Retinal Deficits Precede Cognitive and Motor Deficits in a Rat Model of Type II Diabetes

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PURPOSE. To investigate the temporal appearance of retinal, cognitive, and motor deficits in Goto-Kakizaki (GK) rats, a spontaneously occurring, polygenic model of type II diabetes. GK rats develop impaired insulin secretion at 2 weeks and fasting hyperglycemia at 4 weeks.

METHODS. In male and female GK rats and Wistar controls, glucose tolerance test (hyperglycemia) and electroretinogram (ERG, retinal function) were performed at 4 and 8 weeks of age. Spectral domain-optical coherence tomography (retinal structure) was assessed at 6 weeks. Spatial alternation (cognitive function) and number of entries (exploratory behavior) were assessed via Y-maze at 4, 5, 6, 7, and 8 weeks. Rotarod (motor function) was performed at 4, 6, and 8 weeks.

RESULTS. By 4 weeks, the GK rats exhibited significant glucose intolerance ($P < 0.001$) and retinal deficits, including delays in ERG implicit times (flicker, $P < 0.01$; oscillatory potentials, $P < 0.001$). In addition, the GK rats showed greater ERG amplitudes ($P < 0.001$) and thinner retinas ($P < 0.001$). At 7 weeks, the GK rats showed deficits in cognitive function ($P < 0.001$) and exploratory behavior ($P < 0.01$). However, no motor function deficits were observed by 8 weeks. Interestingly, the male GK rats showed greater hyperglycemia ($P < 0.05$), but the female rats showed greater ERG delays ($P < 0.001$).

CONCLUSIONS. In GK rats, retinal function deficits developed prior to cognitive or motor deficits. Future studies will investigate common mechanistic links, long-term functional and vascular changes, and whether early retinal deficits can predict cognitive dysfunction or late-stage retinal disease.

Keywords: diabetic retinopathy, diabetes, electroretinogram, spatial cognition, goto-kakizaki.

In the United States, more than 30 million people have diabetes,1 a number predicted to increase 35% by 2025.2 Chronic hyperglycemia and accompanying inflammation with diabetes cause complications such as diabetic retinopathy (DR), a leading cause of blindness in working-age adults.3 In the clinic, DR is characterized by retinal ischemia, macular edema, and abnormal neovascularization. However, signs of retinal dysfunction and damage have been observed before the detection of vascular pathology in the clinic.4,5 These early changes in the retinas of diabetic patients and diabetic animals include impairments in night vision,6 contrast sensitivity,6,7 visual acuity (Aung MH, et al. IOVS 2011;52:ARVO E-Abstract 5960),7 and color vision,6 as well as electroretinogram (ERG) deficits such as delayed 30 Hz flicker implicit time6,9 and delayed oscillatory potentials (OPs).10–16 Retinal cells affected include amacrine, Müller, photoreceptor, and ganglion cells.4,17 Because these neural changes in the diabetic retina occur prior to the clinically recognized vascular pathology, they may allow for earlier detection and treatment for DR.

Oxidative stress, hypoxia, lipid abnormalities, and inflammation occur in both the brain and retina with diabetes, suggesting that the disease is damaging both tissues via similar pathways.18–23 Deficits in both cognitive and motor function have been shown to occur with diabetes,24,25 and the presence of DR is associated with cognitive deficits and greater amounts of ischemia in the brain.26 Early or nonproliferative DR (which may indicate chronic hyperglycemia) is associated with structural changes in brain as well as deficits in attention, concentration, and information processing.27 In addition, patients with proliferative DR show increased cortical atrophy as measured by magnetic resonance imaging,28 and severity of vascular DR may be associated with accelerated cognitive decline with age, particularly in men.29 These studies show links between cerebral and retinal vascular complications with diabetes, but it is not known whether early retinal neuronal deficits can be used as an early marker for other complications. Starting interventions at the earliest signs of diabetes-induced retinal dysfunction could prevent the development of cognitive and motor deficits and worsening of retinal deficits, but first, the time course of these deficits must be identified.

Because 90% to 95% of diabetic patients have type II diabetes,1 we chose an animal model of type II diabetes for this research. The GK rat is a non-insulin-dependent, polygenic, nonobese model that develops impaired glucose-induced
insulin secretion by 2 weeks and fasting hyperglycemia by 4 weeks. Retinal and cognitive deficits and reduced motor nerve conduction have been reported in the GK rat. However, the temporal appearance of these deficits and the relationship between them has not been described. We hypothesized that GK rats will exhibit retinal dysfunction prior to cognitive and motor dysfunction.

**Materials and Methods**

**Animals, Diabetes Confirmation, and Experimental Design**

Male and female GK (diabetic) and Wistar (nondiabetic control; Charles River, Wilmington, MA, USA, and in-house breeding; n = 78) rats were housed in shoebox-style cages with chow and water provided ad libitum on a 12:12 light:dark cycle (light onset at 6:00 AM). All procedures were approved by the Atlanta Veterans Affairs Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health guide for the care and use of laboratory animals. One GK rat died during the course of the study and was excluded from our analysis.

The GK rat is a spontaneously occurring, nonobese model of type II diabetes that was developed by selectively interbreeding Wistar rats that showed the highest blood glucose levels during a glucose tolerance test (GTT). As a consequence, GTT responses exhibit the most striking blood glucose differences between GK and wildtype animals. Thus, GTTs were used to confirm hyperglycemia prior to structural and functional assessments (4 and 8 weeks). Body weight was monitored weekly. Unlike rats with streptozotocin (STZ)-induced diabetes, GK rats do not lose weight with diabetes, and thus insulin treatment was not needed.

ERG recordings were performed at the 4- and 8-week timepoints to assess retinal function, and Y-maze testing for spontaneous alternation was performed at 5, 6, 7, and 8 weeks to assess cognitive function and exploratory behavior (see Fig. 1 for experimental timeline). An additional group of GK (n = 7) and Wistar (n = 10) rats were tested on the Y-maze beginning at 4 weeks so that comparisons to 4-week ERG results would be more meaningful. Previously, we had not begun Y-maze testing until 5 weeks because younger animals seem to show a fear response in the maze and exhibit little movement. By handling the rats for 5 minutes prior to starting the test and by starting them at the choice point (as opposed to the back of the first arm), we obtained 4-week Y-maze data in ~85% of the animals. Rats that did not perform on Y-maze at 4 weeks were excluded from the experiment.

In a subset of rats, rotarod testing was performed at 4, 6, and 8 weeks to assess motor function, and spectral-domain-optical coherence tomography (SD-OCT) was performed at 6 weeks to assess in vivo retinal structure. In a second subset of rats, GTT and ERG were performed at 3 weeks to determine whether these changes occurred prior to 4 weeks. Optomotor response was not assessed as both GK and Wistar rats were unresponsive to this test, which is not surprising given that some albino rodents are incapable of tracking and others show greatly reduced tracking (Prusky G, personal communication, 2014).

**Assessment of Hyperglycemia: GTT**

GTTs were used to measure hyperglycemia. Rats were fasted for 6 hours prior to intraperitoneal injections of glucose (2 mg/kg body weight) in dH2O. Blood glucose levels (mg/dL) were monitored at 0, 15, 30, 60, and 120 minutes using a handheld blood glucose meter (FreeStyle Lite; Abbott Diabetes Care, Alameda, CA, USA) and test strips with blood obtained via tail prick. When comparing glucose tolerance in GK rats across time, blood glucose levels were normalized to those of Wistar controls.

**Assessment of Retinal Function: ERG**

After overnight dark adaptation, retinal responses to light were measured in rats via ERG as previously described. Briefly, under dim red light, the rats were anesthetized with ketamine (60 mg/kg) and xylazine (7.5 mg/kg), the corneal surface was anesthetized (0.5% tetracaine), and pupils were dilated (1% tropicamide). Platinum needle ground and reference electrodes were placed in the tail and each cheek, respectively. A gold loop recording electrode was placed on each cornea. Flash stimuli were presented, and electrical responses were recorded using a signal-averaging system (UTAS BigShot; LKC Technologies, Gaithersburg, MD, USA). ERG stimuli consisted of a 6-step protocol of flash stimuli presented in order of increasing luminance. Five dark-adapted responses were recorded to isolate rod-dominated and mixed rod and cone responses (scotopic: -3.0 to 2.1 log cd s/m²). The rats were then light-adapted (30 cd/m²) for 10 minutes to saturate the rod photoreceptors after which responses were recorded to

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**Figure 1.** Experimental timeline and appearance of functional changes. The first observable changes in the GK rat after hyperglycemia are retinal function deficits (4 weeks) with deficits in exploratory behavior, cognitive function, and retinal structure occurring at 6 weeks, and motor coordination deficits and vascular pathology not occurring during the 8-week testing period.
flicker stimuli (2.0 log cd s/m² at 6 Hz) in the presence of the background light to isolate cone pathway function. Afterward, the animals were given yohimbine (2.1 mg/kg) to reverse the effects of xylazine and prevent corneal ulcers.44

ERG data were analyzed offline. The right and left eye responses were averaged for each animal. Amplitudes and implicit times were measured for a-waves, b-waves, and OPs and flicker response for ERG waveforms. Using the ERG system software, OPs were filtered digitally offline (75–500 Hz; EM Version 8.1.2, 2008; LKC Technologies). OPs were analyzed for two flash stimuli, which we previously showed to distinguish early dysfunction in diabetes7–10: −1.9 log cd s/m² (rod pathway function) and 0.7 log cd s/m² (rod-cone pathway).

Assessment of Retinal Structure: SD-OCT
To assess retinal structure, SD-OCT was used as described previously13 to capture in vivo retinal images at 6 weeks. After general anesthesia (using the same methods described in the ERGs) was defined as a successful alternation. The percentage of pupils were dilated (1% tropicamide), and the rats were placed on an adjustable stage. An OCT camera (Bioptigen 4300; Bioptigen, Inc., Morrisville, NC, USA) was used to record a 3-mm radial scan centered at the optic nerve for the right eye only.

Using a customized MATLAB program (MathWorks, Natick, MA, USA), images were marked manually by a trained technician. Layers assessed included the retinal nerve fiber layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, external limiting membrane, inner segments/outer segments, and retinal pigment epithelium (RPE). Individual layer thicknesses were measured by the difference in distance between markers placed on the outer edges of each layer, and total retinal thickness was computed as the distance between the top marker of the retinal nerve fiber layer to the bottom marker of the RPE. Measurements were taken for the first (superior-inferior) and third (nasal-temporal) B-scans. Within each orientation, measurements were taken at 0.5 and 1.2 mm to the left and right of the optic nerve head, resulting in two distances each for the superior, inferior, nasal, and temporal quadrants of the eye.

Analysis of Cognitive and Exploratory Behavior: Y-Maze
To assess short-term spatial memory and exploratory behavior, the Y-maze (San Diego Instruments, San Diego, CA, USA) was used to record spontaneous alternation behavior based on the methods outlined by Maurice and colleagues.46 Briefly, the rats were positioned in one arm of the Y-maze and allowed to explore freely for 8 minutes. The series of arm entries was monitored and recorded. Entering all three arms consecutively was defined as a successful alternation. The percentage of correct alternations / (total number of arm entries − 2) × 100. This number was used to represent cognitive function. The total number of entries during the course of 8 minutes was used to represent exploratory behavior.

Analysis of Motor Function: Rotarod
The rotarod (San Diego Instruments) was used to measure balance and motor coordination, which have been shown to be impaired in diabetic animals.25–27 The rats were placed on a rotating bar, and the latency to fall was recorded. Prior to testing, the animals were allowed 1 minute to acclimate to the stationary rotarod. During testing, the rod began rotating slowly, with the speed gradually accelerating to a maximum of 50 rpm during a 5-minute period. If the rat fell off within the first 15 seconds of rotation, the trial was repeated. The rats underwent four trials per testing day with 5 minutes of rest in between. The three best trials were averaged for each rat.

Statistical Analysis
The results are expressed as mean ± standard error of the mean (SEM). Weight, GTT, ERG (a-wave, b-wave, OP, 4- and 8-week comparisons), OCT, Y-maze, and rotarod results were analyzed using a 2-way repeated measures (RM) ANOVA followed by Holms-Sidak tests for individual comparisons. Flicker ERG results were analyzed using a t-test. Average glucose tolerance results over time and comparisons between male and female GK rats were analyzed using a 1-way ANOVA followed by Holms-Sidak tests for individual comparisons.

RESULTS
GK Rats Showed Reduced Body Weight and Increased Blood Glucose
Although GK rats continued to gain weight over time and did not exhibit weight loss in response to their hyperglycemia, GK rats were ~30% smaller than Wistar controls, as demonstrated by significantly reduced body weights at all timepoints measured from 4 to 8 weeks of age (RM ANOVA main effect of strain, F1,1164 = 26.938, P < 0.001; Fig. 2A). GK rats showed significant increases in fasting blood glucose (the 0-minute timepoint of the GTT; 121.5 mg/dL vs. 85.4 mg/dL, P < 0.05) and progressively worsening glucose tolerance (26%–17%) when compared with Wistar controls (P < 0.001 at 15, 30, and 60 minutes post-glucose administration; RM ANOVA interaction effect, strain × timepoint, F1,1106 = 15.558, P < 0.001; Fig. 2B). Impaired glucose tolerance in GK rats was observed as early as 3 weeks (the earliest point measured, 38% higher, P < 0.001) and worsened significantly over time (78% at 4 weeks and 96% at 8 weeks; RM ANOVA interaction effect, strain × age, F1,87 = 21.9, P < 0.001; Fig. 2C).

GK Rats Showed Increased ERG Amplitudes and Delayed Implicit Times
At 4 weeks of age, GK rats showed increased a-wave and b-wave amplitudes, as shown in representative waveforms (Fig. 3A). When amplitudes were quantified, a-waves were 95% larger and b-waves were 72% larger (RM ANOVA interaction effect; strain × flash intensity; a-wave, F1,159 = 83.828, P < 0.001; b-wave, F1,199 = 31.314, P < 0.001; Figs. 3B, 3C). GK rats also showed increased amplitudes for OPs and flicker ERGs as well as delays in OP and flicker ERG implicit times (Figs. 4A, 4B). When quantified, OP2 amplitudes were 130% larger and flicker amplitudes were 32% larger in 4-week-old GK rats when compared with Wistar controls (OP; RM ANOVA interaction effect; strain × OP, F1,150 = 59.138, P < 0.001; flicker, t-test; t158 = 2.652, P < 0.05; Figs. 4C, 4D). When implicit times were quantified, GK rats exhibited significant delays in OP and flicker ERG implicit times (OP; RM ANOVA interaction effect; strain × OP, F1,150 = 55.868, P < 0.001; flicker, t-test; t158 = 5.055, P < 0.01; Figs. 4E, 4F). In a subset of animals, ERGs were also performed at 3 weeks of age with similar results (data not shown).

From 4 to 8 weeks of age, GK rat ERG amplitudes approached control levels while delays in implicit times persisted. Specifically, in rats that underwent both 4- and 8-week ERGs, a-wave amplitude declined from 86% to 54% (RM
ANOVAs main effect of timepoint, $F_{1,41} = 4.374$, $P < 0.05$; Fig. 3D), b-wave amplitude declined from 81% to 33% (RM ANOVA interaction effect; strain × timepoint, $F_{1,41} = 7.725$, $P < 0.05$; Fig. 3E). OP amplitude declined from 121% to 27% (RM ANOVA interaction effect; strain × timepoint, $F_{1,41} = 15.721$, $P < 0.001$; Fig. 4G), and flicker amplitude declined from 46% to 28% (data not shown). Meanwhile, the implicit time delay did not show significant change between 4 and 8 weeks for either OPs (data not shown) or flicker (RM ANOVA main effect of strain, $F_{1,41} = 24.861$, $P < 0.001$; Fig. 4H).

**GK Rats Had Thinner Retinas Than Wistar Controls**

Representative SD-OCT images reveal thinner retinas in GK rats (Figs. 5A, 5B). At 6 weeks of age, GK rats had thinner retinal nerve fiber layers in the peripheral, but not the central, retina when compared with Wistar controls (RM ANOVA main effect of strain, $F_{1,81} = 8.888$, $P < 0.01$; Fig. 5C). Total retinal thickness was reduced in GK versus Wistar rats as well, both centrally ($P < 0.05$, data not shown) and peripherally, with the greatest differences being observed in the inferior and temporal quadrants of the retina (RM ANOVA interaction effect, strain × quadrant, $F_{1,81} = 6.784$, $P < 0.001$; Fig. 5D). Thinner retinal layers for GK rats were also observed in the central and peripheral RPE ($P < 0.01$ for both), central inner plexiform layer ($P < 0.001$), and central inner nuclear layer ($P < 0.05$; data not shown).

**GK Rats Showed Deficits in Cognitive and Exploratory Behavior, But Not Motor Function**

The GK rats showed significant deficits in cognitive behavior as measured by spontaneous alternation on the Y-maze, beginning at 7 weeks (RM ANOVA interaction effect, strain × age, $F_{1,261} = 2.986$, $P < 0.05$; Fig. 6A). GK rats were indistinguishable from Wistar rats at 4 weeks ($P = 0.694$), a time when ERG deficits were already apparent. The power of the performed test for strain differences was 0.976. Significant deficits in exploratory behavior as measured by number of entries on the Y-maze also occurred at 7 weeks (RM ANOVA interaction effect, strain × age, $F_{1,261} = 10.789$, $P < 0.001$; Fig. 6B). No deficits in motor coordination as measured by rotarod were observed even by 8 weeks (Fig. 6C).

**Greater Hyperglycemia in Male GK Rats; Greater ERG Delays in Female GK Rats**

Male GK rats showed higher levels of hyperglycemia ($P < 0.05$), but female rats showed greater ERG delays for both flicker ($P < 0.001$; Fig. 7) and OPs ($P < 0.05$; data not shown). Meanwhile, male GK rats showed greater reductions in exploratory behavior ($P < 0.05$). No significant sex differences were observed for cognitive behavior or rotarod (Fig. 7).

**DISCUSSION**

**Retinal Changes Appear Prior to Cognitive and Motor Changes in the GK Rat Model of Type II Diabetes**

In GK rats, retinal deficits were the first to appear after hyperglycemia, with significant delays in OP and flicker implicit times being observed at 4 weeks of age. Deficits in spatial cognition and exploratory behavior did not appear until 7 weeks of age, and motor deficits were not observed even at 8 weeks (Fig. 1). In previous studies, our group identified retinal function deficits that preceded retinal vascular pathology in both a rodent model of type I diabetes and in human diabetic patients. If retinal deficits are the earliest functional change after hyperglycemia, then starting treatment at the first sign of retinal dysfunction could prevent or delay cognitive and motor deficits and worsening of retinal deficits. These rodent experiments suggest a similar window in people with diabetes; the discovery of which might result in accelerated treatment of diabetic complications.

It is important to note that we used one test of cognitive function (spontaneous alternation on Y-maze) and one test of motor function (rotarod). Other assays may be more sensitive and could detect changes at earlier timepoints. However, previous studies in GK rats only revealed deficits at later times, including deficits in novel object recognition observed...
at 24 weeks of age, deficits in memory retention observed at 10 weeks, and deficits in olfactory memory at 6 to 10 months.

Supernormal ERG Amplitudes in the GK Rat

In addition to delays in ERG implicit time that are well documented in diabetic retinopathy, we observed ERG amplitudes that were much larger than those of nondiabetic controls. Although supernormal ERG amplitudes are occasionally reported in patients with early-stage diabetic retinopathy, the magnitude of the increases recorded here is unusual. It is possible that because hyperglycemia and impaired insulin secretion begin at 2 weeks in this model, a time when the retina is still developing, the hyperglycemia may alter the

**Figure 3.** GK rats showed increased a-wave and b-wave amplitudes that approached control levels over time. (A) Representative waveforms for dark-adapted ERG (0.7 log cd s/m²). Quantification of dark-adapted a-wave (B) and b-wave amplitudes (C) at 4 weeks of age. Average summed ERG amplitudes normalized to controls for 4 and 8 weeks of age for a-wave (D) and b-wave (E). Black asterisks indicate comparisons between GK and Wistar groups. Red asterisks indicate comparisons between GK groups at different ages. *P < 0.05, **P < 0.01, ***P < 0.001. Results expressed as means ± SEM.
FIGURE 4. GK rats showed increased OP and flicker amplitudes that decrease over time and delayed OP and flicker implicit times that persist to 8 weeks. Representative waveforms for dark-adapted OPs (0.7 log cd s/m²) (A) and light-adapted flicker ERG (B). Quantification of OP (C) and flicker (D) amplitudes and OP (E) and flicker (F) implicit times at 4 weeks of age. Average ERG values normalized to controls for 4 and 8 weeks of age for OP amplitudes (0.7 log cd s/m²) (G) and flicker implicit times (H). Black asterisks indicate comparisons between GK and Wistar groups. Red asterisks indicate comparisons between GK groups at different ages. *P < 0.05, **P < 0.01, ***P < 0.001. Results expressed as means ± SEM.
development of excitatory and inhibitory retinal circuitry such that ERG amplitudes are increased. Indeed, early changes in insulin receptor signaling in the hippocampus were shown to inhibit synaptic maturation and potentially cause cognitive deficits in the GK rat. In addition, decreased inhibition from amacrine cells has been proposed to increase rod bipolar cell signaling in the diabetic retina, suggesting that amacrine cells could be investigated in the GK rat as a target for synaptic change during hyperglycemia in retinal development. Contrary to our results, other groups have reported reduced ERG amplitudes in the GK rat. However, after the GK rat was developed by Goto and Kakizaki in 1975 in Japan, one colony remained in Japan and eventually became the colony sold by Charles River Laboratories, whereas other colonies were started in Europe in the 1990s using stock from the original colony (e.g., the colony that eventually became the animals sold by Taconic Europe, Isaszeg, Hungary). Differences in islet metabolism, islet morphology, and insulin content have been observed among the colonies, and use of rats from different

![Figure 5](image1.png)

**Figure 5.** GK rats had thinner retinal nerve fiber layers and total retinal thicknesses. Representative SD-OCT images from Wistar controls (A) and GK rats (B) at 6 weeks of age. Total retinal thickness was measured from the bottom of the RPE to the top of the RNFL. (C, D) Mean thicknesses for RNFL (C) and total thickness (D) for GK and Wistar rats for all four quadrants (Sup, Superior; Temp, Temporal; Inf, Inferior; Nas, Nasal) of the peripheral retina. **$P < 0.01$, ***$P < 0.001$. Results expressed as means ± SEM. RNFL, retinal nerve fiber layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; ELM, external limiting membrane; IS/OS, inner segment/outer segment layer.

![Figure 6](image2.png)

**Figure 6.** Cognitive and exploratory behavior, but not motor, deficits in GK rats. (A) Cognitive function at 4 to 8 weeks of age as measured by spontaneous alternation on Y-maze. (B) Exploratory behavior at 4 to 8 weeks of age as measured by number of entries on Y-maze. (C) Motor coordination as measured by rotarod latency. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Results expressed as means ± SEM.
Colonies may explain why we observe different ERG phenotypes.

Retinal, Cognitive, and Metabolic Changes in the GK Rat When Compared With Other Diabetes Models

When examining the large ERG amplitude increases observed in GK rats over Wistar rats, it is important to consider whether these increases are caused by larger signal in the GK rats or smaller signal in the Wistar rats. In Figure 6A, we compare the b-wave amplitude data from GK and Wistar rats presented here with b-wave amplitude data from a type I model of diabetes, the STZ rat. In this model, we induced hyperglycemia in 9-week-old Long Evans rats using STZ (100 mg/kg), a toxin that ablates pancreatic beta cells. These data have been presented previously, but have been repurposed to make comparisons with the GK rat. Here, we compare ERGs from 4-week-old GK and Wistar rats with STZ rats that have been hyperglycemic for 4 weeks. We show that b-wave amplitudes are very similar for Long Evans controls and Wistar controls, indicating that the amplitude increases observed in the GK rat are not the result of abnormalities in the Wistar rat. Although significant amplitude increases are observed in the STZ rat as well, the magnitude of increase is larger in the GK rat (71% vs. 31%, on average), and the GK rat amplitudes are significantly larger than the STZ rat amplitudes.

For ERG implicit times, the oscillatory potential delays observed in the GK rats (12%) were similar to the delays observed in the STZ rats (17%; Fig. 6B). Delays in oscillatory potential and flicker ERG implicit times, similar to those found in the GK rats here, are typical of the retinal dysfunction observed in early DR in a type 1 diabetes model. Interestingly, in the STZ rats, delays were observed in OP1, OP2, OP3, and OP4, whereas in the GK rats, delays were observed in only OP3 and OP4. Meanwhile, OP delays were observed with both bright (0.7 log cd s/m²) and dim (~1.9 log cd s/m²) flashes in the GK rats versus dim flash only in the STZ rats.

The GK and STZ rats also showed similar deficits for cognitive and exploratory behavior, with the GK rats showing a 19% decline in spontaneous alternation versus 12% in the STZ rats and the GK rats showing a 19% decline in exploratory behavior versus 20% in the STZ rats (Figs. 8C, 8D). The differences in cognitive and exploratory behavior observed between Wistar controls and Long Evans controls may be the result of age differences. The data presented are from 8-week-old GK and Wistar rats and 17-week-old STZ rats and Long Evans controls (8 weeks posthyperglycemia).

In terms of metabolic phenotype, the GK rats exhibit large impairments in glucose tolerance and a fasting blood glucose of 121.5 mg/dL. For comparison, 250 mg/dL is our fed blood glucose cutoff for inclusion as a type 1 animal in our STZ experiments. As for other type II models, Zucker diabetic fatty rats show fed blood glucose levels of 86 mg/dL at 6 weeks and 200 to 289 mg/dL at 12 to 40 weeks. Otsuka Long-Evans Tokushima Fatty (OLES) rats show fed blood glucose levels of 143 to 168 mg/dL between 10 and 30 weeks and high-fat diet + low-dose STZ rats show fasting blood glucose levels of 140-160 mg/dL. Unlike the STZ rat, the GK rats are not insulin dependent. Unlike other type II models, the GK rats weigh less than their Wistar counterparts and are not obese. However, despite showing reduced weights, the GK rats show changes in lipid profiles, including increased cholesterol levels from 7 weeks though 26 weeks, increased high-density lipoproteins (HDL) levels at 26 but not 18 weeks, increased triglycerides at 12 weeks through 30 weeks, and increased free fatty acids at 18 weeks through 30 weeks. Unfortunately, we did not measure lipid profiles in this experiment, and the studies that investigated lipid profiles did not use timepoints as early as 4 and 8 weeks, the timepoints at which we measured retinal and cognitive function. It is possible that hyperglycemia, as well as hyperlipidemia, contributes to the retinal and cognitive deficits observed here.

Greater Hyperglycemia in Male GK Rats; Greater ERG Delays in Female GK Rats

Male GK rats exhibited significantly higher levels of hyperglycemia than female GK rats. This finding is not surprising given that female rats often require more intensive measures (i.e., a higher percentage of high-fat diet) to induce hyperglycemia. Female animals appear to have a track record for being more resistant to the development of diabetes. Interestingly, despite having reduced hyperglycemia, the female GK rats exhibited greater delays in flicker ERG and OP implicit times. This finding highlights the importance of testing both male and female animals in models of diabetes and in other disease and injury models.

Conclusions

Retinal function deficits developed prior to cognitive or motor deficits in the GK rat model of type II diabetes. Future studies will investigate mechanistic links between retinal and cognitive deficits; assess long-term changes in retinal, cognitive, and motor function and retinal vasculature; and determine whether early retinal deficits can predict cognitive dysfunction and late-stage retinal disease. Future research will also investigate whether starting interventions at the earliest signs of diabetes-induced changes in the retina could prevent or delay other complications, including late-stage retinal vascular changes.

![Figure 7](image.png)

**Figure 7.** Sex differences in GK rats. A comparison of performance on behavioral and functional tests for female and male GK rats versus Wistar controls (sexes combined). GK values were normalized to control values for each test. Flicker, flicker implicit time; Exp, exploratory. *P < 0.05, **P < 0.01, ***P < 0.001. Results expressed as means ± SEM.
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References


