·Basic Research·

Frequency spectrum might act as communication code between retina and visual cortex I

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Abstract

• AIM: To explore changes and possible communication relationship of local potential signals recorded simultaneously from retina and visual cortex I (V1).

• METHODS: Fourteen C57BL/6J mice were measured with pattern electroretinogram (PERG) and pattern visually evoked potential (PVEP) and fast Fourier transform has been used to analyze the frequency components of those signals.

• RESULTS: The amplitude of PERG and PVEP was measured at about 36.7 μV and 112.5 μV respectively and the dominant frequency of PERG and PVEP, however, stay unchanged and both signals do not have second, or otherwise, harmonic generation.

• CONCLUSION: The results suggested that retina encodes visual information in the way of frequency spectrum and then transfers it to primary visual cortex. The primary visual cortex accepts and deciphers the input visual information coded from retina. Frequency spectrum may act as communication code between retina and V1.

• KEYWORDS: pattern electroretinogram; pattern visually evoked potential; fast Fourier transform; retina; visual cortex I
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INTRODUCTION

Physiological experiments and the application of clinical conditions have provided a great deal of evidence that it is retinal ganglion cells (RGCs) that generate the pattern electroretinogram (PERG)[1-7]. PERG consists of two major portions: an early positive component followed by a negative component. The PERG allows researcher conduct the examination of physiological level of the retina and helps the understanding of pathophysiology of various eye diseases and is important in routine opthalmological diagnosis and assessment. For example, the PERG has a major clinical role in checking localized retinal pathology such as in age related macular degeneration, glaucoma, diabetic retinopathy.

In 1934, Adrian and Matthews [8] found that local field potential changes of the occipital electroencephalogram (EEG) could be observed under visual stimulation. That work was the earliest description of visual evoked potential (VEP). Then researchers from all over the world have conducted a wide variety of study to improve methods and theories from the 1970s to present time.

One example is Halliday *et al* [9] used VEP to do clinical study at first time in retrobulbar neuritis patient. Another study was done by Szikla *et al* [10] to localize neural circuits in primary visual pathway by such technique. In the examination of patients, pattern VEP (PVEP) is more generally employed. The reason is that the parameters of latency and amplitudes of PVEP are relatively stable compared to those of flash VEP which were unstable and varied across different people [11,12]. To examine both normal conditions and the pathological conditions of retina and visual cortex, people normally perform PVEP and PERG at same time. For example, if there is loss in central pathway, the PVEP will be found abnormal while the PERG appears normal.

It is important to know the features of PERG and PVEP during visual research and visual diseases diagnosis. Many aspects like spatial and temporal frequency tuning of PERG and PVEP have been revealed[2-7,13], but so far it has not been using fast Fourier transform (FFT) to analyze PERG and PVEP recorded simultaneously to reveal the possible communication code between retina and visual cortex I (V1). One dimension discrete Fourier transform (DFT) analysis is a conventional method that allows researchers to do time-frequency analysis of any wave as a function of time. In

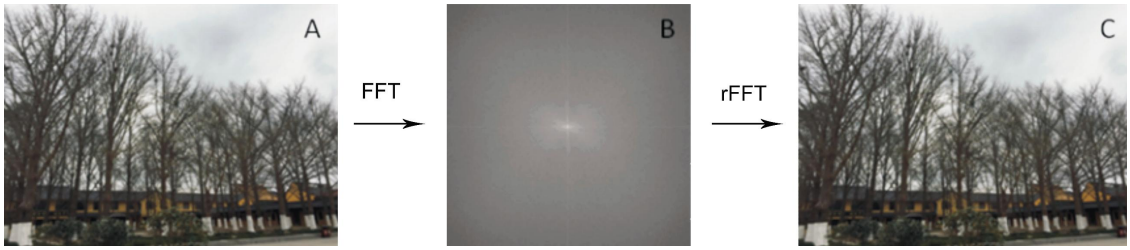

Figure 1 The application of FFT and rFFT in imaging process A: Original picture before FFT processing; B: Image of A after FFT processing; C: Recovered picture of B after a reverse FFT (rFFT) processing. A and C are exactly the same.

digital signal processing field, the function is any quantity or signal which varies over time. The DFT is the most important discrete transform, employed to practice Fourier analysis in many practical applications [14]. Since DFT concerns a limited amount of research data, it can be easily carried out in computers. These implementations usually make use of efficient and exact FFT algorithms [15]. FFT is a useful tool to analyze frequency spectrum of any repeatable wave. In modern digital communication technology, FFT is an efficient processing tool to encode images before they are transported from one place to another no matter how far away they are. FFT transfers image's spatial frequency spectrum to frequency spectrum. After a reverse FFT (rFFT) processing, frequency spectrum can be decoded and transferred to original image again (Figure 1). It is possible for neuroscientists to reconstruct the original visual stimuli just according the frequency spectrum of local potential, such as PERG, PVEP induced out from retina or visual cortex. Similar work has been seen in some research work[16,17]. RGC is the only cell in retina that produces action potentials[18]. And then those action potentials are transferred to primary visual cortex (V1) through lateral geniculate neucleus. In essence, PERG and PEVP are homologous. If PERGs or other natural scenery evoked electroretinograms (ERGs) do not have visual information (code) in it, what shall primary visual cortex recognize and analyze? Analog signal is easy to be interrupted [19], so amplitude of PERG cannot be used as code. Only digital signal, which has been widely used in modern communication field, is stable and possibly be used as code to encode visual information then be transferred from retina to V1. As described above, FFT is an efficient tool to analyze frequency components in PERG and PVEP. Our research has tried to employ this method to explore the interesting hypothesis that frequency spectrum acts as communication code between retina and V1. This might provide useful reference to vision research scientists.

MATERIALS AND METHODS

Animals This study was supported by Tongji University. All experiments carried out abided by the ARVO policy for the Use of Animals in Ophthalmic and Vision Research. Experimental animal (C57BL/6J mice) were bought from Jackson Laboratory (located at Bar Harbor, ME, USA). In present study 14 mice that were 4-month old were used. PERG and VEP were recorded simultaneously under anesthesia.

Surgical Procedure We first weighed the mice and then anesthetized them by intraperitoneal injections (the dose was about 0.5-0.7 mL/kg) of a mixture of ketamine (concentration 42.8 mg/mL), and xylazine (concentration 8.6 mg/mL)[20]. Then, under sterile no bacteria conditions in surgery room, the head bone overlying the monocular zone of V1 of mice of both V1 areas (1.5 mm lateral to the central suture, 1.5 mm anterior to the lamda) was drilled to put a T shaped tiny stainless steel screw (length 2 mm, head width 2 mm) on each side. Each screw was welded together with a Teflon wire and the wire was left in the air for the convenience of recording PVEP (when recording, the naked tip of the wire was connected to one of the two recording electrodes). Dental cement was put around the screws to fix them and also isolate them from the tissue round them. Mice were then set to recover from anesthesia and sent back to their cages. Two weeks later after surgery, PERG and PVEP were recorded at the same time.

Pattern Electroretinogram, Pattern Visual Evoked Potential Recording How PERG recording technique was employed in vision science research has been reported from time to time in some literatures [1,5,21-24]. In current research, mice were firstly put in a cup to get weighed and then according to their weights, they were anesthetized with intraperitoneal injections (the dose was about 0.5-0.7 mL/kg). The injection mixture used consisted of ketamine, whose concentration was 42.8 mg/mL, and xylazine, whose concentration was 8.6 mg/mL. After mice were anesthetized, they were then carefully fixed in a stereo-taxic frame that can make mice have no vision obstacle. While recording, the body temperature of the animal was maintained at 37℃ by employing a heating pad and the whole animal body was kept stable with ear bar and bite bar to avoid vibration. The mice eyes were widely open, and because mice position was lower than the monitor, their optical axes were made laterally and upwardly to the center of the monitor's screen which contained visual stimuli. Silver wire with the diameter 0.25 mm was used to design PERG electrode. The shape of

the electrode was set as a semicircular loop whose radius was about 2 mm. A micromanipulator was employed to help researcher see clearly to place the electrode on the animal's corneal surface around pupil. In order to prevent corneal from dryness, a small drop of balanced saline solution was administrated every 30min. Reference and ground electrodes were both stainless steel needles, the reference electrode was inserted under the skin of the back of the head and, meanwhile, the ground electrode was put under the skin near the tail. Visual stimuli, which was used to induce PERG were shown on a cathode-ray tube display (Sony Multiscan 500; Sony, Park Ridge, NJ, USA), were contrast-reversing horizontal square wave (with the parameters as 100% contrast, spatial frequency 0.05 cyc/deg, mean luminance 50 cd/m^2). Such stimuli were generated by a graphic card which can be programmed according to research needs (VSG Cambridge Research Systems, Rochester, UK).The scanning frequency was 2 reversals per second. Mice pupils were not dilated using atropine. Mice' eyes were not refracted within the distance from monitor to eye because they have focus with a long distance depth [25-27]. Within the viewing distance about 15 to 20 cm, the stimulus range covered approximately an area of 69 ×63 degrees. In this research, three to five continuously recorded PERG responded to 600 contrast reversals each were acquired. And 1800 to 3000 sweeps of the responses were overlapped to check for synchronicity and then averaged to calculate amplitude using a Matlab code (Mathsworks, R2010a). As described before, there are typically a major positive peak at about 90 to 120ms (P100) and a slower negative wave with a relatively flatter trough at approximately 200 to 300ms (N250) inside a PERG waveform [20,22,28]. An example is shown in Figure 2. Because PVEP and PERG are quite correlated, PVEP were recorded simultaneously in the same way (*i.e.* same recording parameters) as PERG (at this time, the recording system were set as two channels recording). Under normal conditions, PERG of left and right eyes and PVEP of left and right hemisphere were exactly the same (equal amplitude and latency). Therefore only PERG of left eye and PVEP of right hemisphere (because most axons from mouse retina go across the chiasm to contralateral visual cortex.) were compared. FFT analysis was carried out using FFT function built in a Matlab (Mathsworks, R2010a) code. This kind of FFT analysis can manifest all frequency components, for example dominant frequency and second harmonic generation, in PERG and PVEP waves.

RESULTS

Amplitude of Pattern Electroretinogram and Pattern Visual Evoked Potential Among fourteen mice, the PERG amplitude was (mean ±SE) 36.71 ±1.54 μV, the PVEP amplitude was (mean±SE) 112.5±4.11 μV. For PVEP, when stimulation was imposed on right eye, the amplitude of left PVEP is much larger than right PVEP. While stimulation was imposed on left eye, the amplitude of right PVEP is much larger than left PVEP. Thus it was known that both PVEP electrodes were put over monocular zone, not binocular zone, of V1 in mice (Figure 3).

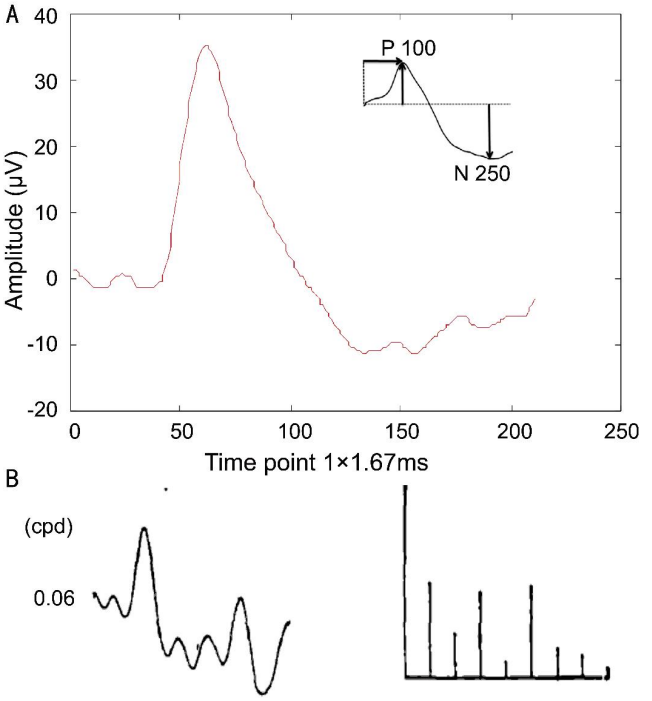

Figure 2 Wave form and frequency spectrum of PERG A: Real time PERG wave and inset (inset was from Yang *et al* [20]) shows a diagram of PERG with the peak at 100ms and trough at about 250ms; B: Vertical lines in right subplot stand for frequency spectrum of left PRG wave after FFT analysis (B was from Yang *et al* [2]).

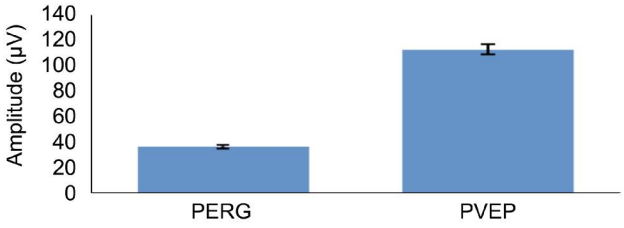

Figure 3 PERG amplitude was about 36.71 ±1.54 μV, the PVEP amplitude was around 112.5±4.11 μV.

Frequency Spectrum of Pattern Electroretinogram, Pattern Visual Evoked Potential was Identical Through FFT analysis to PERG and PVEP recorded concurrently, it was found that the dominant frequency of PERG was 3-4 Hz (on average 3.46±0.1357 Hz, mean±SE), and that of PVEP was also 3-4 Hz (on average 3.5±0.1360 Hz, mean±SE). Dominant frequency of PERG, PVEP stayed unchanged (*t*-test, P=0.79). Both types of wave were similar in frequency components (Figure 4).

DISCUSSION

PERG is a particular kind of ERG acquired reacting to patterned visual stimuli with constant mean luminance (usually contrast-reversing moving gratings or checkerboards

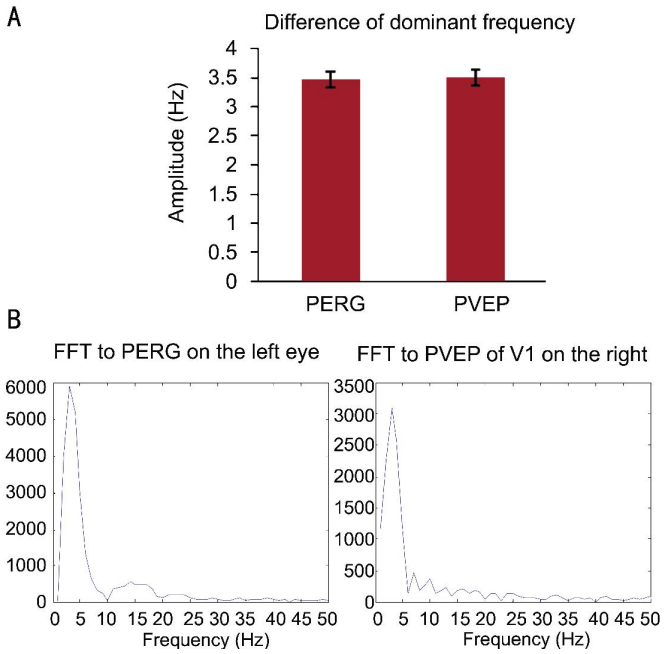

Figure 4 Comparison of frequency components of PERG and PVEP A: The dominant frequency of PERG was 3.46±0.1357 Hz on average, and that of PVEP was 3.5±0.1360 Hz on average; B: Dominant frequency of PERG, PVEP was identical (P=0.79). PERG and PVEP were similar in frequency components.

was employed) whose characteristics are basically unidentical from those of the traditional ERG (defined as flash ERG) in response to diffuse flashes of light. In past several decades, mouse models of optic nerve disease such as glaucoma, optic neuritis *etc*., are being studied at a relatively higher speed to probe specific pathological mechanisms and the effect of neuroprotective therapy. The application of these models may be greatly accelerated by employment of non-invasive recording techniques to track RGC function with methods such as the PERG [1,6,20,23]. But because PERG only samples retinal function of animal and/or human subjects, it cannot tell us about the condition of the central visual pathway. So it is necessary to check the function of visual cortex at the same time employing PVEP. Visual evoked potential is the electrical response as local field potential of visual cortex to visual stimuli, actually occipital EEG. EEG results[29-32] showed that according to the frequency of EEG, there are four kinds of EEG wave. δ wave, frequency <4 Hz; θ wave, frequency is 4-7 Hz; α wave, frequency is 8-15 Hz; β wave, frequency is 16-31 Hz. δ wave was first found in the early 1900s by W. Grey Walter and has been widely used in EEG analysis to track and assess brain activity [33]. Our experiments show, under anesthesia condition, PVEP can be stably detected. Its frequency is 3.5 Hz on average and falls into the scope of δ wave. It is reliable that under anesthesia condition PVEP can be recorded. Similar work has been found in a literature by Yan *et al* [34].

The primary goal of this research was to test relationship between PERG and PVEP in mice recorded simultaneously. After signals transferred from retina to visual cortex, their amplitudes changed from low quantitative level to high quantitative level. But how primary visual cortex recognizes those signals and what kind of codes they are using to communicate was unknown before. According to the results of our research, dominant frequency of PERG, PVEP was identical and both types of wave have similar frequency components, those signals probably have same frequency spectrum. It was suggested that frequency spectrum has been used as codes to transfer visual information from retina to visual cortex. Furthermore, it can possibly be suggested that retina encodes the visual information (like orientation, color, contrast, velocity *etc*.) for primary visual cortex and primary visual cortex accepts and deciphers these kinds of information from retina, gets them processed and then to transfer those processed signals to other areas of brain.

Frequency spectrum is the unchanged signal property of normal PERG and PVEP as response to a fixed visual stimulus. Does it change in sick animals or human patients in their early or middle stage of the retina or visual cortex diseases, which do not have significant symptoms? Thus, it is worthy to do more work using such technology to probe preclinical detection of retina and/or visual cortex diseases.

Also, this work can potentially be used in artificial vision construction. Because specific visual stimulus results in specific frequency spectrum, on the contrary, if a particular frequency spectrum of a certain PERG and/or PVEP is secured, it is possible for researchers to know what the visual stimulus is without seeing it ahead of time. When PERG and/or PVEP are induced out from retina and/or brain, researchers can know what the animal or human subject is viewing just according to the frequency spectrum of the PERG and/or PVEP. Similar work has been conducted in some researches [16,17,35,36]. But how frequency spectrum changes as a function of visual stimuli, it still has much more work to do to explore the mechanism.

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Conflicts of Interest: Yang X, None; **Gong B,** None; **Lu JW,** None.

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