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March 30, 2015

The effects of exercise on diabetic retinopathy and cognitive dysfunction

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Abstract

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Exercise is neuroprotective and neuroregenerative to neuronal tissues. Previously, we have shown that treadmill exercise preserves retinal function in animals that undergo light-induced retinal degeneration, and that the protective effects of exercise were mediated by brain derived neurotrophic factor (BDNF) (Lawson et al., 2014). The purpose of the current study was to determine whether visual and cognitive deficits occurred with early stage diabetes, and whether these deficits could be ameliorated with exercise through a BDNF-mediated mechanism. Wild-type Long Evans rats were injected with streptozotocin (STZ; 100 mg/kg) to induce hyperglycemia (glucose >250 mg/dL), and were compared to non-diabetic controls. Separate cohorts were exercised for 30min on treadmills for 5 days/wk for 8 weeks at either 0 m/min for Inactive groups or 15m/min for Active groups. To evaluate the contributions of BDNF, half of the diabetic rats were injected with a TrkB receptor antagonist, ANA-12, or vehicle 2.5 hours before exercise. Using optokinetic tracking, we found that visual acuity and contrast sensitivity were significantly decreased in diabetic rats and that exercise significantly preserved contrast sensitivity. Similarly, electroretinography using flicker stimuli showed significant delays in diabetic rats that were ameliorated with treadmill running. Furthermore, these protective effects of exercise on visual and retinal function were mediated by BDNF. Cognitive function assessed with a y-maze showed significant deficits with diabetes, while two types of object recognition memory showed no deficiencies. Treadmill running did not benefit cognitive deficits. Exercise is a non-invasive, low cost intervention that may provide benefit to the visual function of diabetic patients.

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Introduction

The prevalence of Type 1 diabetes in the United States is currently 1 in 300 by 18 years of age (Maahs et al., 2010). Diabetes is also the leading cause of new cases of legal blindness in American adults under 65 years old (Herman et al., 1983). While diabetic retinopathy is extremely devastating in terms of quality of life, there is little known about the pathophysiology involved. It takes several years for the detrimental effects of hyperglycemia to be clinically diagnosed based upon vascular malformations. However, irreversible damage may be occurring undetected (Antonetti et al., 2006). Retinal dysfunction occurs relatively early in the disease progression, and this includes neuronal damage to photoreceptors (Fletcher et al., 2007). Diabetes not only has an early detrimental effect on the retina, but it also negatively impacts cognitive functioning. The mechanisms underlying cognitive dysfunction in diabetes are also not well understood. Memory and learning impairments, which are thought to be related to the dysfunction of the hippocampus (Auer et al., 1989a), occur with more frequency in people with Type 1 diabetes than the population at large (McCarthy et al., 2002). Therefore, there is great interest in therapies that could serve as preventative measures to delay the retinal and cognitive decline associated with diabetes.

Exercise may have promise as a therapeutic intervention for diabetic retinopathy and cognitive dysfunction because there is increasing evidence that exercise is neuroprotective in both the CNS and PNS. Previously, exercise has been shown to protect the retina in a light-induced retinal degeneration model (Lawson et al., 2014). Additionally, people who ran several miles every day over several years were at a significantly decreased risk for developing macular degeneration (Williams, 2009). Exercise has also been shown to be protective in cognitive functioning. In a study by Dieuges and colleagues (2014), diabetic rats performed significantly

worse on a spatial memory task than control rats; however, this difference was ameliorated with exercise. Exercise in diabetic rats has been shown to increase IGF-1 and BDNF protein signaling in the hippocampus (Dieuges et al., 2014). Voluntary exercise has also been shown to increase the number of dendritic spines in the hippocampus of diabetic animals (Stranahan et al., 2009). Additionally, in a study by Carro and colleagues (2001), treadmill exercise was shown to protect spatial memory in animals exposed to a hippocampal neurotoxin. In a mouse model of TBI, animals that were preconditioned by exercising voluntarily on running wheels had less neuronal death and thus better cognitive outcomes on a Morris Water Maze task (Zhao et al., 2014). Therefore, exercise may be a relatively easy intervention to slow down the neuronal dysfunction in those who may be at risk for diabetic retinopathy and cognitive dysfunction.

There is reason to believe that the neuroprotective effects of exercise may be mediated by brain-derived neurotrophic factor (BDNF). BDNF plays an important role in the survival and proliferation of neurons through activation of its receptor, tropomyosin receptor kinase B (TrkB) (Hofer & Barde, 1988). Aerobic exercise increases the levels of BDNF (Zoladz et al., 2008) and TrkB receptor signaling (Fang et al., 2013). In a study by Lawson and colleagues (2014), BDNF was found to mediate the protective effects of exercise following light-induced retinal degeneration. BDNF has also been found to be upregulated following exercise in diabetic mice (Stranahan et al., 2009). In this study, we hypothesize that there will be a visual and cognitive decline associated with diabetes, and that this decline will be ameliorated by exercise. We also hypothesized that the protective effects of exercise we see are mediated by BDNF.

Methods

Experimental Design

Male Long Evans rats were randomly assigned into control and diabetic groups. These groups were tested longitudinally to ascertain visual acuity and contrast sensitivity thresholds with optokinetic tracking (OKT), and retinal function was tested with electroretinogram (ERG). Cognitive function was tested with a Y-maze and novel object recognition paradigms. Animals were randomly divided into exercise or inactive treatment groups to determine whether exercise would have protective effects on the retina and brain. Next, BDNF involvement in the protective effects of exercise was tested by inhibiting the TrkB receptor prior to exercise. Animals were sacrificed at 8 weeks following injection of streptozotocin (STZ) or vehicle. Retina, brain, blood, and serum were collected.

Control vs. Diabetic

We randomized adult, male Long Evans rats (325-350 g; Charles River, Wilmington, MA) into 2 groups: Control (n=9) and Diabetic (n=11). On post-natal day 72, rats were injected with vehicle (citrate buffer pH 4.0) or streptozotocin (STZ; 100mg/kg body weight; Sigma-Aldrich, Inc., Milwaukee, WI) via the penile vein. Diabetes was defined as two successive daily blood glucose levels higher than 250 mg/dL after injection of STZ.

Visual Function Testing

Optokinetic Tracking (OKT)

OKT (OptoMotry system; Cerebral Mechanics, Lethbridge, AB, Canada) was tested every 2 weeks for 8 weeks to assess visual acuity and contrast sensitivity. Each rat was placed on

a platform at the center of a virtual-reality chamber composed of four computer monitors that displayed vertical sine wave gratings rotating at 12 deg/s. A video camera positioned above the animal was used to watch in real time for the presence or absence of reflexive head movements (tracking) in response to the projected gratings rotating in the same direction. During visual acuity assessment, the grating started at a spatial frequency of 0.042 cyc/deg with 100% contrast. The visual acuity threshold was determined automatically by the OKT software using a staircase paradigm based on observations of head-tracking reflexes. Similarly, the contrast sensitivity threshold was determined by reducing the contrast of the black and white gradients from 100% in a staircase paradigm until animal head-tracking movements were no longer observed. Contrast sensitivity was measured at the spatial frequency of 0.064 cyc/deg for the study. This was the spatial frequency that elicited the maximum sensitivity obtained from the rats at baseline when a contrast sensitivity curve was assessed across five spatial frequencies as previously described (Aung et al., 2014). Contrast sensitivity was calculated as a reciprocal of the Michelson contrast from the screen's luminance (i.e. $[\text{maximum} + \text{minimum}] / [\text{maximum} - \text{minimum}]$), as previously described (Prusky et al., 2006).

Electroretinography (ERG)

Functional assessments utilizing dark and light-adapted flicker ERGs were measured at the 4 and 8 week time periods to assess cone function. Briefly, rats were anesthetized (ketamine [60 mg/kg] and xylazine [7.5 mg/kg]), pupils dilated (1% tropicamide), and the corneal surface anaesthetized (0.5% tetracaine HCl). Electrical responses were recorded via a gold loop electrode touching the cornea. Rats were exposed to a steady background-adapting field (30 cd/m²) for 10 min to saturate the rod photoreceptors, further isolating the cone pathway function. After the

light-adaptation period, animals were presented with $2.0 \log \text{ cd s/m}^2$ flicker stimuli at 6 Hz in the presence of the background light. Responses were recorded using the same signal averaging system (UTAS bigShot; LKC Technologies, Gaithersburg, MD). After testing, rats received yohimbine (2.1 mg/kg) to reverse the effects of xylazine and to prevent corneal ulcers (Turner and Albassam et al., 2005). For light adapted ERG responses, flicker response implicit times were measured from the trough of the signal after the flash onset to the peak.

Cognitive Testing

Y-maze

The y-maze (San Diego Instruments, San Diego, CA) was utilized every 2 weeks for 8 weeks to test the spatial working memory of the rats and was based upon the methods of Maurice and colleagues (1995). Each rat was placed in one end of the y-maze and was allowed to explore the maze freely for 8 minutes. The series of arm entries were visually recorded. An alternation was defined as entering all 3 arms consecutively. The percentage of spatial alternation was calculated as follows: $\text{number of correct alternations} / (\text{total number of arm entries} - 2) \times 100$.

Novel Object Recognition

Novel object recognition paradigms were used every 2 weeks for 8 weeks to check specific components of long-term memory in the rats, and two versions of this task were used. In the first version of novel object recognition (NOR), object-only memory was tested (Dere et al., 2007). Rats were allowed to habituate to the testing box for 5 minutes. Two different objects were then placed in the box and the rat was allowed to explore them for 3 minutes, which was the study phase. The rat was then removed from the box for a 2 minute retention interval while one of the objects was replaced with a novel object and the other object was replaced with a

duplicate. In the trial phase, the rat was allowed to explore the objects for 3 minutes. The testing session was recorded with a video camera and scored blindly. The configuration of objects was counterbalanced and randomized over trials. Each animal underwent two complete sessions with objects differing from Session 1 to Session 2. A ratio to determine a discrimination index was calculated as follows: (time spent with novel object) / (time spent with novel object + familiar object). In the second version of this novel object recognition paradigm, object-in-location (OIP) memory was tested (Dr. Joseph Manns, Emory University, personal communication). The procedure was the same as mentioned above except 3 objects were placed in the box for the initial study phase, and during the test phase 2 of the objects swapped locations while the third object remained in the same location all using duplicates. A ratio to determine a discrimination index was then calculated as follows: (time spent with swapped object 1 + swapped object 2) / (time spent with the swapped objects + time spent with the unmoved object). Videos were coded and scored blindly by two different individuals to check for reliability.

Exercise Regimens

We randomized adult, male Long Evans rats (325-350 g; Charles River, Wilmington, MA) into 4 groups: Control (n = 9-15) Control + Active (n = 3-10), Diabetic (n = 11-15), Diabetic + Active (n = 6-12). On post-natal day 72, rats were injected with vehicle (citrate buffer pH 4.0) or streptozotocin (STZ; 100mg/kg body weight; Sigma-Aldrich, Inc., Milwaukee, WI) via the penile vein. Diabetes was defined as two successive daily blood glucose levels higher than 250 mg/dL after injection of STZ. Respective members of each group were then placed in individual lanes of either an active or inactive treadmill (Exer-3/6, Columbus Instruments, Columbus, OH). Those assigned to the exercise condition ran on the treadmill at 15 meters/min for 30 minutes/day, 5 days/week for 8 weeks, while those assigned to the inactive treadmill were

placed in an identical treadmill for the same duration of time. Rats received a maximum of 10 shocks from a metal grating at the base of the treadmill (1 Hz, 0.46 mA) during exercise sessions if they stepped off the moving belt.

Inhibition of BDNF Signaling with TrkB Antagonist ANA-12

We randomized adult, male Long Evans rats (325-350 g; Charles River, Wilmington, MA) into 8 groups: Control Active + Vehicle (n=3), Control Active + ANA-12 (n= 2), Control Inactive + Vehicle (n=3), Control Inactive + ANA-12 (n=3), Diabetic Active + Vehicle (n=6-8), Diabetic Active + ANA-12 (n=4-8), Diabetic Inactive + Vehicle (n=4-7), and Diabetic Inactive + ANA-12 (n=1-7). This experimental design was identical to the methodology mentioned above except all groups were either given a dose of ANA-12 (Sigma-Aldrich Inc., Milwaukee, WI; TrkB antagonist; .5 mg/kg) or vehicle (1% DMSO, 16.5% CremophorEL; 16.5% ethanol, 66% Dulbecco's PBS, pH 7.4) 2.5 hours before exercise, which has been shown previously to align peak TrkB inhibition with exercise (Cazorla et al., 2011; Lawson et al., 2014).

Statistical Analyses

We performed one- and two-way repeated measures ANOVAs and Student's t-tests using commercial statistical analysis software (SigmaStat 3.5; Systat Software; Chicago, IL). We set significance at $p < 0.05$ for all analyses and values are expressed as mean \pm sem. The interaction effect of the ANOVA is reported, unless otherwise stated. We performed post-hoc multiple comparisons using the Holm-Sidak method.

Results

Diabetes reduces retina and cognitive function

We examined both visual and retinal function using OKT and ERG. OKT showed significant differences in spatial frequency thresholds between Diabetic and Control groups (Two-way repeated ANOVA, $F(4,89)=5.17$, $p=0.001$; Figure 1A). Diabetic animals had significantly decreased spatial frequency thresholds compared to control animals at 4, 6, and 8 weeks ($p<0.01$; Holm-Sidak post-hoc comparison; Figure 1A). There were also OKT differences in contrast sensitivity between Diabetic and Control groups (Two-way repeated ANOVA, $F(4,100)=27.28$, $p<0.001$; Figure 1B). Diabetic animals had significantly decreased contrast sensitivity thresholds at 2, 4, 6, and 8 weeks ($p<0.01$; Holm-Sidak post-hoc analyses; Figure 1B). For light-adapted flicker responses measured by ERG at 8 weeks post-STZ, the flicker response was significantly delayed in the Diabetic group compared to the Control group (Control 215.0 ± 1.18 , Diabetic 221.3 ± 1.49 ; Student's t-test, $p<0.01$; Figure 1C).

To assess cognitive function, we performed two types of tests: Y-maze for spatial memory (Maurice et al., 1995) and novel object recognition for object-recognition and object-in-place memory (Dere et al., 2007). In the Y-maze spatial alternation task, there was a significant difference in percent spatial alternation between Diabetic and Control animals at all time points, with Diabetic animals performing worse (Two-way repeated ANOVA, main effect of group, $F(1,50)=7.51$, $p=0.01$; Figure 2A). There were no significant differences between groups at any time point in the NOR and OIP testing (Figure 2B; Figure 2C).

Exercise benefits diabetic retinopathy but not cognitive dysfunction

Exercise slowed contrast sensitivity and retinal function loss, but not visual acuity loss. In the exercise treatment groups, we found significant differences in spatial frequency thresholds between Control and Diabetic groups (Two-way repeated ANOVA, $F(12,193)= 2.38$, $p<0.01$; Figure 3A). By 6 and 8 weeks post-STZ, both Diabetic groups had significantly lower spatial frequency thresholds than the Control or Control + Active groups ($p<0.01$; Holm-Sidak post-hoc comparison; Figure 3A) indicating no effect of exercise. However, contrast sensitivity showed significant differences between Diabetic and Control groups with exercise (Two-way repeated ANOVA, $F(12,204)= 8.46$, $p<0.001$; Figure 3B). All Diabetic groups showed significantly decreased contrast sensitivity thresholds relative to the control groups at 4, 6, and 8 weeks ($p<.001$; Holm-Sidak post-hoc analyses; Figure 3B). Importantly, the Active Diabetic group had significantly higher contrast sensitivity thresholds compared to the Inactive Diabetic group at 4, 6, and 8 weeks, indicating a beneficial effect of exercise ($p<0.05$; Holm-Sidak post-hoc analyses; Figure 3B). Delays in flicker implicit time with diabetes were prevented with exercise. In the light-adapted flicker response measured at 8 weeks post-STZ, the Active Diabetic group had significantly faster implicit times that were indistinguishable from the Control and Active Control groups (One-way ANOVA, $F(3,31) = 7.78$, $p=0.001$; Figure 3C).

In the y-maze spatial alternation task, measured at 8 weeks post-STZ, there was a trend for Active Diabetic rats to have higher percent of spatial alternations that were similar to the Control group (One-way ANOVA, $F(3,41)=3.22$, $p<0.05$; Figure 4A). There were no significant differences between any groups in the OIP novel object recognition paradigms at 8 weeks post-STZ with exercise treatment (Figure 4B).

Inhibition of BDNF Signaling with TrkB Antagonist ANA-12

To determine if BDNF was mediating the protective effects of exercise seen in the visual and photoreceptor function, we pre-treated the Active groups with the TrkB receptor antagonist ANA-12. While spatial frequency thresholds were significantly different between Control and all Diabetic groups at 8 weeks post-STZ (Two way repeated ANOVA, $F(16,192)= 3.86$, $p<0.001$ and Holm-Sidak post-hoc analyses, $p<0.01$; Figure 5A) significant differences between Active Diabetic + Vehicle and Active Diabetic + ANA-12 were found at 4 and 8 weeks (Holm-Sidak post-hoc analyses $p<0.01$; Figure 5A). ANA-12 showed a more robust effect on blocking the protective effects of exercise on contrast sensitivity. There were significant differences in contrast sensitivity between Control and all Diabetic groups (Two-way repeated ANOVA, $F(16,183)=2.75$, $p<0.001$; Figure 5B) with additional significant differences between Active Diabetic + Vehicle and Active Diabetic + ANA-12 at 6 weeks (Holm-Sidak post-hoc analyses $p<0.01$; Figure 5B). Importantly, there were no significant differences over time between Active Diabetic+ ANA-12 and Inactive Diabetic groups, indicating that ANA-12 blocked the protective effects of exercise. The protective effect of exercise on retinal function was also mediated by BDNF. ERG flicker implicit time also showed significant differences between Active Diabetic + Vehicle and Active Diabetic + ANA-12 at 8 weeks post-STZ (One way ANOVA, $F(4,52) = 7.94$, $p<0.001$; Holm-Sidak post-hoc analyses, $p<0.01$; Figure 5C). The Active Diabetic + ANA12 group was similar to the Inactive Diabetic groups, indicating that ANA-12 blocked the protective effects of exercise (Figure 5C).

In the y-maze spatial alternation task measured at 8 weeks post-STZ for the ANA-12 treated animals, we did not see the same trends for Diabetic rats having reduced performance compared to Controls (Figure 6A). There were no significant differences in the OIP novel object

recognition paradigms at 8 weeks post-STZ, exercise treatment, and inhibition of BDNF between any groups (Figure 6B).

Discussion

In this study, we assessed whether there were differences in visual and cognitive function between diabetic and control animals, and whether exercise could provide protection against the decline in retinal and cognitive function seen in diabetic animals. We then sought to demonstrate the role of BDNF as a mediator for the protective effects of exercise in both the eye and the brain. We found that there was a significant decline in diabetic visual acuity and contrast sensitivity, delays in flicker implicit time, and decreased spatial working memory. The significant decline in diabetic contrast sensitivity and delays in flicker implicit time was prevented with exercise and was mediated by BDNF.

Early visual deficits in diabetes may predict clinically-diagnosed retinopathy

The early changes in visual and retinal function we see in diabetic animals are consistent with the literature (Aung et al., 2013; Pardue et al., 2014). Similar to these studies, we found changes in visual acuity, contrast sensitivity, and flicker implicit time at 8 weeks post-STZ, indicating that measureable changes in visual function are occurring prior to clinically recognized vascular changes. These results are important because they provide a way to detect visual changes associated with diabetes long before a clinical diagnosis is typically made, which may lead to better outcomes for people with this disease. Although clinically diagnosed as a vascular disease (Cheung et al., 2007), our visual and cognitive testing results indicate that changes in neuronal tissue are also occurring, which agrees with the literature (Pardue et al.,

2014). However, it is unclear whether these neuronal changes are confounded by previous changes in vasculature or are occurring independently.

Hyperglycemia selectively alters cognitive function

While there was a significant difference between Diabetic and Control animals in the y-maze spatial alternation task, there was no significant difference at any time point between any of the Diabetic and Control groups in any novel object paradigm. These results may suggest that y-maze and 2 object novel object recognition (NOR) are mediated by different brain regions with selective susceptibility to hyperglycemic damage. Several studies suggest that the hippocampus does not contribute to short-term object recognition memory, but plays a strong role in spatial working memory. For instance, using localized brain lesions, Mumby and colleagues (2002) found that the rodent hippocampus was not required for the retrieval of object information after a short retention interval of less than 5 minutes. Additionally, almost complete hippocampal lesions produced deficits in object recognition only after 3 hours of retention, while 50-75% lesioned hippocampus produced no deficits, indicating that the hippocampus plays a minimal role in object recognition (Broadbent et al., 2004). Finally, immediate gene expression and electrophysiology after object recognition tasks argue against the role of the hippocampus in object recognition trials with a short delay (Dere et al., 2007). In contrast, the hippocampus has been shown to play a strong role in spatial memory tasks (Burgess et al., 2002), which include spontaneous alternation in a y-maze (Kokkinidis et al., 1976). Because the spatial alternation task relies on the hippocampus while the 2 object novel object paradigm (NOR) does not, this may suggest that the hippocampus is more susceptible to the early pathological changes in diabetes, as suggested by other studies of Type 1 diabetes (Auer et al., 1989b).

It is unclear as to why there were no deficits seen in the object-in-place object recognition task, which is a spatial task that relies on the hippocampus (Dere et al., 2007). Our stark deficits in early visual deficits between Diabetic and Control animals indicate that perhaps the y-maze is a spatial memory task that relies more on vision, as animals rely on visual cues in the room to make alternations. In the OIP task, animals are allowed to freely explore objects with the sense of touch as well as vision, making it less of a visual task than the y-maze. Thus, it is possible that the deficits we see in Diabetic animals compared to Controls in spatial alternation may actually be explained by the early differences in vision. To further investigate this, the y-maze could incorporate differentiating tactile features in each leg of the maze to make the task less dependent on vision.

Exercise protects against early diabetic retinal dysfunction

While other studies have shown exercise to benefit hippocampal proteins in diabetes (Diegues et al., 2014), this study examined whether exercise provides protection against early changes in diabetic visual and retinal function. Exercise has protective effects on the deficits seen in contrast sensitivity and flicker implicit time, but does not seem to have the same effect on visual acuity. This may be because the deficits seen in Diabetic animals compared to Controls in contrast sensitivity and flicker implicit time were so much greater than the deficits seen in visual acuity, and thus there was more function to recover. Additionally, the exercise treatment used in our experiment only spanned the short period of 8 weeks. There is evidence that neuronal changes, such as arborization in the brain, can take up to 12 weeks to occur after implementation of an exercise paradigm in rodents (Stranahan et al., 2009). Similar to the results demonstrating the protective effects of exercise on the light damaged retina were mediated by BDNF (Lawson et al., 2014), we show that BDNF mediates the protective effects of exercise in the diabetic rats.

BDNF seems to be an important mediator for the protective effects of exercise in different retinal disease models, which has implications for the role of BDNF in the health of the retina.

Exercise has minimal benefit to diabetic cognitive dysfunction

It is unclear as to why exercise did not have the same protective effects on diabetic cognitive function as it did on visual and retinal function. As mentioned earlier, this may be because the differences seen in the spatial alternation task between diabetic and control animals may be due to the differences in vision. However, this finding goes against several studies that suggest not only that exercise increases hippocampal dendritic spine density, (Stranahan et al, 2009) but that exercise can also improve the spatial memory decline seen in diabetic animals (Diegues et al., 2014). Again, perhaps the deficit seen in spatial working memory could be lessened if the exercise paradigm was extended past 8 weeks duration, which could be a possible future direction of this study. Additionally, there is evidence that forced exercise may negatively impact protective outcomes. Rats who received a chronic foot shock have been shown to have impaired hippocampal neurogenesis (Dagyte et al., 2009). In the treadmill paradigm used here, the foot shock may have contributed to not finding the same protective effects with exercise on diabetic cognitive function as on visual and photoreceptor function. Additionally, it was peculiar that there was not even a baseline difference between control and diabetic groups in spatial alternation in the inhibition of BDNF signaling experimental paradigm. Because we found a significant deficit in spatial alternation in Diabetic animals compared to Control animals in our results leading up to this experiment, it seems that results of the BDNF inhibition experiments concerning spatial alternation may not be valid, partially because the group numbers are so small in the inhibition of BDNF signaling experiment compared to the groups in the other aspects of the study.

Early diabetic dysfunction in the brain versus retina

Our results indicate that there are no significant correlations between deficits in visual function and cognitive function (data not shown). These results suggest that deficits in the eye do not predict deficits in the brain and vice versa at the time points tested. This finding is contrary to a study that found an inverse relationship with the severity of diabetic retinopathy and cognitive impairment (Crosby-Nwaobi et al., 2013), concluding that cognitive impairment in diabetes may be associated with factors other than microvascular disease. However, this study did not examine neuronal changes in visual function as our study does, and instead focuses on vascular changes. Additionally, our data indicates that visual function may be more susceptible to early diabetes than cognitive function, as there were deficits in all three tests of visual function but there were only deficits in one test of cognitive function. This agrees with other studies of cognition and diabetes, which suggest that cognitive decline associated with diabetes may be associated with aging-related decline (Yeung et al., 2009), while our study is only examining the very early cognitive changes associated with diabetes.

Conclusions

Our data suggest that visual and cognitive dysfunction is associated with early stage diabetes, and that visual dysfunction can be prevented with exercise. Future studies should examine histological and protein quantification to understand the mechanisms underlying the dysfunction, as well as quantify levels of BDNF in the retina and brain. Exercise has promise as a clinically relevant intervention because of its relatively cheap and easy implementation; therefore, our study has exciting clinical significance for those suffering from Type 1 diabetes.

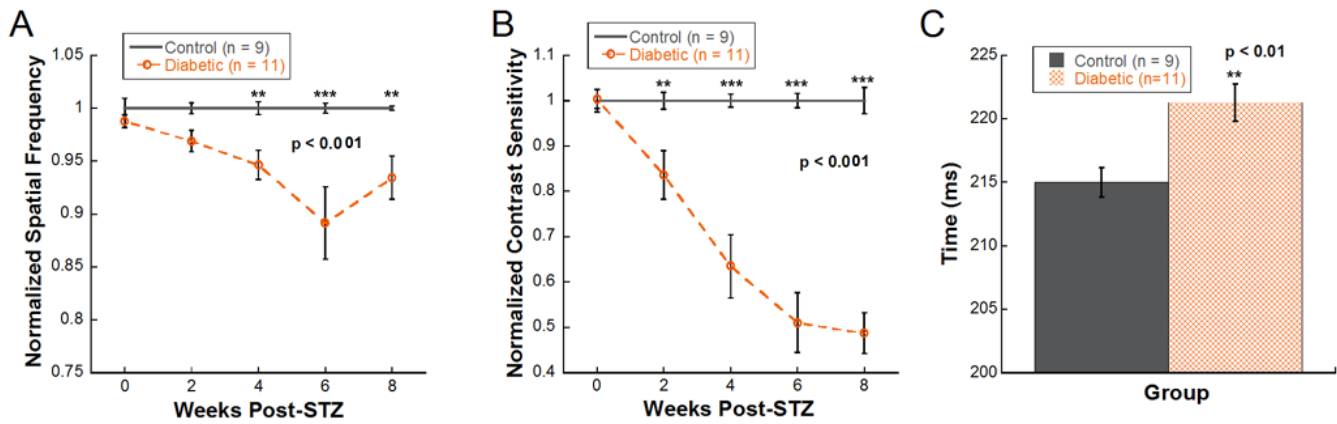


Figure 1. Visual deficits are present in diabetic animals. (A) Diabetic animals had significantly decreased spatial frequency thresholds compared to control animals (Two-way repeated ANOVA, $F(4,89) = 5.17$, $p = 0.001$). (B) Diabetic animals also had significantly decreased contrast sensitivity compared to control animals (Two-way repeated ANOVA, $F(4,100) = 27.28$, $p < 0.001$). (C) At 8 weeks post-STZ, the light-adapted flicker response was significantly delayed in the diabetic group compared to the control group (Control 215.0 ± 1.18 , Diabetic 221.3 ± 1.49 ; Student's t-test, $p < 0.01$). Holm-sidak post-hoc comparisons: ** $p < 0.01$, *** $p < 0.001$

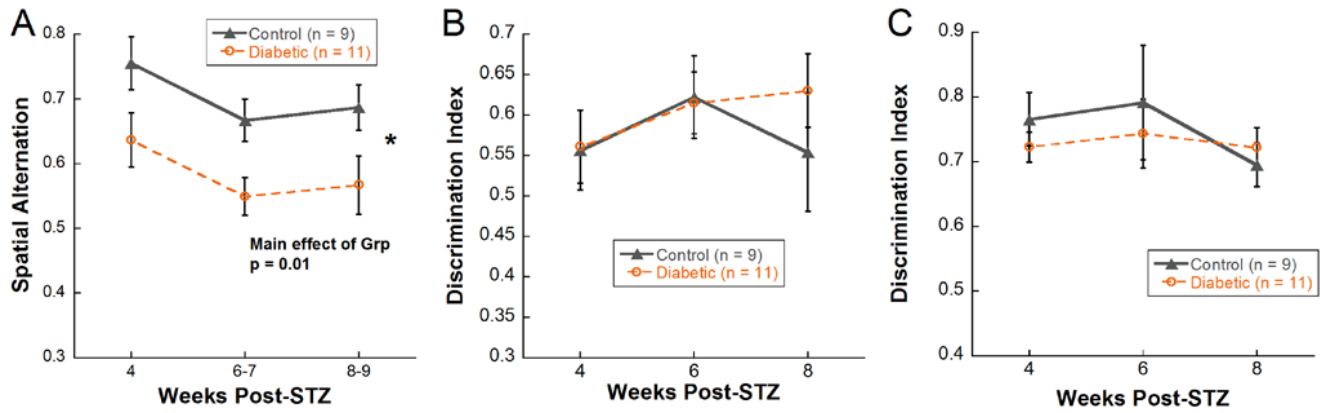


Figure 2. Cognitive deficits in spatial alternation are present in diabetic animals. (A) In the Y-maze spatial alternation task, diabetic animals performed significantly worse at all time points (Two-way repeated ANOVA, main effect of group, $F(1,50)=7.51$, $p=0.01$). There was no difference over time or between groups in the NOR (B) or OIP (C) novel object paradigms.

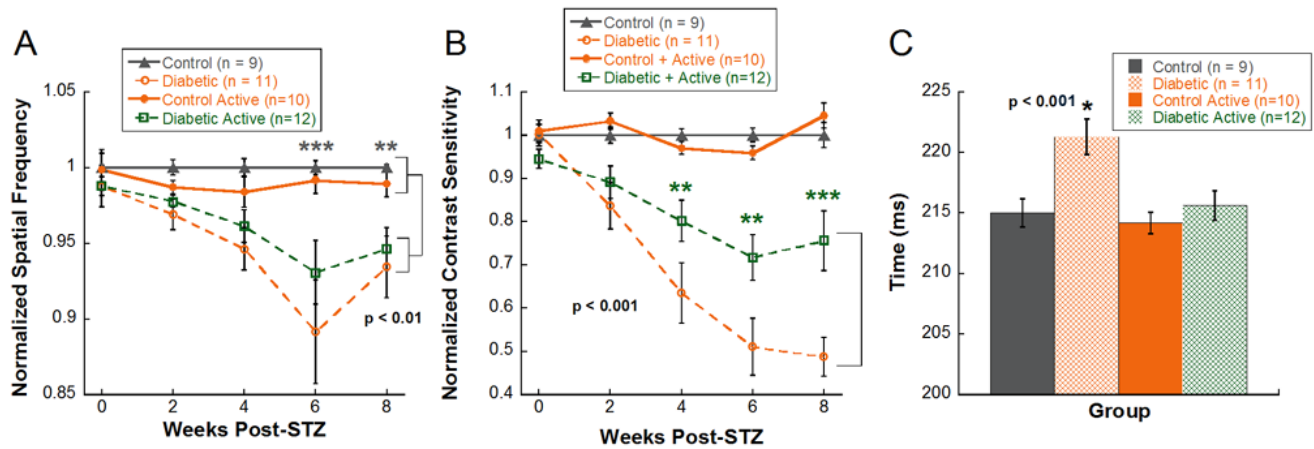


Figure 3. Visual function in diabetic animals was partially protected by exercise. (A) By 6 and 8 weeks post-STZ, both Diabetic groups had significantly lower spatial frequency thresholds than the Control or Active Control groups, ($F(12,193)= 2.38, p<0.01$) although no significant differences between the Diabetic Active and Diabetic group. (B) The Diabetic Active group had significantly higher contrast sensitivity thresholds compared to the Diabetic Inactive group at 4, 6, and 8 weeks, indicating a beneficial effect of exercise (Two-way repeated ANOVA, $F(12,204)= 8.46, p<0.001$) (C) In the light-adapted flicker response measured at 8 weeks post-STZ, the Active Diabetic group had significantly faster implicit times that were indistinguishable from the Control and Control Active groups (One-way ANOVA, $F(3,31) = 7.78, p=0.001$). Holm-sidak post-hoc comparisons: ** $p<0.01$, *** $p<0.001$

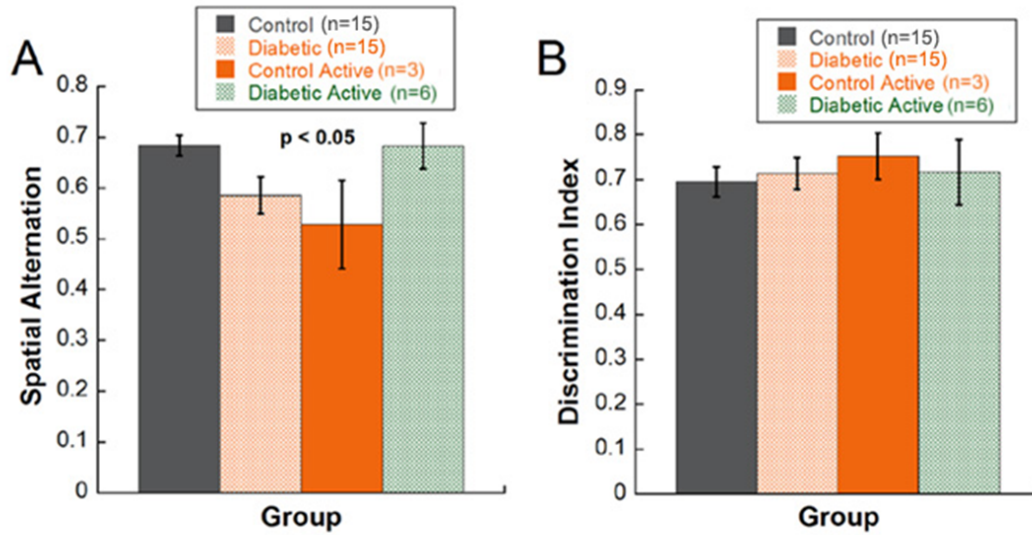


Figure 4. Exercise partially protects against diabetic cognitive dysfunction. (A) In the y-maze spatial alternation task measured at 8 weeks post-STZ, there was a trend for Active Diabetic rats to have a higher percent of spatial alternations that were similar to the Control group (One-way ANOVA, $F(3,41)=3.22$, $p<0.05$). (B) There were no significant differences between any groups in the OIP novel object recognition paradigms with exercise treatment at 8 weeks post-STZ.

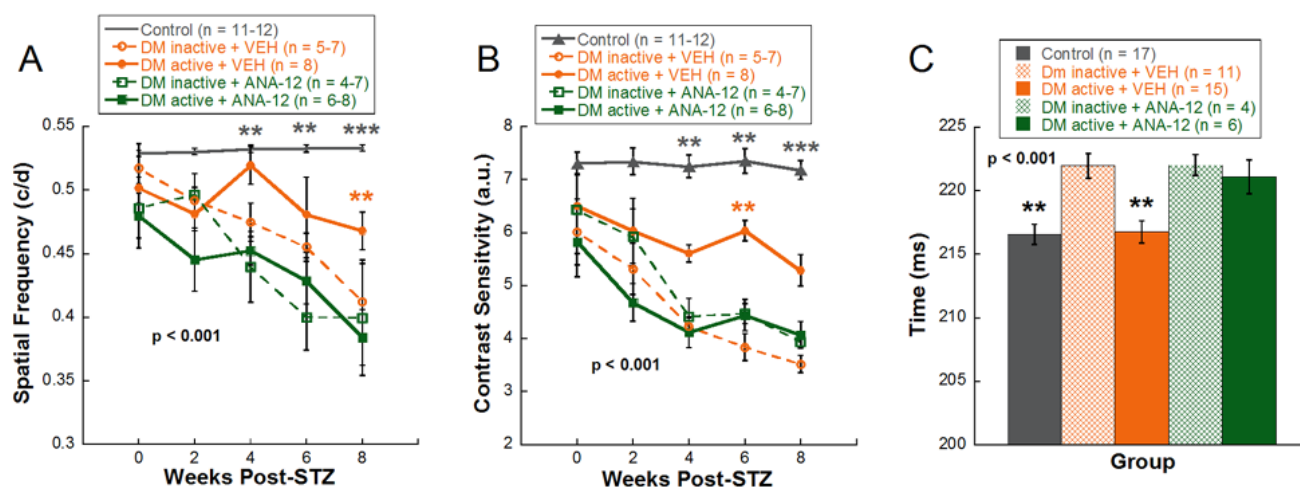


Figure 5. BDNF partially mediates the protective effects of exercise. (A) Spatial frequency thresholds were significantly different between Control and all Diabetic groups at 8 weeks post-STZ, while significant differences between Diabetic Active + Vehicle and Diabetic Active + ANA-12 were found only at 4 and 8 weeks ($F(16,192) = 3.86$, $p < 0.001$). (B) There were significant differences in contrast sensitivity between Control and all Diabetic groups (Two-way repeated ANOVA, $F(16,183) = 2.75$, $p < 0.001$) with additional significant differences between Diabetic Active + Vehicle and Diabetic Active + ANA-12 at 6 weeks. There were no significant differences over time between Active Diabetic + ANA-12 and Inactive Diabetic groups. (C) ERG flicker implicit time also showed significant differences between Diabetic Active + Vehicle and Diabetic Active + ANA-12 at 8 weeks post-STZ (One way ANOVA, $F(4,52) = 7.94$, $p < 0.001$). The Diabetic Active + ANA12 group was statistically similar to the Diabetic Inactive groups. Holm-sidak post-hoc comparisons: ** $p < 0.01$, *** $p < 0.001$

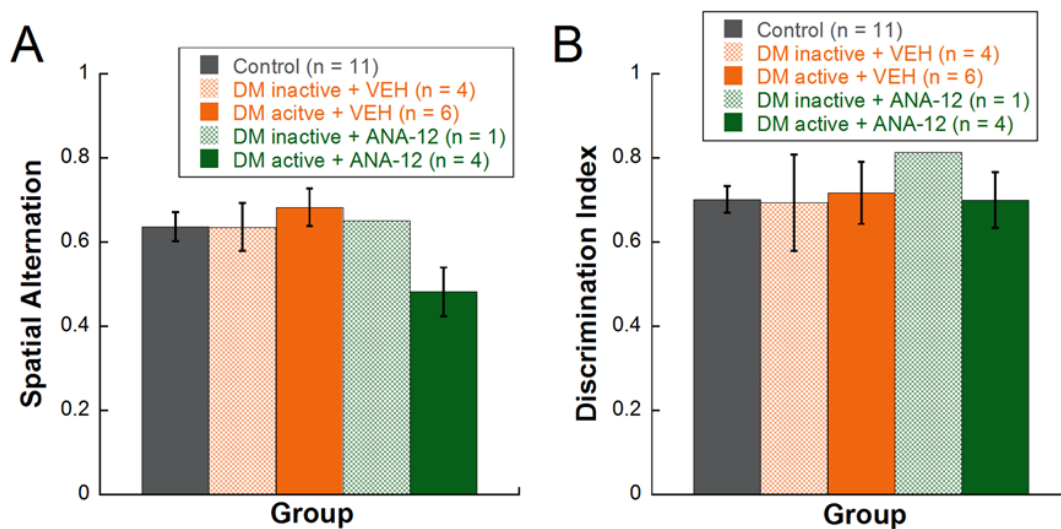


Figure 6. (A) In the y-maze spatial alternation task measured at 8 weeks post-STZ for the ANA-12 treated animals, the same trends for Diabetic rats having reduced performance compared to Controls were not observed. (B) There were no significant differences between groups in the OIP novel object recognition paradigms at 8 weeks post-STZ, exercise treatment, and BDNF inhibition treatment.

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