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Intercellular Calcium Waves in Glia

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ABSTRACT Glial cells are capable of communicating increases in $[Ca^{2+}]_i$ from a single cell to many surrounding cells. These intercellular Ca^{2+} waves have been observed in glia in multiple different preparations, including dissociated brain cell cultures, glial cell lines, organotypic brain slice cultures, and intact retinal preparations. They may occur spontaneously, or in response to a variety of stimuli. Ca^{2+} waves occurring under different conditions in different preparations may have distinctive patterns of initiation and propagation, and distinctive pharmacological characteristics consistent with the involvement of different intracellular and intercellular signaling pathways. This paper presents original data supporting a combination of gap junction and extracellular messenger-mediated signaling in mechanically induced glial Ca^{2+} waves. Additional new observations provide evidence that a rapidly propagated signal may precede the glial Ca^{2+} wave and may mediate rapid glial-neuronal communication. This original data is discussed in the context of a review of the literature and current concepts regarding the potential mechanisms, physiological and pathological roles of this dynamic pattern of glial intercellular signaling. *GLIA 24:39–49, 1998.*

INTRODUCTION

The function of glial cells involves a high level of intercellular coordination. Fluorescence video imaging has recently provided a dramatic visualization of one mechanism for coordination of glial function: intercellular Ca^{2+} waves. Increases in $[Ca^{2+}]_i$ that are propagated in a wave-like fashion from a single cell to many surrounding cells have been observed in multiple glial cell preparations in response to a variety of stimuli. The Ca^{2+} waves that occur under different conditions have both similarities and differences in their temporal and spatial characteristics, and it is now clear that there are multiple forms of Ca^{2+} waves in glia that involve distinct mechanisms of initiation and propagation. Ca^{2+} waves have also been reported in a wide variety of other cell types (Sanderson et al., 1994). This paper focuses exclusively on intercellular Ca^{2+} waves in glial cells, including the role of gap junctions in their communication from cell to cell, messengers involved in their communication, and their potential roles in bi-directional glial neuronal signaling under both physiological and pathological conditions.

MATERIALS AND METHODS

Cell Culture

Primary mixed glial cell cultures were prepared from rat brain using standard techniques as previously described (Charles et al., 1991). In brief, the forebrain was dissected from either 1 day postnatal rats, the meninges were removed, and a cell suspension was obtained by passing the tissue through Nitex mesh. Cells were then plated on glass coverslips in DMEM/F12 with 10% fetal calf serum (FCS) and grown for 7–14 days prior to experimentation. Immunolabeling of these cultures revealed that 40–60% of cells were astrocytes labeled for GFAP, 10–20% were oligodendrocytes labeled for GalC, 10–30% were precursor cells which labeled for A2B5 but not GalC, and 5–10% were microglia labeled for esterase (Charles et al., 1991).

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Primary glial-neuronal cultures were prepared using a modification of the techniques described above. A cell suspension was obtained from embryonic mice (15–17 day gestation), and cells were initially plated on either plastic flasks in DMEM/F12 with 10% fetal calf serum (FCS). The flasks were shaken 6–8 h after initial plating to remove non-adherent cells. The non-adherent cells were then plated onto glass coverslips and grown in DMEM/F12 supplemented with insulin 5 mg/l, transferrin 5 mg/l, and selenium 5 µg/l (Reduser, Upstate Biotechnology), and 5% FCS. This resulted in cultures that were initially composed of 50–75% neurons and 25–50% glial cells (Charles, 1994). Since no mitotic inhibitors were used, the percentage of glial cells increased progressively with age in culture.

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured using a fluorescence imaging system that has previously been described in detail (Charles et al., 1991). In brief, cells on glass coverslips were loaded with fura2 by incubation in 5 µM fura2-AM for 40 min. Cells were then washed and maintained in normal medium for 30 min prior to experimentation. Coverslips were placed on a Nikon Diaphot inverted microscope and excited with a mercury lamp through 340 and 380 nm bandpass filters, and fluorescence at 510 nm was recorded through a 20× or 40× objective with a SIT camera to an optical memory disc recorder. Images were then digitized and subjected to background subtraction and shading correction, after which $[Ca^{2+}]_i$ was calculated on a pixel-by-pixel basis as previously described. Data acquisition and analysis software were written by Dr. Michael Sanderson.

RESULTS

Mechanical stimulation of a single cell in a mixed glial culture in static medium results in a wave of increased $[Ca^{2+}]_i$ that spreads from the point of contact of the pipette throughout the stimulated cell (Fig. 1A; Charles et al., 1991). This is followed by concentric propagation of increased $[Ca^{2+}]_i$ to neighboring cells that occurs at sites of cell-cell contact and that spreads as waves within individual cells. There is often a delay of 0.5–1 s between arrival of the wave at the border of one cell and the initiation of the wave in the adjacent cell.

Rapid perfusion of the extracellular medium (up to 20 ml/min in a chamber holding 0.5 ml) alters the temporal and spatial characteristics of mechanically induced Ca^{2+} waves (Fig. 1B). When a single cell is mechanically stimulated during rapid medium perfusion, the resulting intercellular Ca^{2+} wave is initially concentric (the first 1–5 cells concentrically in all directions), with the same velocity and the same cell-to-cell pattern as that in static medium. However, after 2–4 s of concentric propagation, the wave then "takes off" at a much higher rate in the direction of perfusion, often skipping cells rather than traveling with the same cell-to-cell pattern. The average number of cells in each direction of the concentric component of the wave, including the direction directly opposite the direction of perfusion, was 2.57 ± .97 (n=12 experiments on six different cultures). The wave then traveled to at least 8–10 cells further in the direction of perfusion, usually to the edge of the imaged field.

Ca^{2+} waves induced by mechanical stimulation can be propagated across gaps with no intervening cells. However, we have found that a mechanically-induced Ca^{2+} wave travels significantly faster and farther in areas of cell contact than in cell-free areas (Fig. 1C). We found that the average maximum velocity across cell-free regions that were 50–100 µM wide was 5.43 µm/s (± 1.01 µm/s S.D., n=eight experiments on six different coverslips), whereas the maximum velocity over the same distance in the area where cells were in contact was 11.25 µm/sec (± 2.93 µm/s S.D). We found that the intercellular wave traveled to a maximal distance of 250 ± 41 µm through areas of contacting cells, compared with a maximum distance of 150 ± 22 µm across a 50–100 µm cell free area.

Intercellular Ca^{2+} waves may be preceded by a rapidly propagated signal that does not in itself induce an increase in $[Ca^{2+}]_i$ in most cells. In both primary glial-neuronal cultures (n=5 different cultures) and co-cultures of an immortalized glial cell line with the GT1 neuronal cell line (n=3 different cultures, data not shown), a pattern of glial-neuronal signaling is observed that suggests that rapid communication may precede the intercellular Ca^{2+} wave (Fig. 1D). In each of these preparations, mechanical stimulation of a glial cell induces a change in $[Ca^{2+}]_i$ in distant neurons that precedes by several seconds the arrival of the glial Ca^{2+} waves at sites of contact with these neurons. This pattern of signaling is observed frequently in co-cultures of

Fig. 1. **A:** Mechanically-induced Ca^{2+} wave. Panels represent $[Ca^{2+}]_i$ in field of cells in a mixed glial culture. Mechanical stimulation of a single cell induces an increase in $[Ca^{2+}]_i$ in the stimulated cell that travels in a concentric pattern, at sites of cell-cell contact, to surrounding cells. Scale of images is 320 µm by 300 µm. **B:** Mechanically-induced Ca^{2+} wave with extracellular perfusion. In the same culture as depicted in A, the extracellular medium is perfused rapidly from the top right to the bottom left of the field. Mechanical stimulation of a single cell induces an intercellular Ca^{2+} wave that initially travels concentrically as in A. After 2 s, the wave then travels at a higher velocity in the direction of perfusion, without a distinct cell-cell pattern of communication. Scale of images is 320 µm by 300 µm. **C:** Ca^{2+} wave propagation across a cell-free gap. In this mixed glial culture imaged at lower power, mechanical stimulation of a single cell on the left side of a cell free gap induces a calcium wave that is propagated throughout a monolayer on the same side as the stimulus. After a delay of 30 s, two cells on the opposite side of the gap show an increase in $[Ca^{2+}]_i$. The Ca^{2+} wave travels farther and with a higher maximum velocity through the contacting cells than across the gap. Scale of images is 480 µm by 500 µm. **D:** Rapid glial-neuronal communication. Neuronal cell bodies in this mixed glial-neuronal culture are outlined in white. Mechanical stimulation of a single glial cell induces an immediate increase in $[Ca^{2+}]_i$ in a distant group of neurons, which precedes the arrival of the calcium wave that travels through the glial monolayer. This suggests that a rapidly propagated signal precedes the increase in $[Ca^{2+}]_i$. Scale of images is 380 µm by 360 µm.

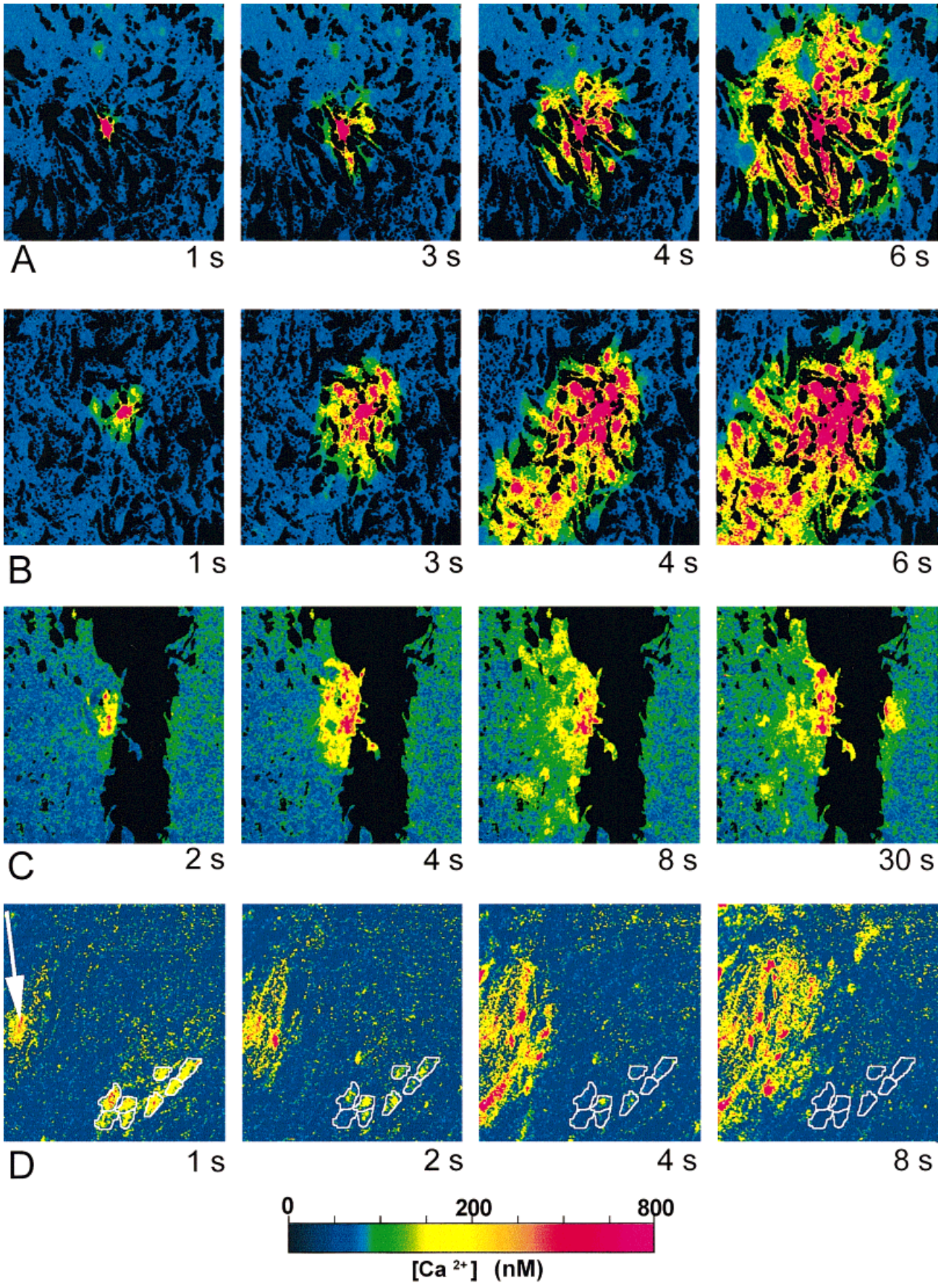

Fig. 1.

TABLE 1. Descriptions of intercellular calcium waves in glia[a]

Reference	Preparation	Stimulus	Conclusion
Cornell-Bell et al., 1990	Hippocampal astrocytes	Glutamate	Intercellular Ca^{2+} waves following bath application of glutamate
Charles et al., 1991	Cortical mixed glia	Mechanical	Intercellular waves induce sustained asynchronous single-cell Ca^{2+} oscillations
Cornell-Bell and Finkbeiner, 1991	Hippocampal astrocytes	Glutamate and glutamate agonists	Different glutamate receptor subtypes involved in different Ca^{2+} responses
Charles et al., 1992	C6 glioma	Mechanical	Intercellular wave propagation correlated with level of transfected connexin43 expression
Dani et al., 1992	Hippocampal slice culture	Electrical, NMDA	Activation of neurons triggers astrocyte Ca^{2+} waves
Enkvist and McCarthy, 1992	Cortical astrocytes	Mechanical	Intercellular propagation of Ca^{2+} waves inhibited by activation of protein kinase C
Finkbeiner, 1992	Hippocampal astrocytes	Glutamate	Intercellular Ca^{2+} waves not effected by perfusion or gap junction inhibitors
Charles et al., 1993	Cortical mixed glia	Mechanical	Intercellular waves propagated via IP3; oscillations dependent upon Ca^{2+}-induced Ca^{2+} release
Kim et al., 1994	Hippocampal astrocytes	Glutamate	Distinguishes metabotropic and ionotropic waves. Ionotropic waves dependent upon extracellular Ca^{2+}
Charles, 1994	Cortical glia/neuron	Mechanical	Bi-directional glial-neuronal Ca^{2+} signaling
Nedergaard, 1994	Forebrain astrocyte/neuron	Focal electrical	Glia-to-neuron communication mediated by gap junctions
Parpura et al., 1994	Cortical astrocyte/neuron	Focal electrical, mechanical	Glia-to-neuron communication mediated via astrocytic release of glutamate
Hassinger et al., 1995	Hippocampal astrocyte/neuron	Electrical	Glia-to-neuron communication mediated by ionotropic glutamate receptor channels
Takeda et al., 1995	Oligodendrocytes	Mechanical	Limited intercellular Ca^{2+} signaling in purified oligodendrocytes
Lee et al., 1995	Human astrocytes	Glutamate	Increased intercellular Ca^{2+} waves in astrocytes from epileptic tissue
Venance et al., 1995	Striatal astrocytes	Mechanical, local glutamate	Intercellular Ca^{2+} waves and gap-junctional communication inhibited by anandamide
Newman and Zahs, 1997	Acutely isolated retina	ATP, electrical, mechanical	Ca^{2+} waves in astrocytes and Muller cells dependent on intracellular Ca^{2+} stores
Venance et al., 1997	Striatal astrocytes	Mechanical, ionomycin, multiple ligands	Ca^{2+} waves induced by multiple ligands involve PLC, IP_3, and gap junctions
Zanotti and Charles, 1997	Cortical mixed glia, astrocytes	Low extracellular Ca^{2+}	Extracellular Ca^{2+} sensing by glial cells evokes intercellular Ca^{2+} waves
Harris-White et al., 1997	Hippocampal slice cultures	Spontaneous NMDA	Curvilinear and spiral Ca^{2+} waves characteristic of an excitable medium

[a]All preparations are dissociated cultures unless otherwise stated.

the immortalized glial cell line and the GT1 neuronal cell line, but infrequently (3/20 stimulations in five different cultures) in the primary glial-neuronal cultures. This rapid glial-neuronal signaling is in contrast to the previously reported neuronal response to glial Ca^{2+} waves, which occurs 0.5–1 s *after* the arrival of the glial Ca^{2+} wave (Charles, 1994).

Intercellular Ca^{2+} waves induced by mechanical stimulation may induce sustained oscillatory changes in $[Ca^{2+}]_i$ in individual cells that continue for as long as 5 min (Fig. 2; Charles et al., 1991). These individual cell Ca^{2+} oscillations may occur as an intracellular waves, but they are only rarely spread from cell to cell, even in adjacent cells that have just communicated a wave. Therefore, intracellular waves and intercellular waves represent distinct phenomena.

DISCUSSION AND REVIEW

Spatial and Temporal Characteristics of Ca^{2+} Waves

Intercellular Ca^{2+} waves in glial cells were first reported by Cornell Bell et al. (1990), who described propagated increases in $[Ca^{2+}]_i$ in astrocytes from rat hippocampus in response to bath application of glutamate. We subsequently described intercellular Ca^{2+} waves in mixed glial cultures from rat cortex in response to mechanical stimulation of a single cell (Charles et al., 1991), and similar waves have since been reported in multiple different glial cell preparations in response to a variety of stimuli (Table 1). These different Ca^{2+} waves have characteristics that indicate distinct mechanisms of propagation. Detailed comparison of glutamate-induced and mechanically induced Ca^{2+} waves highlights some of these differences.

Upon exposure to glutamate, cultured astrocytes show an initial increase in $[Ca^{2+}]_i$ that may be communicated as a wave; this has been referred to as a "spatial spike." Then, after a delay of 30–60 s, intercellular Ca^{2+} waves propagate from multiple single cell foci to surrounding cells (Kim et al., 1994). By contrast, mechanical stimulation of a single cell in a mixed glial or purified astrocyte culture induces a wave of increased $[Ca^{2+}]_i$ that travels from the point of stimulation to involve the entire stimulated cell. After a brief delay (0.5–1 s), the wave is communicated to neighboring cells at points of intercellular contact (Fig. 1A) (Charles

et al., 1991). Both glutamate-induced and mechanically-stimulated waves propagate from a single cell to multiple surrounding cells at a velocity of 10–20 μm/sec. Under both conditions, the waves are communicated at sites of cell-to-cell contact, and the increase in $[Ca^{2+}]_i$ often travels as an intracellular wave within individual cells (Charles et al., 1991; Cornell-Bell et al., 1990). However, Ca^{2+} waves in response to glutamate require extracellular Ca^{2+} and are communicated without pause at the cell borders (Cornell-Bell et al., 1990; Kim et al., 1994). By contrast, mechanically induced Ca^{2+} waves show delays at the borders between cells and do not require extracellular Ca^{2+} (Charles et al., 1991) . Thus, although the temporal and spatial characteristics of the glutamate-induced and mechanically-induced waves are superficially similar, they clearly involve different mechanisms of initiation and propagation.

The Role of Gap Junctions vs. Extracellular Communication

There is extensive evidence that gap junctions are involved in the propagation of Ca^{2+} waves between glial cells. The intercellular communication of glutamate-induced Ca^{2+} waves in astrocytes is blocked by octanol, and the pattern of communication of these waves is not altered by rapid perfusion of the extracellular medium (Finkbeiner, 1992). The intercellular communication of mechanically induced or ionomycin-induced intercellular waves is inhibited by anandamide (Venance et al., 1995) and by 18-α-glycyrrhetinic acid (Venance et al., 1997), both of which were shown in parallel experiments to inhibit gap-junctional coupling. C6 glioma cells, which have low levels of connexin expression and low levels of intercellular dye coupling, show limited propagation of intercellular Ca^{2+} waves induced by mechanical stimulation. By contrast, C6 cells overexpressing connexin43 show an increased propagation of intercellular Ca^{2+} waves that is correlated with the level of connexin expression and the level of intercellular dye coupling (Charles et al., 1992).

While these studies strongly support gap junctional communication as one mechanism for communication of Ca^{2+} waves, they do not rule out other mechanisms. Indeed, the results shown in Figure 1B,C show that Ca^{2+} waves can also be mediated by an extracellular messenger. Similar findings have been previously reported by Hassinger et al. (1996). Detailed analysis of these experiments is revealing. Both the results presented above and those of Hassinger et al. indicate that there is significant propagation of the wave in the direction opposite perfusion. These results show that there is some component of the response that is not altered by perfusion. In addition, the delay in the perfusion-dependent component of the wave shown in Figure 2B suggests that the initial, concentric multicellular response is required to generate a sufficient concentration of an extracellular messenger to elicit the downstream response. This raises the possibility that

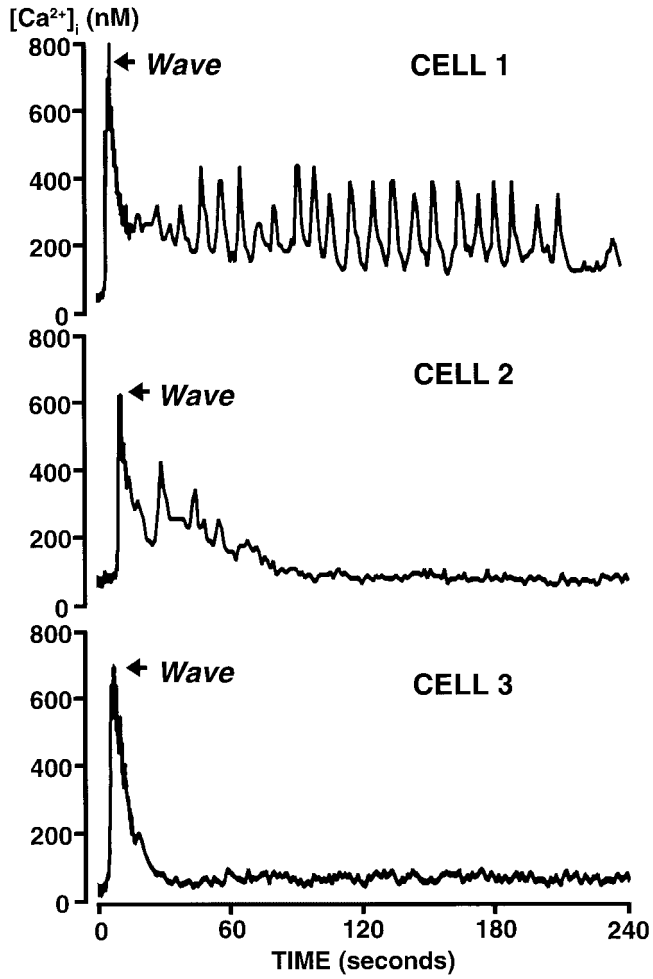

Fig. 2. Patterns of $[Ca^{2+}]_i$ responses to a mechanically induced intercellular Ca^{2+} wave. Tracings represent $[Ca^{2+}]_i$ in three neighboring cells in a mixed glial culture. The initial peak represents an intercellular Ca^{2+} wave induced by mechanical stimulation of a nearby cell. Each cell shows a different pattern of oscillations following the wave; these oscillations are asynchronous in individual cells.

Ca^{2+} waves induced by mechanical stimulation may be initially communicated via gap junctions and subsequently augmented by release of an extracellular messenger. We have also found that intercellular Ca^{2+} waves induced by low extracellular Ca^{2+} are biased by perfusion of the medium, providing evidence for the release of an extracellular messenger in response to this stimulus as well (Zanotti and Charles, 1997).

Analysis of the pattern of communication of Ca^{2+} waves across cell-free regions provides additional information about the role of an extracellular messenger. Hassinger et al. (1996) report that intercellular Ca^{2+} waves induced by electrical stimulation traveled across cell-free lanes in 16 of 35 cases when these lanes were less than 120 μm in width, and never crossed when the lane was greater than 120 μm in width. In addition, Hassinger et al. (1996) report that the velocity of propagation across cell-free lanes was not significantly different than that across regions of confluent cells. By contrast, the results reported above indicate a signifi-

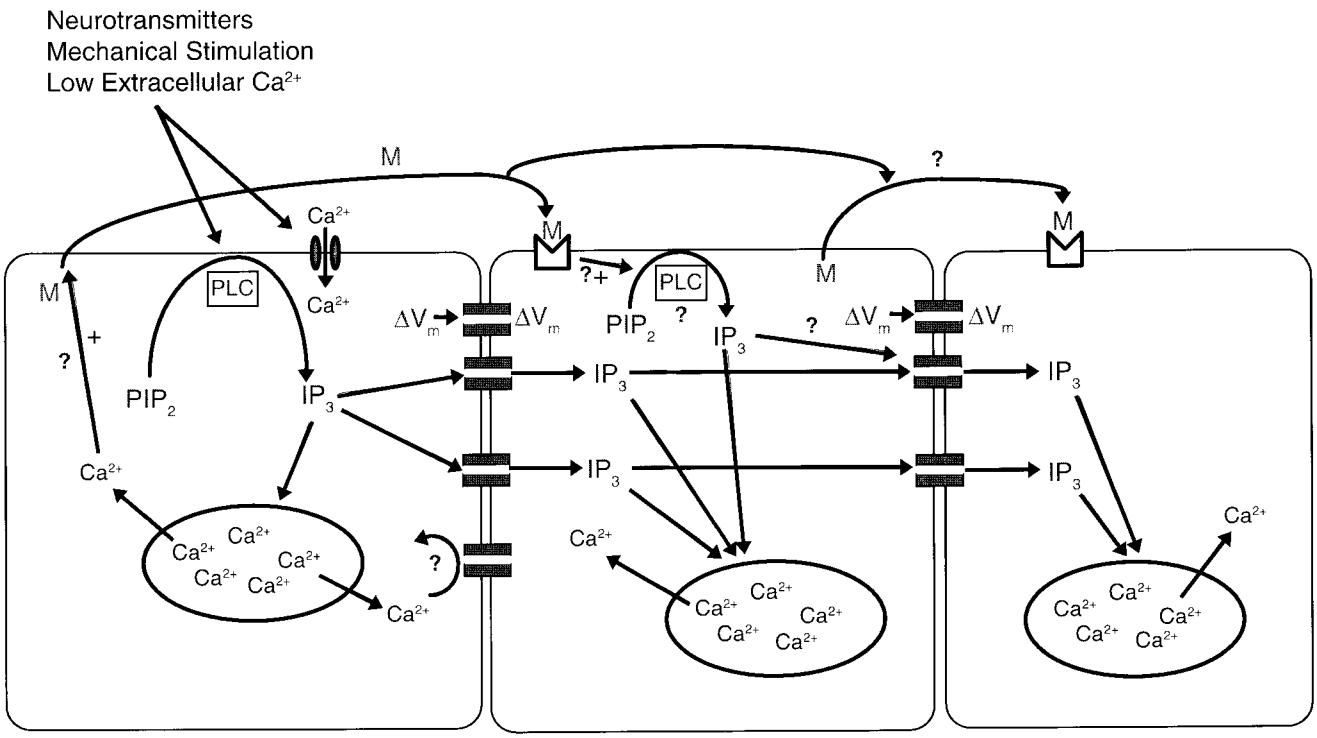

Fig. 3. Schematic model of intercellular Ca^{2+} waves in glia. The model shows the formation of IP_3 in the stimulated cell and the diffusion of IP_3 through gap junctions to the adjacent cell. It also shows the release of an extracellular messenger (M) by the stimulated cell. IP_3 releases Ca^{2+} from intracellular stores in both the stimulated and adjacent cells. Other possible mechanisms suggested but less clearly supported by experimental data are indicated by question marks. These include the stimulation of release of the extracellular messenger by Ca^{2+}, the inability of diffusion of Ca^{2+} itself to mediate intercellular waves, the role of a change in membrane potential in Ca^{2+} waves, and the extent to which the propagation of the wave is regenerated by activation of PLC and release of an extracellular messenger by adjacent cells in addition to the stimulated cell. M, extracellular messenger; PLC, phospholipase C; ΔV_m, change in membrane potential.

cantly higher propagation velocity and distance of propagation in areas of cell contact as compared with cell-free areas. Although the results presented above differ somewhat from those of Hassinger et al., both show that at least some forms of Ca^{2+} waves in mixed glial cultures may involve an extracellular messenger, and both show that the characteristics of wave propagation are different in areas of cell contact compared with areas of no cell contact. Again, it is likely that a combination of gap junctional communication and release of an extracellular messenger may be involved (Fig. 3).

Intracellular Messengers Involved in Propagation of Ca^{2+} Waves

There are multiple potential messengers involved in the communication of Ca^{2+} waves, and these messengers may act individually or in combination. Glutamate-induced Ca^{2+} waves are dependent on extracellular Ca^{2+}; it has been proposed that propagation of these waves involves the sodium-Ca^{2+} exchanger (Kim et al., 1994). By contrast, we and others have reported that mechanically stimulated waves (Charles et al., 1991; Enkvist and McCarthy, 1992; Newman and Zahs, 1997), spontaneous glial intercellular waves in mixed glial-neuronal cultures or in hippocampal slice cultures (Charles et al., 1991; Harris-White et al., 1997), and low-Ca^{2+}-induced intercellular waves (Zanotti and Charles, 1997) do not require extracellular Ca^{2+}. These studies show that some forms of glial Ca^{2+} waves can be generated entirely by the release of Ca^{2+} from intracellular stores. Multiple lines of evidence suggest that Ca^{2+} itself is not the messenger that transmits the response from cell to cell. First, mechanical stimulation in 0 Ca^{2+} medium often does not induce an increase in $[Ca^{2+}]_i$ in the stimulated cell (possibly due to efflux of Ca^{2+} through mechanically activated channels), and yet an intercellular Ca^{2+} wave is still communicated to neighboring cells (Charles et al., 1991). Second, when cells are treated with thapsigargin or the PLC inhibitor U73122, there is still an increase in $[Ca^{2+}]_i$ in the stimulated cell, but this increase in $[Ca^{2+}]_i$ is not propagated to neighboring cells (Charles et al., 1993; Venance et al., 1997). Finally, transient increases in $[Ca^{2+}]_i$ or oscillatory increases in $[Ca^{2+}]_i$ may occur in individual glial cells that reach levels that are equal in amplitude to those seen with intercellular Ca^{2+} waves, and yet there is no communication of these increases in $[Ca^{2+}]_i$ from cell to cell (Charles et al., 1991; Cornell-Bell et al., 1990). These same cells showing non-propagating increases in $[Ca^{2+}]_i$ can participate in propagated intercellular Ca^{2+} waves in response to

mechanical stimulation or glutamate. These results indicate that either there is a different level of conductance of the gap junction channel under the circumstances of asynchronous Ca^{2+} increases vs. communicated intercellular Ca^{2+} waves, or that Ca^{2+} itself is not the primary messenger for intercellular communication of the waves.

The inhibition of Ca^{2+} wave propagation by thapsigargin and U73122 provide evidence that IP_3 is required for intercellular communication of glial Ca^{2+} waves. In tracheal epithelial cells, microinjection of IP_3 in a single cell induces intercellular Ca^{2+} waves similar to those observed in glia (Sanderson et al., 1990) and intercellular propagation of Ca^{2+} waves is blocked by intracellular heparin (an antagonist of the IP_3 receptor) in both tracheal epithelial cells (Boitano et al., 1992) and retinal glia (Newman and Zahs, 1997). These studies provide evidence that IP_3 is a primary messenger involved in the communication of Ca^{2+} waves. Mathematical modeling of Ca^{2+} waves generated by the diffusion of IP3 through gap junctions predicts spatial and temporal patterns of Ca^{2+} signals that are very similar to experimental data from glial cultures (Sneyd et al., 1994, 1995). However, as discussed above, receptor-mediated formation of IP_3 induced by an extracellular messenger may also play a primary role, or augment the diffusion of IP_3 through gap junctions.

The rapid communication of a response from a mechanically stimulated glial cell to distant neurons shows that a signal may be propagated through glial cells that *precedes* the Ca^{2+} wave. While it is possible that this rapid communication occurs via neuronal processes that are not visible by phase-contrast or fluorescence microscopy, another possibility is that a rapidly communicated signal, such as a depolarization spread electrotonically via gap junctions, induces a Ca^{2+} response in neighboring neurons but not in glial cells. This mechanism would indicate the occurrence of gap junctional coupling between at least a subset of glia and neurons (see below). Newman and Zahs (1997) reported that electrical and mechanical stimulation of a single astrocyte in the intact retina induced depolarizations as great as 37 mV in distant astrocytes as measured in the whole-cell current-clamp configuration. Consistent with the observations reported here, these depolarizations either preceded arrival of a Ca^{2+} wave or occurred when waves did not reach the cell that showed a depolarization. Enkvist et al. (1993) reported that depolarization of cells with 50 mM extracellular K did not induce an increase in $[Ca^{2+}]_i$ in glia in their preparation and also did not alter intercellular communication of mechanically induced Ca^{2+} waves. These results, as well as those of Newman et al., show that a change in membrane potential is not directly responsible for a communicated increase in $[Ca^{2+}]_i$. However, Enkvist et al. (1994) also showed that depolarization increased dye coupling in glial cells. It is therefore possible that a propagated depolarization could somehow "prime" cells for the communication of Ca^{2+} waves.

Possible Extracellular Messengers Involved In Ca^{2+} Waves

As discussed above, an extracellular messenger may play a role in the intercellular communication of glial Ca^{2+} waves induced by multiple stimuli. Several candidates for this extracellular messenger have been studied, but none has been definitively shown to mediate glial Ca^{2+} wave communication. Purine nucleotides have been identified as an extracellular messenger in a variety of cell types (Enomoto et al., 1994; Frame and de Feijter, 1997; Osipchuk and Cahalan, 1992), and there is some evidence that they may play a role in glial Ca^{2+} waves. Suramin, a purinergic receptor antagonist, inhibits the extent of intercellular communication of mechanically-induced propagation of glial Ca^{2+} waves at high concentrations (50–100 μM, data not shown). But the actions of this agent are not specific, and therefore they are not conclusive. The ATP agonist 2-methylthio-ATP inhibits intercellular communication of mechanically-induced Ca^{2+} waves, an effect that might be due to desensitization of ATP receptors, or as the authors suggest, to activation of PKC (Enkvist and McCarthy, 1992). Evidence against ATP as a messenger for Ca^{2+} waves is provided by Venance et al. (1995), who reported that the ATP- degrading enzyme apyrase does not alter the intercellular communication of waves. Another potential extracellular messenger is glutamate. Parpura et al. (1995) have shown that there is a Ca^{2+}-dependent release of glutamate from astrocytes that mediates glial-neuronal signaling. However, we have found that Ca^{2+} waves induced by mechanical stimulation can occur in high concentrations of glutamate, suggesting that saturation of the receptor does not affect the waves (Charles et al., 1991). In addition, multiple investigators have shown that Ca^{2+} waves induced by mechanical, electrical, and receptor-mediated stimulation are not blocked by glutamate receptor antagonists (Charles et al., 1991; Enkvist and McCarthy, 1992; Hassinger et al., 1996; Parpura et al., 1994; Venance et al., 1997). While these results do not exclude a role for glutamate in Ca^{2+} wave propagation, they suggest that glutamate receptors are not essential for this process. Another possibility is that glutamate may evoke Ca^{2+} signaling through a glutamate transporter-mediated mechanism. There are multiple other candidates for extracellular messengers involved in glial Ca^{2+} waves, and it is possible that more than one may be involved.

Intracellular Ca^{2+} Waves Are Distinct From Intercellular Ca^{2+} Waves

The simultaneous occurrence of oscillatory intracellular Ca^{2+} waves and propagated intercellular Ca^{2+} waves suggests that Ca^{2+} itself is not the messenger that mediates propagation of intercellular Ca^{2+} waves. Based upon the effects of thapsigargin and dantrolene, we have proposed that IP3 is the messenger that

mediates intercellular communication of waves, whereas Ca^{2+}-induced Ca^{2+} release mediates subsequent single-cell oscillations (Charles et al., 1993). Mathematical modeling based upon IP3 as the messenger that mediates intercellular propagation of Ca^{2+} waves yields multiple patterns of cellular Ca^{2+} oscillations that occur based upon the distance of each cell from the stimulated cell; these patterns are highly consistent with experimental data shown in Fure 2 (Sneyd et al., 1994).

An important implication of the multiple patterns of transient or sustained Ca^{2+} oscillations induced by a single Ca^{2+} wave is that the response of each cell to a wave may have strikingly different characteristics. This may represent a mechanism for individual cells to respond to a common stimulus with distinct patterns of signaling. The amplitude, frequency, and duration of the Ca^{2+} response in individual cells may encode both spatial and temporal information based upon the location and type of the original stimulus.

The Role of Ca^{2+} Waves in Glial-Neuronal Signaling

In addition to providing a mechanism for signaling between glia, intercellular Ca^{2+} waves may also represent a pathway for signaling between glia and neurons. Glial cells in mixed culture or purified astrocyte or oligodendrocyte culture show occasional spontaneous single-cell oscillations, and rare intercellular waves that are limited to a few cells. By contrast, glial cells in culture with neurons show frequent spontaneous oscillations as well as more frequent and more extensive intercellular waves (Charles, 1994). The waves often appear to be initiated at sites of contact with neurons, suggesting that they may be initiated by neuronal-glial communication. However, this glial signaling is not blocked by TTX, showing that ongoing neuronal activity is not required to initiate the process. Dani et al. (1992) have shown that glial Ca^{2+} signaling can be induced by NMDA and stimulation of neuronal pathways in hippocampal slice cultures. We have made similar observations in hippocampal slice cultures, where spiral intercellular Ca^{2+} waves occurring predominantly in astrocytes are induced by bath application of NMDA (Harris-White et al., 1997). These studies indicate that glial Ca^{2+} waves can be evoked by neuronal activity.

Conversely, multiple investigators have also found that glial Ca^{2+} waves can induce changes in neuronal activity (Charles, 1994; Hassinger et al., 1995; Nedergaard, 1994; Parpura et al., 1994). Nedergaard (1994) provides evidence that this signaling between neurons and glial cells occurs via gap junctions. Parpura et al. (1994) provide evidence that glia-to-neuron signaling is mediated by Ca^{2+}-induced release of glutamate. The observations of Hassinger et al. (1995) are consistent with the latter mechanism. As discussed above, we have observed different patterns of glia-to neuron signaling suggesting that both may occur. In both primary neuron-glia cultures from mouse cortex, and in co-cultures of neuronal and glial cell lines, we observe both rapid and delayed response of neurons to Ca^{2+} waves in glia. In the rapid response, the neurons respond to mechanical stimulation of a distant glial cell almost instantaneously, before arrival of the glial Ca^{2+} wave as described above (Fig. 1D). In the delayed pattern of response, the neuron responds 0.5–2 s after the Ca^{2+} wave has arrived to glia immediately adjacent to the neuron. One possible explanation for these distinct temporal patterns of glial-neuronal signaling is that the rapid neuronal response is mediated by depolarization that is spread electronically via gap junctions between glia and neurons, whereas the delayed response is mediated by glial release of an extracellular messenger. Another interesting observation is that spontaneous, single glial cell Ca^{2+} transients rarely if ever induce increases in $[Ca^{2+}]_i$ in neighboring neurons, whereas multicellular Ca^{2+} waves reliably induce increases in $[Ca^{2+}]_i$ in neurons. This discrepancy suggests either distinct messengers, or different concentrations of messengers involved in the single-cell Ca^{2+} transients vs. the multicellular Ca^{2+} waves.

Are Glial Ca^{2+} Waves an Artifact Of Cell Culture?

A question that is frequently raised regarding glial Ca^{2+} waves is the extent to which they occur in the intact nervous system. Technical limitations have prevented a definitive answer to this question. However, recent observations in in vitro preparations with preserved cellular architecture, as well as the correlation of the temporal and spatial characteristics of glial Ca^{2+} signaling with patterns of activity in the intact brain provide indirect evidence for glial Ca^{2+} waves in vivo. The studies by Newman and Zahs (1997) clearly demonstrate the occurrence of glial Ca^{2+} waves in an intact acute retinal preparation that has not been maintained in culture. We and others (Dani et al., 1992; Harris-White et al., 1997) have observed intercellular Ca^{2+} waves in glial cells in hippocampal slice culture preparations in which the typical cellular architecture of the hippocampus is preserved. These studies, while involving slices maintained in culture, do show that intercellular Ca^{2+} waves are not an artifact of dissociation of cells.

Although spatially resolved visualization of cellular $[Ca^{2+}]$ in intact brain preparations has not yet been achieved, studies using laser-doppler imaging, optical intrinsic signal imaging, PET imaging, and EEG techniques have identified changes in blood flow, metabolism, and electrical activity that are propagated with velocity and spatial patterns that are similar to those of glial Ca^{2+} waves observed in culture preparations (Woods et al., 1994; Busch et al., 1995; Lauritzen and Fabricius, 1995). As discussed below, these similarities raise the possibility that glial Ca^{2+} waves are involved

in patterns of signaling in the intact brain observed with other techniques.

Potential Physiological Roles of Glial Ca^{2+} Waves

No physiological role for glial Ca^{2+} waves has been clearly established. However given that an increase in $[Ca^{2+}]_i$ in glial cells may activate ion channels, trigger the release of neuromodulators or trophic factors, or induce changes in glial gene expression, it is easy to speculate that glial Ca^{2+} waves may provide temporal and spatial coordination for these functions. The most immediate question is whether glial Ca^{2+} waves influence neuronal excitability and synaptic activity in vivo. As discussed above, there is now strong evidence that bi-directional glial-neuronal signaling involving glial Ca^{2+} waves occurs in culture preparations. If glial Ca^{2+} waves have similar effects on neuronal activity in vivo, they may represent a mechanism for modulation of neuronal excitability and synaptic signaling that is slow, sustained, spatially organized, and distinct from traditional synaptic interactions.

Regulation of the extracellular environment, particularly extracellular $[K^+]$, is a function that has traditionally been ascribed to glial cells (Janigro et al., 1997; Karwoski et al., 1989; Odette and Newman, 1988; Reichenbach, 1991). Ca^{2+} waves might provide a mechanism for the "spatial buffering" of K^+. A problem with this hypothesis, however, is that an increase in glial $[Ca^{2+}]_i$ could also lead to an *increase* in extracellular K^+, due to opening of Ca^{2+}-activated K^+ channels and subsequent efflux of K^+; such a mechanism might be involved in spreading depression (see below). Another important ionic component of the extracellular environment is Ca^{2+}. We have recently found that glial cells respond to lowered extracellular Ca^{2+} with intercellular Ca^{2+} waves that involve the release of an extracellular messenger (Zanotti and Charles, 1997). We have proposed that this extracellular Ca^{2+} sensing response of glial cells may occur in the setting of excessive neuronal activity, ischemia, or hypoglycemia, where there is a significant decrease in $[Ca^{2+}]$ of the extracellular space (Kristian et al., 1993; Lucke et al., 1995; Puka-Sundvall et al., 1994; Silver and Erecinska, 1992).

Glial Ca^{2+} waves may also play a role in the growth and development of the nervous system. The observation that the extent of propagation of intercellular Ca^{2+} waves in C6 glioma cells overexpressing connexin43 is directly correlated with their rate of proliferation suggests that intercellular Ca^{2+} signaling may be involved in the regulation of glial cell proliferation (Charles et al., 1992). Most of the primary preparations in which glial cell Ca^{2+} waves have been studied are derived from embryonic, perinatal, or immature animals. It is therefore possible that this pattern of signaling is involved in the establishment of the cellular characteristics and connections of the mature nervous system. Yuste and Katz (1995) and Kandler and Katz (1995) have reported spontaneous intercellular Ca^{2+} waves in groups of neurons in the developing cortex, and have suggested that these "domains" of neurons are involved in the establishment of the functional cellular architecture of the cortex. We have observed repetitive, spontaneous, intercellular Ca^{2+} waves in groups of glial cells in both dissociated cortical glial-neuronal cultures (Charles, 1994) as well as in hippocampal slice cultures (Harris-White et al., 1997). It is therefore possible that there may also be "domains" of glial cells that are involved in the development of the cellular architecture of the nervous system.

Several studies suggest that the intercellular communication of glial Ca^{2+} waves may be a target for endogenous signaling molecules in the nervous system. Venance et al. (1995) report that anandamide, an endogenous arachidonic acid derivative that is known to act on cannabinoid receptors, inhibits intercellular coupling and intercellular Ca^{2+} waves in glia. Enkvist and McCarthy (1994) have shown that gap junctional coupling in glia can be altered by exposure to glutamate. Although the functional consequences of these effects on glial intercellular signaling remain uncertain, these studies demonstrate that transmitters whose conventional effects occur through activation of neuronal receptors may also act to modulate glial intercellular Ca^{2+} signaling.

Potential Pathological Roles for Glial Ca^{2+} Waves

A variety of pathological processes in the brain involve slowly propagated changes in activity whose temporal and spatial characteristics are similar to glial Ca^{2+} waves. As discussed above, the possibility that glial Ca^{2+} waves might trigger a propagated change in extracellular ionic concentrations or a propagated release of a neurotransmitter or vasoactive agent provides a hypothetical basis for Ca^{2+} waves in these phenomena. Spreading depression is a propagated excitation followed by a sustained decrease in neuronal activity that may occur in response to a variety of stimuli, including many of the stimuli that initiate glial Ca^{2+} waves (Leao, 1944; Somjen, 1992). Spreading depression propagates at rates of 20–60 μm/sec, which is very close to the rates that have been described for glial Ca^{2+} waves. Spreading depression is blocked by inhibitors of gap junctional communication, raising the possibility that gap junctional signaling between glial cells is involved (Largo et al., 1997; Nedergaard et al., 1995). Additional evidence for a link between spreading depression and glial signaling is the observation that repetitive spreading depression induces changes in GFAP expression in astrocytes (Kraig et al., 1991). Potential mechanisms by which glial Ca^{2+} waves might mediate spreading depression include a propagated increase in extracellular $[K^+]$ or a propagated release of glutamate. However, Largo et al. (1997) also report that fluoroacetate, an inhibitor of glial metabolism, does not

inhibit spreading depression, and therefore conclude that glial activity is not required.

Seizures are characterized by excessive neuronal activity that in some cases propagates slowly from a single focus across multiple territories of normal synaptic connections. The pattern of seizure spread, like that of spreading depression, may in some instances be very similar to that of glial Ca^{2+} waves (Adam et al., 1994; Federico and MacVicar, 1996). Lee et al. (1995) reported that glutamate-induced Ca^{2+} oscillations and intercellular Ca^{2+} waves were more frequent in tissue from epileptic foci as compared with surrounding tissue.

Migraine is another condition that involves wave-like propagation of changes in cellular activity. Migraine aura involves slowly propagated changes in neuronal activity that may be related to spreading depression (Lauritzen, 1994). A decrease in blood flow that spreads slowly across multiple vascular and synaptic territories has been observed in association with migraine (Woods et al., 1994). Since glial cells are known to release vasoactive substances, it is reasonable to suggest that glial Ca^{2+} waves might trigger the propagated release of a vasoactive substance that mediates the spreading hypoperfusion observed in migraine.

Glial Ca^{2+} waves may also play a role in the cellular response to injury in the nervous system. Injury of a single cell consistently evokes intercellular Ca^{2+} waves in glial cells in multiple different preparations. This communicated Ca^{2+} response may therefore coordinate a multicellular response to a localized injury, including release of cytokines and trophic factors, changes in gene expression, and changes in cell morphology.

CONCLUSIONS

Intercellular Ca^{2+} waves are now well established as a pattern of glial cell communication that occurs in response to a variety of stimuli and that may involve multiple mechanisms of inter- and intra-cellular signaling. Current investigation is focused upon the extent to which these waves occur in the intact and the mature brain and spinal cord, and upon their various possible physiological and pathological roles. An increased understanding of this novel pattern of signaling has the potential to provide profound insights into the cellular function of the nervous system.

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REFERENCES

Adam, C., Saint-Hilaire, J.M., and Richer, F. (1994) Temporal and spatial characteristics of intracerebral seizure propagation: Predictive value in surgery for temporal lobe epilepsy. *Epilepsia*, 35: 1065–1072.

Boitano, S., Dirksen, E.R., and Sanderson, M.J. (1992) Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science*, 258:292–295.

Busch, E., Hoehn-Berlage, M., Eis, M., Gyngell, M.L., and Hossmann, K.A. (1995) Simultaneous recording of EEG, DC potential and diffusion-weighted NMR imaging during potassium induced cortical spreading depression in rats. *NMR Biomed*, 8:59–64.

Charles, A.C. (1994) Glia-neuron intercellular calcium signaling. *Dev. Neurosci.*, 16:196–206.

Charles, A.C., Dirksen, E.R., Merrill, J.E., and Sanderson, M.J. (1993) Mechanisms of intercellular calcium signaling in glial cells studied with dantrolene and thapsigargin. *Glia*, 7:134–145.

Charles, A.C., Merrill, J.E., Dirksen, E.R., and Sanderson, M.J. (1991) Intercellular signaling in glial cells: Calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron*, 6:983–992.

Charles, A.C., Naus, C.C., Zhu, D., Kidder, G.M., Dirksen, E.R., and Sanderson, M.J. (1992) Intercellular calcium signaling via gap junctions in glioma cells. *J. Cell. Biol.*, 118:195–201.

Cornell-Bell, A.H. and Finkbeiner, S.M. (1991) Ca2+ waves in astrocytes. *Cell Calcium*, 12:185–204.

Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S., and Smith, S.J. (1990) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science*, 247:470–473.

Dani, J.W., Chernjavsky, A., and Smith, S.J. (1992) Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron*, 8:429–440.

Enkvist, M.O. and McCarthy, K.D. (1992) Activation of protein kinase C blocks astroglial gap junction communication and inhibits the spread of calcium waves. *J. Neurochem.*, 59:519–526.

Enomoto, K., Furuya, K., Yamagishi, S., Oka, T., and Maeno, T. (1994) The increase in the intracellular Ca2+ concentration induced by mechanical stimulation is propagated via release of pyrophosphorylated nucleotides in mammary epithelial cells. *Pflugers Arch.*, 427:533–542.

Federico, P. and MacVicar, B.A. (1996) Imaging the induction and spread of seizure activity in the isolated brain of the guinea pig: the roles of GABA and glutamate receptors. *J. Neurophysiol.*, 76: 3471–3492.

Finkbeiner, S. (1992) Calcium waves in astrocytes-filling in the gaps. *Neuron*, 8:1101–1108.

Frame, M.K. and de Feijter, A.W. (1997) Propagation of mechanically induced intercellular calcium waves via gap junctions and ATP receptors in rat liver epithelial cells. *Exp. Cell Res.*, 230:197–207.

Harris-White, M.E., Zanotti, S.A., Frautschy, S.A., and Charles, A.C. (1997) Spiral intercelular calcium waves in hippocampal slice cultures. *J. Neurophysiol.*, 79:1045–1052.

Hassinger, T.D., Atkinson, P.B., Strecker, G.J., Whalen, L.R., Dudek, F.E., Kossel, A.H., and Kater, S.B. (1995) Evidence for glutamate-mediated activation of hippocampal neurons by glial calcium waves. *J. Neurobiol.*, 28:159–170.

Hassinger, T.D., Guthrie, P.B., Atkinson, P.B., Bennett, M.V., and Kater, S.B. (1996) An extracellular signaling component in propagation of astrocytic calcium waves. *Proc. Natl. Acad. Sci. U.S.A.*, 93:13268–13273.

Janigro, D., Gasparini, S., D' Ambroisio, R., McKhann, G., and DiFrancesco, D. (1997) Reduction of K+ uptake in glia prevents long-term depression maintenance and causes epileptiform activity. *J. Neurosci.*, 17:2813–2824.

Karwoski, C.J., Lu, H.K., and Newman, E.A. (1989) Spatial buffering of light-evoked potassium increases by retinal Muller (glial) cells. *Science*, 244:578–580.

Kim, W.T., Rioult, M.G., and Cornell-Bell, A.H. (1994) Glutamate-induced calcium signaling in astrocytes. *Glia*, 11:173–184.

Kraig, R.P., Dong, L.M., Thisted, R., and Jaeger, C.B. (1991) Spreading depression increases immunohistochemical staining of glial fibrillary acidic protein. *J. Neurosci.*, 11:2187–2198.

Kristian, T., Gido, G., and Siesjo, B.K. (1993) Brain calcium metabolism in hypoglycemic coma. *J. Cereb. Blood Flow Metab.*, 13:955–961.

Largo, C., Tombaugh, G.C., Aitken, P.G., Herreras, O., and Somjen, G.G. (1997) Heptanol but not fluoroacetate prevents the propagation of spreading depression in rat hippocampal slices. *J. Neurophysiol.*, 77:9–16.

Lauritzen, M. (1994) Pathophysiology of the migraine aura: The spreading depression theory. *Brain*, 117:199–210.

Lauritzen, M. and Fabricius, M. (1995) Real time laser-Doppler perfusion imaging of cortical spreading depression in rat neocortex. *Neuroreport*, 6:1271–1273.

Leao, A.A.P. (1944) Spreading depression of activity in cerebral cortex. *J. Neurophysiol.*, 7:359–390.

Lee, S.H., Magge, S., Spencer, D.D., Sontheimer, H., and Cornell-Bell, A.H. (1995) Human epileptic astrocytes exhibit increased gap junction coupling. *Glia*, 15:195–202.

Lucke, A., Kohling, R., Straub, H., Moskopp, D., Wassmann, H., and Speckmann, E.J. (1995) Changes of extracellular calcium concentration induced by application of excitatory amino acids in the human neocortex in vitro. *Brain Res.*, 671:222–226.

Nedergaard, M. (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science*, 263:1768–1771.

Nedergaard, M., Cooper, A.J., and Goldman, S.A. (1995) Gap junctions are required for the propagation of spreading depression. *J. Neurobiol.*, 28:433–444.

Newman, E.A. and Zahs, K.R. (1997) Calcium waves in retinal glial cells. *Science*, 275:844–847.

Odette, L.L. and Newman, E.A. (1988) Model of potassium dynamics in the central nervous system. *Glia*, 1:198–210.

Osipchuk, Y. and Cahalan, M. (1992) Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. *Nature*, 359: 241–244.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S., and Haydon, P.G. (1994) Glutamate–mediated astrocyte-neuron signalling [see comments]. *Nature*, 369:744–747.

Puka-Sundvall, M., Hagberg, H., and Andine, P. (1994) Changes in extracellular calcium concentration in the immature rat cerebral cortex during anoxia are not influenced by MK-801. *Brain Res. Dev. Brain Res.*, 77:146–150.

Reichenbach, A. (1991) Glial K+ permeability and CNS K+ clearance by diffusion and spatial buffering. *Ann. N.Y. Acad. Sci.*, 633:272–286.

Sanderson, M.J., Charles, A.C., Boitano, S., and Dirksen, E.R. (1994) Mechanisms and function of intercellular calcium signaling. *Mol. Cell Endocrinol.*, 98:173–187.

Sanderson, M.J., Charles, A.C., and Dirksen, E.R. (1990) Mechanical stimulation and intercellular communication increases intracellular Ca2+ in epithelial cells. *Cell Reg.*,1:585–596.

Silver, I.A. and Erecinska, M. (1992) Ion homeostasis in rat brain in vivo: Intra- and extracellular [Ca2+] and [H+] in the hippocampus during recovery from short-term, transient ischemia. *J. Cereb. Blood Flow Metab.*, 12:759–772.

Sneyd, J., Charles, A.C., and Sanderson, M.J. (1994) A model for the propagation of intercellular calcium waves. *Am. J. Physiol.*, 266: C293–C302.

Sneyd, J., Wetton, B.T., Charles, A.C., and Sanderson, M.J. (1995) Intercellular calcium waves mediated by diffusion of inositol trisphosphate: A two-dimensional model. *Am. J. Physiol.*, 268:C1537–C1545.

Somjen, G.G., Aitken, P.G., Czeh, G.L., Herreras, O., Jing, J., and Young, J.N. (1992) Mechanism of spreading depression: A review of recent findings and a hypothesis. *Can. J. Physiol. Pharmacol.*, 70 Suppl:S248–S254.

Takeda, M., Nelson, D.J., and Soliven, B. (1995) Calcium signaling in cultured rat oligodendrocytes. *Glia*, 14:225–236.

Venance, L., Piomelli, D., Glowinski, J., and Giaume, C. (1995) Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. *Nature*, 376:590–594.

Venance, L., Stella, N., Glowinski, J., and Giaume, C. (1997) Mechanism involved in initiation and propagation of receptor-induced intercellular calcium signaling in cultured rat astrocytes. *J. Neurosci.*, 17:1981–1992.

Woods, R.P., Iacoboni, M., and Mazziotta, J.C. (1994) Bilateral spreading cerebral hypoperfusion during spontaneous migraine headache. *N. Engl. J. Med.*, 331:1689–1692.

Yuste, R., Nelson, D.A., Rubin, W.W., and Katz, L.C. (1995) Neuronal domains in developing neocortex: mechanisms of coactivation. *Neuron*, 14:7–17.

Zanotti, S.A. and Charles, A.C. (1997) Extracellular calcium sensing by glial cells-low extracellular calcium induces intracellular calcium release and intercellular signaling. *J. Neurochem*, 69:594–602.