# The Selective Benefits of Acute Dosing with Dopamine Receptor Agonists on the Visual Function, Cognitive Function, and Blood Glucose Levels in a Rat Model of Type I Diabetes

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Abstract

**Purpose:** Previous studies have demonstrated that dopamine D1 and D4 receptor (D1R, D4R) agonists selectively improve spatial frequency and contrast sensitivity thresholds, respectively, in healthy and diabetic mice[3,12]. The purpose of this experiment was to investigate the selective benefits of three dopamine receptor agonists (D1R, D2R, D4R) on visual function, cognitive function, and blood glucose in a rat model of Type I diabetes with progression of diabetes.

**Methods:** Streptozotocin (STZ) was used to induce hyperglycemia in 2-month-old male Long–Evans rats (n=17). Outcome measures were acquired before and 30 minutes after an intraperitoneal injection of a single dose of a dopamine receptor agonist. Each agonist was injected 2-3 days apart. After 8, 16 and 20 weeks post-STZ, optomotor response (OMR) assessments were conducted. After 10 weeks post-STZ, blood glucose and Y-maze performance were measured on a subset of animals (n=4 diabetics; 6 controls). The spatial frequency, contrast sensitivity, Y-maze performance, and blood glucose for the diabetic rats and control rats post injection were compared to the baseline values to determine the effect of the acute dosing of each dopamine receptor agonist.

**Results:** At the 8, 16, and 20 week timepoints, spatial frequency and contrast sensitivity thresholds were impaired in diabetic animals compared to controls ($p<0.0001$). At 8 weeks post-STZ, D1R agonist selectively restored spatial frequency thresholds for diabetic rats ($p<0.0001$), but did not alter thresholds in control rats. At 8 weeks post-STZ, the D4R agonist selectively restored contrast sensitivity thresholds for diabetic rats ($p<0.0001$) but did not alter thresholds for control rats. The improvements that we observed at 8 weeks in the diabetic animals with the D1R and D4R agonists were no longer observed at 16 or 20 weeks. The D2R agonist did not demonstrate any effects at any timepoint. There was no significant differences in Y-maze and blood glucose values with agonist treatment.

**Conclusions:** Based on these findings, acute dosing of dopamine receptor agonists selectively benefits visual acuity (D1R) and contrast sensitivity (D4R) at early stages of diabetic retinopathy. Thus, dopamine receptor agonists may provide protective treatment options for early stage visual deficits characteristic of Type I diabetes.

# Introduction

Diabetic retinopathy (DR) is a prominent cause of visual deficits and blindness in the United States and its incidence is predicted to rise[15]. Consequently, there is an important need for the development of early treatments to prevent progression of the disease. Dopamine has recently been identified as a neuroprotective agent for early stages of DR[3]. Furthermore, studies have demonstrated that the retinas of diabetic rodents show dopamine loss[3]. In our lab, we have demonstrated that treating rodents with L-DOPA, a precursor to dopamine that crosses the blood retinal barrier, provides protection against the visual impairments associated with DR[3,14,18,19]. The main objective of this study is to determine the efficacy of using dopamine receptor agonists to treat DR with progression of retinopathy. This study will fill important information gaps about which dopamine treatments are optimal for clinical use in diabetic retinopathy.

Dopamine is prevalent in healthy retinal tissue, serving as a chemical messenger for light adaptation as well as playing a role in circadian rhythm function and vision[21]. Throughout the body, dopamine also stimulates vasodilation in tissue[21]. Since D2 receptors also modulate vascular epithelial growth factors, a decrease in dopamine levels has also been found to enhance the growth of certain tumors and lung cancer[6]. In combination, these findings lead us to believe that a loss of dopamine due to diabetes may potentially contribute to both the neural and vascular deficits seen in DR. In our lab, we have used optomotor response (OMR) and electroretinography (ERG) to demonstrate that treating rodents with L-DOPA during early stages of DR helps to prevent the loss in visual acuity and retinal function[3,14,18,19].

Dopamine is believed to travel through the retina via diffusion and binds to a variety of different cells that contain specific dopamine receptors[21]. Dopamine D1 receptors (D1R) are found in most retinal layers, dopamine D4 receptors (D4R) are found primarily in the photoreceptor layers, and dopamine D2 receptors (D2R) are found primarily on the dopaminergic amacrine cells and inner retinal neurons[21]. A dopamine receptor agonist is a chemical that binds to particular dopamine receptor types to initiate a response. Previous studies have demonstrated that treating diabetic mice with dopamine receptor agonists selectively restored visual impairments[3]. Specifically, the D1R agonist improved spatial frequency, while the D4R agonist improved

contrast sensitivity[3]. These findings are promising because they suggest that even when dopamine levels are depleted, dopamine receptors remain intact. While the dopamine D2 receptor agonist, bromocriptine, has been FDA approved for the treatment of diabetes, it has not yet been studied as a potential treatment for early DR.

In this study, the effects of the dopamine receptor agonists D1R, D2R, and D4R on different aspects of visual function, cognitive function, and blood glucose levels will be examined. It is hypothesized that these dopamine receptor agonists will selectively restore visual deficits in a rat model of Type I diabetes after just one dose (acute effects). This experiment will help to elucidate whether dopamine receptor agonists effectively protect visual function in cases of DR, potentially offering therapeutic benefit without the potential side effects of L-DOPA. In addition, we will examine if dopamine receptor agonists are effective at all stages of DR.

## Literature Review

### Significance of DR

By the year 2025, 35% of the world's population is expected to suffer from diabetes[15]. Due to this increase in the incidence in diabetes, the complications associated with diabetes are expected to burden healthcare systems throughout the world. In the United States alone, treatments for complications associated with diabetes are expected to cost approximately $336 billion per year by 2034[10]. Diabetic retinopathy (DR), one of the more common complications associated with diabetes, is also the primary cause of blindness in working-age adults[16]. Further, on average, approximately 20 years after being diagnosed with diabetes, a majority of people develop DR[16].

### Clinical Definition of DR

Today, DR is clinically detectable by the presence of microaneurysms, dot hemorrhages or other microvascular lesions. When left untreated, the lack of blood flow to the retina results in neovascularization that ultimately leads to blindness[7]. At these late stages of the disease, known as proliferative DR, the only interventions that reduce the chance of blindness, such as pan-retinal laser photocoagulation, vitreo-retinal surgery, or intravitreal injections of anti–vascular

endothelial growth factor antibodies, are expensive and can result in some damage to retinal function[9]. Thus, there is a pressing need for a preventative treatment for this devastating disease.

In order to develop an effective neuroprotective treatment for DR, researchers must first discover methods for diagnosing DR early, before detectable changes in retinal vascularization. Fortunately, studies have demonstrated that neuronal degeneration and deficits can be observed before this clinically observable vascularization[1,4]. Using electroretinograms (ERGs), a tool that enables the monitoring of retinal function, researchers have found that oscillatory potentials (OPs) are delayed or have altered amplitudes in both diabetic rodents and patients[2,18,19]. In addition to changes in ERGs, deficits in visual acuity, specifically night vision, contrast sensitivity, and color vision, have also been observed in diabetic patients before retinal neovascularization[7]. Preliminary studies using the optomotor response have found that diabetic rodent models also demonstrate visual deficits, specifically deficits in spatial frequency and contrast sensitivity, as early as 4 weeks post induction with diabetes[2,3,14]. In combination, these earlier studies have demonstrated that neuronal deficits can be detected before clinically detectible vascularization develops.

**Dopamine in Diabetes**

With this information, more recent studies have focused on introducing neuroprotective agents at the earliest observations of DR. Since the causes of the neuronal changes are not understood, scientists have experimented with different neuroprotective agents potentially lacking in the retinas of diabetic rodent models. In our lab, we have demonstrated that dopamine is reduced in the diabetic retina and increasing dopamine can protect against early neural dysfunction in diabetic rodent models[3], suggesting that a deficiency of dopamine may be responsible for triggering the neuronal changes evident in early DR.

It is reasonable to assume that there may be some dopamine deficiency in the retina since dopamine deficiency underlies several complications associated with diabetes. Cognitive deficits, depression, and Parkinson's disease are all associated with dopamine deficiency in the brain that have been shown to occur more frequency in patients with diabetes[11,17]. Additionally, studies have shown that dopamine deficiency in the kidneys is associated with diabetic

nephropathy, indicating that dopamine deficiency may be prevalent in multiple organ systems in diabetes[22]. Furthermore, the number of dopamine-releasing cells in the retina, also known as dopaminergic amacrine cells, has been shown to be decreased by 16% in later stages of diabetes in diabetic mouse models[8]. These findings have raised the question of whether both increasing levels of dopamine and/or activating dopamine receptors can prevent the neuronal and vascular deficits associated with DR.

**Dopamine Treatments for DR**

In further support of dopamine as an important factor in DR, we have found that dopamine deficiency results in early visual deficits with DR in diabetic rodents using optomotor response testing. In these studies, retinal tissue analysis showed that the type I diabetic rodent model possesses a deficiency in dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite that indicates the turnover of dopamine[3]. Furthermore, we have shown that increasing dopamine with L-DOPA treatment, the precursor for dopamine, results in protection against these deficits[3,14]. Retinal deficits were significantly reduced in diabetic rats treated with L-DOPA compared to the diabetic rats treated with vehicle. L-DOPA in the form of levodopa has been approved by the FDA for Parkinson's patients since the 1960s. In a recent clinical trial in which the ERG results of individuals with diabetes given Sinemet treatments (levodopa plus carbidopa) were compared to the ERG results of controls, the treatment resulted in a reversal of retinal dysfunction, suggesting that the Sinemet treatment may be an exciting potential treatment option for early stage DR[18].

Unfortunately, L-DOPA may not be an ideal option for people who need chronic treatment since it has been associated with side effects such as motor deficits and dyskinesias in patients with Parkinson's disease[13]. Since dopamine receptor agonists, like bromocriptine, are currently being clinically prescribed by doctors for diabetes and the side effects seem to be marginal, it is worth studying whether dopamine receptor agonists produced similar protective benefits to dopamine treatments. A dopamine receptor agonist is a substance that binds to a dopamine receptor type and activates the receptor to generate the biological response. The dopamine receptor agonists D1R and D4R have been shown to have selective effects on visual function in non-diabetic animals[12]. It has also been demonstrated that acutely administering dopamine receptor agonists

(D1R and D4R) to mice at 5 weeks post induction with diabetes selectively restored visual function[3]. Specifically, D1R agonists partially restored spatial frequency and D4 receptor agonists partially restored contrast sensitivity[3]. This suggests that the dopamine receptors are present and functioning in the retina even when dopamine levels are low and that acute dosing of dopamine receptor agonists may serve as a potential treatment option for functional deficits in the diabetic retina.

Given this information, we aim to further assess whether dopamine receptor agonists may provide a protective effect against visual deficits characteristic of type I diabetes by testing the selective benefits of acute dosing with three dopamine receptor agonists (D1R, D2R, D4R) on visual function, cognitive function, and blood glucose in a rat model of type I diabetes at different stage of disease. In line with other studies, we expect to see that acute delivery of dopamine receptor agonists D1R, D4R, and D2R will provide selective benefit to the retinal neurons.

## Methodology

### Animals and diabetes induction

Twenty-nine male Long–Evans rats (Charles River Laboratories) were kept in ventilated cages with food and water and all procedures were approved by the Atlanta VA Institutional Animal Care and Use Committee. Ten rats (2-months old) were injected with a single dose of streptozotocin (STZ: 100 mg/kg; Sigma-Aldrich) dissolved in an eight to one ratio of citrate buffer and 50% glucose solution to induce hyperglycemia. Post-STZ, the blood glucose of these rats was monitored daily using a blood glucose meter and tail-prick blood. Rats were considered to be diabetic once blood glucose levels of greater than 250 mg/dL were observed two days in a row. This group served as the type 1 diabetic group (DM). For the duration of the experiment, the weight and blood glucose of these animals was monitored once a week. If a DM rat was observed to be losing significant weight, an insulin pellet (13 ± 3 mg/pellet) was surgically placed subcutaneously (Linplant; Linshin, Canada) to avert future weight loss without diminishing the hyperglycemia. The rats were maintained for 12 to 20 weeks and then euthanized.

**Dopamine Receptor Agonist Acute Treatment**

Outcome measures (spatial frequency, contrast sensitivity, Y-maze, and blood glucose) were performed on diabetic rats (n=17) and control rats (n=12) before and 30 minutes after an intraperitoneal injection of a single dose of SKF38393 hydrobromide, a D1R agonist (1 mg/kg); bromocriptine mesylate, a D2R agonist (5 mg/kg); or PD168077 maleate, a D4R agonist (1 mg/kg) (Tocris Biosciences). Since the half-lives of these drugs are between 1 and 3 hours, a two-day interval was allotted between treatments to completely ensure that there was no residual effects of the previous treatment[3]. Experimental design is shown in **Figure 1**.

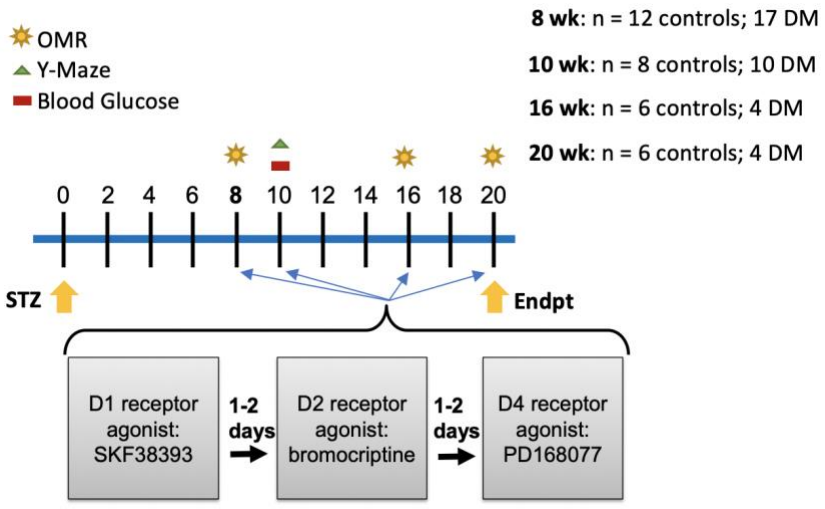


**Figure 1**: Diagram of experimental timeline.

**Analysis of visual function**

At 8, 16, and 20 weeks post-STZ, the virtual optomotor response system (OptoMotry system; Cerebral- Mechanics) was used to measure visual function, specifically contrast sensitivity and spatial frequency thresholds. The system consists of a box in which the four walls are monitors that present vertical lines moving from one side of the screen to the other. The rat was placed in a platform at the center of the 4 monitors and a camera was located above it, allowing for live observation of the animal's visual tracking behavior. The rat was determined to be visually tracking the lines when its head was moving in the same direction and speed as the vertical lines.

To assess for spatial frequency, the black vertical lines were maintained at 100% contrast and started moving at a spatial frequency of 0.042 cycles/degree. The spatial frequency increased

until the rat could no longer visually track the lines. The spatial frequency threshold was the highest spatial frequency that the rat was observed visually tracking the lines three times using a staircase testing paradigm. To assess contrast sensitivity, the spatial frequency was held at 0.064 cycles/degree and the contrast of the vertical lines decreased until the rat could no longer visually track the lines. The contrast sensitivity was then calculated for each animal by taking the luminance of the monitors displaying the vertical lines and calculating the reciprocal of the Michelson contrast [i.e., (maximum + minimum)/(maximum − minimum)], as described previously[20].

**Analysis of cognitive and exploratory behavior – y-maze**

To determine spatial working memory and exploratory behavior, the y-maze (San Diego Instruments, San Diego, CA) was utilized. Rats were positioned in one arm of the y-maze and allowed to explore around the maze (8 min). The number of times the animal entered an arm was recorded. An alternation was defined as an animal entering the arms in a consecutive manner. The spatial alteration behavior (cognitive behavior) was calculated by taking the number of successful alternations and dividing it by the total number of entries minus two and multiplying by one hundred. Exploratory behavior was defined as the total number of arm entries during the 8-minute time window.

**Analysis of blood glucose**

To determine the immediate effects of acute dosing with each dopamine receptor agonist on blood glucose levels, blood glucose was measured using a blood glucose meter that measured from tail-prick blood before and 30 minutes after the dopamine receptor agonist treatment.

**Statistical analysis**

Results are expressed as mean ± standard error of the mean (SEM). Outcome measures were compared pre- and post-agonist between diabetic and control rats at 8, 10, 16, and 20 weeks post-STZ and then all agonists compared across time using three-way repeated ANOVAs followed by a Tukey's multiple comparisons test with mixed effects analysis.

Results

**Selective Effects of Acute Agonist Treatments on Visual Function**

We investigated how selective dopamine receptor agonists could reverse visual deficits in a rat model of type 1 diabetes by performing optomotor response evaluation on a group of 17 diabetic and 12 control rats before and after a single injection of a D1 receptor (D1R) agonist (SKF38393), a D2 receptor agonist (D2R) (5 mg/kg), or a D4 receptor (D4R) agonist (PD168077). At 8 weeks post-STZ, spatial frequency and contrast sensitivity thresholds were significantly worse in the diabetics than the control animals (Two-way ANOVA, main effect of diabetes, spatial frequency $F(1, 27) = 139.6$, $p<0.0001$; contrast sensitivity: $F(1, 26) = 90.89$, $p<0.0001$). In diabetic animals, D1R agonist treatment improved spatial frequency (Multiple comparison, $p<0.0001$), while D4R agonist improved contrast sensitivity (Multiple comparison, $p<0.0001$) (**Figure 2**). To measure the impacts of the agonist treatments during various stages of the disease, optomotor tracking evaluations were also performed at 16- and 20-week time points on a group of 4 diabetic and 6 control rats before and after each agonist treatment. By the 16- and 20-week time points, the same selective benefits on visual function were not observed (**Figure 3**).

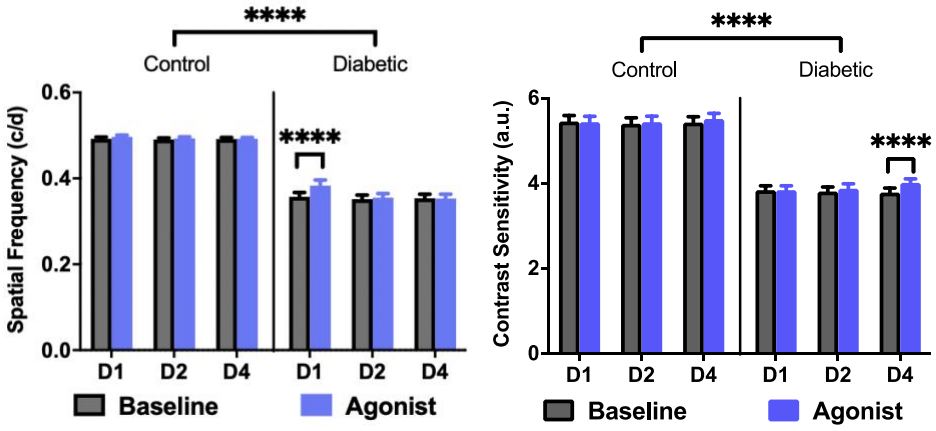


**Figure 2**: **D1R agonist selectively increased spatial frequency while D4R agonist selectively increased contrast sensitivity at 8-week post-STZ.** Baseline and post-agonist spatial frequency (left) and contrast sensitivity (right) thresholds for diabetics (n=17) and controls (n=12) at 8 weeks post-STZ demonstrate selective benefit with D1R and D4R agonists for diabetic rats, respectively. Results expressed as mean ± SEM. Three way ANOVA, ****$p<0.0001$.

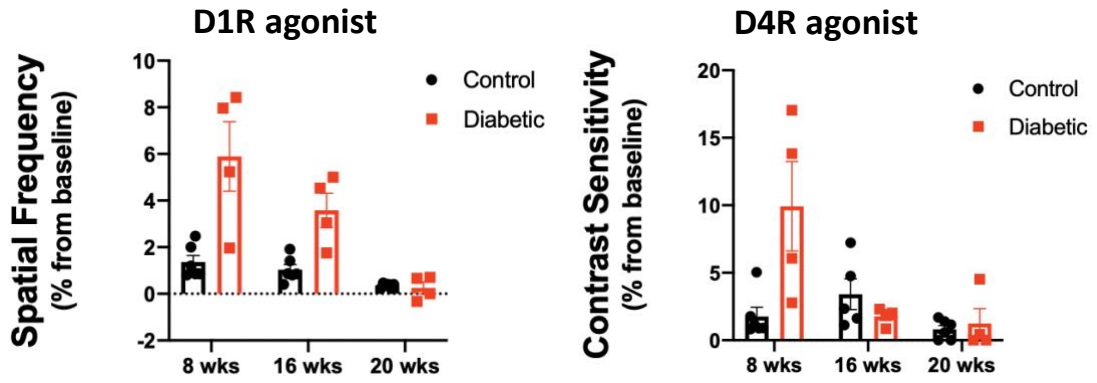


**Figure 3**: **Benefits of dopamine agonists may decline with progression of diabetic retinopathy**. Percent change in spatial frequency (left) and contrast sensitivity (right) thresholds at 8, 16, and 20 weeks post-STZ show that the selective benefits of D1R and D4R agonists decline with progression of the disease. Results expressed as mean ± SEM.

## Effects of Acute Agonist Treatments on Blood Glucose

To assess whether the improvements in visual function due to agonist treatments involved changes in blood glucose, blood glucose analysis was performed on a group of 10 diabetic and 8 control rats before and after an agonist injection. There were no significant differences demonstrated by blood glucose analyses (**Figure 4**).

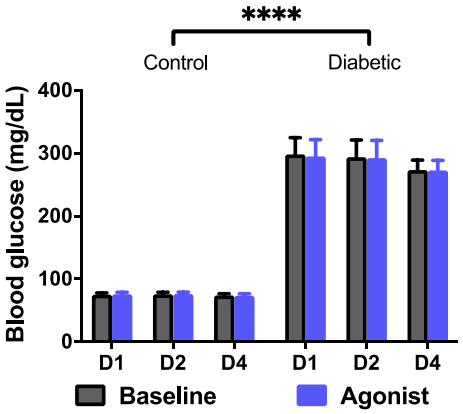


**Figure 4**: **Blood glucose did not change with agonist treatment at 10-weeks post-STZ.**
Baseline and post agonist blood glucose values for diabetics (n=10) and controls (n=8) at 8 weeks post-STZ show agonists do not affect blood glucose levels. Results expressed as mean ± SEM. ****p<0.0001.

### Effects of Acute Agonist Treatments on Blood Glucose

To assess whether agonist treatments impact cognitive function, a Y-maze was conducted on a group of 10 diabetic and 8 control rats before and after each agonist treatment. While diabetic rats displayed reduced spatial alternation and exploratory behavior relative to controls ($p<0.0001$), there was no significant differences between baseline and post-agonist values (**Figure 5**).

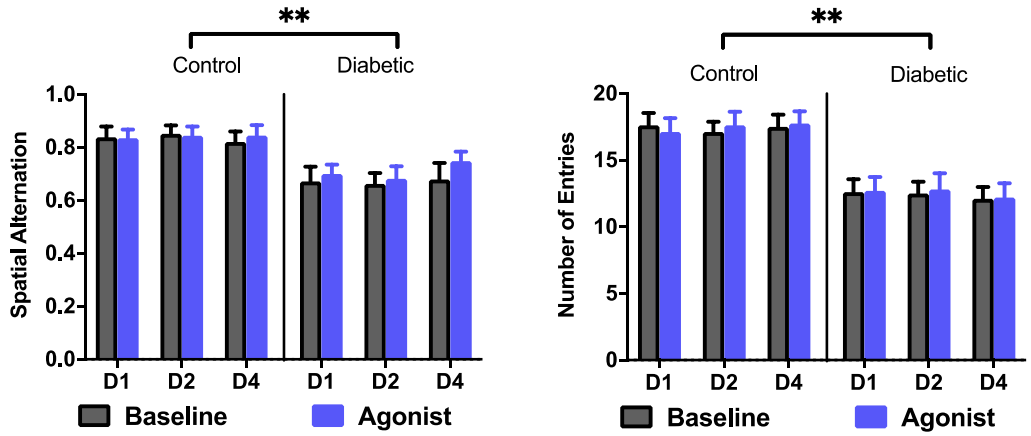


**Figure 5**: **Cognitive function measured with y-maze did not change with agonist treatment at 10-weeks post-STZ.** Baseline and post agonist spatial alternation (left) and exploratory behavior (right) for diabetics (n=10) and controls (n=8). Spatial alternation and exploratory behavior was reduced in diabetic animals; however, agonists do not effect spatial alternation and exploratory behavior. Results expressed as mean ± SEM. **p<0.001.

## Discussion

### Spatial Frequency and Contrast Sensitivity Thresholds Impaired in Type I Diabetic Rats

The present study shows that early stages of DR impact certain aspects of visual function and therefore demonstrates that spatial frequency and contrast sensitivity may serve as useful diagnostic tools for early stages of DR. While some of these visual impairments may be due to the presence of cataracts, previous studies have demonstrated that as early as 4 weeks post-STZ, diabetic animals without cataracts display these visual deficits[2]. These findings suggest that other factors, such as the impact of hyperglycemia on retinal function, contributed to the development of visual deficits over time[2]. Future studies are necessary to identify the exact mechanism that connects these two pathologies.

**Dopamine Receptor Agonists Selectively Restore Visual Function in early stages of Type I Diabetic Rats**

In this study, in diabetic animals, D1R agonist treatment improved spatial frequency, while D4R agonist improved contrast sensitivity. No significant improvements were observed in controls. These results are consistent with the findings in a previous study that showed that acutely administering dopamine receptor agonists (D1R and D4R) to mice at 5 weeks post induction with diabetes selectively restored visual function[3]. These data suggest that the dopamine receptors are present and functioning in the retina even when dopamine levels are low, and that acute dosing of D1R and D4R agonists may serve as potential treatment options for patients with diabetes. Thus, the results of this current study support the idea that diabetes disturbs dopaminergic retinal pathways but keeps the dopaminergic receptors intact. The D2R agonist did not result in any significant improvements in diabetic or control animals. In future experiments, different concentrations of the D2R agonist used should be explored to determine whether the results observed were due to a suboptimal dosage of the drug. By the 16- and 20-week time points, the same selective benefits on visual function were not observed. This suggests that dopamine receptor agonists may only be useful as a treatment for DR in the early stages of the disease. However, it should be noted that since only 4 diabetics and 6 controls remained for these later time-points, future experiments will have to be conducted with more animals to determine whether or not the trends observed remain.

**Blood glucose did not change with acute dopamine receptor agonist treatment**

There were no significant differences demonstrated by blood glucose analyses, suggesting that the improvements in visual function observed at the 8-week timepoint were not due to a sudden change in blood glucose levels. Thus, this data supports the idea that dopamine agonist treatment is acting directly on the retina to restore dopamine and thus function is not acting through a secondary mechanism like blood glucose.

**Cognitive function measured with y-maze did not change with acute dopamine receptor agonist treatment**

While diabetic rats displayed reduced spatial alternation and exploratory behavior relative to controls, indicating impaired cognitive function, there were no significant differences between baseline and post-agonist values. This suggests that dopamine receptor agonists may not restore cognitive function in type I diabetic animals.

## Conclusions

These results demonstrate that D1R agonists improve spatial frequency while D4R agonists improve contrast sensitivity at the early stages of diabetic retinopathy. This finding suggests that diabetes does not change the roles of D1R and D4R in vision, nor does it damage existing dopamine receptors in the retina. This research is clinically relevant because it shows that dopamine receptor agonists may be a helpful treatment in patients with early stages of diabetic retinopathy. This information will hopefully help fill important information gaps about which dopamine treatments are most optimal for clinical use in DR. Since the animal numbers for the later timepoints were smaller than the earlier timepoints, future studies may examine the role of dopamine receptor agonists on late stages of DR, during the appearance of vascular changes, a timepoint that is most clinically relevant to when diabetes is detected in the clinic. Since we did not observe any effects due to the D2 agonist, future studies with different dosages may be conducted to verify that the D2 agonist is not protective.

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