Pupil light reflex in Parkinson's disease patients with and without freezing of gait symptoms

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Abstract:

PURPOSE: Freezing of gait (FOG) is considered as a motor disorder that affects some Parkinson's disease (PD) patients; however, sensory systems may also be involved in FOG. The pupil light reflex (PLR) is a reliable measure of the autonomic nervous system. Different dilation and constriction pupil parameters may be used to investigate the integrity of the autonomic nervous system in PD patients with and without FOG symptoms. This study aimed to look at the integrity of autonomic nervous system and to investigate the nonmotor functions mediated by the cholinergic system in Parkinson's patients with and without FOG symptoms.

METHODS: Constriction and dilation pupil light reflexes were measured by using a handheld pupillometer. Twenty-two patients with FOG symptoms, 25 patients without FOG symptoms, and 25 aged-matched healthy controls participated in this study.

RESULTS: The results showed that most of the constriction parameters and dilation latency of both patient groups differed significantly from healthy controls. FOG patients showed larger pupil size under light condition and larger deficits in constriction latency than nonFOG patients. Both the groups of PD patients had longer dilation latencies than healthy controls.

CONCLUSION: This study suggests that the cholinergic autonomic nervous system is affected in PD patients more than the adrenergic system. FOG patients had larger impairments in nondopaminergic mediated functions such as pupil light reflexes, which suggests that FOG patients have greater impairment in functions that involve cholinergic neurotransmitters.

Keywords:

Autonomic nervous system, freezing of gait, nonmotor, Parkinson's disease, pupil light reflex, sensory

INTRODUCTION

Parkinson's disease (PD) is usually described as a motor disorder disease that affects the central and peripheral nervous systems. Patients with PD are characterized by having body motor dysfunctions such as bradykinesia, muscle rigidity, resting tremor, and postural instability. The main cause of the PD motor disorders is believed to be due to reduction of the dopamine neurotransmitter through cell death within the basal ganglia complex in midbrain.[1-4]

PD patients are also characterized by nonmotor symptoms and signs that are believed to be due to cholinergic system dysfunctions. Deficits in the cortical cholinergic systems are linked to learning and executive functions. Calabresi *et al*.[5] hypothesize that some of the cognitive deficits in PD patients are due to a combination of dopamine and acetylcholine depletion because an increase in dopamine is not sufficient to affect certain cognitive performance, and acetylcholinesterase inhibitors are useful in the treatment of dementia associated with PD. They further hypothesized that at the cellular level, dopamine and acetylcholine interact to produce the synaptic changes associated with learning and memory.[5] This interaction is altered in PD and so these patients experience problems with working memory and learning tasks. Given these findings, it is not surprising that different

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sensory and cognitive functions are impaired along with motor functions in PD,[6] despite James Parkinson's statement in his opening chapter that "the senses and intellect being uninjured" in his detailed description of the disease bearing his name.[7]

Within the autonomic nervous system, acetylcholine dysfunctions include problems in cardiovascular, sexual and urinary gastrointestinal, respiratory, and thermoregulation systems. In the visual system, different parameters of pupil light reflex (PLR) are affected in PD patients.[8-10] Previous studies showed that the constriction latency, amplitude of constriction, maximum constriction velocity (MCV), and maximum constriction acceleration are affected in PD patients. These studies suggest that a dopamine deficiency in the retina or cortex is not responsible for the changes in the different pupillometric parameters because there was no correlation with any other motor symptoms of the disease.[11,12] Furthermore, there are more PLR parameters affected in cognitive impaired PD patients than those patients who have normal cognitive function.[13] PLR parameters of cognitive impaired PD patients were similar to the pupil dysfunction reported in Alzheimer's disease patients. This suggests that both the groups of patients have the same central cholinergic (parasympathetic) deficit in the acetylcholinergic pathways in the frontal lobe.[13,14]

Freezing of gait (FOG) is one of the motor disturbances associated with PD disease. It is defined as discontinuous or interrupted episodes of inability to produce or maintain a forward movement or to make a turn. The episodes usually last a few seconds. Although FOG is considered classically as motor dysfunction in PD patients, it is now hypothesized that impairment of different nonmotor systems may contribute to FOG.[15]

Amboni *et al.* reported that FOG and its severity are associated with frontal cognitive dysfunction and the severity of the frontal cognitive dysfunction, respectively.[16] It is possible that these frontal lobe dysfunctions are a result of an acetylcholine deficit. Furthermore, the deficit in this neurotransmitter may be throughout the central nervous system and so patients who experience gait freezing may show a greater impairment of parasympathetic function (e.g., PLR) than those PD patients who do not experience gait freezing. Since there are no comparisons between FOG PD and non-FOG PD patients using PLR parameters, to our knowledge, we measured various PRL parameters to determine whether FOG PD patients have a more generalized cholinergic deficit, or a deficit in both the parasympathetic and sympathetic systems innervating the eye.

METHODS

Participants

PD patients and healthy controls were recruited from the Sun Life Financial Movement Disorders Research and Rehabilitation Center, Wilfrid Laurier University (Waterloo, ON) database. The subjects gave informed written consent before participating. The study followed the tenets of the Declaration of Helsinki.

The subject groups were as follows:
1. On-medication FOG and non-FOG PD subjects. All subjects met the criteria of PD according to the MDS-UPDRS scale system.[17,18] Patients with other neurological disorders, brain lesions, or concussions were excluded. FOG versus non-FOG subjects were determined based on the FOG questionnaire for PD patients[17,18]
2. Age-matched healthy control group. Subjects free from any neurological disorders, brain damage history, positive history of PD, or concussions.

The exclusion criteria for all participants were a history of diabetes, nystagmus, strabismus, and corrected visual acuity worse than 20/30 at distance or near in either eye. The severity of the disease and the freezing vs. non-freezing patients were determined first by a qualified examiner according to MDS-UPDRS scaling system.[17,18] The cognitive functions of patients and healthy controls were measured according to The Montreal Cognitive Assessment Test (MoCA).[19] Twenty-two FOG PD patients, 25 non-FOG PD patients, and 25 healthy controls participated in this study.

Table 1 shows the mean values (mean ± 1 standard deviation) of different demographic characteristics of the participants and whether the differences were significant between groups.

Procedures

The PLRs parameters were measured using a NeurOptics PLR™ 3000 Pupillometer (NeurOptics, Inc. Irvine, CA, USA). The PLR-3000 is a handheld monocular pupillometer that can measure both pupil constriction and pupil dilation parameters. PLR-3000 records the pupil size using an infrared camera (32 frames/sec) and can measure the pupil size to within ± 0.03 mm.

To measure the pupil constriction parameters, bright stimuli flash against a dark background. To measure pupil dilation parameters, the subjects adapt to a steady light and then it is extinguished for a brief period. Table 2 lists all stimuli characteristics used in this study for the constriction and dilation conditions.

Table 1: Means and standard deviations of the subjects' demographics

Groups	FOG	Non-FOG	Healthy controls	The differences (*P*)
Sample size (*n*) (male/female)	22 (14/8)	25 (19/6)	25 (8/17)	NA
Age (%)	72.3 (6.9)	67.5 (9.4)	70.43 (7.67)	0.059
Cognitive MoCA score (%)	24.95 (4.27)	25.76 (2.18)	26.48 (2.16)	0.221
Severity UPDRS score (%)	22.41 (7.94)	19.96 (9.58)	NA	0.349
Duration of the disease	10.52 (6.6)	8.08 (6.35)	NA	0.203

FOG=Freezing of gait; MoCA=Montreal cognitive assessment; UPDRS=Unified Parkinson's Disease Rating Scale; NA=Not applicable

uW: Microwatts

The measurement began with an explanation and demonstration of the procedure. Next, participants adapted to a darkened room for 5 min. This time allows pupils to expand to the maximum amount. The illuminance on the participants' chair during the dark condition was <0.1 lx. Pupil constriction was measured first for the right eye and then the left eye. This measurement sequence was repeated two more times with 30 s intervals between measurements.

The procedures for dilation measurements were similar to the constriction condition measurements.

If a participant blinked during the measurement, then the data were deleted and another measurement was made. The pupillometer software calculates the various parameters automatically. The data for the three trials were downloaded to a computer for analysis. Table 3 lists PLR parameters that were calculated by the onboard software for constriction and dilation conditions.

Statistical analysis

Constriction and dilation PLRs parameters were measured three times on each eye. The average from the three measurements was calculated for each eye separately. The right and left eye parameters were compared by a paired *t*-test for each group separately. Results showed that none of the PLRs comparisons were statistically significant between eyes for any group. For this reason, the average values of the two eyes were used for further comparisons.

Table 2: Stimuli characteristics used to measure pupil light reflexs

Protocol	Constriction condition	Dilation condition
Definition	Stimuli brighter than background	Stimuli dimmer than the background
Stimulus intensity (uW)	50	0
Background intensity (uW)	0	50
Measurement duration (s)	5	5
Stimulus duration (s)	0.07	1.07

Differences between groups were examined using one-way analysis of variance (ANOVA) with Tukey's *post hoc* tests to examine all pairwise comparisons between groups. The second analysis examined the associations between different PLRs and the severity, duration, and MoCA scores in PD patients by calculating the Pearson correlation coefficients. IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. was used for this data analysis. The criterion of $P < 0.05$ was used to determine statistical significance.
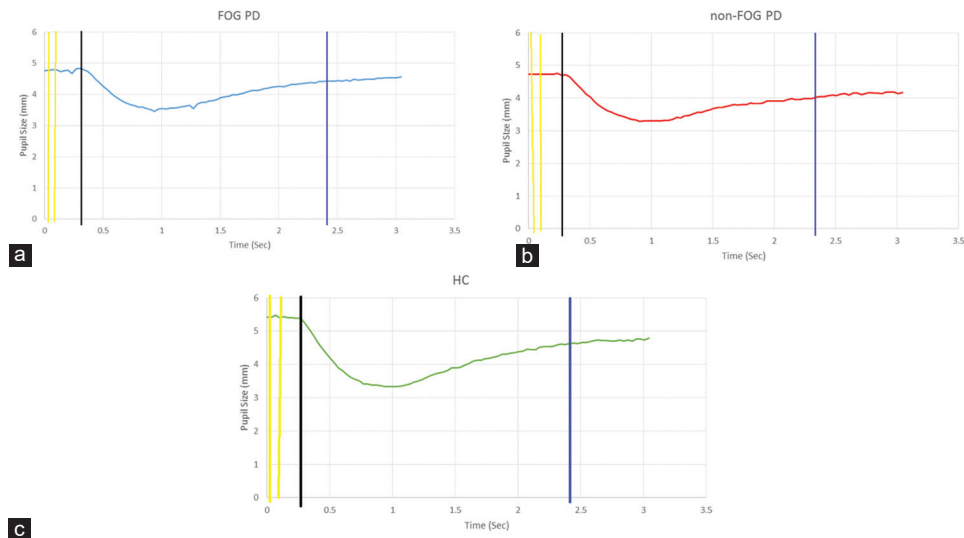
RESULTS

Constriction pupil light reflex

Figure 1 shows representative data of the pupil diameter as a function of time after the eye was stimulated with the white light pulse. Table 4 shows means and standard error of the means of different constriction parameters. Table 5 shows the ANOVA results and the pairwise multiple comparisons between groups for those parameters that showed a significant group effect. There was a significant group effect for all the constriction parameters, except the minimum diameter (End) (F = 2.193, DF = 2, 69, $P = 0.119$), and the re-dilation velocity (re-ADV) (F = 2.112, DF = 2, 69, $P = 0.129$). Pairwise comparisons showed, however, that the group effect was primarily due to the differences between the one, or both, of the PD groups and the HC for most parameters. The only significant difference between the FOG and non-FOG PD was the constriction latency (LAT-C), and T 75%.

Dilation pupil light reflex

Figure 2 shows representative data of the pupil diameter as a function of time after the light was extinguished for 1.03 s. Table 6 lists the means and standard error of the means of different pupil dilation parameters for all groups. One-way ANOVA showed that the differences between groups were statistically significant for all dilation PLR parameters except the dilation percentage of change (Dia %) (F = 1.757, DF = 2, 69, $P = 0.180$) and the dilation velocity (ADV) (F = 1.82,

Table 3: Pupil light reflex parameters for the constriction and dilation measurements

Function	Definition	Unit
Init	Initial dark-adapted pupil size before constriction	mm
End	Pupil diameter at maximum of constriction	mm
Constriction (%)	Init-end/Init	Percentage
LAT-C	Time to onset of constriction	ms
ACV	The average speed of the pupil constriction	mm/s
MCV	The maximum speed of the pupil constriction	mm/s
Re-ADV	The average speed of the pupillary re-dilation after the pupil has reached the peak of constriction	mm/s
75% recovery time (T75%)	The time to reach 75% of the original baseline pupil diameter after the peak of the constriction	s
Init	Initial light adapted pupil size before dilation	mm
End	Pupil diameter at peak of dilation	mm
Dilation (%)	(Init-end)/Init	Percentage
LAT-D	Time to onset of dilation	ms
ADV	The average speed of the pupil dilation	mm/s

LAT-C=Latency of constriction; ACV=Average constriction velocity; MCV=Maximum constriction velocity; LAT-D=Latency of dilation; ADV=Average dilation velocity; Re-ADV=Re-ADV; Init=Initial diameter; End=End diameter

Figure 1: Pupil size response to light stimulus as a function of time in 3 representative participants from each group. The two yellow vertical lines show where the stimulus started and ended, the black vertical line shows the constriction latency, the blue vertical line shows T75%. (a) Freezing of gait Parkinson's disease patient, (b) non-Freezing of gait Parkinson's disease patient, (c) Healthy control subject

Table 4: Means and standard error of means for different constriction pupil light reflexs for all groups

Group	Init (mm)	End (mm)	Amount- constriction (mm)	Constriction (%)	LAT-C (ms)	ACV (mm/s)	MCV (mm/s)	Re-ADV (mm/s)	T75% (s)
FOG (*n*=22)									
Mean	5.22	3.80	1.42	27.81	0.26	2.92	4.15	1.05	1.25
SEM	0.15	0.14	0.07	1.18	0.005	0.10	0.12	0.037	0.09
NonFOG (*n*=25)									
Mean	4.64	3.29	1.32	29.15	0.24	2.95	4.06	1.03	1.50
SEM	0.22	0.18	0.072	1.14	0.01	0.13	0.17	0.07	0.08
HC (*n*=25)									
Mean	5.28	3.59	1.69	32.43	0.21	3.41	4.63	0.90	1.76
SE of Mean	0.20	0.17	0.06	0.96	0.01	0.11	0.14	0.04	0.03

SE=Standard error of mean; FOG=Freezing of gait; Init=Initial diameter; End=End diameter; LAT-C=Latency of constriction; ACV=Average constriction velocity; MCV=Maximum constriction velocity; ADV=Average dilation velocity; Re-ADV=Re-ADV

DF = 2, 69, *P* = 0.169). Table 7 shows the results and the pairwise multiple comparisons between groups for those parameters who showed significant differences between groups.

Relationships between different pupil light reflex parameters and severity, duration, and cognitive abilities of Parkinson's disease patients

Relationships between different PLR parameters with severity (UPDRS score) of the disease, duration of the disease, and the cognitive status (MoCA score) were examined by calculating the Pearson correlation coefficients for FOG and non-FOG PD patient groups separately. Results showed that none of the constriction or dilation PLR parameters correlated significantly with severity, duration, or the cognitive status of the FOG PD patient group. The results for the non-FOG PD patient group were similar for most of the PLR parameters; however, there were two exceptions to this general trend. First, there was a negative and significant correlation ($\rho = -0.428$, *P* = 0.033) between UPDRS score and constriction percent change. That

is, the more severe the disease, the smaller the relative change in pupil size for the non-FOG subjects. Second, there was a negative correlation between MoCA score and T 75% recovery ($\rho = -0.539$, *P* = 0.012). That is, the more cognitive impairment, the longer it takes to re-dilate after the light was extinguished.

DISCUSSION

Nonmotor symptoms due to ANS dysfunctions have been reported in PD patients.[20] Both sympathetic and parasympathetic branches of ANS are known to be affected.[21] Measuring the pupil size under light and dark conditions and measuring different PLR parameters is a relatively easy and noninvasive technique to evaluate the integrity of the ANS sympathetic and parasympathetic pathways.[22]

The main objectives of this study were to examine both constriction and dilation parameters of the PLR to determine whether the cholinergic mediated (parasympathetic) and adrenergic mediated (sympathetic) ANS were differentially affected in FOG PD and non-FOG patients. In addition,
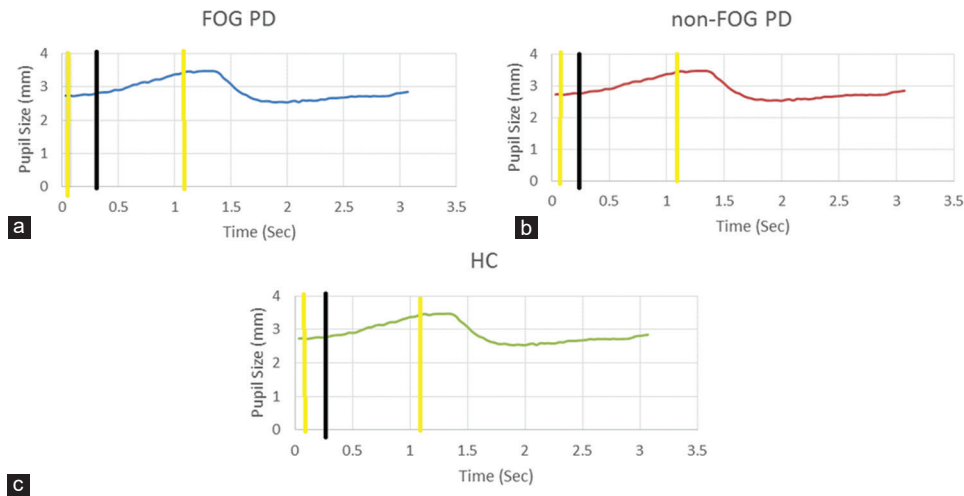
Figure 2: Pupil size response to light stimulus as a function of time in three different participants representing the three subject groups. The two yellow lines show where the stimulus started and ended, the black vertical line shows the dilation latency. (a) Freezing of gait Parkinson's disease patient, (b) non-Freezing of gait Parkinson's disease patient, (c) Healthy control subjects

Table 5: One-way ANOVA tests of constriction pupil light reflexs between groups

Test	ANOVA	DF	*P*	Pairwise multiple comparisons (*P*)		
				Groups	Non-FOG	HC
Initial	3.299	2.69	0.034	FOG	0.1	0.974
				Non-FOG		0.056
Amount-constriction	7.8	2.69	0.001	FOG	0.63	0.021*
				Non-FOG		0.001*
Constriction (%)	4.68	2.69	0.012	FOG	0.672	0.012*
				Non-FOG		0.086
LAT-C	27.128	2.69	<0.001	FOG	0.006*	<0.001*
				Non-FOG		<0.001*
ACV	5.45	2.69	0.006	FOG	0.977	0.014*
				Non-FOG		0.019*
MCV	4.09	2.69	0.021	FOG	0.904	0.084
				Non-FOG		0.024*
T75%	13.652	2.69	<0.001	FOG	0.041*	<0.001*
				Non-FOG		0.022*

*Differences between groups is significant at 0.05 significant level. FOG=Freezing of gait; HC=Healthy controls; LAT-C=Latency of constriction; ACV=Average constriction velocity; MCV=Maximum constriction velocity

the previous clinical results might help disentangle the afferent (sensory) PLR versus efferent (motor) PLR pathways. This information would help us to determine whether the problem originates in the retina or in the central nervous system.

Most of constriction parameters and dilation latency among dilation parameters were significantly different for one or both of PD patient groups compared with healthy controls. Our results were in agreement with previous findings for those common constriction parameters.[11,12] Similar to previous studies, our results suggest that pupil changes could be independent from the dopaminergic deficiency because there was no correlation with any other motor symptoms of the disease except for one case, which could be a spurious correlation.[11,12] In addition,

others have reported that dopaminergic treatment has no effect on different PLR parameters.[23]

Multinomial logistic regressions were conducted for all constriction and dilation PLR parameters separately to determine the best discriminant parameters between groups. The results showed that the final logit regression models adequately fit our data for the constriction parameters (Chi-square test = 96.961, DF = 18, $P < 0.0001$), and for the dilation parameters (Chi-square test = 27.684, DF = 12, $P = 0.006$). Table 8 shows the rank order of different PLR parameters that can best discriminate different subject groups. Except the constriction percentages, the average constriction velocity, and MCV, the other constriction parameters were good discriminators between groups. Dilation latency was the only parameter that could discriminate between groups among dilation parameters. These findings suggest that both parasympathetic and sympathetic ANS pathways were affected in PD patients compared with healthy controls, and the parasympathetic ANS pathway is more affected than the sympathetic ANS pathway in PD patients.

Previous studies showed that MCV and maximum constriction acceleration were the best discriminants among PLR constriction parameters between PD patients and healthy controls.[11,13,14,24] Several reasons could explain different findings of our study compared with the previous studies. First, the temporal resolution of the pupillometric systems was different. The pupillometer in this study had a frame rate of 32 frames per second, whereas the other pupillometric systems were much faster with a frame rate of 263 frames per second.

Second, different studies used different experimental conditions and different stimulus light intensities. We used 50 mW as the stimulus intensity in this study. It could be that this light level was not sufficient to show PLR dysfunctions among some PD patients. Different stimulus intensities can change different

Table 6: Means, standard deviations, and standard error means for different dilation pupil light reflexs for all groups

Group	Initial (mm)	End (mm)	Amount-dilation (mm)	Dilation (%)	LAT-D (ms)	ADV (mm/s)
FOG (*n*=22)						
Mean	2.90	3.43	0.53	19.25	0.40	0.87
SEM	0.09	0.11	0.028	0.92	0.016	0.036
Non-FOG (*n*=25)						
Mean	2.54	2.96	0.41	16.81	0.39	0.74
SEM	0.093	0.10	0.03	1.15	0.010	0.054
HC (*n*=25)						
Mean	2.53	3.02	0.48	19.43	0.34	0.80
SEM	0.06	0.09	0.03	1.20	0.013	0.05

Init=Initial diameter; End=End diameter; LAT-D=Latency of dilation; ADV=Average dilation velocity; FOG=Freezing of gait; HC=Healthy controls; SEM=Standard error of mean

Table 7: One way ANOVA tests of dilation pupil light reflexs between groups

Test	ANOVA	DF	*P*	Pairwise multiple comparisons (*P*)		
				Groups	Non-FOG	HC
Init	5.517	2.69	0.006	FOG	0.014*	0.013*
				Non-FOG		0.99
End	6.049	2.69	0.004	FOG	0.05*	0.018*
				Non-FOG		0.89
Amount-dilation	3.83	2.69	0.027	FOG	0.021*	0.56
				Non-FOG		0.233
LAT-D	5.24	2.69	0.008	FOG	0.75	0.009*
				Non-FOG		0.048*

*Differences between groups is significant at 0.05 significant level. HC=Healthy controls; FOG=Freezing of gait; Init=Initial diameter; End=End diameter; LAT-D=Latency of dilation

PLR responses.[25-27] In addition, the previous studies included PD patients with more severe cases, which could contribute to the difference in results. Another factor is that the previous studies based their conclusions on comparing the ROC curves of the individual parameters. This approach may produce different results from the multinomial logistic regression.

PLR parameters are not solely controlled by the motor responses of ANS. Deficits in the retina or optic nerve could affect PLR as well. Reduction in retinal illuminance levels can reduce light-adapted baseline pupil sizes and produce similar decrements in PLR parameters as found in our study.[25-28] This means that some of the pupil deficits seen in our results could be a result of retinal dysfunction, given the visual acuity and contrast sensitivity losses found in PD subjects.[29]

One PRL parameter deficit that could be either a sensory deficit or parasympathetic motor deficit is the minimum pupil size after constriction (i.e., End).[30] A deficit in either branch of the pathway could produce a larger minimum pupil diameter. Our minimum pupil diameter results do not allow for any further analysis on this parameter because there was no significant difference between groups. This result suggests that the minimum pupil diameter is not a very sensitive parameter for measuring pupil deficits in PD patients.

Another difference that could be due to sensory deficit is a larger initial pupil size under light adaptation (Initial). The larger mean

FOG PD initial pupil size under light conditions suggests that FOG PD group had a larger sensory deficit due to lower retinal inputs. Although a larger pupil size under light suggests sensory deficits, it has been suggested that larger pupil size under light conditions reflects dysfunctions in the parasympathetic (cholinergic) nervous system due to an acetylcholine (ACh) reduction.[31,32] However, this parameter is not considered as a strong indicator of the cholinergic system dysfunction.[24]

A third parameter that is known to reflect the retinal contribution to PLR is the amount of constriction.[13,28,32] Both PD patient groups had a lower amount of constriction compared with healthy controls, which suggests that either their retinal function or optic nerve function was impaired. Constriction latency (LAT-C) is the fourth indicator of the sensory inputs to the pupil responses.[33-35] This parameter was shown to be one of the strongest discriminators between groups among the constriction parameters.

The longer constriction latency (LAT-C) that was found in FOG subjects could be due to deficits in sensory retinal functions. Supporting this evidence is the results from a study by Salter *et al.*[36] They measured constriction PLR parameters using the same device in multiple sclerosis (MS) patients with optical neuritis. Their MS patients had reduced high contrast visual acuity, low contrast visual acuity and contrast sensitivity. In addition, all constriction parameters were found to be significantly affected in MS patients compared with the healthy controls. Moreover, the reduction in constriction percentage, average constriction velocity, and MCV along with the increase in constriction latency found in the MS group was comparable to the changes found in our PD patients results. They also reported that thinning in different retinal layers including total macular volume due to optic neuritis could predict the deficits in different constriction PLR parameters. Lagreze and Kardon also found a correlation between the estimated ganglion cell loss and the relative afferent pupillary defect in optic neuritis.[37] In his review, Simao summarized a number of studies reporting a thinning of the retinal nerve fiber in similar regions of the eye in PD patients.[38] It is possible that this retinal deficit underlies the deficits in pupil function. Nevertheless, he pointed out that the amount of thinning was not correlated with visual function or duration of the disease and so more study about the proposed linkage is required.

Retinal inputs to PLR response are a combination of the signals originating at the rods and cones and the intrinsic response of the intrinsically photosensitive retinal ganglion cells (ipRGCs), which project to the pretectum. Although the role of the ipRGCs in the PLR response is still being studied, it appears that these cells play a major role in maintaining the steady-state size of the pupil.[39] There is evidence that ipRGCs may be damaged in open-angle glaucoma (ONG). The differences between red and blue postillumination pupil responses were reduced in patients with ONG relative to controls. A smaller difference between the postillumination responses is believed to indicate damage to ipRGCs.[40] The input into the ipRGCs includes dopaminergic amacrine cells and so it is possible that the larger mean pupil size under light adaptation found in the FOG-PD arises from reduced dopaminergic inputs into these cells in addition to reduced input from the photoreceptor pathways.

Nevertheless, we cannot completely rule out a motor pathway dysfunction. A lack of correlation between the PRL and VEP latencies was also reported in MS patients when the disease was inactive.[41,42] A study was done on rats found that the number of retinal photoreceptors does not predict the PLRs, which suggests the PLR is not a good indicator of the integrity of retinal photoreceptor cells.[43] Although none of these findings exclude the possibility that the ganglion cells to the pretectum are affected differentially relative to the cells projecting to the LGN, it does raise the question as to whether there is also a motor dysfunction.

The result that the initial pupil size under dark adaptation was smaller in the non-FOG PD group suggests a motor deficit in this group of PD patients. A smaller pupil size in darkness is a sign of either increase in parasympathetic influence or reduction in sympathetic input.[31,32,44]

Nevertheless, this imbalance was not evident in the minimum pupil size during constriction or the during the light-adapted state before the dilation was measured, which suggests that the result could be due to other factors such as attention or general arousal level.[45,46] As to why these levels would be different in the non-FOG subjects is uncertain.

The MCV was slower in the two PD groups, which suggests a parasympathetic deficit.[33-35] However, previous studies have shown that there is a positive and significant relationship between pupil response velocities with the amplitude size change. This means that if constriction amplitude is lower for a certain disease patient, then it is expected that their constriction velocity is slower and there is no new information gained by looking at each parameter.[25,33,47,48] There was a positive and significant relationship between pupil constriction velocities and the amplitude size change and both PD groups also had significantly smaller constriction amplitudes. Linear regression results of MCV versus amplitude of constriction were significant (r ≥ 0.818, *P* > 0.001) for all subject groups.

Scatterplots of these relationships are shown in Figure 3 for all subject groups. All groups showed the expected strong
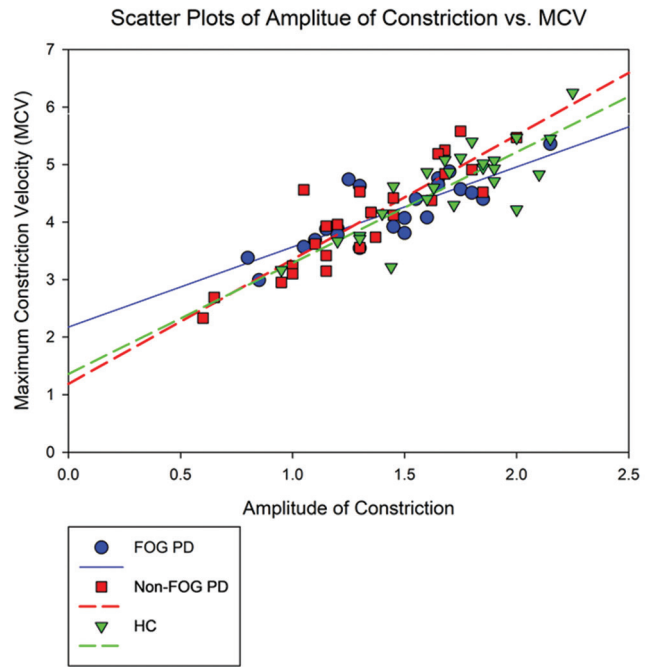
Figure 3: Scatter plots of pupil maximum constriction velocity as a function amplitude of pupil constriction for the subject groups

Table 8: The rank order of different pupil light reflex parameters that can discriminate groups

Test	χ^2	DF	*P*
Constriction parameters			
Intercept	0.276	2	0.871
Re-ADV	20.332	2	0.000*
LAT-C	15.413	2	0.000*
Amount of Constriction	13.754	2	0.001*
End	12.088	2	0.002*
Init	11.732	2	0.003*
T 75% recovery	10.349	2	0.006*
Constriction (%)	3.912	2	0.141
MCV	3.658	2	0.161
ACV	2.182	2	0.336
Dilation parameters			
Intercept	5.289	2	0.071
LAT-D	7.648	2	0.022*
Amount of dilation	3.803	2	0.149
Init	3.225	2	0.199
End	3.181	2	0.204
ADV	0.904	2	0.636
Dilation (%)	0.040	2	0.980

*The parameter shows a significant effect between groups. ADV=Average dilation velocity; Re-ADV=Re-ADV; LAT-D=Latency of dilation; Init=Initial diameter; End=End diameter; ACV=Average constriction velocity; MCV=Maximum constriction velocity; ADV=Average dilation velocity; Init=Initial diameter; End=End diameter

and significant relationships between MCV and constriction amplitude. The FOG PD group has a flatter slope for pupil constriction than the other two groups. The results should be interpreted cautiously because these data are across subjects and not within, but it suggests that the subjects in the FOG

PD group who had a relatively large amplitude of constriction had the slower pupil velocity response. This could indicate a deficit in the parasympathetic motor pathway. The difference in slopes between the groups suggests that measuring the PRL reflexes as a function of light level may help to determine to separate the sensory deficit from any motor deficit.

It may not possible to exclude the motor contribution to the results of constriction latency (LAT-C). Comparisons between FOG and non-FOG PD patient groups regarding this parameter showed FOG PD group had significant LAT compared to non-FOG PD group. The LAT could suggest deficits in either afferent (sensory) or efferent (motor) parasympathetic pathway of ANS. However, constriction latency is not considered as good as MCV to represent the cholinergic (motor) mediated pathway of ANS.[24,34]

Delay in T75% recovery time and slower re-dilation velocity were considered to be strong indicators of motor impairment in the sympathetic pathway of ANS due to adrenergic reduction.[31,34] Unexpectedly, both PD patient groups showed faster recovery time (T75%) and faster re-dilation velocity after pupil constriction (re-ADV) compared to healthy controls, with FOG PD group being faster than non-FOG PD group on these two parameters. Faster T75% and re-ADV that were shown in PD patients could be secondary to less constriction percentages (Con %) that were shown among PD patients compared to healthy controls. That means because both PD patient groups constricted less than healthy controls, then it was expected that their re-dilation recovery time and velocity would be faster. This finding suggests two things. First, both PD patient groups have no obvious motor impairment in the sympathetic pathway of ANS compared to healthy controls. Second, faster T75% and re-ADV in PD patient groups are secondary effects to motor impairment in the parasympathetic pathway of ANS. However, the results of dilation latency (LAT-D) showed that both PD patient groups had significant delay compared to healthy controls. Furthermore, the amount of dilation of non-FOG PD patients was less than the other two groups which still it may not possible to exclude the potential impairment of sympathetic pathway of ANS among PD patients. Similar to our results, it has been found that PD patients had faster but not significant 50% re-dilation recovery time than healthy controls.[49]

Cognitively impaired PD patients have been shown to have more constriction PLR deficits than those patients who have normal cognitive functions (Stergiou *et al.*). The deficits in the cognitive impaired PD patients were similar to the pupil dysfunction reported in Alzheimer's disease patients. This suggests that both groups of patients have the same central cholinergic deficit.[14] It has been shown that FOG and FOG severity are associated with frontal cognitive dysfunction and frontal cognitive dysfunction severity, respectively.[16] Cognitive impairment could be due to degeneration of subcortical regions such as locus coeruleus (LC) in the brain stem. This area is known to be affected in PD and Alzheimer's disease patients.[50]

It is possible that the PLR deficits are due to alterations in the brain stem rather than more centrally or in the peripheral pathways. The LC in the brain stem is one possible site. Pupil size is a good indicator of activity in the LC.[51,52] Rapid changes in the release of acetylcholine (ACh) and adrenaline (NE) occur due to variation activity in LC. The LC activity changes the pupil responses.[53] Since FOG PD patients showed larger impairments on some of sympathetic and parasympathetic PLR parameters, it is possible that adrenergic and cholinergic systems are impaired in FOG PD patients to a greater extent than non-FOG PD patients due to abnormal activities in LC or other autonomic cortical centers.

Although the results confirmed that the PLR was affected in PD, we could not rule out that many of these deficits were due to degraded sensory input from the retina. The general trend in the results was that the deficits reflect a deficit in the parasympathetic pathway, but there are also data suggesting a sympathetic deficit. It is possible that measuring the PRL for different light levels may provide a better understanding of the pupil deficits in PD.

CONCLUSION

Both PD patient groups had pupillary light reflex parameter abnormalities. It was difficult to determine whether the abnormalities were due to impaired sensory input or deficits in the parasympathetic motor input. Nevertheless, the FOG-PD group had larger differences for the parameters that were likely due to sensory impairment, whereas parameters that were likely due to motor deficits were equally affected in both PD groups. There was also evidence that the pupillary sympathetic pathway was affected in PD.

Conflicts of interest

There are no conflicts of interest.

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