HHS Public Access

Author manuscript

Vis Neurosci. Author manuscript; available in PMC 2021 September 16.

Published in final edited form as:

Vis Neurosci. ; 37: E006. doi:10.1017/S095252382000005X.

The effects of early diabetes on inner retinal neurons

Erika D. Eggers, **Teresia A. Carreon**

Departments of Physiology and Biomedical Engineering, University of Arizona, Tucson, AZ

Abstract

Diabetic retinopathy is now well understood as a neurovascular disease. Significant deficits early in diabetes are found in the inner retina that consists of bipolar cells that receive inputs from rod and cone photoreceptors, ganglion cells that receive inputs from bipolar cells and amacrine cells that modulate these connections. These functional deficits can be measured *in vivo* in diabetic humans and animal models using the electro-retinogram (ERG) and behavioral visual testing. Early effects of diabetes on both the human and animal model ERGs are changes to the oscillatory potentials that suggest dysfunctional communication between amacrine cells and bipolar cells as well as ERG measures that suggest ganglion cell dysfunction. These are coupled with changes in contrast sensitivity that suggest inner retinal changes. Mechanistic *in vitro* neuronal studies have suggested that these inner retinal changes are due to decreased inhibition in the retina, potentially due to decreased GABA release, increased glutamate release, and increased excitation of retinal ganglion cells. Inner retinal deficits in dopamine levels have also been observed that can be reversed to limit inner retinal damage. Inner retinal targets present a promising new avenue for therapies for early stage diabetic eye disease.

Keywords

diabetes; inhibition; retina; amacrine cell; ganglion cell

Introduction

Diabetic retinopathy (DR) has long been understood as both a vascular and neuronal disease of the retina. Significant evidence exists showing early neuronal deficits, potentially before any vascular dysfunction, in both diabetic patients and animal models of diabetes (Lynch & Abramoff, 2017). This neurodegeneration and neuronal dysfunction has been shown to be part of the progression of DR in diabetic human and animal models. Various potential neuronal targets have been proposed for therapeutic approaches (Barber & Baccouche, 2017). This has suggested an approach to DR as a neurovascular disease (Abcouwer & Gardner, 2014). In other areas of the nervous system diabetes causes early deficits in excitability (Morgado *et al.*, 2008; Heng *et al.*, 2011), Ca^{2+} signaling (Liu *et al.*, 2012; Zherebitskaya *et al.*, 2012), and glutamate signaling (Gagne *et al.*, 1997; Li *et al.*, 2014) suggesting neuronal specific deficits. In the retina, some of the earliest functional deficits

Corresponding author: Erika Eggers, Department of Physiology and Biomedical Engineering, P.O. Box 245051, University of Arizona, Tucson, AZ 85724, eeggers@arizona.edu.

Author Contributions: Both authors surveyed literature and wrote and edited the manuscript.

found in diabetes are from *in vivo* measurements attributed to inner retinal neurons: bipolar cells that receive inputs from photoreceptors, ganglion cells that receive input from bipolar cells and amacrine cells that modulate this signaling (Juen & Kieselbach, 1990; Parisi & Uccioli, 2001; Kizawa *et al.*, 2006). Here we will review the evidence for early diabetic damage in the inner retina and show evidence for some early mechanistic changes to inner retinal neuronal function.

Changes in *in vivo* inner retinal activity before the development of DR

The electroretinogram (ERG) has been a primary method to measure retinal activity changes in early diabetes, prior to the manifestation of clinical DR. Importantly this method can be used *in vivo* in both humans with diabetes and diabetic animal models. This means that ERG measurements can be repeated over time and could potentially be used as a screening mechanism for early retinal dysfunction. The full-field ERG has several distinct waves that have been attributed to specific cell types in the retina (Wachtmeister & Dowling, 1978; Robson & Frishman, 1998; Green & Kapousta-Bruneau, 1999). Focusing on the inner retina, the b wave primarily reflects the activity of ON bipolar cells that respond to the onset of light (Robson & Frishman, 1998; Green & Kapousta-Bruneau, 1999). The oscillatory potentials (OPs) reflect the interaction between bipolar cells and amacrine cells (Wachtmeister & Dowling, 1978; Green & Kapousta-Bruneau, 1999; McCall *et al.*, 2002). Using different light stimuli the photopic negative response (PhNR) and the scotopic threshold response (STR) reflect primarily ganglion cell activity (Robson & Frishman, 1998; Viswanathan *et al.*, 1999). Further retinal activity (multi-focal and pattern ERG) and visual behavioral measurements (visual acuity and contrast sensitivity) reflect the integration of information in the whole retina. All of these methods have been used to show changes in inner retinal function in early diabetes.

To review these results we will start with the ERG responses that have shown the earliest and most consistent alterations, the OPs. Many studies have shown that OP changes are the earliest retinal activity change present in human diabetic patients, before any DR (Coupland, 1987; Juen & Kieselbach, 1990; Yoshida *et al.*, 1991; Parisi *et al.*, 1997; Luu *et al.*, 2010) and that reduced or slowed OPs can be correlated with later DR development (Simonsen, 1980; Bresnick & Palta, 1987; Vadala *et al.*, 2002; Kizawa *et al.*, 2006). Rat and mouse models of diabetes have shown decreases in OP amplitude (Layton *et al.*, 2007; Zhang *et al.*, 2011) 3–4 weeks after diabetes induction and delayed OP timing (increased implicit time) (Shinoda *et al.*, 2007; Aung *et al.*, 2013; Sergeys *et al.*, 2019) 4–6 weeks after the induction of diabetes. Changes in OPs have been tightly correlated between rat diabetes models and human diabetic patients (Pardue *et al.*, 2014), where rod pathway responses are dysfunctional well before any cone pathway changes (Figure 1). These results strongly suggested either dysfunctional retinal amacrine cells or dysfunction in the communication between retinal amacrine cells and bipolar cells, potentially preferentially affecting the dim light rod pathway.

To determine if bipolar cell activity was specifically affected, we move to the b wave, a reflection of ON bipolar cell activity. Studies have shown that diabetic humans with no DR show no changes in b waves (Vadala *et al.*, 2002; Kizawa *et al.*, 2006). In contrast mouse and

rat diabetes models have shown a decrease in b wave amplitude in between 3–6 weeks after hyperglycemia or diabetes induction (Layton *et al.*, 2007; Zhang *et al.*, 2011; Kur *et al.*, 2016; Lee *et al.*, 2018b) or an increase in implicit time (Aung *et al.*, 2014). This suggests there could be subtle deficits in bipolar cell activity that are observed in animal models that typically have higher blood glucose levels than an average diabetic human. Another possibility, given the strong changes in amacrine cell or amacrine cell/bipolar cell interactions suggested by the OP dysfunction, is that changes in amacrine cell activity are influencing the b wave, as has been shown in previous studies (Naarendorp & Sieving, 1991; Robson *et al.*, 2004; Herrmann *et al.*, 2011; Smith *et al.*, 2015; Travis *et al.*, 2018).

Other, more complex ERG measurements are necessary to measure retinal ganglion cell and coordinated retinal activity. The multi-focal ERG (mfERG), that analyzes activity of small portions of the retina, reflects a combination of inner and outer retina activity (Hood *et al.*, 2002). Multiple studies have shown that the implicit time of mfERGs is delayed in type 1 and 2 diabetic patients with no DR (Bronson-Castain *et al.*, 2012; Dhamdhere *et al.*, 2012; Laron *et al.*, 2012). The PhNR uses specific wavelengths to target retinal ganglion cell activity (Viswanathan *et al.*, 1999). Decreases in the PhNR have been shown in human type 1 diabetes patients with no DR (McFarlane *et al.*, 2012), suggesting ganglion cell dysfunction. Additionally pattern-ERG (PERG), which uses more complicated patterned lights instead of a single flash and captures more ganglion cell activity (Luo & Frishman, 2011) has been used. There is a decrease in PERG responses in type 1 diabetes patients observed as soon as 6 months after diagnosis (Parisi & Uccioli, 2001). In rodent models of diabetes these more complex measurements are difficult, but the scotopic threshold response (STR) has been suggested to reflect ganglion cell activity (Robson & Frishman, 1998). Reduction in the STR has been shown at 4 weeks post diabetes induction (Kohzaki *et al.*, 2008; Liu *et al.*, 2020). Overall these studies strongly suggest that inner retinal activity involving amacrine cells, ganglion cells, and at least inhibitory inputs to bipolar cells are some of the earliest affected areas in the retina.

These changes in inner retinal activity are also reflected in changes in behavioral visual measures. In humans with type 1 and 2 diabetes multiple papers have shown reduced contrast sensitivity with no DR (Di Leo *et al.*, 1992; Dosso *et al.*, 1996; Harris *et al.*, 1996; Lopes de Faria *et al.*, 2001; Katz *et al.*, 2010; Safi *et al.*, 2017). In rodent models visual behavior is more difficult to assess, but many investigators use the optokinetic reflex response, where animals only respond to stimuli that have sufficient contrast or size so that they can distinguish moving bars. Using these measures in rats and mice, contrast sensitivity and visual acuity have been shown to be reduced only 4 weeks after diabetes induction (Kirwin *et al.*, 2011; Aung *et al.*, 2013; Aung *et al.*, 2014; Miller *et al.*, 2018). Together all of these studies suggest important functional changes in inner retinal activity and visual behavior early in diabetes.

Changes in inner retinal structure before the development of DR

Due to advancements in *in vivo* spectral-domain optical coherence tomography (SD-OCT) imaging of the retina (Jaffe & Caprioli, 2004), retinal layer thickness can be measured *in vivo* in diabetic humans and animal models at similar stages of diabetes as the retinal and

visual activity measurements described above. In humans with diabetes but no DR, OCT measurements in vivo have shown smaller total retinal thickness (Bronson-Castain *et al.*, 2012; Chen *et al.*, 2016), retinal nerve fiber layer (RNFL) (Lopes de Faria *et al.*, 2002; Verma *et al.*, 2012; Gundogan *et al.*, 2016), ganglion cell layer (GCL) (Van Dijk *et al.*), and inner retina (Karti *et al.*, 2017). Other studies have not found retinal thinning (Park *et al.*, 2011; Dhamdhere *et al.*, 2012), suggesting that this is something that develops over the course of diabetes (Pinilla *et al.*, 2020). In animal models, OCT measurements in mice found reduced thickness 4–8 weeks post diabetes development/induction (Hombrebueno *et al.*, 2014; Sergeys *et al.*, 2019). However, *in vitro* studies measuring retinal thickness found no changes in retinal thickness in rats at 12 weeks post diabetes induction (Enzsoly *et al.*, 2014) and 10 weeks post diabetes in mice (Martin *et al.*, 2004). Although it is not yet possible to determine if neurons are in the process of dying *in vivo*, several studies have done *in vitro* TUNEL labeling, which labels cells in the process of dying, and caspase-3 labeling, which labels cells that have activated apoptotic pathways. Diabetic rat and mouse models have shown increases in TUNEL (El-Remessy *et al.*, 2006; Gastinger *et al.*, 2006; Hernandez *et al.*, 2013) and caspase-3 (Martin *et al.*, 2004; Scuderi *et al.*, 2014) labeling 2–4 weeks after the induction of diabetes.

At this point *in vivo* measurements cannot count specific cells, but *in vitro* studies have been done to determine if the loss of retinal thickness and increase in cell death markers correlates to early loss of retinal neurons. There are two ways to count cell loss – counting all of the cells in a retinal layer or labeling and counting specific cell types. In studies of early diabetes (4–6 weeks) in rat or mouse diabetes models no loss of total cells in the INL has been shown (Zhang *et al.*, 2011; Moore-Dotson *et al.*, 2016). Some rat and mouse studies have shown the loss of total cells in the GCL 4–6 weeks after diabetes induction (Zeng *et al.*, 2000; Zhang *et al.*, 2011; Yang *et al.*, 2012), but some studies have shown no loss at 6 weeks (Moore-Dotson *et al.*, 2016) or until 10 weeks (Martin *et al.*, 2004).

To clarify these general measurements several studies have labelled specific bipolar cell, amacrine cell or ganglion cell types. Mouse models of diabetes have shown no loss of rod bipolar cells, horizontal cells, GABAergic or glycinergic amacrine cells 6–12 weeks after diabetes (Hombrebueno *et al.*, 2014; Moore-Dotson *et al.*, 2016), but losses of dopaminergic and cholinergic amacrine cells have been reported 16 weeks-6 months after diabetes (Gastinger *et al.*, 2006; Liu *et al.*, 2020). Since the GCL measurements in rodents discussed above contain both ganglion cells and displaced amacrine cells (Jeon *et al.*, 1998), other studies have specifically labeled ganglion cells to measure ganglion cell loss. In diabetic mouse and rat models no loss of ganglion cells was shown after 7–24 weeks of diabetes (Hombrebueno *et al.*, 2014; Enzsoly *et al.*, 2015), but in other studies loss was shown 12–16 weeks after diabetes (Gastinger *et al.*, 2008; Liu *et al.*, 2020). These results suggest that although retinal layer thinning may be an early symptom of diabetic retinal damage, it is probably not the earliest symptom and may not correlate to actual loss of neurons.

Neuronal mechanisms of diabetic inner retinal damage

The measured changes in *in vivo* retinal and visual responses in early diabetes suggest there are specific retinal neuronal deficits caused by early diabetes. General changes in synaptic

function have been suggested by a decrease in the expression of presynaptic proteins from rat retinas after 4 weeks of diabetes: (VanGuilder *et al.*, 2008). Given that the earliest *in vivo* physiological changes strongly suggest dysfunction of inhibition from amacrine cells and excitation to ganglion cells, it is important to understand the changes in both inhibitory and excitatory signaling in the retina. Using *in vitro* preparations this can be studied at a variety of levels. Because the *in vitro* retina will respond physiologically to light, the responses of individual neurons in the retina can be studied in response to light. These responses can then be broken down further to look for deficits in specific neurotransmitter signaling, receptors that mediate the neurotransmitter signaling, and modifiers that affect the synaptic signaling.

Changes in retinal inhibition due to diabetes

The changes in OPs discussed above strongly suggest changes to retinal inhibition, as OPs can be eliminated or changed by modulating inhibitory neurotransmitter levels or receptors (Wachtmeister & Dowling, 1978; Wachtmeister, 1980; Green & Kapousta-Bruneau, 1999; McCall *et al.*, 2002; Moller & Eysteinsson, 2003). Because the retina can be studied with circuits intact *in vitro*, where individual neurons are accessible for recording, several studies have shown specific changes in inhibition evoked from retinal amacrine cells. The majority of studies have investigated the inhibitory input to rod bipolar cells, that receive input from rod photoreceptors, respond to the onset of light, and represent ~50% of the bipolar cells in the human peripheral and rodent retina. Rod bipolar cells receive inputs from GABAergic and glycinergic amacrine cells onto $GABA_A$, $GABA_C$ and glycine type neurotransmitter receptors (Eggers & Lukasiewicz, 2006a). Stimulating the retina physiologically with light, total inhibition and GABAergic inhibition mediated by both the $GABA_A$ and $GABA_C$ receptors on rod bipolar cells were reduced after 6 weeks of diabetes (Moore-Dotson *et al.*, 2016) (Figure 2A–B). A later study showed that this reduction was specific to amacrine cells, as the duration of electrically evoked inhibition and inhibition from rod bipolar cell – amacrine cell feedback was also reduced after 6 weeks of diabetes (Moore-Dotson & Eggers, 2019).

Changes in evoked inhibition could be due to changes in the function or loss of receptors for inhibitory neurotransmitter or in decreases in the release of inhibitory neurotransmitter. One way this can be studied is to determine the effect of activating or blocking inhibitory receptors on the outputs of bipolar cells, since certain circuit connections in the retina are well understood. One previous study measured the effect of inhibition by modulating inhibition while recording the spontaneous release of glutamate from rod bipolar cells onto a type of amacrine cell, the AII amacrine cell. Blocking $GABA_C$ receptors caused an increase in spontaneous release of glutamate onto AII amacrine cells, but after 2–5 weeks of diabetes this effect was decreased (Castilho *et al.*, 2015a), suggesting a decrease in expression or function of $GABA_C$ receptors. However, a different study showed increased sensitivity of rod bipolar cell $GABA_C$ receptors to GABA (Ramsey *et al.*, 2006) after 12 weeks of diabetes. After 6 weeks of diabetes no changes were shown in the amplitude of spontaneous inhibitory synaptic currents (IPSCs) mediated by $GABA_C$ receptors (Moore-Dotson *et al.*, 2016). To date no labeling of $GABA_C$ receptor protein levels has been performed so it is not clear if $GABA_C$ receptor levels are changing. After 6 weeks of diabetes no change was found in $GABA_A$ receptor protein labeling on rod bipolar cells (Moore-Dotson *et al.*, 2016),

although spontaneous IPSCs mediated by $GABA_A$ receptors were increased in amplitude, potentially due to increased frequency of GABA release that will be discussed later. Although no physiological responses of glycinergic inhibition have been measured, after 1 week of diabetes another study showed a significant decrease in glycine receptor α4 subunit (Morales-Calixto *et al.*, 2019). In contrast to the GABA receptor responses measured above in bipolar cells, glycine receptor α4 subunits are in retinal amacrine cells or ganglion cells (Heinze *et al.*, 2007), suggesting there may be a change in inhibition there as well. Collectively these studies suggest that there may be changes in inhibitory receptor function or expression, but the levels might change with duration of diabetes and might not be responsible for the large changes in evoked inhibition.

In contrast, several studies have shown changes in GABA release in diabetes. After two weeks of diabetes a decrease was seen in GABA release evoked by depolarizing amacrine cells with increased K^+ levels (Baptista *et al.*, 2011). Other studies also showed a decrease in the amount of light-evoked GABA release (Moore-Dotson *et al.*, 2016) (Figure 2C–D) and the timing of electrically evoked GABA release (Moore-Dotson & Eggers, 2019) from amacrine cells onto rod bipolar cells after 6 weeks of diabetes. Together these show a decrease in evoked GABA release from amacrine cells. This is supported by a decrease in GAD expression, the enzyme responsible for making GABA in neurons (Honda *et al.*, 1998; Ly *et al.*, 2014) and a decrease in VGAT, the transporter responsible for loading GABA into vesicles, in retinal synaptosomes (Baptista *et al.*, 2011). In contrast, spontaneously released GABA has been shown to increase. The frequency of spontaneous IPSCs mediated by both $GABA_A$ and $GABA_C$ receptors on rod bipolar cells increases after 6 weeks of diabetes (Moore-Dotson *et al.*, 2016). This is supported by other studies that have shown GABA accumulation in the inner retina (Ramsey *et al.*, 2006) and in retinal Muller glial cells (Ishikawa *et al.*, 1996) after 12 weeks of diabetes. Together these results suggest that evoked GABA release is compromised, potentially due to an increase in spontaneous GABA release that depletes amacrine cells of GABA for evoked release.

Changes in inhibition could also be occurring because of dysfunctional Ca^{2+} signaling in amacrine cells. Retinal amacrine cells have relatively slow neurotransmitter release that depends on extended Ca^{2+} signals after a synaptic input (Gleason *et al.*, 1994; Eggers & Lukasiewicz, 2006b; Chavez *et al.*, 2010; Eggers *et al.*, 2013). A previous study showed that after 2–5 weeksof diabetes there was a reduced Ca^{2+} response to glutamate in A17 amacrine cells (Castilho *et al.*, 2015a) (Figure 3). This extended Ca^{2+} signal in amacrine cells is buffered by the slow Ca^{2+} buffer EGTA (Eggers *et al.*, 2013). After 6 weeks of diabetes, EGTA caused a much more significant block of GABAergic inhibition onto rod bipolar cells, suggesting a reduced Ca^{2+} signal in the presynaptic amacrine cells that inhibit rod bipolar cells (Moore-Dotson & Eggers, 2019). Together these studies show specific deficits in inhibitory signaling in the diabetic retina, potentially due to reduced GABA release from amacrine cells.

Changes in retinal excitation due to diabetes

Several studies have also shown potential changes in retinal excitation, either as a result of changing synaptic mechanisms governing excitatory synapses or due to a loss of inhibitory

input. When studying physiological activation of retinal neurons with light after 6 weeks of diabetes, no change was seen in light-evoked excitatory input from photoreceptors to rod bipolar cells, in contrast to the reduced inhibitory input (Moore-Dotson *et al.*, 2016). However, light-evoked excitation to AII amacrine cells increased, potentially as a result of decreased light-evoked inhibition to rod bipolar cells (Figure 2E). Another study found that ON, but not OFF, ganglion cells had increased spontaneous firing after 12–16 weeks of diabetes (Yu *et al.*, 2013). These changes were suggested to be due to changes in synaptic inputs, potentially due to reduced inhibitory inputs onto bipolar cells. Additionally, after 12 weeks of diabetes ON ganglion cells show increased excitability (Cui *et al.*, 2019) that could potentially increase their response to glutamatergic inputs.

There have also been reports of changes in glutamate release and packaging at the synaptic level in early diabetes. Several studies of early diabetes have shown reduced protein or mRNA expression of vesicular glutamate transporters VGlut1 or VGlut2 in the retina (Lau *et al.*, 2013; Ly *et al.*, 2014) or in synaptosomes made from retina (Baptista *et al.*, 2011). In contrast multiple studies have shown increased retinal glutamate levels after 7–12 weeks of diabetes (Lieth *et al.*, 1998; Kowluru *et al.*, 2001; Ali *et al.*, 2019). This could be due to changes in spontaneous release of glutamate. Studies have shown an increase in the frequency of spontaneous EPSCs onto AII and A17 amacrine cells after 2–6 weeks of diabetes (Castilho *et al.*, 2015a; Moore-Dotson *et al.*, 2016). An increase in light-evoked glutamate release from rod bipolar cells onto AII amacrine cells has also been reported (Moore-Dotson *et al.*, 2016) (Figure 2F). It is likely that reduced inhibition to rod bipolar cells contributes to this increased glutamate release, since the ability of tonic inhibition to decrease spontaneous glutamate release from rod bipolar cells is lost in diabetes (Castilho *et al.*, 2015a) and decreased rod bipolar L-IPSCs correlate with increased AII amacrine cell L-EPSCs (Moore-Dotson *et al.*, 2016). However, it is also likely that glutamate release from rod bipolar cells, which occurs by the simultaneous fusion of multiple vesicles (Singer *et al.*, 2004), is increased independent of inhibition, as increased glutamate release occurs in the presence of increased spontaneous inhibition to rod bipolar cells (Moore-Dotson *et al.*, 2016).

Other studies have shown synapse specific changes in glutamate receptors. After 2–5 weeks of diabetes, the function of GluR2 Ca^{2+} permeable AMPA receptors was reduced in synaptic A17 amacrine cell AMPA receptors (Castilho *et al.*, 2015a) and extrasynaptic AII amacrine cell AMPA receptors (Castilho *et al.*, 2015b). In contrast, a separate study showed increased GluR2/3 protein levels in retinal inner plexiform layer (IPL) and GCLs after 2 and 6 weeks of diabetes (Semkova *et al.*, 2010). However, this may have been due to the inability to separately label GluR2 receptors, as a study that could separate them showed that 4–12 weeks after diabetes there were increased protein levels of GluR1, 2/3 and 4, but decreased GluR2 in the whole retina with no changes in mRNA levels (Santiago *et al.*, 2009). After 12 weeks of diabetes another study showed decreased mRNA levels for glutamate receptors and transporters (Lau *et al.*, 2013). NMDA receptor mRNA subunits decreased, GluR4 mRNA levels decreased and no other AMPA subunits changed, with a decrease in all Kainate mRNA subunits. Together these results suggest that glutamate receptor levels are likely changing in early diabetes, but that these changes are receptor subtype specific and could potentially be due to changing glutamate levels.

Changes in retinal dopamine as a neuromodulator in diabetes

Although all retinal amacrine cells release either GABA or glycine (Haverkamp & Wassle, 2000) many of them also co-release other neurotransmitters such as dopamine and acetylcholine (Diamond, 2017). Dopamine is a crucial neuromodulator for the retina, as it is responsible for the process of resetting the sensitivity of the retina to match ambient light conditions through light adaptation (Witkovsky, 2004), in addition to other roles. Several studies have shown that retinal dopamine levels and machinery are lower in the diabetic retina. After 3–12 weeks of diabetes retinal dopamine levels were reduced (Nishimura & Kuriyama, 1985; Aung *et al.*, 2014; Lahouaoui *et al.*, 2016) and reduced dopamine efflux from the retina was also observed (Nishimura & Kuriyama, 1985). Levels of the vesicular monoamine transporter responsible for loading dopamine in vesicles - VMAT2 - were lower by 4 weeks of diabetes (Li *et al.*, 2019). These lowered dopamine levels seem to be important for retinal neuronal dysfunction in diabetes, as supplementation with L-DOPA during diabetes reduced the effect of diabetes on ERG parameters in rats and humans (Kim *et al.*, 2018; Motz *et al.*, 2020) and delayed the significant declines in acuity and contrast sensitivity (Aung *et al.*, 2014). Agonists of the D1 dopamine receptor increased acuity to normal levels and an agonist of the D4 dopamine receptor increased contrast sensitivity after 8 weeks of diabetes (Aung *et al.*, 2014). These reductions in acuity and contrast sensitivity fit with loss of dopamine, as similar deficits are seen in animals where tyrosine hydroxylase – the rate-limiting enzyme for dopamine production – had been knocked out specifically in the retina (Jackson *et al.*, 2012), and these THKO animals looked similar to diabetic animals in retinal and visual function after 6 weeks of diabetes (Aung *et al.*, 2014). Other studies have also shown that the incidence of Parkinson's disease, which results from loss of dopamine in the basal ganglia, is higher in patients with DR (Lee *et al.*, 2018a) and that Parkinson's patients have visual deficits that may be related to reduced retinal dopamine levels (Archibald *et al.*, 2009). These studies suggest that dysfunction in the inner retinal dopamine system may also be important for understanding retinal neuronal dysfunction in diabetes.

DR therapeutic approaches

In this review we have described the effects of diabetes on the inner retina. Although much of the work presented here aims to investigate the early effects of diabetes at the mechanistic and functional level, it is crucial to keep in mind the severity of clinical DR. The use of SD-OCT and ERG, as previously mentioned, have assisted in evaluating damage at the morphological as well as functional level, respectively, in DR patients in the clinic setting. Identification of the type of damage is essential to deciding the treatment option. The management of blood sugar in diabetes is a major tool in improving the disease progression of DR but this does not always prevent retinal damage and patient compliance is difficult. Additionally, the slow nature of DR means that patients do not know they have it until their vision is affected at which point damage has already accrued. The ideal would be to develop treatments that target early retinal damage, such as the damage to the inner retina.

Gold standard for current therapeutic treatments

Laser photocoagulation has remained the gold standard for DR treatment for decades. Panretinal photocoagulation (PRP) refers to the technique that involves burning of the retinal tissue at various areas to seal off leaky blood vessels and inhibit further vessel growth. This technique reduces severe vision damage caused by DR by 50%. However, this treatment option can cause sub-retinal fibrosis and peripheral-vision loss (Chakravarthy & Devanathan, 2018). In addition to this, current treatment options include anti-VEGF antibodies, commonly known as ranibizumab (Lucentis) and bevacizumab (Avastin). They work to inhibit vascular endothelial growth factor (VEGF) to improve visual acuity in DR patients (Nguyen *et al.*, 2012; Brown *et al.*, 2013; Osaadon *et al.*, 2014; Schmidt-Erfurth *et al.*, 2014). Although the functional improvements give anti-VEGF therapies great potential, the effects of long-term treatment with anti-VEGF antibodies are not yet clear. It is also important to note that only 30–40% of individuals respond to anti-VEGF antibody treatment and those that do tend to develop resistance to this treatment if given over a long duration (Gonzalez *et al.*, 2016; Yang *et al.*, 2016). Taken together it is evident that the development of new treatments for DR is needed to delay disease progression and improve overall quality of life for DR patients.

Neuroprotection as a treatment option for DR

The increasing acceptance of DR as a neurodegenerative disease rather than just a microvascular disease has opened an avenue for improved treatment options. Some of the potential treatments that have shown neuroprotection include endogenous substances and antioxidant/anti-inflammatory substances. Glucagon-like peptide 1 (GLP-1) is an endogenous substance that has neuroprotective qualities. An increase in GLP-1 receptors has been observed in diabetic human retinas. In addition, the inhibition of vascular leakage by the increase in GLP-1R activity has been demonstrated through the application of topical administration of GLP-1R agonists in the db/db mouse model (Hernandez *et al.*, 2016a). Investigation into the role that oxidative stress plays in DR has suggested the use of antioxidant/anti-inflammatory substances as treatment options. Flavonoids and carotenoids are classes of antioxidant substances that have been shown to inhibit DR damage induced by increased oxidative stress. There are several specific agents that belong to each class that have altered various pathways in a positive way to reduce inner retinal damage (Hernandez *et al.*, 2016b). Finally, as previously mentioned, investigation into dopamine may hold a promising key to the future of DR therapies. The changes observed in the dopaminergic pathway in the inner retina and previous findings showing the improvement of visual function in diabetic rodent models with the treatment of l-DOPA support the potential of the therapeutic components in this pathway (Seki *et al.*, 2004; Gastinger *et al.*, 2006; Szabadfi *et al.*, 2012; Aung *et al.*, 2014). These substances may soon be an improvement to the current therapies for DR.

Summary

Here we have reviewed the evidence for early inner retinal changes in diabetes and mechanistic changes in inner retinal neurons in early diabetes, summarized in Figure 4. The previous studies discussed above strongly suggest deficits in the rod pathway of the retina.

Previous studies have shown reduced inhibition to rod bipolar cells, likely through decreased GABA release from GABAergic amacrine cells. This leads to an increase in rod pathway output onto AII amacrine cells, which show increased light-evoked and spontaneous activity. These changes also lead to increases in excitatory input and excitability of retinal ganglion cells, the output neurons of the retina. Given that inner retinal neurons are sensitive to excitotoxic damage (Calvo *et al.*, 2020), excitotoxicity due to increased glutamate release could be a further avenue for retinal dysfunction (Lieth *et al.*, 1998; Pulido *et al.*, 2007; Ola *et al.*, 2013; Gu *et al.*, 2014). Although these changes to retinal neuronal signaling may be accompanied by retinal cell death or synapse loss, the studies described suggest that these may be later changes that are potentially specific to individual cell types and not due to mass loss of retinal neurons. Therapeutic strategies described above that target inner retina damage could be an important factor in understanding and reducing visual dysfunction in diabetes.

Acknowledgements:

Disclosures: The authors declare no conflicts of interest regarding the present study.

Grants: This work was supported by the National Eye Institute Grant R01-EY-026027 and NSF-1552184 to EDE.

Acknowledgements: We thank the members of the Eggers laboratory for helpful comments.

References Cited

Abcouwer SF & Gardner TW. (2014). Diabetic retinopathy: loss of neuroretinal adaptation to the diabetic metabolic environment. Ann N Y Acad Sci 1311, 174–190. [PubMed: 24673341]

Ali SA, Zaitone SA, Dessouki AA & Ali AA. (2019). Pregabalin affords retinal neuroprotection in diabetic rats: Suppression of retinal glutamate, microglia cell expression and apoptotic cell death. Exp Eye Res 184, 78–90. [PubMed: 31002823]

Archibald NK, Clarke MP, Mosimann UP & Burn DJ. (2009). The retina in Parkinson's disease. Brain 132, 1128–1145. [PubMed: 19336464]

Aung MH, Kim MK, Olson DE, Thule PM & Pardue MT. (2013). Early visual deficits in streptozotocin-induced diabetic long evans rats. Invest Ophthalmol Vis Sci 54, 1370–1377. [PubMed: 23372054]

Aung MH, Park HN, Han MK, Obertone TS, Abey J, Aseem F, Thule PM, Iuvone PM & Pardue MT. (2014). Dopamine deficiency contributes to early visual dysfunction in a rodent model of type 1 diabetes. J Neurosci 34, 726–736. [PubMed: 24431431]

Baptista FI, Gaspar JM, Cristovao A, Santos PF, Kofalvi A & Ambrosio AF. (2011). Diabetes induces early transient changes in the content of vesicular transporters and no major effects in neurotransmitter release in hippocampus and retina. Brain Res 1383, 257–269. [PubMed: 21281613]

Barber AJ & Baccouche B. (2017). Neurodegeneration in diabetic retinopathy: Potential for novel therapies. Vision Res 139, 82–92. [PubMed: 28988945]

Bresnick GH & Palta M. (1987). Oscillatory potential amplitudes. Relation to severity of diabetic retinopathy. Arch Ophthalmol 105, 929–933. [PubMed: 3606452]

Bronson-Castain KW, Bearse MA Jr., Neuville J, Jonasdottir S, King-Hooper B, Barez S, Schneck ME & Adams AJ. (2012). Early neural and vascular changes in the adolescent type 1 and type 2 diabetic retina. Retina 32, 92–102. [PubMed: 21878857]

Brown DM, Heier JS, Clark WL, Boyer DS, Vitti R, Berliner AJ, Zeitz O, Sandbrink R, Zhu X & Haller JA. (2013). Intravitreal aflibercept injection for macular edema secondary to central retinal vein occlusion: 1-year results from the phase 3 COPERNICUS study. Am J Ophthalmol 155, 429–437 e427. [PubMed: 23218699]

Calvo E, Milla-Navarro S, Ortuno-Lizaran I, Gomez-Vicente V, Cuenca N, De la Villa P & Germain F. (2020). Deleterious Effect of NMDA Plus Kainate on the Inner Retinal Cells and Ganglion Cell Projection of the Mouse. Int J Mol Sci 21.

Castilho A, Ambrosio AF, Hartveit E & Veruki ML. (2015a). Disruption of a neural microcircuit in the rod pathway of the mammalian retina by diabetes mellitus. J Neurosci 35, 5422–5433. [PubMed: 25834065]

Castilho A, Madsen E, Ambrosio AF, Veruki ML & Hartveit E. (2015b). Diabetic hyperglycemia reduces Ca2+ permeability of extrasynaptic AMPA receptors in AII amacrine cells. J Neurophysiol 114, 1545–1553. [PubMed: 26156384]

Chakravarthy H & Devanathan V. (2018). Molecular Mechanisms Mediating Diabetic Retinal Neurodegeneration: Potential Research Avenues and Therapeutic Targets. J Mol Neurosci 66, 445–461. [PubMed: 30293228]

Chavez AE, Grimes WN & Diamond JS. (2010). Mechanisms underlying lateral GABAergic feedback onto rod bipolar cells in rat retina. J Neurosci 30, 2330–2339. [PubMed: 20147559]

Chen Y, Li J, Yan Y & Shen X. (2016). Diabetic macular morphology changes may occur in the early stage of diabetes. BMC ophthalmology 16, 12. [PubMed: 26781272]

Coupland SG. (1987). A comparison of oscillatory potential and pattern electroretinogram measures in diabetic retinopathy. Doc Ophthalmol 66, 207–218. [PubMed: 3428075]

Cui RZ, Wang L, Qiao SN, Wang YC, Wang X, Yuan F, Weng SJ, Yang XL & Zhong YM. (2019). ON-Type Retinal Ganglion Cells are Preferentially Affected in STZ-Induced Diabetic Mice. Invest Ophthalmol Vis Sci 60, 1644–1656. [PubMed: 30995300]

Dhamdhere KP, Bearse MA Jr., Harrison W, Barez S, Schneck ME & Adams AJ. (2012). Associations between local retinal thickness and function in early diabetes. Invest Ophthalmol Vis Sci 53, 6122–6128. [PubMed: 22871830]

Di Leo MA, Caputo S, Falsini B, Porciatti V, Minnella A, Greco AV & Ghirlanda G. (1992). Nonselective loss of contrast sensitivity in visual system testing in early type I diabetes. Diabetes Care 15, 620–625. [PubMed: 1516480]

Diamond JS. (2017). Inhibitory Interneurons in the Retina: Types, Circuitry, and Function. Annu Rev Vis Sci 3, 1–24. [PubMed: 28617659]

Dosso AA, Bonvin ER, Morel Y, Golay A, Assal JP & Leuenberger PM. (1996). Risk factors associated with contrast sensitivity loss in diabetic patients. Graefes Arch Clin Exp Ophthalmol 234, 300–305. [PubMed: 8740250]

Eggers ED, Klein JS & Moore-Dotson JM. (2013). Slow changes in Ca2(+) cause prolonged release from GABAergic retinal amacrine cells. J Neurophysiol 110, 709–719. [PubMed: 23657284]

Eggers ED & Lukasiewicz PD. (2006a). GABA(A), GABA(C) and glycine receptor-mediated inhibition differentially affects light-evoked signalling from mouse retinal rod bipolar cells. J Physiol 572, 215–225. [PubMed: 16439422]

Eggers ED & Lukasiewicz PD. (2006b). Receptor and transmitter release properties set the time course of retinal inhibition. J Neurosci 26, 9413–9425. [PubMed: 16971525]

El-Remessy AB, Al-Shabrawey M, Khalifa Y, Tsai NT, Caldwell RB & Liou GI. (2006). Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. Am J Pathol 168, 235–244. [PubMed: 16400026]

Enzsoly A, Szabo A, Kantor O, David C, Szalay P, Szabo K, Szel A, Nemeth J & Lukats A. (2014). Pathologic alterations of the outer retina in streptozotocin-induced diabetes. Invest Ophthalmol Vis Sci 55, 3686–3699. [PubMed: 24845643]

Enzsoly A, Szabo A, Szabo K, Szel A, Nemeth J & Lukats A. (2015). Novel features of neurodegeneration in the inner retina of early diabetic rats. Histol Histopathol 30, 11602.

Gagne J, Milot M, Gelinas S, Lahsaini A, Trudeau F, Martinoli MG & Massicotte G. (1997). Binding properties of glutamate receptors in streptozotocin-induced diabetes in rats. Diabetes 46, 841–846. [PubMed: 9133553]

Gastinger MJ, Kunselman AR, Conboy EE, Bronson SK & Barber AJ. (2008). Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice. Invest Ophthalmol Vis Sci 49, 2635–2642. [PubMed: 18515593]

Gastinger MJ, Singh RS & Barber AJ. (2006). Loss of cholinergic and dopaminergic amacrine cells in streptozotocin-diabetic rat and Ins2Akita-diabetic mouse retinas. Invest Ophthalmol Vis Sci 47, 3143–3150. [PubMed: 16799061]

Gleason E, Borges S & Wilson M. (1994). Control of transmitter release from retinal amacrine cells by Ca2+ influx and efflux. Neuron 13, 1109–1117. [PubMed: 7524563]

Gonzalez VH, Campbell J, Holekamp NM, Kiss S, Loewenstein A, Augustin AJ, Ma J, Ho AC, Patel V, Whitcup SM & Dugel PU. (2016). Early and Long-Term Responses to Anti-Vascular Endothelial Growth Factor Therapy in Diabetic Macular Edema: Analysis of Protocol I Data. Am J Ophthalmol 172, 72–79. [PubMed: 27644589]

Green DG & Kapousta-Bruneau NV. (1999). A dissection of the electroretinogram from the isolated rat retina with microelectrodes and drugs. Vis Neurosci 16, 727–741. [PubMed: 10431921]

Gu L, Xu H, Wang F, Xu G, Sinha D, Wang J, Xu JY, Tian H, Gao F, Li W, Lu L, Zhang J & Xu GT. (2014). Erythropoietin exerts a neuroprotective function against glutamate neurotoxicity in experimental diabetic retina. Invest Ophthalmol Vis Sci 55, 8208–8222. [PubMed: 25335981]

Gundogan FC, Akay F, Uzun S, Yolcu U, Cagiltay E & Toyran S. (2016). Early Neurodegeneration of the Inner Retinal Layers in Type 1 Diabetes Mellitus. Ophthalmologica 235, 125–132. [PubMed: 26674204]

Harris A, Arend O, Danis RP, Evans D, Wolf S & Martin BJ. (1996). Hyperoxia improves contrast sensitivity in early diabetic retinopathy. Br J Ophthalmol 80, 209–213. [PubMed: 8703857]

Haverkamp S & Wassle H. (2000). Immunocytochemical analysis of the mouse retina. J Comp Neurol 424, 1–23. [PubMed: 10888735]

Heinze L, Harvey RJ, Haverkamp S & Wassle H. (2007). Diversity of glycine receptors in the mouse retina: localization of the alpha4 subunit. J Comp Neurol 500, 693–707. [PubMed: 17154252]

Heng LJ, Yang RH & Jia D. (2011). Diabetes impairs learning performance through affecting membrane excitability of hippocampal pyramidal neurons. Behav Brain Res 224, 250–258. [PubMed: 21722676]

Hernandez C, Bogdanov P, Corraliza L, Garcia-Ramirez M, Sola-Adell C, Arranz JA, Arroba AI, Valverde AM & Simo R. (2016a). Topical Administration of GLP-1 Receptor Agonists Prevents Retinal Neurodegeneration in Experimental Diabetes. Diabetes 65, 172–187. [PubMed: 26384381]

Hernandez C, Dal Monte M, Simo R & Casini G. (2016b). Neuroprotection as a Therapeutic Target for Diabetic Retinopathy. Journal of diabetes research 2016, 9508541. [PubMed: 27123463]

Hernandez C, Garcia-Ramirez M, Corraliza L, Fernandez-Carneado J, Farrera-Sinfreu J, Ponsati B, Gonzalez-Rodriguez A, Valverde AM & Simo R. (2013). Topical administration of somatostatin prevents retinal neurodegeneration in experimental diabetes. Diabetes 62, 2569–2578. [PubMed: 23474487]

Herrmann R, Heflin SJ, Hammond T, Lee B, Wang J, Gainetdinov RR, Caron MG, Eggers ED, Frishman LJ, McCall MA & Arshavsky VY. (2011). Rod vision is controlled by dopamine-dependent sensitization of rod bipolar cells by GABA. Neuron 72, 101–110. [PubMed: 21982372]

Hombrebueno JR, Chen M, Penalva RG & Xu H. (2014). Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse. PLoS One 9, e97970. [PubMed: 24848689]

Honda M, Inoue M, Okada Y & Yamamoto M. (1998). Alteration of the GABAergic neuronal system of the retina and superior colliculus in streptozotocin-induced diabetic rat. Kobe J Med Sci 44, 1–8. [PubMed: 9846053]

Hood DC, Frishman LJ, Saszik S & Viswanathan S. (2002). Retinal origins of the primate multifocal ERG: implications for the human response. Invest Ophthalmol Vis Sci 43, 1673–1685. [PubMed: 11980890]

Ishikawa A, Ishiguro S & Tamai M. (1996). Changes in GABA metabolism in streptozotocin-induced diabetic rat retinas. Curr Eye Res 15, 63–71. [PubMed: 8631205]

Jackson CR, Ruan GX, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM & McMahon DG. (2012). Retinal dopamine mediates multiple dimensions of light-adapted vision. J Neurosci 32, 9359–9368. [PubMed: 22764243]

Jaffe GJ & Caprioli J. (2004). Optical coherence tomography to detect and manage retinal disease and glaucoma. Am J Ophthalmol 137, 156–169. [PubMed: 14700659]

Jeon CJ, Strettoi E & Masland RH. (1998). The major cell populations of the mouse retina. J Neurosci 18, 8936–8946. [PubMed: 9786999]

Juen S & Kieselbach GF. (1990). Electrophysiological changes in juvenile diabetics without retinopathy. Arch Ophthalmol 108, 372–375. [PubMed: 2310337]

Karti O, Nalbantoglu O, Abali S, Ayhan Z, Tunc S, Kusbeci T & Ozkan B. (2017). Retinal Ganglion Cell Loss in Children With Type 1 Diabetes Mellitus Without Diabetic Retinopathy. Ophthalmic Surg Lasers Imaging Retina 48, 473–477. [PubMed: 28613353]

Katz G, Levkovitch-Verbin H, Treister G, Belkin M, Ilany J & Polat U. (2010). Mesopic foveal contrast sensitivity is impaired in diabetic patients without retinopathy. Graefes Arch Clin Exp Ophthalmol 248, 1699–1703. [PubMed: 20499079]

Kim MK, Aung MH, Mees L, Olson DE, Pozdeyev N, Iuvone PM, Thule PM & Pardue MT. (2018). Dopamine Deficiency Mediates Early Rod-Driven Inner Retinal Dysfunction in Diabetic Mice. Invest Ophthalmol Vis Sci 59, 572–581. [PubMed: 29372256]

Kirwin SJ, Kanaly ST, Hansen CR, Cairns BJ, Ren M & Edelman JL. (2011). Retinal gene expression and visually evoked behavior in diabetic long evans rats. Invest Ophthalmol Vis Sci 52, 7654–7663. [PubMed: 21862641]

Kizawa J, Machida S, Kobayashi T, Gotoh Y & Kurosaka D. (2006). Changes of oscillatory potentials and photopic negative response in patients with early diabetic retinopathy. Jpn J Ophthalmol 50, 367–373. [PubMed: 16897223]

Kohzaki K, Vingrys AJ & Bui BV. (2008). Early inner retinal dysfunction in streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci 49, 3595–3604. [PubMed: 18421077]

Kowluru RA, Engerman RL, Case GL & Kern TS. (2001). Retinal glutamate in diabetes and effect of antioxidants. Neurochem Int 38, 385–390. [PubMed: 11222918]

Kur J, Burian MA & Newman EA. (2016). Light adaptation does not prevent early retinal abnormalities in diabetic rats. Sci Rep 6, 21075. [PubMed: 26852722]

Lahouaoui H, Coutanson C, Cooper HM, Bennis M & Dkhissi-Benyahya O. (2016). Diabetic retinopathy alters light-induced clock gene expression and dopamine levels in the mouse retina. Mol Vis 22, 959–969. [PubMed: 27559292]

Laron M, Bearse MA Jr., Bronson-Castain K, Jonasdottir S, B King-Hooper, Barez S, Schneck ME & Adams AJ. (2012). Association between local neuroretinal function and control of adolescent type 1 diabetes. Invest Ophthalmol Vis Sci 53, 7071–7076. [PubMed: 22977133]

Lau JC, Kroes RA, Moskal JR & Linsenmeier RA. (2013). Diabetes changes expression of genes related to glutamate neurotransmission and transport in the Long-Evans rat retina. Mol Vis 19, 1538–1553. [PubMed: 23878504]

Layton CJ, Safa R & Osborne NN. (2007). Oscillatory potentials and the b-Wave: partial masking and interdependence in dark adaptation and diabetes in the rat. Graefes Arch Clin Exp Ophthalmol 245, 1335–1345. [PubMed: 17265029]

Lee SE, Han K, Baek JY, Ko KS, Lee KU, Koh EH & Taskforce Team for Diabetes Fact Sheet of the Korean Diabetes A. (2018a). Association Between Diabetic Retinopathy and Parkinson Disease: The Korean National Health Insurance Service Database. J Clin Endocrinol Metab 103, 3231–3238. [PubMed: 29982669]

Lee VK, Hosking BM, Holeniewska J, Kubala EC, Lundh von Leithner P, Gardner PJ, Foxton RH & Shima DT. (2018b). BTBR ob/ob mouse model of type 2 diabetes exhibits early loss of retinal function and retinal inflammation followed by late vascular changes. Diabetologia 61, 2422–2432. [PubMed: 30094465]

Li J, Chen P, Bao Y, Sun Y, He J & Liu X. (2019). PET Imaging of Vesicular Monoamine Transporter 2 in Early Diabetic Retinopathy Using [(18)F]FP-(+)-DTBZ. Mol Imaging Biol.

Li W, Wang P & Li H. (2014). Upregulation of glutamatergic transmission in anterior cingulate cortex in the diabetic rats with neuropathic pain. Neurosci Lett 568, 29–34. [PubMed: 24686190]

Lieth E, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D & Strother JM. (1998). Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Penn State Retina Research Group. Diabetes 47, 815–820. [PubMed: 9588455]

Liu F, Saul AB, Pichavaram P, Xu Z, Rudraraju M, Somanath PR, Smith SB, Caldwell RB & Narayanan SP. (2020). Pharmacological Inhibition of Spermine Oxidase Reduces Neurodegeneration and Improves Retinal Function in Diabetic Mice. J Clin Med 9.

Liu J, Tu H, Zheng H, Zhang L, Tran TP, Muelleman RL & Li YL. (2012). Alterations of calcium channels and cell excitability in intracardiac ganglion neurons from type 2 diabetic rats. Am J Physiol Cell Physiol 302, C1119–1127. [PubMed: 22189553]

Lopes de Faria JM, Katsumi O, Cagliero E, Nathan D & Hirose T. (2001). Neurovisual abnormalities preceding the retinopathy in patients with long-term type 1 diabetes mellitus. Graefes Arch Clin Exp Ophthalmol 239, 643–648. [PubMed: 11688662]

Lopes de Faria JM, Russ H & Costa VP. (2002). Retinal nerve fibre layer loss in patients with type 1 diabetes mellitus without retinopathy. Br J Ophthalmol 86, 725–728. [PubMed: 12084737]

Luo X & Frishman LJ. (2011). Retinal pathway origins of the pattern electroretinogram (PERG). Invest Ophthalmol Vis Sci 52, 8571–8584. [PubMed: 21948546]

Luu CD, Szental JA, Lee SY, Lavanya R & Wong TY. (2010). Correlation between retinal oscillatory potentials and retinal vascular caliber in type 2 diabetes. Invest Ophthalmol Vis Sci 51, 482–486. [PubMed: 19710418]

Ly A, Scheerer MF, Zukunft S, Muschet C, Merl J, Adamski J, de Angelis MH, Neschen S, Hauck SM & Ueffing M. (2014). Retinal proteome alterations in a mouse model of type 2 diabetes. Diabetologia 57, 192–203. [PubMed: 24078137]

Lynch SK & Abramoff MD. (2017). Diabetic retinopathy is a neurodegenerative disorder. Vision Res 139, 101–107. [PubMed: 28408138]

Martin PM, Roon P, Van Ells TK, Ganapathy V & Smith SB. (2004). Death of retinal neurons in streptozotocin-induced diabetic mice. Invest Ophthalmol Vis Sci 45, 3330–3336. [PubMed: 15326158]

McCall MA, Lukasiewicz PD, Gregg RG & Peachey NS. (2002). Elimination of the r1 subunit abolishes $GABA_C$ receptor expression and alters visual processing in the mouse retina. The Journal of Neuroscience 22, 4163–4174. [PubMed: 12019334]

McFarlane M, Wright T, Stephens D, Nilsson J & Westall CA. (2012). Blue flash ERG PhNR changes associated with poor long-term glycemic control in adolescents with type 1 diabetes. Invest Ophthalmol Vis Sci 53, 741–748. [PubMed: 22222270]

Miller WP, Yang C, Mihailescu ML, Moore JA, Dai W, Barber AJ & Dennis MD. (2018). Deletion of the Akt/mTORC1 Repressor REDD1 Prevents Visual Dysfunction in a Rodent Model of Type 1 Diabetes. Diabetes 67, 110–119. [PubMed: 29074598]

Moller A & Eysteinsson T. (2003). Modulation of the components of the rat dark-adapted electroretinogram by the three subtypes of GABA receptors. Vis Neurosci 20, 535–542. [PubMed: 14977332]

Moore-Dotson JM, Beckman JJ, Mazade RE, Hoon M, Bernstein AS, Romero-Aleshire MJ, Brooks HL & Eggers ED. (2016). Early Retinal Neuronal Dysfunction in Diabetic Mice: Reduced Light-Evoked Inhibition Increases Rod Pathway Signaling. Invest Ophthalmol Vis Sci 57, 1418–1430. [PubMed: 27028063]

Moore-Dotson JM & Eggers ED. (2019). Reductions in Calcium Signaling Limit Inhibition to Diabetic Retinal Rod Bipolar Cells. Invest Ophthalmol Vis Sci 60, 4063–4073. [PubMed: 31560762]

Morales-Calixto E, Velazquez-Flores MA, Sanchez-Chavez G, Ruiz Esparza-Garrido R & Salceda R. (2019). Glycine receptor is differentially expressed in the rat retina at early stages of streptozotocin-induced diabetes. Neurosci Lett 712, 134506. [PubMed: 31541724]

Morgado C, Pinto-Ribeiro F & Tavares I. (2008). Diabetes affects the expression of GABA and potassium chloride cotransporter in the spinal cord: a study in streptozotocin diabetic rats. Neurosci Lett 438, 102–106. [PubMed: 18457921]

Motz CT, Chesler KC, Allen RS, Bales KL, Mees LM, Feola AJ, Maa AY, Olson DE, Thule PM, Iuvone PM, Hendrick AM & Pardue MT. (2020). Novel Detection and Restorative Levodopa Treatment for Preclinical Diabetic Retinopathy. Diabetes 69, 1518–1527. [PubMed: 32051147]

Naarendorp F & Sieving PA. (1991). The scotopic threshold response of the cat ERG is suppressed selectively by GABA and glycine. Vision Res 31, 1–15. [PubMed: 2006543]

Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG, Ehrlich JS, Rise & Group RR. (2012). Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. Ophthalmology 119, 789–801. [PubMed: 22330964]

Nishimura C & Kuriyama K. (1985). Alterations in the retinal dopaminergic neuronal system in rats with streptozotocin-induced diabetes. J Neurochem 45, 448–455. [PubMed: 3925083]

Ola MS, Nawaz MI, Khan HA & Alhomida AS. (2013). Neurodegeneration and neuroprotection in diabetic retinopathy. Int J Mol Sci 14, 2559–2572. [PubMed: 23358247]

Osaadon P, Fagan XJ, Lifshitz T & Levy J. (2014). A review of anti-VEGF agents for proliferative diabetic retinopathy. Eye (Lond) 28, 510–520. [PubMed: 24525867]

Pardue MT, Barnes CS, Kim MK, Aung MH, Amarnath R, Olson DE & Thule PM. (2014). Rodent Hyperglycemia-Induced Inner Retinal Deficits are Mirrored in Human Diabetes. Translational vision science & technology 3, 6.

Parisi V & Uccioli L. (2001). Visual electrophysiological responses in persons with type 1 diabetes. Diabetes Metab Res Rev 17, 12–18. [PubMed: 11241887]

Parisi V, Uccioli L, Monticone G, Parisi L, Manni G, Ippoliti D, Menzinger G & Bucci MG. (1997). Electrophysiological assessment of visual function in IDDM patients. Electroencephalogr Clin Neurophysiol 104, 171–179. [PubMed: 9146484]

Park HY, Kim IT & Park CK. (2011). Early diabetic changes in the nerve fibre layer at the macula detected by spectral domain optical coherence tomography. Br J Ophthalmol 95, 1223–1228. [PubMed: 21216799]

Pinilla I, Idoipe M, Perdices L, Sanchez-Cano A, Acha J, Lopez-Galvez MI, Cuenca N, Abecia E & Orduna-Hospital E. (2020). Changes in Total and Inner Retinal Thicknesses in Type 1 Diabetes with No Retinopathy after 8 Years of Follow-Up. Retina 40, 1379–1386. [PubMed: 31157712]

Pulido JE, Pulido JS, Erie JC, Arroyo J, Bertram K, Lu MJ & Shippy SA. (2007). A role for excitatory amino acids in diabetic eye disease. Exp Diabetes Res 2007, 36150. [PubMed: 17713594]

Ramsey DJ, Ripps H & Qian H. (2006). An electrophysiological study of retinal function in the diabetic female rat. Invest Ophthalmol Vis Sci 47, 5116–5124. [PubMed: 17065533]

Robson JG & Frishman LJ. (1998). Dissecting the dark-adapted electroretinogram. Doc Ophthalmol 95, 187–215. [PubMed: 10532405]

Robson JG, Maeda H, Saszik SM & Frishman LJ. (2004). In vivo studies of signaling in rod pathways of the mouse using the electroretinogram. Vision Res 44, 3253–3268. [PubMed: 15535993]

Safi S, Rahimi A, Raeesi A, Safi H, Aghazadeh Amiri M, Malek M, Yaseri M, Haeri M, Middleton FA, Solessio E & Ahmadieh H. (2017). Contrast sensitivity to spatial gratings in moderate and dim light conditions in patients with diabetes in the absence of diabetic retinopathy. BMJ Open Diabetes Res Care 5, e000408.

Santiago AR, Gaspar JM, Baptista FI, Cristovao AJ, Santos PF, Kamphuis W & Ambrosio AF. (2009). Diabetes changes the levels of ionotropic glutamate receptors in the rat retina. Mol Vis 15, 1620–1630. [PubMed: 19693289]

Schmidt-Erfurth U, Chong V, Loewenstein A, Larsen M, Souied E, Schlingemann R, Eldem B, Mones J, Richard G, Bandello F & European Society of Retina S. (2014). Guidelines for the management of neovascular age-related macular degeneration by the European Society of Retina Specialists (EURETINA). Br J Ophthalmol 98, 1144–1167. [PubMed: 25136079]

Scuderi S, D'Amico AG, Castorina A, Federico C, Marrazzo G, Drago F, Bucolo C & D'Agata V. (2014). Davunetide (NAP) protects the retina against early diabetic injury by reducing apoptotic death. J Mol Neurosci 54, 395–404. [PubMed: 24488575]

Seki M, Tanaka T, Nawa H, Usui T, Fukuchi T, Ikeda K, Abe H & Takei N. (2004). Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factor for dopaminergic amacrine cells. Diabetes 53, 2412–2419. [PubMed: 15331553]

Semkova I, Huemmeke M, Ho MS, Merkl B, Abari E, Paulsson M, Joussen AM & Plomann M. (2010). Retinal localization of the glutamate receptor GluR2 and GluR2-regulating proteins in diabetic rats. Exp Eye Res 90, 244–253. [PubMed: 19878674]

Sergeys J, Etienne I, Van Hove I, Lefevere E, Stalmans I, Feyen JHM, Moons L & Van Bergen T. (2019). Longitudinal In Vivo Characterization of the Streptozotocin-Induced Diabetic Mouse Model: Focus on Early Inner Retinal Responses. Invest Ophthalmol Vis Sci 60, 807–822. [PubMed: 30811545]

Shinoda K, Rejdak R, Schuettauf F, Blatsios G, Volker M, Tanimoto N, Olcay T, Gekeler F, Lehaci C, Naskar R, Zagorski Z & Zrenner E. (2007). Early electroretinographic features of streptozotocin-induced diabetic retinopathy. Clin Experiment Ophthalmol 35, 847–854. [PubMed: 18173414]

Simonsen SE. (1980). The value of the oscillatory potential in selecting juvenile diabetics at risk of developing proliferative retinopathy. Acta Ophthalmol (Copenh) 58, 865–878. [PubMed: 7331773]

Singer JH, Lassova L, Vardi N & Diamond JS. (2004). Coordinated multivesicular release at a mammalian ribbon synapse. Nat Neurosci 7, 826–833. [PubMed: 15235608]

Smith BJ, Cote PD & Tremblay F. (2015). Dopamine modulation of rod pathway signaling by suppression of GABAC feedback to rod-driven depolarizing bipolar cells. Eur J Neurosci 42, 2258–2270. [PubMed: 26080286]

Szabadfi K, Atlasz T, Kiss P, Reglodi D, Szabo A, Kovacs K, Szalontai B, Setalo G Jr., Banki E, Csanaky K, Tamas A & Gabriel R. (2012). Protective effects of the neuropeptide PACAP in diabetic retinopathy. Cell Tissue Res 348, 37–46. [PubMed: 22350850]

Travis AM, Heflin SJ, Hirano AA, Brecha NC & Arshavsky VY. (2018). Dopamine-Dependent Sensitization of Rod Bipolar Cells by GABA Is Conveyed through Wide-Field Amacrine Cells. J Neurosci 38, 723–732. [PubMed: 29217689]

Vadala M, Anastasi M, Lodato G & Cillino S. (2002). Electroretinographic oscillatory potentials in insulin-dependent diabetes patients: A long-term follow-up. Acta Ophthalmol Scand 80, 305–309. [PubMed: 12059871]

Van Dijk HW, Verbraak FD, Kok PH, Garvin MK, Sonka M, Lee K, Devries JH, Michels RP, van Velthoven ME, Schlingemann RO & Abramoff MD. Decreased Retinal Ganglion Cell Layer Thickness in Type 1 Diabetic Patients. Invest Ophthalmol Vis Sci.

VanGuilder HD, Brucklacher RM, Patel K, Ellis RW, Freeman WM & Barber AJ. (2008). Diabetes downregulates presynaptic proteins and reduces basal synapsin I phosphorylation in rat retina. Eur J Neurosci 28, 1–11. [PubMed: 18662330]

Verma A, Raman R, Vaitheeswaran K, Pal SS, Laxmi G, Gupta M, Shekar SC & Sharma T. (2012). Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy? Ophthalmic Res 47, 202–207. [PubMed: 22179629]

Viswanathan S, Frishman LJ, Robson JG, Harwerth RS & Smith EL 3rd. (1999). The photopic negative response of the macaque electroretinogram: reduction by experimental glaucoma. Invest Ophthalmol Vis Sci 40, 1124–1136. [PubMed: 10235545]

Wachtmeister L (1980). Further studies of the chemical sensitivity of the oscillatory potentials of the electroretinogram (ERG) I. GABA- and glycine antagonists. Acta Ophthalmol (Copenh) 58, 712–725. [PubMed: 7211260]

Wachtmeister L & Dowling JE. (1978). The oscillatory potentials of the mudpuppy retina. Invest Ophthalmol Vis Sci 17, 1176–1188. [PubMed: 721390]

Witkovsky P (2004). Dopamine and retinal function. Doc Ophthalmol 108, 17–40. [PubMed: 15104164]

Yang S, Zhao J & Sun X. (2016). Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. Drug Des Devel Ther 10, 1857–1867.

Yang Y, Mao D, Chen X, Zhao L, Tian Q, Liu C & Zhou BL. (2012). Decrease in retinal neuronal cells in streptozotocin-induced diabetic mice. Mol Vis 18, 1411–1420. [PubMed: 22690119]

Yoshida A, Kojima M, Ogasawara H & Ishiko S. (1991). Oscillatory potentials and permeability of the blood-retinal barrier in noninsulin-dependent diabetic patients without retinopathy. Ophthalmology 98, 1266–1271. [PubMed: 1923365]

Yu J, Wang L, Weng SJ, Yang XL, Zhang DQ & Zhong YM. (2013). Hyperactivity of ON-type retinal ganglion cells in streptozotocin-induced diabetic mice. PLoS One 8, e76049. [PubMed: 24069457]

Zeng XX, Ng YK & Ling EA. (2000). Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. Vis Neurosci 17, 463–471. [PubMed: 10910112]

Zhang Y, Zhang J, Wang Q, Lei X, Chu Q, Xu GT & Ye W. (2011). Intravitreal injection of exendin-4 analogue protects retinal cells in early diabetic rats. Invest Ophthalmol Vis Sci 52, 278–285. [PubMed: 20688733]

Zherebitskaya E, Schapansky J, Akude E, Smith DR, Van der Ploeg R, Solovyova N, Verkhratsky A & Fernyhough P. (2012). Sensory neurons derived from diabetic rats have diminished internal Ca2+ stores linked to impaired re-uptake by the endoplasmic reticulum. ASN Neuro 4.

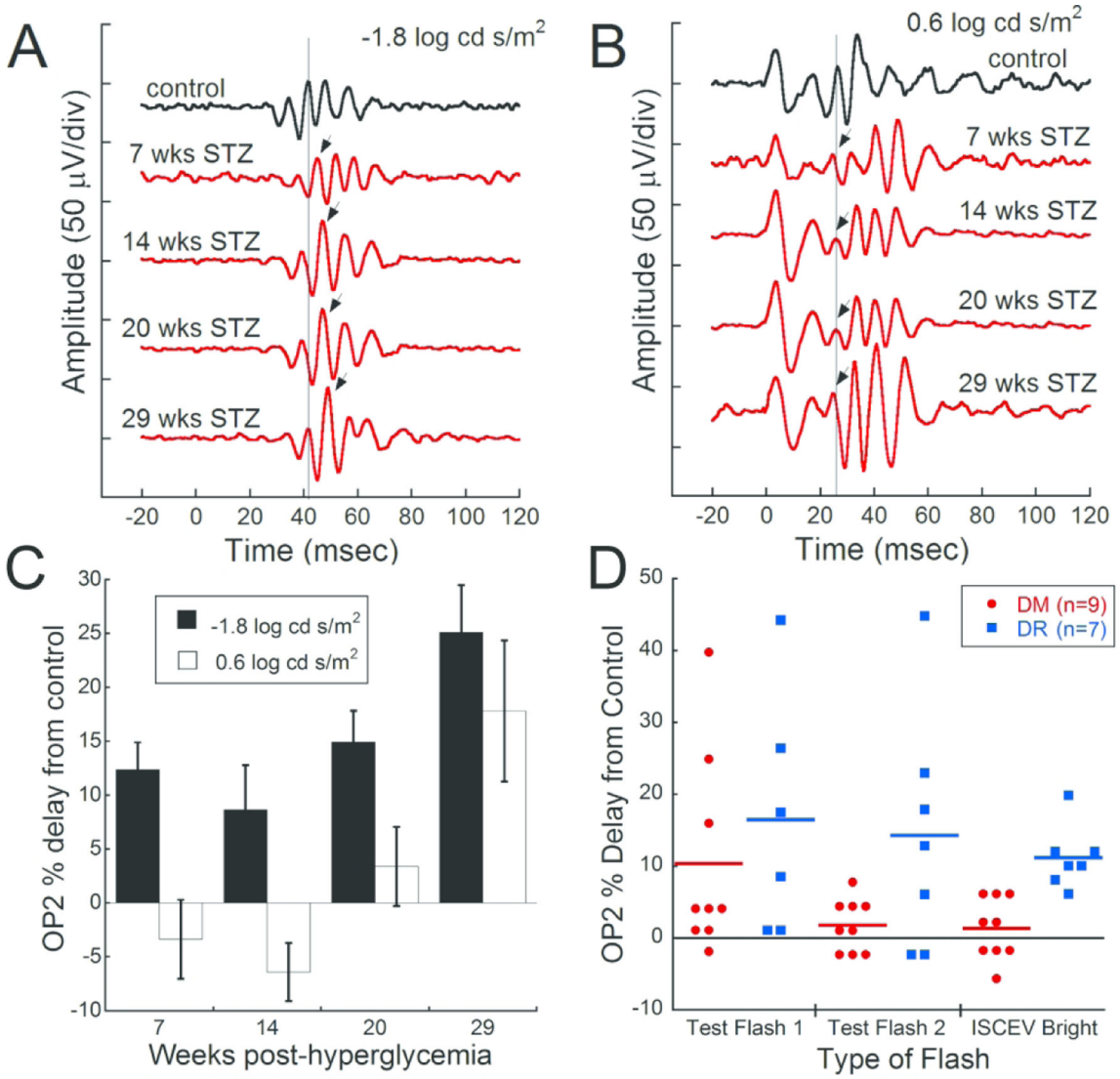

Figure 1.

Similar ERG OP deficits in diabetic human and animal models. Representative OP waveforms from control (*black lines*) and diabetic (*red lines*) rats at each time point in response to dim (A) and bright (B) flash stimuli. The *vertical line* indicates the implicit time of OP2 in the control trace. The *arrows* indicated OP2 in each diabetic waveform. (A) The OPs are progressively more delayed within weeks after STZ exposure in response to a rod-stimulating dim flash. (B) No obvious differences in OP implicit time are detected in response to a bright flash in diabetic rats when compared with controls. ERG OP implicit time delays in response to dim and bright flash stimuli in diabetic rats (C) and diabetic subjects (D). (C) OP2 percent difference in implicit times (diabetic-control) across weeks of hyperglycemia in response to representative dim (1.8 log cd s/m^2) and bright (0.6 log cd s/m^2) flash stimuli in STZ-treated rats. Dim stimuli OP2 implicit times became significantly and progressively delayed compared with bright stimuli across all time points, as indicated by the significant main effect of flash stimuli (two-way ANOVA, $F[1,63] = 20.41$, $P < 0.001$). (D) OP2 percent difference in implicit time (diabetic-control) in DR or DM subjects,

plotted as individual points with the mean indicated by the horizontal line in each column. Test Flash 1 (1.4 log cd s/m^2) revealed a 10% increase in OP2 implicit time in DM subjects, but not in response to the brighter Test Flash 2 (0.82 cd s/m^2) or ISCEV Bright stimulus (0.39 log cd s/m^2). In DR subjects, all flash intensities elicited an OP2 delay of > 12%. Note that one DR subject had no OPs at Test Flash 1.

Figure modified with permission from Pardue et al, 2014, Figs 2, 6.

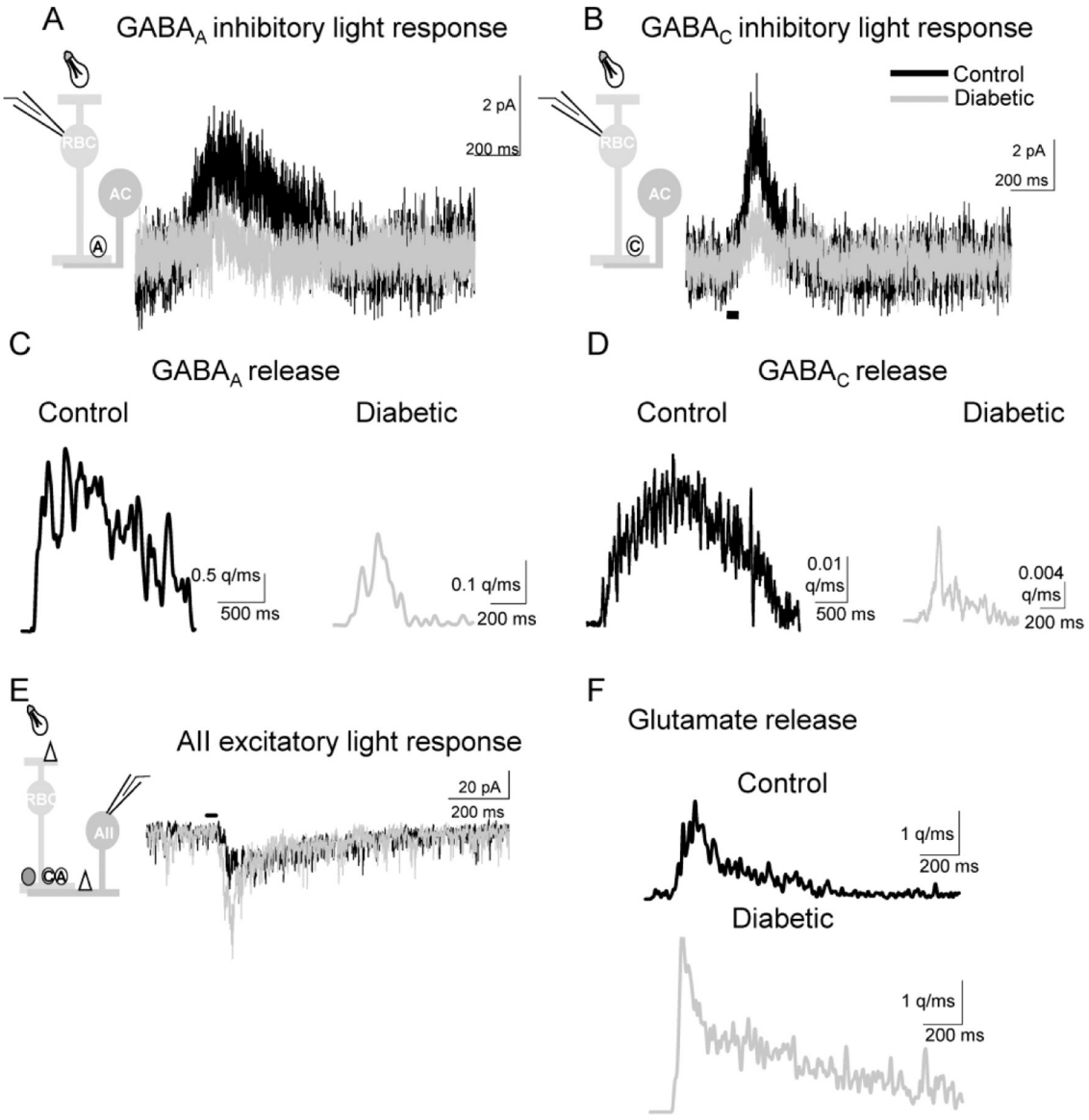

Figure 2.

GABAergic inhibition from amacrine cells to rod bipolar cells is reduced and rod bipolar cell output to AII amacrine cells is increased after 6 weeks in a mouse model of diabetes. A. $GABA_A$ receptor light-evoked inhibitory synaptic currents (L-IPSCs) are reduced in diabetic rod bipolar cells (30 ms light stimulus, $4.75*10^5$ Rh*/rod/sec, black bar). B. L-IPSCs mediated by $GABA_c$ receptors are reduced in diabetic rod bipolar cells (30 ms light stimulus, $4.75*10^5$ Rh*/rod/sec). C-D. GABA release onto rod bipolar cell $GABA_A$ (C) and $GABA_C$ (D) receptors (estimated from deconvolution analysis of GABA receptor-mediated spontaneous (s)IPSCs and L-IPSCs) is reduced in diabetes. E. Light-evoked excitatory post-synaptic currents (L-EPSCs, 30 ms light stimulus, $4.750*10^3$ Rh*/rod/sec) recorded from AII amacrine cells are increased in diabetes. F. Glutamate release (estimated by deconvolution of L-EPSCs and miniature EPSCs) from rod bipolar cells to AII amacrine

cells is increased in diabetes. Figure modified with permission from Moore-Dotson et al, 2016, Figs. 2, 4, 5

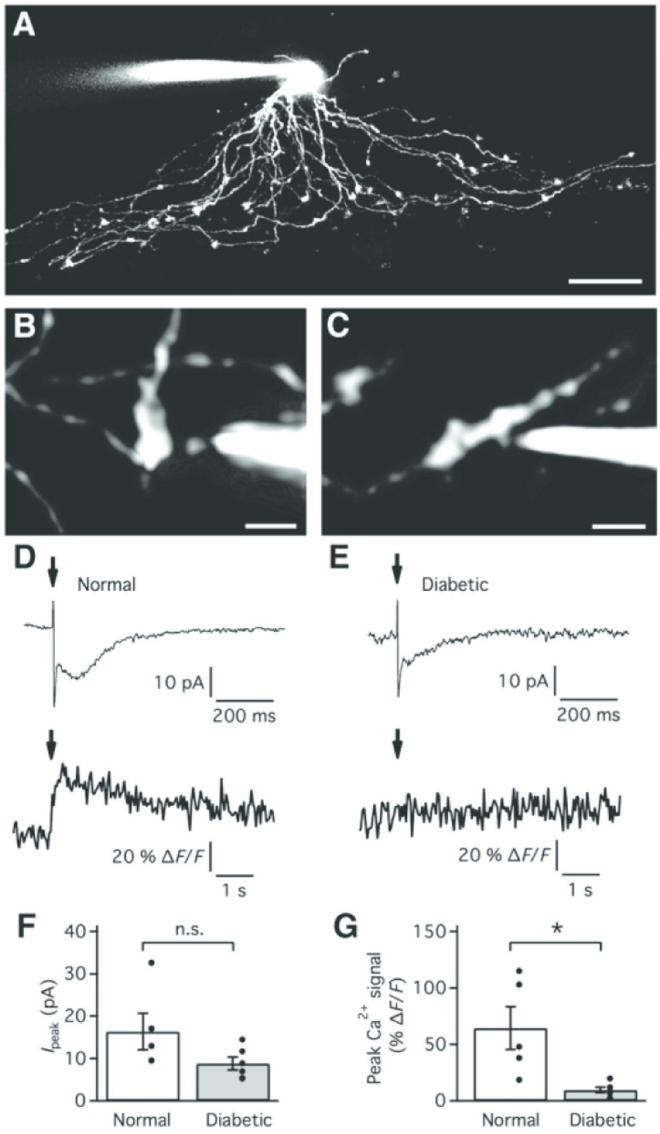

Figure 3.

Reduced Ca^{2+} influx through non-NMDA receptors on A17 amacrine cell varicosities in diabetic animals. A. A17 amacrine cell filled with Alexa Fluor 594 via patch pipette. Maximum intensity projection (along z-axis) generated from two-photon fluorescence image stack after deconvolution (A–C). Scale bar, 20 μm. B, C. Varicosities from two A17 amacrine cells (B, normal, maximum intensity projection of image stack; C, diabetic, single focal plane) filled with Alexa Fluor 594 and the Ca^{2+} indicator dye Oregon Green 488 BAPTA-1 and targeted by fine-tipped pipettes (right) filled with glutamate and Alexa Fluor 594. Scale bars, 2 μm. D, E. Current (top) and Ca^{2+} (bottom) signals in response to brief microiontophoretic pulses of glutamate (D, −400 nA for 1 ms; E, −600 nA for 1 ms; vertical arrows) applied to varicosityin B (from normal animal) or C (from diabetic animal). NMDA receptors blocked by CPP added to the extracellular solution. Each trace, average of five trials. F, G. Peak amplitude of current (F) and Ca^{2+} signals (G) in response to brief

microiontophoretic pulses of glutamate to varicosities from A17 amacrine cells in normal (n = 5 varicosities) and diabetic animals (n = 6 varicosities). Figure modified with permission from Castilho et al, 2015a, Fig. 6

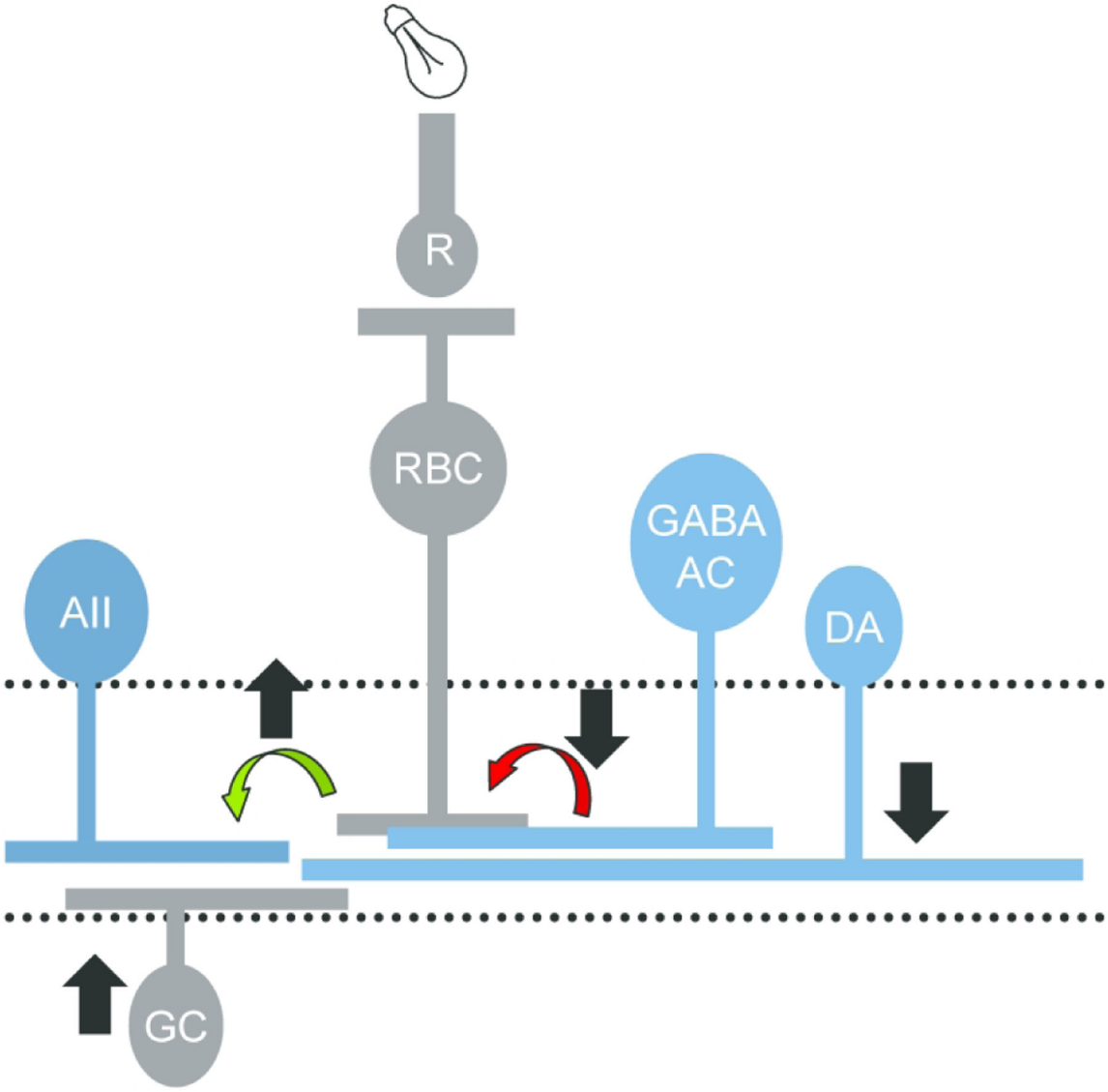

Figure 4.

Changes in inner retinal signaling in early diabetes. The strongest early deficits in diabetes have been shown in the rod pathway that starts with the rod photoreceptor (R) that releases glutamate onto the rod bipolar cell (RBC). Diabetes causes reductions in GABAergic inhibition (GABA AC, red arrow) onto the rod bipolar cell. This also causes an increase in the glutamatergic output from rod bipolar cells onto their output neurons AII (AII) and A17 (not shown) amacrine cells. Ganglion cell (GC) spontaneous activity and excitability is also increased, likely through inputs from the rod pathway. Additionally, dopamine levels from dopaminergic amacrine cells (DA) are decreased in the diabetic retina, leading to functional deficits.