**PARTNERS HUMAN RESEARCH COMMITTEE**

**DETAILED PROTOCOL**

## PRINCIPAL / OVERALL INVESTIGATOR

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## PROTOCOL TITLE

Functional Assessment of the Melanopsin-Containing Retinal Ganglion Cells in Progressive Supranuclear Palsy Using Chromatic Pupillometry

## FUNDING

None

## VERSION DATE

09/18/2017

## I. BACKGROUND AND SIGNIFICANCE

### *a. Historical background*

In 1963, Richardson, Steele and Olszewaki published a landmark clinical report on 8 cases of supranuclear ophthalmoplegia, pseudobulbar palsy, nuchal dystonia and dementia and established the syndrome of heterogeneous system degeneration as a clinicopathological entity now known as progressive supranuclear palsy (PSP) (1).

By 1972, when Steele revisited the subject (2), 73 cases (22 with post-mortem examinations) had been described in the medical literature with examples discovered across the U.S. I encountered approximately 3 to 4 new cases yearly among patients referred for eye movement examinations.

Rare familial clusters have been described in which the heredity pattern is compatible with autosomal dominant transmission with incomplete penetrance (3). Rojo and co-workers have described 12 pathologically-confirmed

pedigrees (4) and noted the variable phenotypical expression of the disease within a single pedigree.

The disease has a characteristic onset in the sixth decade (range 45 to 75 years) with some combination of impaired balance, abrupt falls, visual disturbances, slurred speech, dysphasia and vague changes in personality.

Akinesia is responsible for most of these parkinsonian symptoms described by patients, all of which relate to slowness and difficulty in initiating, implementing, and maintaining motor movements. Akinesia is readily evident during simple observation. When the patient is asked to carry out a specific motor task and it may lead to a misdiagnosis of Parkinson's disease (PD) in an estimated 5% of patients who actually have PSP (5-7).

PSP is caused by a novel silent mutation in exon 10 of the tau gene, and like frontotemporal dementia and Alzheimer's disease, it is a tauopathy (8).

Two distinct phenotypes have been recognized in pathologically proven cases (9). One is called Richardson's syndrome and is characterized by early falls, cognitive decline and vertical gaze abnormalities (10) and a second which more closely resembles PD with rigidity, tremor and some response to treatment with levodopa (9).

Spatial covariance analysis has been used with F-flurodeoxyglucose (FDG) positron emission tomography (PET) to identify the disease-related metabolic patterns that can serve as biomarkers in assessing PD, atypical PD, and PSP (11).

Slowing of voluntary vertical saccades, either down, up or both (10) are a diagnostic marker of PSP (10) and later impairment of voluntary horizontal saccades are characteristic in more than half of the cases (12). A related sign has been the finding of hypometric saccades in response to an optokinetic drum moving vertically in one direction (usually best seen with stripes moving downward) and eventually all voluntary eye movements are lost (12). However, a proportion of PSP patients do not demonstrate these eye signs for a year or more after the onset of the disease. Furthermore, Adams and Victor followed several patients who had no involvement of their eye movements during life but

in whom the typical pathological changes of PSP were found unexpectedly (13) and this has to be kept in mind in follow-up.

Other diagnostic features of PSP are slow hypometric horizontal saccades, selective defects of visual tracking (14), loss of convergence, and disruption of steady gaze by square-wave jerks. Eyelid disorders include reduced blink rate, blepharospasm, repetitive blinking in response to flashlight stimulus (failure to habituate), and impaired initiation of eyelid opening (apraxia) (15). Delayed initiation of eyelid opening in PSP results from metabolic impairment of the motor network and cortical degeneration of the frontal lobes (16). One can, in advanced cases of PSP, visualize, on MRI of the brain, atrophy of the rostral midbrain tegmentum. When detected by mid-sagittal MRI, it looks like the bill of a hummingbird and is referred to as the “hummingbird” sign (17). In PSP, the sign is due to atrophy of the rostral and caudal midbrain tegmentum and to a relative increase in the length of the interpeduncular fossa over that of the anteroposterior diameter of the midbrain tegmentum. Its presence on MRI helps establish the diagnosis (18) (see Figure 1) (17).

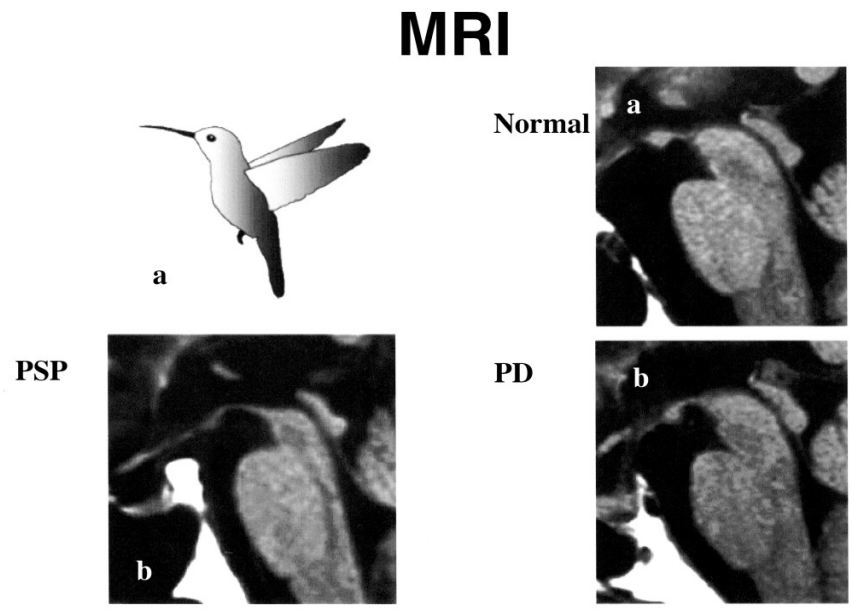


Figure 1. Brain MRI mid-sagittal plain. (A) Normal control. (B) PSP (Progressive supranuclear palsy), PD (Parkinson’s Disease). The region, including the most rostral midbrain, the midbrain

tegmentum, the pontine base and the cerebellum, appears to correspond to the bill, crown, body and wing, respectively, of a hummingbird (e.g., the “hummingbird sign”). (17)

Measurement of the midbrain tegmentum on midsaggital MRI section is also considered a reliable way of differentiating PSP from other Parkinsonian diseases (15, 16).

### *b. Previous pre-clinical or clinical studies leading up to, and supporting, the proposed research*

One of the most absorbing features of the eye is the pupil’s reaction to light (19, 20). Changes in pupil size in response to a light stimulus, the pupil light reflex (PLR), are based on a functional balance between the parasympathetic nervous system and the sympathetic nervous system, and balance between two neurotransmitters - acetylcholine and noradrenaline (19, 20). The method used to optimize pupil measurements, characterize their velocity and determine the sensitivity of pupil latency as a function of the light stimulus is a critically important element in determining the diagnostic potential of the PLR (21).

The PLR is driven by intrinsically photosensitive retinal ganglion cells (ipRGCs) containing melanopsin (22-24). Melanopsin is a novel photo-pigment in the inner retina of both primates and rodents which was discovered only in the last ten years (25-26). Melanopsin has since been found to provide a unique class of ipRGCs with the capability to act as a third set of photoreceptors (27-29), allowing these cells to fire action-potentials on their own when stimulated by light (30-32). Melanopsin-mediated ipRGC photoactivity provides sustained coding of ambient light irradiance for circadian rhythm (33), regulates tonic pupil size and mediates the pupil light reflex hitherto thought to be primarily driven by rods and cones.

Melanopsin-containing ipRGCs show prolonged depolarization after-potential in the dark in response to melanopsin-activating blue light leading to a sustained pupil constriction beyond the offset of light stimulus (34). Lei et al. (35-37) have now demonstrated that melanopsin containing ipRGCs

are selectively sensitive to short wavelength light, with a maximum absorption at around 480nm.

Non-visual light detection and photo transduction occur primarily in a small group of ipRGCs (27, 29-31) and at least three of several subtypes of ipRGC demonstrate distinct anatomic projections with clear functional differences (32-34). One major site of ipRGC projection is the suprachiasmatic nucleus, the central pacemaker for circadian rhythm in the hypothalamus (39-41) (see Figure 2) (41).

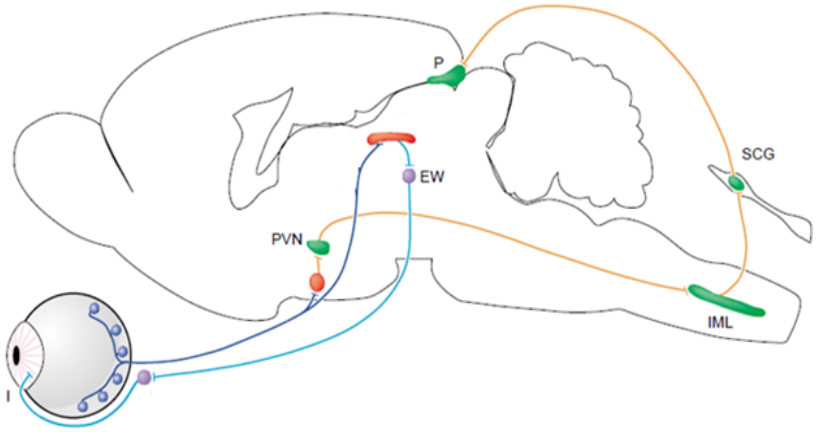


Figure 2. The melanopsin mediated ipRGC pathways projecting to the suprachiasmatic nucleus in the hypothalamus and to the olivary pretectal nucleus in the midbrain. (41)

Another site of ipRGC projection is the olivary pretectal nucleus (OPN), the central integrator of the PLR (42).

The ipRGCs receive or project modulatory signals from and to neighboring cells, including rods and cones which themselves act as local circadian clock cells in the retina (43, 44). The relative contribution of rods, cones, and intrinsic melanopsin to the ipRGC activity at any moment varies with light characteristics, such as wavelength, intensity, and exposure duration (25, 35, 40, 41).

Three main parameters of the PLR are distinguishable on a pupillogram: (1) the immediate pupillary constriction of the dark- or dim light-adapted pupil to the abrupt onset of a light stimulus (transient constriction)

which is largely due to signalling from activated rod-cones; (2) a more sustained state of the pupillary constriction during continuous light stimulation to which all photoreceptive elements may contribute; and (3) under certain conditions an extended phase of pupillary constriction that persists for some time after stimulus light termination (45, 46). This post-stimulus sustained pupil constriction derives primarily from intrinsic melanopsin activation of the ipRGCSs and is specific to the melanopsin signaling pathway. It has been called the 'post-illumination pupil response' (PIPR) (47). When it follows exposure to bright blue light, it is almost entirely attributable to melanopsin ipRGC activity and can therefore be used to estimate functioning of the melanopsin signaling pathway itself (48).

Bergamin and Kardon used a binocular infrared video pupillometer to record the dynamic pupil response and time the onset (latency) of pupil constriction to a light stimulus in normal subjects (mean age 34). Latency to the onset of constriction was defined as the time of maximum constriction acceleration (49). The improved technique developed in their study showed that pupil latency becomes shorter as a function of light intensity in a predictable, non-linear function, and shows less change at higher intensities. This has been confirmed by others (46).

The latency period is composed of two separate mechanisms: an irreducible minimizing latency built into the motor system of the iris, and a variable additional delay due to retinal discharges (from ipRGCs) and their temporal summation in the midbrain (pretectal olivary nucleus) (49). The weaker the stimulus, the longer the latency period (49).

Latency as a function of light intensity is reproducible in a given eye and a similar response is present in the normal right and left eyes. Stergious et al. (47) based their pupillometric study of patients with PD, with and without cognitive impairment, on Bergamin and Kardon's study. The characteristic V-shaped PLR response to three pupillometric parameters is shown in Figure 3: A) Amplitude, B) Maximum constriction velocity, and C) Maximum constriction acceleration (47).

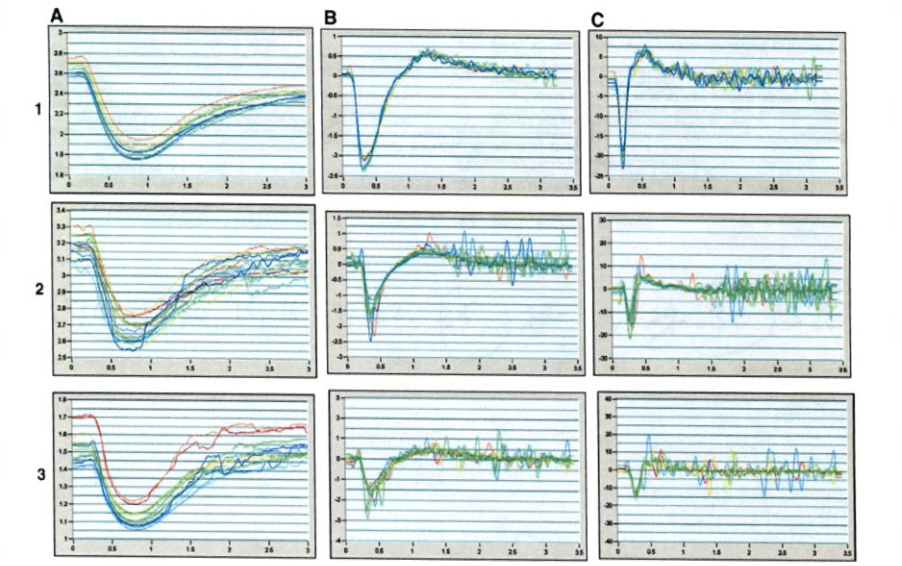


Figure 3. This picture shows the characteristic V-shared PLR response in three pupillometric parameters: A) Amplitude, B) Maximum Constriction Velocity, C) Maximum Constriction Acceleration in three different subjects: 1) a healthy 70-year-old control, 2) a 73-year-old PD patient without cognitive disorders, and 3) a 69-year-old PD patient with coexisting cognitive impairment. All the aforementioned parameters are impaired in both PD patients, especially in the patient with cognitive impairment. (47)

The three parameters were studied in three different subjects: 1) a health 70-year-old control, 2) a 73-year-old PD patient without cognitive impairment, and 3) a 69-year-old PD patient with co-existing cognitive impairment. The parameters were impaired in both PD patients, especially in patient 3 with cognitive impairment (47).

These pupillometric findings were not, however, attributed to the functional impairment or degeneration of amacroinic, dopaminergic cells in the retina. The investigators hypothesized that they were attributable to an additional central cholinergic deficit (47). At the time, the evidence did not exist to attribute these findings to a degenerative process affecting the ipRGCs (44).

### *c. Rationale behind the proposed research and potential benefits to patients and/or society*

The rationale behind using chromatic pupillometry, a novel methodology, as an objective *in vivo* identifier of PSP is based in part on:

1) Clinicopathological correlation between the key clinical signs of a supranuclear gaze palsy with pathological verification that the degenerative process affects the pretectum and rostral midbrain,
2) The melanopsin-signaling pathway from ipRGCs in the eye projects to the OPN in the midbrain,
3) Chromatic pupillometry is a non-invasive technique suitable for elderly subjects with or without dementia.

Because spectral sensitivity or intrinsic melanopsin-mediated phototransduction is narrow with a peak at around 482 nm (short wavelength light) (35-37), there is a higher threshold to light compared with rod-mediated and cone-mediated phototransduction. The activation of ipRGCs has a longer latency, a characteristic pattern of discharge of action potentials and a discharge rate which builds relatively slowly a maximal sustained firing rate that is linearly proportional to light intensity. When the bright light is left on, the firing rate of ipRGCs is remarkably steady and sustained without fatigue or adaptation to the continuous light stimulation. When the light is turned off, ipRGCs do not immediately stop firing but gradually decrease their firing rate until they cease. This long observed sustained pupil constriction, sustained after the cessation of a short wavelength high intensity light stimulus, is now considered a characteristic electrophysiological signature of melanopsin-driven ipRGC intrinsic photoactivity (35-37). Table 1 illustrates the differences in pupil-to-light responses of three types of photoreceptors.

| | CONE | ROD | ipRGC |
|---|---|---|---|
| **Functions** | photopic, central vision | scotopic, peripheral vision | pupillary response, circadian rhythm |
| **Number** | 5 million | 120 million | 4000-12000 |
| **Location** | photoreceptor layer | photoreceptor layer | ganglion cell/inner |

| | (outer retina) | (outer retina) | plexiform layers (inner retina) |
|---|---|---|---|
| **Photopigment** | lodopsin | rhodopsin | melanopsin |
| **Photosensitivity** | low | extremely high | low |
| **Receptive field** | very small | small | very large |
| **Peak absorption wavelength (nm)** | 420 (S), 534(M), 564 (L) | 498 | 482 |
| **Patterns of light pupillary response** | rapid onset, early adaptation | rapid onset, early adaptation | delayed onset and prolonged firing |

Table 1. Properties of Three Types of Photoreceptors: Cones, Rods and melanopsin retinal gangion cells (35-37)

These developments have led to a novel chromatic pupillometry technique to measure the PIPR as an index of intrinsic ipRGC activity. This technique utilizes color stimuli and infrared pupil tracking systems to measure differential pupil responses under continuous light stimulation at selected wavelengths over a range of intensities.

Kankipati et al. (45) showed that the PIPR can be induced *in vivo* in normal human subjects with a chromatic pupillometry technique. Kardon et al. (43) provided evidence that a clinical chromatic pupillometry protocol can assess differentially the contributions of the rod, cone and IPRGC pathways to the pupillary light response. Their test protocol was refined by Park et al. (24) to isolate rod, cone and ipRGC contributions to the pupillary light reflex. In an experimental series, Shaobo Lei and his co-authors (35-37) further developed a comprehensive full-field chromatic pupillometry system to investigate the contributions of rod/cone-driven extrinsic ipRGC activity and melanopsin-driven intrinsic ipRGC activity to the pupillary light reflex. Using this protocol, a robust melanopsin-driven PIPR can be induced with a short flash of blue light of only 400 ms duration, making the test clinically friendly with good test-retest reliability (46). The potential benefit to the patients center on the use of

chromatic pupil responses, especially the blue light induced melanopsin-driven PIPR, to investigate the role of ipRGCs in a wide range of disease populations, including retinitis pigmentosa, achromatopsia, glaucoma and ischemic optic neuropathy. The evidence also suggests that the PIPR correlates with other anatomical and functional parameters in diseases that primarily affect retinal ganglion cells such as glaucoma (50).

## II. SPECIFIC AIMS

The specific aim of this study is to investigate rod, cone and melanopsin driven pupillary light response in individuals with PSP and in age-matched healthy control individuals and individuals with other neurogenerative diseases, using chromatic pupillometry, with special interest in assessing melanopsin-driven PIPR as an identifier for PSP.

This study addresses the following hypotheses:

1) Chromatic pupil responses, including rod/cone-driven rapid phase constriction and melanopsin-driven PIPR, are reduced in subjects with PSP compared to age-matched normal healthy control subjects,
2) Pupil parameters of the melanopsin-driven PIPR are abnormal in PSP subjects without supranuclear palsy, which is indicative of a subclinical physiological deficit of the OPN in the early stages of PSP.

If these hypotheses are upheld, chromatic pupillometry to measure the PIPR promises to be a reliable *in vivo*, non-invasive, convenient and inexpensive technique to detect asymptomatic pupillomotor impairment in advance of diagnostic oculomotor signs and deterioration of cognitive function.

## III. SUBJECT SELECTION

Male and female subjects diagnosed with PSP (age 55 years and over) and followed at the MGH's Gerontology Research Unit (GRU) and/or the MGH's

Frontotemporal Disorders Unit directed by Dr. Brad Dickerson (Department of Neurology) will be recruited for the study. Age-matched healthy control subjects will be recruited from participants enrolled in the Alzheimer's Disease Research Center's (1999p003693) longitudinal research cohort studies that are conducted at the GRU. Age-matched subjects with Parkinson's disease or Alzheimer's disease will also be recruited from those who are enrolled in related research studies conducted in the MGH Neurology Department as a comparison group. For data analyses and cross-validation purposes, subjects with neuroimaging research data will be targeted for enrollment.

No individuals will be excluded from study participation on the basis of sex, race, ethnicity or any other characteristics (e.g., sexual orientation) that bear no effect on the integrity of data for research.

**a) Inclusion Criteria**

1) Individuals that meet the clinical criteria for PSP. Core features include:
   - Recurrent falls and unsteady gait
   - Axial and nuchal rigidity
   - Pseudobulbar palsy
   - Bilateral lid retraction
   - Supranuclear vertical gaze palsy
   - Atrophy of the midbrain tegmentum (the hummingbird sign on brain MRI,

2) Individuals that fit the criteria for the second PSP phenotype (which resembles PD) that has asymmetric findings, tremors and poor responses to treatment with Levodopa,

3) Individuals that meet the clinical criteria for PD with:
   - Progressive bradykinesia
   - Postural instability and frequent falls
   - Festinating gait with loss of associated movements
   - Cogwheel rigidity and mask-like face
   - Rest tremor,

4) Individuals who carry a diagnosis of Alzheimer' disease who present with progressive impairment of memory and cognitive domains such as language and visuospatial perception.

Diagnoses will be confirmed by the review of health/medical records of patients recruited from the Frontotemporal Disorders Unit clinic. In the case of participants recruited from research studies, diagnoses will be confirmed by the review of the research diagnoses indicated on the individuals' research records.

**b)** ***Exclusion Criteria***

1) Individuals who are frail or in questionable health,
2) Individuals with cataracts or with posterior pole ocular pathology such as age-related macular degeneration and optic neuropathies, including open angle high intraocular pressure glaucoma,
3) Individuals with photophobia (i.e., painful light sensitivity) when exposed to bright light, including those with ophthalmological conditions such as keratitis (herpes simplex), uveitis or Achrometopsia,
4) Individuals with advanced dementia with inability to sit erect, hold the eyes open, incontinence,
5) Individuals with epilepsy,
6) Individuals diagnosed with major depression or other severe psychiatric disorders

Study investigators will use their best clinical judgment when selecting potential participants for the study.

## IV. SUBJECT ENROLLMENT

**a)** ***Methods of enrollment***

Potential study participants will first learn about the study from a clinician of the Frontotemporal Disorders Unit or from study staff of related research studies in which these individuals are already enrolled. The health/medical records of patients treated at the Frontotemporal Disorders Unit clinic will be reviewed in order to identify potential participants who meet the inclusion/exclusion criteria of the study. For age-matched control subjects enrolled in protocol 1999p003693, the Center's study staff will ask these subjects if they would be interested in an 'eye-test for detecting brain diseases' study that Dr. Shirley Wray is conducting. If so, subjects will be requested to leave their contact information so that Dr. Wray or one of her study staff may be able to reach them. Individuals from the Frontotemporal Disorders Unit clinic who express interests in learning more about the study and/or to enroll in the study will be referred to the study PI (Dr. Shirley Wray) or research staff for initial screening and the informed consent process.

All potential participants will undergo a brief neuro-opthalmological examination (20-30 minutes) by the study PI prior to the start of the study.

**b)** ***Procedure for informed consent***

Written informed consent for all subjects will be obtained either by a board-certified clinician or by a trained research assistant/study coordinator under the close supervision of the study PI. Informed consent from surrogates for adults who do not have decision-making capacity will only be obtained by a licensed physician investigator. Subjects who are consented by a non-physician investigator will always be given the opportunity to discuss any questions they may have with the study PI. Potential subjects will always be given as much as time as necessary to consider study enrollment. Enrolled subjects will be assigned a randomized study ID and names or other identifiers (except dates of testing) will never be released to anyone who is not an approved study staff on the protocol. All study staff will be trained in research ethics on an ongoing basis.

For individuals who may have impaired decision-making capacities for research participation, their capacity for initial or ongoing consent/assent will be evaluated by our licensed physician-investigators at study enrollment and throughout the study. In line with published guidelines on assessing competency, we generally regard individuals with a Clinical Dementia Rating (CDR) score of 0 or 0.5 as competent to consent for research participation. Individuals with global CDR scores of 1.0 or 2.0 are regarded as having impaired decision-making capacities. For impaired subjects, the PHRC-preferred order of surrogates from whom we will obtain informed consent - based on the 'substituted judgment' of surrogates - will be followed. Study staff will document the relationship of the surrogate to the subject in the latter's research records.

Subjects will not be approached for brain donation/autopsy as part of the study.

## V. STUDY PROCEDURES

### a) *Study visits*

Study visits will be at baseline and approximately at the one-year mark. Subjects will primarily be tested at the MGH Gerontology Research Unit located in Charlestown. Each study test session, including time for dark adaptation, will take no more than 30 minutes. Subjects will not be paid to participate in the study.

### *b) Drugs to be used*

Not applicable.

### *c) Device to be used*

The NeurOptics DP-2000 Multi-Chromatic Desktop Binocular Pupillometer (see Figure 3) will be used to test the subjects in this non-invasive human study. Pupillometry is a well-established clinical

technique in departments of ophthalmic electroretinography to evaluate patients with night blindness and for other diseases of the retina.

DP-2000 is a Class I device and complies with part 15 of the Federal Communications Commission (FCC) Rules.

Dr. Pat Fortune (VP, Market Sectors) of the Partners Innovation Office is processing the necessary agreements with device manufacturer NeurOptics, Inc (Dr. Claudio Privitera, PhD) and external collaborator Dr. Shaobo Lei MD, MSc, PhD, of the University of Toronto regarding Intellectual Property (IP) and related compliance issues in conjunction with the study PI.

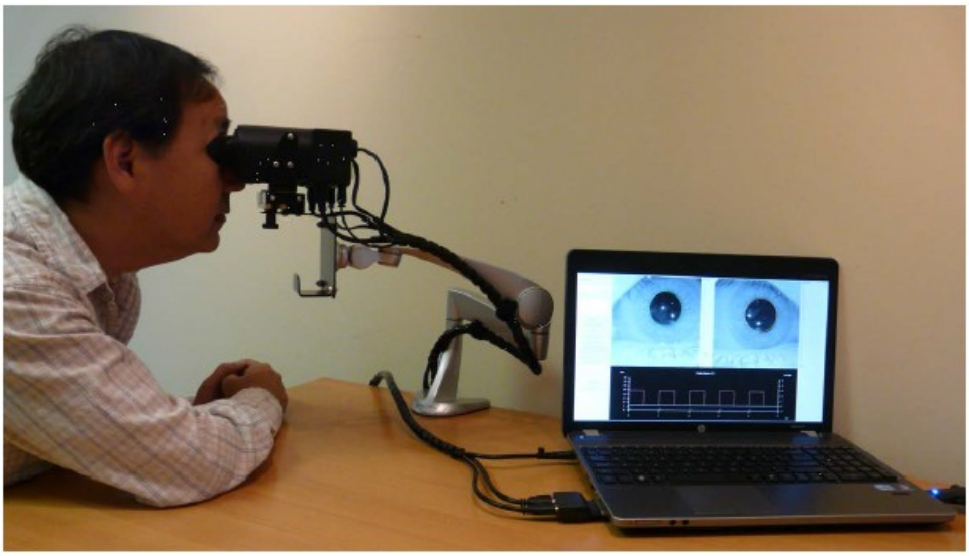

FIGURE 3. The DP-2000 desktop multi-chromatic desktop binocular pupillometer showing the subject's pupils well in focus in the center of the GUI.

The DP-2000 Pupillometer has the following features:

- Binocular dual-camera system measures both eyes at once
- Multi-chromatic (4 ultra-bright color light stimulus: White, green, blue and red)
- Video stream captured at 30 Hz, 0.05mm pixel resolution
- Stimulates direct, consensual, or both eyes simultaneously

- Pupil variables: Latency, diameter at onset and peak of constriction, constriction and dilation velocity, 75% recovery time
- Light intensities defined in Lux or pure radiometric units ($W/m^2$); can be adjusted over ~ 5 log-unit range by increments of 0.1 log-units
- Automatic tracking and pupil detection
- Waveform of light stimulation can be customized using a simple graphical interface or defined in a file using Microsoft Excel™
- Saves pupil profile and light stimulus data in a file (Excel™ compatible) for external/independent analysis

Source: https://neuroptics.com/dp-2000-binocular-pupillometer/

Appendix A contains the pupillary measurement variables.

***d) Pupillometry Testing Procedures***

All subjects and age-matched normal controls will be tested with a Simplified Chromatic Pupillometry Protocol where rod, cone and melanopsin driven pupil responses will be isolated with the following 3 stimulation conditions:

1. 4 lux red light for 1 s (dark-adapted cone response)
2. 4 lux blue light for 1 s (dark-adapted rod response)
3. 200 lux red light for 1 s (cone response)
4. 200 lux blue light for 1 s (melanopsin response)

The test will be done monocularly (if both eyes are eligible, only one eye will be randomly selected to be tested); the non-tested eye will be covered by an eye-patch. After 20 minutes of dark adaptation, condition 1 and 2 will be presented in the dark 3 times at 15 s intervals, then conditions 2-3 will be presented in alternating fashion at 1 minute intervals, for 3 times each. The protocol, including time for dark adaptation, will be approximately 30 minutes or less.

***e) Data to be collected and when the data is to be collected***

Pupil diameter data will be recorded in real time at 30 Hz sample rate. Pupillometry data will be stored on an approved password-protected, malware protection-enabled computer/laptop that is stored in a secured, limited-access testing room located in the GRU. Data will be backed-up on secured drives on a regular basis.

## VI. BIOSTATICIAL ANALYSIS

***a) Specific data variables and pupil measurement variables***

Please see below.

**b) *Study endpoints***

- **Primary endpoints**

1. Maximal Pupil Constriction: the smallest pupil size following light stimulation. This parameter primarily represents rapid phase extrinsic ipRGC activity driven by rods and cones through synaptic input.
2. Post-illumination pupil response (PIPR): measured pupil diameter over a period of 20 seconds, from 10 to 30 seconds after the offset of light stimulation. Previous work found that the cone-driven pupil responses subsided within 10 seconds after the offset of light stimuli, so this measurement is expected to represent “pure” melanopsin-driven ipRGC activity.

- **Secondary endpoints:**

Secondary pupil variables are summarized in Table 2.

| **Broadcast Function** | **Range** |
|---|---|
| Delta= % Change | % of change (Size-MIN)/Size as a% |
| LAT= Latency of constriction | Time of onset of constriction |

| | |
|---|---|
| | following initiation of the light stimulus |
| ACV= Constriction Velocity | Average velocity of how the pupil diameters is constricting measured in millimeters per second |
| MCV= Maximum Constriction Velocity | Maximum velocity of how the pupil diameter is constricting measured millimeters per second |
| ADV= Dilation Velocity | The average pupillary velocity when, after having reached the peak of constriction, the pupil tends to recover and to dilate back to the initial resting size, measured in millimeters per second |
| T75= Time to reach 75% recovery | The time to reach 75% of the original baseline pupil diameter after the peak of the constriction |

Table 2. Secondary pupillary measurement variables

All of the data from the DP-2000 laptop will be de-identified/coded and transferred by secured-file-transfer means to co-investigators Dr. Shaobo Lei, MD, MSc, PhD, Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, ON, Canada and by Dr. Claudio Privitera, PhD, of NeurOptics, Inc for analyzes and measurement of pupil variables.

Dr Lei and Dr. Privitera are experienced clinical/research pupillometric experts and their collaboration is essential to ensure accurate measurements of the pupil variables and to provide advice to the study PI if any adjustment is needed to the research protocol.

No Protected Health Information (PHI) or identifiers will be shared with Dr. Lei and/or Dr. Privitera except for dates of tests.

**c) *Statistical Methods***

The results collected with the help of Drs. Lei and Privitera will be analyzed by our own biostatisticians at MGH. Categorical variables (sex) will be compared with Cochra's Q test. Primary and secondary outcomes will be compared using repeated measure ANOVA. Each ANOVA will have Group as a between-subjects factor (2 levels: AD patients, controls), and Testing Condition (4 levels: conditions 1-4 ) as a repeated within-subject factor. Main effects and interactions will be analyzed further using Tukey's HSD post-hoc tests.

***d) Power analysis (e.g., sample size, evaluable subjects, etc.)***

It is expected that a sample size of approximately 20 will be adequate to detect differences between subjects with PSP and normal healthy age- and sex- matched control subjects. A smaller sample size of approximately 12 subjects with other neurodegenerative diseases will be used as a pilot study group for comparison purposes.

## VII. RISKS AND DISCOMFORTS

***a) Complications of a non-surgical procedure***

Not applicable

***b) Drug side effects***

Not applicable

***c) Device complications/malfunctions***

Care and attention to cleaning and maintenance of equipment will be carried out by the operator. The Pupillometer does not require any regularly schedule maintenance. If the device is not working properly or is damaged, the PI will contact NeurOptics Customer Service.

The Pupillometer will only be used by trained study personnel. If a problem is recognized while operating the device, the device will be removed from use and referred to qualified personnel for servicing. During the testing session, a caregiver may remain with the subject at all times together with the tester.

***d) Complications***

The pupillometry test described is non-contact and non-invasive. Aside from the fact that the bright light may be bothersome for some photophobic subjects, there are no foreseeable physical harms to participating in the study. The test does not induce headache, dizziness, syncope, epilepsy or panic attacks.

**e) *Psychological Risks***

The subject's caregiver will be invited to sit in the room while the subject is dark-adapted and throughout the test. If the subject experiences any feelings of stress, anxiety, discomfort, and an/or fatigue, the patient or the caregiver can stop the test at any time.

**f) *Radiation Risks***

None.

## VIII. POTENTIAL BENEFITS

**a) *Potential benefits to participating individuals***

There are no direct benefits to subjects from participating in this research.

**b) *Potential benefits to society***

The ultimate benefit of this research is the development of a non-invasive test to contribute additional diagnostic data in the evaluation of individuals suspected of having a progressive neurodegenerative disease.

The researchers' observations and experiences will aid in the development of an *in vivo* noninvasive, cost-effective diagnostic chromatic pupillometer to monitor disease-modifying therapies in tau-associated neurodengerative dementia.

## IX. MONITORING AND QUALITY ASSURANCE

The research team will meet regularly to monitor and assure the validity and integrity of the data and adherence to the IRB-approved procedures. Quality assurance, the safety and monitoring of outcomes are ultimately the responsibility of the study PI - Shirley H. Wray, MD, PhD, FRCP. Should an adverse event occur, it will be reported to the IRB in accordance with the IRB's reporting procedures and timelines.

## X. APPENDIX

### *NeurOptics DP-2000 Multi-Chromatic Desktop Binocular Pupillometer*

The video stream infrared device is captured at 30Hz. The video frame is digitized into 640X480 pixels with 8-bit gray level resolution. The profile of light stimulated can be customized using a graphic user interface (GUI). Pupillary variables are automatically analyzed, reported graphically in a special window and saved into a results file. Light stimulation has four different chromatic options: Red (622 nm), Green (528 nm), Blue (463 nm) and White. Light is emitted through a diffusing screen (approx. 50° x 35° of visual angle).

The system includes a laptop that has specific technical characteristics and cannot be replaced with a different model. Pupillary distance can be adjusted to fit the subject's eyes by turning the knob located below the camera. Camera height can be adjusted be pressing the button on the arm and lifting the camera to the desired position.

All possible options to operate the program are listed on the left extreme side of the GUI. The live video from the two cameras is displayed in the same GUI. Pupil tracking is represented by a circle fitting the contour of the pupil – the

subject's left eye is displayed on the right and the subject's right eye is displayed on the left. This is to maintain the natural perspective of the examiner facing the subject during the examination. The left pupil (OS) is tracked with a yellow circle and the right pupil (OD) with a green circle. This association (left-yellow and right-green) is also used for display purposes. Light stimulus waveform and the corresponding pupil response dynamics are shown together in the graph located at the bottom of the GUI. Note: the software version can be checked by pressing the Version button.

***Subject ID***

The first two windows on the top of the list serve to record a subject's randomized ID number and information regarding the current measurement. This information is attached to the master data result file produced at the end of the measurement and will be used for defining the name of the result file. The ID number of each subject will allow the operator to re-check previous studies as needed.

***Edit Stimulus Profile***

The Edit Stimulus Profile button serves to define and to save a new light stimulus profile or to load a predefined profile. The basic unit of a profile is a light step during which a light pulse is flashed to the eye and then turned off. Light pulses can be repeated a number of times, thereby forming a section. Pulses and/or sections can be concatenated in order to define the final light stimulus profile. The Edit Stimulus Profile GUI is used to define all the elements of the light stimulus profile.

***Preparing the subject for the scan and checking the tracking***

The subject will be dark-adapted, sitting in a dark room for 3 minutes with the tester, and with their caregiver if requested. They will then be positioned close to the camera, with the eye in front of the lens, visible and well in focus in the center of the video display in the main GUI. It is advisable to slightly rotate

the two cameras (as opposed to keeping the cameras perfectly straight and parallel) for a better fit to the subject's eyes. A green circle should automatically track the right eye (displayed on the left) and a yellow circle tracks the left (displayed on the right). It is very important to carefully check the accuracy of the tracking before initiating the test. If the circles are not precisely around the pupil *or* there is no circle *or* there is only a red circle moving erratically around the video frames and tacking other portions of the image *or* the green (or Yellow) circle turns occasionally into red – all of these are signs of bad tracking and a few corrective steps are necessary before initiating the measurement.

***Right IR – Left IR***

Each camera has two pairs of IR LEDs (0 and 1) that can be toggled using the IR buttons for the left and right channel. This can be useful for small pupils, if one of the IR corneal reflections is overlapping on the pupil, disrupting the tracking. Switching to the other pair of IRs can, often, resolves the problem and improves the visibility of small pupils.

Results obtained from the Pupillometer scan will be used for information or scientific research investigation only, not for clinical diagnostic purposes. The subject's ID will be recorded on the main screen to enable recall of subject data.

## XI. REFERENCES

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