Electrical synapses between AII amacrine cells in the retina: Function and modulation

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ABSTRACT

Adaptation enables the visual system to operate across a large range of background light intensities. There is evidence that one component of this adaptation is mediated by modulation of gap junctions functioning as electrical synapses, thereby tuning and functionally optimizing specific retinal microcircuits and pathways. The AII amacrine cell is an interneuron found in most mammalian retinas and plays a crucial role for processing visual signals in starlight, twilight and daylight. AII amacrine cells are connected to each other by gap junctions, potentially serving as a substrate for signal averaging and noise reduction, and there is evidence that the strength of electrical coupling is modulated by the level of background light. Whereas there is extensive knowledge concerning the retinal microcircuits that involve the AII amacrine cell, it is less clear which signaling pathways and intracellular transduction mechanisms are involved in modulating the junctional conductance between electrically coupled AII amacrine cells. Here we review the current state of knowledge, with a focus on recent evidence that suggests that the modulatory control involves activity-dependent changes in the phosphorylation of the gap junction channels between AII amacrine cells, potentially linked to their intracellular Ca^{2+} dynamics.

Contents

1. Introduction

The performance of rod vision is quite remarkable (Hecht et al., 1942). The absorption of a single photon in just one of the several thousand rod photoreceptors within the receptive field of a ganglion cell, leads to a change in the firing rate of that ganglion cell (Mastronarde, 1983). This sensitivity is made possible by the amplification of visual signals in rods and further amplification and convergence within the downstream pathway. The ability of the visual system to operate across a large range of background light intensities, from starlight to bright sunlight, represents a cardinal example of sensory adaptation. This adaptation takes place not only at the level of photoreceptors, but also involves functional optimization of multi-purpose retinal microcircuits. There is evidence that such optimization can correspond to modulation of gap junction coupling, thereby functionally tuning the circuits for the changing light intensity, with the gap junctions functioning as electrical synapses (Bloomfield and Völgyi, 2009; Massey, 2009). While such tuning is thought to take place both in the outer and inner retina, corresponding to electrical synapses located in the outer and inner plexiform layers (OPL and IPL), respectively, particular focus has been directed towards elucidating the cellular and molecular mechanisms that mediate the tuning of the gap junction coupled network of AII amacrine cells, how this tuning might be related to activity-dependent synaptic input and how such tuning modifies synaptic integration. Activity-dependent plasticity of electrical synapses is an increasingly important theme in neuroscience (Haas et al., 2011; Hestrin, 2011; Landisman and Connors, 2005; Pereda et al., 2004; Zsiros and Maccaferri, 2008) and it is important to increase our understanding of how modulating the strength of electrical synapses can optimize the signal processing in the CNS. The gap junction coupling between neurons (and many other cells) is mediated by specialized channel proteins termed connexins (reviewed by Söhl et al., 2005) where post-translational modification by phosphorylation can modulate important functional properties (reviewed by Moreno and Lau, 2007). As with other areas of the CNS, progress in our understanding of electrical synaptic transmission in the retina is to a large extent hampered by the difficulties associated

with investigating the structure and function of intact neural circuits under natural conditions. Here we focus on electrical coupling of the AII amacrine cell, a retinal interneuron that plays a central role in visual signal processing in starlight, twilight and daylight and that seems to be found in the retina of all mammals.

Fig. 1 near here

2. Neuronal networks in the retina and visual coding

During a 24 hour day and night cycle, our eyes are exposed to intensities of light that vary by a factor of ~10^{12} (Valberg, 2005). Our vision is fully operative throughout this range, even though the spike rate of retinal ganglion cells varies by only a factor of 10^2 (reviewed by Demb, 2010). The ability of the ganglion cells to cover this range is made possible through a series of mechanisms, both at the level of photoreceptors and at post-receptoral levels. First, phototransduction is mediated by two types of photoreceptors, rods and cones, each with a different sensitivity to light (Fig. 1). At the lowest light intensities (starlight, corresponding to scotopic vision), each rod will only capture a single photon perhaps once a minute, while at the highest intensities (daylight, corresponding to photopic vision), each cone may capture hundreds or even thousands of photons every second. Second, the sensitivity of the transduction process itself can be adjusted in response to changes in the ambient light intensity (reviewed by Fain et al., 2001). Third, rod and cone photoreceptors connect to two fundamentally different circuits in the retina: the rod and cone pathways (Fig. 1; reviewed by Bloomfield and Dacheux, 2001). The two pathways share key cellular components, however, and there is evidence that the switching between alternate processing pathways, active at different ambient light intensities, is mediated by regulating the strength of coupling of gap junctions serving as electrical synapses (Bloomfield and Völgyi, 2009; Massey, 2009). A particularly intriguing aspect of the functional importance of these electrical synapses is that they act both as primary connections in specific pathways and as substrates for signal averaging and noise reduction.

2.1. Rod and cone pathways in the mammalian retina

Like microcircuits elsewhere in the CNS, retinal microcircuits are complex, but have the advantage that their function can be interpreted in a clear physiological context. Indeed, without an understanding of signal processing at the systems level, we cannot properly interpret the functional role of the elementary molecular and cellular components. Rod and cone photoreceptors make glutamatergic ribbon synapses with rod bipolar (RB) and cone bipolar (CB) cells, respectively, in the OPL. Rod bipolar cells constitute a single type (but see Pang et al. (2010) for a different view), whereas cone bipolar cells can be divided into 8-10 different populations with specific synaptic outputs in the IPL (Wässle, 2004). Rod bipolar cells receive their input via the specific metabotropic glutamate receptor mGluR6 (Numura et al., 1994) and depolarize in response to the onset of a light stimulus. Cone bipolar cells are either ON-cone bipolar cells that depolarize to onset of a light stimulus or OFF-cone bipolar cells that depolarize to offset of a light stimulus. ON- and OFF-cone bipolar cells receive input mediated by mGluRs and ionotropic glutamate receptors (iGluRs), respectively (reviewed by Copenhagen, 2004). This dichotomy in postsynaptic mechanisms generates the ON and OFF pathways of the visual system. In turn, ON- and OFF-cone bipolar cells contact ON- and OFF-ganglion cells, respectively, via iGluRs in excitatory ribbon synapses. In contrast, rod bipolar cells make ribbon synapses with two different types of amacrine cells, termed AII and A17. A17 amacrines provide GABAergic feedback onto the rod bipolar cell axon terminals (Chávez et al., 2006; Hartveit, 1999).

Fig. 2 near here

3. The AII amacrine cell in the mammalian retina

The AII amacrine cell is the key cellular component in the link between the rod and cone pathways (Fig. 1). It is a narrow-field, bistratified, axon-less interneuron with a dendritic morphology that supports a distinct spatial segregation of synaptic inputs and outputs. The cell body of an AII amacrine is of medium size and located at and across the border between the inner nuclear layer (INL) and the IPL (Fig. 2). The AII amacrine cell has a relatively thick primary dendrite that tapers as it descends into the

IPL and divides into lobular appendages in sublamina *a* and arboreal dendrites in sublamina *b* of the IPL (Fig. 1, 2). Quantitative estimates indicate that the AII amacrines are the most numerous amacrine cells in the mammalian retina (MacNeil and Masland, 1998). The AII amacrine has been found in all mammalian retinas examined and, to the extent investigated, the details of its cellular and network properties are remarkably conserved among different species (reviewed by Wässle and Boycott, 1991). These features of the AII amacrine make it one of the most studied interneurons in the retina. Indeed, a recent study utilizing a connectomics approach focused on the connections of the AII, referring to it as "the gold standard for neural reconstruction" (Anderson et al., 2011).

3.1. Chemical synaptic connections involving AII amacrine cells

3.1.1. Connections between AII amacrine cells and bipolar and OFF-ganglion cells

AII amacrine cells are postsynaptic to rod bipolar cells at dyad synapses in the inner (proximal) part of the IPL where varicosities from A17 amacrine cells comprise the other postsynaptic element (Famiglietti and Kolb, 1975; Kolb 1979; Kolb and Famiglietti 1974; Strettoi et al., 1990, 1992). The synaptic input from rod bipolar cells is located at the arboreal dendrites in sublamina *b* of the IPL. At the EM level, the presynaptic elements are classical ribbon synapses (Kolb 1979; Sterling et al., 1988; Strettoi et al., 1990, 1992). The synaptic input is mediated by iGluRs of the AMPA-type (GluARs) (Singer and Diamond, 2003; Singer et al., 2004; Veruki et al., 2003, Veruki et al., 2006) and immunocytochemical studies have identified the presence of GluA2/3 and GluA4 AMPA receptor subunits postsynaptically (Ghosh et al., 2001; Li et al., 2002). A recent study suggests that AII amacrines may also receive input at the arboreal dendrites from ribbon synapses of one type of ON-cone bipolar cell (Anderson et al., 2011).

AII amacrines are also postsynaptic to (some) OFF-cone bipolar cells at dyad synapses in the outer part of the IPL where the other postsynaptic element is typically the dendritic process of a ganglion cell or that of another amacrine cell (McGuire et al., 1984; Strettoi et al. 1992, 1994; Tsukamoto et al., 2001). The synaptic input from OFF-

cone bipolar cells is located at the lobular appendages in sublamina *a* of the IPL. At the EM level, the presynaptic elements are ribbon synapses here as well. Although it has not been examined extensively, electrophysiological recordings suggest that the input is mediated by iGluRs (Veruki et al., 2003; see also Xin and Bloomfield, 1999). The AII amacrine lobular appendages can also be presynaptic to the axon terminals of OFF-cone bipolar cells from which they receive input and to dendrites of OFF-ganglion cells (McGuire et al., 1984; Strettoi et al., 1992, 1994; Tsukamoto et al., 2001). At the EM level, the lobular appendages contain synaptic vesicles (Strettoi et al., 1992) and there is evidence that the transmission is glycinergic (Pourcho and Goebel, 1985; Sassoè-Pognetto et al., 1994).

3.1.2. Connections between AII amacrines and other amacrine cells

Ultrastructural investigations have revealed that AII amacrine cells are postsynaptic to other types of amacrine cells throughout the IPL (Strettoi et al., 1992), but the specific identity of the presynaptic amacrine cells is not known, except that they are not other AII amacrine cells as there is no physiological evidence for chemical synapses between these cells (Veruki and Hartveit, 2002a). Strettoi et al. (1992) found evidence that the presynaptic inputs corresponded to two morphologically distinct types, potentially corresponding to glycinergic and GABAergic amacrine cells. One specific type of amacrine input targets the cell body region and originates from dopaminergic cells (Kolb et al., 1990; Voigt and Wässle, 1987) which might use GABA (Wässle and Chun, 1988) and/or glutamate (Anderson et al., 2011) as a co-transmitter. Processes from the dopaminergic amacrine cells form ring-like structures around the cell bodies / apical dendrites of AII amacrines and ultrastructural examination has demonstrated the presence of specialized synaptic contacts (Kolb et al., 1990; Voigt and Wässle, 1987; Wässle et al., 1993). This innervation is of particular interest because of the evidence for dopaminergic modulation of tracer coupling between AII amacrine cells (Hampson et al., 1992; see below). There is evidence for both GABA and glycine receptors on AII amacrine cells (Boos et al., 1993; Gill et al., 2006; Weiss et al., 2008; Zhou and Dacheux,

2004), but so far only glycine receptors have been demonstrated to mediate direct synaptic inputs (Gill et al., 2006; Weiss et al., 2008).

3.2 Gap junction connections involving AII amacrine cells

3.2.1. Homologous gap junctions between AII amacrine cells

The first evidence for potential electrical synapses between AII amacrine cells came from ultrastructural studies of cat retina (Famiglietti and Kolb, 1975; Kolb and Famiglietti, 1974). These studies defined the AII amacrine cells as such and demonstrated several of their synaptic contacts. The homologous gap junctions are located between arboreal dendrites in the innermost part of the IPL (stratum 5; S5; Chun et al., 1993; Kolb, 1979; Sterling, 1983; Strettoi et al., 1992). The gap junctions have been described as small and symmetric and tend to be located between arboreal dendrites that are postsynaptic to the same rod bipolar axon terminal (Strettoi et al., 1992).

3.2.2. Heterologous gap junctions between AII amacrines and ON-cone bipolar cells

As for the homologous junctions between AII amacrines, the first evidence for electrical synapses between AII amacrines and ON-cone bipolar cells also came from ultrastructural studies in cat retina (Famiglietti and Kolb, 1975; Kolb, 1979; Kolb and Famiglietti 1974), but has since been verified for several other mammalian retinas (Chun et al., 1993; Massey and Mills, 1999b; Tsukamoto et al., 2001; Wässle et al., 1995). Ultrastructurally, the heterologous gap junctions differ from those interconnecting AII amacrines by an asymmetric structure, specifically a layer of "fluffy material" on the cytoplasmic side of the AII amacrine (Kolb, 1979; Strettoi et al., 1992). The AII amacrine - ON-cone bipolar gap junctions are found in sublamina *b*, concentrated in S3 and S4, of the IPL.

3.2.3. Connexins involved in gap junctions of AII amacrine cells

It is well established that the homologous gap junctions between the arboreal dendrites

of AII amacrine cells contain the neuronal gap junction protein connexin 36 (Cx36) (Deans et al., 2002; Feigenspan et al, 2001; Mills et al., 2001) and tracer coupling between AII amacrine cells is virtually abolished between AII amacrine cells in Cx36-deficient ("knock-out"; KO) mice (Deans et al., 2002). Cx36 gap junction channels display very low voltage-sensitivity and a low single-channel conductance (~15 pS; Srinivas et al., 1999; Teubner et al., 2000). As far as we know, Cx36 KO mice have not been examined for electrical coupling with dual whole-cell recording of neighboring AII amacrine cells.

With respect to the heterologous gap junctions between AII amacrine cells and ON-cone bipolar cells, tracer coupling (see below) is strongly reduced in Cx36 KO mice (Deans et al., 2002) suggesting that the channels could be homotypic Cx36 channels. This seems not to be the case, however, and the current state of knowledge suggests that they contain Cx36 on the AII amacrine side and either Cx36 or Cx45 on the bipolar side (Dedek et al., 2006; Han and Massey, 2005; Lin et al., 2005; Maxeiner et al., 2005). It is still unclear, however, how the individual connexons are assembled on the bipolar side, i.e., to which extent there are both homomeric Cx36 and Cx45 connexons and heteromeric Cx36-Cx45 connexons. It is also unclear how these types of connexons can assemble with Cx36 connexons on the AII amacrine side to make functional gap junction channels.

3.3. Intrinsic conductances of AII amacrine cells

In the first physiological study of AII amacrine cells using an *in vitro* slice preparation of rat retina, Boos et al. (1993) found evidence for voltage-gated I_{Na} and I_K. The presence of I_{Na} has been verified in several later studies, as has the involvement of this current in the ability of AII amacrine cells to generate low-amplitude spikes (Boos et al., 1993; Cembrowski et al., 2012; Tamalu and Watanabe, 2007; Veruki and Hartveit, 2002a, b). Immunocytochemistry and *in situ* hybridization studies suggest that the channels are of the $Na_v1.1$ type, with an intriguing localization at a specific lobular appendage (Cembrowski et al., 2012; Kaneko and Watanabe, 2007; Wu et al., 2011),

seemingly corresponding to the "morphologically distinct processes" identified in an earlier study by van Wart et al. (2005). Physiological evidence for expression of voltage-gated I_{Ca} was provided by Habermann et al. (2003) and imaging experiments suggested a preferential location in the region of the cell body and lobular appendages, consistent with a role in mediating transmitter release in the chemical synapses with OFF-cone bipolar cells and ganglion cells.

4. Electrical coupling between AII amacrine cells

4.1. Evidence from tracer coupling

The presence of gap junctions between cells can be detected by the consequent presence of chemical coupling, i.e. the diffusion of small molecules through the gap junction channels. When fluorescent dyes such as Lucifer yellow are used (e.g. Hampson et al., 1994), the coupling is termed dye coupling, and when non-fluorescent compounds are used, the coupling is termed tracer coupling. The most frequently used tracer compounds are biocytin (Horikawa and Armstrong, 1988) and Neurobiotin (Kita and Armstrong, 1991). They can be visualized by reacting with streptavidin-biotinylated-HRP (horseradish peroxidase) complex and diaminobenzidine (e.g. Vaney, 1991) or fluorochrome-conjugated streptavidin (e.g. Mills et al., 2001). Whereas several types of gap junction channels are permeable to Lucifer Yellow, no diffusion of this dye can be observed after injection into an AII amacrine cell (Vaney, 1985, 1991). The first evidence for chemical diffusion between AII cells was reported after injection of Neurobiotin (Vaney, 1991), suggesting that the molecular composition of the gap junctions between AII amacrines is different from that of Lucifer Yellow-permeable gap junctions between horizontal cells in the mammalian retina (Hampson et al., 1994; Vaney, 1991), as has been confirmed in later studies. After injection of Neurobiotin into a single AII amacrine, the tracer diffuses into the network of neighboring AII amacrines and ON-cone bipolar cells (Bloomfield et al., 1997; Hampson et al., 1992; Mills and Massey, 1995; Vaney, 1991), as expected from the ultrastructural data for gap junctions between these cells. With longer diffusion times, the tracer will diffuse more

extensively across the retina and fill a larger number of cells. When sufficient Neurobiotin has diffused into a coupled cell, it can be identified as either an AII amacrine cell or an (ON-cone) bipolar cell, but tracer coupling has in general not been able to identify the specific types of ON-cone bipolar cells coupled via gap junctions to AII amacrine cells (but see Massey and Mills, 1999b; Petrides and Trexler, 2008).

4.2. Evidence from visual receptive field measurements

Because AII amacrine cells are connected to each other via gap junctions, it is expected that electrical coupling would enable the transmission of visually evoked signals such that the visual receptive field would be larger than the lateral extent of the dendritic tree. Alternatively, a larger receptive field could simply be a consequence of spatial summation of receptive fields of presynaptic cells (cf. Vaney, 1994). Initial measurements of receptive fields indicated that the receptive field size of an AII amacrine was about the same size as the dendritic field size (Dacheux and Raviola, 1986; Nelson, 1982). In a later study (Bloomfield et al., 1997), however, receptive field measurements were performed under different conditions of light adaptation and for some ambient light levels, receptive fields were considerably larger than the dendritic field size, consistent with the presence of electrical coupling. The additional observation of concomitant changes in receptive field size and extent of tracer coupling at different background light levels suggested that the strength of gap junction coupling between AII amacrine cells is physiologically modulated (see below).

4.3. Evidence from electrophysiological recording of coupled cell pairs

Certain aspects of the tracer coupling method are unsatisfactory when compared to simultaneous electrophysiological recording from coupled cell pairs (Connors, 2009; see also Bennett, 1966, 1977 for an historical perspective). First, although the presence of tracer or dye coupling in general correlates well with the presence of electrical coupling, the two can be dissociated from each other, at least under certain circumstances (Eckert, 2006; Ek-Vitorin et al., 2006; Ek-Vitorin and Burt, 2012). Second,

it is very difficult, in practice basically impossible, to extract values for electrical conductance from measurements of tracer coupling (cf. Mills and Massey, 1998). Such values are important because a given conductance has a clear physiological interpretation and measuring junctional conductance can provide essential information with respect to the functional role of gap junctional communication. Third, it is difficult to compare the extent of tracer coupling quantitatively between different research groups and laboratories. Fourth, changes in tracer coupling may not reflect corresponding changes in electrical coupling (e.g. Ek-Vitorin et al., 2006). Finally, for studies of plasticity and modulatory control, tracer coupling does not allow on-line measurements of the strength of coupling and cells cannot serve as their own controls.

With the advent of the tight-seal, whole-cell recording technique (Hamill et al., 1981), it became possible to use single-electrode voltage-clamp recording and small mammalian cells for such investigations. Dual, whole-cell, voltage-clamp recordings between pairs of electrically coupled cells can be used to measure junctional conductances both at the single-channel level and at the macroscopic level (Galarreta and Hestrin, 1999; Gibson et al., 1999; Neyton and Trautmann, 1985). Apart from the indirect evidence from tracer coupling via chemical diffusion and visual receptive field measurements, the first direct evidence for functional electrical synapses between AII amacrine cells was obtained in our laboratory by dual patch-clamp recording from pairs of AII amacrine cells in rat retinal slices (Veruki and Hartveit, 2002a). The recordings demonstrated bidirectional, non-rectifying electrical coupling between these cells, with an average junctional conductance (G_j) of ~700 pS (range from about 300 to about 1500 pS). The coupling displayed low-pass transmission characteristics, typical of electrical synapses (Bennett, 1966, 1977; Connors and Long, 2004). The conductance was large enough that action potentials in one AII cell could evoke distinct responses (electrical postsynaptic potentials; electrical PSPs) in coupled cells. The electrical coupling can mediate precise synchronization of subthreshold membrane potential fluctuations and spiking that could serve as a substrate for coincidence detection in visual processing (Veruki and Hartveit, 2002a). Using dynamic clamp

electrophysiology, we have also demonstrated that the magnitude of the junctional conductance quantitatively predicts the observed coupling characteristics (Veruki et al., 2008). Paired recordings in slices have also been used to examine the electrical coupling between AII amacrine cells and ON-cone bipolar cells (Trexler et al., 2005; Veruki and Hartveit, 2002b). The homologous, as well as the heterologous coupling, can be reversibly blocked by meclofenamic acid (Veruki and Hartveit, 2009).

4.4. Functional role of electrical coupling involving AII amacrines

The two different sets of gap junction connections that AII amacrine cells make, homologous with other AII amacrines and heterologous with ON-cone bipolar cells, each have a different function. While the former are thought to act as a substrate for signal averaging and noise reduction (see below), the latter are thought to act as connections that link the rod and cone pathways. During daylight vision, visual signals from cone photoreceptors flow via cone bipolar cells to ganglion cells. Through the electrical coupling of ON-cone bipolar cells to AII amacrine cells, the ON pathway can inhibit the OFF pathway via the glycinergic synapses from AII amacrine cells to axon terminals of OFF-cone bipolar cells and dendrites of OFF-ganglion cells, thereby mediating what is termed "crossover inhibition" (Manookin et al., 2008; Münch et al., 2009; Murphy and Rieke 2008). When ambient light intensity falls below cone threshold, the cone pathway becomes non-functional. Rod photoreceptor visual signals now flow via rod bipolar cells to AII amacrine cells and couple into both the ON and OFF cone pathways: via sign-conserving electrical synapses to ON-cone bipolar cells and via sign-inverting inhibitory synapses to OFF-cone bipolar cells and OFF-ganglion cells (Fig. 1). The two alternative rod pathways, dependent on gap junctional coupling between rod and cone photoreceptors and chemical synapses between rod photoreceptors and specific types of OFF-bipolar cells (not illustrated in Fig. 1) have been reviewed in detail elsewhere (Bloomfield and Völgyi, 2009) and are not discussed further here.

5. Tuning the circuits: Modulation of electrical coupling between AII amacrine cells

The connectivity schemes of the scotopic and photopic pathways are conceptually simple, but reveal neither the complex functional dynamics nor the requirement for tuning the circuits for optimal performance. There is evidence that such tuning involves modulation of gap junction coupling and serves to functionally optimize the circuits for the background light intensity (Bloomfield and Völgyi, 2009; Massey, 2009). Tuning is thought to take place both in the OPL, for coupling between horizontal cells (Hampson et al., 1994) and between photoreceptors (Ribelayga et al., 2008), as well as in the IPL. In the IPL, there is evidence that the electrical synapses that couple AII amacrine cells are regulated, potentially via dopamine released from specific amacrine cells. In general, dopamine-mediated changes in coupling have been interpreted as reflecting the action of dopamine as a chemical messenger for light adaptation in the retina (Witkovsky, 2004). For coupling of fish horizontal cells , there is strong evidence for a modulatory role of dopamine (Lasater and Dowling, 1985), acting via the cAMP - protein kinase A (PKA) pathway (DeVries and Schwartz, 1989; Lasater, 1987) to change the conductance of the gap junction proteins (McMahon et al., 1989) and this work has been an important source of inspiration for similar investigations of AII amacrine cells.

5.1. Evidence for modulation from tracer coupling

Tracer coupling between AII amacrine cells can be modulated by pharmacological manipulations or changes in the state of light adaptation. The first pharmacological evidence was obtained for activation of D1-type dopamine receptors in rabbit retina (Hampson et al., 1992; Mills and Massey, 1995; Xia and Mills, 2004) and has since been verified for mouse retina (Urschel et al., 2006). The strength or extent of coupling in these studies is measured by intracellular injection of tracer into a single cell, waiting a fixed time period, and then counting the number of cells to which the tracer diffused during a fixed time period. Alternatively, the area of the array of cells to which tracer diffused is measured. Compared to control conditions, dopamine and D1-type agonists

reduced the number of tracer-coupled cells and the action was blocked by D1-type antagonists. When tracer coupling was examined as a function of light adaptation, the extent of coupling was low in both darkness and under light-adapted conditions and highest at intermediate intensities (Bloomfield et al., 1997). Taken at face value, these observations suggest that if dopamine modulates coupling, it is most likely not the only modulator.

5.2. Evidence for modulation from visual receptive field measurements

When visual receptive fields of AII amacrine cells were measured under different conditions of light adaptation, from absolute darkness to low photopic levels, the smallest size was measured in absolute darkness and at low photopic levels (Bloomfield and Völgyi, 2004). At high scotopic and mesopic levels, the receptive field size was larger. These results were consistent with the hypothesis that the strength of electrical coupling between AII amacrine cells depended on the state of light adaptation, but the mechanisms responsible for the changes were not investigated. Because release of dopamine in the retina is highest during light-adapted conditions (Witkovsky, 2004), coupling would be expected to be strongest under dark-adapted conditions and weakest under light-adapted conditions. If the reduction in coupling observed at photopic levels was mediated by increasing concentrations of dopamine, it is unclear how the reduction in coupling in absolute darkness can be explained.

Fig. 3 near here

Fig. 4 near here

5.3. Evidence for modulation from electrophysiological recording of coupled cell pairs

To our knowledge, there is as of today no direct evidence that dopamine, or any other neurotransmitter or neuromodulator, can modulate the junctional conductance between electrically coupled AII amacrine cells. We have observed, however, that the junctional conductance can increase during longer-lasting whole-cell recordings between pairs of electrically coupled AII amacrines (Fig. 3; Veruki et al., 2008), suggesting that the conductance can be influenced by modulatory mechanisms. Over a

30 - 90 min recording period, the junctional conductance could increase up to ~3000 pS. The increase occurs spontaneously and in a time-dependent manner that is reminiscent of a similar "run-up" observed for the strength of coupling between Cx36-expressing Neuro2A cells in culture (Zoidl et al., 2002). For AII amacrines, the time-dependent increase is only observed in whole-cell recordings with low-resistance pipettes (~5 - 7 MΩ; Veruki et al., 2008). When similar experiments were performed with higher-resistance pipettes (25 - 35 MΩ), using discontinuous, single-electrode voltage clamp (DSEVC) amplifiers to obtain adequate voltage clamp (Veruki et al., 2008; Hartveit and Veruki, 2010), an increase was not observed. Based on these observations, it was hypothesized that the time-dependent increase could be linked to intracellular washout and perturbation of a modulatory control system. Somewhat surprisingly, however, preliminary experiments with higher-resistance pipettes have been unable to demonstrate an effect of either D1-type agonists or antagonists on the junctional conductance between AII amacrines (Fig. 4; see also Demb and Singer, 2012). To our knowledge, there are no reports of whole-cell recording from coupled cell pairs where changes in junctional conductance have been followed under varying conditions of light adaptation.

5.4. Functional role of modulating electrical coupling between AII amacrines

Inspired by the evidence for electrical coupling between photoreceptors, theoretical work suggested that electrical coupling could be important for reducing noise from visual signals (Lamb and Simon, 1976). Although the electrical coupling between AII amacrines and ON-cone bipolar cells is generally interpreted as a primary connection of a specific pathway, the prevalence of electrical synapses in the retina is thought to occur because signal averaging and noise reduction are important strategies in early visual processing (reviewed by Massey, 2009). That the extent of tracer coupling of AII amacrines, as a function of light adaptation, displays an inverted U-shape (Bloomfield et al., 1997), has been interpreted to suggest that in darkness the relative uncoupling of AII amacrines is a mechanism to preserve single-photon responses. With increasing

background illumination, an increase in coupling would allow for summation of coincident signals and an improved signal-to-noise ratio. Under photopic conditions, uncoupling of AII amacrines may increase the spatial resolution of "crossover inhibition", i.e. the inhibition of OFF-bipolar and OFF-ganglion cells by ON-cone bipolar cells via AII amacrine cells. Theoretical studies with computer simulations using simplified networks of reduced models of AII amacrine cells are consistent with the idea that electrical coupling can be a mechanism for signal averaging and noise reduction (Smith and Vardi, 1995; Vardi and Smith, 1996). Although there is little experimental evidence that directly supports the above interpretation, it is consistent with two observations made when electrical coupling between AII amacrines was eliminated in Cx36 KO mice. First, the most sensitive ganglion cells functionally disappeared, reflecting loss of the high-sensitivity rod signals (Völgyi et al., 2004). Second, Dunn et al. (2006) found that there was an approximately twofold increase in noise in the absence of electrical coupling when they compared visual responses of AII amacrines in wild-type and Cx36 KO mice.

6. Effector mechanisms for gap junction modulation

6.1. Phosphorylation of Cx36 in gap junctions between AII amacrines

Hampson et al. (1992) suggested that the effects of dopamine and dopaminergic agonists and antagonists on the extent of tracer coupling of AII amacrines were mediated by changes in the phosphorylation of the connexin(s) involved and that the reduction of coupling was mediated by D1-type dopamine receptors and activation of the cAMP - PKA pathway. While a later study by Mills and Massey (1995) confirmed the basic observations of Hampson et al. (1992), neither study provided any direct evidence for a mechanism of modulatory control. Urschel et al. (2006) found evidence that Cx36 could be phosphorylated by PKA at specific sites and suggested that this would correspond to decreased gap junction conductance.

Recent evidence suggests, however, that the strength of tracer coupling is directly related to the degree of Cx36 phosphorylation (Kothmann et al., 2009). Using

an antibody that is specific for Cx36 phosphorylated at Ser293, these authors found a positive correlation between the level of phosphorylation and the extent of tracer coupling. Using the level of Ser293 phosphorylation as an index of the coupling strength, Kothmann et al. (2009) used a series of pharmacological tools to decipher the intracellular transduction pathway after D1-type dopamine receptor agonist or antagonist stimulation. They concluded that D1-type receptor-driven uncoupling of the AII network results from PKA activation of protein phosphatase 2A (PP2A) and subsequent dephosphorylation of Cx36 at Ser293 (Kothmann et al., 2009), i.e., phosphorylation of Cx36 would correspond to increased gap junction conductance.

These disparate results raise a series of questions that have yet to be addressed. First, what is the evidence that the effect of dopamine and D1-type agonists/antagonists on tracer coupling is mediated by direct action on AII amacrine cells? It is problematic that D1-type receptors have not been found on these cells (Veruki and Wässle, 1996; see also review by Demb and Singer, 2012). On the other hand, DARPP-32 (dopamine and cAMP-regulated phosphoprotein), an important target for dopamine and PKA, is expressed in AII amacrine cells (Partida et al., 2004; Witkovsky et al., 2007), suggesting that the AII could be a target of dopamine in the retina. Second, if D1-type receptor activation drives dephosphorylation of Cx36 in AII amacrines, which mechanisms are then responsible for driving the phosphorylation of Cx36, potentially leading to increased coupling strength? In light of recent evidence that Ca^{2+}/calmodulin-dependent kinase II (CaMKII) can bind directly to and phosphorylate Cx36 (Alev et al., 2008), it is tempting to speculate that activity-mediated Ca^{2+} influx can be involved in the modulatory control of coupling.

6.2. Potential involvement of CaMKII in modulatory control of gap junctions between AII amacrine cells

Following up their work on phosphorylation of Cx36 at Ser293, Kothmann and coworkers recently published an abstract where they tested the hypothesis that presynaptic bipolar cell activity drives Cx36 phosphorylation and AII amacrine

coupling (Kothmann et al., 2010). The authors reported immunocytochemical colocalization of Cx36, CaMKII and the type 1 NMDA receptor subunit (GluN1) in processes of AII amacrine cells. CPP (an NMDA receptor antagonist), KN-93 (a CaMKII inhibitor), and dark adaptation all reduced the phosphorylation of Cx36 at Ser293. Similarly, exposure to photopic light or pharmacological activation of ON bipolar cells (by application of CPPG, an antagonist of the mGluR6 receptor in the OPL) increased the phosphorylation of Cx36.

If these results can be confirmed, they suggest a Ca^{2+} dependent regulatory mechanism, potentially similar to the one postulated for the nervous system of goldfish where Ca^{2+} influx through NMDA receptors can modulate gap junctions in brain stem neurons via activation of CaMKII (Pereda et al., 1998, 2004). Molecular studies suggest that Cx36 can be phosphorylated both by CaMKII and PKA (Alev et al., 2008; Urschel et al., 2006), strengthening the potential similarity with goldfish electrical synapses and their modulatory control (Cachope et al., 2007). On this background, it seems pertinent to review the potential sources of Ca^{2+} influx in AII amacrine cells (Fig. 5).

Fig. 5 near here

6.3. Sources of Ca^{2+} influx and Ca^{2+} dynamics and signaling in AII amacrine cells

6.3.1. AMPA receptors in AII amacrine cells

The chemical synapses between rod bipolar cell axon terminals and AII amacrine cells are located at the arboreal dendrites, in close proximity to the electrical synapses between AII amacrines (Strettoi et al., 1992). AII amacrines express GluARs with significant Ca^{2+} permeability (Mørkve et al., 2002), but with no or only mild inward rectification (Mørkve et al., 2002; Singer and Diamond, 2003; Veruki et al., 2003), suggesting that they are Ca^{2+} permeable GluARs (CP-GluARs; Diamond, 2011; Dingledine et al., 1999) of a potentially unique type (Bowie, 2012). Using the technique of ultrafast application of glutamate to outside-out patches revealed functional properties similar to those of spontaneous excitatory postsynaptic currents (spEPSCs) in AII amacrines (Veruki et al., 2003). The spEPSCs display extremely rapid kinetics (rise-time ~340 μs, τ_{decay} ~760 μs), suggesting that the decay is determined by the

deactivation kinetics of the GluARs. These electrophysiological data suggest that GluARs on AIIs lack the GluA2 subunit and contain the GluA4 subunit, consistent with immunocytochemical data (Ghosh et al., 2001; Li et al., 2002). Taken together, there is good evidence that these GluARs are indeed CP-GluARs, but a functional role for the Ca^{2+} influx is still missing.

It is not known if the inputs from rod bipolar cells and OFF-cone bipolar cells are mediated by the same type of glutamate receptors. The input from OFF-cone bipolar cells to AII amacrine cells can be detected in dual recordings of synaptically connected pairs (Veruki et al., 2003) and preliminary pharmacological experiments suggest that it utilizes GluARs. The Ca^{2+} permeability and kinetics of the receptors have not yet been directly investigated.

6.3.2. NMDA receptors in AII amacrine cells

Our laboratory obtained the first evidence for the expression of NMDA receptors (GluNRs) on AII amacrines in a physiological study with whole-cell recording of cells in rat retinal slices (Hartveit and Veruki, 1997). However, dual recordings of pairs of synaptically connected rod bipolar and AII amacrine cells suggest that GluNRs do not contribute to the evoked EPSC (Singer and Diamond, 2003; Trexler et al., 2005; Veruki et al., 2003) and immunocytochemical investigations have not detected GluNRs at the synapses between rod bipolars and AII amacrines (Fletcher et al., 2000). Thus, it is likely that GluNRs have a peri- or extrasynaptic localization. Supporting this is immunocytochemical evidence for a co-localization of the GluNR1 subunit with Cx36 at some distance from kinesin II-labeled synaptic ribbons (Kothmann et al., 2010). This suggests that GluNRs could be involved in modulatory regulation of the electrical synapses and that extrasynaptic receptors could be activated by spillover of glutamate escaping from the synaptic cleft after release from bipolar cells. Veruki et al. (2006) found evidence for spillover of glutamate between neighboring rod bipolar cells and that ambient and transiently released glutamate can activate a glutamate transporter on rod bipolar axon terminals (see also Wersinger et al., 2006). This could imply that

ambient levels of glutamate might be sufficient to activate GluNRs on AII amacrines and contribute to regulating the junctional conductance of their electrical synapses.

6.3.3. Voltage-gated Ca^{2+} channels in AII amacrine cells

As reviewed above, the available evidence suggests that voltage-gated Ca^{2+} channels are located in the region of the cell body and lobular appendages of AII amacrines where they presumably are involved in release of the neurotransmitter glycine onto postsynaptic sites of OFF-cone bipolar cells and OFF-ganglion cells (Fig. 5; Habermann et al., 2003), suggesting that they are not located in a position to influence the gap junction molecules in the arboreal dendrites.

6.3.4. Ca^{2+} dynamics in AII amacrine cells

If influx of Ca^{2+} plays a functional role in regulating the junctional conductance of the electrical synapses between AII amacrines, it becomes important to consider not only the potential sources of Ca^{2+} influx, but also intracellular mechanisms of localization and clearance. In general, Ca^{2+} ions play important roles in a variety of functions of excitable cells and it has recently become possible to understand and characterize the subcellular Ca^{2+} dynamics in individual neurons (Carter and Sabatini, 2008; Helmchen, 2008). The peak and duration of a Ca^{2+} signal are two important factors that regulate downstream Ca^{2+} dependent processes. Specificity of signaling can be achieved and maintained via Ca^{2+} nano- or microdomains, i.e., the tight physical coupling between a Ca^{2+} source and a downstream signaling pathway. Whereas there are at least three possible sources for Ca^{2+} influx in AII amacrines (CP-GluARs, GluNRs and voltage-gated Ca^{2+} channels), the expression of mobile, cytoplasmic Ca^{2+} buffers (parvalbumin, calretinin, calbindin) in these cells (Casini et al., 1995; Goebel and Pourcho, 1997; Massey and Mills, 1999a; Wässle et al., 1993, 1995) could be important mechanisms for spatially localizing Ca^{2+} signals. In addition to Ca^{2+} influx and buffering, Ca^{2+} dynamics are also influenced by Ca^{2+} clearance, with two acknowledged mechanisms in dendrites: uptake of Ca^{2+} into intracellular stores via (sarco)endoplasmic reticulum

Ca^{2+}-ATPase (SERCA) pumps and extrusion of Ca^{2+} through the plasma membrane via a Ca^{2+}-ATPase and a Na^{+}/Ca^{2+} exchanger (Clapham, 2007; Goldberg and Yuste, 2005). Currently, nothing is known for AII amacrine cells with respect to the relative importance of any of these mechanisms for localizing, integrating and terminating Ca^{2+} signals. Determining the downstream targets of Ca^{2+} signals in these cells and their potential importance for regulating gap junction communication are important goals for future investigations.

7. Conclusion

AII amacrine cells are retinal interneurons that play a crucial role for visual signal processing at starlight, twilight and daylight. Connected by homologous gap junctions composed of Cx36, they form an electrically coupled network that plays an important functional role in signal averaging and noise reduction. Through a combination of their electrical and chemical synapses, AII amacrines participate in intricate, multi-purpose microcircuits. Whereas there is good evidence that modulation of the strength of electrical coupling in the network of AII amacrines is an important mechanism of circuit adaptation that tunes the retina for optimal performance, it is less clear how this modulation is mediated at the cellular and molecular levels. Increasing evidence suggests that the change in coupling strength corresponds to a change in phosphorylation of Cx36 and that activity-dependent modulation could be expressed as an interplay between retinal dopaminergic and glutamatergic signaling. Future experiments that address important questions of glutamate spillover and activity-dependent intracellular Ca^{2+} dynamics seem necessary for deciphering the mechanisms involved.

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Figure legends

Fig. 1 - Schematic overview of pathways and circuits of the mammalian retina highlighting the chemical (excitatory, inhibitory) and electrical synapses made by the AII amacrine cell. R, rods; C, cones; RB, rod bipolar cell; ON-CB, ON-bipolar cell; OFF-CB, OFF-cone bipolar cell; AII, AII amacrine cell; L, lobular appendage; A, arboreal dendrite; ON-GC, ON-ganglion cell; OFF-GC, OFF-ganglion cell.

Fig. 2 - Morphological characteristics of the AII amacrine cell. Left: *in vitro* slice preparation of rat retina, infrared differential interference contrast videomicrograph of an AII amacrine cell in a retinal slice. Arrow points towards the cell body. Right: composite fluorescence photomicrograph of an AII amacrine cell filled with Lucifer Yellow. Scale bars, 20 μm (adapted from Mørkve et al., 2002).

Fig. 3 - Junctional conductance (G_j) as a function of recording time for electrically coupled pairs of AII amacrine cells recorded in in vitro rat retinal slices with low-resistance (5 - 7 MΩ; ○; n = 6 cell pairs) or higher-resistance (24 - 30 MΩ; ●; n = 5 cell pairs) pipettes. Notice time-dependent increase of G_j for cells recorded with low-resistance pipettes and stable G_j for cells recorded with higher-resistance pipettes (adapted from Veruki et al., 2008).

Fig. 4 - Lack of effect of dopamine D1-type receptor agonist and antagonist on junctional conductance (G_j) between electrically coupled pairs of AII amacrine cells. A. G_j as a function of time for a cell pair recorded in voltage clamp with high-resistance electrodes and DSEVC amplifiers (see Veruki et al. (2008) for details). G_j is calculated as the average of the conductance values measured for each direction of coupling (with voltage pulses applied to either cell 1 or cell 2, cartoon inset). D1-type receptor agonist SKF 38393 (200 ∝M) was applied in the extracellular perfusing solution during the period indicated by the horizontal dashed line (duration ~10 min). B. same as in A,

except D1-type receptor antagonist SCH 23390 (20 ∝M) was applied in the extracellular perfusing solution during the period indicated by the horizontal dashed line (duration ~25 min).

Fig. 5 - Schematic overview of subcellular location of potential sources of Ca^{2+} influx in AII amacrine cells in relation to microcircuits involving presynaptic rod bipolar cells (RB), pre- and postsynaptic OFF-cone bipolar cells (OFF-CB) and electrical coupling between AII amacrines. *Glu*, glutamate (released by rod bipolar and OFF-cone bipolar cells). *Gly*, glycine (released by AII amacrine cells). Approximate location of CP-GluAR, GluNR and VGCC as indicated by stars. Variable resistor indicates electrical synapse with modulated conductance to neighboring AII amacrine cells. Morphologically reconstructed AII amacrine cell from *in vitro* retinal slice preparation. Cell was filled with biocytin during a short (5–10 min) period of whole cell recording (adapted from Veruki et al., 2010).

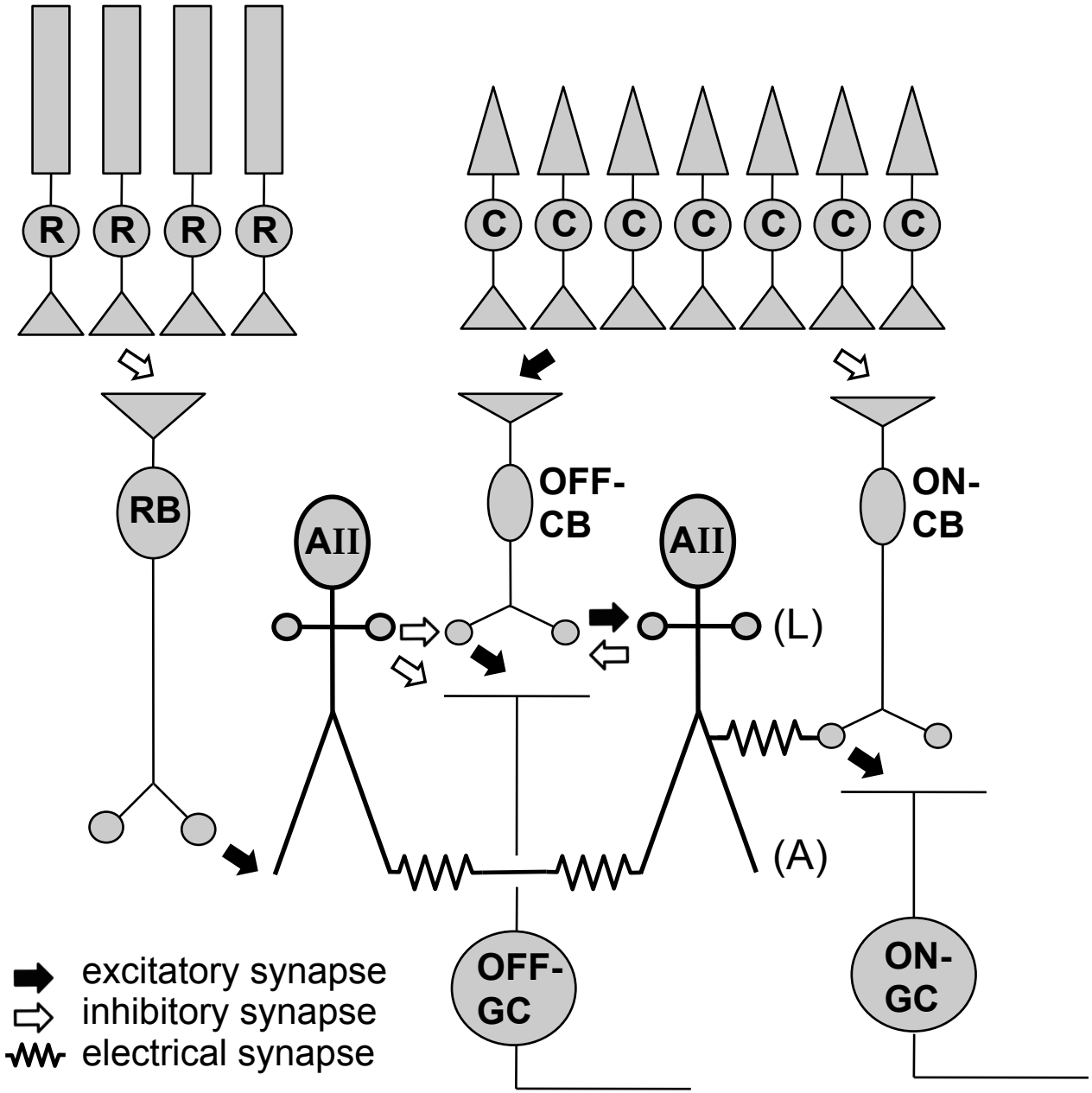


Figure 1 (Hartveit and Veruki)

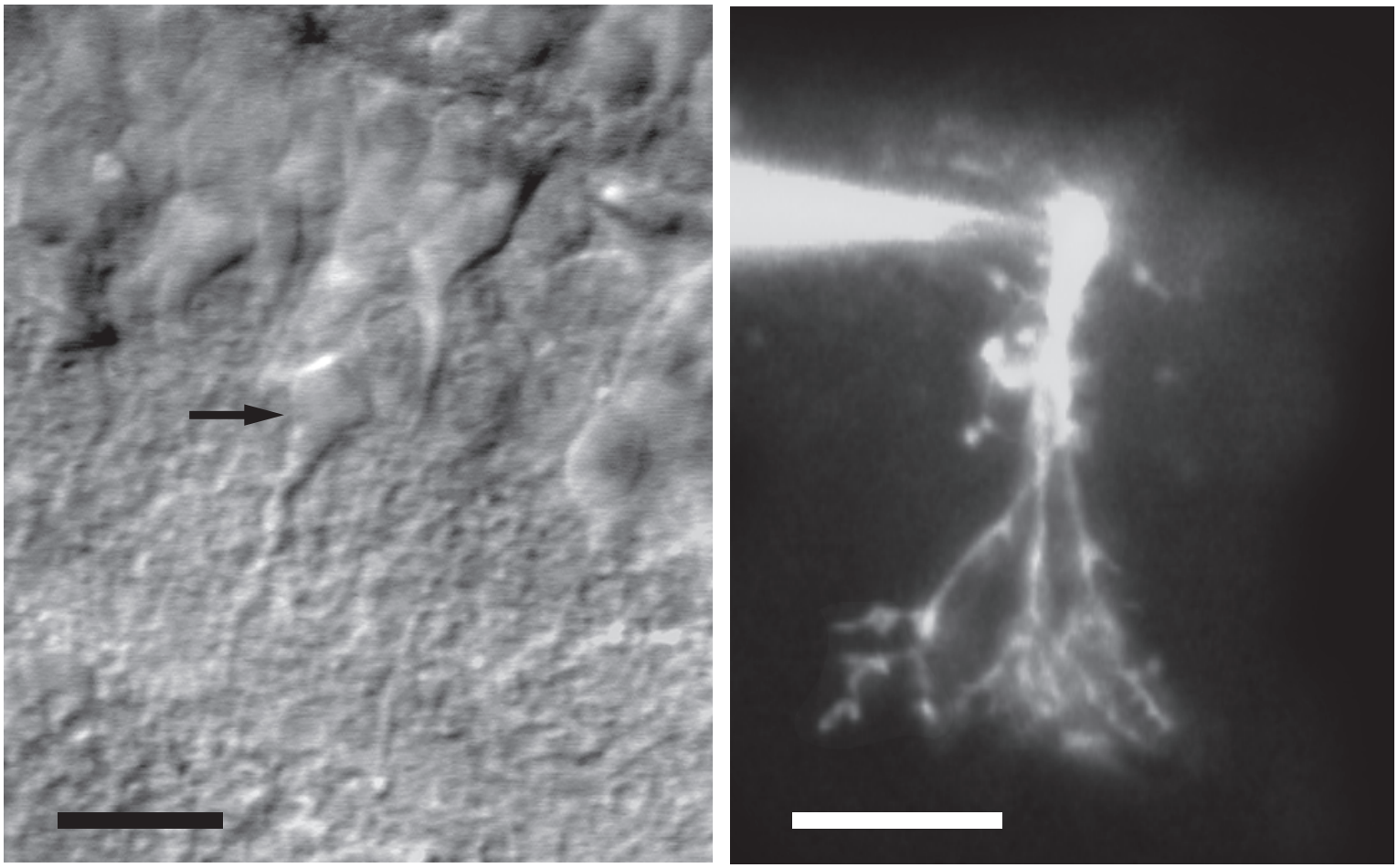


Figure 2 (Hartveit and Veruki)

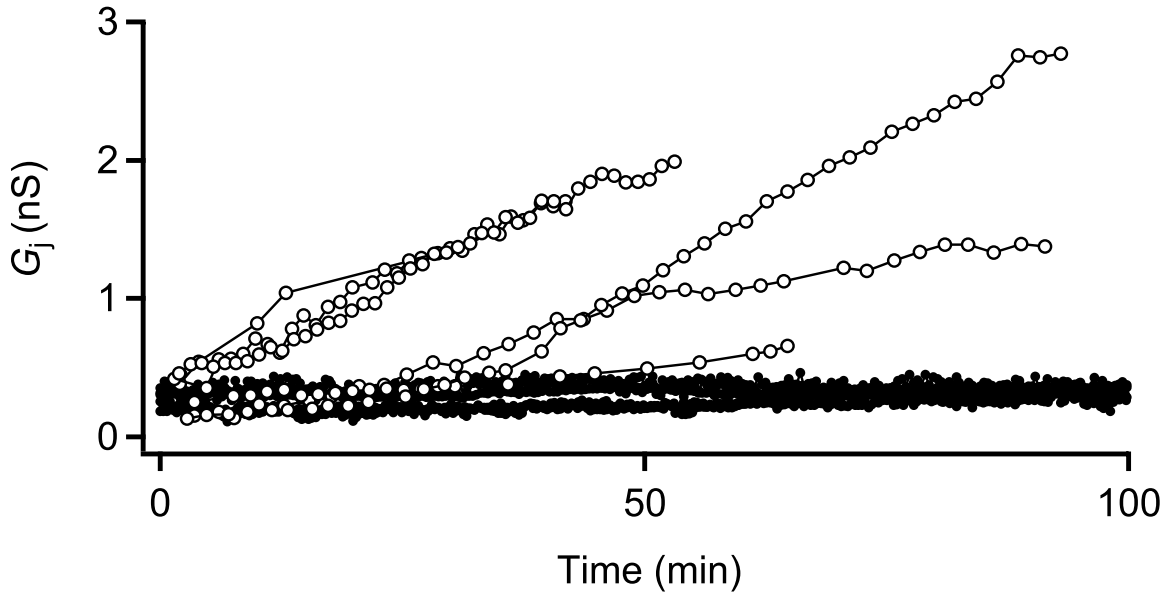
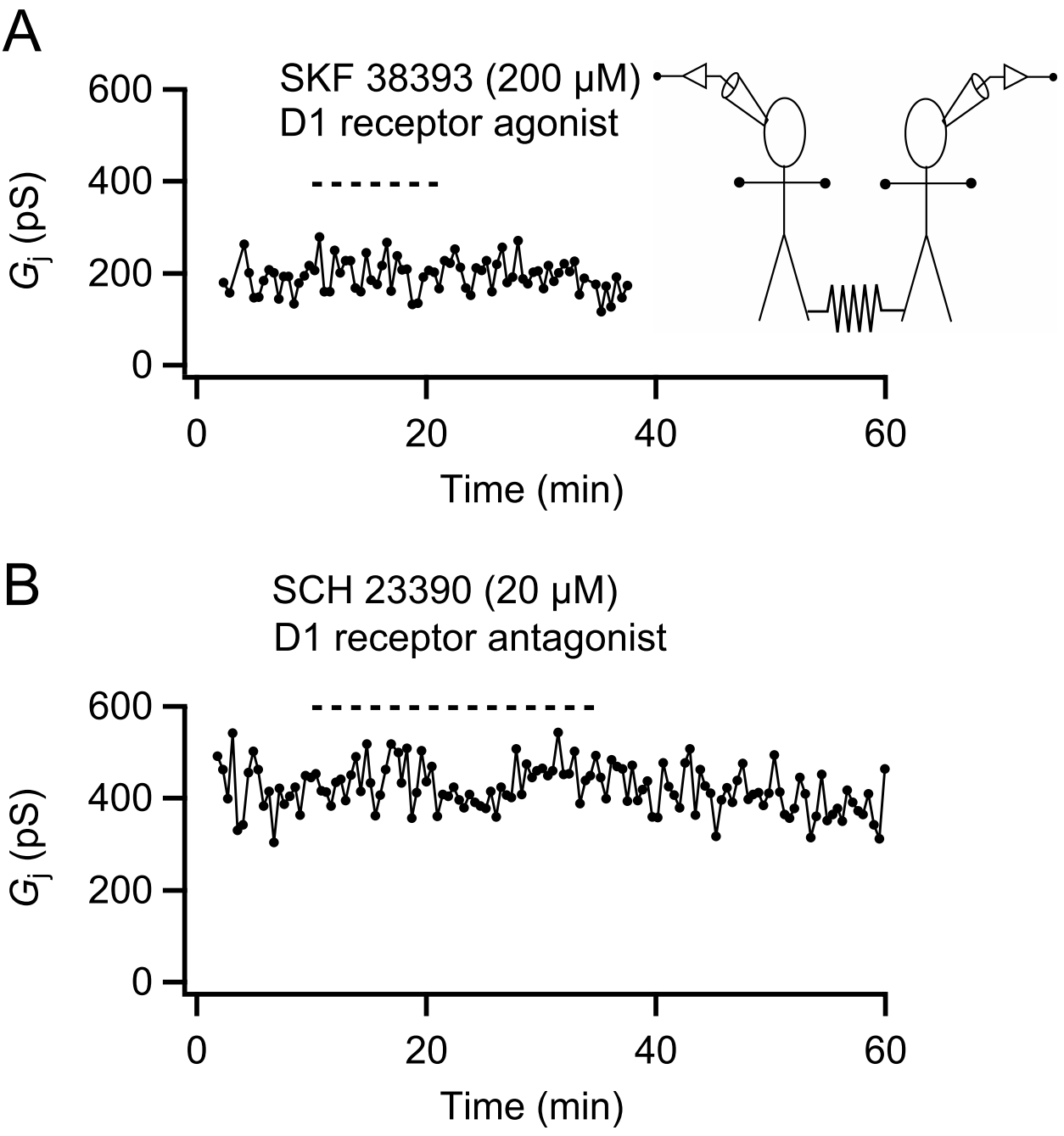


Figure 3 (Hartveit and Veruki)



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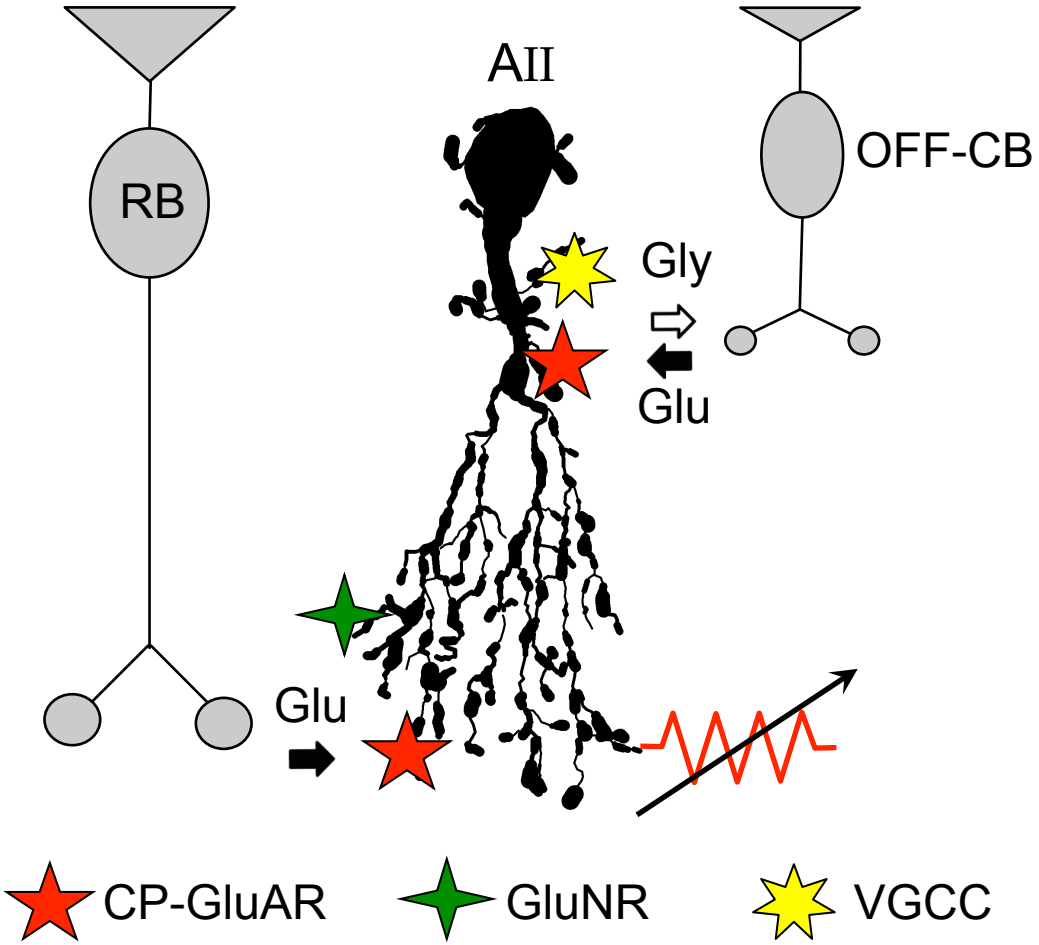

Figure 5 (Hartveit and Veruki)