear Cell Strands**

As a control, we assessed the characteristics of microscopic impulse propagation in 30–60-μm wide linear cell strands. Mean conduction velocity in these strands was 0.38 ± 0.03 m/s with maximal upstroke velocities of 138 ± 19 V/s (mean ± SD; n = 6). A typical example of such a recording is depicted in Fig. 2. The overall conduction velocity, as measured along eight contiguous detectors, was 0.39 m/s while individual activation delays between adjacent photodiodes ranged from 18 to 54 μs (38.5 ± 14.4 μs, mean ± SD; n = 7), which represent local conduction velocities between 0.28 and 0.83 m/s. Variation of similar magnitude was found in all six preparations and ranged from 17 to 68 μs (40 ± 17 μs, mean ± SD; n = 44), corresponding to conduction velocities between 0.20 and 0.90 m/s. Impulse propagation on a microscopic scale in cell strands three to four cells across, therefore, was never entirely uniform, but was characterized by small local advances or delays of activation. To distinguish these baseline fluctuations from larger delays causing
delayed propagation, we considered abnormally increased activation delays to have occurred when detector-to-detector activation delays exceeded twice the mean of the remaining activation delays in a given experiment.

In most cases, wherever delayed propagation was encountered in a linear cell strand, its occurrence could be related to inhomogeneities in the microanatomy of the strand. In these instances, a local decrease in cell density caused a narrowing of the conducting pathway, leading to a local slowing of conduction. An example of this type of nonuniform conduction is illustrated in Fig. 3. In contrast to the previous example, upstrokes were no longer evenly spaced, but were grouped around a large activation delay of 510 μs occurring between detectors 6 and 7. This delay corresponds to an apparent local slowing of conduction to 0.03 m/s, and it coincides with a partial discontinuity (local narrowing) in the cell strand, as illustrated in Fig. 3 A. The shapes of the upstrokes close to the large activation delay exhibited major distortions: during the period of delayed activation of detector 7, detector 6 showed an initially decreased action potential amplitude, which coincided temporally with a prepotential at the region downstream from activation. This, together with the finding that the action potential amplitude recorded by detector 6 showed a secondary increase upon full activation of the region downstream (release of the polarizing clamp on the region upstream), indicates that the distortions of the action potential upstroke were caused by bidirectional electrotonic interaction between the segments separated by the narrowing of the strand.

Impulse Propagation across an Abrupt Two-dimensional Expansion of Excitable Tissue

To assess the impact of a sudden increase in the current load on the propagating action potential, we patterned the growth of myocytes in such a way that a thin linear
cell strand merged abruptly with a large rectangular cell monolayer. We expected this geometry to exhibit characteristics of microscopic impulse propagation similar to those modeled previously for current-to-load mismatch situations (Joyner, 1981; Joyner et al., 1984; Leon and Roberge, 1991), i.e., increased activation delays at the site of the expansion, asymmetric propagation, and unidirectional block.

An example of impulse conduction across such an abrupt expansion is illustrated in Fig. 4. While Fig. 4 A shows the spatial arrangement of the detectors with respect to the shape of the preparation, Fig. 4 B shows the changes in the shape of the upstroke portion of the action potential as the activation wavefront passed through the sudden expansion. Beginning ~150 µm upstream from the expansion, the amplitude of the propagating signal became increasingly depressed, changing over gradually into marked prepotentials as recorded by the last two detectors of the row (detectors 11 and 12). During this gradual transformation, action potential upstrokes in close proximity to the abrupt expansion displayed two distinct phases: an initial, gradually diminishing, and progressively slower rising phase was, after a plateau lasting ~1 ms, followed by a second rise that was increasingly faster and of greater amplitude as the wavefront progressed into the large cell area. In this example, successfully propagating action potentials alternated with conduction blocks. Fig. 4 C shows the pattern of electrical activity during a failure of impulse propagation recorded immediately (200 ms) before the signals shown in Fig. 4 B. Each signal was scaled with the same factor used for normalization of the signals in Fig. 4 B, permitting the direct comparison of the amplitudes of both the successful and the blocked response. The rising phase of the set of signals obtained during block matches very closely the shape of the first phase of the propagated action potential.
upstrokes as shown in Fig. 4 B. This similarity is further illustrated by the spatial evolution of the amplitudes of the successful and the blocked propagation as shown in Fig. 4 D. The amplitudes of both the initial phase of a successfully propagating impulse, as well as the amplitude of the blocked response, decreased in a sigmoidal fashion, falling off to ~75% proximal to the abrupt expansion and declining further to 20% of a full-response 360 μm into the expansion. This similarity demonstrates

![Figure 4](image)

that the spatio-temporal evolution of the first phase of the upstroke of successfully propagating impulses is entirely determined by the electrotonic interaction between the activated proximal cell strand and the as yet still well-polarized large distal cell area (i.e., the downstream, preactivated region imposes a polarizing clamp on the transmembrane potential of the region upstream). Conversely, the second phase,
which was present only during successful propagation, could be explained by the delayed release of this "clamp" during distal activation, which caused a secondary depolarization, supported by the electrotonic interaction in the upstream direction. Fig. 4 E illustrates the spatial distribution of the activation delays during successful propagation. Beginning at ~200 μs, activation delays start to increase 180 μm proximal to the expansion, and reach a maximum of 2,800 μs 120 μm distal to the expansion. Thereafter, they decrease, returning to preexpansion values 180 μm further downstream.

Additional experiments carried out with 11 preparations of comparable geometries (diameter of thin strands: 40–80 μm; diameter of expanded region: 1,500–3,500 μm) revealed propagation characteristics similar to those depicted in Fig. 4 in the majority (eight) of cases. In three cases, however, a distinctly different pattern of propagation could be observed, and an example of this type is illustrated in Fig. 5. While the total delay occurring during successful propagation in the region of the abrupt expansion (black dot) was comparable to that shown in the previous figure, the distance over which this development was much shorter, and only a single recording site, situated just upstream from the expansion, exhibited an upstroke with two phases. Downstream from this site, action potential upstrokes were preceded by a small prepotential of decreasing amplitude as the impulse propagated further into the large cell area. During 44 consecutive stimulations, the preparation initially displayed a fixed 2:1 block changing over to complete block after the 16th stimulation. The spatio-temporal evolution of the signal obtained during conduction block (Fig. 5 C) shows, in contrast to the characteristics of the blocked response in Fig. 4, not a gradual, but, rather, an abrupt decline in the region of the expansion (black dot). This is further illustrated by the spatial evolution of the signal amplitudes as shown in Fig. 5 D: falling off to ~75% of a full response at the site of the expansion, the amplitude drops to 25% within two detector widths (120 μm), disappearing altogether after a further 240 μm. The spatial distribution of activation delays during successful propagation also differed markedly from the profile shown in Fig. 4: the spatial extent of disproportionately large activation delays was limited to 120 μm, with the largest delay occurring at the site of the expansion. These delays were preceded by the shortest of all activation delays in the upstream direction, and they were also followed by the shortest value in the downstream direction. All the characteristics of conduction observed in these minority cases, i.e., (a) delayed propagation occurring within a very short distance (120 μm, equivalent to one to two cell lengths); (b) signal amplitudes declining abruptly in the case of the blocked response; (c) decrease in activation delay and (detailed values not shown) in maximal upstroke velocity in front of the region producing the delay, suggest, by analogy with the example shown in Fig. 3, the presence of a localized increase in longitudinal resistance in the transition region, which determined, in concert with the abrupt increase in electrical load, the characteristics of the propagation delay. Because we could not identify any clear morphological correlate for this suggested increase in longitudinal resistance (as we could in the case shown in Fig. 3), we are compelled to speculate that it was the result of partial electrical uncoupling at the site of the tissue expansion.
Action Potential Shape during Successful Propagation and during Block

Even though it was generally difficult to judge the overall shape of an optically recorded action potential because of the concomitant contraction, a few recordings were nearly completely free of this artifact, and they permitted the comparison of action potential durations during successful propagation and during block. The recordings shown in Fig. 6 are samples from a train of action potentials elicited at an interstimulus interval of 100 ms in a 80-μm wide cell strand merging with a 3,500-μm wide strand. With the exception of the recording obtained from site 8 (bright debris...
sticking to the region imaged and moving with each contraction), the action potential shapes were virtually devoid of motion artifacts. During the first excitation wave \((A)\), the recording sites 2–4, 120–300 \(\mu\text{m}\) upstream from the abrupt expansion, exhibit an action potential shape reminiscent of the so-called "spike-and-dome" configuration described for action potentials recorded at the transition between Purkinje fibers and the myocardium (Matsuda et al., 1967; Sasyniuk and Mendez, 1971). Along the remaining recording sites upstream to the expansion (4–6), the amplitude of the spike gradually diminished before converting, in the large cell area, into a small but

![Figure 6](image)

**Figure 6.** Propagation of a series of action potentials across an abrupt expansion of excitable tissue. The panel on the left schematically illustrates the tissue geometry (not drawn to scale). Numbers above and below indicate the actual widths of the thin and the wide regions, respectively. The preparation was stimulated in the thin region at 10 Hz. The numbers in the scheme denote the position of individual detectors, which monitored tissue patches measuring 60 × 60 \(\mu\text{m}\). The cascade of traces to the right shows the optical signals recorded by each of the detectors (corresponding horizontal position) during a train of four consecutive action potentials \((A–D)\). For clarity of presentation, and to emphasize the spatial dependence of the change in shape of the optical signals, the signals are shifted by a constant value to the right.

still clearly visible hump within the fast upstroke. These signals, in expanded form, shared identical characteristics with the signals obtained during the successful propagation illustrated in Fig. 4 B. The second propagated action potential \((B)\) displayed identical shapes with a slightly decreased spike width, indicative of a smaller delay encountered by the wavefront at the expansion. The third impulse \((C)\) was blocked at the transition to the large cell area. Starting in the thin cell strand, the shape of the blocked signal changed from a triangular form with an increasing rate of initial repolarization (detectors 1–7; action potential duration at 90% repolariza-
tion = 70% of propagated response) to a subthreshold electrotonic response (detectors 9–12). Note that the amplitude of the blocked response is, in accordance with the results shown in Fig. 4, identical to the amplitude of the initial spike occurring during successful propagation (identical scaling factors). This demonstrates once again that the spatio-temporal evolution of the shape of the initial spike is determined entirely by electrotonic interaction with the passive load provided by the distal tissue expansion, which, in its resting state, exerts a "polarizing clamp" on the tissue upstream. Furthermore, this interaction induces a marked abbreviation of the action potentials well beyond the site of expansion in the upstream direction, which could be explained by an increased membrane resistance during repolarization (Sasyniuk and Mendez, 1971) and/or by complex electrotonic interactions during the repolarization process, which tend to have a larger spatial extent than those expected from passive electrotonic interactions alone (Joyner, 1986). On the other hand, during

**Figure 7.** Rectification of conduction across an abrupt expansion of excitable tissue.  
(A) Positioning of individual detector elements with respect to a schematic drawing of the preparation. Each detector recorded electrical activity from an area measuring 60 × 60 μm in the object plane. The gray stippled area indicates the general shape of the preparation (not drawn to scale; actual widths are given above the drawing). (B) The schematic drawing on top indicates that stimulation of the preparation was from the left on the thin cell strand. Below, normalized action potential upstrokes for a successfully propagating impulse as recorded by consecutive detectors along the activation path (1–12) are displayed superimposed (ordinate to the right). (C) Spatial pattern of activation delays determined from the signals in B (ordinate to the right; abscissa oriented according to the sequence of curves in B). (D) The schematic drawing on top indicates that stimulation of the preparation was from the right, in the large cell area. Below, normalized action potential upstrokes as recorded by consecutive detectors (12–1) during successful propagation along the preparation are displayed superimposed (ordinate to the left). (E) Spatial pattern of activation delays determined from the signals in D (ordinate to the left; abscissa oriented according to the sequence of curves in D).
successful propagation, comparison of action potential duration upstream from the expansion shows that the signal widths were prolonged by an amount equal to the total delay encountered at the transition because of electrotonic interactions with the activated distal tissue expansion, which acts, in this instance, as a depolarizing clamp on the tissue upstream.

**Rectification of Conduction and Unidirectional Block**

It may be expected that conduction in these geometrically asymmetric preparations will also be asymmetric, depending on the direction of stimulation, as a result of the reversal of the current-to-load (source-to-sink) situation (Joyner, 1981). This assumption was tested in a series of experiments where the preparations were stimulated by either of two electrodes placed at opposite locations with respect to the abrupt expansion. The result from one of these experiments is shown in Fig. 7. The cycle length between double stimulations was 1,200 ms, while the interstimulus interval between left-sided and right-sided stimulation was 150 ms. Fig. 7B shows the superimposed action potential upstrokes recorded during propagation from the narrow to the wide cell strand, while Fig. 7C illustrates the corresponding activation delays calculated from contiguous detectors. Propagation slows down as the action potential reaches the site of expansion, exhibiting a maximal activation delay close to 800 μs between detectors 6 and 7. Thereafter, conduction velocity transiently increases again before it encounters a second disproportionately large activation delay of 600 μs between detectors 10 and 11. While the shapes of the upstrokes in the vicinity of the expansion are clearly similar to those described in Fig. 4 (graded...
development of the delay), the second delay is confined to a single increased activation delay between detectors 10 and 11, with 10 exhibiting a reduced initial spike amplitude coincident with a prepotential in 11. The characteristics of the second delay are, therefore, similar to those depicted in Fig. 3, and they are suggestive of a locally increased longitudinal resistance. Upon stimulation from the right (Fig. 7 D), the delayed activation in the region of geometrical asymmetry completely disappears, while the delay between detectors 10 and 11 remains fixed at ~600 μs (Fig. 7 E). In this instance, consistent with the opposite direction of stimulation, detector 11 shows a reduced initial spike amplitude, while detector 10 exhibits just a hint of a prepotential. These results illustrate in a single experiment, that, on one hand, conduction across an abrupt expansion of excitable tissue is

![Figure 9. Impulse propagation across a gradual expansion of excitable tissue.](image)

rectified, while, on the other, delayed activation occurring in a symmetrical region of the cellular pattern, which is most probably the result of a localized increase in axial resistance, is not influenced by the direction of stimulation.

While we never found a preparation exhibiting a fixed unidirectional block, this phenomenon could be elicited in a few experiments by critically raising the stimulation rate. An example of this type of conduction disturbance is depicted in Fig. 8. The preparation was stimulated alternately from the left and from the right with an interstimulus interval of 150 ms. While the excitation elicited in the narrow strand failed to activate the expanded region (sequence of action potentials on the left), propagation in the reverse direction was clearly successful (sequence of action potentials on the right), exemplifying a unidirectional conduction block.
The Effect of Different Geometries of Expansions on Impulse Propagation

Since we are able to define arbitrarily the two-dimensional geometry of the patterned growth cell ensembles, we tried to examine the idea that the size of the current load, specified by form alone, is a significant factor determining the delay encountered by the propagating action potential as it crosses the site of expansion in a structure consisting of cells with identical membrane properties. We reduced the electrical load of the expansion by half, compared with the standard rectangular patterns, by designing a funnel having an angle of 90° between the sides, which served as a connecting junction between a narrow and a wide cell strand. Alternatively, we increased the electrical load by half, compared to the standard pattern, by connecting the narrow cell strand to a rectangular pattern designed with an incisure at the site of the connection (see Fig. 1).

The characteristics of propagation across a funneled junction are depicted in Fig. 9. In most cases, the activation wavefront entered the funnel without any appreciable delays, indicating uniform propagation across the gradual expansion. A completely different picture was seen in experiments with expansions having incisures, which represent an increased current load for activation. This geometry gave rise to the largest activation delays of all three patterns, and a typical example is depicted in Fig. 10. Approaching the site of the expansion, the amplitudes of the action potentials recorded from the narrow strand are gradually depressed, leading to marked prepotentials in the large cell mass, which, after a long delay of ~4 ms (apparent slowing of conduction to 0.02 m/s), evolve into a propagating action potential.

A summary of determinations of activation delays measured at the sites of expansion of excitable tissue are given in Table I for the three different expansion geometries explored. The results were all obtained at a spatial resolution of 60 × 60 μm from preparations that differed only in the shape of the connection of the thin
strand to the wide strand, while all other parameters possibly influencing propagation across the expansion were kept constant, i.e., all narrow strands were 50 μm wide, the broad expansions measured ≥1,500 μm across, and the interstimulus interval was ≥400 ms. Linear strands with the dimensions of our narrow strands always exhibited uniform propagation. In the majority of the cases (five out of six), funnels behaved like linear strands, showing no delayed activation at the site of the expansion. In the case of the rectangular expansion, four out of six preparations examined displayed a modest delay at the junctional region of ≤500 μs or less (corresponding to a decrease of conduction velocity to 0.12 m/s), while the remaining two exhibited delays ranging between 500 and 1,000 μs. Delays >1,000 μs or a constant block were seen in all the cases where expansions had incisures.

**DISCUSSION**

The present work represents the first attempt to analyze transmembrane voltage changes in cardiac tissue of controlled geometry in a spatial and temporal domain that has, until now, been restricted to computer simulations. By combining patterned growth of monolayer cultures of neonatal rat heart cells with multiple site optical recording of transmembrane voltage (MSORTV), we have been able to investigate the dependence of action potential propagation on the geometry of the underlying excitable tissue.

As a basis for interpreting our data pertaining to impulse propagation across tissue expansions, we first assessed the characteristics of microscopic conduction in linear strands of myocytes three to four cells across, and found that activation delays measured at 15-μm intervals along the middle of the cell cable were not completely uniform but ranged, e.g., in the case of the representative experiment described in Results, between 18 and 54 μs. While a small part of this variability may be ascribed to inhomogeneity in the temporal response of the amplifiers (maximally 10 μs), the major part of the differences seemed to be real. They were reminiscent of locally increased activation delays at the sites of cell-to-cell appositions as measured earlier in cell strands consisting of a linear chain of single myocytes (Rohr and Salzberg, 1992b). In those experiments, the simple architecture of the cell strands allowed us to determine unequivocally the conduction velocity along single cells and across cell-to-cell junctions. Although a similar direct proof was not possible in the present experiments, because of the more complex architecture of the multiple cell wide strands ("bands"), it is tempting to speculate that the variation in activation delays depended on the discrete nature of the preparations here as well. The finding that

| Type of pattern | None | <500 μs | 500-1,000 μs | >1,000 μs | Block |
|----------------|------|---------|--------------|-----------|-------|
| Linear         | 5    |         |              |           |       |
| Funnel         | 5    | 1       |              |           |       |
| Rectangular    | 4    | 2       |              |           |       |
| Incised        | 1    | 2       | 1            |           |       |
the maximal delays found in the present experiments were smaller than those determined in single-cell chains could be explained by the presence of lateral cell coupling in the cell bands, which tends to decrease the effects of the recurrent increases in longitudinal resistance by offering the excitatory current a collateral path.

In contrast to the comparatively small fluctuations in activation delays observed in regularly shaped cell bands, impulse propagation across two-dimensional tissue expansions exhibited prominent discontinuities at the site of the expansion. The size of these discontinuities was clearly dependent on the shape of the expansion: while propagation in the majority of funnel shaped expansions was continuous, it was characterized by delays as long as 1 ms in the case of rectangular expansions and even larger delays were observed in the preparations where the expansion was given an incised shape. This dependence can be explained by the geometry-related increase in the electrical load, whose relative magnitudes were in the ratio 1:2:3 for the funnel, rectangular and incised shape, respectively.

Delayed propagation occurring at the site of a tissue expansion was always accompanied by the development of a marked distortion of the action potential upstroke. The most extreme distortions were characterized by an initial fast upstroke ending in a plateau at 50% of the full signal amplitude, from which, after a delay, a secondary, slower depolarization carried the potential to its full amplitude ("50/50 double-hump" upstrokes). This form of the action potential signal is reminiscent of the shapes described by Mendez and colleagues (1970) in terminal Purkinje fibers. Our spatially resolved measurements complement their single point measurements, and demonstrate that the distortions develop gradually over a distance of several hundred microns, indicative of electrotonic interaction among the cells. While the amplitude of the initial component decreased in a sigmoidal fashion across the expansion in the downstream direction, the second component displayed a contrary evolution. The origin of these interactions could be partially dissected in those cases where propagation failed intermittently at high rates of stimulation. These blocks were marked by a virtually unchanged initial signal component, as evidenced, e.g., by a nearly identical spatial decay of the amplitude of the initial component along the preparation for both the blocked and the successful propagation. On the other hand, the secondary depolarization completely vanished during block, suggesting that it resulted entirely from an electrotonic response directed upstream from the delayed activation of the expanded tissue area. Very similar sequences of distorted action potential upstroke shapes, assessed on a cellular scale, were reported earlier from computer simulations of abrupt expansions of cardiac tissue and axons (Joyner et al., 1984). The major differences between these simulations and our findings relate to the locations of the maximal decreases in the conduction and upstroke velocities with respect to the abrupt expansion. While we consistently found that the maximal slowing of conduction and upstroke velocity occurred within the expansion, the simulations predicted the occurrence of these conduction minima proximal to the expansion. It may be argued that this difference results from the mode of calculation of the activation times. While activation times in the simulations were equated with the moment of maximal inward current, we derived them from the time of 50% depolarization, which, considering the dramatic changes in action potential shapes in the transition region, results in a certain ambiguity. However, the most extreme
distortions of the action potential upstrokes in the simulations always occurred just upstream from the abrupt expansion, while we always observed these 50/50 double humps within the expanded tissue region itself. It seems, therefore, that this specific simulation model did not entirely fit the spatial evolution of the signals observed in our monolayer cultures. This conclusion is further supported by the recent findings of two-dimensional simulations (as opposed to the one-dimensional model of Joyner) of abruptly expanding cardiac tissue, which show that both the maximal decline in conduction and in upstroke velocity occur within the region of expanded tissue (Leon and Roberge, 1991).

In addition to the typical distortions of the upstroke in the vicinity of an abrupt tissue expansion, the overall shape of the propagating action potential in these regions was characteristically altered as well. During delayed propagation, action potentials recorded in the narrow afferent strand were characterized by a notch in the early repolarization phase resulting in a spike and dome configuration as described by Matsuda et al. (1967). This configuration is identical to a temporally contracted version of the upstroke distortions described above and became apparent 300 μm upstream from the site of the expansion. The width of the spike close to the expansion was an approximate measure of the delay encountered by the propagating action potential, because the depolarizing phase of the spike was determined by the activation of the thin cell strand, while the end of the short initial repolarizing phase (brought about by the "dome") indicated delayed activation in the expanded region. This dome, which was the result of upstream electrotonic interaction, prolonged the action potential recorded in the narrow strand upstream from the expansion to an extent, which was roughly proportional to the size of the delay. A prominent decrease in action potential duration was found when propagation across the expansion failed. In these cases, the signals recorded in the thin afferent cell strands assumed a triangular shape resulting in an abbreviation of the signal duration, measured at 50% repolarization, by as much as 50%. This abbreviation was in agreement with action potential shortenings described earlier for blocked responses in a variety of experimental settings (Mendez et al., 1969, Cranefield, Klein, and Hoffman, 1971, De La Fuente et al., 1971). The possible impact of this shortening results from the concomitant truncation of the refractory period, which would tend to favor the generation of reentry in a given network (Sasyniuk and Mendez, 1971).

In contrast to these commonly observed characteristics of propagation across abrupt rectangular expansions, three isolated cases revealed a completely different propagation pattern. While the total delay across the abrupt expansion was of comparable magnitude, it did not develop gradually, but arose within a very short distance of 60–120 μm, i.e., within the length of one to two cells. This was similar to the spatial extent of the delay observed in the example of the thinned linear cell strand. Although we did not find any analogous morphological correlates of the delay in these three cases of abrupt expansions, the absence of any major electrotonic interaction, either downstream or upstream, was compatible with the existence of a locally increased longitudinal resistance of unknown origin. This explanation was further supported by the finding that the decrease in the amplitude of the blocked response was accelerated in the region of the delay, and that the conduction velocity and the maximal upstroke velocity displayed a transient increase just upstream from
the region producing the delay, which was reminiscent of an action potential approaching the sealed end of a semi-infinite cable.

More direct evidence pertaining to the question of whether activation delays were caused by a large electrical load or by a locally increased longitudinal resistance was obtained during alternate stimulations of the preparations from the side of the narrow strand or the side of the expansion. Using this protocol, delayed propagation based on an increased electrical load resulting from a tissue expansion disappeared completely upon stimulation from the side of the expansion, while delays, which were most probably based on locally increased longitudinal resistances, remained largely unaffected by changing the direction of stimulation.

One of the aims of this study, viz., the design of a structure exhibiting fixed unidirectional block, was surprisingly difficult to achieve. Blocks could only be induced by critically raising the stimulation frequency or by preilluminating for a short time, the junction between narrow and large cell area. The latter approach, which resulted in a variable degree of phototoxic damage to the exposed tissue area, was not pursued further because of its unphysiological character. Based on previous simulations (Joyner et al., 1984), block can be expected for cylindrical preparations expanding by a factor >1.9. For our experimental system, this value needed to be corrected upwards because the preparations were flat, resulting in a reduced electrical load of an expanded region as compared to cylinders. Nevertheless, much higher expansion factors were obtained in our experiments, which is best illustrated by the presence of successful propagation at stimulation rates of \( \leq 3 \) Hz in those extreme cases \((n = 5; \text{data not shown})\), where very thin cell strands \((\text{diameter of } 15-30 \ \mu m; \text{one to two cells across})\) were connected to large rectangular areas, \( \geq 1,400 \ \mu m \) wide. This means that an expansion by a factor as large as 90 still permitted successful, although delayed, propagation in the two-dimensional case. Even if this figure is corrected downward by assuming that only the region within a single assumed space constant \((360 \ \mu m, \text{Jongsma and van Rijn, 1972})\) is electrotonically relevant to the action potential approaching the expanding region, the resulting factor of 24 is still an order of magnitude higher than the simulation result. Based on these findings, we were forced to the conclusion that it was virtually impossible, using the rectangular type of expansion, to produce a pattern exhibiting a fixed unidirectional block at low stimulation rates. Besides recognizing that impulse propagation is very stable in these preparations, there are two essential caveats: \((a)\) the insertion of the thin cell strands into the rectangular tissue expansion was most often accompanied by a slight fan-out of the cell(s) just adjacent to the expansion and this "minimal funnel," according to the hypothesis of Mendez and colleagues for the case of the PVJ (Mendez et al., 1970), might have facilitated conduction across the transition to a certain extent; \((b)\) while the cells were elongated and aligned in parallel in the narrow cell strands, their shape was more compact and their long axes were distributed at random in the large expansions, which made it likely that the space constant in the expanded region was comparatively small. The resulting combination of a large space constant in the thin strand and a smaller space constant in the expanded region, then, might be expected to favor successful propagation across the transition. In this context, one might speculate that a similar mechanism also facilitates conduction from the Purkinje fibers to the ventricles, because the longitudinal axes of
the cells in the former tissue also diverge, resulting in a situation where the Purkinje fiber "sees" a smaller electrotonic load.

In conclusion, we have been able to characterize impulse propagation at the cellular level in two-dimensional excitable structures that have precisely defined geometries. The application of MSORTV to patterned growth heart cell cultures permits the systematic investigation of the effects of geometry per se, manifested as electrical load, on the properties of the advancing action potential as it encounters an abrupt change in tissue dimensions. This experimental system complements and extends computer simulations of, e.g., the Purkinje fiber–ventricular junction, and avoids the complexities of ill-defined tissue geometries or local differences in active membrane properties that bedevil whole tissue studies. We have shown that the magnitude of the propagation delays imposed at the site of a sudden expansion of the excitable tissue is a function of the shape of the transition region, expressed as the electrical load presented to the approaching action potential, and we have found evidence that the complex distortions of the action potential upstrokes occurring in the region of the maximal delay result entirely from electrotonic interactions in both upstream and downstream directions. An important question is the degree to which these distortions may affect the timecourse and magnitude of ionic currents in the transition zones, a question that we hope can be resolved by combining MSORTV with optical measurements of ion activities in these patterned growth cell cultures.

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