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Facilitating and nonfacilitating synapses on pyramidal cells: A correlation between physiology and morphology

(synaptic facilitation/piriform cortex/synaptic vesicles)

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ABSTRACT **Pyramidal cells in piriform cortex receive excitatory inputs from two different sources that are segregated onto adjacent segments of their apical dendrites. The present studies show that excitatory postsynaptic potentials (EPSPs) evoked by primary olfactory tract afferents that terminate on distal apical segments display paired shock facilitation whereas ESPSs evoked by intrinsic association fibers that terminate on proximal apical segments do not. An ultrastructural comparison of the presynaptic elements of these two fiber systems has revealed that the facilitating olfactory tract afferent synapses have a much lower packing density of synaptic vesicles than do the nonfacilitating association fiber synapses. Further, a search of the literature has revealed that where both morphological and physiological data are available for the same synapses, this same correlation appears to apply. We propose a hypothesis to account for this correlation based on synaptic vesicles to buffer internal calcium and the biochemical characteristics of preterminal calcium-dependent mechanisms affecting the number of vesicles available for release.**

At many synapses, the second of an identical pair of appropriately timed presynaptic activations evokes a larger excitatory postsynaptic potential (EPSP) than the first (1–4). This synaptic property, termed paired shock facilitation, has been demonstrated in numerous peripheral and central nervous system synapses. However, there are also synapses that do not facilitate. The factors underlying this difference in the physiological characteristics of different synapses are as yet unknown, but presumably a fundamental mechanism regulating neurotransmitter release is involved. We report here the existence of a system that appears to be well suited for study of the mechanism of paired shock facilitation. Using *in vitro* brain slices of olfactory (piriform) cortex, we have found that the apical dendrites of single pyramidal cells are contacted by facilitating and nonfacilitating synapses with each type spatially segregated from the other and arising from different fiber systems. Specifically, EPSPs evoked by primary olfactory tract afferents that terminate on distal apical dendritic segments display paired shock facilitation, whereas EPSPs evoked by intrinsic association fibers that terminate on proximal apical segments do not (see Fig. 1). Previous ultrastructural studies comparing the presynaptic elements of these two fiber systems showed that the facilitating olfactory tract afferent synapses have a much lower packing density of synaptic vesicles than do the nonfacilitating association fiber synapses. Further, a search of the literature has revealed that where both morphological and physiological data are available, this same correlation appears to apply for other excitatory synapses. We therefore propose a hypothesis that may account for this correlation based on the ability of synaptic vesicles to buffer internal calcium and therefore affect the ability of subsequent vesicles to be mobilized for release. These results have been presented previously (5).

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METHODS

The present experiments were performed by using an *in vitro* brain slice preparation of the piriform cortex of albino Sprague–Dawley rats. Slices (300 μm thick) were cut perpendicular to the laminar organization of the cortex (Fig. 1) and maintained *in vitro* using standard techniques (6). Boundaries between cortical layers Ia, Ib, II, and III (Fig. 1) can be readily visualized by transmitted light in this slice preparation, allowing stimulating and recording electrodes to be placed accurately within any layer. Afferents from the lateral olfactory tract or association fibers were activated by using tungsten microelectrodes, insulated except at the tip, which had been placed in layer Ia or Ib, respectively (see Fig. 1). Very low stimulation shock strength was used to minimize inhibitory and disynaptic excitatory responses. Intracellular recordings were made from cells in layer II (see Fig. 1) with micropipettes filled with 2–4 M potassium acetate (60- to 80-MΩ resistance). In one experiment, three cells that displayed the response characteristics described in this report were stained by intracellular injection of horseradish peroxidase as in our previous *in vivo* study (7). All were found to be pyramidal cells with apical dendrites extending through layers Ia and Ib. Potential recording, intracellular current injection, and data analysis were as described (7). Only those cells with stable resting potentials of at least −65 mV were studied.

RESULTS

Fig. 2 illustrates the major finding of these experiments. When layer Ia was stimulated with paired shocks having an interstimulus interval between 10 and 200 ms (20 ms in Fig. 2*A*), the second EPSP was markedly facilitated. This result is in agreement with previous reports based on extracellular recording methods (8–12). However, when identically timed paired stimuli were given to layer Ib (Fig. 2*C*), no facilitation of the second EPSP was seen. Fig. 3 compares the amplitudes of the EPSPs evoked in one cell by individual paired trials of layer Ia and Ib stimulation and demonstrates no overlap in the behavior of these two types of synapses with respect to facilitation.

Though synaptic facilitation would seem the most likely explanation for the layer Ia paired stimulation effects, several possible alternative explanations for enhancement of EPSPs by preceding stimuli also need to be considered. First, layer Ia activation could evoke an apparent increase in response amplitude by increasing postsynaptic membrane resistance.

Abbreviation: EPSP, excitatory postsynaptic potential.

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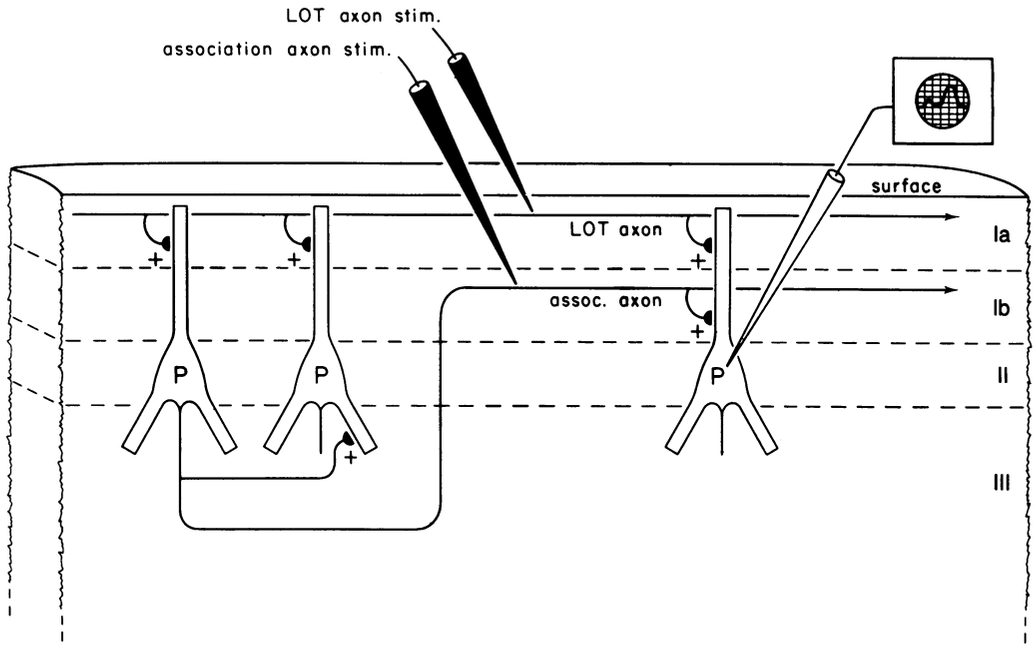

FIG. 1. Schematic diagram of a piriform cortex slice showing the positions of stimulating (black) and recording (white) electrodes and the relevant cortical lamination, fiber systems, and excitatory synaptic connections. LOT, lateral olfactory tract (afferent fibers from the olfactory bulb); P, pyramidal cell.

Although a local dendritic resistance change cannot be completely ruled out, the experiment illustrated in Fig. 2*B* strongly contradicts this alternative explanation by showing that layer Ia stimulation does not enhance the EPSP evoked by layer Ib stimulation in adjacent dendritic segments. A second way in which layer Ia stimulation could produce a larger second Ia EPSP is if olfactory tract afferents have lowered thresholds for reactivation after a first shock. However, previously reported experiments have specifically ruled out this possibility for the *in vitro* piriform cortex slice preparation by showing that the amplitude of submaximal lateral olfactory tract extracellular spike potentials are unaffected by conditioning volleys (10).

Inhibitory current shunting effects must also be considered as a possible explanation for the present results. For example, an increase in membrane conductance following layer Ib stimulation could negate the effect of facilitation on a second layer Ib stimulation. This possibility was ruled out in several ways. First, it was demonstrated that layer Ib stimulation had no effect on subsequent layer Ia evoked EPSPs (Fig. 2*D*). If an increase in membrane conductance resulted from layer Ib activation, it would have been apparent in this experiment because layer Ib synapses lie between layer Ia synapses and the recording site (see Fig. 1). It is also possible that layer Ib stimulation activates an inhibitory effect at or near the level of the cell body that could mask paired shock facilitation. This was ruled out by direct measurement of conductance by intracellular current injection (Fig. 4). Finally, we have recorded paired EPSPs after perfusing the slice with medium containing 100 μM picrotoxin, which is known to block chloride-mediated inhibitory postsynaptic potentials in piriform cortex (7, 13). Although this treatment resulted in apparent seizure activity at high shock strengths, with low shock strengths the facilitatory properties of layer Ia and Ib synapses were unchanged.

To establish clearly the existence of synaptic facilitation, it is also necessary to rule out possible excitatory or disinhibitory multisynaptic effects. Though apparent multisynaptic effects are generated *in vivo* and *in vitro* with moderate to high shock strengths (7, 11, 14), at the low strengths used in the present studies the smooth monophasic shapes of layer Ia and Ib evoked EPSPs suggest an exclusive monosynaptic origin. However, to further rule out multisynaptic effects, we made a fine cut under layer II in two experiments to section all pyramidal cell association axons (see Fig. 1). After 1–2 hr of recovery, we could still record −65 to −70 mV resting potentials from cells in layer II. This procedure appeared to remove the multisynaptic components seen in intact slices because high shock strengths increased EPSP amplitudes without changing their shapes. After undercutting, both high and low shock strengths evoked responses to paired layer Ia and Ib stimulation that were identical to those evoked by low shock strengths in nonundercut slices.

Based on these control procedures that rule out the most likely alternative explanations, we conclude that olfactory tract afferent synapses display synaptic facilitation, whereas association fiber synapses on the same pyramidal cells do not facilitate.

DISCUSSION

Recent experiments in this laboratory have taken advantage of the different origins of the layer Ia and Ib fiber systems to make a comparison of the ultrastructure of the synapses associated with each (15). This comparison revealed that the density and distribution of synaptic vesicles in the presynaptic terminals of these two systems is one of their most strikingly different features. The nonfacilitating association fiber synapses in layer Ib have a much higher packing density of vesicles than the facilitating olfactory bulb afferent terminals in layer Ia. This difference is especially apparent in the area adjacent to the presynaptic density, where vesicles in layer Ib synapses are found in compact clusters, whereas those in layer Ia synapses are more sparsely and evenly distributed. Although this synaptic morphology was described by using autoradiographic axonal transport methods in opossum piriform cortex, studies with degeneration methods suggest that a similar difference is present in the rat (16).

What makes this result particularly intriguing is that a subsequent survey of the literature reveals that this association of sparse vesicles with facilitating synapses and packed vesicles with nonfacilitating synapses is found in other

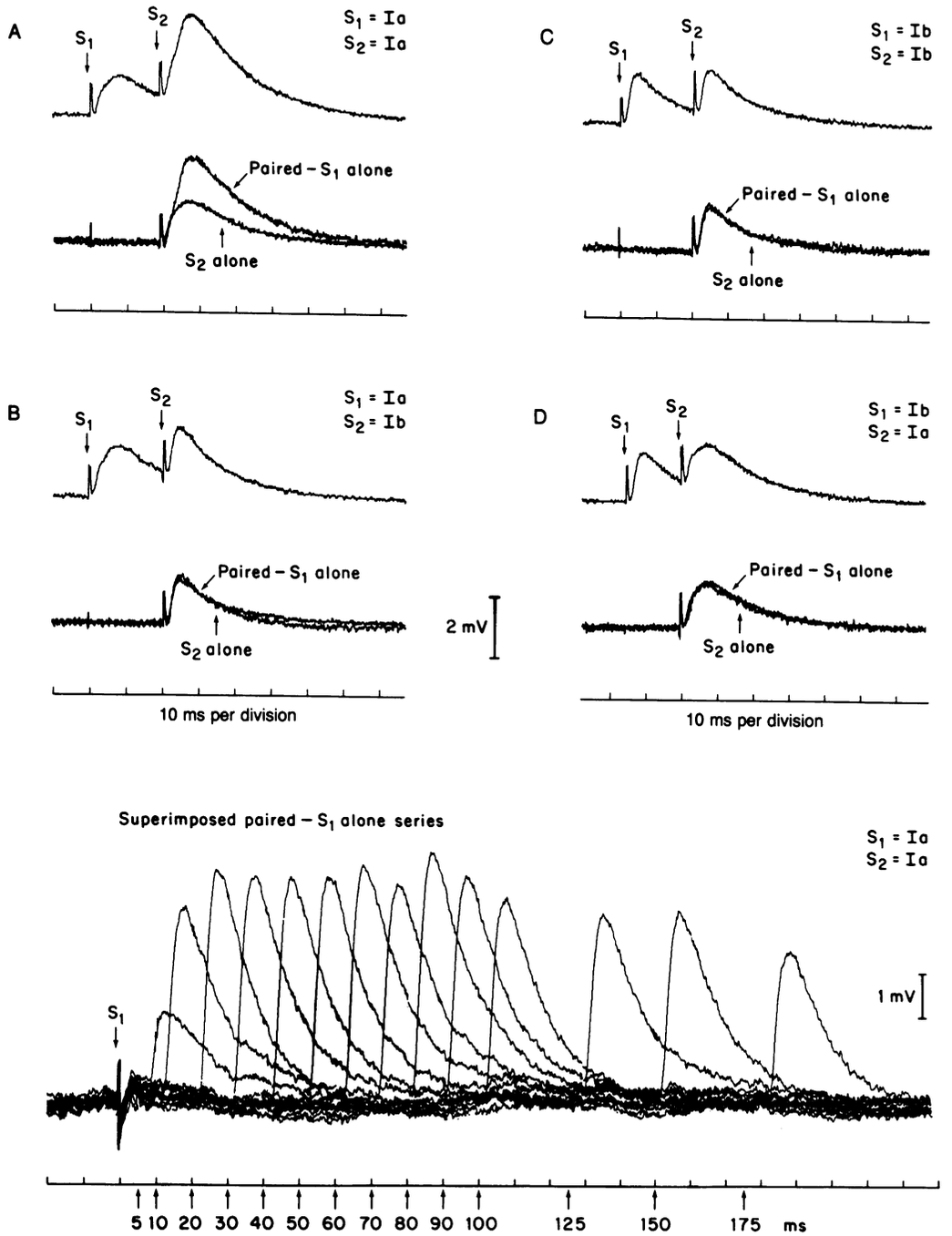

FIG. 2. Comparison of the synaptic effects on a neuron recorded in layer II of different paired combinations of layer Ia and/or layer Ib stimuli. For the sets of intracellular records shown in *A–D*, the uppermost trace is the average of several responses to the indicated stimulus pair, whereas the lower trace shows averaged control responses to the second stimulus alone superimposed on the averaged response to the second stimulus when it is preceded by the first. In the latter trace, the tail of the first response has been removed by subtraction. The voltage and time scales are the same for all records. The series of superimposed averaged traces at the bottom of the figure show the time course of layer Ia-evoked facilitation. The arrows below the time line indicate the interstimulus delay between pairs for each superimposed record. The response to the first stimulus alone is not shown but was similar in amplitude to the response evoked after a 175-ms delay.

synapses in which both physiological and morphological data are available. For example, in the torpedine electric organs of *Narcius brasilienis*, the main electric organ synapses do not facilitate, whereas the accessory electric organ synapses do (17, 18). Correspondingly, the nonfacilitating synapses of the main organ have 75% more vesicles located next to the presynaptic junctional area than do the facilitating synapses of the accessory organ. A second and particularly interesting example is found in the dorsal spinal cord, where group Ia afferent synapses have very dense vesicle packing (19) and physiological recordings from spinocerebellar tract neurons on which these fibers synapse (20) reveal no facilitation in group Ia afferent evoked EPSPs (21). On the other hand, group Ia synapses in the ventral cord have less dense vesicle packing (19, 22), and the EPSPs they evoke in motoneurons do facilitate (21, 23, 24). Furthermore, synapses made by different branches of a single identified group Ia axon display this different morphology in the ventral and dorsal horn (19), raising the possibility that vesicle density and synaptic function may be regulated locally, perhaps by the postsynaptic cell. Equally intriguing is the result of physiological experiments in which the facilitating properties of individual

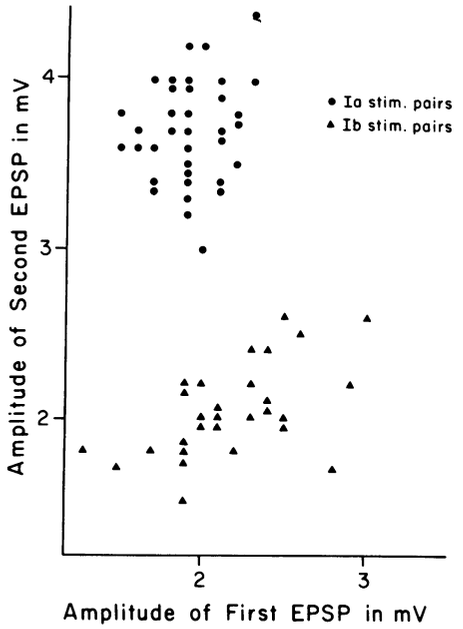

FIG. 3. Relative amplitude of EPSPs evoked by individual paired layer Ia and Ib stimulation trials in a single neuron (average records shown in Fig. 2 *A* and *C*). Note that in every trial the second of two layer Ia-evoked EPSPs was 1.5–2.0 times greater in amplitude than the first.

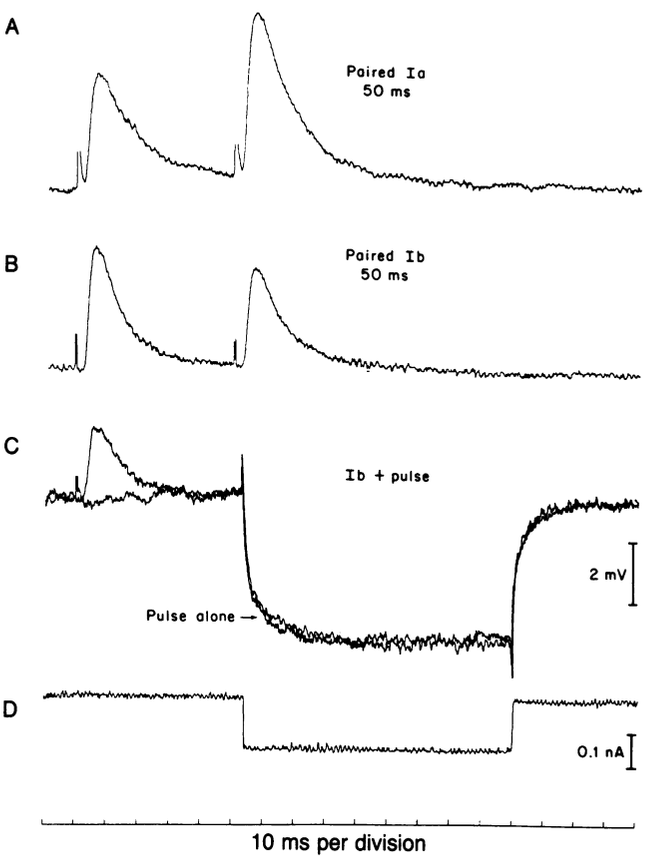

FIG. 4. Demonstration of the lack of conductance change following layer Ib stimulation. (*A* and *B*) Intracellular response of a neuron recorded in layer II to identical paired layer Ia (*A*) and Ib (*B*) stimuli. (*C*) Response recorded to a single layer Ib stimulus followed by an intracellular current injection occurring at the same latency as the second EPSP in records *A* and *B*. The response to a control current pulse has been superimposed. The lack of a significant membrane conductance change at the time of the second EPSP is revealed by the similar time constants for the rising phases of the voltage changes resulting from the control and post-EPSP current pulses. (*D*) Current monitor record. All responses were averages and were recorded from the same cell. Voltage scales for *A–C* are identical.

group Ia synapses on motoneurons in the ventral spinal cord were studied (22, 23). These synapses were found to vary in their degree of facilitation, a result which we would hypothesize may parallel variabilities in vesicle packing densities also seen in these synapses (19, 22).

Though the results presented in this paper do not directly address the biochemical mechanism underlying synaptic facilitation, the observed inverse relationship between the occurrence of facilitation and the density of synaptic vesicles when coupled with other biochemical results does suggest a working hypothesis. Strong physiological evidence suggests that facilitation like that seen in layer Ia synapses is mediated by a presynaptic mechanism involving calcium (1–4, 25–27) that produces an increase in the amount of transmitter released (4, 25). Further, recent modeling efforts have emphasized the important role played by intraterminal calcium buffering in kinetics of transmitter release (28–33). These results are interesting in light of our own because of the several lines of evidence indicating that synaptic vesicles can act as strong buffers of cytosolic calcium (17, 34–39). Given the packed vesicle morphology of layer Ib synapses and calcium uptake by presynaptic vesicles, we would expect a substantially increased calcium buffering capacity in these synapses. This is especially so near the presynaptic density where the most densely packed vesicles are seen and calcium channels are presumably located (40, 41). The resulting decrease in the stimulus-induced internal free calcium in these synapses would be expected to result in less calcium being available to trigger the recruitment, positioning, or other readying of vesicles for release by subsequent depolarizations. In contrast, the lesser calcium buffering capacity that may result from the relatively lower density of presynaptic vesicles in layer Ia synapses could result in more postdepolarization calcium-dependent activity.

Recent studies of synapsin I, a vesicle-associated protein found in large concentrations in presynaptic endings, have illuminated one such calcium-dependent mechanism (42, 43). Specifically, it has been proposed that kinases activated by a transient stimulus-induced increase in internal calcium would phosphorylate synapsin I, resulting in its dissociation from synaptic vesicles. Without synapsin I, vesicles may rapidly move to a release-ready position close to the synaptic cleft. Based on this mechanism, we propose that the facilitated EPSP resulting from the second of two shocks is a direct result of an increase in the number of vesicles properly positioned for release as a result of the first shock. Accordingly, we would predict that following a single activation of afferent fibers, layer Ia synapses would undergo a transient 200-ms increase in the number of vesicles adjacent to the synaptic cleft. Note that even without the buffering of calcium by presynaptic vesicles, such a mechanism would be expected to have less of an influence on the layer Ib synapses, whose vesicles are already in a highly packed state. Based on this reasoning, any experimental manipulation that reduces the number of vesicles available for release at nonfacilitating synapses would be expected to increase the ability of these synapses to facilitate. Exactly this result has been reported in *N. brasilienis*, in which high frequency (tetanic) bursts of afferent stimulation deplete presynaptic vesicles and result in a subsequent facilitation of EPSPs in the normally nonfacilitating packed vesicle synapses of the main electric organ (18) [similar result in neuromuscular junction (44)]. In the central nervous system, normally nonfacilitating synapses with packed synaptic vesicles also have been shown to facilitate following tetanic stimulation (45–47).

If the ability of synapses to facilitate is directly linked to the number of vesicles available for release, then another prediction that can be made is that, all other things being equal, individual nonfacilitating layer Ib synapses should produce more transmitter release per single presynaptic activation than should individual layer Ia synapses. Unfortunately, this kind of information is difficult to obtain in central synapses, but in the crayfish opener neuromuscular junction preparation, where facilitating and nonfacilitating synapses can be directly compared while controlling for postsynaptic effects, it has been shown that nonfacilitating synapses do in fact release more quanta per single impulse than do facilitating synapses (28, 48). All of these results, we believe, lead to the conclusion that the morphologies of presynaptic terminals may provide an important clue to their physiological characteristics. Further, if the morphologies of these synapses are directly related to facilitation in the way that has been suggested, then how these morphologies are established, maintained, and changed by synaptic activity becomes an important question for future studies.

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