

The diverse roles of ribbon synapses in sensory neurotransmission

Gary Matthews* and Paul Fuchs†

Abstract | Sensory synapses of the visual and auditory systems must faithfully encode a wide dynamic range of graded signals, and must be capable of sustained transmitter release over long periods of time. Functionally and morphologically, these sensory synapses are unique: their active zones are specialized in several ways for sustained, rapid vesicle exocytosis, but their most striking feature is an organelle called the synaptic ribbon, which is a proteinaceous structure that extends into the cytoplasm at the active zone and tethers a large pool of releasable vesicles. But precisely how does the ribbon function to support tonic release at these synapses? Recent genetic and biophysical advances have begun to open the ‘black box’ of the synaptic ribbon with some surprising findings and promise to resolve its function in vision and hearing.

Changes in our external environment are detected by sensory receptor cells, which transduce sensory stimuli into an electrical signal that is graded depending on the stimulus intensity. In vision, balance and hearing, synapses of the receptor cells are unusual because they function tonically — that is, they transmit graded information with high fidelity across a broad range of stimulus intensities and for long periods of time, which places stringent demands on their release machinery. Rod photoreceptor cells of the retina can detect single photons but are nevertheless able to signal changes in light intensity over a range of $\sim 10^5$. Auditory hair cells are also exquisitely sensitive to small mechanical displacement and yet can signal sound intensity over six orders of magnitude. To these requirements must be added the remarkably high, sustained rates of release provided by some ribbon synapses, driving several hundred action potentials per second in cochlear afferent neurons. Contrast this with neurotransmission at conventional synapses, which is tightly phase-locked to the occurrence of presynaptic action potentials, allowing the resulting highly episodic release to be supported by relatively small pools of readily releasable synaptic vesicles.

To cover this wide dynamic range and sustained release, the synaptic outputs of these sensory neurons have evolved specialized machinery — the synaptic ribbon — at the active zones, where synaptic vesicle exocytosis occurs. Besides auditory hair cells and retinal photoreceptor cells, other types of primary sensory neurons have ribbon synapses as well, including pineal

photoreceptors, electroreceptors, and hair cells of vestibular organs and the lateral line system. In the visual system, ribbons are also found at the output synapses of the second-order retinal bipolar neurons, which signal by means of graded changes in membrane potential, similar to photoreceptors and hair cells. Indeed, synaptic ribbons are found wherever transmission requires graded modulation of ongoing vesicle exocytosis.

Ribbons vary considerably in size, shape and number in different cell types, but they share the common characteristic of having numerous glutamate-containing synaptic vesicles attached to their surface by filamentous tethers. Because ribbons seem to be exclusive to synapses where graded depolarization drives sustained neurotransmitter release, it is generally thought that ribbons increase the pool of vesicles available to support continuous transmission, by serving as a storehouse for release-ready vesicles and perhaps as a transport and/or timing system for delivering those vesicles to the plasma membrane. Consistent with this view, the vesicles associated with ribbons do not exchange with cytoplasmic vesicle pools in unstimulated synapses but do turn over rapidly when calcium channels open. As we will see, ribbons may have the additional role — and possibly primary role in some instances — of synchronizing the release of multiple vesicles at stimulus onset. In this Review, we focus first on the structure of ribbon synapses, including both presynaptic and postsynaptic features, then discuss what is known about the functional roles

*Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, New York 11794-5230, USA.

†The Center for Hearing and Balance, Otolaryngology, Head and Neck Surgery and the Center for Sensory Biology, Johns Hopkins University School of Medicine, Ross 818, 720 Rutland Avenue Baltimore, Maryland 21205, USA.

Correspondence to G.M. and P.F.

e-mails:

Gary.C.Matthews@sunysb.edu;

pfuchs1@jhmi.edu

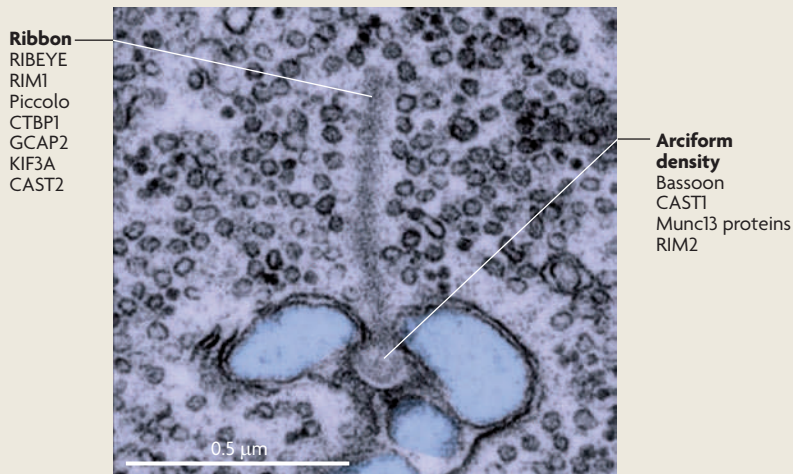
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of ribbons at retinal synapses and in hair cells of the inner ear. We end by briefly describing some of the remaining questions about how ribbon synapses work, as well as approaches to providing answers.

Box 1 | Molecular composition of ribbon synapses



An increasing array of proteins have been identified at ribbon active zones (see the figure), including many that are also present at conventional, non-ribbon active zones. Among the latter is the cytomatrix protein bassoon and various proteins that are involved in the synaptic vesicle cycle⁵⁰. Bassoon is thought to be an important component of the arciform density because bassoon immunoreactivity is located at the boundary between the ribbon and the plasma membrane^{79–81}. Bassoon binds to the main structural protein of the ribbon, RIBEYE⁸⁰, and ribbons fail to anchor in photoreceptors and hair cells when bassoon is functionally disrupted^{1,13}. The ribbon protein RIBEYE consists of an A domain that is unique to ribbons and a carboxy-terminal B domain that is mostly identical to the transcriptional co-repressor C-terminal binding protein 2 (CTBP2)⁸². RIBEYE self-assembles via interaction sites in both the A and B domains to form complexes that resemble rudimentary ribbons⁸³. Based on known protein interactions of CTBP2, the identity between RIBEYE B domain and CTBP2 has been used to design a fluorescent peptide that binds RIBEYE and renders ribbons visible in living cells²⁹. This has proved to be useful for studies of ribbon function, as well as for photoinactivation of ribbons³⁹. To date, RIBEYE is the only protein known to be unique to the ribbon itself, but in freeze-fracture studies the surface of the ribbon is studded with particles of various sizes⁸⁴, which suggests that a number of different proteins are part of the ribbon. Among those identified so far are RAB3 interacting protein 1 (RIM1)⁸⁵ (involved in priming vesicles for exocytosis), kinesin-like protein KIF3A⁸⁶ (a component of kinesin motor complexes), piccolo⁸⁷ (a component of the cytomatrix at the active zone), CTBP1 (REF. 80) (a transcriptional regulator that might bind to the B domain of RIBEYE) and guanylyl cyclase-activating protein 2 (GCAP2)⁸⁸ (a calcium-binding protein). In addition to proteins associated with the ribbon, ribbon synapses express specific isoforms of the SNARE protein syntaxin 3B^{89,90} and the SNARE-associated complexins 3 and 4 (REF. 91). In cochlear hair cells a C2 domain transmembrane protein, otoferlin, is required for normal transmitter release, and is mutated in a form of human deafness⁹². The precise functions of these ribbon-specific components are not yet certain (see REF. 50 for a recent review). The calcium sensor for release is unknown. Although otoferlin⁹² and a variant of synaptotagmin⁵⁹ have been suggested to act in cochlear hair cells, at least for otoferlin this is not certain¹² and it seems likely that different ribbon synapses have differently composed sensors, because the calcium sensitivity of release varies across cell types. In photoreceptors^{6,33,93}, the calcium sensor has high affinity and low cooperativity, which would allow random openings of individual calcium channels to trigger release at a rate sufficient for tonic signalling to postsynaptic targets during steady depolarization. By contrast, cochlear hair cells⁹⁴ have a sensor with high cooperativity and low affinity — features that are suited for highly synchronous release. CAST1, CAZ-associated structural protein 1.

The structure of ribbon synapses

Ribbon synapses confer a capacity for sustained, graded vesicle release in sensory receptor cells. However, the diverse types of stimuli and wide dynamic range over which they need to operate are reflected in the wide variation in the size, shape and functionality of ribbons across cell types as well as the number of tethered vesicles. The study of retinal and auditory synapses has revealed that ribbon morphology is tailored to the needs of particular synapses for particular rates of ongoing vesicle exocytosis.

Retinal photoreceptor ribbons. In photoreceptors, ribbons are planar structures, which when cut transversely appear in electron microscopy images as an electron-dense bar, ~30 nm thick, extending vertically from the plasma membrane into the cytoplasm (BOX 1; FIG. 1a). This long, narrow appearance in single transverse sections gives ribbons their name, but it can be misleading about their true three-dimensional shape (FIG. 1b). In reality, photoreceptor ribbons are plate-like structures that in rods can run several microns parallel to the plasma membrane, curving around the deep, invaginating pocket that contains the dendrites of postsynaptic horizontal and bipolar cells. The ribbon is attached at its base to the presynaptic membrane by an electron-dense structure called the arciform density (FIG. 1a), which keeps the ribbon on a leash and hovering just above the plasma membrane. The cytomatrix protein bassoon (BOX 1) is thought to be an important component of the leash, and ribbons float free in the cytoplasm of the photoreceptor terminal when bassoon is functionally disrupted¹.

The large ribbons of photoreceptor cells can harbour thousands of synaptic vesicles, tethered to both surfaces of the plate by fine filaments. Unfortunately, the molecular composition of these filaments is not yet known, although this information would be very useful in deciphering ribbon function. The large size of the tethered pool of vesicles might be required because photoreceptor synapses have release rates of up to hundreds of vesicles per second², sustained for long periods. In addition to the vesicles attached to the face of the ribbon at a distance from the plasma membrane, a subset of tethered vesicles at the base of the ribbon (FIG. 1) also touch the plasma membrane, much like the 'docked' vesicles of conventional active zones. The calcium channels that drive exocytosis (BOX 2) are arrayed linearly, parallel to the long axis of the ribbon, just beside the arciform density. This means that these docked vesicles are poised for very rapid release when the channels open and admit a puff of calcium. The molecular machinery of release itself, such as SNARE complexes, is for the most part similar at ribbon-type and conventional active zones, although there are ribbon-specific isoforms of some components (BOX 1).

The postsynaptic architecture of photoreceptor ribbon synapses is quite complex, because the output of a single photoreceptor cell must be distributed to multiple parallel pathways that simultaneously process different types of visual information. To achieve this, glutamate released at ribbon active zones reaches several different

postsynaptic targets, including dendrites of horizontal cells and several types of bipolar neurons, that are located at progressively greater distances from the ribbon and express ionotropic or metabotropic glutamate receptors with appropriately tuned kinetics and affinity³. This ‘engineered spillover’ of glutamate to multiple targets at different distances introduces variable temporal filtering, so that targets near the site of vesicle fusion respond rapidly and transiently, whereas those farther away provide slower, integrated signals. As a result, different postsynaptic cells carry different temporal bandwidths of visual information⁴, providing the basis for separate visual pathways that are concerned with sustained versus transient aspects of visual stimuli.

Retinal bipolar cell ribbons. The ribbons of retinal bipolar neurons (FIG. 1c) are typically much smaller than the microns-long rod photoreceptor ribbons. For example,

planar ribbons of the giant synaptic terminal of goldfish bipolar neurons are ~150 nm tall and ~400 nm long⁵. Although bipolar cell ribbons are small, there are many in a single cell, ranging from 30 to >100 ribbons depending on the species and the subtype of bipolar neuron. As a result of their small size, single bipolar cell ribbons tether at most ~100 vesicles, of which ~20 are docked. This smaller vesicle pool translates into a more transient synaptic output of bipolar neurons, which serves to accentuate changes in light intensity. Interestingly, cone ribbons are also smaller than those of rods, and they may function similarly to provide a transient burst of release when illumination dims⁶. Each bipolar cell ribbon typically communicates with a pair of postsynaptic processes, which are symmetrically placed in close contact with the active zone (FIG. 1c). A given ribbon may contact a pair of amacrine cell processes, a ganglion cell dendrite and an amacrine cell process or, more rarely, a

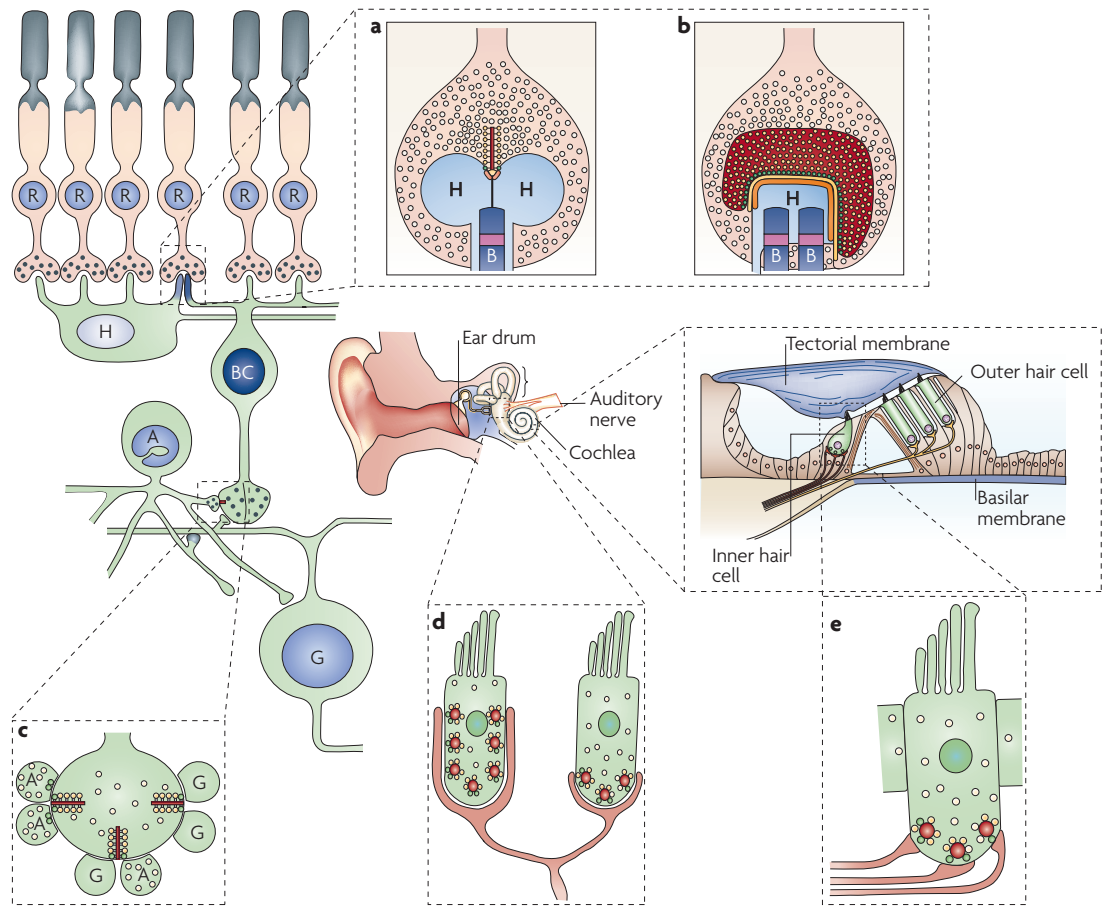


Figure 1 | Diversity of ribbon morphology and postsynaptic architecture in different cell types. **a, b** | Schematic diagram of transverse (**a**) and *en-face* (**b**) views of a mammalian photoreceptor ribbon synapse¹¹⁷ in the retina. **c** | Synaptic arrangement at bipolar cell ribbons. **d** | Synaptic arrangement at ribbons of cochlear inner hair cells. **e** | Vestibular afferents receive synaptic inputs from multiple ribbons of multiple hair cells. The locations of cochlear inner hair cells and the vestibular apparatus are shown schematically. Synaptic ribbons are shown in dark red, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors in orange and the arciform density in yellow. Vesicles attached to ribbon are shown by yellow circles and docked vesicles by green circles. Horizontal cell dendrites (H) are shown in light blue, ON bipolar cell dendrites (B) in dark blue and mGluR6 receptors in purple. A, amacrine cell process; BC, bipolar cell; G, ganglion cell dendrite; R, rod photoreceptor cell. Parts **a** and **b** are reproduced, with permission, from REF. 117 © (1995) Cell Press. Parts **c** and **d** are reproduced, with permission, from REF. 3 © (2005) Cell Press.

Freeze-fracture

A technique for ‘three-dimensional’ imaging of cellular ultrastructure by coating the surface of fractured tissue with electron-dense material.

SNARE

Soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein (SNAP) receptor.

ON bipolar cell

Bipolar cells are classed as either ON or OFF depending on how they respond to glutamate released in their vicinity by photoreceptors. ON bipolar cells respond to a lowering of released glutamate by depolarizing, and OFF bipolar cells respond to this change by becoming hyperpolarized.

Box 2 | Specific subtypes of L-type calcium channels drive release at ribbon synapses.

Ribbon synapses require calcium channels that remain active during prolonged depolarization and respond rapidly to changes in membrane potential. Accordingly, calcium currents recorded from ribbon synapses exhibit relatively little inactivation^{53,95}, activate rapidly upon depolarization and deactivate rapidly upon repolarization^{96,97}. Various lines of evidence indicate that either voltage-dependent L-type calcium channel subunit alpha 1D (Ca_v1.3) or Ca_v1.4 L-type calcium channels drive release at ribbon synapses, depending on cell type. First, the presynaptic calcium current at ribbon synapses is dihydropyridine-sensitive, indicating that the channels are L-type^{96,98,99} and that blocking this current with dihydropyridines reduces glutamate release in bipolar cells⁹⁸, photoreceptors¹⁰⁰ and hair cells^{101,102}. Second, mutations in Ca_v1.4 cause rod and cone dysfunction and visual impairment in humans^{103,104} and mice¹⁰⁵, immunostaining for Ca_v1.4 is tightly co-localized with synaptic ribbons in photoreceptor terminals^{106,107} and calcium influx is reduced by 90% in photoreceptor synaptic terminals of Ca_v1.4 knockout mice¹⁰⁵. Third, interaction with calcium-binding protein 4 (CABP4) shifts the activation range of Ca_v1.4 (REF. 108) to better align with the resting potential of photoreceptor cells in darkness (about -40 mV), and loss of CABP4 in mice or humans strongly impairs photoreceptor neurotransmission^{108,109}. This reinforces the central role of Ca_v1.4 in driving synaptic vesicle exocytosis at photoreceptor ribbon synapses. Fourth, cochlear hair cells strongly express Ca_v1.3 (REF. 110), and Ca_v1.3 knockout mice are deaf⁵¹ because of greatly reduced calcium currents and calcium-triggered exocytosis in cochlear hair cells^{111,112}. Although vestibular function has been reported to be normal in Ca_v1.3-null mice, zebrafish with a mutation in an orthologue of Ca_v1.3 have both auditory and vestibular deficits. In general, the evidence indicates that Ca_v1.3 channels are important for neurotransmitter release at auditory hair cell synapses, whereas Ca_v1.4 is primary at photoreceptor synapses. However, Ca_v1.3 α-subunits have also been identified at retinal ribbon synapses, in both photoreceptors and bipolar cells, and it is possible that this subtype may play a role in the retina as well as in the cochlea. The conventional wisdom is that members of the P/Q and N subgroups of calcium channels drive neurotransmitter release at synapses, but it is clear that Ca_v1, L-type α-subunits can also support neurotransmitter release when the demands of the release process require them.

pair of ganglion cell dendrites⁷. This dyad arrangement is considerably simpler than the complicated architecture of the postsynaptic elements at photoreceptor synapses, but it nevertheless also serves to distribute the output of a single ribbon to more than one functional target.

Hair cell ribbons. In hair cells, synaptic ribbons (also called dense bodies) are morphologically diverse: they can be spherical, planar or oblong in shape, and variable in size, tethering from 20–400 vesicles⁸. Even within one hair cell, individual ribbons can vary in size and shape. Although those in non-mammalian hair cells can be somewhat larger (200–400 nm in diameter), ribbons in mammalian hair cells are ≤200 nm in width, closer in size to bipolar cell ribbons than to photoreceptor ribbons. Mammalian cochlear ribbons have 100–200 tethered synaptic vesicles and associated voltage-gated calcium channels that are estimated to range from approximately 100–200 per ribbon^{9–11}. As with retinal ribbons, a subset of vesicles attached to ribbons (~12, as estimated by electron tomography¹²) are docked at the plasma membrane in hair cells, but in the case of spherical ribbon the docked vesicles can be sandwiched between the ribbon and the plasma membrane (FIG. 1d). Even though hair cells have no visible arciform density like that of photoreceptors, ribbons float free in hair cells when bassoon is functionally knocked out¹³, indicating that the anchoring mechanism is similar in both cell types. Calcium entry through voltage-gated channels occurs near ribbons in hair cells^{14,15}, so it is likely that the docked vesicles fuse rapidly at the onset of a depolarizing stimulus. The number of vesicles in this ‘readily releasable pool’ estimated from physiological measurements never differs by more than a factor of two from the morphologically defined number of docked vesicles in hair cells of various vertebrates¹⁶. Increasing attention is being paid to differences in structure and function among hair cells

located at different points along the cochlea. Ribbons in the chicken basilar papilla (part of the hearing organ) vary in size and vesicle capacity along the tonotopic axis, with hair cells in the high-frequency region having larger ribbons and correspondingly larger calcium currents¹⁷. Growing evidence suggests that calcium channel numbers can vary among ribbons within a single mammalian cochlear hair cell^{10,15}, which could contribute to marked differences in sensitivity of individual postsynaptic afferent fibres to sound stimuli. There is also considerable variety in afferent innervation of hair cells in different end organs and species⁸. Vestibular afferents can branch to contact several presynaptic hair cells (FIG. 1d) and may receive transmitter released from dozens or even hundreds of synaptic ribbons. At the other extreme, the solitary dendrite of a type I afferent is usually postsynaptic to a single ribbon of a single inner hair cell in the mammalian cochlea (FIG. 1e).

Functional roles of retinal ribbon synapses

Ribbons play several parts in neurotransmission, whose relative importance varies in different cell types, mirroring the diversity in ribbon structure. Like conventional synapses, ribbon synapses release vesicles immediately when calcium channels open, but they also support re-supply of vesicles so that release continues during sustained depolarization. In addition, increasing evidence indicates that ribbons coordinate simultaneous fusion of multiple vesicles, which is essential when precise timing relationships must be maintained, such as in audition. Recent work provides new insights into how ribbons might accomplish these varied tasks.

Synaptic vesicles on ribbons contribute to neurotransmission. Vesicles in the cytoplasmic pool at ribbon synapses are highly mobile^{18–20}, but those tethered to ribbons are not^{18,21}, raising the question of whether the

Tonotopic

The mapping of tones of different frequencies onto space along a receptive surface, such as the mammalian cochlea, or onto different spatial locations within a brain nucleus that processes auditory information.

Capacitance

Electrical measure of charge-storing capacity. Cell membranes behave as electrical capacitors because the insulating lipid separates two electrically conductive salt solutions. The capacitance of a cell is proportional to the cell's surface area and thus serves as an index of membrane addition and retrieval.

Cytomatrix at the active zone

The complex of membrane-associated and cytoplasmic proteins that provide structural organization for the many components required to dock, prime and fuse synaptic vesicles at presynaptic active zones.

ribbon-tethered vesicles have a role in neurotransmitter release. It seems that vesicles attached to ribbons do participate in the synaptic vesicle cycle upon depolarization, because vesicles labelled with activity-dependent extracellular markers are observed among the ribbon-attached pool in electron microscopy^{18,22–25}. In addition, turnover of ribbon-associated vesicles has been observed directly using fluorescence imaging in living bipolar neurons^{18,21}, and uptake of fluorescent dye into newly endocytosed vesicles after stimulation occurs preferentially near ribbons²⁶, demonstrating selective fusion and retrieval of vesicles at ribbons. Furthermore, electron microscopy shows fewer synaptic vesicles associated with ribbons in stimulated synapses^{6,20,27,28}. In some cases, the depletion of vesicles at stimulated ribbons is strongest near the plasma membrane^{6,20,28}, consistent with selective release of vesicles that are nearest to calcium channels. Calcium influx through voltage-gated channels in bipolar cell terminals occurs at 'hot spots' that coincide with ribbons^{14,29}, and when calcium channels open, capacitance measurements indicate immediate exocytosis of a subset of vesicles within ~1 ms³⁰, followed by slower release (within ~200 ms) of a larger vesicle pool. Postsynaptic recordings at retinal ribbon synapses also reveal two kinetically distinct components of release^{31–34}. The size of the fast component correlates with the total number of vesicles docked at the plasma membrane at ribbon active zones, as defined by electron microscopy. There is also good agreement between the size of the total releasable pool and the number of vesicles tethered to all of the ribbons in goldfish bipolar cell terminals⁵, as well as in salamander rods³³. Taken together, the evidence is consistent with a model in which fast, transient release at ribbons is supported by the lowest row of docked vesicles, followed by slower, sustained release of vesicles tethered in higher rows on the ribbon. In this view, ribbons could be considered a more elaborate version of the cytomatrix at the active zone of conventional synapses (BOX 3), which also serves to bring releasable vesicles into close proximity with calcium channels at the release site.

Box 3 | Insights into ribbon synapse function from conventional synapses

Active zones of conventional, non-ribbon synapses are also thought to have mechanisms to capture and orient synaptic vesicles at release sites. This is perhaps most striking at the frog neuromuscular junction, where two rows of synaptic vesicles are held at the presynaptic membrane just across from high-density clusters of postsynaptic acetylcholine receptors. Electron tomography shows that this precise geometry is maintained by a network of 'beams' that run parallel to the active zone and 'ribs' that extend to the side from the beams and contact synaptic vesicles¹¹³. This vesicle-tethering network is reminiscent of the arrangement of vesicles on ribbons, except that the tethering machinery runs parallel to the membrane instead of perpendicular to it, as it does at ribbon synapses. At cortical synapses, electron tomography also reveals an elaborate polyhedral matrix of active zone material, extending from the plasma membrane into the cytoplasm and contacting both docked and undocked synaptic vesicles¹¹⁴, whereas similar experiments on cryopreserved hippocampal synapses show a more extended mesh of filaments that extend from the active zone and interconnect synaptic vesicles in the terminal¹¹⁵. Therefore, synaptic ribbons could be viewed as an extreme example of the more general need for presynaptic machinery to facilitate the supply of release-ready vesicles to the active zone¹¹⁶.

Although the correspondence between vesicle pools, as defined by capacitance measurements, and anatomically defined populations of ribbon-associated vesicles is suggestive, it is by no means conclusive. Measurements of membrane capacitance reflect total surface area and so may not accurately report vesicle fusion specifically at active zones. For example, prolonged depolarization can elevate calcium levels throughout the cell and trigger exocytosis of synaptic vesicles or other structures at sites distant from the ribbon^{21,35,36}. However, the exocytosis reported by capacitance changes results in glutamate release within striking distance of postsynaptic receptors. This is because direct comparison of presynaptic capacitance changes and simultaneously recorded postsynaptic responses^{31,33,37} reveals close correspondence between the two estimates for the number of vesicles contributing to the fast and slow components of release. Furthermore, experiments based on analysis of postsynaptic responses rather than presynaptic capacitance at cone–horizontal cell synapses indicated that the fast component corresponds to docked vesicles at ribbons, and the sustained component to the rest of the ribbon-tethered population³⁴. Despite these indications that the ribbon contributes both the fast and slow components of release, disruption of ribbons by knockout of bassoon¹³ or by natural circadian regulation³⁸ leaves the sustained component largely intact, whereas it eliminates the fast component. This suggests that something other than ribbons accounts for sustained release. To resolve these conflicting conclusions, it would be useful to knock out ribbon function acutely at a single synapse while monitoring the effect on the rapid and sustained components of release in paired recordings from synaptically coupled neurons. For example, photo-ablation of fluorescently labelled ribbons³⁹ is a promising way to resolve the issue.

Models of vesicle fusion at ribbons. Assuming for the moment that the vesicles that are attached to higher rows on ribbons account for sustained release, how do they fuse when calcium channels open? A commonly held notion is that the ribbon provides a surface along which vesicles move towards the plasma membrane, either actively or passively, to replace those that fused previously at the base of the ribbon (FIG. 2a; progressive fusion). If so, then the movement is evidently calcium dependent, because the sustained component of exocytosis is blocked by millimolar levels of the calcium buffer ethylene glycol tetra-acetic acid (EGTA)^{30,40–43}. By contrast, EGTA does not affect the fast component, which is consistent with the fast component being due to fusion of docked vesicles that are located very near calcium channels. In the progressive fusion model, newly arrived vesicles would require some time to dock and assemble fusion complexes before that particular release site would again be release-competent. In the meantime, other docked vesicles could take up the slack and release their neurotransmitter (FIG. 2a) to sustain glutamate signalling to the postsynaptic elements. This could account for the fact that rod photoreceptor ribbons extend a large distance along the active zone: their size provides a sufficiently large number of release sites for docked, primed vesicles to maintain the requisite rate of ongoing vesicle fusion.

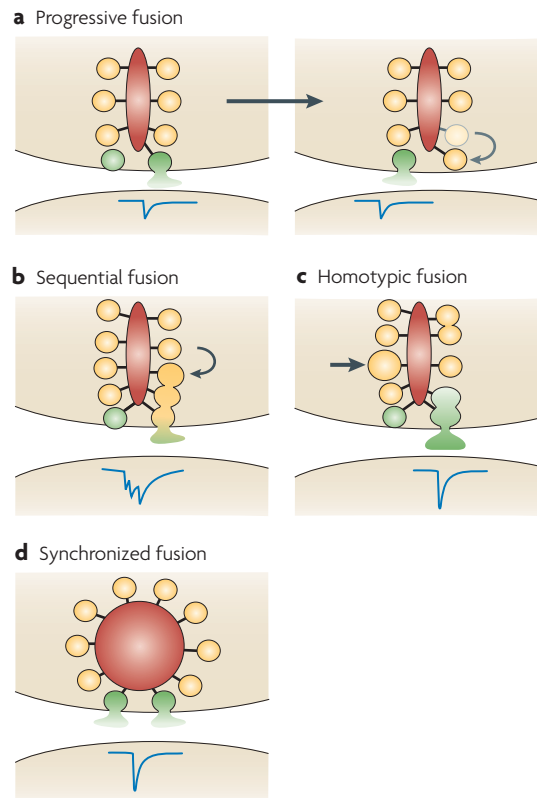


Figure 2 | Modes of synaptic vesicle fusion at ribbon synapses. Green vesicles represent vesicles that are docked at the plasma membrane (shown by a black line) at release sites. **a** | Progressive fusion, in which primed vesicles docked at different release sites fuse progressively during maintained depolarization, each producing an independent unitary postsynaptic current. Vesicles in higher rows move along the ribbon to replenish empty release sites. **b, c** | Two forms of compound fusion. In sequential fusion (**b**), vesicles fuse in sequence up the face of the ribbon, starting with the vesicles docked at the plasma membrane. This produces a burst of quantal postsynaptic currents in rapid sequence. Vesicles attached to the ribbon might fuse with each other (**c**). The resulting larger bolus of glutamate produces a larger postsynaptic current. **d** | Synchronized fusion of multiple docked vesicles, which results in a multiquantal postsynaptic current. The postsynaptic currents are shown by blue traces.

Alternatively, the ribbon might serve as a substrate for compound fusion, in which vesicles attached to the ribbon fuse with each other. This could occur in two ways, which are not mutually exclusive. In one scenario, vesicles at the base of the ribbon release their contents first — accounting for the fast component of exocytosis — but do not collapse fully into the plasma membrane. Then, vesicles in higher rows fuse successively, releasing their neurotransmitter through the previously fused vesicles (FIG. 2b; sequential fusion). The filaments that tether vesicles would serve to hold vesicles in the proper configuration, so that a wave of calcium-triggered exocytosis sweeps up the ribbon, generating the slower component of exocytosis. In frog saccular hair cells, sequential fusion has been proposed to account for

the fact that brief depolarization triggers exocytosis of many more vesicles than are docked at the plasma membrane⁴⁴. In general then, sequential fusion of this type might allow single release sites to be reused more rapidly than in the progressive fusion model, which could be an advantage for smaller ribbons that lack the large pool of docked vesicles found in photoreceptor synapses. In another scenario for compound fusion, ribbon-attached vesicles fuse among themselves, but before fusion with the plasma membrane. This form of compound fusion results in larger vesicles being attached to the ribbon (FIG. 2c; homotypic fusion), which then release a larger bolus of glutamate when they fuse at the plasma membrane during depolarization. Electron microscopy studies of bipolar cell ribbons have indeed revealed a subset of larger vesicles attached to ribbons after stimulation²⁷, consistent with vesicle-vesicle fusion on the ribbon during calcium influx. In addition, large pleiomorphic infoldings of the plasma membrane are attached to the base of the ribbon in repetitively stimulated bipolar cell ribbons, as is expected for compound exocytosis²⁷. Definitive evidence for compound exocytosis in its various forms is still lacking, and more direct studies of living ribbon synapses are required. Of course, it is also possible that the multiple modes of vesicle fusion coexist in different proportions depending on stimulation conditions.

Postsynaptic recordings at ribbon synapses of hair cells and retinal bipolar neurons suggest that another function of ribbons is to coordinate the simultaneous fusion of multiple vesicles at the active zone. For example, in the retina, large synaptic events consistent with synchronous release of multiple quanta were observed in simultaneous presynaptic and postsynaptic recordings from pairs of bipolar neurons and amacrine cells⁴⁵. These were interpreted to reflect synchronized fusion of multiple synaptic vesicles (FIG. 2d; synchronized fusion), but such large events could also occur from homotypic pre-fusion of vesicles (FIG. 2c). Recordings from afferent fibres that receive inputs from hair cells also show that ‘unitary’ postsynaptic currents correspond to simultaneous fusion of several synaptic vesicles, as discussed below. How ribbons might coordinate the fusion of independent vesicles (if that is indeed the case) remains mysterious, but in the auditory system such synchronization is likely to be a major functional role of ribbons, because it promotes the precisely timed activation of afferent neurons that is required for encoding speech patterns and for sound localization.

Functional roles of hair cell ribbons

As in the retina, ribbon synapses provide tonic transmitter release from sensory cells of the inner ear. Most notably, each type I afferent neuron in the mammalian cochlea is usually postsynaptic to a single ribbon in a single inner hair cell. It is remarkable that the timing, intensity and frequency content of sound is transmitted to the brain through these solitary, submicroscopic contacts. It is little wonder that considerable attention has been paid to hair cell synapses in the cochlea and elsewhere. These efforts over the past decade have provided surprising insights into ribbon function, including the

Pleiomorphic
Varied in shape.

variable reliance on calcium influx at different synapses and the ability to spontaneously release multiple vesicles. Provocative as these findings are, our understanding of hair cell ribbon function remains far from complete. Fortunately the endpoint is certain — to explain the signalling of afferent neurons that is driven by transmitter release from the ribbon. The following sections briefly summarize the requirements for afferent signalling, discuss the evidence for our emerging picture of ribbon function and point to what a future synthesis should include.

Afferent activity driven by hair cell ribbons. Mechano-transduction by sensory hair cells occurs with minimal delay, allowing the cochlea to signal stimulus frequencies exceeding 100 kHz in some mammals. The hair cell's membrane potential cannot vary at these ultrasonic frequencies. However, phase-locked transmitter release from hair cells can drive afferent firing at frequencies up to 5 kHz. At the other extreme, vestibular epithelia signal continuously to provide information on head position and movements that occur over the span of seconds. In fact, all hair cells drive continuous 'spontaneous' afferent activity that is modulated by a stimulus. These synaptic transfer functions arise from intimate, but still unresolved, relations between voltage-gated calcium channels and the vesicular release machinery, evidence for which is summarized below.

Afferent activation in the mammalian cochlea presents additional challenges. Strikingly, the 10–30 individual afferents that contact a single inner hair cell can have different spontaneous rates, acoustic thresholds and dynamic ranges⁴⁶. Although postsynaptic membrane properties⁴⁷ and modulation by lateral efferents must also be considered, it is likely that functional variations among ribbons contribute to differential afferent signalling for a given change in hair cell membrane potential. Certainly, individual ribbons within individual hair cells differ in size, shape and the number of vesicles associated with them⁴⁸. Also, the number of voltage-gated calcium channels may vary between hair cell ribbons^{8,17,49}. Confocal measurement with fluorescent calcium indicators has shown that peak influx varies substantially between different 'hotspots' in one cochlear hair cell during depolarizing voltage commands, suggesting that the maximum number of open channels was varied¹⁵. The calcium hotspots were co-localized with a fluorescent 'ribbon-binding' peptide and ribbon size correlated with hotspot amplitude. Variations in calcium channel number per active zone could account for differences in spontaneous rate, as well as the acoustic threshold (depolarization threshold) of individual afferent neurons contacting an inner hair cell.

Calcium channel gating at hair cell ribbons. Although our understanding of most ribbon proteins remains in progress⁵⁰ (BOX 1), it is certain that the voltage-dependent L-type calcium channel subunit alpha 1D (Ca_v1.3) is of key importance for transmitter release from cochlear hair cells. Indeed, release begins with the voltage gating of these channels, as shown by a detailed study using

voltage-clamp recording⁵¹. Whole-cell and single-channel measurements have shown that these channels are activated near the resting membrane potential of the hair cell, -60 mV ($V_{1/2}$ -35 to -40 mV), have open times of a fraction of a millisecond and show only modest, slow inactivation^{52,53}. These gating properties meet the dual demands of precise temporal coding and continuous, steady-state activity. Single-channel open probability in mammalian inner hair cells was reported to reach a maximum of 0.15, so that only a small number of the 100–200 channels at an active zone may be open for any release event¹¹. Given this limitation, a variation in total channel number is still more likely to significantly alter the release statistics for each active zone.

Ribbon function assayed by presynaptic membrane capacitance. The presynaptic function of hair cells has been studied most extensively using voltage-evoked changes in membrane capacitance⁵⁴, serving as a proxy for transmitter release. Capacitance measurements have established basic parameters, including estimates of the number and rates of vesicular release. In vertebrate hair cells, the size of the earliest component of release, the 'readily releasable pool', lies somewhere between that of the membrane-docked¹³ and ribbon-tethered vesicle pool^{42,44}, and variance analysis of capacitance changes in cochlear hair cells have suggested that the majority of release events in mouse inner hair cells involved single vesicles⁵⁵. Capacitance measurements have also been employed to describe differences in synaptic release by hair cells from different positions along the cochlea's tonotopic axis⁵⁶, as a function of development^{57,58} and as a test of molecular mechanisms^{59,60} (see REF. 16 for additional examples). These studies have revealed interesting variations in the relationship between calcium influx and membrane capacitance. For example, with the onset of hearing during the second postnatal week in rodents, calcium currents of inner hair cells become smaller but produce relatively larger increases in capacitance^{43,61}. This increase in release efficiency may result from a spatially close association between calcium channels and synaptic vesicles, the postulated 'nanodomain' (REF. 9).

Ribbon function assayed by postsynaptic currents. Physiologists depend heavily on intracellular recordings from postsynaptic cells to understand transmitter release. Such recordings have recently become prevalent in the study of hair cell transmission, although pioneering efforts in fish⁶², reptiles⁶³ and mammals⁶⁴ provided important early signposts. Whole-cell patch clamp recordings from afferent terminals at the base of inner hair cells in the immature cochlea (up to the twelfth postnatal day) revealed randomly timed, rapid glutamatergic excitatory postsynaptic currents (EPSCs) and provided early evidence for multivesicular release⁶⁵, as did recordings from a frog auditory organ — the amphibian papilla⁶⁶. In both cases these 'spontaneous' (in the sense that presynaptic membrane potential was constant) individual EPSCs were much larger and more variable than expected for the release of single vesicles — ranging more than tenfold in amplitude, with a coefficient of

Voltage-clamp

A fundamental electrophysiological technique for measuring ionic currents in cells while 'clamping' the membrane potential to prevent changes caused by those currents.

variation up to 0.95 (REF. 65) — and some had irregular, inflected ('multiphasic') waveforms as though they were composed of smaller units. The hypothesis for multivesicular release was based on these observations and on a correspondence between the smallest responses and glutamatergic 'minis' in the CNS, as well as on consistency with estimates from capacitance recordings^{43,61}. These assumptions have been strengthened by observations of classical minis that are thought to form larger, multivesicular EPSCs at frog hair cell synapses⁶⁷ (see below). Multivesicular release has also been proposed to explain the similarly large and variably-sized EPSCs in retinal bipolar cell synapses^{45,68}, that are composed from univesicular minis⁶⁹. If larger EPSCs indeed result from the synchronized release of multiple vesicles, then this occurs without a coordinating presynaptic action potential to evoke release, in contrast to what occurs at 'phasic' synapses. The mechanism for multivesicular release is unknown and remains under active investigation. Likewise, it is still uncertain as to whether multivesicular release operates at all ribbon synapses under all conditions. Both frog hair cells⁶⁷ and mouse retinal bipolar cells⁶⁹ regularly release single vesicles when the membrane potential is at, or negative to, the resting membrane potential. Also, as previously noted, variance analysis of membrane capacitance fits with the assumption that mostly single vesicle release occurs in mammalian cochlear hair cells⁵⁵.

Paired recordings from hair cells and afferents in both the frog⁶⁶ and the mammalian cochlea⁷⁰ further defined the synaptic transfer function. At solitary mammalian synapses, release rates were low enough to resolve individual EPSCs, and the low release rates indicated that the transfer function resulted entirely from changes in EPSC frequency without changes in average amplitude⁷⁰. The somewhat surprising implication, based on the assumption that release of single vesicles produced the smallest EPSCs, is that the number of vesicles that make up a multivesicular EPSC is independent of calcium influx⁷⁰. This conclusion was further supported by the observation that EPSC frequency (presynaptic hair cell membrane potential at -29 mV), but not amplitude, varied as a function of calcium buffer concentration⁷⁰, with equally large EPSCs under all conditions. Strikingly, this held true even when EGTA was substituted with BAPTA, a calcium-ion chelating agent whose more rapid binding normally interrupts calcium-dependent activities. To date, however, this finding has not been reported for other ribbon synapses. Small — presumably univesicular — events were observed in adult frog hair cell afferents when the presynaptic hair cell was held at -80 or -90 mV. These had a Gaussian distribution with a coefficient of variation (0.29), as expected for univesicular events⁶⁷. As the hair cell was depolarized to membrane potentials between -75 and -63 mV, the average EPSC amplitude grew larger, leading to the conclusion that both frequency and amplitude of EPSCs are calcium-dependent at this synapse. Similarly, the retinal bipolar to amacrine cell synapse seems to switch from univesicular to multivesicular release as the calcium channel open probability rises⁶⁹.

Initial descriptions of multivesicular release from hair cells were made in immature cochlear tissue (that is, tissue taken before hearing onset at postnatal day 12), prompting concerns that this is a transient feature of ribbon function⁶⁷. Studies on cochleas from older rats (postnatal days 19–60) have extended the original observations⁷¹. Mature EPSCs were faster and larger, with more symmetrical amplitude distributions in some recordings. Using the foregoing assumptions for the response to a single vesicle, the average mature EPSC was estimated to result from the release of between 7 and 9 vesicles. As in young synapses, the mean and distribution of EPSC amplitudes did not vary with release probability. Continued study of hair cells with differing synaptic transfer functions will help further in defining the range of mechanisms operated by the ribbons. For example, numerous outer hair cells contact the extended dendritic arbor of type II afferents in the cochlea to produce small, apparently monophasic EPSCs⁷². Although the effects of cable loss on the spread of signals along the extended type II dendrite remain to be determined, the overall rate of release seems to be much lower than that from ribbons of inner hair cells. It will be of interest to quantify ultrastructural details that may underlie these functional differences.

Implications for hair cell ribbon function. Our current view of hair cell ribbon function is based largely on two types of experiment: voltage-driven changes in presynaptic membrane capacitance of the hair cell and recordings from afferent dendrites of EPSCs. The chief distinction is that capacitance measurements report the summed behaviour of all the ribbons in that cell, whereas EPSCs can result from release at a single ribbon. Postsynaptic EPSCs provide evidence suggesting that individual hair cell ribbon synapses often release multiple vesicles simultaneously, as reconfirmed recently^{12,67,71}. By contrast, capacitance measurements suggest that cochlear hair cell ribbons most commonly release single vesicles⁵⁵. These different insights regarding vesicle release in mammalian cochlear hair cell ribbons eventually must be merged, as has recently been achieved for hair cells of the frog amphibian papilla⁶⁷. In particular, it will be useful to establish conditions for quantifying univesicular EPSCs from cochlear hair cell ribbons.

Both presynaptic capacitance and postsynaptic EPSCs have revealed that transmitter release (in some cases) rises linearly with increasing calcium influx. Combined with the effects of varied calcium buffering this has led to the postulate of a 'nanodomain' for vesicular release, in which calcium flux through a single channel can drive the fusion of a closely associated synaptic vesicle^{9,66,67,70}. This postulate has received recent support at the retinal bipolar–amacrine cell synapse, where single-vesicle EPSCs were produced under conditions in which only a single calcium channel was likely to be open at any ribbon⁶⁹. It is less certain, however, how multiple vesicles would be coordinately released at single ribbons as calcium channel gating probability rises. More challenging still is the task of coupling a nanodomain mechanism of gating to the observation that EPSC

'Mini'

Shorthand for miniature postsynaptic current, which is the current produced by spontaneous or evoked exocytosis of a single synaptic vesicle.

Cable loss

The decrement of 'passive' voltage signals along neuronal processes.

amplitude is independent of release probability at rat cochlear hair cell synapses^{65,70,71}, as has also been observed in the mouse¹².

The detection of univesicular EPSCs at low release probability in ribbon synapses of bipolar cells and hair cells may provide a way forward to new hypotheses. Perhaps the ribbon operates in the nanodomain mode at very low calcium channel open probabilities but switches into an as-yet-undefined multivesicular release mode as the probability of calcium channel opening rises. Conceivably, this higher probability release mode could then depend on a 'cloud' of calcium, as has been proposed for the gating of calcium-sensitive potassium channels in hair cells⁷³. The frequency at which that condition is met will depend on the mean open time of calcium channels and local calcium buffering, but is fundamentally a binomial prediction based on the number of channels and their mean open probability. Mean open time seems to be invariant with open probability¹¹ and therefore will not alter the extent of calcium 'overlap' by itself. Thus, for a given ribbon with a fixed number of calcium channels, EPSC frequency will rise with open probability and fall with stronger calcium buffering, as has been observed at hair cell synapses in rats⁷⁰ and frogs⁶⁷.

A satisfying corollary of this proposal is that synaptic strength should vary with the number of calcium channels that are clustered at each ribbon^{10,15} (FIG. 3), in keeping with substantially different activity patterns of afferents contacting an individual inner hair cell⁴⁶. With more channels, the threshold calcium for fusion would be reached at lower open probabilities (that is, more-negative membrane potentials). Intrinsic variations in release efficiency⁷¹ could add additional range to these synapses. Continued refinements in imaging technology, coupled with postsynaptic recordings, could provide much needed information on the relationship between stochastic calcium channel gating and EPSC amplitude at individual ribbon synapses.

The role of ribbon function in afferent firing patterns. Does the literature on afferent recordings help to refine hypotheses of ribbon function? On the one hand, spontaneous activity in cochlear afferent fibres ranges from ~1 Hz to over 100 Hz, requiring that some ribbons produce EPSCs that are large enough to exceed the threshold at these same frequencies. Grant *et al.*⁷¹ proposed that ribbons with only large monophasic EPSCs could drive higher frequency activity — most EPSPs being supra-threshold. On the other hand, ribbons releasing a large fraction of smaller amplitude, multiphasic EPSCs would produce more subthreshold responses and so drive lower levels of afferent activity. On this basis, calcium channel numbers and gating could be identical at each ribbon, but the spontaneous rate and acoustic threshold of afferent fibres would be determined by the ratio of monophasic to multiphasic events at each synapse.

Another relevant aspect of afferent function is the relatively low cycle-by-cycle probability of acoustically driven activity. For example, during phase-locking to a 1-kHz tone (the best frequency for the afferent), the probability of firing on a cycle-by-cycle basis ranged from

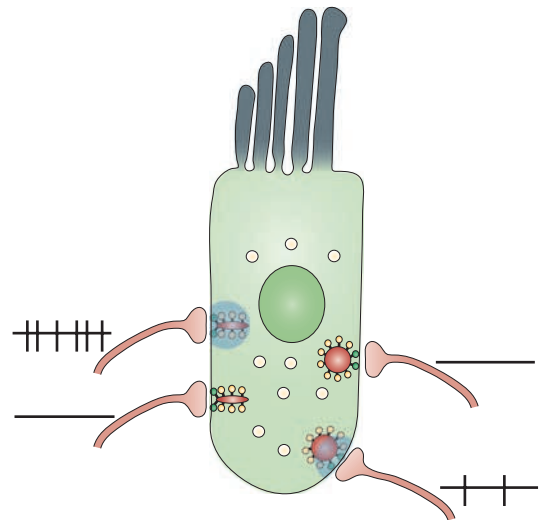


Figure 3 | Variable ribbon function within one cochlear inner hair cell. 'Low threshold' ribbons may have greater numbers of calcium channels, release transmitter more frequently at a given membrane potential and so trigger more afferent action potentials (top left trace; action potentials in afferent fibres are shown by black lines). 'High threshold' ribbons may have fewer calcium channels and therefore require greater depolarization to cause equivalent levels of afferent activity (bottom right trace). Alternatively, variations in vesicular fusion could produce similar outcomes. The overall probability of transmission is relatively low, so not every synapse will be active at a given point in time (bottom left and top right traces). Calcium influx is shown by the blue cloud. Vesicles attached to ribbons are shown by yellow circles and docked vesicles by green circles.

only 0.04 to a maximum of 0.15 (REF. 74). This is reminiscent of the low gating probability described for hair cell calcium channels¹¹. If the probability of release arises largely from the gating probability of calcium channels, an additional implication is that vesicle docking and priming do not lower the release probability further. Indeed, vesicle recruitment at ribbons seems to be fast and inexhaustible⁷⁵. Multivesicular release may be an adaptation to ensure precise timing⁷⁶ and indeed, first spike latency to acoustic stimuli was degraded in mice with reduced numbers of ribbons⁷⁷. Finally, as discussed earlier⁷⁸, the phase-constancy of afferent firing for different sound intensities⁷⁴ is consistent with the observation that average EPSC amplitude is invariant during hair cell depolarization^{65,70,71}: the afferent fibre is depolarized to threshold at the same rate (the same average synaptic amplitude) regardless of the acoustic intensity (regardless of hair cell depolarization).

Conclusion

Although we know a great deal about ribbon synapses, mysteries still abound. Do ribbons account for the rapid burst of release at the onset of depolarization, for sustained release during prolonged depolarization or for both? If synaptic vesicles attached to the ribbon participate in transmitter release, how do the vesicles in higher

Phase-locking
Precise timing between two signals. In the auditory system, phase-locking refers to coordination between action potentials in auditory neurons, and the cycles of a tonal stimulus.

rows reach the plasma membrane in order to fuse? Do vesicles move on the surface of the ribbon and, if so, is this an active process or simple diffusion? Do vesicles fuse with other vesicles on the ribbon? How are ribbons able to coordinate nearly simultaneous fusion of multiple vesicles? All of these fundamental questions remain to be answered and new approaches are needed to answer them — such as high-resolution optical imaging in living cells or ultrastructural methods to catch the vesicles in the act, and ways to alter ribbon function in real time during electrophysiological experiments.

It should also be kept in mind that not all ribbons may operate in the same way and, as studies advance, it will be important to discriminate between general features and unique specializations. After all, ribbons vary enormously in size and postsynaptic architecture, and so the relative importance of fast synchronous release versus slow sustained release is likely to vary as well, depending on cell type and on the functional context of the synapse. In this regard, ribbon synapses are no different from conventional synapses, which also vary widely in their functional properties.

- Dick, O. *et al.* The presynaptic active zone protein bassoon is essential for photoreceptor ribbon synapse formation in the retina. *Neuron* **37**, 775–786 (2003).
- Heidelberger, R., Thoreson, W. B. & Witkovsky, P. Synaptic transmission at retinal ribbon synapses. *Prog. Retin. Eye Res.* **24**, 682–720 (2005).
- Sterling, P. & Matthews, G. Structure and function of ribbon synapses. *Trends Neurosci.* **28**, 20–29 (2005).
- DeVries, S. H., Li, W. & Saszik, S. Parallel processing in two transmitter microenvironments at the cone photoreceptor synapse. *Neuron* **50**, 735–748 (2006).
- von Gersdorff, H., Vardi, E., Matthews, G. & Sterling, P. Evidence that vesicles on the synaptic ribbon of retinal bipolar neurons can be rapidly released. *Neuron* **16**, 1221–1227 (1996).
- Jackman, S. L. *et al.* Role of the synaptic ribbon in transmitting the cone light response. *Nature Neurosci.* **12**, 303–310 (2009).
This study characterized release from cone photoreceptor ribbon synapses and suggested that ribbons operate in a partially depleted state, which serves to accentuate transient responses to dimming of illumination.
- Calkins, D. J., Schein, S. J., Tsukamoto, Y. & Sterling, P. M. and L cones in macaque fovea connect to midgen ganglion cells by different numbers of excitatory synapses. *Nature* **371**, 70–72 (1994).
- Moser, T., Brandt, A. & Lysakowski, A. Hair cell ribbon synapses. *Cell Tissue Res.* **326**, 347–359 (2006).
- Brandt, A., Khimich, D. & Moser, T. Few Cav1.3 channels regulate the exocytosis of a synaptic vesicle at the hair cell ribbon synapse. *J. Neurosci.* **25**, 11577–11585 (2005).
- Meyer, A. C. *et al.* Tuning of synapse number, structure and function in the cochlea. *Nature Neurosci.* **12**, 444–453 (2009).
This study examined variations in ribbon number, size and intracellular distribution in inner hair cells along the cochlea's tonotopic axis. Fluorescent indicators revealed substantial variability in calcium 'hotspots', even within single inner hair cells.
- Zampini, V. *et al.* Elementary properties of Cav1.3 Ca²⁺ channels expressed in mouse cochlear inner hair cells. *J. Physiol.* **588**, 187–199 (2010).
In this paper, single-channel recording from inner hair cells of the mouse cochlea provided additional estimates of the numbers of channels associated with each active zone (~180 per ribbon) and the maximum open probability (~0.15).
- Pangrsic, T. *et al.* Hearing requires otoferlin-dependent efficient replenishment of synaptic vesicles in hair cells. *Nature Neurosci.* **13**, 869–876 (2010).
- Khimich, D. *et al.* Hair cell synaptic ribbons are essential for synchronous auditory signalling. *Nature* **434**, 889–894 (2005).
- Zenisek, D., Davila, V., Wan, L. & Almers, W. Imaging calcium entry sites and ribbon structures in two presynaptic cells. *J. Neurosci.* **23**, 2538–2548 (2003).
- Frank, T., Khimich, D., Neef, A. & Moser, T. Mechanisms contributing to synaptic Ca²⁺ signals and their heterogeneity in hair cells. *Proc. Natl Acad. Sci. USA* **106**, 4483–4488 (2009).
Fluorescent calcium indicators and calcium channel immunolabeling were used in this study to chart 'active zones' in cochlear inner hair cells. The covariation in the number of calcium channels and ribbon size found in this study may provide a mechanism for diversifying the activity of postsynaptic afferent neurons.
- Nouvian, R., Beutner, D., Parsons, T. D. & Moser, T. Structure and function of the hair cell ribbon synapse. *J. Membr. Biol.* **209**, 153–165 (2006).
- Martinez-Dunst, C., Michaels, R. L. & Fuchs, P. A. Release sites and calcium channels in hair cells of the chick's cochlea. *J. Neurosci.* **17**, 9133–9144 (1997).
- LoGiudice, L., Sterling, P. & Matthews, G. Mobility and turnover of vesicles at the synaptic ribbon. *J. Neurosci.* **28**, 3150–3158 (2008).
- Rea, R. *et al.* Streamlined synaptic vesicle cycle in cone photoreceptor terminals. *Neuron* **41**, 755–766 (2004).
- Holt, M., Cooke, A., Neef, A. & Lagnado, L. High mobility of vesicles supports continuous exocytosis at a ribbon synapse. *Curr. Biol.* **14**, 173–183 (2004).
- Zenisek, D. Vesicle association and exocytosis at ribbon and extraribbon sites in retinal bipolar cell presynaptic terminals. *Proc. Natl Acad. Sci. USA* **105**, 4922–4927 (2008).
Together with reference 18, this paper provided evidence for turnover of ribbon-associated synaptic vesicles during depolarization of retinal bipolar neurons.
- Schaeffer SF & Raviola, E. Membrane recycling in the cone cell endings of the turtle retina. *J. Cell Biol.* **79**, 802–825 (1978).
- Townes-Anderson, E., MacLeish, P. R. & Raviola, E. Rod cells dissociated from mature salamander retina, ultrastructure and uptake of horseradish peroxidase. *J. Cell Biol.* **100**, 175–188 (1985).
- Siegel, J. H. & Brownell, W. E. Synaptic and Golgi membrane recycling in cochlear hair cells. *J. Neurocytol.* **15**, 311–328 (1986).
- Paillart, C., Li, J., Matthews, G. & Sterling, P. Exocytosis and vesicle recycling at a ribbon synapse. *J. Neurosci.* **23**, 4092–4099 (2003).
- LoGiudice, L., Sterling, P. & Matthews, G. Vesicle recycling at ribbon synapses in the finely branched axon terminals of mouse retinal bipolar neurons. *Neuroscience* **164**, 1546–1556 (2009).
- Matthews, G. & Sterling, P. Evidence that vesicles undergo compound fusion on the synaptic ribbon. *J. Neurosci.* **28**, 5403–5411 (2008).
- Lenzi, D., Crum, J., Ellisman, M. H. & Roberts, W. M. Depolarization redistributes synaptic membrane and creates a gradient of vesicles on the synaptic body at a ribbon synapse. *Neuron* **36**, 649–659 (2002).
- Zenisek, D., Horst, N. K., Merrifield, C., Sterling, P. & Matthews, G. Visualizing synaptic ribbons in the living cell. *J. Neurosci.* **24**, 9752–9759 (2004).
This study characterized a fluorescent peptide that binds to RIBEYE, the principal protein component of ribbons, and that allows ribbons to be localized in single synaptic terminals for live-cell imaging combined with electrophysiology.
- Mennerick, S. & Matthews, G. Ultrafast exocytosis elicited by calcium current in synaptic terminals of retinal bipolar neurons. *Neuron* **17**, 1241–1249 (1996).
- von Gersdorff, H., Sakaba, T., Berglund, K. & Tachibana, M. Submillisecond kinetics of glutamate release from a sensory synapse. *Neuron* **21**, 1177–1188 (1998).
- Singer, J. H. & Diamond, J. S. Sustained Ca²⁺ entry elicits transient postsynaptic currents at a retinal bipolar synapse. *J. Neurosci.* **23**, 10923–10933 (2003).
- Thoreson, W. B., Rabl, K., Townes-Anderson, E. & Heidelberger, R. A highly Ca²⁺-sensitive pool of vesicles contributes to linearity at the rod photoreceptor ribbon synapse. *Neuron* **42**, 595–605 (2004).
- Bartoletti, T. M., Babai, N. & Thoreson, W. B. Vesicle pool size at the salamander cone ribbon synapse. *J. Neurophysiol.* **103**, 419–425 (2010).
- Midorikawa, M., Tsukamoto, Y., Berglund, K., Ishii, M. & Tachibana, M. Different roles of ribbon-associated and ribbonfree active zones in retinal bipolar cells. *Nature Neurosci.* **10**, 1268–1276 (2007).
- Coggins, M. R., Grabner, C. P., Almers, W. & Zenisek, D. Stimulated exocytosis of endosomes in goldfish retinal bipolar neurons. *J. Physiol.* **584**, 853–865 (2007).
- Palmer, M. J. Characterisation of bipolar cell synaptic transmission in goldfish retina using paired recordings. *J. Physiol.* **588**, 1489–1498 (2010).
- Hull, C., Studholme, K., Yazulla, S. & von Gersdorff, H. Diurnal changes in exocytosis and the number of synaptic ribbons at active zones of an ON-type bipolar cell terminal. *J. Neurophysiol.* **96**, 2025–2033 (2006).
- Snellman, J. & Zenisek, D. Photodamage of the ribbon disrupts coordination of multivesicular release and blocks vesicle replenishment at the rod bipolar cell synapse in mouse. *Soc. Neurosci. Abstr.* **522.7** (2009).
- Innocenti, B. & Heidelberger, R. Mechanisms contributing to tonic release at the cone photoreceptor ribbon synapse. *J. Neurophysiol.* **99**, 25–36 (2008).
- Moser, T. & Beutner, D. Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. *Proc. Natl Acad. Sci. USA* **97**, 883–888 (2000).
- Spassova, M. A. *et al.* Evidence that rapid vesicle replenishment of the synaptic ribbon mediates recovery from short-term adaptation at the hair cell afferent synapse. *J. Assoc. Res. Otolaryngol.* **5**, 376–390 (2004).
- Johnson, S. L., Marcotti, W. & Kros, C. J. Increase in efficiency and reduction in Ca²⁺ dependence of exocytosis during development of mouse inner hair cells. *J. Physiol.* **563**, 177–191 (2005).
- Edmonds, B. W., Gregory, F. D. & Schweizer, F. E. Evidence that fast exocytosis can be predominantly mediated by vesicles not docked at active zones in frog saccular hair cells. *J. Physiol.* **560**, 439–450 (2004).
- Singer, J. H., Lassoova, L., Vardi, N. & Diamond, J. S. Coordinated multivesicular release at a mammalian ribbon synapse. *Nature Neurosci.* **7**, 826–833 (2004).
- Lieberman, M. C. Single-neuron labeling in the cat auditory nerve. *Science* **216**, 1239–1241 (1982).
- Yi, E., Roux, I. & Glowatzki, E. Dendritic HCN channels shape excitatory postsynaptic potentials at the inner hair cell afferent synapse in the mammalian cochlea. *J. Neurophysiol.* **103**, 2532–2543 (2010).
- Lieberman, M. C., Dodds, L. W. & Pierce, S. Afferent and efferent innervation of the cat cochlea, quantitative analysis with light and electron microscopy. *J. Comp. Neurol.* **301**, 443–460 (1990).
- Wu, Y. C., Tucker, T. & Fettiplace, R. A theoretical study of calcium microdomains in turtle hair cells. *Biophys. J.* **71**, 2256–2275 (1996).
- Zanazzi, G. & Matthews, G. The molecular architecture of ribbon presynaptic terminals. *Mol. Neurobiol.* **39**, 130–148 (2009).
- Platzer, J. *et al.* Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca²⁺ channels. *Cell* **102**, 89–97 (2000).
- Nouvian, R. Temperature enhances exocytosis efficiency at the mouse inner hair cell ribbon synapse. *J. Physiol.* **584**, 535–542 (2007).
- Grant, L. & Fuchs, P. Calcium, calmodulin-dependent inactivation of calcium channels in inner hair cells of the rat cochlea. *J. Neurophysiol.* **99**, 2183–2193 (2008).
- Parsons, T. D., Lenzi, D., Almers, W. & Roberts, W. M. Calcium-triggered exocytosis and endocytosis in an isolated presynaptic cell, capacitance measurements in saccular hair cells. *Neuron* **13**, 875–883 (1994).
- Neef, A., Khimich, D., Piri, P., Riedel, D., Wolf, F. & Moser, T. Probing the mechanism of exocytosis at the hair cell ribbon synapse. *J. Neurosci.* **27**, 12933–12944 (2007).

56. Johnson, S. L., Forge, A., Knipper, M., Munkner, S. & Marcotti, W. Tonotopic variation in the calcium dependence of neurotransmitter release and vesicle pool replenishment at mammalian auditory ribbon synapses. *J. Neurosci.* **28**, 7670–7678 (2008).
57. Beurg, M. *et al.* Calcium- and otoferlin-dependent exocytosis by immature outer hair cells. *J. Neurosci.* **28**, 1798–1803 (2008).
58. Johnson, S. L., Franz, C., Knipper, M. & Marcotti, W. Functional maturation of the exocytotic machinery at gerbil hair cell ribbon synapses. *J. Physiol.* **587**, 1715–1726 (2009).
59. Johnson, S. L. *et al.* Synaptotagmin IV determines the linear Ca²⁺ dependence of vesicle fusion at auditory ribbon synapses. *Nature Neurosci.* **13**, 45–52 (2010).
60. Dulon, D., Safieddine, S., Jones, S. M. & Petit, C. Otoferlin is critical for a highly sensitive and linear calcium-dependent exocytosis at vestibular hair cell ribbon synapses. *J. Neurosci.* **29**, 10474–10487 (2009).
61. Beutner, D. & Moser, T. The presynaptic function of mouse cochlear inner hair cells during development of hearing. *J. Neurosci.* **21**, 4593–4599 (2001).
62. Furukawa, T., Hayashida, Y. & Matsuura, S. Quantal analysis of the size of excitatory post-synaptic potentials at synapses between hair cells and afferent nerve fibres in goldfish. *J. Physiol.* **276**, 211–226 (1978).
63. Crawford, A. C. & Fettiplace, R. The frequency selectivity of auditory nerve fibres and hair cells in the cochlea of the turtle. *J. Physiol.* **306**, 79–125 (1980).
64. Siegel, J. H. Spontaneous synaptic potentials from afferent terminals in the guinea pig cochlea. *Hear. Res.* **59**, 85–92 (1992).
65. Glowatzki, E. & Fuchs, P. A. Transmitter release at the hair cell ribbon synapse. *Nature Neurosci.* **5**, 147–154 (2002).
66. Keen, E. C. & Hudspeth, A. J. Transfer characteristics of the hair cell's afferent synapse. *Proc. Natl Acad. Sci. USA* **103**, 5537–5542 (2006).
67. Li, G. L., Keen, E., Andor-Ardo, D., Hudspeth, A. J. & von Gersdorff, H. The unitary event underlying multiquantal EPSCs at a hair cell's ribbon synapse. *J. Neurosci.* **29**, 7558–7568 (2009).
- In this study, paired presynaptic and postsynaptic recordings in the frog amphibian papilla were used to establish the response to a single vesicle. The larger postsynaptic responses that occurred with depolarization of the hair cell are presumed to be composed of multiple, simultaneously-released vesicles.**
68. Maple, B. R., Werblin, F. S. & Wu, S. M. Miniature excitatory postsynaptic currents in bipolar cells of the tiger salamander retina. *Vision Res.* **34**, 2357–2362 (1994).
69. Jarsky, T., Tian, M. & Singer, J. H. Nanodomain control of exocytosis is responsible for the signaling capability of a retinal ribbon synapse. *J. Neurosci.* **30**, 11885–11895 (2010).
- In this study, paired recordings from bipolar cells and postsynaptic amacrine cells revealed small (single vesicle) release events when presynaptic calcium channel gating was rare. With presynaptic depolarization larger, multivesicular release events occurred.**
70. Goutman, J. D. & Glowatzki, E. Time course and calcium dependence of transmitter release at a single ribbon synapse. *Proc. Natl Acad. Sci. USA* **104**, 16341–16346 (2007).
- This study showed that paired recordings from inner hair cells and type I afferent dendrites describe a 'linear' synaptic transfer function. This is explained by an increase in release probability, with no change in the average amplitude of postsynaptic currents.**
71. Grant, L., Yi, E. & Glowatzki, E. Two modes of release shape the postsynaptic response at the inner hair cell ribbon synapse. *J. Neurosci.* **30**, 4210–4220 (2010).
- In this study, postsynaptic currents were recorded in dendrites of type I cochlear afferents from older rats (up to 2 months of age). As at younger synapses, postsynaptic currents were widely variable in size and the amplitude distribution was unchanged by hair cell depolarization. The fraction of smaller 'multiphasic' currents varied between synapses, but on average were fewer than in younger synapses.**
72. Weisz, C., Glowatzki, E. & Fuchs, P. The postsynaptic function of type II cochlear afferents. *Nature* **461**, 1126–1129 (2009).
73. Roberts, W. M. Localization of calcium signals by a mobile calcium buffer in frog saccular hair cells. *J. Neurosci.* **14**, 3246–3262 (1994).
74. Rose, J. E., Brugge, J. F., Anderson, D. J. & Hind, J. E. Phase-locked response to low-frequency tones in single auditory nerve fibers of the squirrel monkey. *J. Neurophysiol.* **30**, 769–793 (1967).
75. Griesinger, C. B., Richards, C. D. & Ashmore, J. F. Fast vesicle replenishment allows infatigable signalling at the first auditory synapse. *Nature* **434**, 212–215 (2005).
76. Wittig, J. H. & Parsons, T. D. Synaptic ribbon enables temporal precision of hair cell afferent synapse by increasing the number of readily releasable vesicles: a modeling study. *J. Neurophysiol.* **100**, 1724–1739 (2008).
77. Buran, B. N. *et al.* Onset coding is degraded in auditory nerve fibers from mutant mice lacking synaptic ribbons. *J. Neurosci.* **30**, 7587–7597 (2010).
78. Fuchs, P. A. Time and intensity coding at the hair cell's ribbon synapse. *J. Physiol.* **566**, 7–12 (2005).
79. Brandstätter, J. H., Fletcher, E. L., Garner, C. C., Gundelfinger, E. D. & Wässle, H. Differential expression of the presynaptic cytomatrix protein bassoon among ribbon synapses in the mammalian retina. *Eur. J. Neurosci.* **11**, 3683–3693 (1999).
80. tom Dieck, S. *et al.* Molecular dissection of the photoreceptor ribbon synapse, physical interaction of Bassoon and RIBEYE is essential for the assembly of the ribbon complex. *J. Cell Biol.* **168**, 825–836 (2005).
81. Deguchi-Tawarada, M. *et al.* Active zone protein CAST is a component of conventional and ribbon synapses in mouse retina. *J. Comp. Neurol.* **495**, 480–496 (2006).
82. Schmitz, F. A., Königstorfer, A. & Südhof, T. C. RIBEYE, a component of synaptic ribbons, a protein's journey through evolution provides insight into synaptic ribbon function. *Neuron* **28**, 857–872 (2000).
83. Magupalli, V. G. *et al.* Multiple RIBEYE–RIBEYE interactions create a dynamic scaffold for the formation of synaptic ribbons. *J. Neurosci.* **28**, 7954–7967 (2008).
84. Usukura, J. & Yamada, E. Ultrastructure of the synaptic ribbons in photoreceptor cells of *Rana catesbeiana* revealed by freeze-etching and freeze-substitution. *Cell Tissue Res.* **247**, 483–488 (1987).
85. Wang, Y., Okamoto, M., Schmitz, F., Hofmann, K. & Südhof, T. C. Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. *Nature* **388**, 593–598 (1997).
86. Muresan, V., Lyass, A. & Schnapp, B. J. The kinesin motor KIF3A is a component of the presynaptic ribbon in vertebrate photoreceptors. *J. Neurosci.* **19**, 1027–1037 (1999).
87. Dick, O., Hack, L., Altrock, W. D., Garner, C. C., Gundelfinger, E. D. & Brandstätter, J. H. Localization of the presynaptic cytomatrix protein Piccolo at ribbon and conventional synapses in the rat retina, comparison with Bassoon. *J. Comp. Neurol.* **439**, 224–234 (2001).
88. Venkatesan, J. K. *et al.* Nicotinamide adenine dinucleotide-dependent binding of the neuronal Ca²⁺ sensor protein GCAP2 to photoreceptor synaptic ribbons. *J. Neurosci.* **30**, 6559–6576 (2010).
89. Curtis, L. B. *et al.* Syntaxin 3b is a t-SNARE specific for ribbon synapses of the retina. *J. Comp. Neurol.* **510**, 550–559 (2008).
90. Curtis, L. *et al.* Syntaxin 3B is essential for the exocytosis of synaptic vesicles in ribbon synapses of the retina. *Neuroscience* **166**, 832–841 (2010).
91. Reim, K. *et al.* Structurally and functionally unique complexins at retinal ribbon synapses. *J. Cell Biol.* **169**, 669–680 (2005).
92. Roux, I. *et al.* Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* **127**, 277–289 (2006).
93. Duncan, G., Rabl, K., Gemp, I., Heidelberger, R. & Thoreson, W. B. Quantitative analysis of synaptic release at the photoreceptor synapse. *Biophys. J.* **98**, 2102–2110 (2010).
94. Beutner, D., Voets, T., Neher, E. & Moser, T. Calcium dependence of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse. *Neuron* **29**, 681–690 (2001).
95. von Gersdorff, H. & Matthews, G. Calcium-dependent inactivation of calcium current in synaptic terminals of retinal bipolar neurons. *J. Neurosci.* **16**, 115–122 (1996).
96. Zidanic, M. & Fuchs, P. A. Kinetic analysis of barium currents in chick cochlear hair cells. *Biophys. J.* **68**, 1323–1336 (1995).
97. Mennerick, S. & Matthews, G. Rapid calcium-current kinetics in synaptic terminals of goldfish retinal bipolar neurons. *Vis. Neurosci.* **15**, 1051–1056 (1998).
98. Tachibana, M., Okada, T., Arimura, T., Kobayashi, K. & Piccolino, M. Dihydropyridine-sensitive calcium current mediates neurotransmitter release from bipolar cells of the goldfish retina. *J. Neurosci.* **13**, 2898–2909 (1993).
99. Heidelberger, R. & Matthews, G. Calcium influx and calcium current in single synaptic terminals of goldfish retinal bipolar neurons. *J. Physiol.* **447**, 235–256 (1992).
100. Schmitz, Y. & Witkovsky, P. Glutamate release by the intact light-responsive photoreceptor layer of the *Xenopus* retina. *J. Neurosci. Methods* **68**, 55–60 (1996).
101. Robertson, D. & Paki, B. Role of L-type Ca²⁺ channels in transmitter release from mammalian inner hair cells. II. Single-neuron activity. *J. Neurophysiol.* **87**, 2734–2740 (2002).
102. Zhang, S. Y., Robertson, D., Yates, G. & Everett, A. Role of L-type Ca²⁺ channels in transmitter release from mammalian inner hair cells. I. Cross sound-evoked potentials. *J. Neurophysiol.* **82**, 3307–3315 (1999).
103. Bech-Hansen, N. T. *et al.* Loss-of-function mutations in a calcium-channel $\alpha 1$ -subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nature Genet.* **19**, 264–267 (1998).
104. Strom, T. M. *et al.* An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nature Genet.* **19**, 260–263 (1998).
105. Mansergh, F. *et al.* Mutation of the calcium channel gene *Cacna1f* disrupts calcium signaling, synaptic transmission and cellular organization in mouse retina. *Hum. Mol. Genet.* **14**, 3035–3046 (2005).
106. Morgans, C. W. Localization of the α_{1E} calcium channel subunit in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **42**, 2414–2418 (2001).
107. Wässle, H., Haverkamp, S., Grünert, U. & Morgans, C. W. In *The Neural Basis of Early Vision*. (ed. Kaneko, A.) 19–38 (Springer Verlag, Tokyo, 2003).
108. Haeseleer, F. *et al.* Essential role of Ca²⁺-binding protein 4, a Cav1.4 channel regulator, in photoreceptor synaptic function. *Nature Neurosci.* **7**, 1079–1087 (2004).
109. Zeitz, C. *et al.* Mutations in *CABP4*, the gene encoding the Ca²⁺-binding protein 4, cause autosomal recessive night blindness. *Am. J. Hum. Genet.* **79**, 657–667 (2006).
110. Kollmar, R., Montgomerie, L. G., Fak, J., Henry, L. J. & Hudspeth, A. J. Predominance of the $\alpha 1D$ subunit in L-type voltage-gated Ca²⁺ channels of hair cells in the chicken's cochlea. *Proc. Natl Acad. Sci. USA* **94**, 14883–14888 (1997).
111. Michna, M. *et al.* Cav1.3 ($\alpha 1D$) Ca²⁺ currents in neonatal outer hair cells of mice. *J. Physiol.* **553**, 747–758 (2003).
112. Brandt, A., Striessnig, J. & Moser, T. Cav1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. *J. Neurosci.* **23**, 10832–10840 (2003).
113. Harlow, M. L., Ress, D., Stoschek, A., Marshall, R. M. & McMahan, U. J. The architecture of active zone material at the frog's neuromuscular junction. *Nature* **409**, 479–484 (2001).
114. Zampighi, G. *et al.* Conical electron tomography of a chemical synapse, polyhedral cages dock vesicles to the active zone. *J. Neurosci.* **28**, 4151–4160 (2008).
115. Siksou, L. *et al.* Three-dimensional architecture of presynaptic terminal cytomatrix. *J. Neurosci.* **27**, 6868–6877 (2007).
116. Zhai, R. G. & Bellen, H. J. The architecture of the active zone in the presynaptic nerve terminal. *Physiology* **19**, 262–270 (2004).
117. Rao-Mirotnik, R., Harkins, A. B., Buchsbaum, G. & Sterling, P. Mammalian rod terminal, architecture of a binary synapse. *Neuron* **14**, 561–569 (1995).

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

Gary Matthews' homepage: <http://neurobiology.informatics.sunysb.edu/graduate/faculty/matthews.html>
Paul Fuchs' homepage: <http://neuroscience.jhu.edu/PaulFuchs.php>

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