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THE EFFECT OF STIMULUS PATTERN, COLOR COMBINATION AND FLICKER FREQUENCY ON STEADY-STATE VISUAL EVOKED POTENTIALS TOPOGRAPHY

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Abstract. *We investigated color/luminance characteristics and flicker frequency characteristics from steady-state visual evoked potentials (SSVEPs) topography on sinusoidal patterns and square wave patterns using black-and-white flicker stimulus and isoluminant color combination stimulus. Flicker frequencies of 6-18 Hz to black-and-white flicker stimuli and isoluminant color combination stimuli (red/blue, red/green) were adopted. Each of the stimuli and stimulus patterns were sinusoidal and square wave patterns. The study subjects were 20 healthy, young adult males (9 for monochrome and 11 for colors). Their topographical characteristics were evaluated by the maximum amplitude, central point coordinates, and the ratio of the activated area from the VEP averaged waveform in each recorded area. No significant difference in any of the characteristic parameters between stimulus patterns existed. The isoluminant color combination stimuli had a significantly smaller maximum amplitude compared with the black-and-white flicker stimulus; the central point coordinates and the ratio of the activated area changed considerably. Significant changes in characteristic parameters were also seen between stimulus frequencies. It is suggested that the position and distribution of the neuronal population in the active primary visual cortex differ according to color, luminance, and flicker frequency. However, it is considered that even if the stimulus patterns are different, the active primary visual cortex neuronal population does not change.*
Keywords: Steady-state visual evoked potentials (SSVEPs), Topography, Color, Luminance, Flicker frequency

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1. **Introduction.** Steady-state visual evoked potentials (SSVEPs) are seen maximumly at the visual cortex by the visual stimulus with flicker frequency of 3.5 Hz or higher [1]. The first harmonic component (1F) and the second harmonic component (2F) primarily appear corresponding to the value of flicker frequency in the recording of SSVEPs. The origination area of the SSVEPs for flash stimulus and flicker stimulus is widely reported as the primary visual cortex (V1) [2, 3, 4, 5, 6, 7, 9, 10, 11, 12]. However, the motion sensitivity cortex (V5/MT) [4], parieto-occipital area [5, 6], and lateral extrastriate area [7] have also been reported as the origination area. Therefore, SSVEPs for flicker stimulus would include the activity in the neuronal population of the lower visual cortex such as V1.

SSVEP is one of effective approaches for visual system analyses in the brain and many stimulus conditions have been presented [8]. The SSVEPs reaction changes according to the presented stimulus conditions [1, 8]. Flicker frequency [12], color [13, 14], and isoluminant color combination [15, 16, 17] have been reported as stimulus conditions that affect the amplitude of the SSVEPs. Strasburger et al. [18] reported spatial tuning of SSVEPs amplitude for pattern reversal stimuli was different from that for on-off modulation stimuli. Rudvin and Valberg [19] reported that the characteristics of SSVEPs amplitude for red-green grating reversing at different temporal frequency differ among respective levels of luminance contrast. Furthermore, it has been reported that SSVEPs amplitude flicker frequency characteristics are different for continually changing sinusoidal patterns and discretely changing square wave patterns in the case of black and white flicker stimulus and color combination stimulus [20, 21].

In visual information processing, the active neuronal population in the visual cortex depends on stimulus conditions such as color, brightness, and flicker frequency [22]. For this reason, the impact of stimulus conditions on SSVEPs topographical distribution has also been investigated [9, 10, 11]. Skrandies [9] reported that the center of gravity of the SSVEPs topographical distribution changes according to flicker frequency, and Muller et al. [10] reported that when the flicker frequency becomes high, the source of SSVEPs changes to the anterior and ventromedial occipital cortex. Keil et al. [11] have reported that the SSVEPs amplitudes for both luminance stimulus and color stimulus are maximized in the posterior area greater than that of the color stimulus. It has also been reported that, in case of stimulus patterns, from research using functional magnetic resonance imaging (fMRI), activity is observed around V1 and V5/MT in case of sinusoidal wave patterns and activity is only observed for V1 for square wave patterns [23]. In addition, Floriano et al. [24] analyzed the intensity of SSVEP responses at temporal and occipital area under several stimulus conditions (white/black, red/green and blue/green). However, it remains unclear whether SSVEPs topographical distribution flicker frequency characteristics and color/brightness characteristics change when stimulus patterns differ.

This paper proposes a simple topography analysis method for characterizing SSVEP among different stimulus conditions such as luminance, color combination, frequency and brightness pattern. Three chromatic combinations (white/black, red/blue and red/green), 2 brightness pattern (sinusoidal and square) and 14 flicker frequencies were employed as conditions of visual stimuli. Amplitudes of 1F and 2F components were then obtained from the power spectrum averaged VEP waveform. Maximum amplitude, central point coordinates and the ratio of activated area were calculated as characteristic parameters. Next, those parameters distributions (mean and standard deviation) for respective conditions were compared. Tendency of the characteristic parameters of the topography was not the same, but no significant differences were seen. However, differences among the chromatic combinations and the flicker frequencies were larger than those between brightness patterns. Those facts suggest that the neural distribution for color, luminance and frequency are fundamentally different, and the same neural group activates for different brightness patterns. The proposed analysis method for SSVEP topography is simple as compared with other ones, but is regarded as appropriate for analyzing limited brain area activity.

2. Method.

2.1. Subject and data acquisition.

The study subjects were men aged 21-24, of which nine received achromatic flicker stimulus and 11 received isoluminant chromatic flicker stimulus. No participants had color vision deficiencies. The intention to record was explained to the subjects in advance, and recording took place after informed consent was obtained. This study was approved by the ethics committee in the International University of Health and Welfare.

The stimuli were presented on a cathode ray tube (CRT) monitor using the pattern generator (VSG Three, Cambridge Research System, U.K.). At a visual angle of 10°, the viewing distance was 57 cm. CIE 1931 color coordinates were $x = 0.308$, $y = 0.342$ (white); $x = 0.391$, $y = 0.347$ (black); $x = 0.620$, $y = 0.353$ (red); $x = 0.166$, $y = 0.162$ (blue); $x = 0.290$, $y = 0.621$ (green). Visual stimuli were surrounded by a homogeneous background containing a mixture of color combination. The mean luminance of the visual stimuli and homogeneous background was 87.7 cd/m^2. Before the experiment, subjects viewed each color combination stimulus alternating at 18 Hz to establish psychophysical isoluminance, and adjusted the relative luminance to minimize perception of flicker. The stimulus patterns were two types of sinusoidal wave patterns wherein luminance and color continuously changed with discretely changing square wave patterns. Fourteen stimulus frequencies, i.e., 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 15, and 18 Hz were adopted, and the stimulus presentation time was 50 s. The subjects sat on a chair in a dark room, and gazed at a fixation point (visual angle of 0.2°) in a center of the monitor with their dominant eye [20, 21]. The Neurofax EEG-1100 (Nihon-Kohoden Cooperation, Japan) was used to record electroencephalograms (EEGs) at the International University of Health and Welfare, Fukuoka, Japan. According to the International 10-20 system, electrodes were attached to nine portions, Oz, O1, O2, Pz, P3, P4, T5, T6, and Cz, and EEGs were recorded under the following conditions: the referential electrode for deriving EEGs was Fz, the band path filter bandwidth was 0.53-60 Hz, and the sampling frequency was 200 Hz.

2.2. Characteristic parameters.

In some past SSVEPs topographical distribution analysis, the parameters of amplitude and center of gravity have been used to evaluate the characteristics such as the size of the SSVEPs reaction and the central area of activity [5, 6, 7, 9]. In this study, the characteristic parameters from the amplitude of SSVEPs in each area were obtained, and the differences in neuronal population in the visual cortex according to the stimulus conditions were evaluated.

The stimulus-locked averaging method was used for each stimulus condition and the averaged waveforms for the recorded area (i) were obtained. Then, the power spectrum was derived according to fast Fourier transform in relation to the averaged waveform. Based on the obtained power spectrum, the sum of frequency components in the flicker frequency ±0.5 Hz band (1F component) was defined as $S_1(i)$, and the sum of frequency components of twice the flicker frequency ±0.5 Hz band (2F component) was set to $S_2(i)$. From $S_1(i)$ and $S_2(i)$, the amplitude values of the 1F and 2F components were respectively calculated as follows [25].

$$A_1(i) = 4\sqrt{S_1(i)} \quad [\mu\text{V}] \tag{1}$$

$$A_2(i) = 4\sqrt{S_2(i)} \quad [\mu\text{V}] \tag{2}$$

Based on the 1F and 2F components amplitude values for each part obtained from (1) and (2), the parameters that express the topographical distribution characteristics were derived. First, we allocated the SSVEPs amplitude in each area on a 5×5 grid, as shown in Figure 1. The actual distance between electrodes differs for each area; however, similar to other methods [9, 26], equidistance is assumed to estimate the topographical distribution projected onto a secondary flat surface. For convenience, with Oz as the point of origin, the distance between blocks was set to 20 (the unit is dimensionless).

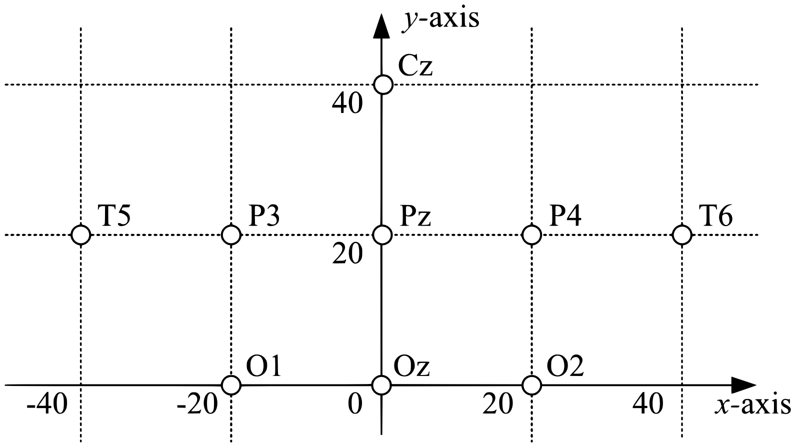

FIGURE 1. Rearrangement of the electrode placement by the 10-20 system onto a two-dimensional plane

The maximum amplitude, central point, and the ratio of the activated area were defined as the characteristic parameters. The maximum amplitude A^m is the maximum amplitude for all recorded points. The central point is defined as the coordinate position (x^c, y^c) obtained from the amplitude values for all points. 1F components were obtained from the amplitude $A_1(i)$ for part i, its x coordinate $x(i)$, and y coordinate $y(i)$.

$$x_1^c = \sum_i x(i)A_1(i) \bigg/ \sum_i A_1(i) \tag{3}$$

$$y_1^c = \sum_i y(i)A_1(i) \bigg/ \sum_i A_1(i) \tag{4}$$

The central point for the 2F component was calculated using the same procedure. The ratio of the activated area is defined as the ratio of all recorded parts, the amplitude of which makes up 50% or more of the maximum amplitude, to the total number of parts. In concrete terms, with the number of all recorded parts being N_a, the number of parts with an amplitude of 50% or more than the 1F component maximum value A_1^m as N_1, the ratio of the activated area for the 1F component is expressed as follows.

$$R_1 = \frac{N_1}{N_a} \times 100 \quad [\%] \tag{5}$$

The ratio of the activated area of the 2F harmonic component was also calculated according to the same procedure for that of 1F component.

3. **Result.**

3.1. **Characteristics in relation to color combinations.** Figure 2 shows the mean value for the characteristic parameters related to sinusoidal and square wave patterns for black and white flicker stimulus (Bk/W), and isoluminant color combinations stimuli red/blue (R/B) and red/green (R/G). With the top layer as (i) the 1F component and the second layer as (ii) the 2F component, from the left, this figure shows the (a) maximum amplitude, (b) central point x coordinates, (c) central point y coordinates, and (d) the ratio of the activated area. Each figure shows the average mean and standard deviation for the sinusoidal wave pattern (sin) and square wave pattern (sq) for Bk/W, R/B, and R/G. The differences between each group were evaluated using two-way analysis of variance (ANOVA) with stimulus pattern and color combination as factors.

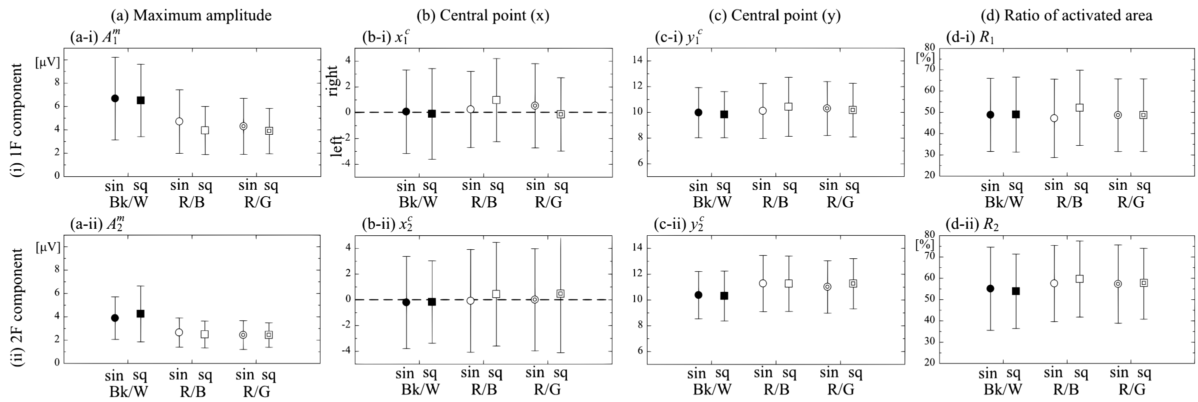

FIGURE 2. Characteristic parameters of SSVEPs topography for stimulus patterns and color combination

The (a-i) maximum amplitude for the 1F components was greater for Bk/W than that for R/B and R/G with any stimulus pattern, and a significant difference was observed ($p < 0.001$). In terms of (b-i) the central point x coordinates, (c-i) central point y coordinates, and (d-i) the ratio of the activated area, no significant difference was observed between either the color combinations or the stimulus patterns.

For the 2F components, (a-ii) the maximum amplitude was greater for Bk/W than R/B and R/G, and a significant difference was observed ($p < 0.001$). In terms of (c-ii) the central point y coordinates, Bk/W was closer to the posterior area than R/B and R/G, and a significant difference was observed ($p < 0.001$). In terms of (d-ii) the ratio of the activated area, R/B and R/G were greater than Bk/W, and a significant difference was observed ($p < 0.001$). No significant difference was observed in any of the characteristic parameters between the stimulus patterns.

3.2. **Characteristics in relation to flicker frequency.** In a previous study [20], in relation to flicker frequency for flicker stimulus, differences tended to exist between sinusoidal and square wave patterns when the SSVEPs amplitude was a flicker frequency less than 8 Hz, between 8 and 12 Hz, or greater than 12 Hz. Therefore, frequency bandwidth was defined into three parts, low as 6-7.5 Hz, middle as 8-12 Hz, and high as 15-18 Hz, and the characteristics were then evaluated. Figure 3 shows the average mean of the characteristic parameters for sinusoidal and square wave patterns in each flicker frequency band. These figures can be viewed the same way as those in Figure 2. As described in Section 3.1, the differences between each group were evaluated using two-way ANOVA with two factors (stimulus pattern and flicker frequency).

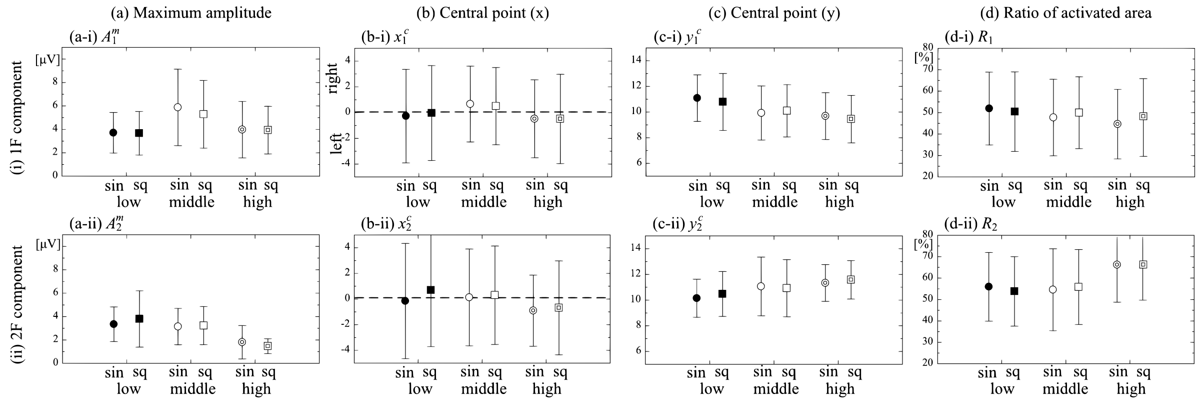

FIGURE 3. Characteristic parameters of SSVEPs topography for stimulus patterns and flicker frequency

In terms of the 1F components, the (a-i) maximum amplitude was more significant in the case of middle frequency stimulus than that in the case of low and high frequency stimulus ($p < 0.001$). The (c-i) center point y coordinates changed to the occipital area with increased flicker frequency ($p < 0.001$). No significant differences were observed for (b-i) the center point x coordinates and (d-i) the ratio of the activated area between stimulus frequencies. Furthermore, no significant differences were observed between the stimulus patterns for any of the characteristic parameters.

In terms of the 2F components, (a-ii) the maximum amplitude decreased significantly with increasing flicker frequency ($p < 0.001$). For (c-ii) the center point y coordinates, they separated significantly from the occipital area as flicker frequency increased ($p < 0.001$). In terms of (d-ii) the ratio of the activated area, there was a significant increase in the high frequency stimulus compared with that in the low and middle frequency stimuli ($p < 0.001$). There was no significant difference observed between the stimulus patterns for any characteristic parameters.

4. Discussion.

4.1. Analysis method of topographical characteristics. The proposed method employed the EEG records obtained from total 9 electrodes. Then, the maximum amplitude, the central point coordinate and the ratio of activated area have been calculated as topographical characteristics of SSVEPs. In recent studies on SSVEP topography, Cottereau et al. [27] investigated the source estimation by combining EEG, MEG and fMRI. Peterson et al. [28] used the SSVEP approach to analyze the working memory activity. In addition, Dipole analysis [10] and LORETA anasysis [5, 6] have been widely used. Those studies bring more detailed information for brain source localization. However, those methodologies require the special measurement equipment and/or enormous calculation cost for obtaining the result. Some calculation methods for optimization and estimation have no robustness for the noise contamination. In contrast, the proposed calculation method is simple and only the limited EEG information is necessary. In this study, the target area of the brain is not the whole head but is located at V1 in the occipital area. Therefore, the proposed method is considered to be usable for analyzing the topographical characteristics of SSVEPs for fundamental stimulus conditions such as luminance, color and frequency.

4.2. Recorded area. In the previous studies, in terms of the activated point in relation to visual stimulus, Schmolesky et al. [2] reported that the area 17 neuronal population participates in monkey SSVEPs in response to flash stimulation. Rager and Singer [3] reported that, in cats, the areas 17 and 18 contribute to SSVEPs. In addition, in the human SSVEPs amplitude topographical distribution, it has been reported by Skrandies [9] that the maximum amplitude point is the occipital area. Kim et al. [7] reported that, in 1F components, it is the medial occipital area, and in 2F components, it is the contralateral area. Kim et al. [7] also reported that it is the posterior area. From SSVEPs signal source estimation, Pastor et al. reported that, with LORETA analysis, the signal source for 1F components was detected in V1, and for 2F components, it was detected in the parieto-occipital area [5, 6]. Muller et al. [10] reported that through steady state visual evoked magnetic field dipole analysis, the dipole was observed in the areas 17 and 18. In studies using fMRI dipole analysis, Di Russo et al. [4] reported that the main dipole was located in V1, and dipoles could be observed in V5/MT as well. Thus, it can be said that the neuronal population around V1 primarily contributes to SSVEPs in relation to visual stimulus. Therefore, in relation to this record, it is expected that these will be the main areas in which response appears.

In a study examining the correspondence of the Broadmann's cortical map and the International 10-20 system [29], Oz corresponds to the area 17. O1 and O2 correspond to the areas 18 and 19, respectively, P3 and P4 correspond to the area 39, and T5 and T6 correspond to the areas 27 and 37, respectively. These results show that, since activity was recorded from V1 in the area 17 and the area covering the surrounding area, it can be evaluated in V1 and the surrounding area. As V5/MT, wherein activity is reported in relation to visual stimulation, is the intersection between the areas 19 and 37 [30], it is considered that with this record, a response can be obtained from P3 and P4. In this study, recorded electrodes were not covered with the whole area of the scalp; consequently the detail analysis of topographical distribution was limited. However, the differences in V1 and its surrounding can be evaluated correctly.

4.3. **Characteristic parameters.** In previous studies, SSVEPs topographical distribution characteristics were expressed on the basis of amplitude and center of gravity, and changes in SSVEPs were evaluated in relation to stimulus conditions [5, 6, 7, 9]. In relation to amplitude, Pastor et al. evaluated the flicker frequency characteristics using the average of the amplitude of the SSVEPs at O1, O2, and Oz electrodes [5, 6]. Kim et al. [7] evaluated the differences in the 1F and 2F components using the posterior area SSVEPs amplitude. In terms of the center of gravity, Pastor et al. [6] calculated the center of gravity using only posterior area data wherein the SSVEPs reaction is maximized from the results using LORETA analysis, and they evaluated the differences in the activated area for the 1F and 2F components. Furthermore, Skrandies [9] investigated the relationship between flicker frequency and the activated area using the positive area and negative area at the time at which the global field power from the SSVEPs for each record point is maximized.

In this study, we calculated the maximum amplitude, central point, and ratio of the activated area as the characteristic parameters expressing the SSVEPs topographical distribution characteristics. Among these, the maximum amplitude and central point are the same as the characteristic parameters defined in previous studies [5, 6, 7, 9]. In addition, we defined the characteristic parameters that express the activation area, and these were set to areas with a maximum amplitude of 50% or greater. Change of the activation area has not been investigated until now; however, from Figure 3, we can see that there is a significant change in the activation area depending on the stimulus conditions. From this, it is considered that the characteristic parameters that express the activation area are effective in the analysis of SSVEPs topographical distribution.

4.4. **Characteristics related to stimulus patterns.** Fawcett et al. [23] investigated the magnetoencephalographic (MEG) frequency components in relation to a hemi visual field checkerboard stimulus in terms of event-related synchronization (ERS) and event-related desynchronization (ERD). When the checkerboard stimulus is a square wave pattern, the ERS peak is observed in the contralateral medial occipital cortex. On the other hand, for a sinusoidal pattern, it is reported that the ERS peak is observed in the contralateral medial occipital cortex and the area around V5/MT [23]. Under the recording conditions of Fawcett et al. [23], the square wave pattern only presented stimulus in the right visual field; however, in the sinusoidal pattern, after presenting to the left visual field, it was presented to the right visual field. If two spatially different luminous points are presented discretely, the subject perceives apparent motion [31]. Furthermore, the neuronal population around V5/MT responds to the speed according to the flicker frequency [32]. Thus, in the case of the sinusoidal pattern reported in [23], ERD in the area around V5/MT may occur based on the stimulus paradigm. Both from Figure 2 and Figure 3, it is considered that, as no significant differences were observed between stimulus patterns among all characteristic parameters, the active neuronal population is the same even if the stimulus patterns differ. Therefore, it is thought that the difference in the SSVEPs amplitude flicker frequency characteristics observed in previous studies [20, 21] reflects the differences in the responsiveness to V1 neuronal population stimulus patterns.

4.5. **Characteristics related to color combinations.** In Figure 2, significant differences are observed between luminance stimulus and color combination stimulus in terms of (a-i), (a-ii) maximum amplitude, (c-ii) center point y coordinates, and (d-ii) the ratio of the activated area. Keil et al. [11] have reported that SSVEPs in relation to both color stimulus and luminance stimulus are maximized in the posterior area, and the amplitude significantly increases with the luminance stimulus. Since Keil et al. [11] have not performed detailed investigation into the differences in the activation area, it is impossible to perform a direct comparison; however, differences in amplitude support these results. Visual information converted from light to electrical signals in the retinal cone cells is split into the two paths of magnocellular (M) layer and parvocellular (P) layer from the retinal cone cells [22]. The M-pathway is thought to contribute to luminance information and the P-pathway to color information [22]. Furthermore, Johnson et al. [33] reported that, in V1, three neuronal populations exist: the neuronal population responding to luminance stimuli, the neuronal population responding to color stimuli, and the neuronal population responding to both luminance and color stimuli. Therefore, the current results can be considered to reflect the differences in the neuronal populations responding to luminance and color in V1. Furthermore, Johnson et al. [33] stated that the ratio of each neuronal population is luminance : luminance-color : color = 5 : 3 : 1. Thus, the differences in the maximum amplitude based on color combinations in these results are considered to reflect the fact that there are more neuronal populations activated by luminance stimuli than color stimuli.

4.6. **Characteristics related to flicker frequency.** In Figure 3, the (c-i) 1F component center point y coordinates and (c-ii) the 2F component center point y coordinates change on the basis of the flicker frequency. In relation to SSVEPs topographical distribution changes due to flicker frequency, Muller et al. [10] investigated the activated area in relation to flicker stimuli using MEG dipole analysis. They mentioned that dipoles were observed in areas 17 and 18 with a flicker frequency of 6 and 11.2 Hz, and more medial in case 15.2 Hz [10]. Furthermore, Skrandies et al. [9] reported significant changes in the 2F component's center of gravity in relation to a black and white checkerboard stimuli.

Regan [12] reported three types of SSVEPs in relation to flicker frequency comprising peaks at 10 Hz (low frequency VEP), 16 Hz (middle frequency VEP), and 40-50 Hz (high frequency VEP), with the low frequency VEP activating a wider area than the visual field and the areas 18 and 19 being activated by the middle frequency VEP. This is supported by the results of our study. Furthermore, changes in the center point in relation to flicker frequency differed in relation to 1F and 2F harmonic components. Pastor et al. reported that the results of SSVEPs LORETA analysis in relation to flicker stimulus showed that the source was observed in V1 in the case of 1F components and in the parieto-occipital area in the case of 2F components [5, 6]. Kim et al. [7] reported that the maximum amplitude in SSVEPs 1F components in relation to hemifield flicker stimulus was observed in V1, whereas the maximum amplitude for 2F components was located in the lateral extrastriate cortex. This is supported by the results of our study, and it is considered that the active neuronal populations differ for 1F and 2F components and that this contributes to the processing of different visual field information.

5. **Conclusion.** In this study, we have investigated the SSVEPs topographical distribution characteristics for sinusoidal and square wave patterns using black and white flicker stimulus and isoluminant color combination stimulus. No differences were seen in the topographical distribution characteristics in terms of color or flicker frequency between stimulus patterns. From this, we can conclude that the variation in the active neuronal populations according to color, luminance, and flicker frequency represents the fact that reactivity in the same neuronal populations differs when the stimulus pattern is different.

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