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Characterizing the Relationship between Eye Movement Parameters and Cognitive Functions in Non-demented Parkinson's Disease Patients with Eye Tracking

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Dr. Ronald Myers
Senior Science Editor, Journal of Visualized Experiments
1 Alewife Center Suite 200 Cambridge MA 02140

28th March 2019

Dear Dr. Myers,

We are a group of researchers in neuropsychiatry based in Hong Kong, China. We would appreciate your consideration of an article, "Characterizing the Relationship between Eye Movement Parameters and Cognitive Functions in Non-demented Parkinson's Disease Patients with Eye Tracking" for publication in the Journal of Visualized Experiments.

Parkinson's disease is a chronic debilitating neurodegenerative disease that patients suffer from a variety of cognitive deficits on top of the motor symptoms. Our current study explored the clinical utility of eye tracking technology as an adjunctive biomarker of cognitive functions in Parkinson's disease. In the experiment, we used a visual search task coupled with an eye tracker to capture metrics of saccade and fixation to establish correlations with individual cognitive functions in a case-control study of non-demented Parkinson's disease patients.

The experimental design allowed us to study in-depth on the relationship between eye-movement metrics and cognitive functions on a phenomenological level. With our existing knowledge of cognitive neuroscience and physiology of eye movement control, we are able to infer postulations to the observed results. For example, we found that visual fixation duration correlates negatively with performances in visual memory, verbal recognition memory, and semantic verbal fluency. We postulate a possible common confluence at the temporal and parietal region of the brain with a cholinergic type of deficit that accounted for the observed correlations. The findings encourage us to further explore the longitudinal relationship between the two functions. The paradigm was highly tolerable to our subjects and it has the potential to be applied transdiagnostically in other neurocognitive disorders to explore similar research question. Therefore, we would like to share our experimental design and set-up with researchers working in a similar field.

This paper is not under consideration for publication elsewhere. All co-authors have read the manuscript. Thank you very much for your time in considering our paper.

Yours sincerely,

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TITLE:

Characterizing the Relationship Between Eye Movement Parameters and Cognitive Functions in Non-Demented Parkinson's Disease Patients with Eye Tracking

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KEYWORDS:

Parkinson's disease, cognition, cognitive impairment, eye tracking, eye movement, saccade, visual fixation

SUMMARY:

Here, we present a protocol to study the relationship between the eye movement parameters and cognitive functions in non-demented Parkinson's disease patients. The experiment used an eye tracker to measure the saccadic amplitude and fixation duration in a visual search task. The correlation with performance in multi-domain cognitive tasks was subsequently measured.

ABSTRACT:

Cognitive impairment is a common phenomenon in Parkinson's disease that has implications on the prognosis. A simple, noninvasive and objective proxy measurement of cognitive function in Parkinson's disease will be helpful in detecting early cognitive decline. As a physiological metric, eye movement parameter is not confounded by the subject's attributes and intelligence and can function as a proxy marker if it correlates with cognitive functions. To this end, this study explored the relationship between the eye movement parameters and performance in cognitive tests in multiple domains. In the experiment, a visual search task with eye tracking was set up, where subjects were asked to look for a number embedded in an array of alphabets scattered randomly on a computer screen. The differentiation between the number and the alphabet is an overlearned task such that the confounding effect of cognitive ability on the eye movement parameters is minimized. The average saccadic amplitude and fixation duration were captured and calculated during the visual search task. The cognitive assessment battery covered domains of frontal-executive functions, attention, verbal and visual memory. It was found that prolonged fixation duration was associated with poorer performance in verbal fluency, visual and verbal memory, allowing further exploration on the use of eye movement parameters as proxy markers for cognitive function in Parkinson's disease patients. The experimental paradigm has been found

to be highly tolerable in our group of Parkinson's disease patients and could be applied transdiagnostically to other disease entities for similar research questions.

INTRODUCTION:

Parkinson's disease is classically a motor disorder; yet, the disease is also associated with cognitive deficits, and progression into dementia is common[1]. The pathophysiology of cognitive impairment in Parkinson's disease is not well understood. It is thought to be related to alpha-synuclein deposition in the cortical area based on Braak's staging[2]. It was also proposed that a dual syndrome of degeneration of the dopaminergic and the cholinergic system leads to different cognitive deficits with prognostic implication[3]. More research is needed to further elucidate the exact mechanisms involved in cognitive impairment in Parkinson's disease. On the clinical aspect, the presence of cognitive impairment has a significant impact on prognosis[4,5]. Assessment of cognitive function in clinical practice is, therefore, essential. However, a lengthy cognitive assessment is limited by patients' mental and motor conditions. Therefore, a noninvasive and simple measurement that can reflect the disease's burden on cognitive function is needed.

The eye movement abnormalities are widely described detectable signs of Parkinson's disease from its early stages[6], yet the pathophysiology is even less well-characterized than that of cognitive impairment. The generation of eye movement is through a transformation of the visual sensory input, subserved by an intertwined cortical and subcortical network, into signals to the oculomotor nuclei in the brainstem for effect[7]. Involvement of Parkinson's disease pathologies in these networks may lead to observable eye movement abnormalities. There is, perhaps overlapping of neuroanatomical structures that govern the control of eye movement and cognitive function. Furthermore, there have been studies examining the relationship between saccadic eye movement and cognitive function in other neurodegenerative disorders[8]. On such grounds, it is worthwhile to explore the use of eye movement parameters as a proxy marker of cognitive functions in Parkinson's disease. One cross-sectional study[9] showed that reduced saccadic amplitude and longer fixation duration was associated with the severity of global cognitive impairment in Parkinson's disease. However, there is a lack of data on the correlation between eye movement parameters and specific cognitive domains. The significance and need of measurement of specific cognitive domains, rather than a general cognitive state, is that individual cognitive domain informs differential prognostic information in Parkinson's disease[3] and they are subserved by different neural networks. The aim of this study is to explore the specific relationship between eye movement metrics and different cognitive functions. This is the first step to establish a foundation on which the development of biomarkers of cognitive decline in Parkinson's disease using eye tracking technology could be built.

The experimental paradigm presented is composed of 2 major parts: the cognitive assessment and the eye tracking task. The cognitive assessment battery encompassed a range of cognitive functions, including attention and working memory, executive function, language, verbal memory and visuospatial function. The choice of these 5 cognitive domains is based on the Movement Disorder Society Task Force Guidelines for the mild cognitive impairment in Parkinson's disease[10], and a set of locally available cognitive tests were selected to build the assessment battery. In a previous similar eye tracking study on Parkinson's disease cognition

mentioned[9], the author extracted the eye movement parameters while the subjects were engaged in visual cognitive tasks, where the parameters may potentially be influenced by the subject's cognitive ability. As this study aimed to assess the correlation between the eye movement parameters and different cognitive domains, the potential confounding effect of cognitive abilities on the eye parameters must be addressed. In this regards, a visual search task, adapted from another eye tracking study on Alzheimer's disease[11], was employed to capture the eye movement parameters of the subjects. During the task, subjects had to search for a single number on a computer screen among multiple alphabet distracters. This task would elicit the alternate use of saccadic eye movement and visual fixation, the abnormalities of which are described widely in Parkinson's disease. The identification and differentiation of number and alphabet is an overlearned task where the demand for cognitive functions is only minimal and would, therefore, be suitable to answer the research question of this study. A computer program was developed based on the specifications and design as stated by Rösler et al[11]. in their original study to be run within the in-built software of our eye tracker. An in-house algorithm for classification and analysis of the eye tracking data was also developed for this study.

PROTOCOL:

This research project was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CREC Ref. No.: 2015.263).

1. Participants recruitment and baseline assessment

1.1. Recruit Parkinson's disease patients aged less than or equal to 70 from a neurology specialist clinic with the diagnosis made based on the United Kingdom Parkinson's Disease Society (UKPDS) Brain Bank Diagnostic Criteria[12].

1.1.1. Exclude subjects with psychiatric illnesses, ophthalmological diseases that would impair eye movement, or other neurological disorders. Also, exclude cases using anticholinergics as they are known to affect cognitive performance and eye movement.

1.2. Recruit healthy controls on a 1:1 basis matched by sex, age, and education.

1.3. Obtain informed consent from the subject.

1.4. Conduct a clinical diagnostic interview with the subject and, if available, their relatives, to exclude dementia and screen for cognitive impairment with Mental State Examination (MMSE)[13] and Montreal Cognitive Assessment (MoCA)[14]. Exclude dementia cases from the study or if the subject's scores of either MMSE or MoCA is <22/30.

1.5. Assess the visual acuity with a Snellen chart. Exclude the subject if the visual acuity is less than 20/40.

1.6. Assess motor severity and staging of Parkinson's disease using the Unified Parkinson's

Disease Rating Scale (UPDRS) Part II & III[15] and Modified Hoehn and Yahr (H&Y) Staging[16], respectively. Also, obtain information about the current medications taken by the subject.

1.7. Assess the depressive mood state by the Beck Depression Inventory-II (BDI-II)[17].

2. Experimental setup

2.1. Conduct the experiment in a quiet room with an adequate light source.

2.2. Conduct the experiment for Parkinson's disease subjects when they are on medication with optimal motor function.

2.3. Prepare the setup that consists of a screen-based eye tracker, a computer, a mouse, a standard keyboard, a chin rest, and cognitive assessment tools (**Table of Materials**).

2.4. Use an eye tracker with a sampling rate of at least 300 Hz.

2.5. Place the chin rest 60 cm in front of the eye tracker screen.

3. The flow of the cognitive assessment and the visual search task

3.1. Carry out the Chinese Categorical Verbal Fluency Test[18]. Instruct the subject to name as many animals as possible in a minute. Record the number of answers and perseverative error. Then repeat the same in the category of fruits and vegetables.

3.2. Conduct the registration part (Trial 1, 2 and 3) of the Hong Kong List Learning Test (HKLLT)[19] by reading out a pre-defined 16-vocabulary word list and instruct the subject to remember them. Afterwards ask the subject to do free recall of the word list and record the answer (Trial 1).

3.2.1. Repeat step 3.2 twice for Trial 2 and Trial 3.

3.3. Wait 10 min and 30 min after the registration part of the HKLLT for the 10- and 30-min delay recall.

3.4. Before the 10 min delayed recall of the HKLLT, perform the Pattern Recognition Memory (PRM) from Cambridge Neuropsychological Test Automated Battery (CANTAB)[20] (**Table of Material**).

3.4.1. Using the tablet computer, present 24 visual patterns, one at a time, at the center of the screen. Instruct the subject to remember the pattern.

3.4.2. After the presentation, in a 2-choice force discrimination paradigm, instruct the subject to choose the pattern that he/she can recognize.

3.5. Perform the 10 min delay recall of HKLLT by asking the subject to do free recall of the 16-vocabulary word list.

3.6. Before the 30 min delayed recall of the HKLLT, perform Spatial Span (SSP) from CANTAB[20].

3.6.1. Use the tablet computer to show a pattern of white boxes which change in color, one by one, in variable sequences.

3.6.2. Afterwards instruct the subject to touch the boxes in the same sequence they were presented and record the spatial span length that the subject could attain as the difficulty (number of boxes change in color) of the task increases.

3.7. Carry out the 30 min delay recall by asking the subject to do free recall of the 16-vocabulary word list.

3.7.1. Conduct the recognition and discrimination part of the HKLLT by reading out another pre-defined 32-vocabulary word list, of which half of the vocabularies are from the original word list in 3.2. Instruct the subject to determine whether each vocabulary read out is from the original word list or not.

3.8. Allow the subject to rest quietly if they finish the tasks in 3.4 and 3.6 before the 10- and 30-min delay recall, respectively.

3.9. Perform the Stocking of Cambridge (SOC) from CANTAB[20].

3.9.1. Using the tablet computer, present 20 scenarios of two parallel displays of 3 balls held in 3 vertical stockings, of which the arrangement of the balls in the displays varies in each scenario.

3.9.2. Instruct the subject to determine, in each scenario, the least number of moves required to rearrange the balls in the lower display in order to copy the pattern shown in the upper display. Record the mean number of choices to correct answer.

3.10. Perform the Stroop Test[21].

3.10.1. Give the subject 3 cards consecutively; the first card contains dots printed in different colors, the second card contains Chinese characters printed in different colors while the last card has Chinese characters denoting different colors (e.g., Chinese words of "blue", "yellow", "green", or "red") but printed in a color not denoted by the name (e.g., the word "red" printed in blue ink).

3.10.2. Ask the subject to read out the printed color of the dots/Chinese characters as quickly as possible and record the time required for each card (T1, T2, and T3).

3.10.3. Calculate the interference index with the formula (T3-T1)/T1.

3.11. Proceed to the visual search task after completing the cognitive tests.

NOTE: Do not carry out any verbal cognitive task after the registration part of HKLLT until the end of the whole HKLLT (3.7) to prevent interference effect on verbal memory performance.

4. Visual search task

4.1. Position the subject on a chair and place their chin on the chin rest with their forehead against a bar to minimize head movement. Align the subject's eyes to approximately the center of the computer screen. Begin by clicking the **Start Recording** button in the computer program.

4.2. Calibration

4.2.1. Calibrate the eye tracker with the in-built calibration program by clicking the **Start** button in the calibration interface.

4.2.2. Ask the subject to gaze at a red dot moving across the screen with 9 fixation points, while keeping the head still.

4.2.3. Check for the quality of the calibration by viewing the calibration plot (**Figure 1**). Make sure that the length of the green lines, which represent the error vectors, fall within the grey circles for an acceptable quality of the calibration. Redo the calibration if there is any missing point or the green lines fall outside the grey circles. Click **Accept** to proceed to the visual search task.

4.3. Instruction

4.3.1. Provide verbal instruction to the subject and start with 5 practice runs to familiarize the subject with the task.

4.3.2. Instruct the subject to fixate their gaze on the central fixation cross at the beginning of each trial. Then, press **Enter** on the keyboard to begin a trial, at which the computer screen will display a single number and 79 distracter alphabets scattered randomly (**Figure 2**).

4.3.3. Instruct the subject to look as quickly as possible for the number and then simultaneously click on the mouse and state the number aloud as soon as the number is located.

4.3.4. Cross check if the number stated is correct or not.

4.3.5. Administer a total of 40 trials after the 5 practice runs.

4.4. Design of the trial images in the visual search task

NOTE: The program code, written in PHP, for this section can be found in **Supplement File 1**.

4.4.1. Use the numbers 4, 6, 7 and 9 exclusively (**Supplementary File 1** - Line 5).

NOTE: The pilot study[11] showed that these numbers are most easily discriminated from the alphabets.

4.4.2. Ensure that the location of the target number is randomized from trial to trial with the rule that it could not be in the same visual quadrant for more than three successive trials (**Supplementary File 1** - Line 48-52).

4.4.3. Do not use ambiguous alphabets such as "I" and "O" (**Supplementary File 1** - Line 76-78).

4.4.4. Set the size of the fixation cross, alphabets, and numbers at 0.85° visual angle (equivalent to around 0.9 cm on a 23 inches computer screen).

NOTE: Numbers and alphabets are used because these are easily recognizable visual stimuli yet require foveation for identification.

4.4.5. Allow a time lapse of 1.5 s after the investigator has pressed **Enter** in 4.3.2 and before the display of the central fixation cross is switched to a trial image to begin a trial (**Supplementary File 2** - Line 71; 156-158).

4.4.6. Ensure that the screen will go blank with the fixation cross reappearing as the mouse is clicked or after 10 s have elapsed since the beginning of a trial, whichever that is earlier (**Supplementary File 2** - Line 72; 162-180).

4.4.7. As the task is finished, generate a .csv file that contains the timestamps of the beginning and the end of each trial (**Supplementary File 2** – line 48-59; 199-208). Use this file in the data analysis in section 5.

5. Eye tracking data processing and analysis

5.1. In the **Replay** section of the computer program, check the Samples Percentage of the eyes during the visual search task (**Figure 3**). Discard the subject's data if more than 20% missing data are observed.

NOTE: Samples Percentage denotes the percentage of time the eyes are successfully located by the eye tracker during the visual search task.

5.2. Click on the **Play** button for the recording to check the quality of the data by eyeballing the visualized scan path video generated (**Figure 4**). Discard the whole subject's data if it is grossly erroneous (**Figure 5**).

5.3. Discard any trial(s) in which the subject pressed the mouse accidentally and prematurely.

5.4. In the **Data Export** section of the program, select **GazePointX (ADCSpx)** and **GazePointY (ADCSpx)** and the subject of interest (**Figure 6**). Click **Export Data** to export each subject's data and save as a .csv file. The file contains the x and y coordinates of the subject's eyes position on the computer screen, in pixels, at every time point.

5.5. Use the Visual Search Analyzer and in the interface (**Figure 7**), select the data exported in 5.4 as the input of **Eye Data** and the .csv file generated in 4.4.7 as the input of **Action data**. Select **ST DBScan** as the classification algorithm and click on **Run**. Then, click on **Summary** to generate a spreadsheet file containing the mean saccade amplitude and the mean fixation duration of the subject.

5.6. Design of the visual search analyzer

NOTE: The coding for the design of the analyzer could be found at https://github.com/lab-viso-limited/visual-search-analyzer. Its program code can be found in **Supplementary File 3.**

5.6.1. Program the analyzer such that it extracts and analyzes only the data from the beginning to the end of the trial (i.e., from the display of the number and alphabets until the mouse is clicked or 10 s have elapsed), using the .csv file generated in 4.4.7 (**Supplementary File 3** - Line 6-173).

5.6.2. Program the analyzer such that it fills in the data loss due to eye-blinking by averaging the x and y coordinates of the gaze point immediately before and after the blinking (**Supplementary File 3** - Line 176-260).

5.6.3. Program the analyzer such that it classifies the raw data into either saccade or fixation by using the algorithm developed based on ST-DBSCAN[22] (Program code in **Supplementary File 4)**.

REPRESENTATIVE RESULTS:

The full result of this study is available in the original paper published[23]. Parkinson's disease subjects (n = 67) were recruited and completed the assessment. However, 5 cases failed to complete the visual search task as they wore progressive lens incompatible with the eye tracker and their data was discarded. The mean age of the subjects was 58.9 years (SD = 7.5 years) with a male to female ratio of 1.7:1. 62 healthy age-, sex-, and education-matched controls were recruited for comparison.

Cognitive and eye movement parameters

Consistent with other previous studies[24], the Parkinson's disease group showed poorer performance in multiple cognitive tasks as compared to the control group (**Table 1**). Using the in-house algorithm for classification of the visual search task data, fixations and saccades are identified and extracted for calculation and analysis. It was found that the disease group had a

smaller mean saccadic amplitude (16.36° ± 2.36) as compared to controls (17.27° ± 2.49; p = 0.037). The mean fixation duration was not significantly different between the groups (216.58 ms ± 31.64 vs, 211.59 ms ± 24.90; p = 0.331) (**Table 2**).

Correlation between eye movement parameters and cognitive function

After adjusting for covariates, there were negative correlations found between the mean fixation duration and the performance in verbal recognition memory score (Recognition and Discrimination scores; F = 5.843, t = -2.417, p = 0.017 and F = 12.771, t = -3.574, p = 0.001, respectively), pattern recognition memory (F = 5.505, t = -2.346, p = 0.021) and categorical verbal fluency test in the categories of fruit (F = 5.647, t = -2.376, p = 0.009) and vegetable (F = 9.744, t = -3.122, p = 0.002). (**Table 3**). However, there was no significant interaction found in these correlations between the disease and control group, suggesting that the correlations are not specific to the disease group. It is speculated that as the control of visual fixation and the correlated cognitive functions commonly involve temporal and parietal regions of the brain with a predominantly cholinergic basis, pathological changes to these neuroanatomical and biochemical mechanisms may explain the findings.

FIGURE AND TABLE LEGENDS:

Figure 1: A calibration plot of the eye tracker. The plot shows the result of the calibration. The length of each green line indicates the difference between the gaze point calculated by the eye tracker and the actual dot position. As all the green lines fall within the grey circles and there is no missing point, the quality of this calibration is acceptable.

Figure 2: An example of a trial of the visual search task. Display of a non-linear array of 80 stimulus items, of which there is 1 number among 79 distracter alphabets.

Figure 3: The interface to check the overall sampling percentage. In the Replay section of the computer program, the Samples Percentage, which denotes the percentage of time that the eyes are successfully located by the eye tracker during the visual search task, could be checked for each subject.

Figure 4: An example of a visualized scan path from the visual search task. The scan path during this trial was visualized, with the red straight lines representing the saccadic eye movement and the red dots for visual fixations. Note that the end of each visual fixation is followed by a saccade and vice versa in a normal scan path.

Figure 5: An example of a grossly erroneous visualized scan path. This example of a grossly erroneous scan path is taken from a subject wearing a pair of incompatible progressive lens. In contrast to the normal scan path in **Figure 4**, the red lines (saccade) run in zigzag and fall out of the computer screen. The fixation points are not on either the alphabets or the number.

Figure 6: The data export interface in the computer program. This shows the interface where the subject and the kind of the eye tracking data captured can be selected for data export. In our experimental paradigm, the x and y coordinate, in pixels, of the eyes position on the screen at

every time point will be used for data analysis.

Figure 7: The interface of the Visual Search Analyzer. This shows the interface of the in-house analysis program for eye tracking data.

Table 1: Comparison of cognitive scores between two groups using independent sample t-test. MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; *– $p<0.05$ [a] – mean choices to correct; [b] – scores transformed into z-score; [c] – percentage correct; [d] – span length. This table has been reproduced from[23].

Table 2: Comparison of eye tracking parameters between two groups using independent sample t-test. * - $p < 0.05$. This table has been modified from[23].

Table 3: Correlations between cognitive scores and eye-tracking parameters using General Linear Model: Significant findings only. * - $p < 0.05$. This table has been reproduced from[23].

Supplemental File 1: Codes related to the trial image design.

Supplemental File 2: Codes related to the actual run of the visual search task.

Supplemental File 3: Codes related to the software (e.g., analyzer program).

Supplemental File 4: Codes related to the ST-DBSCAN algorithm used for classifying eye movement metrics.

DISCUSSION:

The protocol presented above was designed as the first part of a longitudinal study in exploring the potential clinical utility of eye movement parameters as surrogate markers for cognitive functions in Parkinson's disease. While there are studies that examine more classical eye tracking paradigms such as self-paced saccade, reflexive saccade, and anti-saccade[25,26,27], a visual search task was used in this study to measure eye movement parameters. As discussed, the design of this visual search task is of paramount importance as it must minimize the known confounding effect of a cognitive ability on the performance of the eye tracking task, as it may affect the eye movement parameters recorded. An example of which would be the effect of frontal executive functions on the saccadic latency[28]. The critical issue in the design would be the random scattering of the number and alphabets and varying quadrants of the number's location, making it more difficult to use cognitive strategies to enhance the performance of the task. Together with an average of roughly 650 saccades measured in 40 trials per subject, the average saccade amplitude calculated represents more of a physiological ability of the eye to generate saccade. In accordance with previous literature, it was found that the saccade amplitude is smaller in Parkinson's disease patients[29,30]. The choice of parameters extracted from the eye tracking task also needs to be taken care of with respect to the issue of the potential confounding effect by cognition. For example, parameters such as the speed of finding the number, error rate, and accuracy, which are a direct measurement of attention and processing speed, were not used.

Another critical step for this study is to ascertain the validity of the algorithm that was used in the classification of eye movement parameter. There exist numerous ways of classifying eye tracking data into saccade and fixation: velocity-based, dispersion-based algorithm and so on[31]. Each of these algorithms has its own pros and cons and there is no gold standard for doing so such that one has to also take into consideration the specifications of the eye tracker used and the design of eye tracking task to determine the best way of classifying the data. For this study, an in-house, density-based clustering algorithm, developed based on ST-DBSCAN[22], was used. The research team has cross-validated the validity of this classification algorithm against manual classification in a pilot study before applying the algorithm to the data of this study. The computer program incorporating the algorithm would automatically splice out and classify the data within the trials, from the moment the trial starts (with the alphabets and number appearing on the screen) to the end (that the subject clicks on the mouse or 10 s has lapsed) so that no non-trial data recorded (e.g., during the display of the fixation cross) will be analyzed to contaminate the results.

The use of domain-specific cognitive tests in this study allow correlations of the eye movement parameters with individual cognitive function performance. As discussed, this has significance over using general overall cognitive measures as the neural circuitry and biochemical basis for each cognitive function are different. The contemporary knowledge on the neural mechanisms of eye movement control and individual cognitive functions allow us to make inference and interpretation of the results found. For example, the significant negative correlations of fixation duration with temporal-, parietal-, and cholinergic-based cognitive functions are of particular interest as impairment of these functions may predict the development of dementia[3]. Detailed discussions of the scientific basis that explain the correlations can be found in the original paper published[23].

The battery of cognitive examination and the visual search task were highly tolerable to the subjects of this study. Requiring roughly 1.5 h to complete the entire battery, none of the subjects were unable to finish because of fatigue or physical discomfort. The visual search task consisted of 40 trials and took only around 5-10 min to complete. The noninvasive, simple and quick nature of the task makes it suitable as a screening tool if supported by more robust data. This paradigm could also be applied transdiagnostically in other neurocognitive disorders to answer similar research questions. One major practical limitation encountered in this protocol is the incompatibility of the eye tracker in subjects wearing certain progressive lens, as presbyopia is not an uncommon condition in the elderly. Eyelid apraxia and blepharospasm are also seen in Parkinson's disease[32] and sufferers of these conditions may not be able to complete the task.

As an explorative and cross-sectional study, the design of the study does not allow us to infer any definite neuroanatomical and biochemical basis that explains the results found. The interpretations of the results were mostly based on independent knowledge on the physiologies of cognitive functions and eye movement control and, therefore, remained as postulations. The longitudinal data on how these parameters may change over time during the neurodegenerative process is unknown. Yet, it is worthwhile to have a follow-up study to investigate the predictive

values of the baseline eye movement parameters on cognitive impairment development. Future studies should incorporate neuroimaging to address the neurostructural underpinnings for more solid support of any postulation, without which further development of eye tracking as a proxy marker of cognitive function will not be possible.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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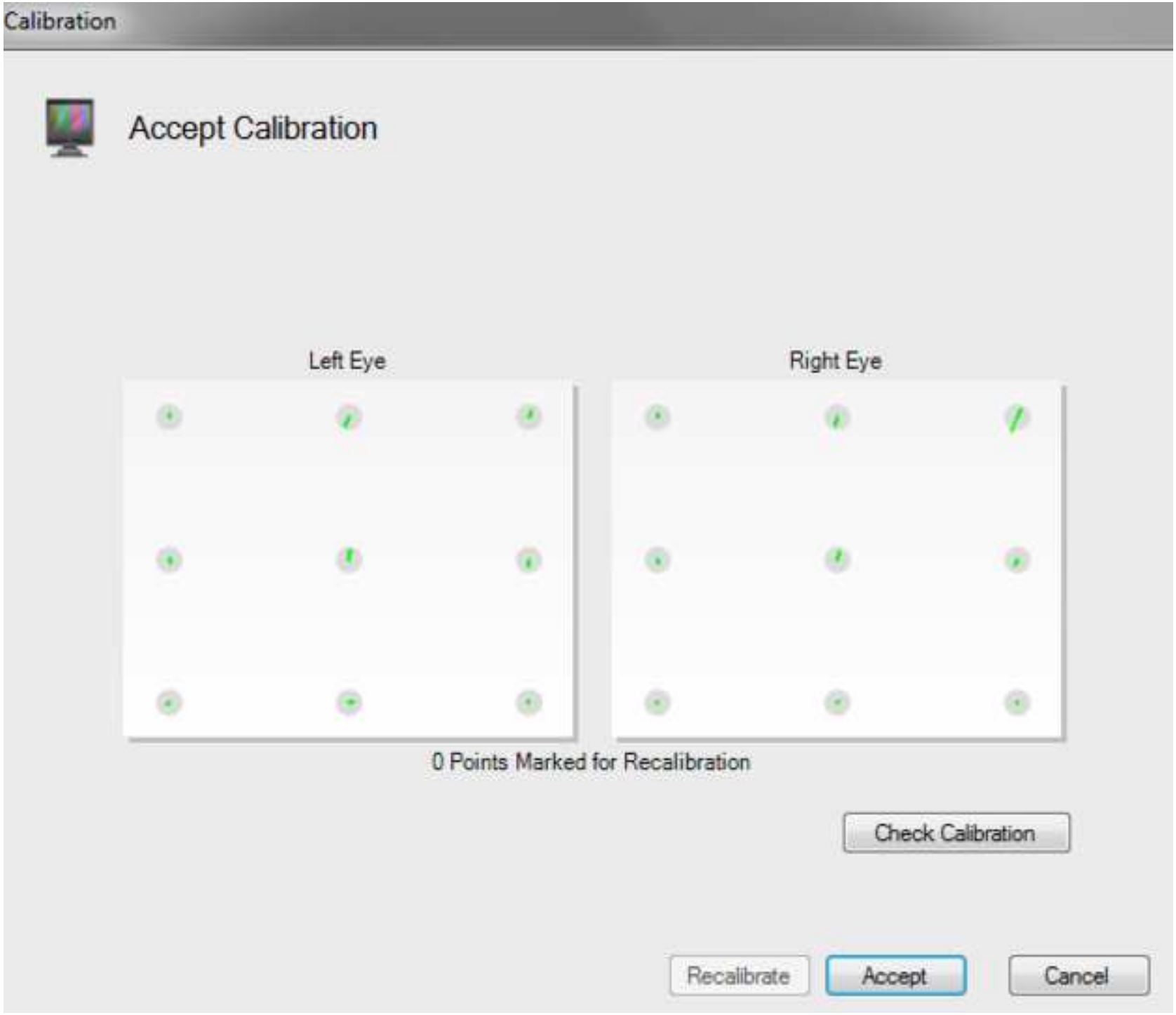
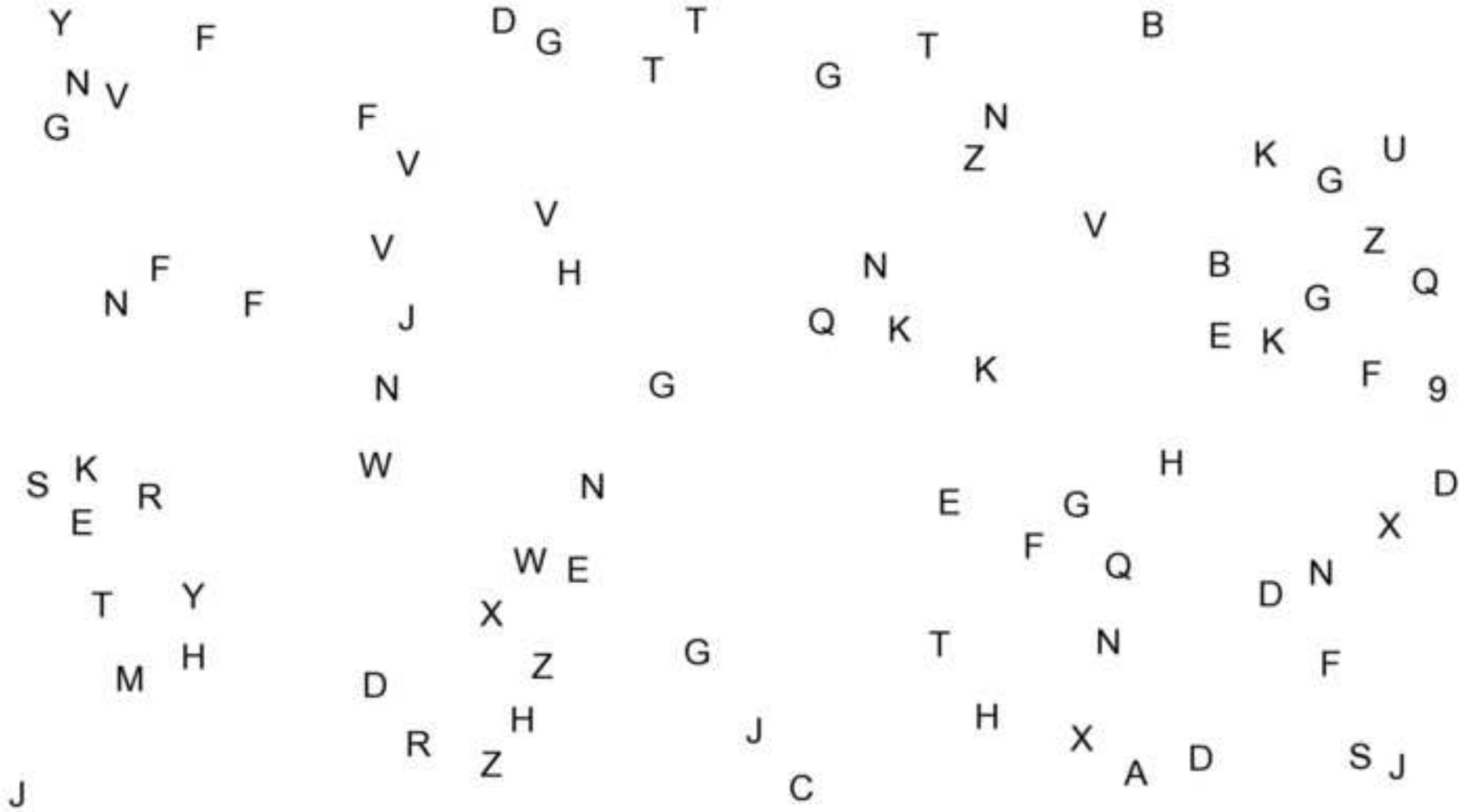


Figure 2



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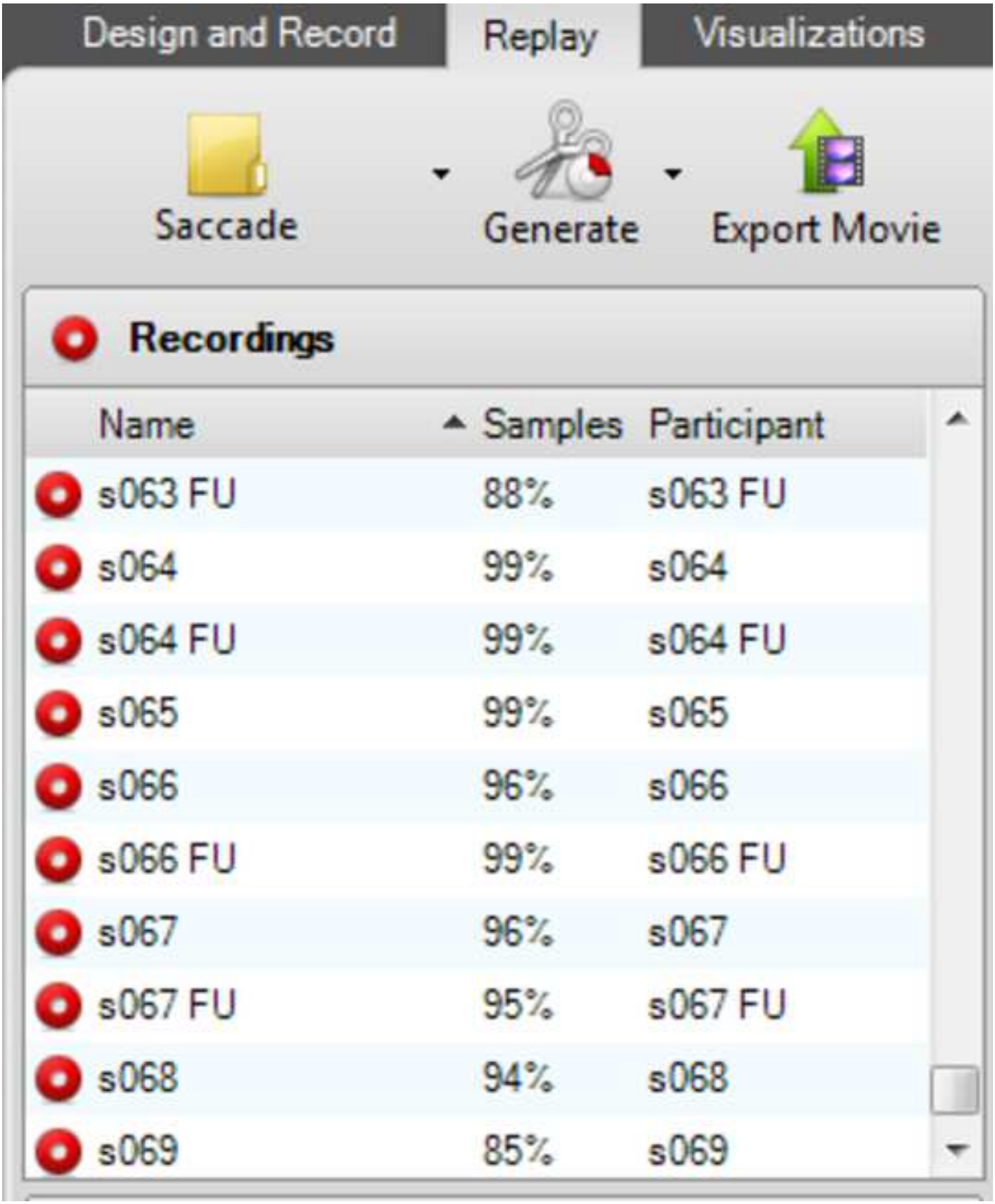

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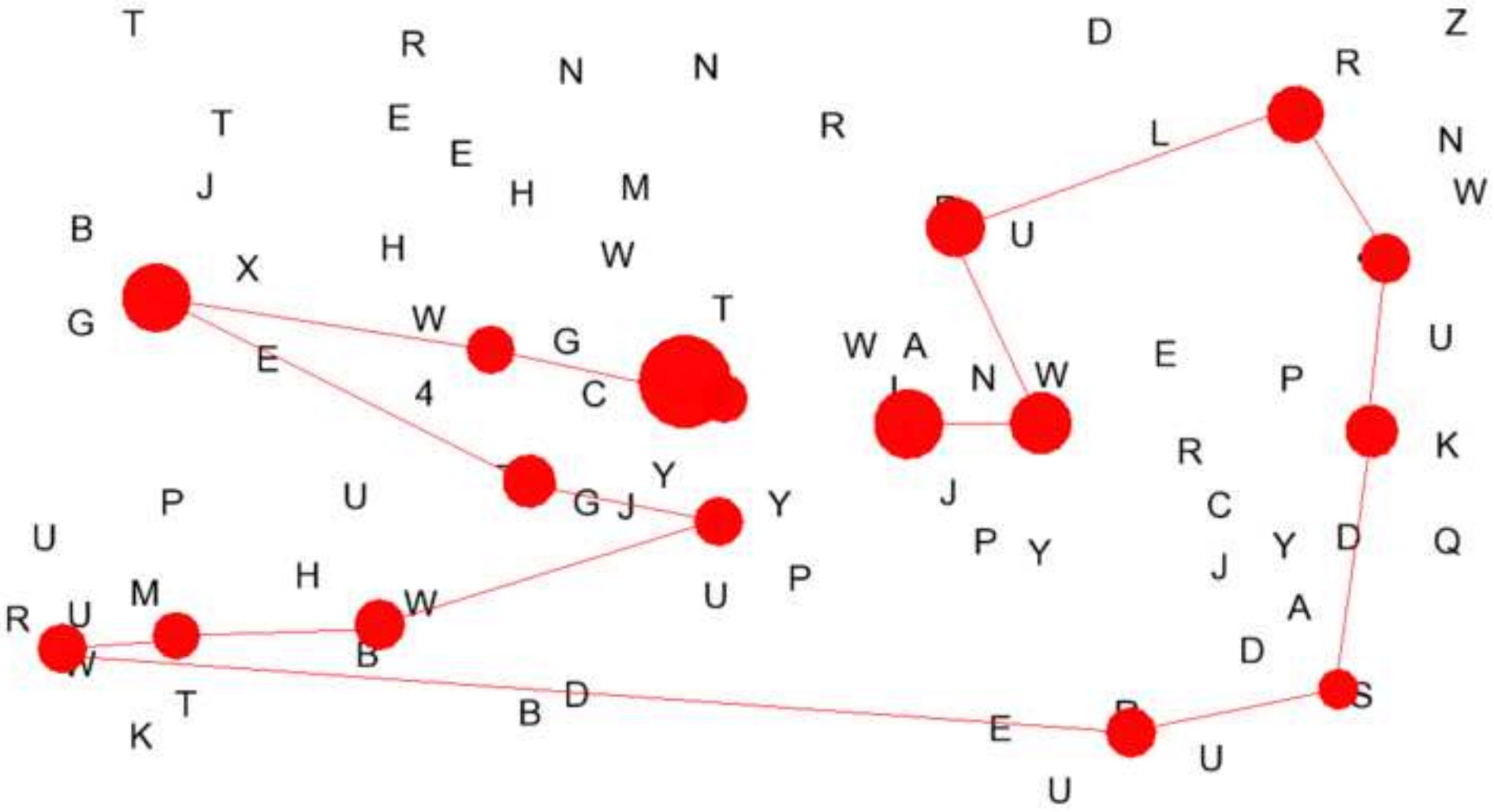

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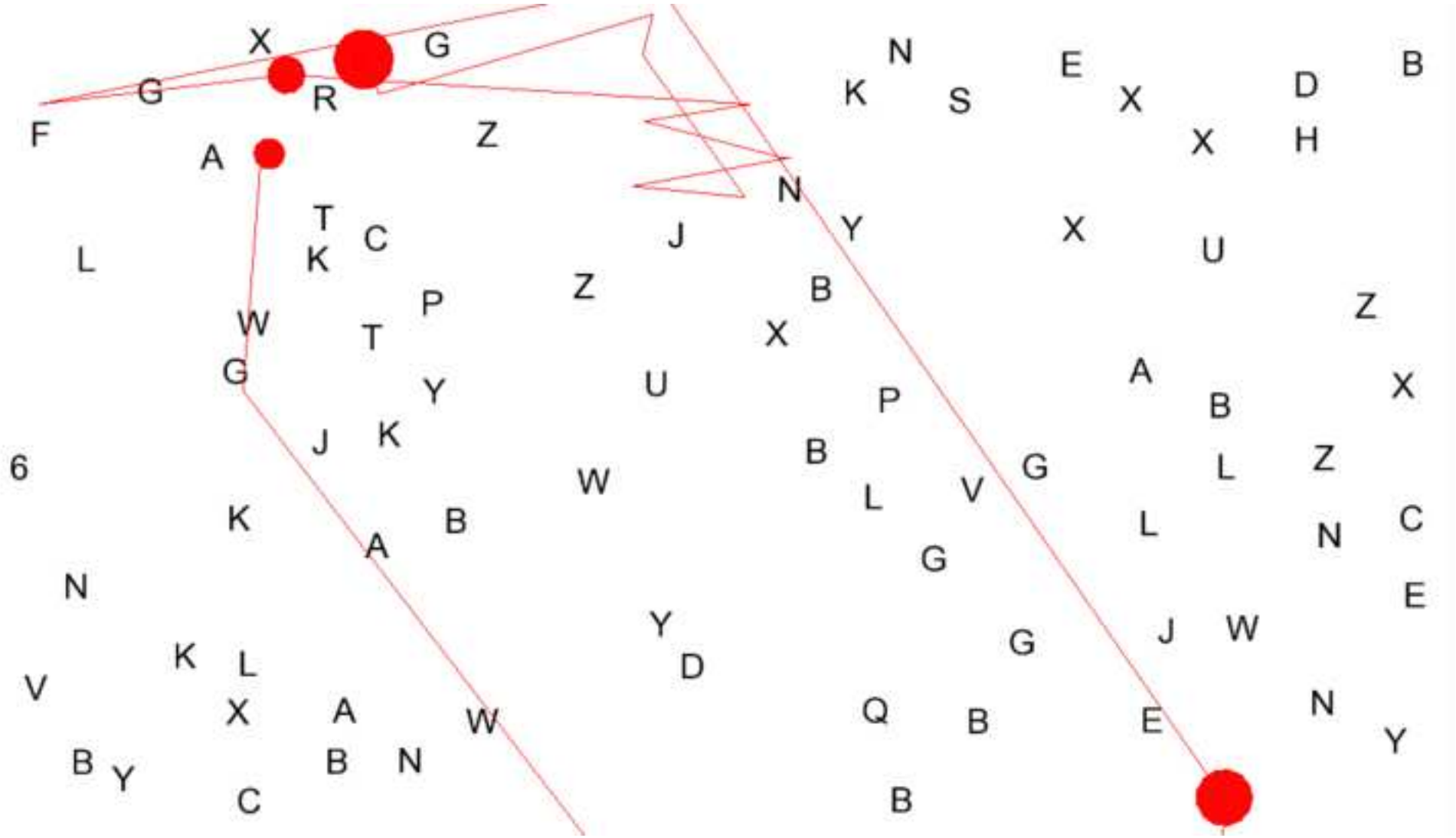

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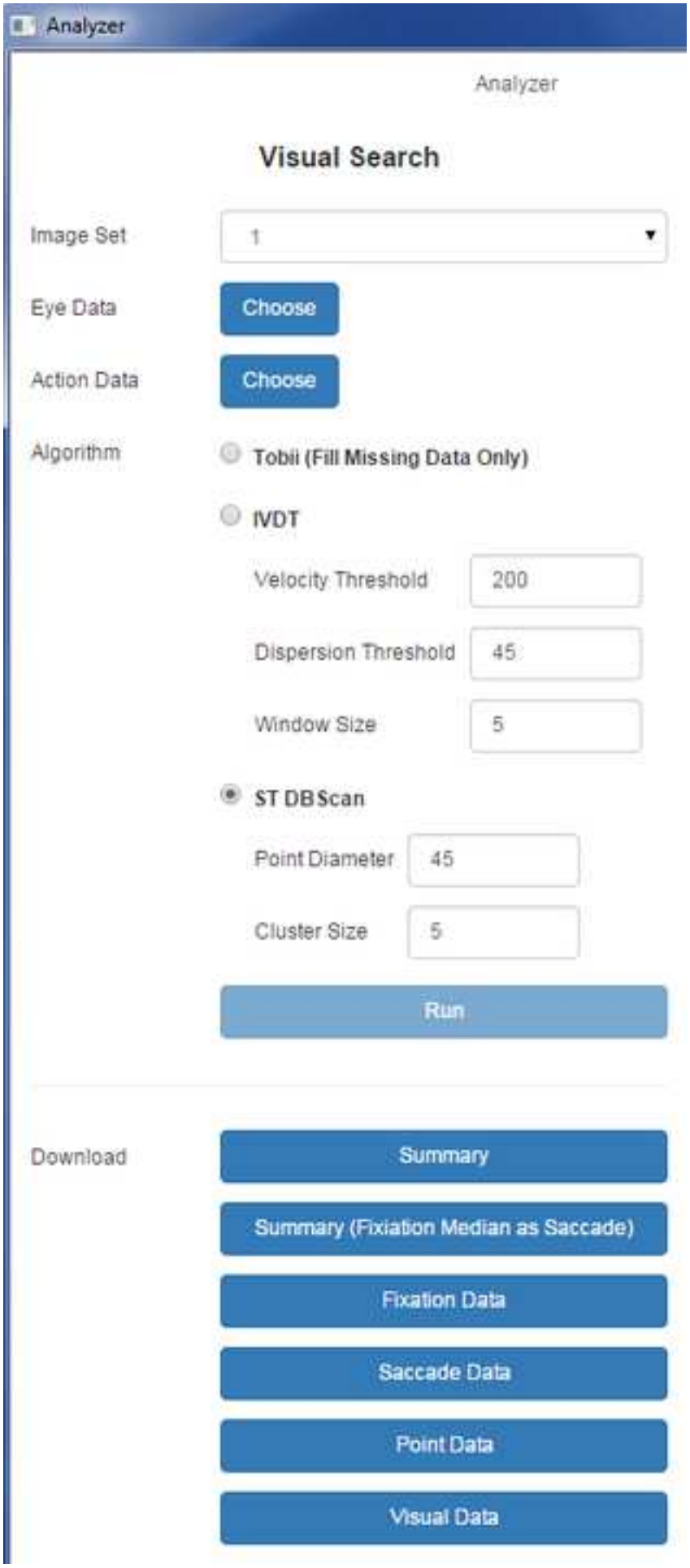

	Control group	Parkinson's group	p-value
Global Cognitive scales			
MMSE	28.53 (1.63)	28 (1.84)	0.09
MoCA	27.10 (2.25)	26 (2.34)	0.009*
Specific Cognitive tests – Frontal executive & Frontal-temporal			
Stocking of Cambridge[a]	1.16 (0.14)	1.24 (0.19)	0.018*
Stroop test[b]	1.24 (1.77)	1.36 (1.65)	0.697
Verbal fluency - animal[b]	0.92 (1.47)	0.26 (1.31)	0.01*
Verbal fluency - fruit[b]	-0.71 (0.74)	-1.01 (0.79)	0.028*
Verbal fluency - vegetable[b]	-0.66 (1.04)	-1.11 (0.90)	0.011*
Specific Cognitive tests – Verbal Memory (Hong Kong List Learning Test)			
Total learning[b]	0.03 (0.90)	-0.30 (0.87)	0.037*
10 minutes delay free recall[b]	-0.17 (0.90)	-0.44 (1.10)	0.131
30 minutes delay free recall[b]	-0.19 (0.90)	-0.39 (1.04)	0.206
Recognition score[b]	0.10 (1.00)	0.15 (0.73)	0.722
Discrimination score[b]	-0.05 (1.02)	-0.13 (0.97)	0.636
Specific Cognitive tests – Visual spatial memory			
Pattern recognition memory[c]	91.33 (9.40)	87.77 (10.20)	0.045*
Specific Cognitive tests – Attention/Working memory			
Spatial span[d]	6.15 (1.10)	5.65 (1.17)	0.016*

Table 2

	Control group	Parkinson's disease group	p-value
Mean fixation duration, in miliseconds (SD) [Range]	211.59 (24.90) [165.77 - 264.63]	216.58 (31.64) [145.43-312.68]	0.331
Mean saccadic amplitude, in degrees (SD) [Range]	17.27 (2.49) [13.34 - 22.99]	16.36 (2.36) [11.66-23.20]	0.037*

Table 3

Source	Dependent Variable	df	F	B
Mean fixation duration	Verbal fluency - fuit	1	5.647	-0.006
	Verbal fluency - vegetable	1	9.744	-0.009
	Recognition score	1	5.843	-0.007
	Discrimination score	1	12.771	-0.011
	Pattern recognition memory	1	5.505	-0.071

Beta	Std. Error	t	p-value
-0.227	0.002	-2.376	0.009*
-0.288	0.003	-3.122	0.002*
-0.215	0.003	-2.417	0.017*
-0.314	0.003	-3.574	0.001*
-0.215	0.03	-2.346	0.021*

Name of Material/Equipment	Company	Catalog Number
Computer	Intel	
Computerized cognitive assessment tool	CANTAB	CANTAB Research Suite
Eye Movement Analyzer	Lab Viso Limited	
Eye tracker	Tobii	Tx300
Hong Kong List Leanrning Test	Department of Psychology, The Chinese University of Hong Kong	The Hong Kong List Learning Test (HKLLT) 2nd Edition
Stroop test	Laboratory of Neuropsychology, The University of Hong Kong	Neuropsychological Measures: Normative Data for Chinese, Second Edition (Revised)
Tobii Studio	Tobii	Tobii Studio version 3.2.2
Visual Search Task	Lab Viso Limited	

Comments/Description

Contains Pattern Recognition Memory, Spatial Span, and Stockings of Cambridge
https://github.com/lab-viso-limited/visual-search-analyzer
23 inch computer screen with resolution of 1920x1080, Sampling rate at 300Hz

Computer programme for running the visual search task
https://www.labviso.com/#products

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11th June 2019

Dear Dr. Bajaj,

We would once again like to express our gratitude of the editorial board for the editing work and comments to our manuscript. The changes that we have made are as follows.

1. In line 102 we have removed "Tobii Studio" from the manuscript as suggested.

2. For step 1.4 – 1.7 we have now stated the cutoff score of MMSE/MoCA and the visual acuity. The BDI was just a tool for capturing the depressive symptoms and it was used as a covariate in statistical analysis and there is no cut off/exclusion.

3. Added citation to 1.6, 3.6, 3.9, 3.10. Citation to the Table of Material was added in 3.4 after CANTAB was first mentioned. For the Note after 3.11, the "verbal cognitive task" here is a general term of any cognitive task that involves a strong component of verbal ability. It does not specifically refer to a particular cognitive task and therefore no citation is added here.

4. We have revised the phrasing of point 3.9 – essentially the "problems" are actually scenarios that the subject has to determine the number of moves required to rearrange the balls of the lower display to match that of the upper.

5. All the subjects completing section 3 will proceed to section 4. We have added a point 3.11 to specify that.

6. We have added several figures for better visualisation of the visual search task. First of all the visualisation of the quality of the calibration process is added as Figure 1. There was also an expansion on the description of how to do so on 4.2.3. Secondly, to check for if there are more than 20% missing data, we would use the Samples rate in the Tobii Studio to see how the percentage of the time that the eye position is successfully located by the eye tracker, visualisation of the interface is now shown in Figure 3 and stated in 5.1. Figure 4 and 5 are examples of a typcial normal visualised scanpath and a grossly erroneous one respectively and they were mentioned in 5.2. Figure 6 was added to describe the data export process in Tobii Studio with an expansion of the text in 5.4. Lastly, Figure 7 shows the interface of our in-house Visual Search Analyzer.

7. For the programming and coding, we have upload the coding files for the design of the visual search task and how the number and alphabets and number are randomised (Supplementary file 1 & 2 respectively). Alternatively we have also made the programmes (both the task and the Analyzer) available online: https://www.labviso.com/#products and would like to seek for the editor's comment on which way would suit the Journal better. For the Analyser we have also put it on Github (https://github.com/lab-viso-limited/Eye-Movement-Analyzer) which shows the coding for the analyzer.

8. We have expanded the section 5 substantially and adding point 4.4.3 to detail the data export and analysis procedure using the Tobii Studio and our own Analyzer.

Once again, thank you for the opportunity to submit our manuscript to JOVE and the effort by the editoral board and reviewers.
Please let us know if there are any issues that require further clarification.

Yours sincerely,

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