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TITLE:

Assessing Early Stage Open-Angle Glaucoma in Patients by Isolated-Check Visual Evoked Potential

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KEYWORDS:

visual evoked potential, isolated-check, retinal ganglion cell, magnocellular cells, M-cells, pathway, open-angle glaucoma, signal-to-noise ratio, cross-sectional study

SUMMARY:

The isolated-check visual evoked potential (icVEP) method is implemented here to assess the magnocellular ON pathway that is initially damaged in glaucoma. The study shows standard operative procedures using icVEP to obtain reliable results. It is proved to serve as a useful objective diagnosing technology for the early detection of glaucoma.

ABSTRACT:

Recently, the isolated-check visual evoked potential (icVEP) technique was designed and has been reported to detect glaucomatous damage earlier and faster. It creates low spatial frequency/high temporal frequency bright stimuli and records cortical activity initiated primarily by afferents in the magnocellular ON pathway. This pathway contains neurons with larger volumes and axonal diameters, and it is preferentially damaged in early glaucoma, which can result in visual field loss. The study presented here uses standard operative procedures (SOP) of icVEP to obtain reliable results. It can detect visual function loss using a signal-to-noise ratio (SNR) corresponding to the defects of retinal nerve fiber layer (RNFL) in early stage open-angle glaucoma (OAG). A setting of 10 Hz and condition of 15% positive-contrast (bright) are selected to differentiate OAG patients and control subjects, with each check containing eight runs. Each run persists for 2 s (for 20 total cycles). A flowchart is constructed, which consists of pupil size and intraocular pressure over a 30 min rest period before each examination. Additionally, the testing order of eyes is performed to obtain reliable electroencephalographic signals. VEPs are recorded and analyzed automatically by software, and SNRs are derived based on a multivariate statistic.

An SNR of ≤ 1 is considered abnormal. A receiver-operating-characteristic (ROC) curve is applied to analyze the accuracy of group classification. Then, the SOP is applied in a cross-sectional study, showing that icVEP can detect glaucomatous visual function abnormality in the central visual field in the form of SNR. This value also correlates with the thickness thinning of RNFL and produces high classification accuracy for early stage OAG. Thus, it serves as a useful and objective diagnostic technology for the early detection of glaucoma.

INTRODUCTION:

Open-angle glaucoma (OAG) is a chronic, irreversible disease and one of the leading causes of blindness. Previous studies have shown that visual field tests, which are the current gold standard for glaucomatous visual loss detection, are based on conventional standard automated perimetry (SAP) cannot detect early glaucomatous functional loss until 20%–40% of retinal ganglion cells (RGCs) are damaged[1,2]. Furthermore, SAP has also been shown to have only moderate test-retest reliability, because it is a subjective psychophysical test and a time-consuming task for patients[3].

Objective electrophysiological visual field functional measures have better test-retest reliability when detecting glaucoma. Such measures include the multifocal visual evoked potential (mfVEP) and pattern electroretinogram (pERG). However, the pERG cannot provide topographic information, and the mfVEP is more time-consuming than SAP[4-8]. Fortunately, the isolated-check visual evoked potential (icVEP) was recently designed as an additional technique to detect glaucomatous damage earlier and faster[9].

In the retina, there are several RGC subpopulations such as magnocellular cells (M-cells), parvocellular cells (P-cells), and bistratified cells. They represent parallel pathways for visual information being transmitted to the brain (**Figure 1**)[9,10]. To govern the separate perceptions of brightness and darkness, the dichotomy of ON and OFF pathways has been established[11,12]. Magnocellular ON (M-ON) cells are considerably larger than magnocellular OFF (M-OFF) cells, while M-cells are considerably larger than P-cells in humans[13,14]. The M-cell pathway mainly conveys low spatial frequency/high temporal frequency information[15]. Thus, cells involved in the M-ON pathway are sensitive to low levels of luminance contrast and not sensitive to chromatic information with larger diameter axons, which are preferentially damaged in early glaucoma[16,17]. Therefore, the icVEP produces low spatial frequency/high temporal frequency bright stimuli and records cortical activity primarily initiated by afferents (such as those found in the M-ON pathway) for the early detection of glaucoma[18-23].

PROTOCOL:

The study was approved by the Ethics Committee Review Board of Peking University Third Hospital and conformed to the Declaration of Helsinki.

1. Settings

NOTE: The icVEP hardware necessitates a reexamination of stimulus conditions for favoring the M-ON pathway using a standard video card with an 8-bit digital-to-analog converter per electron gun.

1.1. Click the **Setting** button and ensure that the frame rate is 60 Hz, luminance of the display's static background is 51 cd/m^2, and total cycles are 20.

1.2. To differentiate OAG patients and control subjects, ensure the following conditions: sinusoidal temporal signals of 10 Hz (six frames per cycle) and 15% positive-contrast (bright).

2. Examination

2.1. Click the **IC** button to ensure that the spatial pattern is a 24 x 24 array of isolated-checks to subtend a 11° visual field, with a 2 x 2 array fixation cross without sinusoidal temporal signals, in order to cue facilitated and careful fixation on the center of the screen (**Figure 2**)[9].

2.2. Place the gold cup electrodes filled with electrolytic water-soluble paste at the following midline sites on the scalp based on the international 10-20 system (**Figure 3**)[24]. Ensure that the testing distance is 114 cm.

2.3. Click the **Start** button. One run last for 2 s: the first second of this period presents half of the test contrast level (7.50%) as an adaptation condition, and the following second presents the full test contrast (15.0%).

2.4. Note the prompt from the program and repeat the run when noise is detected and when the electroencephalography (EEG) epoch is rejected.

2.5. Note the EEG data that is displayed on the operator's monitor when the run is determined to be valid and when the operator is prompted to either accept or reject the data based on reliability.

3. Automatic data processing using software

NOTE: The data is calculated by a discrete Fourier transform after EEG signals are recorded.

3.1. Note that once the data is accepted, the program will instruct the operator to initiate the next run until a set of eight valid runs are accumulated.

3.2. Note that each run produces a fundamental frequency component (FFC), and if one of

the FFCs is an outlier relative to the remaining seven, the program will discard that FFC and will prompt the operator to repeat the run until eight qualified runs are collected.

3.3. Wait for the program to calculate the mean FFC and radius of a 95% confidence circle using the T^2_{circ} statistic[25] that is automatically produced from the eight FFCs within a few seconds.

3.4. Ensure that the individual and mean FFC values, confidence circle, and signal-to-noise ratio (SNR) are automatically displayed on the monitor within less than 1 min after the end of the test (**Figure 4A**).

4. Flowchart for assessing reliability of results

4.1. Ensure that the refractive error is corrected to adapt for a distance of 114 cm.

4.2. Ensure that intraocular pressure (IOP) is ≤ 30 mmHg on the day of examination.

4.3. Ensure that pupil diameters are ≥ 2 mm and without mydriasis.

4.4. Ensure that each subject rest and is quiet ≥ 30 min before examination.

4.5. To avoid the influence of a study curve, first check the right eye, then left eye; then, check the right and left eyes again, and record this second result.

4.6. Initiate a retest after at least a 30 min rest when the R-value (nosing ring radius) between both eyes shows a difference of > 0.2, which means the result is unreliable as mood swings.

REPRESENTATIVE RESULTS:

Recent studies showed that the accuracy of icVEP for glaucoma diagnosis ranges from 91%–100%[9,22,26]. Cross-sectional studies in China are presented here to further evaluate the potential diagnostic value of icVEP for early stage OAG.

Subjects

Subjects were OAG patients and healthy volunteers recruited by the Department of Ophthalmology, Peking University Third Hospital during 2015 and 2016. Inclusion criteria for OAG patients included the following: 25–75 years of age; best-corrected visual acuity (BCVA) < 0.3 (logarithm of the minimum angle of resolution, log MAR); spherical refraction between -6 and +3 diopters; and transparent ocular media. Additionally, patients showed the presence of OAG (subjects with open-angle, visual field defects corresponding to glaucomatous optic neuropathy [GON], and having normal or elevated IOP without secondary causes), in which the IOP was medically well-controlled and had reliable visual field test results (false positive errors ≤ 20%, false negative errors ≤ 20%, fixation losses ≤

30%) that showed early glaucomatous visual field defects on SAP.

Inclusion criteria for control subjects included the following: no ocular abnormalities, especially no GON in any eye; and a normal IOP that was never elevated over 21 mmHg. Exclusion criteria included the following: diabetes or any other systemic disease; history of ocular or neurologic disease; unequal pupil diameters and pupil diameters of < 2.0 mm; poor fixation; current use of medications that can affect visual field sensitivity (i.e., ethambutol, hydroxychloroquine, chlorpromazine); and previous history of intraocular surgery or refractory surgery.

Examinations for OAG diagnosis

For all patients, spectacle corrections were used to decrease possible effects of a blur on visual field sensitivity. At least two reliable SAP tests were performed by the Humphrey Field Analyzer II 30-2 SITA standard program at baseline. The second reliable visual field result obtained was used in this study to minimize learning effects[27]. An early stage of glaucomatous visual field loss was defined as a mean deviation (MD) of ≥ -6.00 dB, and with at least one of the following: 1) there existed a cluster of ≥ 3 points in an expected location of the visual field depressed < 5% level, at least one of which was < 1% level on pattern deviation plot; 2) corrected pattern standard deviation or pattern standard deviation significant were at $p < 0.05$; 3) glaucoma hemifield test result was "outside normal limits"[28].

Baseline examination consisted of tests for visual acuity and refraction, pupil-diameter-measurement with a ruler in natural light, slit-lamp biomicroscopy, gonioscopy, Goldmann applanation tonometry (GAT), and dilated stereoscopic fundus examination in all subjects.

The baseline IOP was measured by GAT during glaucoma service (8 A.M. to 11 A.M. local time) on the day after receiving icVEP test reports. Each patient was also subjected to a central corneal thickness (CCT) measurement using ultrasound pachymetry under topical anesthesia[29]. An average of five consecutive readings was recorded.

Stereoscopic fundus photographs were obtained from each patient after pupil dilation and evaluated in a masked fashion by two experienced doctors. Discrepancies between the two doctors were either resolved by consensus or adjudication of a third experienced doctor. GON was defined as at least one of the following: 1) the rim-to-disc ratio was < 0.1 in the upper or lower rims; 2) there existed retinal nerve fiber layer (RNFL) defects; 3) optic disc showed splint hemorrhages[30,31].

Each patient was also subjected to an optical coherence tomography (OCT) test to confirm RNFL defects corresponding to both stereoscopic photographs and HFA results. The changing of RNFL thickness in temporal superior (TS) quadrant and temporal inferior (TI) quadrant were calculated as follows: changing of RNFL thickness = RNFL thickness value - standard value from database of normal people (**Figure 4B**).

Statistical analysis

One eye was randomly selected to be analyzed when both eyes met the inclusion criteria. All the data needed to be established within 3 months for each subject. The SPSS 22.0 statistical package with statistical tests was used as follows: independent sample t-test was used for normally distributed variables; Mann-Whitney U test was used for numeric variables that were not normally distributed; and binomial variables were compared with a Chi-squared test or Fisher's exact test, when necessary. Receiver-operating-characteristic (ROC) curve analysis was used to estimate prediction accuracy for the presence of glaucomatous damage[32]. Pearson correlation coefficient was used to analyze correlations between SNR and parameters on OCT as well as between SNR and abnormalities in the central 11° field on SAP. If $p < 0.05$, differences were considered significant.

Results

A total of 44 OAG patients and 39 control subjects were included with complete data. None of these subjects complained during the icVEP test. All 83 subjects were Chinese (48 males and 35 females) with an average age of 48.54 ± 16.70 years old (range of 25–74 years). No statistical differences existed in age, sex, right/left eye, BCVA, spherical equivalent, or pupil diameter between patients and controls (**Table 1**, $p > 0.05$), but SNR was significantly lower in patients than in controls (**Table 1**, $p < 0.05$).

Regarding the icVEP results, there were 30 eyes of early OAG patients that were SNR-positive (68.18%) and only two eyes in the control group (5.13%). Using an SNR criterion of 1, icVEP showed a sensitivity of 68.18% and specificity of 94.87% for diagnosing early OAG (calculating an accuracy of 67/83 [80.72%]). However, ROC analysis indicated that an *a priori* SNR criterion of 0.93 was optimal for discrimination between patients and control subjects (**Figure 5**). Using an SNR criterion of 0.93, the specificity of the test reached 100% with a sensitivity of 65.90% (calculating an accuracy of 82.10%).

For the patients, abnormalities in the central 11° visual field test (HFA, pattern deviation, central 16 test points; **Figure 4C**) were calculated by the numbers of abnormal points with different possibility criteria. With a criterion level of $p < 0.5$, the amount of abnormal test points in the central 11° visual field was significantly negatively correlated with SNR ($p < 0.05$, $r = -0.332$, **Table 2**). Thickness changing of RNFL in the temporal superior quadrant was significantly positively correlated with SNR ($p < 0.05$, $r = 0.370$, **Table 2**), while SAP-MD, SAP-MD of the other eye, thickness changing of RNFL in the temporal inferior quadrant, and baseline IOP and CCT were all not correlated with SNR ($p > 0.05$, **Table 3**).

FIGURE AND TABLE LEGENDS:

Figure 1: Representation of the isolated-check visual evoked potential evaluating the M-cell pathway. Layers 1 and 2 are involved in the magnocellular pathway. Layers 3, 4, 5, and 6 are involved in the parvocellular pathway. The spaces between these six layers are involved in the bistratified cell pathway. RGC = retinal ganglion cell.

Figure 2: Bright conditions (positive-contrast) on the screen of isolated-check visual evoked potential. This figure has been modified from a previous publication[24].

Figure 3: Diagram of isolated-check visual evoked potential examination. GND = grounding electrode; Cz = central midline electrode; Pz = parietal midline electrode; Oz occipital midline electrode. This figure has been modified from a previous publication[24].

Figure 4: Typical results from an early stage open angle glaucoma patient. (**A**) Abnormal isolated-check visual evoked potential results. (**B**) Outcomes of peripapillary retinal nerve fiber layer thickness (RNFLT) classification on the report of optical coherence tomography. Changing of RNFLT = RNFLT value (black number). The standard value from a database of normal subjects. (green number in brackets). G = global; N = nasal; T = temporal; NS = nasal superior; TS = temporal superior; NI = nasal inferior; TI = temporal inferior. (**C**) Central 16 test points of pattern deviation on Humphrey Field Analyzer 30-2 SITA program corresponding to the central 11° visual field. This figure has been modified from a previous publication[24].

Figure 5: ROC curve. Shown is a ROC curve (blue) for data collected from the signal-to-noise ratios of isolated-check visual evoked potential in open-angle glaucoma patients and control subjects.

Table 1: Clinical characteristics of OAG patients and control subjects at baseline.

Table 2: Correlation between icVEP-SNR and abnormalities in central 11° visual field of SAP in open-angle glaucoma patients.

Table 3: Correlation between icVEP-SNR and relating factors in patients with open-angle glaucoma.

DISCUSSION:

Different settings of icVEP can stimulate different M-cell pathways and create different EEG signals. Under conditions of high temporal frequency (15 Hz) luminance contrast of icVEP (16% positive-contrast), a study involving 15 OAG patients and 14 normal observers showed a sensitivity of 73.33% and specificity of 100%[22]. However, one-half of these patients had advanced OAG. Therefore, for early stage OAG, the sensitivity could not be estimated due to the small sample size.

Tsai's study showed a sensitivity of 78% (conditions of 15% positive-contrast and 10 Hz temporal modulation) and specificity of 100%, with an accuracy of 94% from the ROC curve. These results improved upon Greenstein's study because of the lower contrast and spatial frequency found in earlier glaucoma patients. Nevertheless, there were less than 11 early stage OAG patients among 18 glaucoma patients (17 open-angle, 1 angle-closure) and 16

controls in the study[9].

In the current study, the OAG patients were only those in early stages and included a much larger sample size, which suggests that icVEP is indeed useful for detecting OAG in the “real” early-stage. About 70% of early stage OAG eyes were detected by icVEP, and the SNR of patients was greatly different than that of normal subjects.

A recent study showed that pupil size can affect icVEP results in normal subjects. icVEP values were influenced by pupillary constriction and dilation as well as optical blur[33]. This suggests that when obtaining icVEP measurements, the influence of pupil size and optical blur should be kept in mind for accurate interpretations. In the current study, pupil size was measured, and it was ensured that all values fell in the normal range. Furthermore, all EEG signals may have been affected by emotions, which yields mostly false positive errors. The current study ensured an IOP of ≤ 30mmHg on the day of examination to avoid mood swings caused by high pressure. All patients rested for ≥ 30 min before each examination, and reexamination was also performed to avoid mood effects.

SNR was defined as the ratio of the mean amplitude of the FFC to the radius of the 95% confidence circle. An SNR of > 1 indicated a significant response at the 0.05 level, which implied normal electrophysiological activity in the optic nerve. An SNR of ≤ 1 indicated a response similar to or weaker than the background noise at the 0.05 level, implying abnormal electrophysiological activity in the optic nerve. However, an SNR of 0.93 was optimal for discrimination of early stage OAG patients and control subjects in the current study using a ROC curve. Therefore, a SNR criterion of 0.93 may distinguish the severity of GON in early stage OAG patients for this study.

More than 50% M cells were in the macular region; thus, if the fovea was stimulated, there was likely a strong signal resulting in SNR > 1. Therefore, the 2 x 2 array fixation cross on the center of the screen without sinusoidal temporal signals was able to cue-facilitate careful fixation as well as avoid false negative errors with poor fixation[34]. Moreover, recent SD-OCT studies proved that RGCs in the macular region become damaged even in early stages of glaucoma, because proteolysis and secondary axotomy after damage to the optic nerve head may result in RGC apoptosis[35-38].

Analysis of central 16 test points in the current study based on pattern deviations in HFA corresponded to the 5°–10° of Bjerrum areas, where almost one-half of M-cells are distributed[10-14]. This study showed the numbers of abnormal test points in which different possibility criteria were negatively correlated with SNR (negative R-value); though, only when $p < 0.5\%$ was the correlation significant, suggesting that icVEP was able to detect functional abnormalities and reflect the severity of central visual field loss in early stage OAG.

It has been reported that the responses to stimulation of the P-cell and M-ON pathway are severely disrupted in early stages of glaucoma, even without functional involvement of the

central visual field test[26]. However, a limitation of this study is that the icVEP test requires patients with a BCVA value of larger than 0.3, spherical refraction between -6 and +3 diopters, and transparent ocular media. The study only shows the usefulness of icVEP in early OAG eyes with better visual acuity. Therefore, further studies are needed to create better stimulations and define more accurate criteria for OAG eyes with poorer visual acuity. This will help determine if the icVEP can serve as the optimal functional test for discriminating glaucoma suspects as well as pre-perimetrical and early-stages of OAG. Furthermore, another limitation is that the study does not account for differences between dominant and non-dominant eyes. Differences between those pathways and testing of these two eyes may affect the EEG signals. Above all, the flowchart will be improved after further studies are performed.

In summary, icVEP is able to detect glaucomatous visual function abnormalities in almost 70% of early stage OAG patients, with a specificity about 95%. The measured functions correlate with both the severity of central 11 °visual field loss of standard automated perimetry and decreases in RNFL thickness as detected by OCT. Therefore, icVEP can serve as a useful and objective electrophysiological visual field functional test for diagnosing early stage OAG.

DISCLOSURES:

All the authors have nothing to disclose.

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Figure1

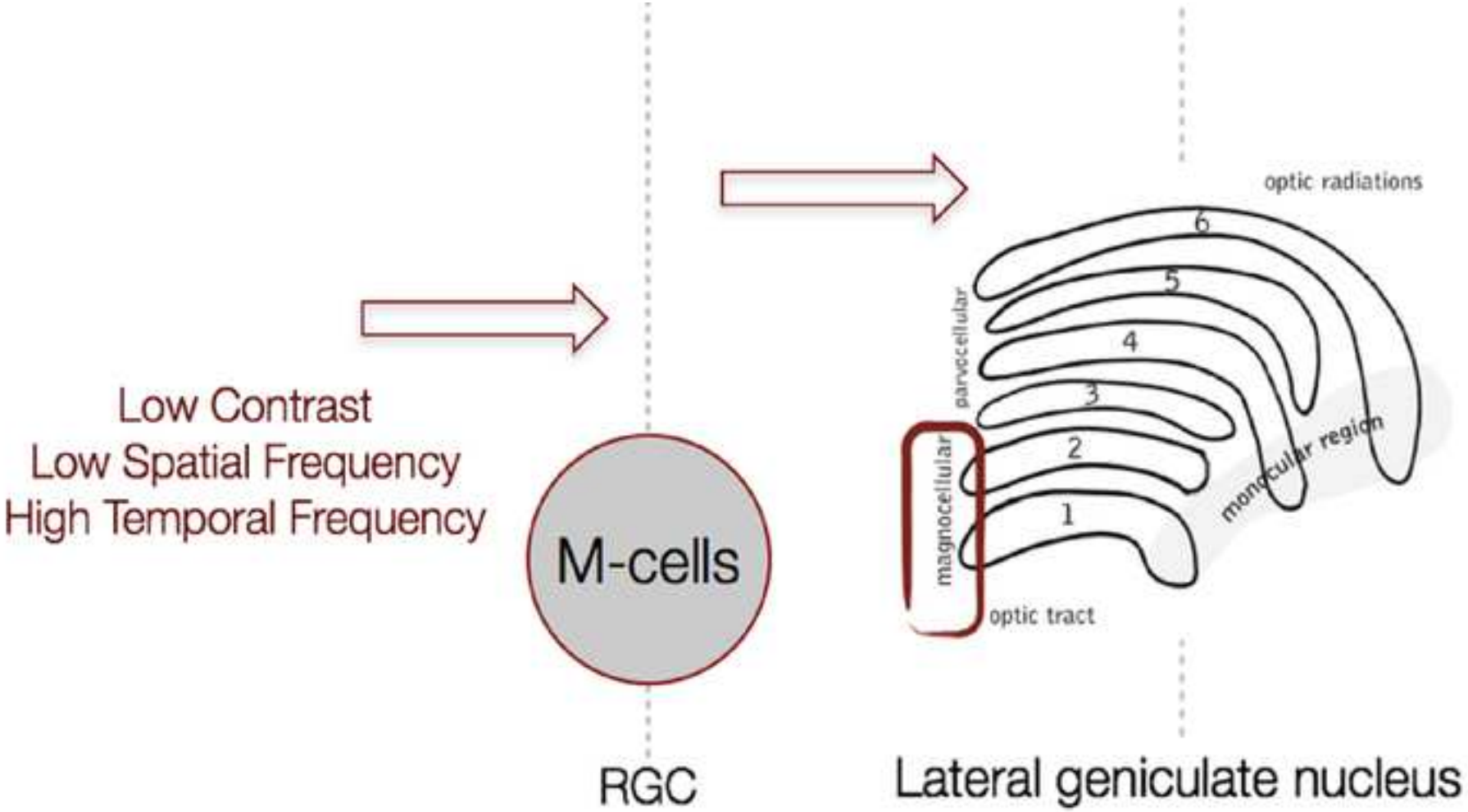

Figure2

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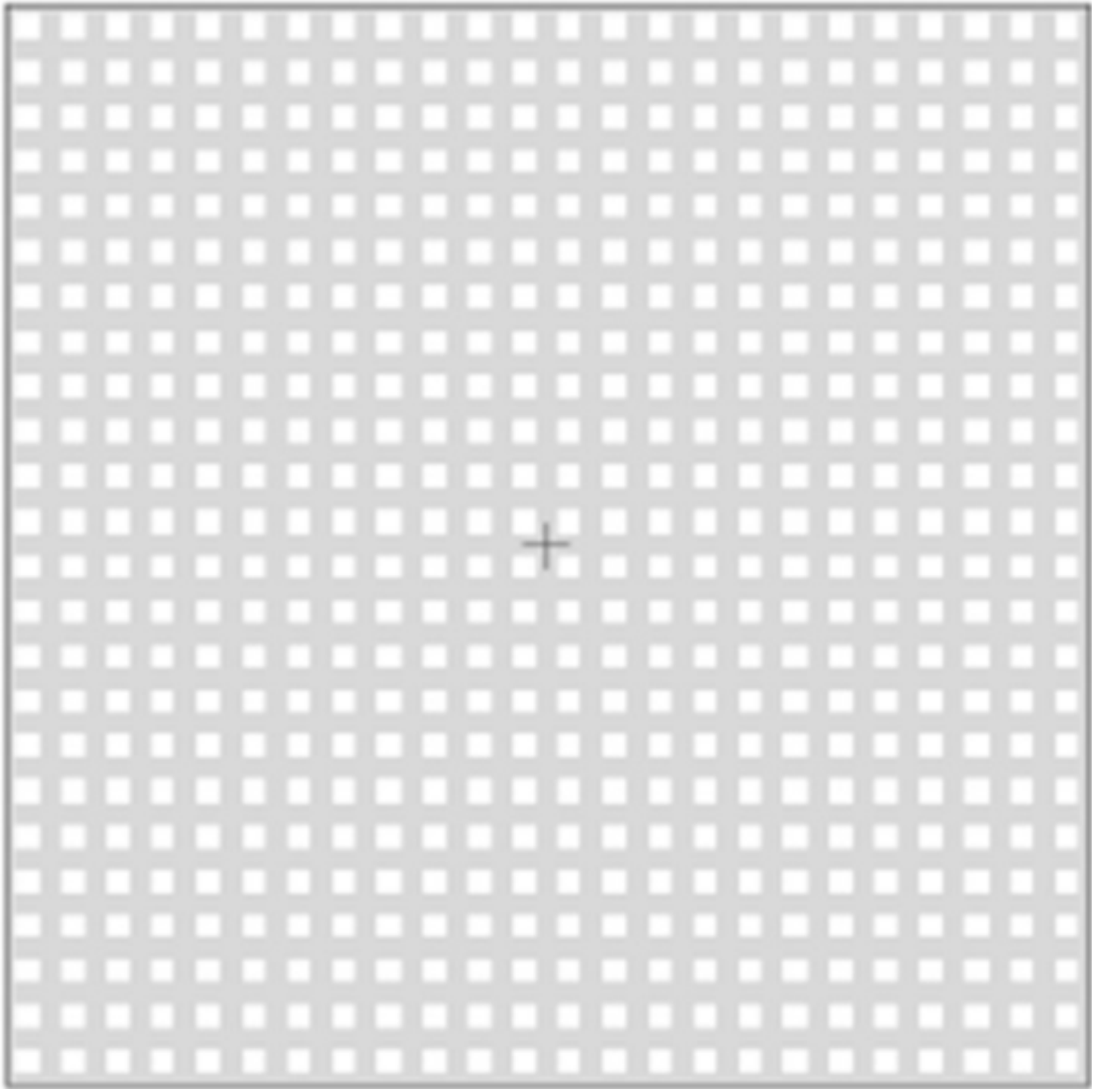

Figure3

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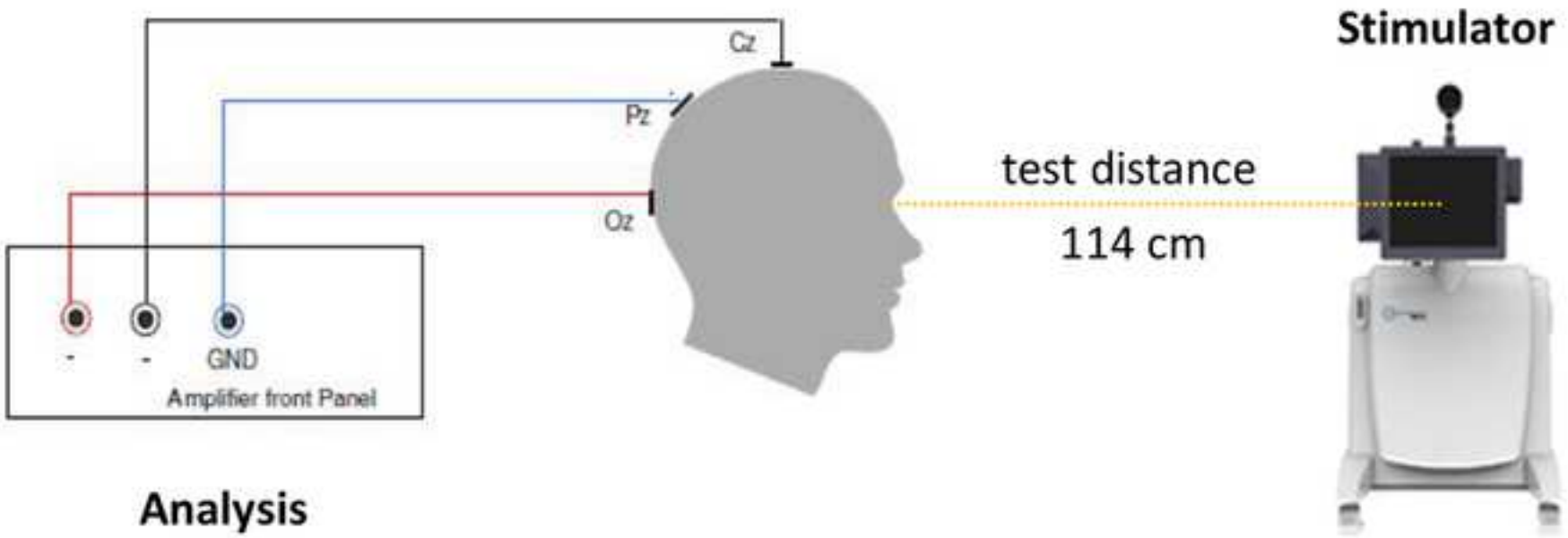

Figure4

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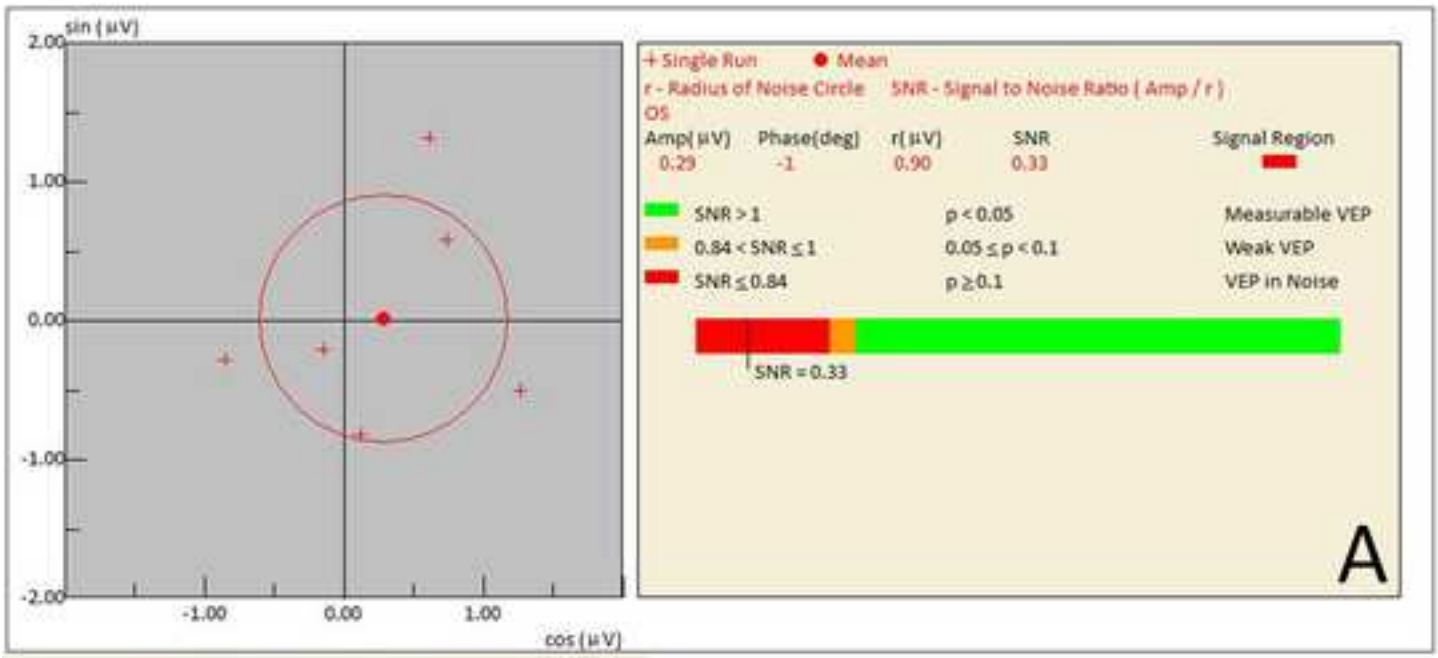

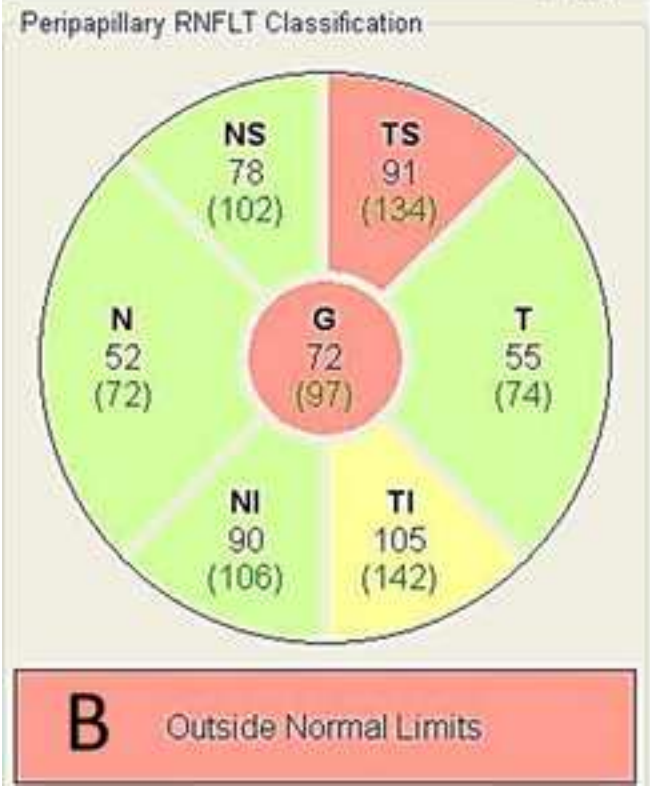

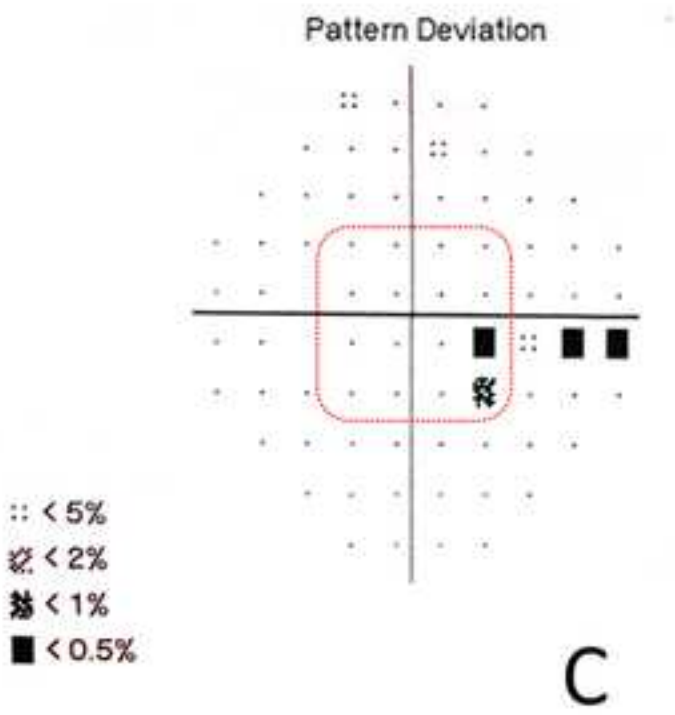

Figure5

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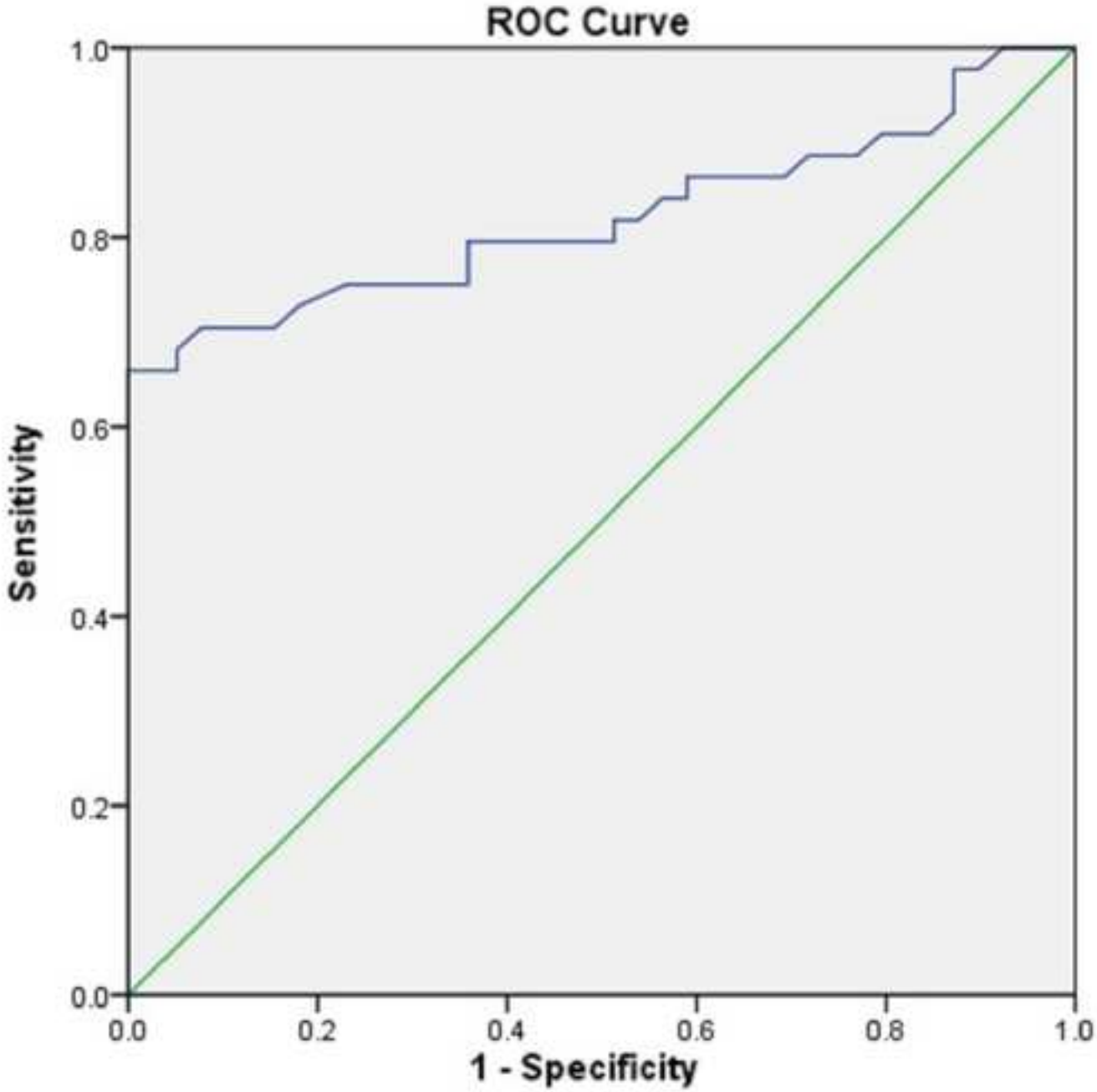

Table 1. Clinical characteristics of the OAG patients and control subjects at baseline

	OAG patients (n=44)	Control subjects (n=39)	p
Age (year)	51.59±14.98	44.72±16.88	0.053*
Sex (male/female)	28/16	20/19	0.175$
Right eyes / Left eyes	20/24	19/20	0.770$
BCVA (log MAR)	0.04±0.06	0.01±0.04	0.093#
Spherical Equivalent(D)	-1.80±2.16	-1.30±2.00	0.276#
Pupil Diameters (mm)	3.43±0.50	3.46±0.51	0.789#
icVEP-SNR	0.85±0.53	1.44±0.57	0.000#

*Independent-sample t test, $Chi-square test, #Mann-Whitney U test

OAG: open-angle glaucoma, BCVA: best-corrected visual acuity; log MAR: logarithm of the minimum angle of resolution; icVEP: isolated-check visual evoked potential; SNR: signal-to-noise ratio

Table 2. Correlations between icVEP-SNR and the abnormalities in central 11° angle glaucoma patients.

Numbers of abnormal test points	Mean ± Std (n=44)	*r*
When P<5%	4.20±2.60	-0.264
When P<2%	2.83±2.34	-0.298
When P<1%	2.08±2.12	-0.266
When P<0.5%	1.48±1.80	-0.332

*Pearson correlation test

icVEP: isolated-check visual evoked potential; SNR: signal-to-noise ratio; SAP: standa

visual field of SAP of open-

p*
0.099
0.061
0.097
0.037

ard automated perimetry

Table 3. Correlations between icVEP-SNR and relating factors for patients with open

	Mean ± Std (n=44)	*r*
SAP-MD (dB)	-3.83±1.26	0.115
SAP-MD of the other eye (dB)	-4.86±3.94	-0.15
OCT-Thickness changing of RNFL (μm)		
Temporal Superior quadrant	-39.31±29.89	0.37
Temporal Inferior quadrant	-43.64±29.83	-0.22
Baseline IOP (mmHg)	15.48±2.80	-0.121
CCT (μm)	523.24±29.64	0.171

*Pearson correlation test

icVEP: isolated-check visual evoked potential; SNR: signal-to-noise ratio; SAP: standard auto
(HFA 30-2 SITA); MD: mean deviation; OCT: optical coherence tomography; RNFL: retinal r
intraocular pressure; CCT: central corneal thickness

ı-angle glaucoma.

p*
0.457
0.33
0.016
0.161
0.435
0.333

mated perimetry
ıerve fiber layer; IOP:

Name of Material/Equipment	Company	Catalog Number	Comments/Description
CR-2 AF Digital Non-Mydriatic Retinal Camera	Canon U.S.A., Inc., Melville, NY, USA		Stereoscopic fundus photographs
DGH 500 PachetteTM	DGH Technology, Exton, PA, USA		ultrasound pachymetry
HFA II 750i	Carl Zeiss Meditec Inc., Dublin, German		Humphrey Field Analyzer II
Neucodia novel electrophysiological instrument	Huzhou Medconova Medical Technology Co.Ltd., Zhejiang province, P.R. China		icVEP
Spectralis SD-OCT	Heidelberg Engineering, Heidelberg, Germany		OCT

Reviewers' comments:

Reviewer #1:

Summary:

A new technique, isolated-check visual evoked potential (icVEP) was designed and reported to detect glaucomatous damage earlier. It created low spatial frequency/high temporal frequency bright stimulus, recording cortical activities initiated primarily by afferents in the magnocellular ON cell pathway. The study was raised to present standard operative procedures (SOP) of icVEP to obtain reliable results, and to prove it can detect visual function loss by signal-to-noise-ratio corresponding to the defects of retinal nerve fiber layer (RNFL) in early-stage of open-angle glaucoma (OAG). VEPs were recorded and analyzed automatically by the software and signalto-noise ratios (SNR) were derived based on a multivariate statistic. Eyes that yielded an SNR≤1 were considered as abnormal. Receiver-operating-characteristic (ROC) curve analysis was used to estimate the accuracy of group classification. So far, the SOP had been applicated in cross-sectional studies, showing icVEP could detect glaucomatous visual function loss in central visual field by the form of SNR, which was also correlated with the thickness thinning of RNFL and produced high classification accuracy for early-stage of OAG.

The manuscript is interesting, but authors must make some changes to make it pleasing to the reader.

Please review both the acronyms (some are lost) and the English language. Yes, I've revised them. Thank you!

The whole manuscript must be reviewed and lightened by some sentences. Yes, I've lightened them. Thank you!

Please, the purpose of the research must be better highlighted in the Introduction Section. Yes, I've revised the purpose. Thank you!

The explanations of the figures and tables must be improved. The reader must understand through the legend what is present in the figures and in the tables. Yes, I've revised them. Thank you!

Please, table 1 the "Diagnosis (POAG/NTG)" data. Do you need it? I've revised it. Thank you!

The Discussion section also needs a makeover for make the section lighter and more pleasant. For example, I propose to add 2 references to expand and enrich the manuscript:
- Role of Protease-Inhibitors in Ocular Diseases. Pescosolido N, et al. Molecules. 2014 Dec 8;19(12):20557-20569.
- Multifocal and pattern-reversal visual evoked potentials vs. automated perimetry frequency-doubling technology matrix in optic neuritis. Nebbioso M, et al. Indian J Ophthalmol. 2013 Feb;61(2):59-64. Yes, I've added them. Thank you very much!

Reviewers' comments:

Reviewer #2:

Manuscript Summary:

This clinical study details results of another trial investigating the isolated check visual evoked potential testing in patients with early stage open angle glaucoma.

Major Concerns:

N/A

Thank you very much!

Minor Concerns:

The manuscript requires editorial review.

I have revised the manuscript by the comments of the editorial. Thank you!

Reviewers' comments:

Reviewer #3:

Manuscript Summary:

Interesting study with strong potential clinical applications. Manuscript well written but could be linguistically improved.

Major Concerns:

1. The difference in age between POAG and controls is almost statistically significant in this study (p=0.053). Do the authors believe that the younger age of controls may have affected results? Yes, it's a very good question. But we found they were similar in BCVA and we excluded diabetes or any other systemic disease and the history of ocular or neurologic disease, so we thought they all had good macular function to do icVEP. Therefore, the age was different but not significantly different and we thought it might not affect icVEP results. Thank you!

2. Did the authors find any differences in their electrophysiological scores between POAG and NTG? If so, this could support the possibility of a mechanical effect of the IOP on neuronal activity. We found similar SNR results between POAG and NTG because they were all well controlled by medications without any significant difference in baseline IOP. Thank you!

3. The authors measured central corneal thickness (CCT) in this study but do not report on results nor if they adjusted the IOP readings based on CCT results. It would be interesting to know if patients with thinner corneas have more compromised electrophysiological findings, perhaps because of higher real IOP, compared to patients with thicker corneas. Yes, that an interesting question. Thank you very much! In our study, we just analyzed CCT as an independent factor and we mainly thought it might indicate something about the tolerance of optic nerve. Moreover, all the patients were well controlled by medications with IOP in almost middle-teen which might reach the target IOP for these early stage OAG.

4. What was the average time per patient for the completion of icVEP in this study? This information would be useful in order to assess the practical value of icVEP for glaucoma diagnosis in the everyday clinical proactice. Thank you for your good question! Line 30, 89, 114, 130 of 60673_R0.doc showed that the test protocol was just 2 seconds for each run, 8 runs for each eye. So, the test protocol was only 32 seconds for both eye if we could get reliable result from at least 16 runs. However, before test or repeat of test, there must be a 30 min rest, and adding the time of putting on the electrodes the examination time might from 3min to 36 min.

5. Line 132, 292flow chart: A limit of 30mmHg chosen as a cut-off level for inclusion in the study to avoid "mood swings" which could affect the EEG signals. Is there any literature information to support this point? If so, the authors should add the reference. We were sorry that we did not find any literature. However, in our clinic, we observed that if OAG patients got IOP over 30mmHg they might feel anxious even uncomfortable. And your suggestions might encourage us to held further study to explore the relationship between IOP and "mood swings" in glaucoma patients. Thank you very much!

Minor Concerns:

1. Lines 65-67: "Therefore, cells enrolled in M-ON pathway were sensitive to low levels of luminance contrast but rather insensitive to chromatic information and with larger diameter axons, which could be preferentially damaged in early glaucoma and might result in visual field loss". Do the authors imply that the larger diameter of M-ON axons renders them more sensitive to risk factors, such as higher intraocular pressure (IOP), for the development of glaucoma, perhaps because of an increased mechanical compressive effect of the IOP on the larger axons? Yes, M-cells were larger also with larger axons which could be more sensitive to risk factors such as IOP, TLCP (trans-lamina cribrosa pressure), ischemia, et al. Thank you!

2. Line 158: please replace the term "control observers" with the term "control subjects". Yes, I have replaced all. Thank you!

3. The legend of Figure 1 should be expanded to better explain the separate layers in the lateral geniculate nucleus. Yes, I have revised the legend. Thank you!

4. Lines 162-163: "current use of a medication that could affect visual field sensitivity". The authors should provide more information on the list of medications they would consider affecting visual field sensitivity. Yes, I have added the list. Thank you!

Reviewers' comments:

Reviewer #4:

Manuscript Summary:

accept without changes

Thank you very much!

Editorial comments:

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Yes, I've done.
5. Please use a single space between numerical values and their units. Yes, I've done.
6. Please use h, min, s for time units. Yes, I've done.
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8. All tables should be uploaded separately to your Editorial Manager account. Yes, I've done.
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3. Step 3.4.2: Please write this step in the imperative tense. Yes, I have revised it.
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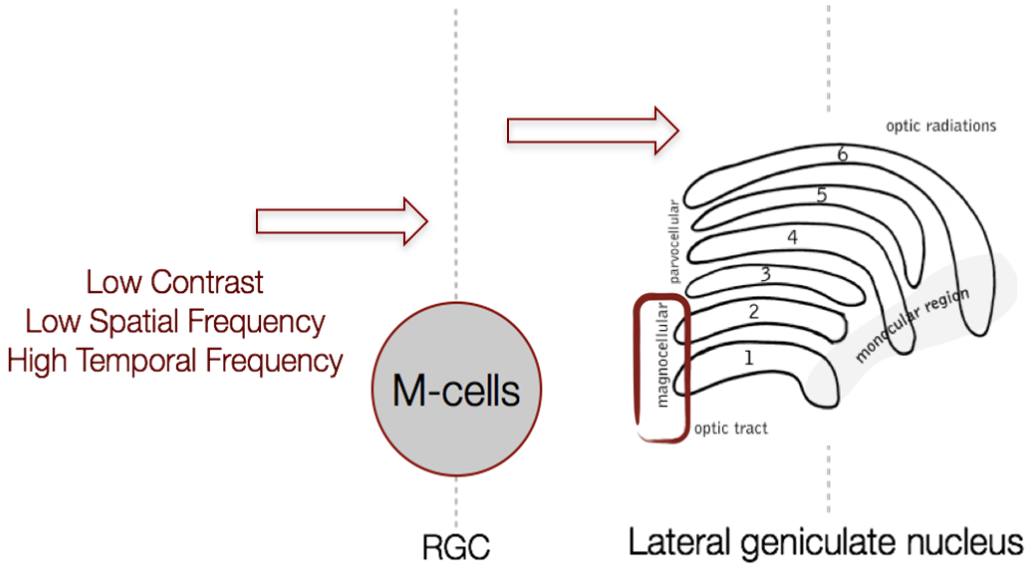

Figure 1. Theory of isolated-check visual evoked potential cheking the magnocellular (M-cells) pathway. 1 and 2 layers were enrolled in magnocellular pathway; 3, 4, 5, 6 layers were enrolled in parvocellular pathway; and the spaces among these 6 layers were enrolled in bistratified cells pathway. RGC: retinal ganglion cell.

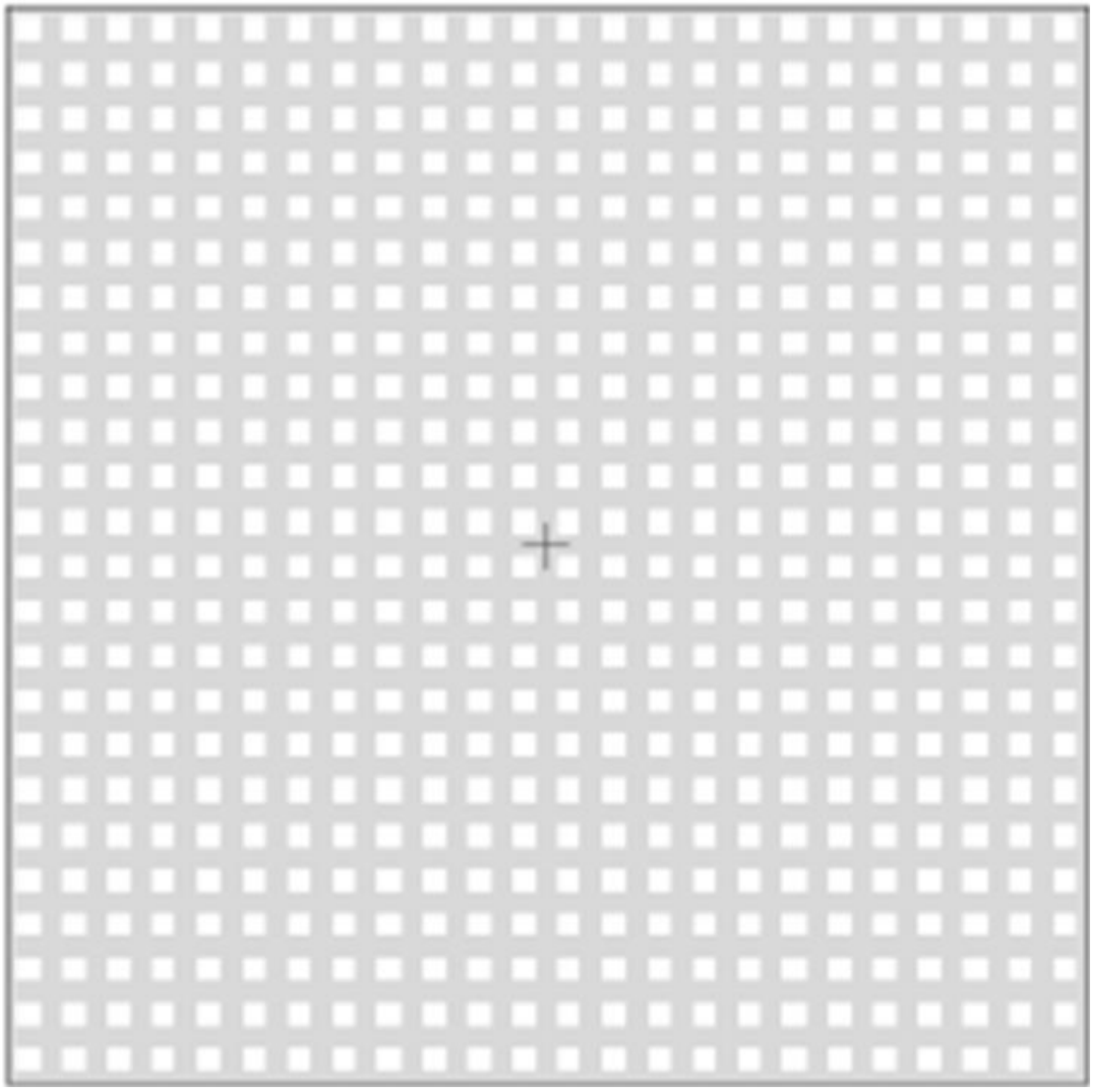

Figure 2. Example of bright conditions (positive-contrast) on the screen of isolated-check visual evoked potential. This figure has been modified from [Xiang F, Lingling W, Xia D, Tong D, Aihua D. Applications of Isolated-Check Visual Evoked Potential in Early Stage of Open-Angle Glaucoma Patients. Chinese Medical Journal. 131(20):2439-46(2018). DOI: 10.4103/0366-6999.243564.].

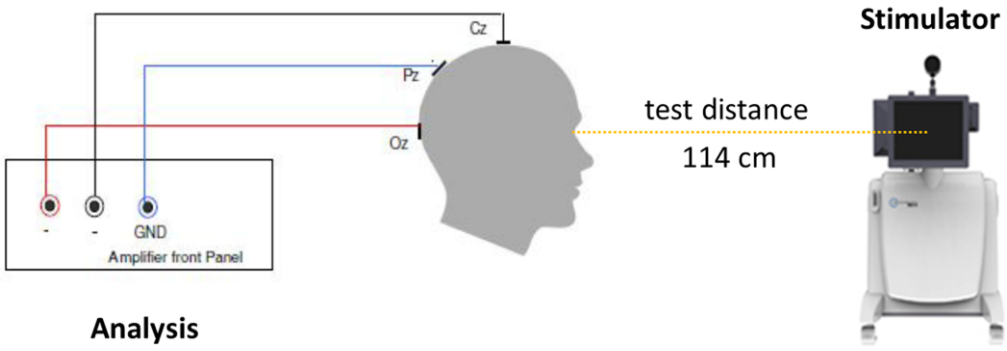

Figure 3. The diagrammatic sketch of examination for the isolated-check visual evoked potential. GND: Grounding electrode; Cz: Central midline electrode; Pz: Parietal midline electrode; Oz: Occipital midline electrode. This figure has been modified from [Xiang F, Lingling W, Xia D, Tong D, Aihua D. Applications of Isolated-Check Visual Evoked Potential in Early Stage of Open-Angle Glaucoma Patients. Chinese Medical Journal. 131(20):2439-46(2018). DOI: 10.4103/0366-6999.243564.].

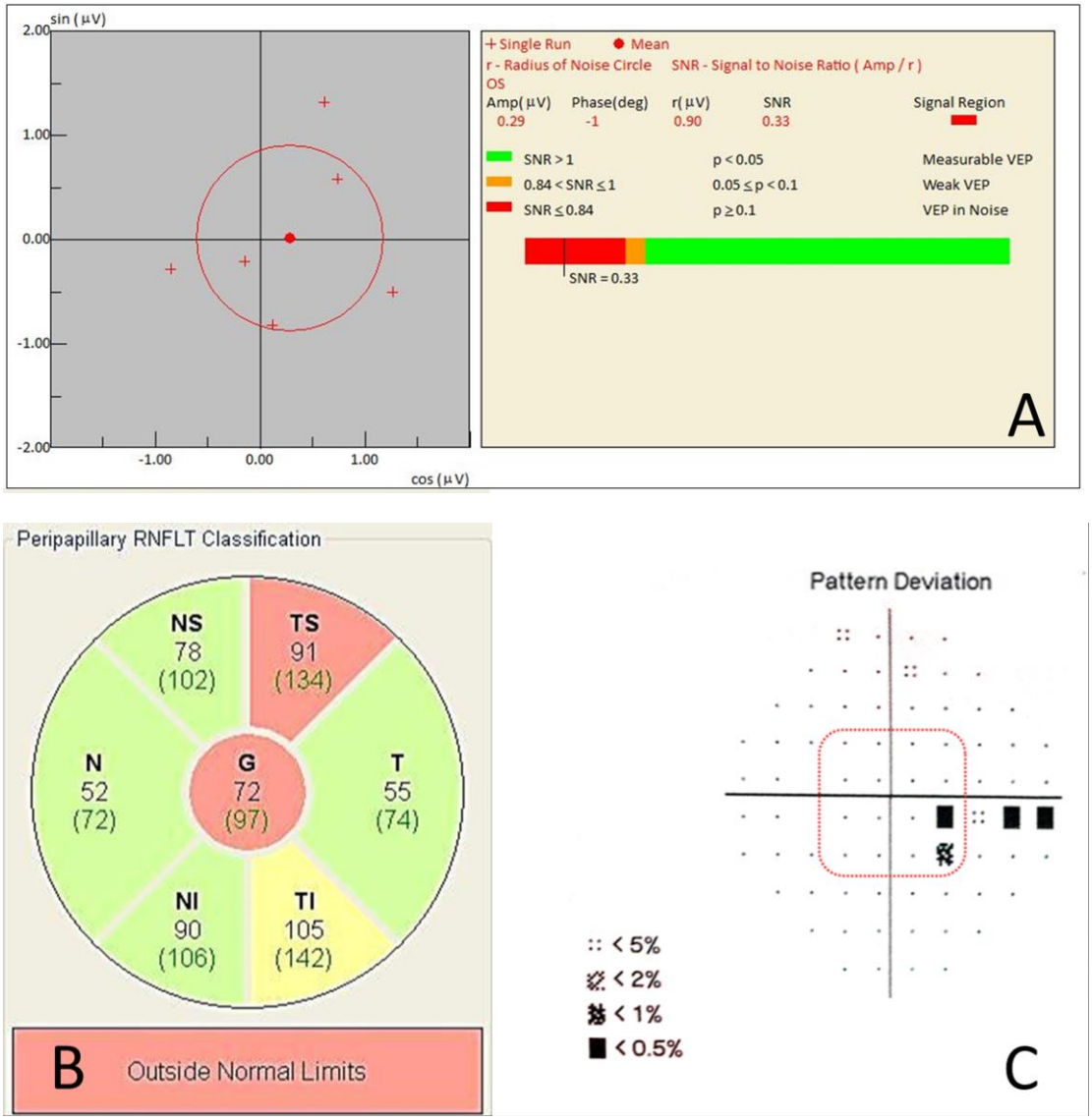

Figure 4. Typical results of an early-stage open angle glaucoma patient: A) Abnormal isolated-check visual evoked potential results; B) Outcomes of peripapillary retinal nerve fiber layer thickness (RNFLT) classification on the report of optical coherence tomography. Changing of RNFLT = RNFLT value (black number) – The standard value from database of normal people (green number in brackets). G: Global; N: Nasal; T: Temporal; NS: Nasal superior; TS: Temporal superior; NI: Nasal inferior; TI: Temporal inferior; C) Central 16 test points of pattern deviation on Humphrey Field Analyzer 30-2 SITA program corresponding to the central 11°visual field. This figure has been modified from [Xiang F, Lingling W, Xia D, Tong D, Aihua D. Applications of Isolated-Check Visual Evoked Potential in Early Stage of Open-Angle Glaucoma Patients. Chinese Medical Journal. 131(20):2439-46(2018). DOI: 10.4103/0366-6999.243564.].

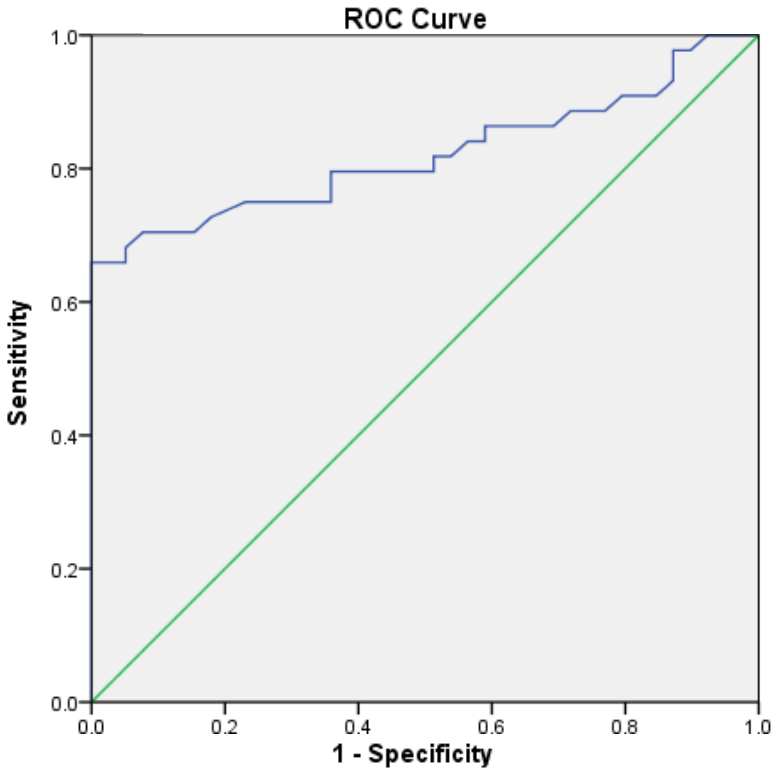

Figure 5. Receiver-operating characteristic (ROC) curve for data collected from the signal-to-noise ratios of isolated-check visual evoked potential of open-angle glaucoma patients and control subjects.

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