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Synaptic Release at Mammalian Bipolar Cell Terminals

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Abstract

Bipolar cells play a vital role in the transfer of visual information across the vertebrate retina. The synaptic output of these neurons is regulated by factors that are extrinsic and intrinsic. Relatively little is known about the intrinsic factors that regulate neurotransmitter exocytosis. Much of what we know about intrinsic presynaptic mechanisms that regulate glutamate release has come from the study of the unusually large and accessible synaptic terminal of the goldfish rod-dominant bipolar cell, the Mb1 bipolar cell. However, over the past several years, examination of presynaptic mechanisms governing neurotransmitter release has been extended to the mammalian rod bipolar cell. In this review, we discuss the recent advances in our understanding of synaptic vesicle dynamics and neurotransmitter release in rodent rod bipolar cells and consider how these properties help shape the synaptic output of the mammalian retina.

Keywords

rod bipolar cell; exocytosis; endocytosis; ribbon synapse; synaptic vesicle

Introduction

In the vertebrate retina, bipolar cells provide the major pathway through which visual information encoded by photoreceptors is transmitted from the outer retina to the inner retina and ultimately, to the output neurons of the retina, the ganglion cells. While their dendrites reside in the outer plexiform layer, the synaptic terminals of bipolar cells lie in the inner plexiform layer (Figure 1A), where they are exposed to a variety of circuit interactions that modulate synaptic output. The large and accessible terminals of the rod-dominant Mb1 bipolar cells of the goldfish retina have proven an excellent model system for studying presynaptic mechanisms. Data obtained from these neurons have helped lay a foundation for our current understanding of presynaptic mechanisms, from calcium entry and the roles of presynaptic calcium, to synaptic vesicle trafficking and neurotransmitter exocytosis, and the role of the synaptic ribbon. Recently, several groups have extended the study of presynaptic mechanisms to the rod bipolar cell of the rodent retina, which has a different connectivity than the Mb1 bipolar cell and experiences different synaptic demands.

Here, we review the current literature on synaptic signaling and neurotransmitter release in the mammalian rod bipolar cell. We compare and contrast results obtained from paired recordings of synaptically-connected rod bipolar cells and third-order neurons, which report

on transmitter release detected by postsynaptic glutamate receptors, with those obtained using membrane capacitance measurements, which provide a direct, presynaptic assay of synaptic vesicle fusion. Where instructive, properties of the mammalian rod bipolar cell will be compared with those of the well-studied rod-dominant Mb1 bipolar cell of the goldfish retina.

Basic Physiology and Synaptic Organization

The mammalian rod bipolar cell is part of a high-sensitivity neural pathway specialized for scotopic vision. In contrast to the Mb1 bipolar cell which receives inputs from both rod and cone photoreceptors and makes chemical synapses directly onto third-order neurons (Stell et al., 1977; Ishida et al., 1980; Joselevitch & Kamermans, 2007), the mammalian rod bipolar cell typically receives inputs exclusively from rod photoreceptors (but see also Pang et al., 2010) and indirectly provides outputs to ganglion cells via a unique mammalian circuit between the rod bipolar cell, AII amacrine cells, and cone bipolar cells. This circuit, which grafts the rod pathway onto the cone pathway at the level of cone bipolar cell axon terminals (Kolb & Famiglietti, 1974; Kolb, 1979; Bloomfield & Dacheux, 2001; Tsukamoto et al., 2001; Veruki & Hartveit, 2002), is essential for mammalian scotopic vision. Defects in this pathway are associated with loss of rod-driven, on-center responses in ganglion cells (Deans et al., 2002) and night vision deficits in humans (Berson & Lessell, 1988; Alexander et al., 1992; Bech-Hansen et al., 1998; Strom et al., 1998; see also Fitzgerald et al., 1994).

The visual response of bipolar cells is predominantly sustained (Nelson, 1982; Saito & Kujiraoka, 1982; Kolb & Nelson, 1983; Dacheux & Raviola, 1986; Bloomfield & Xin, 2000; Euler & Masland, 2000; Pang et al., 2004; Joselevitch & Kamermans, 2007); these neurons encode changes in illumination with a graded change in membrane potential (Dowling & Werblin, 1969; Kaneko, 1973), although under certain conditions, the light response might become regenerative (Burrone & Lagnado, 1997; Zenisek & Matthews, 1998; Protti et al., 2000; Ma & Pan, 2003; Palmer, 2006; Hu et al., 2009). In contrast to the largely sustained light response of the rod bipolar cell, the light response of the postsynaptic AII amacrine cell exhibits a mixture of transient and sustained components (Nelson, 1982; Dacheux & Raviola, 1986; Bloomfield & Dacheux, 2001). While the transient transformation may be sculpted by local circuit interactions such as GABAergic feedback (Tachibana & Kaneko, 1987; Maguire et al., 1989; Heidelberger & Matthews, 1991; Maple & Wu, 1998; Vigh & von Gersdoff, 2005; Eggers & Lukasiewicz, 2006; Chavez et al., 2010), the transient component of the AII amacrine cell light response may also arise from intrinsic properties of rod bipolar cell glutamate release.

Rod bipolar cells release the excitatory neurotransmitter glutamate via calcium-dependent exocytosis at ribbon-style active zones (Dowling & Boycott, 1966; Dowling, 1987). Conventional synapses are rare, although they have been reported in some species (Miller et al., 2001; Midorikawa et al., 2007). The synaptic ribbon tethers synaptic vesicles near sites of fusion. The morphology, molecular architecture and general function of synaptic ribbons, also found in some primary sensory neurons such as photoreceptors, has been the topic of numerous recent reviews (Heidelberger et al., 2005; Sterling & Matthews, 2005; tom Dieck & Brandstatter, 2006; Wu et al., 2007; Schmitz, 2009; Zanazzi & Matthews, 2009). An overall picture emerges in which vesicles that are tethered near the base of a synaptic ribbon and close to the presynaptic plasma membrane, wherein calcium channels and fusion machinery components cluster, contribute to the fastest component of glutamate release. Vesicles located further up the ribbon may contribute to a second, larger and more sustained component of release. Both components of release reflect the fusion of vesicles that have undergone all ATP-dependent priming steps required for fusion (Heidelberger, 1998; Heidelberger et al., 2002a). One or both populations may be refilled from those many

vesicles that are not associated with synaptic ribbons but are freely-diffusible in the synaptic terminal cytosol (Zenisek et al., 2003; Holt et al., 2004; LoGiudice et al., 2008). In addition, some release may occur outside of ribbon-style active zones (Midorikawa et al., 2007; Zenisek, 2008), although the magnitude of this release and its role in synaptic communication is not yet clear.

In the Mb1 bipolar cell, exocytosis is triggered via calcium entry through L-type voltage-gated calcium channels (Tachibana & Okada, 1991; Tachibana et al., 1993). These channels are preferentially located in the synaptic terminal (Heidelberger & Matthews, 1992; Tachibana et al., 1993) and have been identified as the $Ca_v1.3$ ($\alpha(1D)$) subunit (Logiudice et al., 2006). This type of L-type calcium channel is less sensitive to dihydropyridine agonists and antagonists, rapidly activates at a relatively negative voltage range, and shows a substantially slower rate of calcium-dependent inactivation than other L-type calcium channels (von Gersdorff & Matthews, 1996; Koschak et al., 2001; Xu & Lipscombe, 2001; Logiudice et al., 2006). The latter properties help maintain graded exocytosis during a prolonged stimulus (Heidelberger & Matthews, 1992; von Gersdorff et al., 1996; Sakaba et al., 1997; Mennerick & Matthews, 1998; Protti & Llano, 1998; Xu & Lipscombe, 2001; Zenisek et al., 2003; Logiudice et al., 2006).

Calcium entry through both L- and T-type calcium channels have been proposed to drive exocytosis in mammalian rod bipolar cells (de la Villa et al., 1998; Hartveit, 1999; Pan, 2000; Pan et al., 2001; Hu et al., 2009). However, the primary source of calcium that triggers release in rats and mice maybe different. In the rat rod bipolar terminal, the T-type calcium currents are prominent (Pan, 2000; Pan et al., 2001; Hu et al., 2009), although the L-type $Ca_v1.2$ ($\alpha(1C)$) and/or $Ca_v1.3$ subunit have also been shown to be expressed at low levels (Kamphuis & Hendriksen, 1998; Xu et al., 2002). The T-type calcium current in a rat rod bipolar cell terminal is atypical, as it exhibits both transient and sustained components (Pan, 2000). This channel also has an extremely slow activation and slow time dependent inactivation kinetics (Pan, 2000; Pan et al., 2001; Singer & Diamond, 2003), possibly arising from a splicing variant, mutant or other modification of the $Ca_v3.3$ ($\alpha(1I)$) Ca^{2+} channel subunit (Pan, 2000; Hu et al., 2009). Although the T-type calcium current was originally suggested to drive the transient release of glutamate in the rat rod bipolar cell (Pan et al., 2001), a subsequent study reported transient release in the presence of sustained calcium influx when T-type channels were not activated (Singer and Diamond, 2003). Activation of T-type calcium channels resulted in larger postsynaptic currents at the rat rod bipolar cell-AII amacrine cell synapse, consistent with a role in boosting glutamatergic synaptic transmission (Singer and Diamond, 2003).

In mouse rod bipolar cell terminal, the L-type $Ca_v1.2$, $Ca_v1.3$ and $Ca_v1.4$ ($\alpha(1F)$) channels are expressed (Satoh et al., 1998; Berntson et al., 2003; Baumann et al., 2004). The major subunit, however, is $Ca_v1.4$ (Berntson et al., 2003; Baumann et al., 2004), which shows slow voltage-dependent inactivation and lacks calcium-dependent calcium channel inactivation (Koschak et al., 2003; Baumann et al., 2004; McRory et al., 2004; Wahl-Schott et al., 2006). The properties of this channel are ideal for supporting continuous calcium signaling during prolonged depolarization in the light. Mutations in $Cav1.4$ channels, which are also present on rod photoreceptor terminals (Morgans et al., 2005), are associated with incomplete X-linked congenital stationary night blindness in humans (CSNB2) (Bech-Hansen et al., 1998; Strom et al., 1998). Mouse bipolar cells have also been reported to express a transient T-type I_{Ca} (Kaneko et al., 1989; Kaneko et al., 1991) but there is evidence to suggest that these channels are not present in the synaptic endings (Satoh et al., 1998). Thus, in contrast to the rat, the T-type calcium channel may not be positioned in the mouse rod bipolar cell to drive exocytosis.

Synaptic Vesicle Pools

The concept of synaptic vesicle pools, first introduced in the general exocytosis literature, is based upon the physiological ability to identify distinct components of release. These components possess distinct kinetic properties, magnitudes, and proximity to calcium channels. The latter is evidenced by differential sensitivity to the calcium buffers EGTA and BAPTA, which have similar binding affinities for calcium ions but different on-rates (Adler et al., 1991; Borst & Sakmann, 1996; Mennerick & Matthews, 1996; Sakaba et al., 1997; Moser & Beutner, 2000; Burrone et al., 2002). The naming of these pools has been largely descriptive, with the first pool described in a particular neuron under a particular set of conditions being given the name “releasable pool” or “readily-releasable pool” and subsequent pools being named relative to the first. Thus, some authors have referred to a particular pool as the releasable pool while others have called the same pool the reserve pool depending upon the assay. The commonly used abbreviations for the pools such as “RP” and “RRP” are also confusing as the former may be used to denote either the reserve pool or the releasable pool and the latter, the rapidly-releasing pool or the readily-releasable pool. In the bipolar cell literature, this loose nomenclature has led to some consternation, although in actuality, there is remarkable agreement across laboratories and experimental approaches. Here, we fall back on nomenclature derived from capacitance measurements in other systems and refer to the pool that fuses with properties consistent with vesicles being already docked and primed at the active zone and located closest to calcium channels, as the rapid or rapidly-releasing pool, and the slower releasable pool, which has undergone all ATP-dependent priming steps (Heidelberger, 1998; Heidelberger et al., 2002a), is released within several hundred milliseconds, and is similar in size to the remainder of the ribbon-tethered pool, as the releasable pool. We refer to those vesicles that reside in the cytoplasm, are not attached to synaptic ribbons, and are not recruited to fuse in response to a single one second, maximal depolarization as the cytoplasmic reserve or reserve pool. Properties of the different vesicle pools of rod-driven bipolar cells are summarized in Table 1.

The Rapidly Releasing Pool

A depolarizing voltage step applied to a presynaptic rat rod bipolar cell evokes an excitatory postsynaptic synaptic current (EPSC) in a postsynaptic AII amacrine cell (Figure 1B). This EPSC consists of a prominent fast, transient component followed by a small, more sustained component (Singer & Diamond, 2003; Trexler et al., 2005; Snellman et al., 2009). The transient component was observed under exogenous calcium buffering conditions expected to block all release except that which occurs in the close proximity of an open calcium channel. The activation of this component was limited by the time course of calcium channel activation (Singer & Diamond, 2003), similar to the rapidly-releasing pool of the Mb1 bipolar cell (Mennerick & Matthews, 1996, 1998). The transient component was depleted with a time constant of a few milliseconds (Singer & Diamond, 2006; Olstedal & Hartveit, 2010), also consistent with a rapid pool (Mennerick & Matthews, 1996). Thus, the transient component of the EPSC is likely to result from the exocytosis of neurotransmitter from a limited pool of rapidly-releasing vesicles. The magnitude of the EPSC transient was estimated to reflect the release of glutamate from about 70 vesicles per terminal, or about 7 vesicles per synaptic ribbon, given 10 ribbon synapses between a rod bipolar cell and AII amacrine cell (Singer & Diamond, 2006).

Another way to study synaptic vesicle pools is via membrane capacitance measurements. Membrane capacitance measurements permit changes in surface area indicative of membrane addition and retrieval to be monitored as an index of exocytosis and endocytosis (Lindau & Neher, 1988; Gillis, 1995). Almost a decade ago, Pan and colleagues used this approach in the isolated synaptic terminals of the rat bipolar cell to show that activation of

the T-type calcium channel could trigger exocytosis in this neuron (Pan et al., 2001). As with the Mb1 bipolar cell (Mennerick et al., 1997; Palmer et al., 2003), the capacitance approach can be applied to rat rod bipolar cells embedded in a retinal slice provided that the methodology is modified to account for the complex morphology of an intact rat rod bipolar cell (Oltedal et al., 2007; Oltedal & Hartveit, 2010). The capacitance approach has also been successfully used to study synaptic vesicle dynamics in isolated, intact mouse rod bipolar cells under carefully-controlled conditions (Zhou et al., 2006; Wan et al., 2008; Wan et al., 2010). Extra care is warranted when employing capacitance techniques in the mammalian rod bipolar cell when compared with the goldfish Mb1 bipolar cell. This is due to the smaller size of a mammalian rod bipolar cell synaptic terminal, which can hinder direct terminal recordings, particularly in the mouse, and the comparatively long, thin axon connecting the soma and terminal.

Estimates of the magnitude of the rapidly releasing pool made with capacitance measurements are similar between mouse and rat, although that of the mouse is a little smaller. The rapid pool is about 10 fF in the rat (Oltedal & Hartveit, 2010) and approximately 7 fF in mouse (Wan et al., 2008; Wan et al., 2010). As expected for a rapid pool (Mennerick & Matthews, 1996), the rate of exocytosis of this component of release is limited by the activation time course of the presynaptic calcium channels (Oltedal & Hartveit, 2010).

To relate the magnitude of the rapid pool to number of vesicles or vesicles released/synaptic ribbon requires knowledge of the vesicle surface area. Synaptic vesicles in mouse rod bipolar cells have a mean diameter about 30 – 40 nm in fixed tissue (Spiwox-Becker et al., 2001; LoGiudice et al., 2008; Wan et al., 2010), somewhat larger than that reported for Mb1 bipolar cells (Lagnado et al., 1996; von Gersdorff et al., 1996). If corrected for systematic underestimation in thin sections (Abercrombie, 1946), synaptic vesicle diameter in the mouse rod bipolar cell rises to about 50 nm (Wan et al., 2010). If a specific membrane capacitance of 9 fF/ μm^2 is assumed (Gentet et al., 2000), a 7 – 10 fF rapid pool would represent about 100 – 400 vesicles per cell, assuming an average vesicle diameter of 30 – 50 nm. Given that there are approximately 35 synaptic ribbons per rodent rod bipolar cell (Singer et al., 2004; Wan et al., 2008), this corresponds to a rapid pool in the rodent rod bipolar cell of between 3 and 11 vesicles per synaptic ribbon, in general agreement with the estimate of 7 vesicles per synaptic ribbon obtained from analysis of the EPSC transient (Singer & Diamond, 2006).

It is important to note that there is some play in the numbers, particularly with respect to the total number of rapidly-releasing vesicles. Capacitance measurements suggest upwards of 100 vesicles in the rapid pool per cell, while the EPSC transient calculations suggest less than 100. While this discrepancy could reflect the possibility that not all fused vesicles release glutamate or that not all released glutamate is detected by postsynaptic receptors, it may also simply reflect the many assumptions and uncertainties inherent in the calculations. Ultrastructural analysis of the rodent rod bipolar cell synaptic ribbon may provide some answers. Taken as a whole, the data support the hypothesis that the transient component of the EPSC reflects fusion of vesicles that belong to the rapidly-releasing pool of vesicles, although, as will be discussed in a subsequent section, the shape of the transient may be influenced by factors that modulate synaptic vesicle dynamics.

The Releasable Pool

In the Mb1 bipolar cell of the goldfish, a 5:1 relationship between the magnitude of the releasable pool and the rapid pool has been noted (Sterling & Matthews, 2005). This relationship led Singer and Diamond to extrapolate from the magnitude of the EPSC

transient, representing the rapid pool, a releasable pool of approximately 35 vesicles in the rat rod bipolar cell (Singer & Diamond, 2006). More recently, the magnitude of the releasable pool has been directly assayed using capacitance measurements in both mouse and rat rod bipolar cells. In the isolated mouse rod bipolar cell, this pool is approximately 30 fF (Zhou et al., 2006; Wan et al., 2008), and like the similarly-characterized releasable pool of the Mb1 bipolar cell and other ribbon-style synapses (von Gersdorff & Matthews, 1994a, 1997; Moser & Beutner, 2000; Heidelberger et al., 2002a; Eisen et al., 2004; Rabl et al., 2005), this pool in the mouse rod bipolar cell was depleted with a time constant of a few hundred milliseconds (Wan et al., 2008). Also as in the Mb1 bipolar cell (Mennerick & Matthews, 1996; von Gersdorff et al., 1996), this pool is roughly five times the ≈ 7 fF estimate of the rapid pool obtained from a pulse-duration plot (Wan et al., 2008). With respect to vesicle number, the results suggest a releasable pool of vesicles between 425 and 1200 vesicles per terminal and between 12 and 35 vesicles per synaptic ribbon, for a 30 – 50 nm range of vesicle diameters (see above). In the rat rod bipolar cell, capacitance measurements indicate a releasable pool of 50 – 60 fF (Oltegal & Hartveit, 2010), which is about five times their estimate of the magnitude of the rat rapid pool obtained with capacitance measurements. The 50–60 fF corresponds to 700 – 2400 vesicles, depending upon the assumed diameter and approximately 20 – 69 vesicles per synaptic ribbon.

It has been suggested that because the upper limit for the estimate of releasable vesicles in the rat rod bipolar cell exceeds five times the 7 vesicle per ribbon rapid pool estimate of Singer et al (2006), this could indicate that more vesicles are fusing than are tethered to synaptic ribbons (Oltegal & Hartveit, 2010). Extra-ribbon release has been reported in optical studies of synaptic vesicle fusion in Mb1 bipolar cells (Zenisek et al., 2004; Coggins et al., 2007; Midorikawa et al., 2007; Zenisek, 2008). However, the magnitude of the proposed extra-ribbon release, and its significance to synaptic signaling is not well-delineated. In addition, there is the added complication that ribbons can detach from the plasma membrane (Zenisek et al., 2004; Midorikawa et al., 2007). Given the assumptions inherent in converting capacitance to vesicle number or postsynaptic responses to the number of fused vesicles per ribbon, it would be prudent to await ultrastructural analysis of a ribbon-style active zone in a rodent rod bipolar cell before firmly concluding that there must be extra-ribbon release based solely upon a comparison of results obtained with different approaches. Taken at face value, the numbers do suggest that the synaptic ribbons of the rat rod bipolar cell may tether roughly twice as many vesicles as that of the mouse rod bipolar cell. It will be interesting to see whether this prediction will be confirmed by ultrastructural examination.

The Cytoplasmic Reserve Pool

In addition to the rapid and releasable pools, bipolar cells also contain a large number of cytoplasmic vesicles that are not tethered to synaptic ribbons (von Gersdorff et al., 1996). These cytoplasmic vesicles are much more mobile than those of the conventional synapse and of ribbon-tethered vesicles (Zenisek et al., 2003; Holt et al., 2004; LoGiudice et al., 2008). This enhanced mobility helps support the quick replenishment of releasable pools (Rea et al., 2004; LoGiudice et al., 2008). The reason underlying enhanced mobility is not clear, but one possibility is that it reflects the ribbon-specific lack of synapsin, a synaptic vesicle phosphoprotein thought to attach vesicles to the actin cytoskeleton (Mandell et al., 1990; Mandell et al., 1992; Von Kriegstein et al., 1999; but see Gaffield & Betz 2007). In the Mb1 bipolar cell, there are approximately 1000 vesicles in the cytoplasmic reserve for every vesicle on a the synaptic ribbon (von Gersdorff et al., 1996). The magnitude of this pool is not yet known for the rod bipolar cell. However, if the density of vesicles per terminal is similar to that of the Mb1 bipolar cell, this would suggest a reserve pool in the mouse rod bipolar cell that is > 100 fold smaller (Logiudice et al., 2009; Wan et al., 2010).

Refilling of Vesicle Pools and the Role of Calcium

In much of the secretory literature, a paired-pulse paradigm is used to study refilling of a depleted pool. The first pulse is of a magnitude and duration known to deplete a particular pool. The second pulse in the pair is given at a variable time point after the first to assess the state of refilling of the previously depleted pool. Using this type of static assay, the releasable pool in the Mb1 bipolar neuron was found to refill with a time constant of approximately 7–8 s (von Gersdorff & Matthews, 1997; Heidelberger et al., 2002a; Hull et al., 2006). Refilling of the rapid pool when assayed with a paired-pulse paradigm also exhibited a time constant of a few seconds ($\tau \approx 4$ s; Mennerick and Matthews, 1996), reflecting the rate of recovery when calcium channels are closed. However, a small, fast component of refilling with a time constant of a few hundred milliseconds has been noted when examining recovery of the releasable pool; this component has been attributed to the refilling of the rapid pool when calcium channels are open (Mennerick & Matthews, 1996). Consistent with a calcium-dependent mechanism, this component is inhibited by intracellular addition of the calcium buffer EGTA (Gomis et al., 1999; see also Sakaba et al., 1997). Together, these observations support that in the Mb1 bipolar cell, as has been described for other neurons and secretory cells, there is calcium-accelerated refilling pool of the rapidly-releasing pool (von Ruden & Neher, 1993; Dittman & Regehr, 1998; Stevens & Wesseling, 1998; Wang & Kaczmarek, 1998; Gomis et al., 1999; Sakaba & Neher, 2001).

In the mammalian rod bipolar cell, refilling of vesicle pools has also been assayed using a paired-pulse protocol. In the rat, recovery from paired-pulse depression of the rapidly-releasing pool was examined by monitoring the recovery of the transient component of the EPSC. The time constant of recovery is approximately 4 s; refilling was faster following a depleting stimulus of a larger magnitude, suggestive of calcium-accelerated refilling (Singer and Diamond 2006). In the mouse rod bipolar cell, recovery from paired-pulse depression has been assayed using capacitance measurements. Like the Mb1 bipolar cell, the mouse rod bipolar cell releasable pool refills with a time constant of about 7 s (Figure 2A). In addition, a small fast component of refilling is present that may reflect calcium-accelerated filling of the rapid pool (Wan et al., 2008). Consistent with this possibility, in response to a brief stimulus train in which each pulse is of a magnitude sufficient to deplete the rapid pool, the evoked cumulative capacitance increase rises more rapidly and to a higher level when the presynaptic calcium concentration is elevated (Figure 2B; see also Wan et al., 2010). Together, these lines of evidence support the hypothesis that, as in the Mb1 bipolar cell and other neurons, refilling of the rapid pool is accelerated by elevated calcium in the mammalian rod bipolar cell.

Endocytosis & Synaptic Vesicle recycling

Following exocytosis, fused vesicles are retrieved from the plasma membrane through a process called endocytosis. In neurons, this process is compensatory, meaning that the endocytosis that follows calcium-evoked exocytosis restores the cell surface area back to baseline. Multiple pathways have been suggested to participate in compensatory endocytosis. These include clathrin-mediated retrieval of single vesicles, bulk retrieval of large cisternae, and kiss-and-run retrieval, which is the rapid reversal of a pore-like connection between the vesicle and plasma membrane. The first two, but not the latter, may also require the activity of dynamin, a GTPase essential for membrane scission (Koenig & Ikeda, 1989; Shupliakov et al., 1997; Marks et al., 2001; Yamashita et al., 2005; Lou et al., 2008). Significant advances in our general understanding of endocytosis mechanisms have recently been reviewed (Rizzoli & Betz, 2005; LoGiudice & Matthews, 2007; Wu et al., 2007; Smith et al., 2008; Dittman & Ryan, 2009; Royle & Lagnado, 2010).

In the Mb1 bipolar cell, capacitance studies have identified two modes of compensatory endocytosis. The fast mode of membrane retrieval has a time constant of a few seconds or less, while the slower phase has a time constant in the 10–20 s range (von Gersdorff and Matthews, 1994a; Neves & Lagnado, 1999; Heidelberger et al., 2002b; Hull & von Gersdorff, 2004). Both components may be noted following a single stimulus, and the balance may shift from fast to slow in the presence of elevated presynaptic calcium concentration (von Gersdorff & Matthews, 1994b; Neves & Lagnado, 1999; Heidelberger, 2001). With strong stimuli, a calcium-dependent delay in the onset of endocytosis is also observed (von Gersdorff & Matthews, 1994b; Neves & Lagnado, 1999; Heidelberger 2001).

The fast and slow modes of endocytosis in the Mb1 bipolar cell are thought to reflect distinct mechanistic pathways of membrane retrieval. First, these two pathways are differentially-sensitive to blockade by elevations in hydrostatic pressure. Elevated hydrostatic pressure selectively inhibits the slow phase of retrieval, sparing the fast (Heidelberger et al., 2002b). Only the slow phase of endocytosis is inhibited by peptides that interfere with clathrin-dependent membrane retrieval (Jockusch et al., 2005). Thus, the slow mode, but not the fast, has been attributed to the clathrin-dependent retrieval of vesicles following their full collapse. The molecular pathway underlying the fast mode is not yet clear. Optical techniques have failed to detect evidence of kiss and run retrieval in the Mb1 bipolar neuron (Zenisek et al., 2002; Llobet et al., 2003), suggesting that the fast mode might represent a mechanism other than kiss and run. The fast mode is selectively inhibited by elevated internal chloride ions (Hull & von Gersdorff, 2004), but like the slow mode, it requires nucleotide hydrolysis (Heidelberger, 2001; Heidelberger et al., 2002a; Jockusch et al., 2005). Thus, the fast mode of endocytosis may represent dynamin-dependent, clathrin-independent membrane retrieval. Interestingly, introduction of non-hydrolyzable GTP analogs into the Mb1 terminal was found to inhibit both fast and slow modes of endocytosis following a relatively mild stimulus (Jockusch et al., 2005), but not after a stimulus that depleted the entire releasable pool (Heidelberger, 2001). Although other explanations are possible, this differential effect may reflect an activity-dependent transition between dynamin-dependent and dynamin-independent rapid and slow endocytosis, as reported recently in the calyx of Held (Xu et al., 2008). Following very strong or prolonged exocytosis, endocytosis in the Mb1 bipolar cell can also occur by a form of bulk retrieval that does not involve clathrin-coated vesicles or pits (Holt et al., 2003; Paillart et al., 2003). Inhibitors of PI-3-kinase block bulk retrieval, but not the fast and slow modes (Holt et al., 2003), indicating that bulk membrane retrieval may occur by a different biochemical pathway (Clayton et al., 2010).

In the mammalian rod bipolar cell, calcium-evoked exocytosis is followed by compensatory endocytosis. Reminiscent of endocytosis in the Mb1 bipolar neuron, in both the mouse and rat rod bipolar cell, the time course of endocytosis is prolonged with stronger stimuli or high presynaptic calcium loads (Wan et al., 2008; Olstedal & Hartveit, 2010). In addition, the brief delay between the succession of exocytosis and the onset of endocytosis is prolonged with larger presynaptic calcium loads. In response to a single depolarization of a few hundred milliseconds to a second in duration, an average time constant of membrane retrieval of ≈ 7 s was reported in the mouse rod bipolar cell, with a range of 2–21 s depending upon the depolarization-evoked rise in the presynaptic calcium concentration. In rat rod bipolar cell, the time constant of retrieval averaged ≈ 17 s, although values as low as ≈ 1 s were noted, depending upon the stimulus. A separate, fast phase of retrieval was not discernable in either species, although the existence of one cannot be excluded due to resolution issues and the possibility that some amount of exocytosis may occur following calcium channel closure (e.g., Figure 3, inset). We do note that in both species, the time constant of recovery is faster following milder stimulations, (Wan et al., 2008; Olstedal & Hartveit, 2010). These shorter time constants are similar to those of the fast phase of endocytosis reported in other neurons

and could indicate the presence a fast mode of endocytosis that is limited in capacity. Future experiments will be needed to resolve this issue.

Recently, using a combination of electrophysiology, confocal imaging and electron microscopy, LoGiudice et al. (2009) reported that exocytosis was followed by the retrieval of single, clathrin-coated vesicles. Retrieval was dynamin-dependent, as evidenced by the fact that terminals treated with a potent inhibitor of dynamin function called dynasore had more vesicles attached to the plasma membrane after stimulation (LoGiudice et al., 2009). Using membrane capacitance measurements to study membrane retrieval, we have shown directly that compensatory endocytosis is inhibited by dynasore in the mouse rod bipolar cell (Figure 3). Dynasore had no effect on the magnitude of the stimulus-evoked calcium transient or on the recovery of the presynaptic calcium concentration to baseline (not shown), however, resting calcium was mildly elevated in the presence of dynasore (control: 43 ± 9 nM, $n=16$; dynasore: 83 ± 21 nM, $n=14$; $P = 0.008$, Mann-Whitney Rank Sum Test). Whether this elevation is sufficient to impede the recovery of membrane capacitance in the mouse rod bipolar cell is not known, but it is well below the levels that impede endocytosis in the Mb1 bipolar cell (von Gersdorff & Matthews, 1994b; Rouze & Schwartz, 1998). Thus, it seems reasonable to provisionally postulate that the dominant mode of compensatory endocytosis under the conditions tested is both dynamin- and clathrin-dependent in the mammalian rod bipolar cell.

Synaptic vesicle dynamics and the postsynaptic response

It is generally agreed that an understanding of synaptic vesicle dynamics is an essential part of understanding how the pattern of neurotransmitter release is determined and thus, how presynaptic properties dictate the postsynaptic response. For the rod bipolar cell to AII amacrine cell synapse, the evidence presented above suggests that the transient component of the EPSC corresponds to the exocytosis of neurotransmitter from a small, rapid pool of vesicles. The structure of the ribbon, combined with recent optical work (Zenisek et al., 2000; Zenisek et al., 2004; LoGiudice et al., 2008), provides support for the hypothesis that vesicle replenishment at ribbon-style active zones is important for on-going release and that these replacement vesicles can come from higher rows of vesicles tethered to the synaptic ribbons.

A related question is the extent to which the experiments used to probe the vesicle pools recapitulate the light-driven responses of the postsynaptic AII amacrine cells. While the light responses of third-order neurons have both transient and sustained components, the transient component is not nearly as prominent under physiological conditions as it is under the types of experimental protocols presented above. A clue to understanding this apparent discrepancy comes from earlier work in the Mb1 bipolar cell in which a depolarization-dependent delay in glutamate exocytosis was reported (von Gersdorff et al., 1998). Activation of a glutamate-gated current attributable to fusion of vesicles in the rapid pool was more rapid in onset, peaked faster and was larger when evoked by a strong depolarizing voltage step. This current was delayed in onset, peaked more slowly, and was smaller when evoked by a voltage step to a less positive potential (von Gersdorff et al., 1998). Similarly, even when the voltage response of the Mb1 bipolar cell is regenerative, spike latency decreases with light intensity (Protti et al., 2000). Thus, stimulus strength has the ability to alter the kinetics of membrane depolarization, glutamate release and the shape of the EPSC, presumably via its effect on calcium channel activity and associated changes in the presynaptic calcium concentration.

In an elegant study, Snellman and colleagues reflected upon the fact that in most studies of mammalian rod bipolar cell of exocytosis, a depolarizing voltage step was used to evoke

calcium-triggered glutamate release. The essentially instantaneous depolarization drives a concerted opening of calcium channels, resulting in a relatively rapid rise in the presynaptic calcium concentration. Because the rate of vesicle fusion is steeply calcium-dependent (Heidelberger et al., 1994), such stimuli will trigger the fast fusion of vesicles in the rapid pool. By contrast, the membrane response of the mammalian rod bipolar cell to light is not instantaneous, but may take 100 ms or more to peak (Euler & Masland, 2000; Berntson et al., 2004; Trexler et al., 2005). Snellman et al. (2009) varied the properties of the stimuli given to the rod bipolar cell and demonstrated that the shape of the AII amacrine cell EPSC is profoundly influenced by the rate at which the rod bipolar cell is depolarized. Faster membrane depolarizations were associated with a pronounced transient component of the EPSC, while slower rates of membrane depolarization favored more sustained release.

The effect of rate can generally be understood by looking at a simple computer simulation of the occupancy state of the rapid pool (Figure 1C). In this simulation, we compare the time course of depletion of the rapid pool when the fusion rate is changed ten-fold, keeping all other starting parameters constant. With a fusion rate comparable to that of a non-neuronal secretory cell or asynchronous release in a neuron (Heinemann et al., 1994; Sun et al., 2007), the rapid pool empties over a period of tens of milliseconds (Figure 1C; black trace; fusion rate = 100 s^{-1}). With a fusion rate of 1000 s^{-1} (Figure 1C; red trace), which is similar in magnitude to the maximal fusion rates of neurons (Heidelberger et al., 1994; Bollmann et al., 2000; Schneggenburger & Neher, 2000; Beutner et al., 2001; Duncan et al., 2010), the pool becomes depleted within a few milliseconds, indicative of a fast bolus of glutamate release.

In a similar vein, it is important to note that all of the studies on bipolar cell synaptic vesicle dynamics cited above were performed at room temperature. From a mechanistic viewpoint, this facilitates direct comparisons across cells from different species. However, many aspects of synaptic vesicle dynamics may be temperature-sensitive, and thus caution should be used when extrapolating data obtained at room temperature to physiological temperature. For example, studies in other glutamatergic neurons have indicated that the rate of pool refilling is two or three times faster at physiological temperature than at room temperature (Dinkelacker et al., 2000; Pyott & Rosenmund, 2002; Kushmerick et al., 2006). It is therefore conceivable that at physiological temperatures, the rates of pool refilling in the mammalian rod bipolar cell will be faster than those reported above and in Table 1, by a factor of two or three. The simulation in Figure 1C (dashed trace) shows that a three-fold increase in the refilling rate delays the depletion of the rapid pool. The predicted response at physiological temperature would therefore be less transient in nature than that observed at room temperature. While this simulation is overly simplistic, it serves to highlight the importance of both synaptic vesicle dynamics and temperature-dependent kinetics in shaping synaptic output.

Concluding Remarks

Many of the basic features of synaptic vesicle pools and their dynamics have now been characterized for the mammalian rod bipolar cell. The different vesicle pools, their relationship to each other and the interplay between pools, and the kinetics of vesicle transitions all have the ability to sculpt the pattern of neurotransmitter release. An important goal for the future will be to understand the contributions of synaptic vesicle dynamics to neurotransmitter release under physiological conditions. Furthermore, given that several of the steps in the exocytosis pathway are regulated by calcium, a related topic is the extent to which synaptic vesicle dynamics change with changes in background illumination that may alter steady state calcium levels. Might the rod bipolar cell secretory machinery adapt to steady illumination and prolonged elevation of the presynaptic calcium concentration, as has

been postulated in a recent study (Wan et al., 2010)? If so, what advantage does such adaptation provide? Does it preserve the gain of the synapse or change it? Finally, the move into the mammalian system, especially the mouse rod bipolar cell, along with recent explorations into zebrafish bipolar cell physiology (reviewed in Connaughton, 2011), will open up the field to the identification and study of presynaptic molecules that modulate the exocytotic pathway, and hence synaptic transmission from bipolar cells to second- and third-order neurons.

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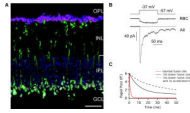


Figure 1. Rod bipolar cells of the mammalian retina

A) A vertical section of a mouse retina triple-labeled for PKC- α (a rod bipolar cell marker; green), SV2 (a synaptic vesicle protein; red), and VGLuT1 (a vesicular glutamate transporter; blue). A single optical section is shown. VGLuT1 and SV2 are selectively found in the outer and inner plexiform layers (OPL and IPL), as expected for proteins located on synaptic vesicles. Photoreceptor terminals, which label for SV2 and VGLuT1 but not PKC- α , are seen as magenta. Rod bipolar cell dendrites, somata and axons, which express PKC- α , are seen as green. Rod bipolar cell terminals, located in the distal IPL, express all three markers and are seen as white. In the IPL, not all SV2 puncta co-localize with VGLuT1 in the IPL, consistent with the presence of both glutamatergic and non-glutamatergic synapses. Scale bar: 20 μm .

B) A paired recording from a synaptically-coupled rod bipolar and AII amacrine cell in a retinal slice from the mouse retina. A 100 ms depolarization evokes a sustained Ca^{2+} current in the presynaptic rod bipolar cell. The postsynaptic AII amacrine cell EPSC exhibits both transient and sustained components. *Journal of Physiology* 587/11 by Snellman et al., 2009 by John Wiley and Sons reproduced with permission from John Wiley and Sons via Copyright Clearance Center. C) A simple two-step simulation (Innocenti & Heidelberger, 2008) depicting the occupancy state of the rapid vesicle pool, which underlies the transient component of the EPSC. With a maximal fusion rate of 1000 s^{-1} , the rapid pool is quickly depleted (red line), consistent with a prominent EPSC transient component. With a fusion rate of 100 s^{-1} , the depletion of the rapid pool is slower (black line), suggestive of a smaller, broader EPSC transient. Increasing the refilling rate, as might occur when increasing the temperature from ambient room temperature to physiological temperature (Dinkelacker et al., 2000; Pyott & Rosenmund, 2002; Kushmerick et al., 2006), reduces the speed with which the rapid pool is depleted. Dashed line shows a simulation with a release rate of 100 s^{-1} and a three-times faster refilling rate than that of the solid black line.

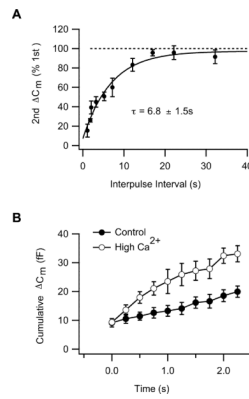


Figure 2. Recovery from depression and the role of presynaptic calcium

(A) Recovery of the releasable pool from depression was measured in the isolated mouse rod bipolar cell using a paired-pulse paradigm. The second capacitance response as a percentage of the first is plotted against the interpulse interval. The time course of refilling is described by a single exponential function (curve) given by the equation $Y=Y_0+A_1e^{(-x/\tau)}$ where $Y_0 = 97.3 \pm 5.0$, $A_1=90.2 \pm 6.3$, $\tau = 6.8 \pm 1.5$ s. The non-zero intercept suggests the presence of a fast component of refilling. Reproduced with permission from Wan et al. (2008), copyright Cambridge University Press.

(B) Elevated presynaptic Ca^{2+} enhances the rate of refilling of the rapid pool in the mouse rod bipolar cell. The cumulative exocytosis, ΔC_m , evoked by a brief 4Hz stimulus train (Wan et al., 2010), is plotted as a function of time for each pulse in the train. Each pulse in the train was sufficient to deplete the rapid pool. Filled circles represent data from control cells loaded with standard internal solution (containing 0.5 mM EGTA and no added Ca^{2+}). Open circles represent data from cells loaded with internal solution containing 0.2 mM EGTA and 0.15 mM Ca^{2+} . With the latter solutions, cells had an average resting Ca^{2+} of ≈ 115 nM compared with ≈ 35 nM in controls and higher stimulus-evoked rises in Ca^{2+} (Figure 7A, Wan et al., 2010). Data replotted from Wan et al., 2010.

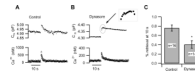


Figure 3. Dynasore inhibits compensatory endocytosis in the mouse rod bipolar cell

(A) Representative example of exocytosis followed by endocytosis evoked by a 500 ms depolarization in a mouse rod bipolar cell.

(B) Representative example of the response to a 500 ms depolarization in a mouse rod bipolar cell treated with 160 μM dynasore. Note the slow time course of membrane recovery. Inset shows a small rise in membrane capacitance that occurs after the closure of calcium channels.

(C) The extent of membrane retrieval 10 s after calcium channel closure is significantly reduced in rod bipolar cells treated with dynasore ($p < 0.05$). Ten seconds after calcium channel closure, control cells retrieved $\approx 76\%$ of membrane added via stimulus-evoked exocytosis. By contrast, the extent of membrane retrieval in this same interval was reduced to $\approx 40\%$ in cells exposed to dynasore. In 2/14 cells, dynasore fully blocked endocytosis, whereas all control cells exhibited membrane recovery. Error bars represent SEM. (Wan and Heidelberger, unpublished data)

Table 1

Synaptic vesicle pools of rod-driven bipolar cells

	Mb1 BC	Rat RBC	Mouse RBC
Ribbon number	40 – 60 ¹	36 ²	23 – 43 ^{3,4,5}
Vesicle diameter	29 – 36 nm ^{1,6}	?	30 – 50 nm ^{7,8,9}
Vesicles per ribbon	≈ 110 ¹	20 – 70 ^{10,11}	12 – 35 ³
Rapid pool			
size (fF)	≈ 30 ^{1,12,13}	≈ 10 ¹¹	7 – 10 ^{3,9}
refilling time constant	$\tau \approx 4 - 12$ s ^{13,14} $\tau \approx 400$ ms ^{(a)13}	$\tau \approx 4$ s ¹⁰ $\tau \approx 132$ ms ^{(b)10}	?
Releasable pool			
size (fF)	150 ^{1,15}	50 – 60 ¹¹	≈ 30 ^{3,16}
refilling time constant	8 s ^{17,18,19}	?	7 s ³
Cytoplasmic reserve pool			
size (# vesicles/cell)	400,000 – 1,000,000 ¹	?	5,000 – 10,000 ^{(c)7,9}
Endocytosis time constant	Fast 1 – 2 s ¹⁵ Slow 10 – 20 s ^{12,20,21}	0.7 – 17 s ¹¹	2 – 21 s ^{3,4,22}

BC: bipolar cell, RBC: rod bipolar cell. Only key references are included in this table.

(a) Refilling during high Ca²⁺.

(b) Refilling during ongoing release.

(c) Estimated.

¹ von Gersdorff, et al., 1996

² Singer, et al., 2004

³ Wan, et al., 2008

⁴ LoGiudice, et al., 2008

⁵ Tsukamoto, et al., 2001

⁶ Lagnado, et al., 1996

⁷ Logiudice, et al., 2009

⁸ Spiwoks-Becker, et al., 2001

⁹ Wan, et al., 2010

¹⁰ Singer and Diamond, 2006

¹¹ Olstedal and Hartveit, 2010

¹² Neves and Lagnado, 1999

¹³ Mennerick and Matthews, 1996

¹⁴Palmer, et al., 2003

¹⁵von Gersdorff and Matthews, 1994a

¹⁶Zhou, et al., 2006

¹⁷Hull, et al., 2006

¹⁸Heidelberg, et al., 2002a

¹⁹von Gersdorff and Matthews, 1997

²⁰Hull and von Gersdorff, 2004

²¹Heidelberg, et al., 2002b

²²Llobet, et al., 2003