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Ca^{2+} influx and neurotransmitter release at ribbon synapses

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Abstract

Ca^{2+} influx through voltage-gated Ca^{2+} channels triggers the release of neurotransmitters at presynaptic terminals. Some sensory receptor cells in the peripheral auditory and visual systems have specialized synapses that express an electron-dense organelle called a synaptic ribbon. Like conventional synapses, ribbon synapses exhibit SNARE-mediated exocytosis, clathrin-mediated endocytosis, and short-term plasticity. However, unlike non-ribbon synapses, voltage-gated L-type Ca^{2+} channel opening at ribbon synapses triggers a form of multiquantal release that can be highly synchronous. Furthermore, ribbon synapses appear to be specialized for fast and high throughput exocytosis controlled by graded membrane potential changes. Here we will discuss some of the basic aspects of synaptic transmission at different types of ribbon synapses, and we will emphasize recent evidence that auditory and retinal ribbon synapses have marked differences. This will lead us to suggest that ribbon synapses are specialized for particular operating ranges and frequencies of stimulation. We propose that different types of ribbon synapses transfer diverse rates of sensory information by expressing a particular repertoire of critical components, and by placing them at precise and strategic locations, so that a continuous supply of primed vesicles and Ca^{2+} influx leads to fast, accurate, and ongoing exocytosis.

Keywords

ribbon synapses; calcium channels; exocytosis; synaptic transmission; transmitter release; short-term plasticity; calcium microdomains

Introduction

The synapses between auditory hair cells and afferent fibers are the first chemical synapse where transmission of the signals involved in hearing starts. Hair cells in the cochlea detect graded sound wave signals and convert them into bioelectrical signals, namely the opening of Ca^{2+} channels and exocytosis. Release of glutamate onto the afferent fibers then leads of excitatory synaptic currents (EPSCs) that depolarize the afferent fibber, thus triggering all-or-none spikes. A similar form of analog-to-digital conversion of sensory signals also occurs in the retina, where graded light and dark signals are detected in photoreceptors and eventually converted to all-or-none spikes at ganglion cell axons. This transduction and encoding of incoming sensory information seems to require ribbon synapses at the first excitatory synapse of the auditory and visual systems, and even at the second (bipolar cell) excitatory synapse of the retina. Unlike most neuronal synapses, the distinctive characteristics of hair cells, photoreceptors and retinal bipolar cells are that they are able to

manage both tonic and graded release of synaptic vesicles at ribbon-type active zones without the necessity of action potential firing.

The molecular basis for Ca^{2+}-dependent exocytosis at ribbon synapses

Specialized organelles, called synaptic ribbons, are found in auditory and vestibular hair cells as well as the lateral line of teleosts. Ribbon synapses are also present in photoreceptor terminals and bipolar cell terminals in the vertebrate retina, and in electroreceptors in some marine vertebrates. Ribbons are electron-dense discs that tether a halo of synaptic vesicles via fine filaments [1]. The shapes of ribbons are varied; some are spherical, ellipsoidal or bar shaped depending on cell type, species, and developmental stages (Figure 1) [2, 3]. Ribbons may play a role in delivering vesicles to release sites and they probably contribute to create conditions that favor prominent multivesicular release [4–6]. Previous studies show that hair cells and photoreceptors lacking a scaffolding protein called bassoon also lack ribbons anchored to their plasma membrane at active zones, and show severe deficits of synaptic transmission in the retina [7] and cochlear inner hair cells [8]. This suggests that the ribbons may be required for the fast and precise release of synaptic vesicles during the onset of a stimulus and for the continuous high-rate of release during the stimulus [9]. However, the exact role of the synaptic ribbons remains elusive since some non-ribbon (conventional) synapses, like mossy fibers in the cerebellum, also can release large amounts of vesicles in a fast and continuous manner [10].

Although ribbon synapses and non-ribbon synapses function differently, the molecular composition of ribbon synapses appears to be mostly conserved and similar to that of non-ribbon synapses. Syntaxins, SNAP25, and synaptobrevin/VAMP (the three core SNARE proteins) are found in hair cells [11, 12] and in the photoreceptors and bipolar cells [1]. However, some synaptic proteins are unique to ribbons, such as RIBEYE, a novel protein with homology to both a transcription repressor factor (CtBP2) and a NADH dehydrogenase [13]. RIBEYE has been used as a useful tool to specifically target the synaptic ribbon with fluorescence [14–17]. In addition, ribbon synapses lack certain proteins that are found in non-ribbon synapses, such as the synapsin family of proteins. Synapsins link vesicles to the actin cytoskeleton in non-ribbon synapses and they appear to be absent from ribbon synapses. Synaptotagmins 1 and 2, the main putative Ca^{2+}-sensitive mediators of fast and synchronous release in the brain have not been found in the adult cochlea [11], but are present in photoreceptors and some bipolar cells. Instead, otoferlin is the proposed Ca^{2+} sensor for exocytosis in adult cochlear hair cells [18, 19, 119], though it may also have a Ca^{2+}-dependent vesicle recruitment function [20, 119]. However, inner hair cells in adult hypothyroid rats, which apparently do not express otoferlin, still can release transmitter, so otoferlin's exact role is still under investigation [21, 22]. Recent studies have also shown that the expression of synaptotagmins in mammalian hair cells depends on their developmental stage [22]. In addition, hair cells lack complexins [23, 121], which are expressed in retinal ribbon synapses [24]. Therefore, the composition of ribbon synapses in hair cells differs remarkably from that in the retina, and from that of non-ribbon synapses, though there are also many fundamental similarities.

Ca^{2+} channels and spatial-temporal profile of Ca^{2+} influx

The essential role of Ca^{2+} in triggering neurotransmitter release has been well established [25, 26]. Basically, Ca^{2+} influx through open voltage-gated Ca^{2+} channels located at the active zone of a presynaptic terminal triggers neurotransmitter release via synaptic vesicle exocytosis [27]. In conventional synapses, voltage-gated Ca^{2+} channels open when an action potential invades the presynaptic terminal and depolarizes the membrane potential. In

contrast, graded membrane potential changes in ribbon synapses trigger transmitter release without sodium-based action potentials.

Among several types of voltage-gated Ca^{2+} channels (L-, N-, P/Q-, T- and R-type), the P/Q-type channel and the N-type channels mainly control transmitter release at most CNS bouton-type synapses and at the neuromuscular junction [28]. However, at ribbon synapses, the L-type channel is instead critical for neurotransmitter release, though some hair cells show multiple Ca^{2+} channel types [29]. Interestingly, Ca^{2+} currents show a very negative voltage activation range and a rapid gating in hair cells [6, 30, 113, 114]. Ca^{2+}-dependent inactivation of Ca^{2+} currents has been observed in frog and turtle auditory hair cells [6, 114].

The entry of Ca^{2+} through voltage-gated Ca^{2+} channels increases the intracellular Ca^{2+} concentration transiently in a spatially restricted “microdomain” of the active zone [31, 32]. The microdomain concept can explain the rapid onset and cessation of neurotransmitter release in fast synaptic transmission. In non-ribbon synapses, such as calyx of Held, the Ca^{2+} sensor for neurotransmitter release is thought to be positioned very close to these Ca^{2+} channels at some active zones, so that transmitter release occurs within less than a millisecond [33]. In fact, the delay between Ca^{2+} influx and the fusion of synaptic vesicles at different synapses can be as little as 60–350 μsec. This suggests that Ca^{2+} diffuses less than 100 nm to trigger the exocytosis of synaptic vesicles [34–36]. Given the homeostatic Ca^{2+} handling mechanisms (Ca^{2+} pumps, Ca^{2+} exchangers and Ca^{2+} buffers) present within nerve terminals, free Ca^{2+} cannot diffuse great distances in the nerve terminal, unless all these mechanisms are saturated by large Ca^{2+} influx loads.

The microdomain model predicts the existence of temporally and spatially restricted increases of intracellular Ca^{2+}, so called Ca^{2+} hotspots. In hair cells, such Ca^{2+} hotspots have been detected along the basolateral membrane of the hair cell where ribbons are located [37–39], whereas regions distal from the ribbons show only small and slow increases of Ca^{2+} signals, which are not coupled with the stimulus (Figure 2 and 3). This tight temporal and spatial regulation of intracellular Ca^{2+} requires strong Ca^{2+} clearance mechanisms such as a variety of Ca^{2+} ATP-ase pumps and Ca^{2+} exchangers in addition to a large concentration of mobile Ca^{2+} buffers. Mobile Ca^{2+} buffers can reduce free Ca^{2+} at the synapse on a fast time scale by binding Ca^{2+} ions that enter the cell and then shuttling them away from the active zone [40]. In contrast, fixed Ca^{2+} buffers may prolong the duration of the Ca^{2+} transients after rapidly binding the free Ca^{2+} and then releasing it with a delay [41]. Mobile Ca^{2+} buffers such as calretinin and parvalbumin help to spatially restrict and localize free Ca^{2+} ions within the bullfrog saccular hair cell [42–44]. High concentration of an exogenous Ca^{2+} buffer (for example 10 mM BAPTA) greatly restricts the spread of Ca^{2+} to the presynaptic dense body area (Figure 3E). Nevertheless, large EPSCs can still be observed at the postsynaptic afferent fiber with relatively mild depolarizations of the hair cell (Figure 3F). This suggests that the Ca^{2+} rise underneath the ribbon is sufficient to trigger large EPSCs and transiently overwhelms the various Ca^{2+} pumps, Ca^{2+} buffers, and intracellular Ca^{2+} stores that function to remove cytoplasmic Ca^{2+}. Plasma membrane Ca^{2+} ATPases, Na/Ca exchangers, the sarcoplasmic-endoplasmic reticulum Ca^{2+} pumps (SERCAs), and Ca^{2+} uptake into mitochondria play important roles in Ca^{2+} removal at different ribbon-type synapses [37, 45–48].

The Ca^{2+}-sensor molecules for exocytosis at ribbon synapses

At the individual vesicle level, there are several steps that bring synaptic vesicles into close apposition with presynaptic Ca^{2+} channels. Vesicles do not traffic through the cytoplasm randomly and most synaptic vesicles in presynaptic terminals are not immediately available

for fusion. Cytoskeletal elements, such as actin, myosin and motor proteins, may play important roles in the transport of vesicles. The disassembly of the actin network may control the transit of vesicles from the reserve pool to the readily releasable pool and this process can be regulated by Ca^{2+} and ATP [49–51]. A complex of proteins, called the "exocyst" interacts with the cytoskeleton to target vesicles to the active zone. Rab-3 may regulate the alignment of vesicles in the docking area and assist in the displacement of n-sec-1 from syntaxin (this step is often called tethering). Then, syntaxin and SNAP25 bind to VAMP to form the core SNARE complex. This step "docks" a vesicle to the release site at the active zone, which contains the presynaptic Ca^{2+} channels. Ca^{2+} binding to the Ca^{2+} sensor for exocytosis is thought to cause vesicle fusion [52]. After vesicle fusion, cytoplasmic NSF/α-SNAP are thought to dissociate the tight SNARE core complex so that vesicles and their associated membrane proteins can be recycled [50, 53]. However, there is recent evidence that vesicle release at ribbon synapses may use atypical processes in comparison with non-ribbon synapses. A convincing study has shown that the typical neuronal SNARE proteins, which are specifically cleaved by neurotoxins, do not seem to be involved in the exocytosis process of mouse inner hair cells [120]. It is possible that the structure of the SNARE core complex may be more dynamic and the SNARE complex may be less stable (or less tightly coupled) in hair cell ribbon synapses [121, 122]. This may permit a more continuous and higher fusion rate at hair cells, which are epithelial cells that are not derived from the neural crest during development, and thus may contain non-typical SNARE molecules.

Based on its ability to bind to Ca^{2+}, and to link Ca^{2+} influx and vesicle fusion, the best candidate Ca^{2+} sensor for transmitter release is synaptotagmin in non-ribbon synapses. Synaptotagmin is a vesicle membrane protein with two cytoplasmic Ca^{2+}-binding motifs, called C2 domains (the C2A and C2B domains) [54, 55]. Synaptotagmin 1 is thought to function as a Ca^{2+} sensor regulating the fast, phasic exocytosis of synaptic vesicles [55]. Despite incomplete information, synaptotagmin 2 seems functionally similar to synaptotagmin 1 [56, 57], but synaptotagmin 5–7, 10 appear to be related with asynchronous release of transmitter [56]. Synaptotagmin 4 is predominantly expressed postsynaptically and may function in the release of retrograde signals that enhance presynaptic release [58]. Synaptotagmin 1 has 5 Ca^{2+} binding sites; the C2A domain binds 3 Ca^{2+} ions and the C2B domain binds 2 Ca^{2+} ions [59]. Both C2 domains have low intrinsic affinity for Ca^{2+}. The affinity for Ca^{2+} of C2 domains strongly increases into physiological ranges when the C2 domains bind to phospholipids in the plasma membrane. Under these phospholipid binding conditions, the overall Ca^{2+} affinity of the C2 domains increases up to 5000 fold (K_D = 5–50 μM), because additional coordination sites for Ca^{2+} are probably provided by the negatively charged phospholipid headgroups [60, 61]. Other synaptic molecules binding with synaptotagmin, such as syntaxin, SNAP-25, and intracellular domains of the voltage-gated Ca^{2+} channel, also affect Ca^{2+}-binding affinity of C2 domains [62]. The Ca^{2+} binding affinities described above may be relative values rather than absolute, because they depend on the composition of the phospholipid membranes [60, 61]. Depending on the exact lipid composition of the fusion sites, the real Ca^{2+} affinities may vary by a factor of 2–4 [61]. A previous study measured the response time of the synaptotagmin C2 domain and demonstrated that the synaptotagmin C2A domains could respond rapidly to both increases and decreases in Ca^{2+} concentration, which was fast enough to fit known rates for Ca^{2+}-triggered vesicle fusion. They also showed that Ca^{2+} binding triggered synaptotagmin penetration into the membrane and led to a simultaneous binding to the SNARE complex [63].

The precise dependence of transmitter release on Ca^{2+} concentration varies among different synapses. However, in many non-ribbon synapses, a nonlinear relationship, such as a third to fourth order cooperative relationship, between Ca^{2+} concentration and transmitter release is

observed across various synapses and species, including the squid giant synapse [34, 64], the crayfish neuromuscular junction [65], the frog neuromuscular junction [26, 66], the calyx of Held [67, 68] and hippocampal CA3-CA1 synapses [69]. The number of Ca^{2+} channels that contribute to the release of a single vesicle also seems different among various synapses [33, 34, 70–72]. Two models have been proposed: the single-channel "nanodomain" hypothesis, which suggests that Ca^{2+} influx through a single Ca^{2+} channel triggers the release of a vesicle, and the "overlapping microdomain" hypothesis, which suggests that multiple Ca^{2+} channels contribute to the fusion of a single vesicle and overlapping Ca^{2+} microdomains are required to trigger release. Based on the non-linear dependence of transmitter release on Ca^{2+} entry, as well as the tight temporal and spatial relationship between Ca^{2+} and transmitter release, even slight modifications of presynaptic Ca^{2+} influx rates would be expected to significantly affect transmitter release.

While many non-ribbon synapses have shown a fourth order power-law dependence of release on Ca^{2+}, hair cell ribbon synapses have shown a linear or sublinear Ca^{2+} dependence of neurotransmitter release [73–77], and this changes depending on developmental maturation. A similar linear relationship between Ca^{2+} influx and transmitter release is also found at the photoreceptor synapse [78]. At hair cells this linear dependence may be due to the unique Ca^{2+} sensor, otoferlin, plus the presence of synaptotagmin 4, and to other isoforms of synaptotagmins at retinal ribbon synapses [22, 78]. A linear function may be expected if the exocytosis of a single vesicle is triggered by the Ca^{2+} influx through a single nearby Ca^{2+} channel, which results in no cooperativity among Ca^{2+} channels [79, 80]. However, recently, it has been proposed that this linearity may result from the summing across multiple active zones, which individually have a supralinear Ca^{2+} dependence of exocytosis [81].

The number of Ca^{2+} channels per synaptic ribbon

Voltage-gated Ca^{2+} channels are clustered at the synaptic ribbon active zone so that Ca^{2+} entry is highly localized to particular regions of the plasma membrane in mouse inner hair cells [15], and in frog [40] and turtle hair cells [37]. Importantly, the amplitude and voltage dependence of the individual Ca^{2+} microdomains vary considerably within a single mouse inner hair cell [15]. This suggests that the individual active zones may have different numbers of Ca^{2+} channels and this may lead to differences in transmitter release rates and spontaneous spike rates [15]. Freeze-fracture studies suggest that clusters of Ca^{2+} channels were located approximately 100 – 200 nm from the sites of exocytosis underneath the synaptic ribbon [116]. The total numbers of Ca^{2+} channel in hair cells are estimated to 1700 in mouse inner hair cells [79] and 2000 – 3000 in frog auditory hair cells [6]. The total number of Ca^{2+} channels in each active zone is about 90 in frog saccular hair cells [116] and about 80 in mouse cochlear inner hair cells [79]. Assuming that all Ca^{2+} channels are clustered close to ribbons, there are approximately 40 – 50 Ca^{2+} channels per synaptic ribbon in frog auditory hair cells from the amphibian papilla [6]. The release of a single synaptic vesicle is regulated by only a few nearby Ca^{2+} channels [79]. In fact, the opening of a single Ca^{2+} channel may trigger the simultaneous release of up 5–6 vesicles (or quanta) at bullfrog hair cells, because large EPSC events were observed even when the hair cell was voltage-clamped to about −70 mV, a membrane potential where very a few Ca^{2+} channels are open [6, 115].

Vesicle pools and endocytosis at ribbon synapses

Synaptic vesicles belong to distinct releasable pools, which show different fusion competency as demonstrated by the multiple kinetic components of exocytosis [82]. This may result from different states of molecular readiness for exocytosis based on the

interaction of proteins participating in vesicle fusion. It may also reflect the physical distance of release sites (or docked vesicles) from Ca^{2+} channels [83, 84]. Although various synapses show a wide range in their ability to release neurotransmitter, most presynaptic terminals contain vesicles in a “readily releasable pool”, which can be released quickly upon stimulation. Vesicles in a large “reserve pool” are more reluctant and can only be mobilized more slowly in response to prolonged or strong stimulation. In addition, intermediate vesicle pools may contain vesicles released more slowly than vesicles of the readily releasable pool, but which can release faster than vesicles from the reserve pool. Some of these pools may correspond to morphologically defined vesicle pools. For example, synaptic vesicles docked to the presynaptic membrane appear to be the readily releasable pool at hippocampal synapses [85]. At ribbon synapses, the readily releasable pool can include vesicles tethered to the ribbons as well as the docked vesicles [6, 86, 87].

To maintain sustained release for prolonged periods of time, released vesicles should be quickly retrieved by endocytosis and eventually reloaded into vesicle pools [88]. Endocytosis is critical for synaptic function at non-ribbon synapses [89]. This process includes recovery of vesicle membrane after fusion, appropriate sorting of proteins needed for vesicle fusion, and refilling of neurotransmitter, all of which depend on temperature and developmental maturation [90]. It is still not clear how many different mechanisms for endocytosis exist in neurons. The fastest possible mode (a “kiss-and-run” type of vesicle cycling, also termed “flicker fusion”) suggests a fast closer of the fusion pore, and the refilling of empty vesicle sacs with neurotransmitter, after a transient fusion of the vesicle with the plasma membrane [91]. The slow pathway of endocytosis is generally thought to go through classical clathrin-mediated endocytosis [92]. It is uncertain how the different modes of endocytosis are chosen, how they depend on Ca^{2+}, and if clathrin-mediated endocytosis may be sufficiently fast at synapses to mediate most of the physiologically relevant endocytosis needed to maintain a steady nerve terminal and cellular surface area.

Short-term plasticity at ribbon synapses

Physiological activity patterns lead to changes in synaptic strength at all types of synapses. Some synapses show increased neurotransmitter release with repeated stimulation, whereas other synapses a decrease in neurotransmitter release. These alterations in synaptic strength are often called “facilitation” for increases, or “depression” for decreases. The mechanisms that underlie these short-term processes may vary at different synapses, and the observed behavior is often a mix of the offsetting strengths of these influences. In general, at synapses with a high probability of release, depression dominates, while synapses with a low release probability tend to show facilitation [93]. While manipulating the release probability by changing the extracellular Ca^{2+} concentration can alter the form of short-term plasticity that dominates at a particular synapse, there are physiologically relevant manipulations that can change these plastic events as well. Possible physiologically relevant regulation sites for short-term synaptic plasticity include changes in the waveform of the presynaptic action potential [94], changes in Ca^{2+} influx [95], the size of the readily releasable pool [96], neuromodulators [97], and internal Ca^{2+} stores [98]. It is widely accepted that residual Ca^{2+} from previous stimulation causes activity-dependent synaptic facilitation, though the underlying molecular mechanisms of these dynamic changes in synaptic strength are not well understood. One possible mechanism for this is saturation of local Ca^{2+} buffers during the first action potential in a pair of stimuli [99]. In contrast, depletion of the vesicles within the readily releasable pool can cause synaptic depression [100]. In addition, postsynaptic receptors can be desensitized by a long exposure to neurotransmitter that reduces postsynaptic currents [101].

Many presynaptic proteins including synaptotagmin, synapsin, synaptophysin, and munc 13-1, and native Ca^{2+} binding proteins, such as frequenin and piccolo, can influence short-term plasticity [102–106]. Despite our current detailed molecular understanding of presynaptic events surrounding transmitter release, there is no general consensus as to the molecular mechanisms that underlie some of the particular phases of short-term plasticity. Much attention has been focused on trying to implicate various Ca^{2+} binding proteins found in synaptic terminals. As there are many mechanisms that may lead to short-term synaptic plasticity, at most synapses multiple mechanisms probably interact in complex ways to generate the plastic physiological patterns of synaptic transmission [107].

Interestingly, many ribbon synapses, such as retinal bipolar cells of the goldfish, rod bipolar cells in the rat retina, photoreceptors of salamanders, frog saccular hair cells and cochlear inner hair cells of the mouse, show only short-term depression (Figure 4). These studies used a variety of experimental stimulation protocols, such as different durations and amplitudes of depolarization, and different durations of inter-pulse intervals, external Ca^{2+} concentrations, internal Ca^{2+} buffers, and holding potentials [86, 87, 108–112]. So it is hard to compare the detailed results from all these different ribbon-type synapses.

Facilitation has been observed at ribbon synapses, namely in auditory hair cell synapses in rats [117], bullfrogs [77] and in the *in vivo* chinchilla auditory nerve fiber using sound stimuli [118]. In adult bullfrog amphibian papilla hair cells, the recovery rate from paired-pulse depression was surprisingly fast (about ten milliseconds; Figure 4A–D). A clear form of paired-pulse facilitation was also observed, although depression dominated with a longer depolarizing pulse duration [77]. Likewise, the recovery from paired-pulse depression in acutely isolated frog saccular hair cells is very fast with a time constant of 29 ms [110]. In chick hair cell synapses, sound-evoked adaptation quickly recovers (τ = 70 ms) [87], and a rapid calcium-dependent vesicle supply mechanism allows sustained high rates of exocytosis in more mature hair cells [119]. Mouse inner hair cells show a biphasic recovery (τ_f = 140 ms and τ_s = 3 s) from depletion of the readily releasable pool [108]. By contrast, goldfish retinal bipolar cell terminals show a relatively slow double-exponential recovery from paired-pulse depression (τ_f = 1.03 s and τ_s = 11.8 s; Figure 4F). Interestingly, the Ca^{2+} currents evoked by a pair of stimuli show facilitation in bipolar cell terminals (Figure 4E), whereas in hair cell ribbon synapses they show inactivation (Figure 4A). Recovery from paired-pulse depression in rat rod bipolar cell synapses is also relatively slow (τ = 4 s) [111].

In conclusion, several recent studies indicate that recovery from depression at auditory hair cell synapses is generally faster than at retinal ribbon synapses. Perhaps hair-cell synaptic ribbons, and their surrounding vesicle mobilization machinery, are tailored for fast and efficient replenishment of depleted vesicle pools, so that these synapses can transmit faithfully the phase-locked timing of high frequency sound signals [6, 77]. The remarkable speed and continuous release in the processing of ongoing auditory signals in hair cell synapses may require a fast and calcium-dependent recruitment of vesicles towards the synaptic ribbons and this results in a fast time course of recovery from depression. Such stringent requirements for speed and precise timing in the release process may not be necessary in the retinal ribbon synapses since visual signals are intrinsically slower and rate limited by the relatively slow phototransduction cascade in rods and cones [77]. Different ribbon synapses thus have remarkably different properties that seem to be tailored to their kinetic operating ranges and rate limited to match their respective physiological sensory transduction processes.

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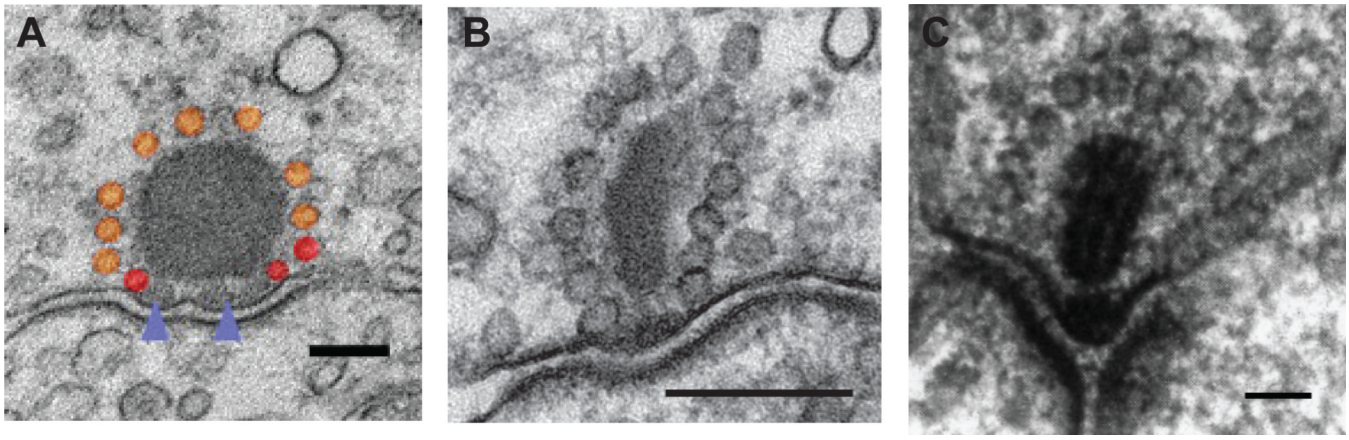

Figure 1.

Morphology of ribbon synapses. A halo of synaptic vesicles surround the electron dense synaptic ribbon. The ribbon can be plate-like, ellipsoidal, or spherical.

A. Representative section of adult bullfrog amphibian papilla hair cell synapse imaged at 40000× (scale bar, 100 nm). Ribbon-attached vesicles (orange), immediately-releasable vesicles (red; vesicles docked on the membrane), ribbon anchoring sites (purple arrowheads). Modified with permission from Graydon et al., 2011 [6].

B. A mature (p14 – 17) mouse inner hair cell synapse (scale bar, 200 nm) viewed by electron microscopy. Modified from Neef et al., 2007 [3].

C. Ultrastructure of goldfish retina bipolar cell synapse (scale bar, 50 nm). The arciform density resides below the base of the synaptic ribbon. Modified from von Gersdorff et al., 1996 [84].

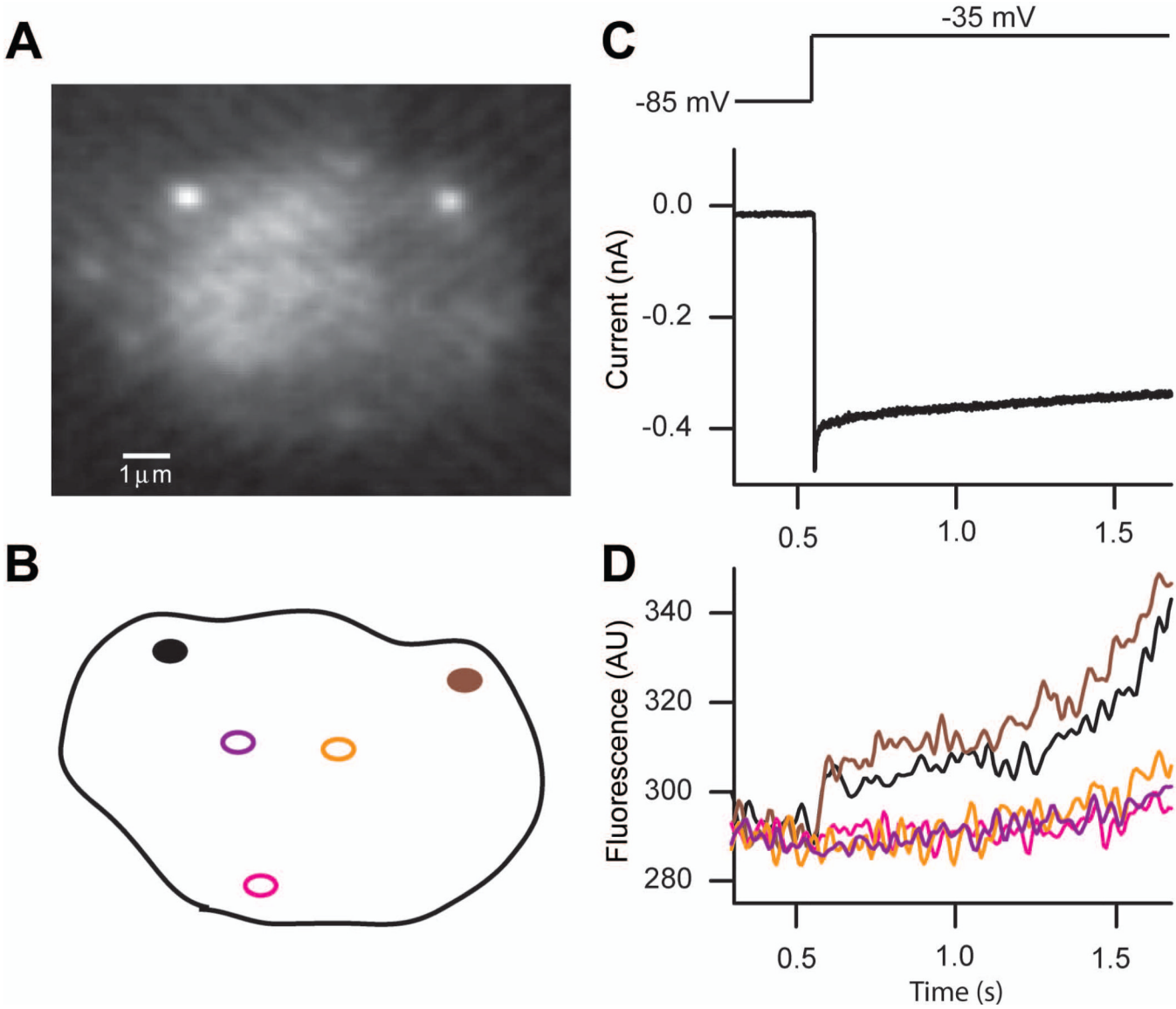

Figure 2.

High-speed confocal Ca^{2+} imaging of presynaptic dense bodies in auditory hair cells from the turtle's papilla.

A. Fluorescence image of presynaptic dense bodies with rhodamine-tagged ctbp2-terminal binding peptide (scale bar: 1 µm).

B. Regions selected for Ca^{2+} imaging.

C. A depolarizing stimulus from −85 mV to −35 mV (top) on the hair cell evoked a large L-type Ca^{2+} current.

D. The fluorescent signal shows a rapid initial increase with subsequent slow rise of the internal Ca^{2+} concentration. Regions far from the synapse show little initial response followed by a slow slight increase. This indicates Ca^{2+} channels are clustered at the synaptic ribbon sites.

Modified from Schnee et al., 2011[39].

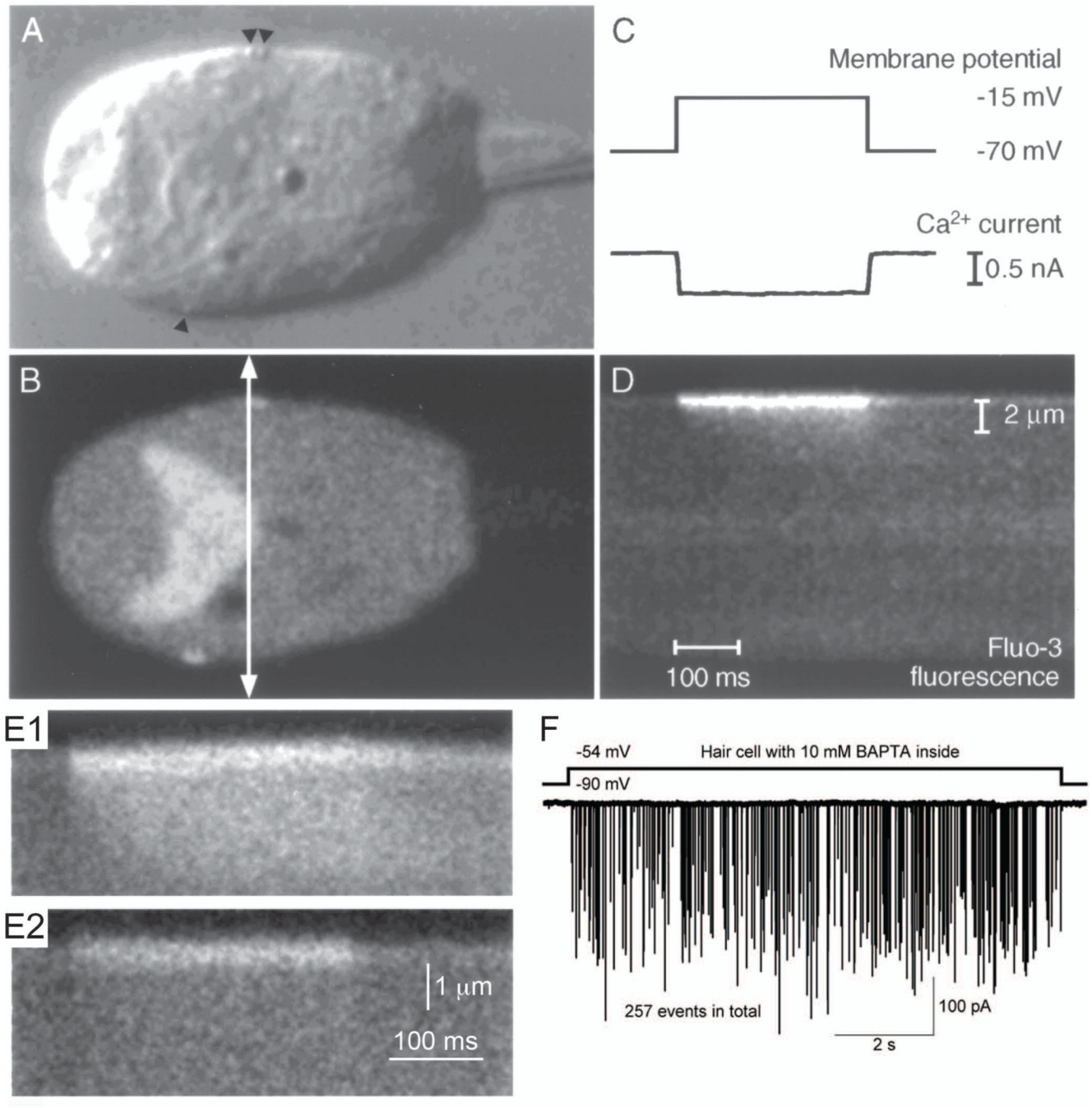

Figure 3.

Presynaptic Ca^{2+} entry in frog hair cells.

A. Image of a hair cell using differential interference contrast light microscopy. Arrowheads indicate the locations of presynaptic ribbons.

B. Epifluorescence image of the same hair cell with A, which is loaded with 200 μM fluo-3. The fluorescence image shows strong fluorescence signals at the positions of ribbons. The arrow indicates the transect scanned in D.

C. While the image was acquired, the hair cell was depolarized from −70 mV to −15 mV for 300 ms (upper trace). Ca^{2+} current was evoked by this stimulus (lower trace).

D. Time course of Ca^{2+} entry in isolated hair cell loaded with 200 μM fluo-3. The line-scan feature of a laser-scanning confocal microscope with high temporal resolution generated a two-dimensional image. One axis is distance and the other axis is time. A transect (400 nm) across the hair cell (arrow in B) was repeatedly scanned every 2 ms for a period including the depolarization in C. Within a few milliseconds of a depolarization's onset, fluorescence signals increased in restricted region, which was close to the presynaptic membrane, then signals spreaded gradually to the inside of the cell. The spatial scale bar in D applies to A and B. The temporal scale bar in D applies to C.

E. Mobile Ca^{2+} buffer affects temporal and spatial changes in fluo-3 fluorescence. Hair cells were loaded with 200 μM fluo-3 (E1) or 200 μM fluo-3 plus 10 mM BAPTA (E2). The spread of Ca^{2+} was restricted to the presynaptic dense body by high concentration of mobile Ca^{2+} buffer (E2).

A – E: isolated frog saccular hair cells (modified from Issa and Hudspeth, 1996 [40]).

F. EPSCs from paired recordings from the bullfrog amphibian papilla hair cell synapse. Presynaptic hair cell dialyzed with 10 mM BAPTA was depolarized from −90 mV to −54 mV. The afferent fiber shows large amplitude EPSCs during the depolarizing pulse. Modified from Graydon et al., 2011 [6].

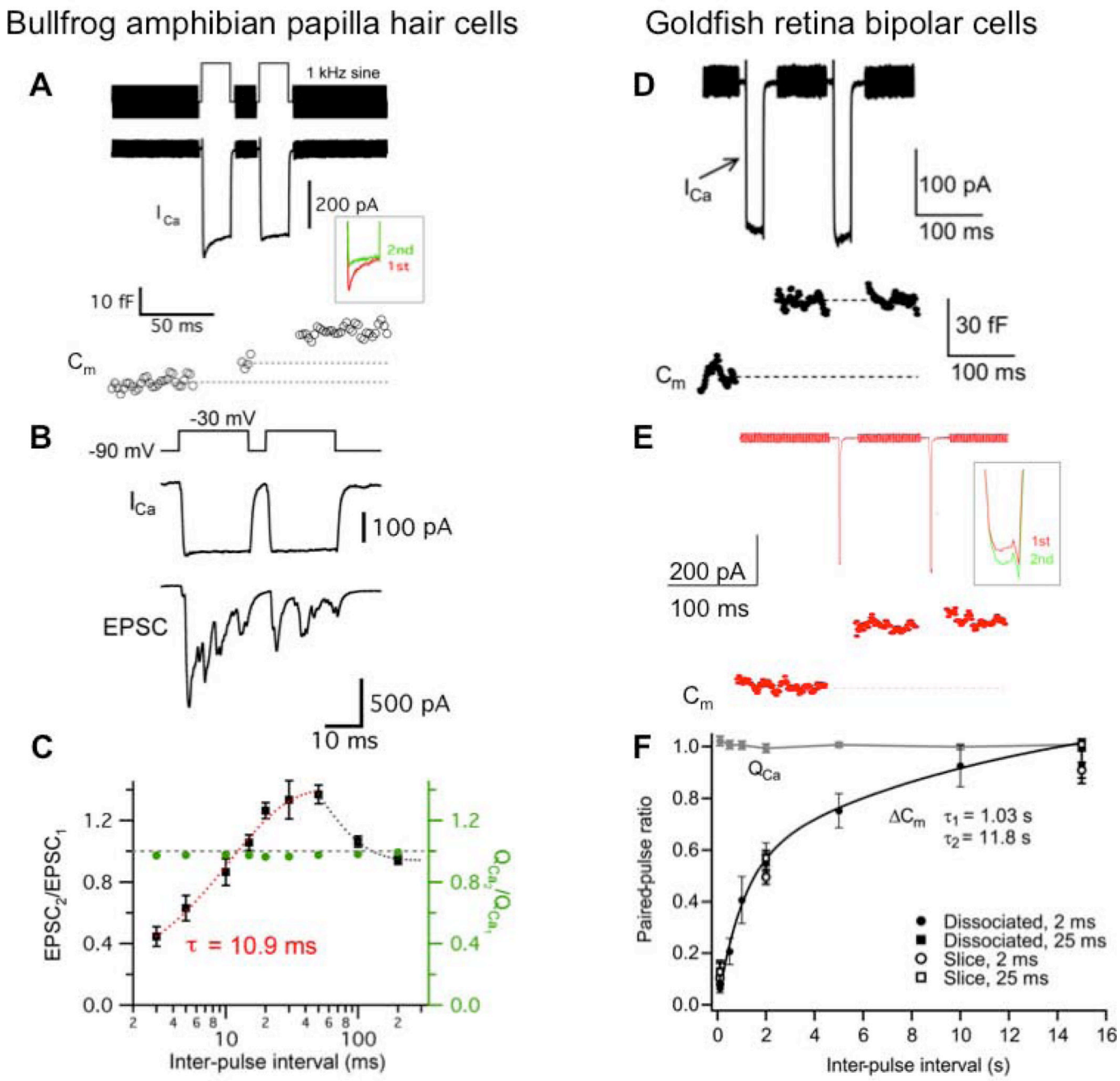

Figure 4.

Short-term plasticity at bullfrog auditory hair cell synapses (A – C) and goldfish retina bipolar cell synapses (D – F).

A. Ca^{2+} current (I_{Ca}) and membrane capacitance (C_m) evoked by a pair of 20 ms depolarization from −90 mV to −30 mV with 20 ms interpulse interval shows paired-pulse facilitation in the ratio of the C_m jumps. Note that I_{Ca} shows a slow form of calcium-dependent inactivation (inset).

B. When a hair cell was depolarized from −90 mV to −30 mV for 20 ms with 5 ms interpulse interval, EPSC was recorded from the connected afferent fiber terminal. The ratio of EPSC ($EPSC_2/EPSC_1$) shows paired-pulse depression.

C. The relationship between EPSC peak ratios and interpulse intervals. Hair cells were depolarized from −90 mV to −30 mV for 20 ms with various interpulse intervals (n = 4 – 8). The EPSC paired-pulse ratio ($EPSC_2/EPSC_1$) recovered exponentially with a single exponential fit from 3 to 50 ms intervals (τ = 10.9 ms; red dashed curve). The gray dashed line indicates that the ratio is 1 ($EPSC_2 = EPSC_1$). The ratio of Ca^{2+} charge (Q_{Ca2}/Q_S) was relatively constant and close to 1 (0.96 – 0.99).
D. I_{Ca} and C_m were evoked by a pair of 25 ms pulses from −60 mV to −10 mV with 100 ms interpulse interval in dissociated goldfish retina bipolar cells. The ratio of C_m shows strong paired-pulse depression.
E. A bipolar cell was depolarized by a pair of 2 ms pulses from −60 mV to −10 mV with 100 ms interpulse interval. Note that although I_{Ca} shows slight calcium-dependent facilitation in amplitude (inset), the ratio of C_m jumps shows strong paired-pulse depression due to vesicle pool depletion.
F. Paired-pulse ratio ($\Delta C_{m2}/(\Delta C_{m1}$) and the ratio of Ca^{2+} charge (Q_{Ca}) with pairs of 2 ms or 25 ms pulses in dissociated bipolar cell terminals or terminals in retinal slices. Paired-pulse ratio recovered with fast time constant (τ = 1.03 s; 43 %) and slow time constant (τ = 11.8 s; 57 %). The black line is a double exponential fit. Under all conditions, the rates of recovery from depression were similar.
A–C: from Cho et al., 2011 [77], D–E: Modified from Palmer et al., 2003 [109].