Original article

Application of omni-purpose electric devices to electrophysiological student practices at the Department of Orthoptics and Visual Sciences, Niigata University of Health and Welfare

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Abstract

Electrophysiological examinations, such as electroencephalogram (EEG) and electrooculogram (EOG), are important for objective assessments of human visual functions. Therefore, students training in orthoptics must understand the principles of electrophysiological recordings, which comprise differential amplification and time-locked averaging. However, clinical-use EEG apparatuses are expensive and much encapsulated. We believed that a simpler, cost-effective electrophysiological recording system would be useful for undergraduate education in our department. Today, monolithic instrumentation amplifiers are commercially available at low-prices. They have the common mode rejection ratios>120 dB, high ($\geqq 10^{10}\ \Omega$) input impedances, and very low noise levels. For time-precise visual stimulation, an Arduino-compatible microcontroller with an on-board three-color light-emitting diode was employed. Using these omni-purpose devices, we developed a simple undergraduate practice system for electrophysiological recordings. With the system, even inexperienced students can record visually evoked potentials (VEPs) in a common, non-shield room. The signal quality of recorded VEPs was high enough to demonstrate P2 and N2 peaks in student practices. The system is also applicable to EOG recording. Thus our practice system can help students gain the essential knowledge and experiences of electrophysiological examinations in orthoptics.

Introduction

In the fields of ophthalmology and orthoptics, in spite of recently achieved morphological inspection utilizing laser interferometry [1,2], electrophysiological examinations including electroencephalogram (EEG) and electrooculogram (EOG) still have great importance in objectively assessing the human visual functions [3]. However, EEG recording systems for full medial applications are expensive (*e.g.*, a set of Nihon-Kohden EEG-1250 costs approximately 5 million yen [4]) and has more channels than the minimal requirements for understanding visually evoked potentials (VEPs). In addition, the main parts of these apparatuses are encapsulated within the set and cannot be seen from outside. Thus, a simpler, cost-effective electrophysiological recording system is needed for undergraduate education in our department.

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As shown in Table 1, monolithic integrated instrumentation amplifiers consisting of two or three operational amplifiers (op-amps) within a single package have been used since the late 1980's. Currently, versatile instrumentation amplifiers are commercially available at very low prices (approximate retail price of INA128 is 750 yen[5]). With closely matched built-in laser-trimmed resistors, these instrumentation amplifiers provide much higher (>120 dB) common-mode rejection ratios (CMRRs) than the monolithic op-amps widely used in the past for electrophysiological recordings, in addition to high ($\geqq 10^{10}\ \Omega$) input impedances. Because of the precise cancellation between the positive and negative inputs, differential amplifiers with high CMRR are highly resistant to external noises, such as noises from power lines. Therefore, when using integrated instrumentation amplifiers, even inexperienced students are able to record VEPs in common, unshielded rooms.

Another challenge is how to perform time-locked visual stimulation. Recently, numbers of easy-to-program microcontrollers have been introduced. A microcontroller is a small all-in-one microcomputer consisting of a central processing unit and the necessary peripheral circuits and is useful for real-time applications. Moreover, some of microcontrollers have a video graphic array output or on-board light-emitting diodes (LEDs) as well as digital input–output (I/O) lines, which are very convenient for visual stimulation for VEP recordings.

In this study, we developed a simple EEG and EOG recording system for student practice using two low-cost omni-purpose devices: a high-precision instrumentation amplifier for electrical recording and an Arduino-compatible microcontroller with an on-board three-color LED for displaying the visual target.

Materials and Methods

1. Subjects

This study was performed as a part of the study approved by the research ethics committee of Niigata University of Health and Welfare (Number: 17827-170605). Eight healthy volunteers participated in the study. All participants were from the Department of Orthoptics and Visual Sciences. Five were female undergraduates, two were male undergraduates, and one was a male teacher. All recordings with our system were performed by students (mainly undergraduate authors S.T. and E.N.).

Table 1. Specifications of representative monolithic operational / instrumentation amplifiers [5] [13-16].

Model	Release year	Input	Type	Input resistance* (Ω)	Input capacitance (pF)	CMRR (dB)	Noise (nV/√Hz) at 1 kHz
μA741	1969	Bipolar	Omni-purpose operational amplifier	2×10^{6} (typical)	1.2	90 (typical)	NA
LF356	1974	FET	Omni-purpose operational amplifier	10^{12} (typical)	3	100 (typical)	12
INA110	1988	FET	Omni-purpose instrumentation amplifier	5×10^{12} (typical)	6	110 (typical) 100 (minimal)	10
INA128	1995	FET	Omni-purpose instrumentation amplifier	10^{10} (typical)	2	130 (typical) 120 (minimal)	8

*: in INA110 and INA128, the impedance of the differential inputs are shown.

2. Recording system

The recording setup is shown in Figures 1A and 1B. Two head amplifiers (head-amps) were made using a monolithic instrumentation amplifier (INA128, Texas Instruments, TX, U.S.A.). Each head-amp was connected to one of two preamplifiers (AVB-10 and AVB-11A, Nihon-Kohden, Tokyo, Japan) of an oscilloscope (VC-10, Nihon-Kohden, Tokyo, Japan). As shown in Figure 1C, the gain of the head-amps was × 19.5 (Rgain: 2.7 kΩ), while the required gains for both preamplifiers were × 20. Therefore, the recorded voltage in each channel was 2.5% smaller than the true value. The underestimation was so small that we ignored it in this study. The signals from head-amps were band-pass filtered (0.5−100 Hz for EEGs, 0.08−100 Hz for EOGs) by the preamplifiers and fed to an analog-digital converter (ADC) (PCIe-6323, National Instruments, TX, U.S.A.) of a personal computer. No further signal conditioning apparatuses (*e.g.*, hum-filters and electromyogram filters) were inserted. The sampling frequency was 500 Hz. The data acquisition software was written in LabVIEW (National Instruments, TX, U.S.A.).

Visual stimulation was performed using a battery-driven microcontroller (GR-COTTON, Renesas Electronics, Tokyo, Japan), which consists of an Arduino-compatible microcontroller chip (RL78/G13, Renesas Electronics, Tokyo, Japan) and a three-color LED [6]. Trigger signals from the GR-COTTON and a pushbutton were stored via digital I/Os of the ADC.

3. Electrode locations

EEGs were recorded from O1 and O2 according to the international 10/20 system[7] via EEG disk electrodes (NE-102A, Nihon-Kohden, Tokyo, Japan). Reference electrodes were located at A1 and A2 for O1 and O2, respectively. In EOG recording, the EEG disk electrodes were placed at the right and left sides of both eyes. In all cases, the ground electrode was attached to the forehead.

4. Stimulation

The GR-COTTON on-board LED was used for visual stimulation. The red, green, and blue elements of the LED were turned on simultaneously to create white light. Stimulus duration was 50 ms and stimulation interval was 1 s. The subject looked at the LED with an eye (6: right, 2: left) at a distance of 15–20 cm (Figure 1D) in the darkened practice room. The other eye was covered by a gauze eye patch. Alpha-blocking experiments were performed in six of the eight subjects. The subject alternately closed and opened both eyes approximately every 10 s in the normally lighted-practice room and pressed a pushbutton with the eyes closed.

The GR-COTTON was also used to evaluate the latencies and amplitudes of saccadic eye movements. As shown in Figure 1B, the GR-COTTON was placed at the center and a stationary visual target was placed on each side with a distance of 10 or 20 cm. The distance from the subject to the GR-COTTON was 56.7 cm. Initially, the on-board LED was green, giving the fixation point, and then it turned red or blue for 50 ms at pseudo-randomized intervals (between 1 s and 2 s). The subject was instructed to look at the correct target (red: right, blue: left) as quickly as possible after the LED color was changed.

5. Data analysis and statistics

Frequency-domain analyses were performed for every trial by applying Gabor wavelets (sigma = 1) or Fast Fourier Transformation using a 256-ms time window with the Hanning window function. In analyzing VEPs, the time window was slid by 20 ms within a peristimulus period (-400−600 ms to the LED onset). The power spectra were averaged over trials and divided by total power (averaged over the peristimulus period) to cancel out the 1/f properties of EEG. In analyzing alpha-blocking, the time window for frequency-domain analyses was slid by 200 ms from 6 s before the button press to 6 s after the button press and no normalization

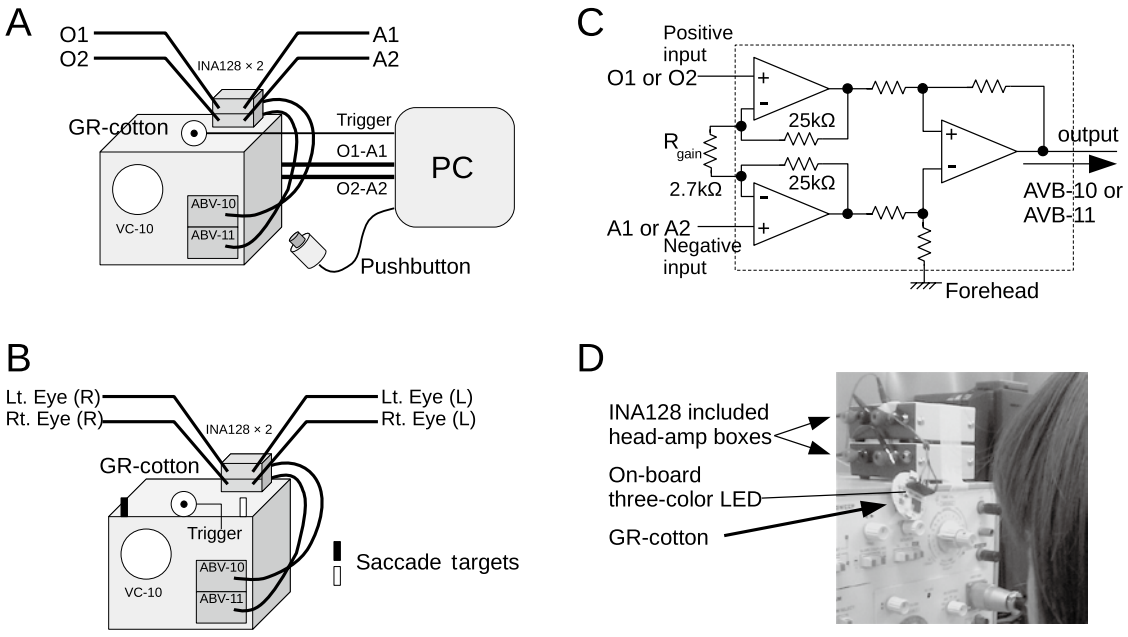

Figure 1.
A, B: Recording set-ups using omni-purpose devices for (A) VEP recording practices and (B) EOG recording practices.
C: A diagram of the head-amplifier. The dashed box indicates the inner parts of an INA128 [16]. The power for the INA128 was supplied by AVB-10 or AVB-11.
D: a photograph of a VEP recording practice in an unshielded room. The GR-COTTON is seen on the front panel of the oscilloscope in front of the subject, and three-color LED is at the center. The practice room is not darkened for display purposes.

was performed. Spectrogram analyses were performed with custom-made softwares written in FreePascal/Lazarus (http://www.lazarus-ide.org/). Statistical analyses were performed with free statistical software GNU R (http://www.r-project.org/) on Macintosh computers. Results are expressed as means ± standard deviation unless otherwise mentioned.

6. Comparison with a clinical examination device

To compare the performance of our recording system with results of a clinical examination device, flash VEP recording with LE-4000 (Tomey, Nagoya, Japan) was performed in one subject. The recording and reference electrodes were placed at Oz and A1, respectively, and the ground electrode was placed at A2. The visual stimulus was 2-Hz ganzfeld white flash to each eye. Recording with LE-4000 was performed by author U.H.

Results

1. Alpha-blocking

To confirm the EEG recording ability of our system, alpha-blocking [8,9] was examined in six subjects. A sample time-course is shown in Figure 2A. At both hemispheres, alpha-waves of approximately 50−60 μV were frequently observed during eye closing. After eye opening, however, alpha-waves diminished and the faster waves became prompt. Diminishing alpha-band

power after eye opening was clearly observed by Gabor wavelet analyses (Figure 2B). The alpha-blocking was distinguishable in the pooled data as well (Figure 2C). To quantify the alpha-blocking, we averaged alpha-band powers at the eye-closing period (5−1 s before button release) and the eye-opening period (1−5 s after button release). Then the alpha-band power ratios were calculated by dividing the latter by the former. In the pooled data (Figure 2D), the alpha-band power ratios were significantly less than one ($p = 1.84\times10^{-5}$ and 0.000925 for O1 and O2, respectively, *t*-test), suggesting that the alpha-blocking phenomenon was appropriately reproduced by our system. In addition, Figures 2A and 2B also indicate that the noise level of our system was sufficiently low for student practice and even research, because no normalization nor hum-filtering were performed on these data.

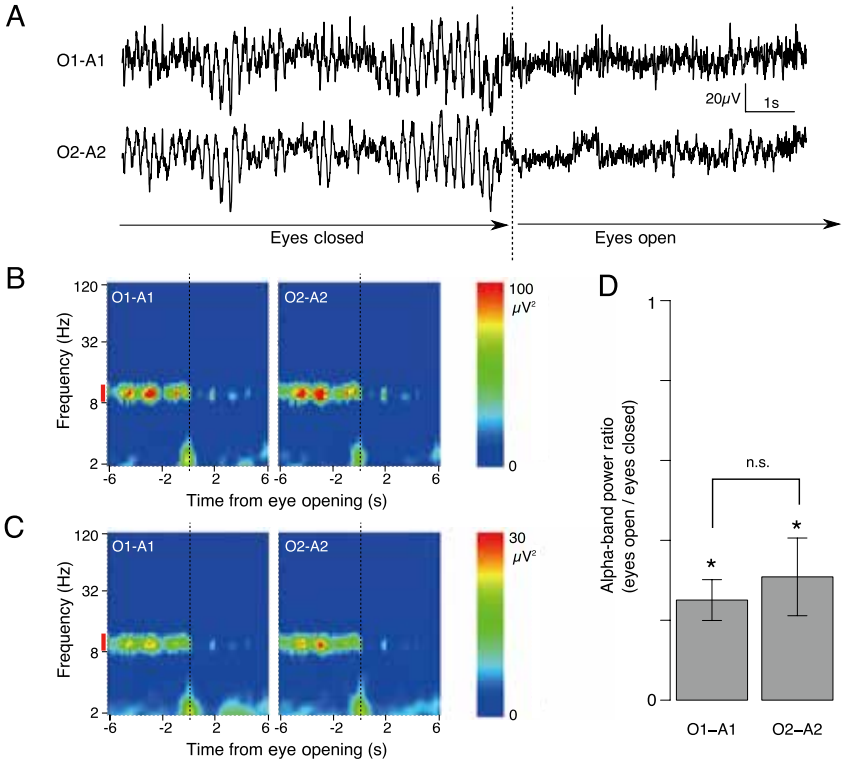

Figure 2. Alpha-blocking recorded using our system constructed with omni-purpose devices.

A: Sample EEGs recorded from O1 (upper trace) and O2 (lower trace). Vertical dashed line indicates the time of button release (*i.e.*, eye opening). These are the raw data and no digital filtering was performed.

B: Time-frequency representations (TRFs) of power spectra of O1 (left panel) or O2 (right panel) signals. Abscissae: Time from eye opening. Ordinates: frequency. Vertical dashed lines indicate the time of button release. Red line indicates the alpha band. Six trials are averaged.

C: The average power from six subjects is shown in the same format as B.

D: Average alpha-blocking ratios (relative alpha band power in the eye-opening period to that in the eye-closing period; see text) for O1 and O2. *: $p < 0.001$, *t*-test. Results are expressed as means ± standard error of mean.

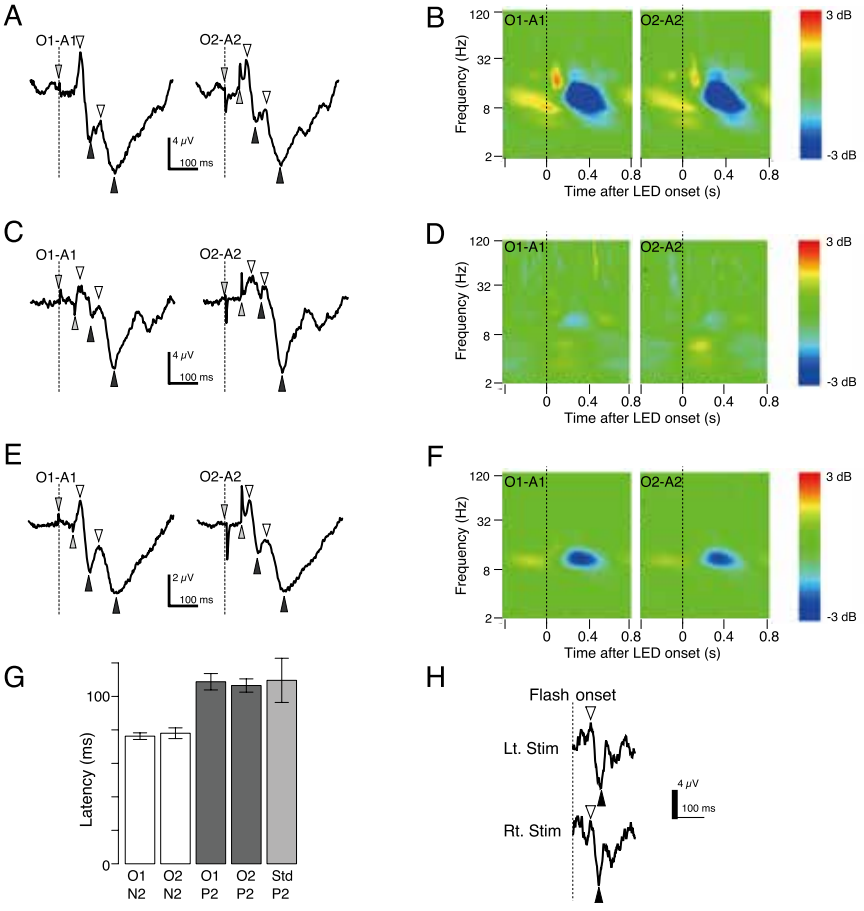

Figure 3. Examples of flash VEPs recorded using our system constructed with omni-purpose devices.

A: VEPs recorded from (left) O1 and (right) O2 of a subject. Data from 855 trials were averaged. No digital filtering was performed. Downward deflection indicates positive potential. Vertical dashed lines indicate the LED onset. Grey arrowheads: Artifacts of the LED onset and offset. White arrowheads: N2 and N3. Black arrowheads: P2 and P3.

B: Peristimulus time TRFs of normalized power calculated using Gabor wavelets. Vertical dashed lines indicate the LED onset. Negative color scales are saturated for display purposes. The minimal values are −4.93 dB and −4.23 dB for O1 and O2, respectively, at 13.2 Hz, 0.29 s after LED onset.

C, D: Data from another subject are shown in the same formats as A and B. Data from 856 trials were averaged. In A-D, the left eye was occluded.

E, F: Average VEPs and TRFs from eight subjects are shown in the same formats as A and B, respectively. In F, the power were averaged over eight subjects at first, then divided by the average peristimulus power.

G: Mean latencies of (white) N2 and (dark gray) P2 for O1 and O2. Light gray: previously reported normal P2 latency of flash VEPs[10].

H: VEPs recorded from Cz to flash stimuli to the (upper) right or (lower) left eye using LE-4000. The vertical dashed line indicates the flash onset. The data were obtained from the same subject as C and D. According to the common EEG format, the print-out is displayed upside down. Data from 128 trials were averaged for each trace. White arrowheads: N2. Black arrowheads: P2. The thermal array outputs on the data sheet from LE-4000 was manually digitized to obtain the traces.

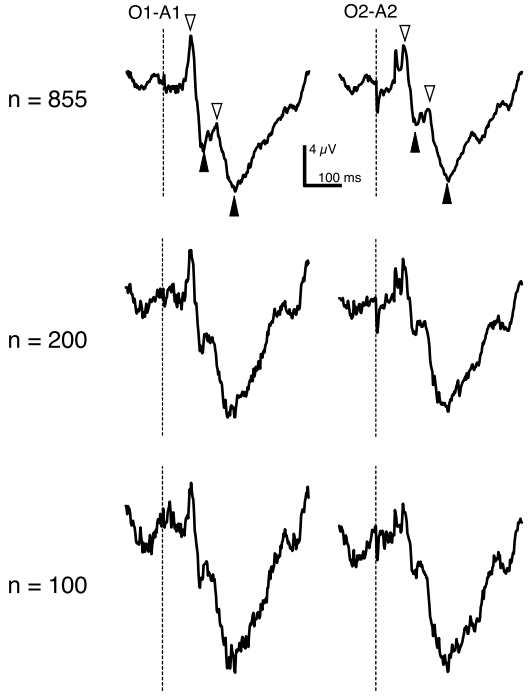

Figure 4. Effects of smaller trial numbers. Data were same for Figure 3A, except that only first (mid) 200, or (bottom) 100 trials were used for averaging. No digital filtering was performed.

2. Visually Evoked Potentials

As shown in Figure 3, VEPs were successfully recorded using our system. The data for Figure 3C were obtained in a preliminarily tried VEP practice by a trainee, who had not experienced EEG recordings previously. Because we used 50-ms stimulation, N1 and P1 were masked by the artifacts, but N2 and P2 could be distinguished in all subjects. As shown in Figure 3E, N2 and P2 are distinguishable in the pooled VEP, since the latencies of N2 and P2 distributed within small ranges (74−80 and 100−116 ms, respectively) (G). The P2 latencies recorded in this study were comparable with previously reported normal latency for flash VEP (109.6 ± 13.2 ms [10]), suggesting that our system is applicable for VEP practices in our department as well as a clinical-used equipment (H, the data were obtained from the same subject as C). In Figure 3B, event-related beta desynchronization (EBD) was clearly observed after the LED onset in both hemispheres. In the pooled average (F), EBD after the LED onset was prominent, even though EBD was small in a subject (D) with VEP waveforms (C) resembled those from the subject with a large EBD (A).

In these recordings, the visual stimuli were repeated > 800 times and responses were averaged to reduce noises, taking approximately 15 min for each subject. If smaller stimulation numbers were enough to obtain adequate results, the practice could be performed in a shorter period of time. To evaluate the effect of trial number reduction, we recalculated average VEPs from smaller numbers of trials using data for Figures 3A and 3C. As shown in Figure 4, N2 and P2 were distinguishable even in VEPs averaged over the first 100 trials. This result suggests that 100−200 repeats would be enough if the electrodes are carefully fixed to the body.

3. Versatility of the system

Our system was originally planned for setting-up an EOG practice in our department. For example, using a GR-COTTON as a fixation point and a commander, the latencies of saccadic eye movements were measured (Figure 5) with our system.

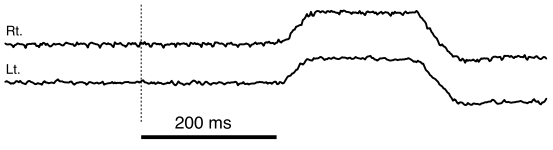

Figure 5. Examples of horizontal EOGs recorded from (upper) right and (lower) left eyes of a subject in a single trial. Vertical dashed line indicates the time when the LED turned red. With a latency of approximately 0.2 s, rightward conjugate (conjunctive) eye movements were observed. No digital filtering was performed.

Discussion

We constructed a simple VEP recording system with low-cost omni-purpose devices for student practices in the Department of Orthoptics and Visual Sciences. With the exception of the visual stimulation procedures, this system conforms to the guideline of the American Clinical Neurophysiology Society [11]. The noise level and CMRR of first-stage amplifier strongly affect the noise figure of the whole system. Using monolithic instrumentation amplifiers with very high CMRRs and low noises (Table 1) for the head-amplifiers, undergraduate students in our department could record EEGs in an unshielded-practice room (Figures 2 and 3). The quality of recorded VEPs to the small on-board LED was comparable to those recorded using a clinical-use device with a ganzfeld visual stimulator (Figures 3 and 4). The long-latency peaks, especially the P3, recorded with our system had different time-courses to those recorded with the clinical-use device (Figures 3C and 3H). The most possible explanation for the discrepancy may be contamination of the off-response that evoked after the on-response by 50 ms in this study, while effect of different reference electrode locations (A1 and A2 in our system for O1 and O2, respectively, A1 for Oz in the clinical-use device) could not be ruled out. The contamination of the off-response can be eliminated using the longer (> 200 ms) "on" duration, at the expense of the longer recording time for the whole practice.

Moreover, in our system, raw EEG waveforms are continuously displayed on the oscilloscope and the PC monitor. VEP itself is a very weak signal and averaging is necessary, and event-related changes of brain functions can be demonstrated through alpha-blocking in real time (Figure 2). Additionally, the simplicity of the recording system may help students understand the importance of good contacts between the skin and the electrodes. Even with high-CMRR amplifiers, hums may appear on the oscilloscope if skin cleaning is insufficient. After cleaning and re-applying of the electrode, hums will reduce immediately. Such trainings may help students engaging in electrophysiological examinations at hospitals or clinics to understand the principles of electrophysiological recordings, which consist of differential amplification and time-locked averaging. Likewise, the system can be applied to EOG practices (Figure 5).

In this study, we used preamplifiers of an oscilloscope as band-pass filters and power supplies because of their availability. Without them, analog filters would be made of op-amps. We utilized a microcontroller, GR-COTTON, for visual stimulation. The "sketches" (programs) for GR-COTTON were written in C++ language in a web-based developing environment that makes cross-compilation on desktop computers quite easy. Recently, some microcontrollers introduced 16-bit ADCs, which may be able to build robust and lower-cost recording systems without personal computers.

Interestingly, EBDs were observed as repeatedly flashing LED, though EBD has been reported to be involved in higher perceptual functions, such as speech and memory [12]. Because the stimulation interval was fixed in this study, some anticipatory mechanisms might be involved in the EBDs. Further studies with many participants and EEG channels are required to reveal relationships between the EBDs observed in this study and visual cortical functions.

Acknowledgments

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Conflicts of interest

No potential conflicts of interest are disclosed.

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16. Specification sheet of INA128. Available from: http://www.ti.com/lit/ds/symlink/ina128.pdf (accessed June 15, 2017)