Chemical synaptic activity modulates nearby electrical synapses

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Most electrically coupled neurons also receive numerous chemical synaptic inputs. Whereas chemical synapses are known to be highly dynamic, gap junction-mediated electrical transmission often is considered to be less modifiable and variable. By using simultaneous pre- and postsynaptic recordings, we demonstrate at single mixed electrical and chemical synapses that fast chemical transmission interacts with gap junctions within the same ending to regulate their conductance. Such localized interaction is activity-dependent and could account for the large variation in strength of electrical coupling at auditory afferent synapses terminating on the Mauthner cell lateral dendrite. Thus, interactions between chemical and electrical synapses can regulate the degree of electrical coupling, making it possible for a given neuron to independently modify coupling at different electrical synapses with its neighbors.

gap junction | Mauthner | postsynaptic density (PSD) | plasticity | long-term potentiation | long-term depression

The recent cloning of a neuron-specific gap junction gene and *in situ* hybridization and immunolabeling of its mRNA and protein have greatly expanded the known distribution of electrical transmission in mammalian CNS (1–3). Whereas the mechanisms regulating the strength of chemical transmission have been the focus of extensive research, significantly less is known about the plastic properties and modifiability of electrical synapses. Generally, axosomatic and axodendritic gap junctions are associated with presynaptic vesicles clustered at active zones opposed to postsynaptic densities (PSDs) characteristic of chemical transmission (4–6); the close approximation of the two modes of transmission raises the possibility that they could interact by short-range cytoplasmic signaling. We tested for interactions at the “large myelinated club endings” on the Mauthner (M-) cell; these endings form mixed synapses with both gap junctions and chemical active zones and can have both chemical and electrical components in their synaptic potentials (Fig. 1A). Club endings (7) are terminations of auditory afferents and comprise a morphologically relatively homogeneous group of terminals, which are tightly clustered on the distal portion of lateral dendrite of the M-cell (8). The M-cells are a pair of large reticulospinal neurons that mediate tail flip escape responses, which are readily evoked by sound (9). Consistent with the morphological observations, extracellular stimulation of a population of these afferents in the posterior branch of the eighth nerve evokes a mixed excitatory postsynaptic potential (EPSP) in the dendrite composed of a fast electrical component followed by a chemical component mediated by glutamate (10–12; Fig. 1B). Strikingly, patterned high-frequency stimulation of the eighth nerve induces potentiation not only of the glutamatergic response but also of the electrical potential, and this potentiation usually persists for the remainder of an experiment (up to 2 h; refs. 13 and 14). Activity-dependent depression of both electrical and chemical components also has been reported (15). Because potentiation of each component requires *N*-methyl-D-aspartate receptor activation (13), it has been suggested that the observed activity-dependent modification of the electrical component

depends on interaction with the chemically transmitting region(s) of the same synapse (14, 16).

To directly determine the existence and spatial extent of such a relation between chemical and electrical synapses, we obtained multiple paired recordings between single afferent fibers and the same M-cell dendrite. We were able to explore the distribution of synaptic efficacy, which varied among afferents over a wide range. We show that electrical transmission at individual club endings is modulated locally by the activity of the chemically transmitting areas within the same contact and suggest that the observed variability of junctional conductance among terminals arises from different histories of activation by sounds.

Materials and Methods

Electrophysiology. For these experiments, goldfish (*Carassius auratus*) were used and the surgical and recording techniques were similar to those described (12). Individual eighth-nerve afferents ($n = 140$) were penetrated either outside the brain during simultaneous recordings with the M-cell's lateral dendrite (Fig. 1A), or less often, intracranially close to the dendrite (Fig. 2B). Recording electrodes were filled with 2.5 M KCl (35–45 M Ω). Afferents with electrical synapses on the M-cell were identified by the presence of electrotonic coupling potentials when the M-cell antidromic spike was evoked by stimulating the spinal cord (Fig. 2B). For simultaneous recordings, a second electrode (5 M KAc, 4–12 M Ω) was inserted in the M-cell lateral dendrite, 350–400 μ m from the axon cap of the cell. A bipolar stimulating electrode placed on the posterior eighth nerve distal to the recording site was used to activate eighth-nerve fibers (Fig. 1A). Resting and action potentials of afferents averaged -71 ± 0.5 mV (SEM, $n = 140$) and 81.3 ± 1.3 mV (SEM), respectively. The M-cell resting potential averaged -78.7 ± 2.5 mV (SEM, $n = 95$). To obtain activity-dependent potentiation, discontinuous tetanic stimulation of the posterior eighth nerve was used, consisting of trains of four to six pulses at 500 Hz applied every 2 sec during 4 min at a stimulating strength sufficient to occasionally activate the M-cell (13, 14, 16). Tetanization of individual afferents was produced by passing pulses of depolarizing current (1–2 nA for 10 ms) that evoked a burst of five to six presynaptic action potentials at ≈ 500 Hz, every 2 sec during 4 min. Student's *t* test was used to assess statistical significance of the results.

Dye Coupling. For anatomical identification or dye coupling evaluation, auditory afferents and M-cells were intracellularly injected with the tracer Neurobiotin (cation M_r : 286; Vector Laboratories). The electrodes were filled with a 4% solution of Neurobiotin in 2.5 M KCl, and this solution was iontophoretically injected into the recorded afferent or the M-cell's soma (400-ms pulses of 50 nA for 20 min), visualized with avidin-

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Abbreviations: PSD, postsynaptic density; M-cell, Mauthner cell; EPSP, excitatory postsynaptic potential.

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Fig. 1. Electrical EPSPs evoked by stimulation of different afferent fibers differ dramatically in amplitude. (A) Experimental arrangement showing eighth nerve and afferent fibers ending on the ipsilateral M-cell lateral dendrite (*, mixed synapse). Eighth-nerve, antidromic (AD), and pre- and postsynaptic electrodes are indicated. (B) Graded eighth-nerve stimulation evokes graded, mixed electrical and chemical EPSPs (superimposed traces). Here and elsewhere, unless otherwise indicated, traces represent the average of at least 20 individual responses. (C) Unitary electrical EPSPs (1–4, *Upper*) evoked by stimulation of four afferents (*Lower*) obtained sequentially while recording in the same fish at the same dendritic site ($\approx 400 \mu\text{m}$ from soma). The unitary electrical EPSPs (Post) show much greater variability in amplitude than the presynaptic action potentials (Pre). Only one of these four unitary EPSPs exhibited a clear chemical component (trace 1). (D) Histogram showing the amplitude distribution of the unitary electrical EPSPs ($n = 95$; 45 fish).

conjugated diaminobenzidine or FITC and examined under transmitted light or fluorescent optical and confocal (Bio-Rad) microscopes. To avoid unintentional extracellular leakage of Neurobiotin around the club endings during coupling experiments, the injections were performed in the soma, $\approx 400 \mu\text{m}$ from the club endings. After the injections, waiting periods of 1 to 2 h were used to allow diffusion of the dye.

Results

Because auditory afferents are differentially activated by sound (17) and therefore likely subject to different levels of activation, electrical synapses between these terminals might be expected to exhibit different junctional conductances due to differences in potentiation/depotentiation (15). Four lines of evidence confirm this expectation. (i) Unitary electrical EPSPs recorded at

Fig. 2. Electrical synapses at neighboring terminals differ in junctional conductance. (A) Unitary electrical EPSP amplitude was not correlated with difference in time (Δt) between the peaks of the presynaptic spike and the unitary electrical EPSP ($n = 95$). (B) Intraterminal recordings (club ending) obtained after an initial recording of the antidromic spike in the M-cell lateral dendrite (M-cell, *Left*) showed that the amplitude of the antidromic coupling potential also varied in amplitude (1–4, *Right*). The amplitude varied from 0.9 to 7 mV, averaging 4.7 ± 0.28 mV (SEM, $n = 21$). (C–F) Transfer of Neurobiotin at neighboring club endings differs dramatically, indicating that they differ in permeability. (C) View of the bifurcation of the lateral dendrite (LD, dark branches) obtained with Nomarski optics and revealing differences of labeling between two neighboring club endings. Labeled (arrowhead) and unlabeled (arrow) fibers terminate in the same area of lateral dendrite. (D) Tetanic stimulation that causes LTP of the electrical EPSPs increases the number of labeled terminals. The plot summarizes data from five experiments (empty circles) in which Neurobiotin was injected into both M-cells, but only one eighth nerve was tetanized [TET (abscissa), tetanized side; non-TET (ordinate), control side; red circle and lines show means and SDs]. Both M-cells were injected sequentially within an interval of only 5–10 min using the same injection parameters. (E and F) Images of the distal portions of the lateral dendrites of the left (E) and right (F) M-cells from one such experiment where the left eighth nerve was tetanized. No terminals were detected on the nontetanized side in the same $50\text{-}\mu\text{m}$ section (image is in pseudocolor to improve contrast).

Fig. 3. Properties of chemical transmission in unitary and population responses. (A) Selected individual traces from a unitary EPSP in which a chemical component could be detected (presynaptic spikes are not illustrated in these examples). The unitary chemical EPSPs varied in delay and time course, and there were occasional failures of transmission (traces 1 and 5). (B) A chemical component was always detected when a population of eighth-nerve fibers was simultaneously activated (Population, gray bar), but, in contrast, chemical transmission was detected only in 14% of the unitary responses evoked by single-fiber stimulation (Unitary, black bar). (C) Chemical transmission at single club endings is facilitated by coactivation of afferents. Superimposed traces represent population synaptic potentials obtained at different stimulation strengths. The chemical components evoked by weak stimuli (that activate fewer afferents) were significantly smaller than those produced by stronger stimuli. (Inset) The ratio of the amplitudes of the chemical and electrical components (Chem./Elec.) increase with the size of the electrical component (Elec.), an indicator of the number of stimulated fibers.

$\approx 400 \mu\text{m}$ from the soma dramatically vary in amplitude (Fig. 1C), as demonstrated by multiple simultaneous pre- and postsynaptic recordings obtained sequentially from individual afferents (up to seven) and the same M-cell lateral dendrite. The amplitude of these orthodromic responses varied from 0.25 to 2 mV and averaged 0.74 ± 0.04 mV (SEM, $n = 95$; Fig. 1D). The observed variability is likely to represent differences in junctional conductance rather than the relative positions of the different afferent terminals with respect to the intradendritic recording electrode, because no correlation was found between the amplitude of the electrical EPSP and the time between the peaks of the presynaptic spike and the postsynaptic response (Fig. 2A). (ii) The amplitude of the antidromic coupling potential produced by the antidromic M-cell action potential also varied in amplitude as demonstrated by multiple and sequential intraterminal recordings within a radius of 40–60 μm from the lateral dendrite (Fig. 2B), and these amplitudes were correlated with those of the orthodromic responses of the same synapses (not illustrated, but see Fig. 4D). (iii) Camera lucida reconstructions of afferents injected with Neurobiotin showed that both large and small electrical potentials corresponded to club endings, terminating as single terminals at the distal portion of the M-cell lateral dendrite, and no correlation was found between electrical EPSP amplitude and either fiber diameter (a larger terminal might contain more gap junction plaques) or the amplitude of the presynaptic action potential (Fig. 7, which is published as supporting information on the PNAS web site, www.pnas.org). (iv) Tracer coupling to the afferents after injection of the M-cell with Neurobiotin varied markedly between

Fig. 4. Potentiation and depression induced in unitary EPSPs by tetanic stimulation of the eighth nerve. (A) High-frequency stimulation of the eighth nerve (TET) evoked enhancement of both components of the population response (Left) and of a unitary electrical EPSP (Right; no chemical component was detected in the unitary response either before or after the tetanus). (B) Plot summarizing the relationship between tetanus-induced changes in the population electrical EPSP (ordinate) and a unitary electrical EPSP evoked by stimulation of a single fiber in the same fish (abscissa). Potentiation of the electrical and chemical components of the population response averaged $46.9 \pm 13.9\%$ (SEM) and $76.7 \pm 25.6\%$ (SEM) of control, respectively ($n = 12$). In the same experiments, unitary EPSPs were potentiated in five cases, depressed in three, and unaffected in two. In the two cases in which there was depression of the population EPSP, there was no change in the unitary potentials. (C) One of the examples in which the population electrical EPSPs were potentiated (Left) but a unitary EPSP (Right) was depressed. (D) Depression of the unitary electrical EPSP was accompanied by decrease in transmission of the antidromic M-cell spike from dendrite to terminal.

neighboring club endings; unlabeled afferents and afferents showing different degrees of staining were observed in the same dendritic area (Fig. 2C). Only a small number of club endings (6–24) were detected by transfer after injection of the tracer Neurobiotin into the M-cells. The number of labeled afferents represented only 5–25% of the estimated population (≈ 100), suggesting that gap junction permeability at club endings is generally low and that most fibers did not accumulate enough Neurobiotin to produce a visible reaction product. To test whether dye-coupling methods are sensitive to functional differences between neighboring club endings, we asked whether high-frequency tetanic stimulation of the posterior eighth nerve (known to increase the overall conductance of the electrical synapses at these terminals during electrical recordings; refs. 13 and 14) can also increase the number of labeled terminals. For this purpose, Neurobiotin was injected in both M-cell somata, and then the posterior eighth nerve on one side was stimulated tetanically. We found that almost twice as many terminals were detected on the stimulated side. Labeled terminals averaged 21.8 ± 1.5 (SD) for the nontetanzed side (non-TET) and 10.14 ± 5.3 (SD) for the tetanized side ($n = 5$ fish; Fig. 2D). Fig. 2E and F illustrates the contrast between the two sides in one such experiment.

Eighth-nerve stimulation that activated many afferents always evoked a distinct chemical component (Fig. 1B), whereas in confirmation of a previous report (18), most unitary EPSPs (86%, $n = 115$) in paired recordings during the same experiments lacked any detectable chemical component (Fig. 1C). Unitary chemical EPSPs showed, when present, kinetics similar to those observed in population responses (Figs. 1B and C and 3A and C). Most if not all chemically silent club endings can be induced to release transmitter by presynaptic spike broadening (18); thus, the striking discrepancy between population and unitary responses (Fig. 3B) within the same fish suggests that these afferents release neurotransmitter with higher probability

Fig. 5. Eighth-nerve stimulation, which activates a chemical component, is necessary to evoke modifications of the electrical component of unitary EPSPs. (A) Effect of eighth-nerve tetanization on the amplitude of a unitary electrical EPSP. In this example, the unitary potential (Left, control) was increased (Right, after, 12 min and 25 min) by the tetanus (Center, shaded). (B) Tetanization of an individual afferent (Center, shaded, Intracellular TET), which did not evoke a chemical component of the EPSP, did not lead to changes in the amplitude of electrical component (Left, control; Right, after). Horizontal bars in A Right and B Right indicate control amplitudes. (C) Graph summarizing the data obtained for the two stimulating protocols ($n = 12$). Intracellular tetanization had little effect on the unitary electrical EPSPs (filled circles; data from 8 fish). Eighth-nerve tetanization evoked both increases and decreases in the unitary electrical components (same data as in Fig. 4B; 12 fish). Although average changes in amplitude were similar for both protocols, a variance test demonstrated that these populations were different (t test, $P < 0.0001$).

when activated simultaneously. The existence of this property was explored by measuring the amplitude of chemical responses produced by the activation of different numbers of fibers. Accordingly, the chemical components evoked by weak stimuli (that activate fewer afferents) were smaller in amplitude compared with the electrical components than those produced by stronger stimuli (Fig. 3C). Thus, more reliable chemical transmission appears to involve cooperativity of a number of these fibers. Because the increased reliability occurs with the synaptic delay between presynaptic volley and postsynaptic chemical component, the interaction is likely to be electrical and involve larger or longer-lasting presynaptic spikes.

To determine whether activity-dependent modifications could contribute to the wide variability in electrical coupling among club endings, we evaluated the effect of high-frequency (tetanic) stimulation on the size of the unitary EPSPs. The amplitude of the synaptic response to stimulation of a population of fibers (eighth-nerve stimulation) and the amplitude of the unitary response evoked by one of the participating afferents (see Fig. 1A–C) were measured before and after discontinuous tetanic stimulation of the posterior eighth nerve (trains of four to six pulses at 500 Hz were applied every 2 sec during 4 min; test PSPs were evoked every 2 sec; stable baseline recordings of at least 5 min were obtained before tetanic stimulation; the amplitudes of synaptic responses before and after potentiation were determined by averaging at least 20 individual responses). Previous physiological and pharmacological evidence showed that this discontinuous stimulating pattern is essential to evoke potentiation; continuous tetanic stimulation, which rapidly depresses the chemical component, does not cause potentiation (14). Bursting facilitates release of glutamate and allows temporal summation of the synaptic responses for the postsynaptic depolarization presumably necessary to relieve Mg^{2+} block of the N -methyl-D-aspartate receptors, whose activation is necessary for potentiation (12, 14). In most cases, tetanization induced enhancement of both components of the population synaptic response (Fig. 4A and B) that was observed immediately after the tetanus and lasted for the remainder of the recording session (for the time course of these forms of potentiation see ref. 13, 14,

and 16). This enhancement was generally paralleled by equally lasting enhancement in unitary electrical synaptic potentials (Fig. 4A). However, for a fraction of the fibers, the amplitude of the unitary electrical EPSP was unaffected (Fig. 4B) or depressed, which changes were also stable over time. Thus, the electrical component at some endings is depressed, and potentiation and depression of the electrical component coexist within the population of afferents. Fig. 4C and D illustrates one of the examples in which the unitary EPSP was depressed while the electrically mediated population response and, presumably, the electrically mediated responses of most of the terminal connections were potentiated. In contradiction to our expectation, the mean change of the electrical component of the population response was greater than the mean change obtained for the single unit responses [$46.9 \pm 13.9\%$ (SEM) and $7.9 \pm 25.6\%$ (SEM), respectively, $n = 12$]. This difference may be due to cooperativity in orthodromic propagation of action potentials in the afferents, which accounts for the larger chemical component in multifiber EPSPs. Alternatively, the difference may have arisen by chance due to the relatively small number of unitary responses sampled or by bias introduced through selection of afferents based on their relative position on the posterior eighth-nerve root. In contrast to the modifications of electrical coupling, we did not observe an increased incidence of chemical components in single-fiber EPSPs after tetanic stimulation. Only one unitary response showed both electrical and chemical components before tetanization (Fig. 5A). After tetanization the electrical component was potentiated at the need of the tetanus and the chemical component showed a modest and delayed potentiation.

Previous pharmacological data showed the requirement for the chemical component in potentiation (13, 14). For club endings that lack a chemical component when stimulated in isolation, tetanic stimulation of that fiber should be ineffective in inducing activity-dependent changes in electrical transmission at that terminal, although tetanic stimulation of the eighth nerve (where the chemical component is increased) could potentiate it. We asked whether activity restricted to a single fiber can induce changes in electrical coupling. For single-fiber tetanization, we

reproduced the pattern of presynaptic activity observed during eighth-nerve tetanic stimulation by delivering a depolarizing current pulse of sufficient intensity to evoke a burst of five to six presynaptic action potentials at ≈ 500 Hz. In contrast with eighth-nerve tetanization (Fig. 5A), tetanization of an individual afferent did not induce significant changes in coupling (Fig. 5B). Fig. 5C compares the amplitude of unitary electrical EPSPs of these single fibers (all lacking a chemical component) expressed as a percentage of their control values after tetanic stimulation of the single fibers and of another 12 single fibers after stimulation of the eighth nerve.

The requirement for chemical transmission in activity-dependent changes was confirmed in experiments in which single-fiber tetanization was paired with dendritic depolarization. Pairing of single-fiber tetanization with postsynaptic depolarizing current pulses in the postsynaptic M-cell dendrite (50 nA, which depolarized the dendrite to about -15 mV) did not lead to changes in the amplitude of the unitary electrical EPSPs (unitary EPSP averaged $94.1 \pm 4.2\%$ (SEM) of their amplitude at control, $n = 3$; Fig. 8A, which is published as supporting information on the PNAS web site). Because the depolarization did not lead to generation of a chemical component in the EPSP, the lack of potentiation is not surprising. In contrast, if a chemical component of the unitary EPSPs was induced by prior presynaptic injection of 4-aminopyridine (4-AP), single-fiber tetanic stimulation paired with postsynaptic depolarization did cause changes in the electrical component (-40 to 27% , $n = 6$). Both potentiation and depression were induced in different fibers in the same fish; pairing led to a potentiation of $\approx 27\%$ for one fiber and a depression of 20% for a second fiber (Fig. 8B, which is published as supporting information on the PNAS web site).

Consistent with our hypothesis that local rather than widespread interactions between chemical and electrical synapses are responsible for modifications of junctional conductance, the changes induced by the same presynaptic activity pattern differ across the population of afferents and include both depression and potentiation. To test for terminal-specificity of modification of electrical coupling in a more direct way, we determined whether activity in a group of terminals induces changes in a neighboring *inactive* terminal (Fig. 6). The amplitude of the electrical component of the synaptic response to stimulation of a population of fibers and the amplitude of the unitary electrical synaptic potential evoked by one of the participating afferents were measured (Fig. 6A Right and B). Then, the posterior eighth nerve was stimulated tetanically while blocking impulses in the single presynaptic fiber by delivering a hyperpolarizing pulse of the same duration as the stimulus train (-15 to -30 nA, 15-ms duration) through the presynaptic recording electrode (Fig. 6A Center, shaded). Eighth-nerve tetanic stimulation enhanced the electrical component of the population EPSP (Fig. 6B), but the unitary electrical EPSP was unaffected (Fig. 6A Left and C). The plot of Fig. 6D summarizes the results obtained for five terminals, in which eighth-nerve stimulation induced a potentiation of $178.2 \pm 15\%$ (SEM) of control, whereas the single-fiber electrical components were essentially unaffected. These results suggest that a signal from activated chemical synapses that is potentiating electrical transmission at many club endings does not spread the distance between club endings to potentiate transmission at the inactive synapses in the same dendrite.

Discussion

In this article we provide evidence for the existence of local interactions between chemical and electrical transmission sites in single club endings. Activity-dependent modulation of electrical coupling requires activity of nearby chemically receptive zone(s) in the same synapse and, although we cannot rule out a contribution from neighboring terminals, these data suggest that there

Fig. 6. Modulation of single-fiber EPSPs by eighth-nerve stimulation requires activity of the potentiated fiber. (A) The synaptic response to stimulation of a population of fibers and the amplitude of the unitary EPSP evoked by one of the participating afferents was measured before and after high-frequency tetanic stimulation of the posterior eighth nerve (note the difference in the amplitude calibration with respect to the unitary EPSPs). (Left) Control. Unitary electrical EPSP (Upper) evoked by stimulation a single fiber (Lower). (Center) Shaded. The eighth nerve was stimulated tetanically, while hyperpolarizing the presynaptic fiber (-15 nA) to block its action potential. (Right) After. The tetanus did not evoke a change in the unitary EPSP or presynaptic spike recorded at 2 and 30 min after the tetanus. (B and C) Superimposed traces of averaged population and single-fiber responses in control and 2 and 30 min after the tetanus. Although eighth-nerve tetanic stimulation evoked marked changes in the amplitude of both components of the population EPSPs, no change occurred in the amplitude of responses evoked by stimulation of the single fiber that was blocked during the tetanus. (D) The graph summarizes the effects of eighth-nerve tetanic stimulation on population EPSPs and unitary responses mediated by the blocked single fiber after the same tetanus. The population responses were potentiated to $178.2 \pm 15\%$ of control (SEM, open circles; filled circle and line are the mean and SEM, $n = 5$). The single-fiber responses remained at $95.4 \pm 2.2\%$ of their control amplitudes after stimulation (SEM, open circles superimposed and obscured by the filled circle at the mean, $n = 5$). (E) Electrical synapses at club endings differ in conductance (indicated by different colors). Activity of neighboring chemically transmitting regions affects conductance via a PSD-mediated mechanism (arrows). This mechanism is local and does not reach gap junctions at neighboring endings.

is a critical distance for such regulatory control (Fig. 6E). Anatomical data suggest that this "critical distance" is at least a few nanometers (distance between a given PSD and the closest gap junction at a club ending; ref. 8) but necessarily less than ≈ 5 μ m, the distance between the closest neighboring terminals on the surface of the M-cell's lateral dendrite (7). This conclusion is supported by previous findings where, rather than the existence of a generalized signaling mechanism, the differential blocking by Ca^{2+} buffers with different binding kinetics together with the involvement of Ca^{2+} /calmodulin-dependent kinase II (a postsynaptic density-localized kinase with activation proper-

ties suited to detect Ca^{2+} rises; ref. 19) raised the possibility that a major determinant of gap-junctional conductance at each of the terminals is its own individual chemical synaptic activity operating postsynaptically via local calcium signaling (16). Because PSDs are considered to be “signaling machines” that delicately regulate the strength of chemical synaptic transmission (20), taken together with our previous data (16) the present experiment supports the hypothesis that their modulatory roles also includes regulation of nearby gap junctions. Although this modulatory role is local, cooperativity, presumably electrical, with other fibers appears necessary to elicit the chemical component of the EPSPs and to obtain sufficient postsynaptic depolarization to permit Ca^{2+} influx at the *N*-methyl-D-aspartate receptors.

We speculate that the wide range in degree of electrical coupling within the population of single terminals reflects the history of activation of the afferents as they were differentially stimulated by sounds (17). Similarly, whether tetanic stimulation evokes depression or potentiation could be related to differences in the activation history of each afferent. Both depression and potentiation need to coexist for the control of synaptic gain at these terminals. The resemblance of some of the mechanisms for induction of synaptic potentiation at the M-cell (16) to those proposed for hippocampal and neocortical synapses (21, 22) suggests that a concept similar to the “sliding threshold” for potentiation vs. depression (21) would explain why some terminals in the M-cell undergo potentiation whereas others are depressed by the same tetanus. As a result of exposure to sounds

with differing frequency content, the threshold for potentiation (or “modification threshold”; ref. 18) could be heterogeneously distributed across the population of afferents. Indeed, evidence supporting this model has been obtained from these M-cell synapses (15).

The PSD-mediated signaling described here (see also ref. 16) can be relevant not only to M-cell mixed synapses (where it is easier to study) but also to situations in which PSDs belonging to chemical synapses are situated close to dendrodendritic or axodendritic gap junctions formed with other cells. Although mixed synapses have been reported in the mammalian CNS (23, 24), dendrodendritic synapses containing gap junctions rarely have colocalized chemically transmitting zones. However, dendrodendritic gap junctions are generally near to chemical synapses on the same cells (6). Recent freeze-fracture studies on different mammalian CNS neurons (6) revealed that PSDs are located at distances from gap junction plaques comparable to those in the club endings (8). Such an arrangement suggests that this form of gap junction modulation could constitute a widespread property of electrical synapses, relevant to structures such as the retina, inferior olive, olfactory bulb, hippocampus, and neocortex where glutamatergic transmission and gap junctions coexist. Moreover, other ion channels might be similarly regulated by PSD-mediated signaling.

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