Review

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Spikes and ribbon synapses in early vision

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Image processing begins in the retina, where neurons respond with graded voltage changes that must be converted into spikes. This conversion from 'analog' to 'digital' coding is a fundamental transformation carried out by the visual system, but the mechanisms are still not well understood. Recent work demonstrates that, in vertebrates, graded-to-spiking conversion of the visual signal begins in the axonal system of bipolar cells (BCs), which transmit visual information through ribbon-type synapses specialized for responding to graded voltage signals. Here, we explore the evidence for and against the idea that ribbon synapses also transmit digital information. We then discuss the potential costs and benefits of digitization at different stages of visual pathways in vertebrates and invertebrates.

Introduction

Sensory systems encode physical stimuli that vary continuously, such as the loudness or frequency of sound or the intensity of light. The receptor cells that sense these forms of energy represent the intensity of the stimulus through changes in membrane potential, an analog representation that varies as a continuous function of stimulus amplitude. The subsequent transmission of this information to the brain typically requires this analog signal to undergo a fundamental transformation: digitization into action potentials (APs or spikes, see Glossary). The amplitude of APs is relatively fixed; thus, information is mainly contained in their temporal sequence. The necessity for digitization arises from fundamental properties of neuronal signal conduction. Left to spread passively, graded voltage signals rapidly become smaller and slower as they move from their point of origin. By contrast, APs involve regenerative mechanisms, allowing signals to be transmitted along axons over large distances while maintaining reliability and temporal precision.

Analog-to-digital (A–D) conversion occurs at different stages of pathways for different sensory modalities (Figure 1). Some primary receptors can immediately generate spikes for transmission through their long axons, such as mechanoreceptors in the skin and olfactory receptors in the nasal epithelium. Other mechanosensitive neurons, such as hair cells of vertebrate auditory and vestibular systems, generate analog signals that are only transmitted as far as a synapse located in the main cellular compartment, with A–D conversion occurring in the secondary afferent neuron. Why should different sensory systems carry out A–D conversion at different stages? How does this conversion occur and what is it good for? How are the synapses that transmit these signals suited to their task? In this review, we discuss these questions by making comparisons between the early visual system of vertebrates and insects.

Circuits carrying out the first stages of visual processing

The vertebrate retina is the window of the brain onto the visual world and a beautiful neural circuit [1,2]. Here, photoreceptors (PRs) convert light into graded changes in membrane potential for transmission to secondary neurons, the retinal BCs, through ribbon synapses. BCs in turn form excitatory connections with retinal ganglion cells (RGCs), which deliver the results of retinal processing to the brain as a spike code. At each synaptic stage of this vertical pathway, visual signals are shaped and modulated by complex interactions with inhibitory interneurons: horizontal cells in the outer retina and amacrine cells (ACs) in the inner retina. Notably, several AC types also respond to visual stimuli with regenerative depolarizations, including

Glossary

Action potential (AP): a highly stereotype, all-or-nothing depolarizing voltage transient, with a clear refractory period, driven predominately by sodium influx, as used by most neurons and described by the Hodgkin–Huxley model.
Damped voltage oscillation: an electrical resonance generated by the balanced interplay of voltage-activated inward and outward currents, for example, mediated by calcium and potassium channels. Imbalance of the currents either suppresses the oscillation, or converts depolarizing phases into spikes.
Digital signals: voltages that have near-constant amplitude and are clocked with a certain speed (cycles/s). Used here as shorthand to depict pulse trains of voltages in the form of APs.
Rebound spike: a single spike elicited after release from (strong) hyperpolarization.
Spike: we use 'spike' as a collective term to denote a stereotype, fast regenerative depolarizing voltage transient with a clear voltage threshold that is supported by either sodium and/or calcium currents. 'Spikes' include both APs and spikelets.
Spikelet: a spikelet can have variable amplitudes and is supported by either calcium and/or sodium currents.

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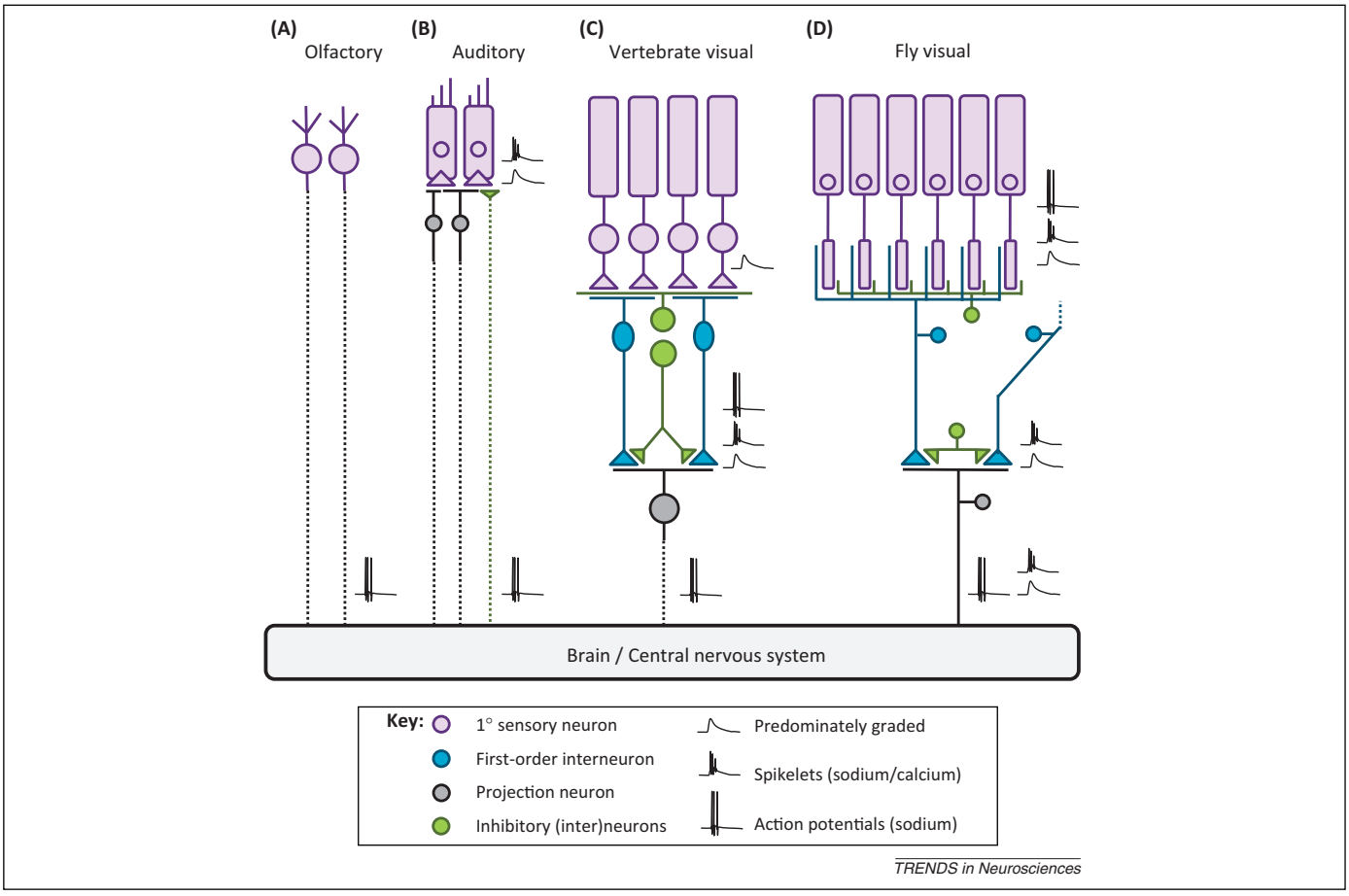

Figure 1. Analog–digital (A–D) conversion in different sensory systems. Different sensory systems implement A–D conversion at different processing stages. **(A)** Olfactory receptor neurons feature long axons that generate spikes themselves. **(B)** Hair cells forward a graded and/or spiking signal to spiking afferents and, depending on the system, they can receive efferent inhibition. **(C)** In the early vertebrate visual system, photoreceptors (PRs) forward visual information via bipolar cells (BCs, blue) to retinal ganglion cells (RGCs, gray), the spiking output neurons of the retina. Horizontal cells and amacrine cells (green) provide lateral inhibitory connectivity in two synaptic layers. **(D)** Similarly, invertebrate R1–R6 PRs connect via lamina neurons (blue) to transmedullary neurons (TM; gray), and inhibitory connections (green) provide lateral feedback in two layers. In both vertebrates and invertebrates, PRs usually use a predominately graded mode of signal encoding, although there are a few exceptions. Secondary neurons in the sensory periphery can use a combination of spiking and graded modes of transmission. Projection neurons are all spiking neurons in the case of vertebrates (i.e., RGCs, but can use different graded and spiking modes of transmission in invertebrates (TMs).

full-blown APs [3]. Traditionally, BCs have been considered nonspiking neurons that drive transmitter release at their axon terminals through purely graded potentials [4,5]. However, recent evidence indicates that this picture is a simplification and that BCs in a range of species are also capable of generating spikes [6–10].

A similar overall organization exists in the compound eyes of insects, as exemplified by flies [11–14]. Here, graded PR signals from six PRs (R1–6) in each retinal module (ommatidium) converge to large monopolar cells (LMCs), which in turn provide input to transmedullary cells (TMs). Two other PRs (R7/R8) provide direct inputs to TMs. Depending on the type of neuron, both LMC and TM neurons generate a mixture of graded and spiking signals (e.g., [15,16]). TMs provide input to a wide range of visual interneurons in the lobula and lobula plate complex, which, again depending on the type of neuron, generate graded, mixed, or spiking visual responses [12,13]. Similarly, in the accessory visual system (ocelli), graded PR signals are transmitted to L-neurons, which can generate spikes in a manner similar to LMCs in the compound eye (e.g., [17]). As in the vertebrate retina, synaptic transmission in the compound eye is modified by inhibitory inputs at all synaptic stages, most notably at the PR–LMC synapses [18]. Therefore, the propensity to generate graded and spiking responses in early visual neurons of both vertebrates and invertebrates is highly diverse, with various parallel pathways using different forms of A-D conversion at different processing stages (Figure 1).

How are analog and digital signals transmitted from one neuron to another? Intriguingly, the first sensory synapses transmitting information about light and sound are set apart from 'conventional' synapses by an unusual organelle, the ribbon, which aggregates vesicles close to the active zone (Figure 2). At conventional synapses, an AP lasting a few milliseconds triggers a transient burst of vesicle fusion. Ribbon synapses share many fundamental properties with conventional synapses and can also release vesicles in short, fast bursts. However, they also support a continuous mode of transmitter release, the rate of which varies continuously with graded changes in membrane potential. In vertebrates, ribbons exist in PRs and bipolar cells of the retina, and in mechanosensitive hair cells of cochlea and vestibular organs of balance, as well as in the lateral line of fish (reviewed in [19–23]). Notably, spikes and resonances have also been reported in hair cells (Box 1). Many insects have comparable

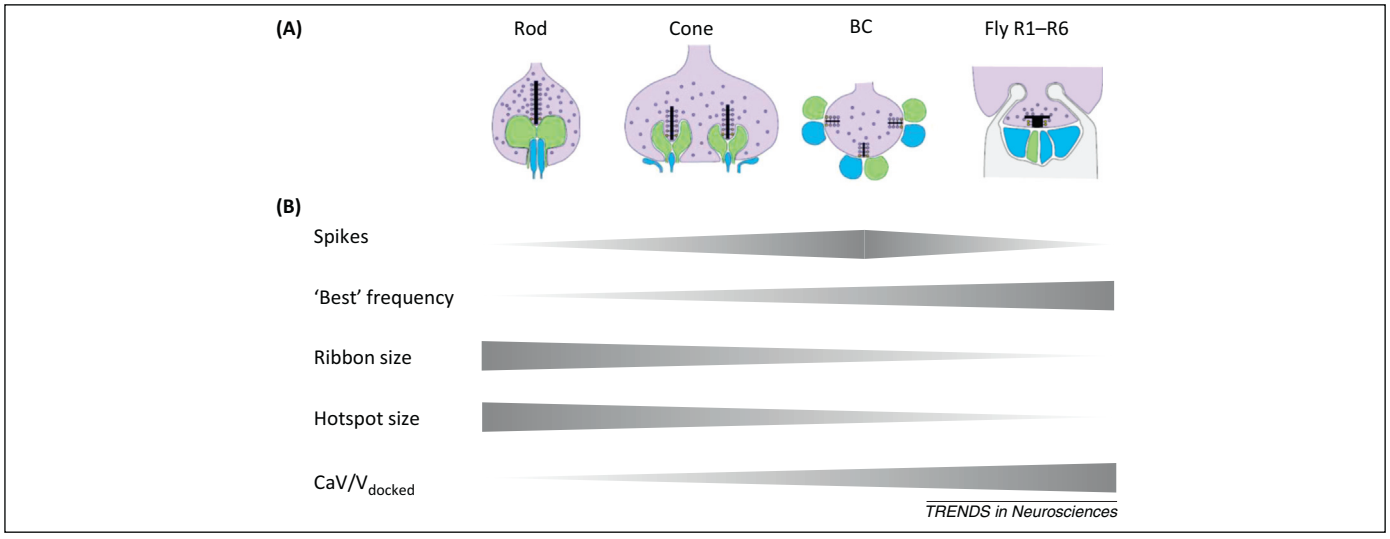

Figure 2. Ribbon synapses in visual systems. **(A)** Schematic representations of ribbon-type synapses. Rod and cone photoreceptors (PRs) and bipolar cells (BC) of the vertebrate visual system, and fly R1–R6 PR synapses. Ribbons are shown in black, inhibitory feedback interneurons in green, afferent neurons in blue, and glial cells in gray. **(B)** Voltage spikes are most notably a feature of some types of BC, which typically exhibit peak transmission at 5–15 Hz. The 'best frequency' of rods is significantly lower and they transmit through larger ribbons containing more vesicles and fewer voltage-activated calcium channels (CaV) per docked vesicle (V_{docked}). A tendency for neurons operating at lower frequencies to transmit through larger ribbons containing more vesicles also seems to occur in the auditory system (Table 1, main text). Note that, although the different neurons can be sorted by their 'best frequency', there is significant overlap in their operating ranges. Adapted from [19,24] (A).

synaptic structures that aggregate vesicles at the active zone, such as the 'T-bars' in insect PRs (reviewed in [24]).

In the first part of this review, we examine the evidence for and against the idea that ribbon synapses can transmit both analog and digital information. In the second part, we discuss the potential costs and benefits of digitization at different stages of visual pathways.

Ribbon synapses and transmission of spikes

Although it has generally been thought that sensory neurons transmitting through ribbon synapses are purely graded, it is now clear that many are also capable of producing regenerative voltage signals, either as damped oscillations, spikelets, and/or as full-fledged fast APs. Notably, these signals are often initiated by the same voltage-activated calcium channels that control neurotransmitter release and, therefore, are intimately linked to the molecular structure and function of the ribbon synapse. The difficulty has been to establish whether spiking activity is a normal feature of sensory processing. Depending on the recording conditions, the ability of a neuron to spike might be enhanced or abolished, and so the evidence must be carefully examined.

Vertebrate PRs

Rod and cone PRs can generate spikes in a range of species, from amphibians to humans, but the recording conditions have tended to be unusual [24–30]. For example, calcium spikes recorded in isolated PRs [25–27] necessarily occur in the absence of inhibitory feedback from horizontal cells. In retinal explants, spikes in PRs have only been observed following the block of K^+ currents [28], upon 'squeezing' into suction pipettes [29], or following release from strong hyperpolarization [30]. Similarly, the probability of spike-like transients recorded optically in mouse cones increased with decreasing quality of tissue slice preparation [31]. To date, there is no good evidence that vertebrate PRs encode light with spikes *in vivo*.

Retinal bipolar cells

The traditional view that BCs are purely graded neurons was first challenged by the demonstration of regenerative potentials in a particular type of ON BC in the goldfish, the Mb1, which receives input from both rods and cones [32,33]. Importantly, these spikes could be elicited by light stimulation in retinal slices [6]. Mb1s have very large terminals, enabling the functional properties of these ribbon synapses to be studied using a variety of imaging techniques and electrophysiological approaches. These experiments have demonstrated that the regenerative 'engine' of Mb1 bipolar cells is located directly within the axonal terminal, which expresses a high density of L-type Ca^{2+} channels and closely coupled Ca^{2+}-dependent K^+ (BK) channels [6,34]. However, spiking in BCs is not unique to fish: two types of BC in the rat retina can generate spikes through TTX-sensitive Na^+ channels upon current injection [7,35], and one type of ON BC in ground squirrel generates Na^+ spikes triggered by light [10]. Indeed, most BCs in fish [36] and approximately half of BCs in rat exhibit clear Na^+ currents [37], and Na^+ channels in transient BCs of the salamander retina enhance visual responses recorded at the RGC level [38].

Although the electrophysiological evidence for spikes in bipolar cells has accumulated gradually, the notion that they are used to encode light has been slow to take hold.

Box 1. Spikes and active resonances in hair cells

Many hair cells generate spikes and electrical resonance from active currents for sensory tuning, amplification, and activity-dependent mapping to postsynaptic partners before the onset of hearing. For example, auditory hair cells of lower vertebrates often use active electrical tuning mechanisms to shape their frequency selectivity (reviewed in [66]). In addition, many hair cells are capable of generating spikes (e.g., in the bullfrog sacculus [67,79]), but their role during physiological processing remains unclear (for a discussion, see [79]). Spikes in mammalian IHCs appear to be restricted to developmental periods before the onset of hearing [80].

This is understandable because electrophysiology necessarily requires upsetting the retinal tissue by, for instance, slicing it. Recently, however, a less invasive and more direct approach has been used to monitor the activity of BC terminals *in vivo*: multiphoton imaging of calcium reporter proteins in zebrafish. Using this approach, Dreosti and colleagues [8,9] analyzed light-evoked Ca^{2+} signals in BC terminals of larval zebrafish and found that most terminals generated events that were highly reminiscent of Ca^{2+} transients evoked by an underlying voltage spike. These Ca^{2+} transients were larger than those associated with graded depolarizations, indicating that they would drive neurotransmitter release at higher rates. Notably, these Ca^{2+} transients occurred in a variety of different functional types of BCs, including ON, OFF, transient, and sustained BCs. Using Ca^{2+} imaging, spikes were recently also recorded in at least three types of mouse BCs [39]. Taken together, this evidence indicates that spikes are used to encode light in a substantial fraction of BC types across vertebrates.

Insect compound eyes

Most insect PRs signal with graded voltages, but some clearly generate spikes *in vivo*. PRs in honeybee drones generate full-blown APs [40], whereas the axons of cockroach PRs seem to transmit both graded and spiking signals [41,42]. Even fly PRs may generate repetitive slow spiking activity, when the dampening K^+ channels are inhibited, and this activity is transmitted across the synapse to LMCs [43]. Beyond PRs, a subclass of fly first-order interneurons (L3) may generate spikes in response to depolarizing input [16], although they seem to also transmit the highly band-pass filtered graded signals all the way to their axon terminals [44]. Analogously, L-neurons of the ocellar eyes can generate (rebound) spikes, but also pass along the graded component to the next processing stage (reviewed in [45]). Deeper in the brain, the TM neurons generate both spikes and graded potentials. In the lobular plate, the large motion tangential neurons use spikes, graded potentials, or a mixture of both, depending on cell type (reviewed in [13]).

How might a ribbon synapse deal with a spike?

Most synapses in the vertebrate brain are small, perhaps a micron in diameter, containing just a single active zone. Although there may be approximately 100 vesicles in the synaptic terminal, only a handful are docked at the active zone ready to respond to an increase in local Ca^{2+} concentration. These small synapses are rather unreliable: the arrival of a single spike does not necessarily trigger transmission, in part because high levels of Ca^{2+} (tens to hundreds of micromolar) are required to trigger vesicle fusion.

Table 1. Properties of ribbon synapses[a,b]

	Rod	Cone	BCs	HC frog sacculus	HC turtle cochlea	IHC mammal	Fly R1–R6
Typical frequency range (Hz)	<5	<10	<30	<200; best, ~50	<1000; best, ~200	>1000	<300
Spikes and/or resonance	Graded	Graded	Graded, spikes, resonance	Graded, spikes, resonance	Graded, spikes, resonance	Graded	Graded
Ribbons							
Ribbon shape	Planar	Planar	Platelet	Spheroid	Spheroid	Elliptical and/or platelet	T shaped
Number of ribbons	Mammals, 1–2; salamander, 5–10	10–50	Sustained, 30–50; transient, 100–120	15–20	20–60	10–25	*Drosophila*, 50; housefly, 200
Size: length × height (nm)	>1000 × 200–500	200–1000 × 150–250	400 × 150–230	200–450[2]	200–270[2]	<200 × 200–600	220–360 × 130–170
Vesicles							
Vesicles ribbon^{-1}	600–700	100–300	70–110	300–400	–	100–200	900
Docked vesicles ribbon^{-1}	100–130	20–60	10–20	40–200	30–55	10–15	–
Transmitter	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Glutamate	Histamine
Calcium channels							
Channels ribbon^{-1}	>300	>300	30–50	80–100	20–40	80–200	–
Channel type	Mainly L-type Ca^{2+} 1.4	Mainly L-type Ca^{2+} 1.4	Mainly L-type Ca^{2+} 1.4	Mainly L-type Ca^{2+} 1.3	Mainly L-type Ca^{2+} 1.3	Mainly L-type Ca^{2+} 1.3	Cacophony
Micro/nanodomain (nm)	>1000	>1000	600–800	300–1000	–	20–40	–
Release kinetics							
$\tau_{release}$ fast (ms)	20–45	3	0.5–4	2–10	18–46	10	–
$\tau_{release}$ slow (ms)	1000	500	150–300	–	–	>1000	–
Sustained release (vesicles s^{-1} ribbon^{-1})	>150	10–80	20	80–100	600–800 fF/s	96–223 fF/s	100

[a]Definition of terms: typical frequency range, best frequency of synapses (across types of neuron and across species); $\tau_{release}$ (fast) and (slow), time constant of release kinetics during RRP depletion (fast) and ongoing activity (slow); sustained release, typical release rates during ongoing signaling.

[b]References: Rods [19,81–86]; Cones [19,82–85,87–91]; BCs [19,32,46,48,49,51,52,90,92–97]; HC frog [21,49,50,79,93,98,99]; HC turtle [21,100]; IHC mammal [19,21,49,101–103]; T-Bar [54,55,82,104,105].

It appears that, for synapses that transmit the first sensory information, evolution has deviated from this 'conventional' synapse design. Several structural and functional differences allow ribbon synapses to support continuous neurotransmitter release and thereby signal graded changes in membrane potential: (i) terminals tend to be large, containing many thousands of vesicles; (ii) the presynaptic compartment typically contains multiple active zones; (iii) active zones have a ribbon attached, holding a large pool of vesicles just next to the fusion sites; (iv) vesicles in the cytoplasm are more mobile than those in conventional synapses, allowing an efficient resupply of vesicles to ribbon and active zone; (v) vesicle fusion can be triggered by low (submicromolar) levels of calcium; (vi) Ca^{2+} channels at the active zone are usually of the L-type and display little inactivation, and (vii) Ca^{2+} influx also accelerates the processes that supply vesicles for release (Table 1).

There is, however, a second mode of vesicle release in ribbon synapses that is at least as rapid as in conventional synapses [46]. For instance, in BCs, a Ca^{2+} spike can trigger the fusion of >20 vesicles at a single active zone [47]. This extremely fast mechanism probably reflects exocytosis of vesicles of a 'rapidly releasable pool' (RRP) primed for release, and allows the synapse to transmit information about a stimulus with very short lag and with high temporal precision.

Can a ribbon synapse that transmits information about graded changes in membrane potential also signal the arrival of a spike? The answer is yes. Direct injection of a spike waveform into Mb1 terminals resulted in a marked increase in membrane capacitance, confirming that they can activate the synaptic release machinery strongly [47]: the number of vesicles released is roughly equivalent to the total number of vesicles docked at the active zone under the ribbon. This dual mode of transmission is made possible by the processes that supply vesicles to the active zone. Electron microscopy studies of ribbons in BCs [19,48,49] and hair cells [20,50] indicate that the RRP is refilled efficiently and is only partially depleted during ongoing activity. As a result, a regenerative voltage signal causing a sudden, large influx of Ca^{2+} will be able to act on a sizeable pool of vesicles with the potential to fuse very rapidly. Once depleted, the RRP in fish bipolar cells appears to be refilled in two phases, a fast one with a time constant of a few hundred milliseconds, and a slower one of tens of seconds [51,52]. The number of vesicles released by a spike is therefore expected to depend on the state of the synaptic terminal at the time the spike arrives [47,53] (Figure 3). Thus, in addition to accentuating release associated with particularly effective stimuli, spikes in ribbon synapses may serve to suppress vesicle release driven by subthreshold activity following a spike. Moreover, depletion of the RRP through the large influx of calcium associated with the arrival of the spike may limit the frequency response of the individual synapse. Notably, spikes in BC synapses vary widely in amplitude and waveform. Clearly, it will be important to study the interplay of Ca^{2+} influx driven by different waveform spiking and graded processes and their impact on vesicle release.

The spiking properties of some insect PRs suggest that T-bar 'ribbon' synapses are also capable of transmitting both analog and digital signals. Although we have little information about the relative efficiency of these two modes of transmission in invertebrates, continuous transmitter release in PRs has been demonstrated [11,54]. Several properties of T-bar synapses indicate that they will support both slow continuous release and fast bursts of pulsatile release, including the presence of a large number of vesicles and multiple vesicle release sites.

Potential costs and benefits of spikes in early vision

Synapses continuously responding to graded changes in membrane potential typically transmit information at higher overall rates than do synapses driven by spikes [55,56]. One might therefore suggest that spikes do not occur in PRs because this would lead to the loss of too much visual information before it can be acted on by the rest of

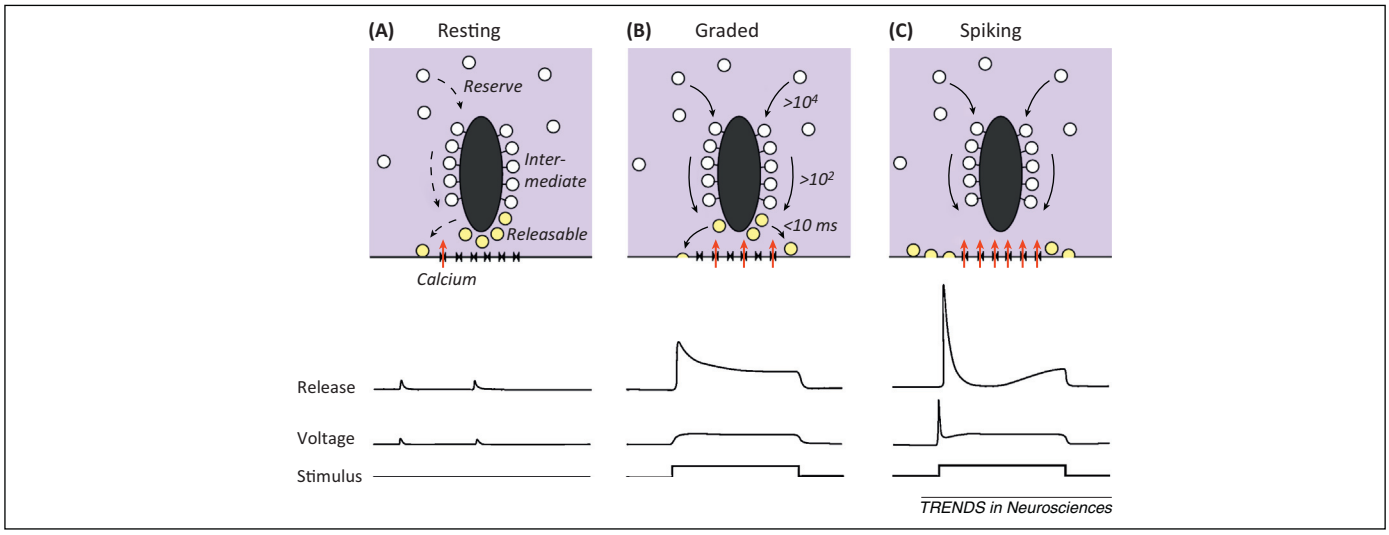

Figure 3. Graded and spiking signals driving a ribbon synapse. **(A)** When hyperpolarized (rest), ribbon synapses exhibit low spontaneous rates of release, resulting in a fully stocked ribbon. (**B**) Graded depolarizing drive opens a small proportion of the calcium channels, which are located beneath the ribbon. Fusion of docked vesicles occurs at a relatively low rate, and complete depletion of this pool is prevented by processes that supply new vesicles to release sites, resulting in a sustained mode of release. **(C)** A large transient depolarization, such as a spike, opens many calcium channels and triggers the fusion of all releasable pool within few milliseconds. As a result, release is briefly depressed following a spike.

the retinal circuit. The second-order neurons, BCs in vertebrates and LMCs in insects, sum inputs from highly overlapping sets of PRs [4,5] and this may allow subthreshold signals to be preserved for transmission to the inner retina (or the insect medulla) through ribbon synapses transmitting graded signals. The generation of spikes (or active resonances) would use the same synapses to accentuate selectively the transmission of specific features in the visual input. Individual BCs exhibit relatively low spike rates (often <1 Hz), so features encoded through spikes would necessarily be transmitted at low bandwidth. In the following, we discuss some of the metabolic and computational consequences of using digital and analog forms of signaling in early visual systems.

Spikes as a noise filter towards high temporal precision

Vertebrate BC spikes can lock onto stimulus modulations with a temporal precision of a few milliseconds [9,10], a precision indistinguishable from that recorded in postsynaptic RGCs [57]. How is this precision achieved? One possible answer may lie in the very low BC spike rates that result from a relatively high spiking threshold. BC spikes may be triggered best if a strong stimulus-driven depolarization is further increased by an additional positive voltage deflection in synaptic noise. As a result, they sparsely encode only the largest depolarizing events; that is, only when the BC is near to be 'optimally' driven, with spike probability defined by a Poisson process modulated by the underlying membrane voltage. Notably, a conceptually related process likely acts as noise filter at the level of rectifying rod PR terminals operating near the visual threshold [58] (see also [59]).

Encoding of fast changes in the visual scene

Depending on the state of the synapse, a single BC spike can elicit the nearly instantaneous release of the entire RRP (see above). Because spikes appear to be prominent in 'transient' BC types [38,39], it is tempting to speculate that spikes are used in visual computations that require high temporal precision [56], such as temporal edge detection, or to boost high-frequency stimulus components (e.g., [16]). Moreover, depending on the threshold, spikes may contribute to reducing response latency [33] and/or, given a sufficient signal-to-noise ratio, to boosting sensitivity at low stimulus contrast. However, BC spikes are unreliable: most visually driven depolarizing events fail to elicit a spike [9]. Boosting response reliability therefore requires a substantial degree of pooling, such that the 'spiking strategy' appears most appropriate for postsynaptic neurons that receive many independent inputs (i.e., feature large dendritic fields). In the mammalian retina, the largest RGCs (e.g., 'alpha-like' cells in mouse, parasol cells in primate, Y-cells in cat, and brisk transient cells in guinea pig) with dendritic field diameters of >250 μm are known to encode rapid changes in the visual scene [60–62]. They integrate inputs from several hundreds of individual BCs across several thousands of synaptic contacts. Moreover, these RGCs generally feature large, highly nonlinear receptive fields (RFs) [61,63]. In brief, their specific properties render these RGCs as likely candidates to be primarily driven by spiking BCs.

By contrast, the 'midget pathway' that is specific to the primate retina contains RGCs with very small dendritic fields, at the extreme with 1:1:1 connectivity between cone PRs, midget BCs, and midget RGCs in the foveal center [4]. As a consequence, stochastic spiking in a midget BC would result in unreliable encoding of changes occurring within its RF. In line with this notion, midget BCs stratify towards the borders of the inner plexiform layer (IPL) of the retina, where, in nonprimate mammals at least [39,64], synaptic output from BCs is predominantly sustained and probably nonspiking. By contrast, the mouse RGC with the smallest dendritic field is the W3 cell, which stratifies in the center of the IPL [65], suggesting that it is predominately driven by spiking BCs, which in mouse also mainly stratify at this depth [39]. The latter is supported by the observation that W3 cells exhibit highly transient responses to sudden light changes, a functional property that could be explained by strongly rectifying excitatory inputs. Although the RF diameter of this RGC is comparatively small (approximately 100 μm), its dendritic stratification covers two to three neighboring IPL sublaminae, possibly to improve response reliability by gathering inputs from multiple types of spiking BC [65]. The vertebrate retina contains approximately 20 different types of RGC, each featuring a different dendritic stratification pattern within the IPL. As a consequence, these cells gather excitatory input across a diverse spectrum of both spiking and nonspiking BCs. Therefore, to understand retinal signal processing, it will be crucial to examine how these different inputs are combined in the different RCG type towards the extraction of specific visual features.

Resonance generating active tuning

Rather than generating full-blown APs, some types of BC appear to generate 'damped voltage oscillations' [6,9,32]. Such oscillations can result from balancing of voltage-activated inward and outward currents (i.e., L-type Ca^{2+} channels and BK channels) and are a well-known feature of other ribbon-type synapses, most notably of hair cells in the auditory and vestibular systems of lower vertebrates [66]. In general, any form of damped oscillation necessarily imparts a bandpass tuning onto the synapse [67], as famously used by auditory hair cells of the turtle [66]. Therefore, the generation of damped voltage oscillations in the axon terminals of retinal BCs may represent an important ingredient towards shaping the frequency response of BC output, a function usually ascribed to temporal tuning in BC dendrites through different glutamate receptors, ionic conductances, and contact morphologies to PRs [68–72]. An interplay of Ca^{2+} and small-conductance Ca^{2+}-activated K^+ channels (SK) channels in fly PR terminals seems to serve a similar function [73].

Lessons from compound eyes

The ultimate reason that primary sensory cells and part of the postsynaptic interneurons code sensory information in the form of graded potentials may lie in metabolic aspects of information transmission. Pushing through the same high information rate in axons requires approximately ten times more energy in the form of spikes than with graded potentials [74]. This metabolic constraint seems to reserve

Box 2. Outstanding questions

- Is there an overall principle with respect to ribbon structure that is directly related to the temporal structure of the stimulus?
- How do ribbons and T-bars deal with a combined spiking and graded drive? To what extent can spikes suppress release driven by a subsequent subthreshold process?
- Which types of retinal BC and LMC generate spikes, and what are the specific retinal circuits operating with a spiking and/or graded drive?
- How do different types of RGC and TM neurons integrate spiking and nonspiking inputs?
- How do inhibitory inputs and neuromodulators control and shape spike generation?
- In retinal BCs, where exactly in the terminal system are spikes initiated: each individual bouton or more centrally at the main branching point of axons? Do spikes contribute to synchronizing or desynchronizing different synaptic terminals belonging to the same BC?

spiking for predominately sparse coding in the central nervous system [75] and when signals need to be faithfully relayed over long distances. An important reason for 'accepting' the metabolic costs and using spikes in addition to graded signals in the peripheral sensory system may be that it allows two regimes of precision: the graded mode for controlling the overall average activity of the postsynaptic neurons, and the spiking mode for accurate timing. Support for this view comes, for instance, from a subset of motion detection neurons in the fly lobular plate, where the voltage in the presynaptic terminals of vertical system (VS) cells show both graded and spiking responses, which correlate with the responses of the postsynaptic V1 cells in different temporal regimes [76]. Spikes in sensory neurons may also not need to be 'fully fledged' APs, in the sense that their sole purpose is to boost certain frequency components in the transmitted signals, as has been suggested for fly L3 interneurons [16] and for honeybee drone PRs [77]. The most intriguing advantage of using actual APs for coding in insect vision may be related to vision in the dark: both the PRs of the cockroach compound eye and those in the ocelli of the nocturnal bee *Megalopta* sp. [78] generate APs. Here, the spikes riding on top of graded polarizations ensure that large signals in PRs are preferentially transmitted relative to the baseline resulting in a sparse but less noisy signal. Postsynaptic neurons pool over many such inputs to 'rescue' the sparse signal [41], in line with the idea that vertebrate RGCs enhance response fidelity by pooling across many spiking BC inputs [9]. This combination of graded and spiking signals has the additional advantage that it allows PRs to be (relatively) noisy; in fact, the thresholding mechanism was shown to be more effective at dim illumination [41].

Concluding remarks

Many sensory systems in both vertebrates and invertebrates use a mixture of graded potentials and spikes, resulting in the start of digitization of the analog stimulus if not in the primary neurons then often in the secondary neurons. In the early visual system, this dual-mode signal transmission may reflect the different computational needs, including preserving important sensory information, reliable signalling, highlighting and extracting strong and fast changes in the visual world, and keeping the metabolic costs at a minimum. In the light of the striking heterogeneity of A–D 'conversion' in parallel visual pathways, it will be important to understand the specific role(s) of spikes and graded potentials in the context of encoding different features in the visual scene (Box 2).

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