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Analysis for Extracted Features of Pupil Light Reflex to Chromatic Stimuli in Alzheimer's Patients

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Abstract

INTRODUCTION: Some Alzheimer's Disease (AD) patients respond to chromatic light stimulus, which may influence intrinsically photosensitive retinal ganglion cells (ipRGCs), due to factors common to both AD and Age-Related Macular Disease (AMD).
OBJECTIVES: In this study, short light pulses of three colours were introduced to a novel diagnostic procesure for AD patients such as classification techniques using waveform features of pupil light reflexs (PLRs), and their prediction performances were evaluated.
METHOD: PLRs to 1s pulses of red, blue and white stimuli shown at high and low photopic levels followed by a 7s restoration process were recorded after the stimuli were shown to 7 AD patients and 12 non-AD participants (aged 42-89). Features of waveform shapes of PLRs in 5 dimensions and 15 features of PLRs were extracted.
RESULTS: In a classification analysis, most non-AD participants were correctly identified using the same level of performance we reported when PLRs for red and blue stimuli were used to measure the performance of AD patients. There were significant differences in some of the features of PLRs extracted from the two groups (AD and non-AD participants), particularly with the features for blue light stimuli in high brightness, which produced significant reactions in AD patients. The classification performance of using 15 features of the response to blue light stimuli was the highest among responses for all three colours, and was higher than the performance using the procedure in the previous study. Also, a few of the features extracted using the three colours of stimuli changed significantly across age ranges (70 and under, 71-80, and over 80), so these may indicate factors related to ageing.
CONCLUSION: These results confirm that some specific features of PLRs, in particular the response to blue light, can indicate the existence of AD in patients. Also, a few of the features may reveal factors related to ageing during evaluations which use PLRs. This evidence may help in the better understanding of features of PLRs.

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1. Introduction

The pupil light reflex (PLR), which produces changes in pupil diameter in response to a light pulse of white or green, has been introduced to diagnose Alzheimer's Disease (AD) [1, 2]. In addition to this, the recent discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs) [3, 4] reveals the possibility of using various new procedures to diagnose diseases related to retinal conditions by using a shorter light wavelength, such as blue light [5–7]. For example, PLRs related to ipRGCs can be used to detect symptoms of Age-Related Macular Degeneration (AMD) [8]. Also, the phenomenon has been used to diagnostic procedures for diseases [9–11]. Additionally, some critical studies

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have suggested that common sources may be the origin of both AD and AMD diseases [12–15]. The details of these investigations will be summarised in the next section. However, because most AD patients are elderly, the influence of ageing on PLRs should be evaluated carefully.

The authors have been studying a diagnostic procedure for detecting AD symptoms using PLRs of various types of light pulses and observing the conditions the light pulses produce [16]. Though these results show the possibility of aiding the diagnosis of the disease, a more flexible procedure is required. In particular, a simple and effective assessment procedure should be designed for elderly people, in order to reduce the workload during the observation. Therefore, the authors have developed a novel diagnostic procedure for AD patients such as classification techniques using waveform features of pupil light reflexes (PLRs), and their prediction performances were evaluated.

In this paper, the possibility of detecting Alzheimer's Disease in patients using a set of features of PLRs in response to chromatic stimuli is examined. The PLRs of both AD patients and healthy control participants were observed using stimuli consisting of white, red and blue light to activate ipRGCs and conventional retinal ganglion cells. The following topics are addressed:

1. The features of PLRs for chromatic stimuli shown at two brightness levels to healthy and AD patients are compared.

2. The influence of participant's age on PLRs in responses to the stimuli is measured and analysed.

3. The possibility of detecting AD in patients using the features of PLRs is examined. The contributing features are also extracted.

As a result, the possibility of developing a new diagnostic procedure is discussed.

2. Related works

2.1. Alzheimer's disease (AD)

Clinical features of Alzheimer's disease (AD) include progressive cognitive decline, affecting memory, learning, language, deterioration of sleep and disturbances of normal circadian rhythms [17, 18]. Alzheimer's disease affects approximately 10% of people aged 65 or over. It is a chronic, progressive neurodegenerative disease characterised by changes in many cognitive and behavioural aspects that significantly interfere with function in everyday life.

One model of AD formation is that many factors combine to cause the accumulation of amyloid β (Aβ) in the brain, which in turn causes synaptic dysfunction, tangle formation and neuronal death. These pathology conditions are found in the central nervous system, as well as retina (especially mRGC) [19] and in the locus coeruleus (LC) [20–22].

There is widespread agreement on the need for identification of an early stage of AD using minimally-invasive (or even non-invasive), inexpensive screening test. In addition to the debilitating symptoms endured by AD patients, this disease imposes a huge social and economic load on the society.

2.2. PLRs with AD patients

PLRs have been used with AD patients, and the influences of light intensity [23], light pulsing [24], and the resulting behaviours [25, 26] have been examined using the response to some forms of light. In these studies, the characteristics of PLRs of AD patients, comparable AD-like participants such as people with mild cognitive impairment (MCI) [27] and healthy, age-matched control subjects, were compared. Since PLRs of elderly peoples are influenced by factors concerning age, dynamical features of pupil responses such as amplitude, latency and others are affected [28]. This is the reason for the requirement of preparing age-matched subjects. Though there are some differences between AD patients and other subjects [26], an appropriate extraction procedure for AD patients that considers the factor of age needs to be created.

2.3. PLRs based on ipRGCs

The initial research into diagnosing AD patient was conducted before ipRGCs were discovered, unfortunately. As many recent studies have indicated, another type of PLR which responds to the melanopsin-based ipRGCs has been discovered [29, 30]. This phenomenon can be used as a disease-detecting diagnostic procedure [5, 6], since the PLR behaviours of ipRGCs is different from conventional reactions which are based on the rod and cone system [9, 31]. These detailed differences in PLR mechanisms in response to chromatic stimuli at several brightness levels have been discussed [9, 32]. In addition to the behavioural differences of ganglion cells, ipRGCs are scattered across the retina, while the conventional ganglion cells, such as the rod and cone system, are located in specific areas. By using these differences in PLR behaviour, PLRs in response to chromatic stimuli have been observed in patients with retinal diseases such as Pigmentosa or Glaucoma [7, 10, 11]. Also, this phenomenon was used with AMD patients, and a diagnostic procedure has also been discussed [8]. The patients do not recognise the influence of AMD when the diseased area appears at the periphery of the retina. In this case, the cone-based PLRs may not be influenced, but ipRGC-based

PLRs will be influenced in regards to the difference in their distribution. Therefore, the differences in the two types of PLRs may suggest a retinal condition as the possible reason for the size of the damaged area caused by AMD. To examined the differences, several features of waveform shapes of PLRs in response to red and blue light stimuli were compared using discrimination analysis [8].

2.4. Possibility of diagnose using PLRs with ipRGCs

The previous studies revealed some significant differences in features of PLRs between AD patients and healthy, age-matched control healthy subjects. Modern day statistical analysis offers the possibility of identifying potential patients. To obtain accurate results, appropriate feature extraction and a robust discriminant technique is required.

While some diagnostic procedures have been developed using ipRGC-based PLRs, conventional PLRs in response to white lights [1] and green lights (wavelength = 585nm) [2] are still used to diagnose AD patients when the influence of age is not consideration.

These studies are also examining whether typical features of PLRs can be bio-markers for AD patients [26, 27, 33]. In the case of AMD patients, the retinal conditions influence PLR waveforms, in particular the differences between blue and red light stimuli. Recently, in addition to amyloid β accumulation, retinal ganglion cell and nerve fiber layer loss [34], retinal bio-markers such as retinal vascular conditions can now be used as indices of symptoms for AD patients.

Interestingly, the early stages of AD diseases show histopathological changes in the structure of one of the types of RGCs, namely ipRGCs. In addition, it is very interesting that in AD, ipRGCs are the first to be destroyed even when the remaining RGCs remain pathologically unchanged.

Current AD patient studies suggest that the presence of amyloid β (Aβ) influences the disease [35, 36], and also that this material, in particular the retinal pigment epithelium (RPE), is a common risk factor for AMD [12]. Using these two approaches, the detailed mechanisms of both diseases have been discussed by medical researchers [13–15]. The results have shown that due to the condition of the retinal, PLR responses may reflect both AMD and AD.

Therefore, a diagnostic procedure that has been developed for AMD can be used as the common diagnostic procedure for detecting symptoms of AD using PLR waveform shapes. This is a quick and simple procedure. The performance of preliminary procedures has produced results that are insufficient [16]. An improved procedure using ipRGC-based PLRs should be developed, and the diagnostic performance examined together with conventional methods.

Table 1. Number of subjects by age

Label	Age	Control	Patient
L	≤ 70	5	3
M	71~80	5	3
H	81≤	2	1

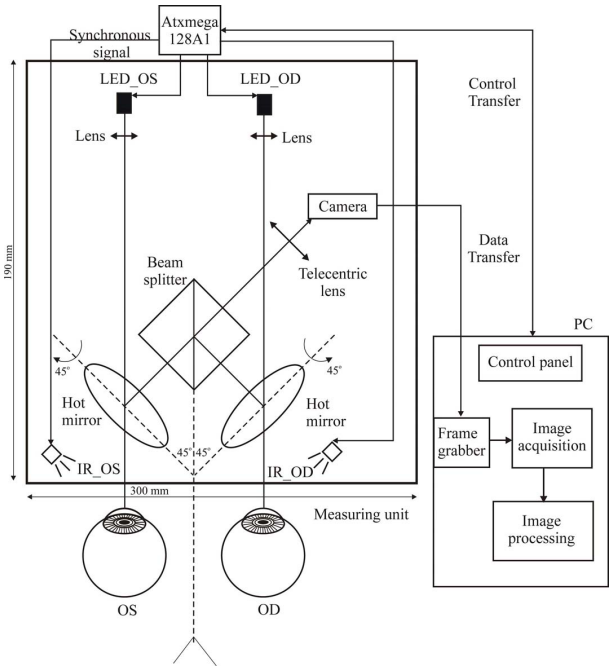

Figure 1. Diagram of measuring equipment [37]

3. Method

3.1. Participants

A conventional PLR experiment was performed using 19 participants (42~89 years old, mean age:70.6), 12 of which were healthy individuals with normal vision (the control group: 62~89 years old, mean age:72.1) and 7 who were patients with Alzheimer's Disease (AD Patients: 42~84 years old, mean age:68.1) who had already been diagnosed by medical doctors. It was not easy to invite volunteers who were aged over 80. The age levels are summarised in Table 1.

The experiment confirmed by the local ethical committee of the Wrocław Medical University which was established in accordance with the the Declaration of Helsinki. Patients and healthy controls gave informed written consent on paper before the experiment, and debriefed afterwards about the purpose of the experiment.

3.2. PLR measurement

The stimuli consisted of three chromatic lights, red (635nm), blue (470nm) and white (CIE x:0.28, y:0.31), at two levels of brightness (10 and 100 cd/m^2). These

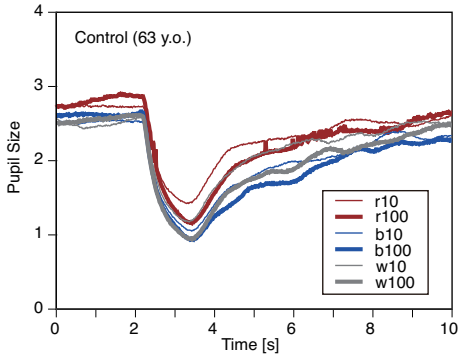

Figure 2. An example of PLRs for a control group subject

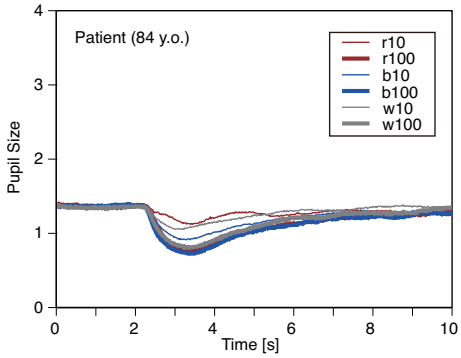

Figure 3. An example of PLRs for an AD patient

stimuli were labelled as r10, r100, b10, b100, w10 and w100.

The duration of observations was 10 seconds, with the first 2s being a prestimulus phase as a rest period, followed by a 1s light pulse and 7s as a restoration phase. Pupil diameters were measured in mm at 60Hz using a system developed by some of the authors, which is illustrated in Figure 1 [37]. Figure 1 shows a diagram of the measuring unit, which is installed on an ophthalmological slit lamp base as the image acquisition module, and it consists of the infrared (IR) illumination, the optical path, and a single camera with a frame grabber. The illumination part consists of four IR light-emitting diodes (LEDs), two for the left eye (OS)(IR_OS) and two for the right eye (OD)(IR_OD). The IR light is reflected by the corneal surface, transmitted by the optical paths, and projected onto a high-speed high-resolution camera. The PC stores the acquired data as a sequence of grey-scale pupil images in bmp format. The image processing module estimates the measured pupil parameters frame by frame, individually for the left and the right eyes. Finally, the obtained pupil dynamic waveforms are stored. PLRs for each stimulus were observed in single trials using a repeated-measure design.

Examples of measurements for a healthy individual are shown in Figure 2, and for an AD patient in Figure 3. In these figures, PLRs are illustrated in response to 6 stimuli, namely the 3 colours and two levels of brightness.

4. Feature extractions from PLRs

In comparing the waveforms of PLRs between two Figures 2 and 3, the information from the PLRs may represent some of the features of AD patients. Two types of feature representations need to be considered in order to extract the specific reactions of the two approaches.

4.1. Fourier descriptors for PLR waveform shapes

PLR's waveform shapes can be represented using Discrete Fourier Transform (DFT) [38, 39]. Discrete Fourier Transform (DFT) was applied to all responses. The signal $x(n)$ can be noted as an equation (1) using DFT [40].

$$x(n) = a_0 + \sum_{k=1}^{N/2} (a(k)cos(2\pi k \frac{t(n)}{N\Delta}) + b(k)sin(2\pi k \frac{t(n)}{N\Delta})) \quad (1)$$

$$a_0 = X(1)/N$$
$$a(k) = 2\ real(X(k+1))/N$$
$$b(k) = 2\ imag(X(k+1))/N$$

Some of the magnitudes of the coefficients in the early orders of the series, $a(k)$ and $b(k)$, and the DC component (a_0) were employed to form feature vectors of the waveform shapes [8]. The magnitudes of the coefficients, including a_0, $FD_i(i = 0, \ldots, 5)$, can be noted as follows:

$$f = [FD_0, FD_1, \ldots, FD_5] \quad (2)$$

The features are affected by individual factors, so that a standardised feature using the component, FD_1, which is converted from the above vector as follows [39]:

$$f = [\frac{FD_2}{FD_1}, \frac{FD_3}{FD_1}, \ldots, \frac{FD_5}{FD_1}] \quad (3)$$

As a result, the features of the waveform shapes can be noted as a vector consisting of 5 components, including the DC component.

4.2. PLR feature definitions

A typical PLR waveform shape is illustrated in Figure 4. In the figure, the light pulse overlaps for a period of 2~3 seconds. As the figure illustrates, there are pupillary response delays due to the shrinking of pupil and its restoration to normal size.

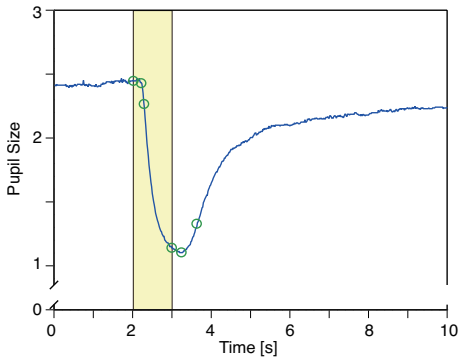

Figure 4. Feature extraction from PLR responses

Table 2. Definitions of PLR features

Variable	Definition & notes
ps_base	Mean of the pupil size for time before light pulse
ps_lon	Pupil size where light pulse on
ps_loff	Pupil size where light pulse off
ps_min	Minimum pupil size
RA	Range of pupil size (ps_lon - ps_min)
v_con	Max amplitude of pupil constriction velocity
v_rest	Max amplitude of pupil re-constriction velocity
ac_max	Min amplitude of pupil acceleration
t_delay	Pupil response delay
t_min	Time when "ps_min" appears
t_v_con	Time when "v_con" appears
t_v_rest	Time when "v_rest" appears
int_con	Integration of constriction phase
int_rest	Integration of restoration phase
int	Overall integration (int_con + int_rest)

Some features which are extracted to specify the PLR response, and these variable features are summarised in Table 2. They are pupil size, velocity of pupillary change, duration of change, and integration of the waveform. These features are calculated for each PLR response.

4.3. Prediction procedure

Regarding the differences between Figures 2 and 3, PLRs may be influenced by disease in patients with AD. This possibility is examined by introducing classification analysis using a Random forest technique.

The number of variables used to train the model for the data sets was considered carefully. The number of variables sampled was optimised using the tuning function of the "randomForest" package for R [41].

The results of a two-class classification were evaluated using a Random forest technique, which consists of training the model using a part of the assigned data set, and conducting classification prediction using the model and the remaining data. The results produced an average of ten training and prediction trials, in order to evaluate classification performance. Prediction performance was evaluated using error rates for each class of healthy and patient participants, and for the overall error rate.

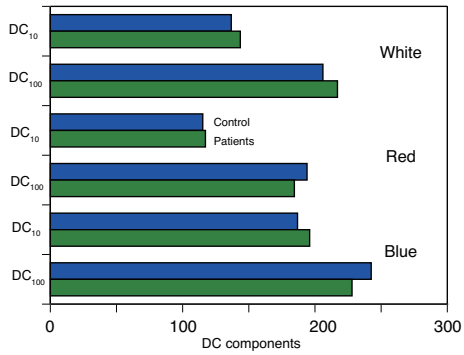

Figure 5. Comparison of DC components between stimulus conditions

5. Results

5.1. Features comparisons in PLRs

Features of PLRs are defined in the previous section. These features are compared between the two groups of healthy control and AD patient participants.

Features of waveform shapes. The features of PLR waveform shapes for all PLRs throughout the 10s observation period were extracted using the procedure mentioned in the above section.

Since the waveform shapes in Figures 2 and 3 looked different, the features were compared. The major features are the DC components such as the overall magnitude of the pupillary changes. Figure 5 shows the means of the DC components for the three colours at the two levels of brightness (10, 100cd/m^2).

The results confirm that the mean amplitudes for blue lights are the highest, and that the mean amplitudes decrease in the order of white to red light. Also, the brightness of the stimuli affects the mean amplitudes of all colour conditions independently. In a comparison of the means of the two groups of healthy control and AD patients, there are no significant differences. The overall amplitudes of waveform shapes are comparable between the two groups. This result coincides with the previous study [16].

The four dimensional components were produced by using the extraction procedure for the waveform shapes. Though each component of every stimulus condition was compared between the two groups, there were no significant differences except for one feature in the b10 condition. This result suggests that AD patients cannot be detected using the differences of the features.

Comparison of extracted features between the two groups. The extracted features from each stimulus were compared between the two groups. The results are summarised

Table 3. Means of PLR features

Feature	b10(N=19)		b100(N=17)		r10(N=18)		r100(N=19)		w10(N=19)		w100(N=19)	
Variable	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient
ps_base	19.88	16.69	**20.51**	**14.21**	**20.16**	**14.37**	19.59	16.41	19.2	16.40	19.20	16.46
ps_lon	20.24	16.72	**20.81**	**14.18**	**20.29**	**14.22**	20.00	16.26	19.56	16.54	19.45	16.46
ps_loff	10.90	8.02	**9.76**	**6.76**	13.02	9.29	10.15	7.68	11.61	8.80	9.14	7.03
ps_min	10.68	7.59	**9.20**	**5.88**	12.75	8.96	9.76	7.21	11.31	8.56	8.77	6.29
RA	9.56	9.13	**11.60**	**8.30**	7.53	5.27	10.24	9.05	8.26	8.08	10.69	10.17
v_con	-0.46	-0.47	**-0.51**	**-0.31**	**-0.36**	**-0.22**	-0.44	-0.37	-0.40	-0.40	-0.48	-0.42
v_rest	0.13	0.10	**0.18**	**0.08**	**0.13**	**0.08**	0.16	0.10	0.14	0.10	0.14	0.11
ac_max	-0.06	-0.07	**-0.07**	**-0.04**	**-0.05**	**-0.03**	-0.06	-0.05	-0.06	-0.05	-0.07	-0.06
t_delay	0.24	0.26	0.23	0.24	0.25	0.26	0.24	0.25	0.26	0.28	0.23	0.23
t_min	**1.13**	**1.31**	**1.30**	**1.43**	1.09	1.26	1.22	1.36	1.10	1.22	**1.26**	**1.41**
t_v_con	0.34	0.35	**0.33**	**0.36**	0.35	0.39	0.37	0.37	0.37	0.38	0.32	0.33
t_v_rest	1.84	1.78	1.91	2.14	1.65	2.06	1.88	2.11	1.67	1.96	1.74	1.98
int_con	293.1	277.0	**345.0**	**211.0**	**230.0**	**144.5**	301.5	260	249.4	241.6	330.5	300.3
int_rest	748.5	823.0	1015.9	802.7	532.1	444.1	849.8	796.9	612.6	652.3	906.9	941.7
int	1041.6	1100	1361	1014	762.1	588.7	1151.3	1056.9	862.0	893.9	1237.3	1242.0

Table 4. Age affected Features (list of significant variables)

		age levels		
Stimulus	Variable	L	M	H
b10	ac_max	-.11	-.05	-.04
b100	ps_min	8.0	6.9	10.9
r10	t_v_con	0.37	0.36	0.43
r100	t_delay	0.23	0.24	0.31
	int_con	381.4	249.5	176.3
w10	RA	11.2	7.9	4.7
	v_con	-.64	-.33	-.25
	ac_max	-.09	-.04	-.03
w100	RA	14.3	9.6	7.6
	v_con	-.64	-.39	-.33
	t_v_con	0.28	0.34	0.36
	int_con	459.6	280.8	217.9
	int_rest	1279.1	853.7	659.8
	int	1738.8	1134.5	877.7

Age factor is significant ($p<0.05$)

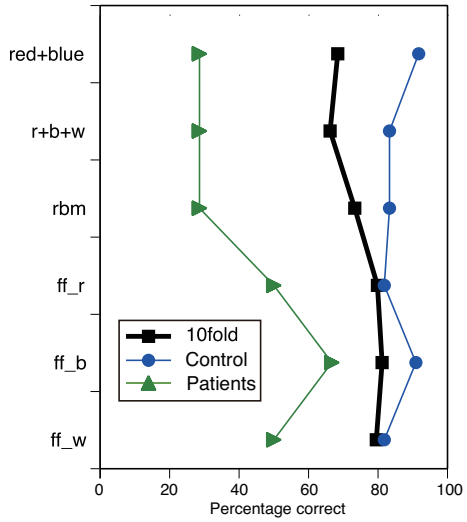

Figure 6. Comparison of classification performances

in Table 3. A statistical test, known as a t-test, was applied to these values. When there was a significant difference ($p < 0.05$) between pairs of values, the values are displayed in bold face. As the table shows, there are many significant pairs for the b100 and r10 conditions, but few significant pairs for the white stimulus. In regards to the significant differences for the b100 condition, such as pupil size, velocity and acceleration of pupillary change, the pupil size for AD patients is relatively small, and responds slowly.

All participants are elderly, and in addition to being AD patients, their ages may affect pupil responses. The effect of two factors (participant group and age level) on pupillary changes was examined using two-way ANOVA. The variables with deviations which contribute most significantly were selected ($p < 0.05$) , and are summarised in Table 4 using means across age levels. These means change along with age levels. Most variables selected are related to velocity and time delay. Since there are few significant interactions between the two factors, there are no interactions between two factors in regards to features of PLRs. In addition, most significant differences between age levels appeared for white stimulus and most differences between the two groups occurred when w100 light was used. These results may be related to the mechanism of the PLR, and thus a detailed analysis of this will be a topic of our further study.

5.2. Prediction performance

Prediction using features of waveform shapes of PLRs. The possibility of extracting patients with AD was examined using 5 dimensional features of waveform shapes for a stimulus condition [16]. Some data sets were prepared as combinations of features for stimulus condition consisting of a combination of features for red, blue and white colours, at two levels of brightness ($10cd/m^2$, $100cd/m^2$).

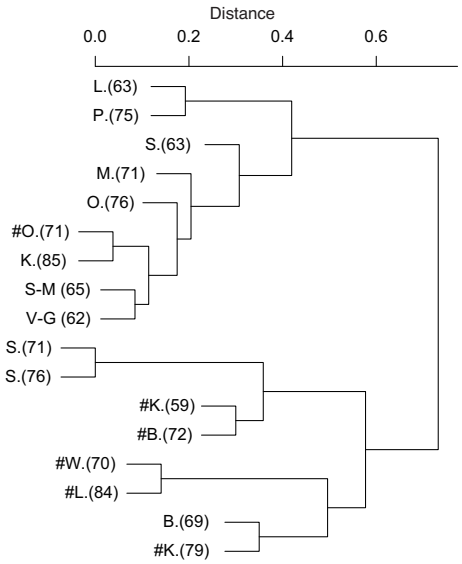

Figure 7. Dendrogram of participants using features of b100 condition; mark # are patients

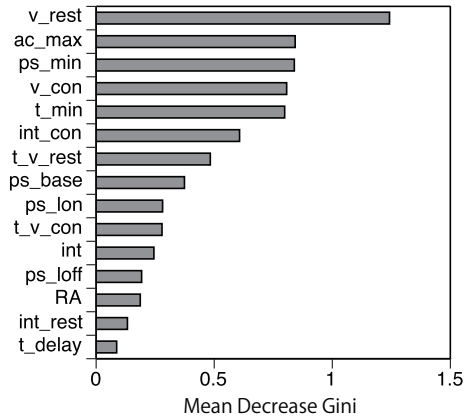

Figure 8. Contribution weights in features of b100 condition for the prediciton

The combinations were all conditions for red and blue light stimuli at two levels of brightness (red+blue), all conditions for the three light stimuli at two levels of brightness (r+b+w), and selected combinations of the three conditions such as [r10, b10, b100] (rbw). As mentioned in the Method section, a Random forest technique, which is based on a bootstrap method [42], was applied to the data sets as the number of samples is quite limited. Performance was summarised using mean correct ratios of the overall 10-hold cross validations for patients and for healthy control groups. The results are indicated in the upper panel of Figure 6. The overall discrimination performance is around 70~80%, and for the selected feature combinations it is slightly higher than for the other two conditions. Prediction performance for AD patients stays at lower levels though the levels for healthy control group for controlled healthy group are over 80%. The overall prediction performance is comparable with the previous preliminary experiment where the duration of light pulse was 10 seconds. This evidence shows that the prediction performances between two durations of light pulses (10 seconds and 1 second) is comparable. In both cases, the prediction performance for AD patients is quite low, and thus additional improvement is necessary.

Prediction using features of PLRs. The features of PLRs defined in Table 2 were used in the prediction, and so was the Random forest technique. To evaluate the effectiveness of stimulus colours, prediction performance was compared using a set of one of three colours in two levels of brightness ($10cd/m^2$, $100cd/m^2$). This performance is summarised in Figure 6 for three colour conditions (ff_r: red colour stimuli, ff_b: blue colour stimuli, ff_w: white colour stimuli). The performances using features of PLRs improved the percentage of correct for AD patients. The 10-fold cross-validations of the overall performance are higher than are the ones which use the features of waveform shapes. Though the percentage correct predictions for AD patients increased, it remained at the level of chance. The performance (10fold) for blue stimuli (ff_b) is the highest of the six conditions. Also, the percentages for both the control and AD patients are the highest. As shown in Table 3, there are significant differences between AD patients and the healthy control group. The feature values for blue lights may affect prediction performance. In addition, the performance using the b100 condition is at the same level as the performance using the two levels of blue light.

To present the actual prediction performance, the nineteen participants are placed in clusters using the PLR features of the b100 condition. Many significant differences were observed, as Table 3 shows. Hierarchical cluster analysis using an averaging method was applied to features of the b100 condition, and a dendrogram of the results was created. The dendrogram is illustrated in Figure 7. The labels in the figure show the participants and their ages, and the subjects marked with an "#" are AD patients. The lower cluster includes 5 of the 6 patients, but 3 healthy participants are also in the same cluster. It is not easy to predict perfectly which patients are which, but the results show the possibility of extracting AD patients using the performance.

To measure the degree of contributions of features of PLRs, the level of importance of the variables extracted for prediction in the b100 condition is summarised in Figure 8. This figure shows the importance of the variables which were introduced to

the prediction using the Random forest method. The largest contribution is *v_rest* (Max. amplitude of pupil re-constriction velocity). As following the order of the degree of contribution, *v_con* (Max. amplitude of pupil constriction velocity), *ac_max* (Min. amplitude of pupil acceleration), *ps_min* (Minimum pupil size) and it's time *t_min*. Most variables represent pupil constriction behaviour.

As these features are based on blue light stimulus at a high level of brightness, the pupil response of ipRGCs based reactions may provide information about patients. Further extraction of typical features may improve prediction performance. However, as it is not easy to gather a large enough sample of patients, other types of prediction procedures should be considered. They will be the subjects of our further study.

6. Discussion

The values of features of PLRs defined above were compared between AD patients and healthy control subjects, as shown in Table 3. The results of t-tests show that the most significant differences appeared when b100 (blue $100cd/m^2$) and r10 (red $10cd/m^2$) were used. There were few differences when white light stimuli was used. In particular, significant differences in pupil diameters were observed for light pulse, speeds and acceleration of the change in response to b100 stimulus. For AD patients, this result suggests that pupil diameters under light pulse and their change in speed are relatively small, and the responses are delayed.

As mentioned above, both being an AD patient and factors related to ageing may influence pupil response. An analysis of the factors related to ageing was conducted on the features of PLRs, and the results are summarised in Table 4. The results show the gradual influence of age in accordance with the age levels (L,M,H). Significant differences are observed in *v_con* (Max. amplitude of pupil constriction velocity), and their delays such as *t_v_con* (Time when "*v_con*" appears), *t_delay* (Pupil response delay). The differences may have occurred because the size of the pupil constriction is relatively small. In addition, most factors of interactions between ageing factors and the two groups of subjects (AD patients and the healthy control group) were not significant. Both factors operate independently of each other.

As mentioned above, for white light pulse there are significant differences in ageing factors for features of PLRs, although significant differences between the two groups of subjects appeared when blue light was used. PLRs for blue light which result mainly from ipRGCs, can be used to evaluate the ageing factor independently. This kind of response behaviour may be caused by a mechanism in PLRs which concerns ipRGCs and conventional rod-cone systems.

Prediction performance using the PLR features extracted was better than the performance using features of PLR waveform shapes. In particular, the performance using features of PLRs in response to blue light stimuli ($10cd/m^2$, $100cd/m^2$) was the highest. Once again, the pupil responses for blue light, such as PLRs based on ipRGCs, reflects some of the features specific to AD patients. Though prediction performance was not perfect, as Figure 7 shows, it can be used as a reference.

In a detailed analysis of prediction performance using the blue light stimulus b100, the importance of features of PLRs was evaluated, as Figure 8 shows. As the contributing variables suggest, a more sophisticated prediction procedure needs to be developed. This will be a subject of our further study.

In addition to the above discussion, the number of participants in this study might be small scale comparing with the general classification tasks. On the other hand, there are much deviations on the features of elderly people or AD patients, then the larger sample size may not resolve the individual difference issues. During the assessment for classification performances, 10 fold validation index is employed in order to consider validation of the procedure. If there were insufficient estimations for the trained classifiers, they can be improved step by step with sample size growing. Therefore, more precise models will be developed along with our study progress. More detailed discussion will be a subject of our further study.

7. Conclusion

This paper confirms the possibility of detecting AD patients using a set of features of PLRs in response to chromatic stimuli. For conventional PLR studies, the stimuli consist of green and white light. For some applications with AD patients, PLRs based on ipRGCs used red and blue lights. These features were extracted and measured using the Fourier descriptor technique for PLR waveform shapes, and specific features of PLRs were also defined.

1. The differences in features of PLRs between the two groups of participant were compared. In the results, there were no differences in Fourier descriptors, but some significant differences in features of PLRs were produced for the b100 and the r10 experimental conditions.

2. PLRs for factors related to ageing were compared, and there were some significant differences in features of PLRs in regards to response delay and range of pupillary change. These differences were

observed mainly when a white light stimulus was used.

3. Prediction analysis for AD patients was conducted using the Random forest technique, and most participants in the healthy control group were estimated correctly. The estimation performance was the highest when the features of PLRs for blue lights were employed. This confirms the contribution of ipRGCs to the prediction of AD patients.

4. The variables which contributed to the highest levels of correct estimation were identified. They represented the temporal behaviours of PLRs.

More accurate prediction procedures and mechanisms for prediction will be subjects of our further study.

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