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Yiğit Tuncel, Toygun Başaklar and Yusuf Ziya Ider
1 Electrical and Electronics Engineering Department, Bilkent University, 06800 Ankara, Turkey
1 Author to whom any correspondence should be addressed.
E-mail: tuncel@ee.bilkent.edu.tr, basaklar@ee.bilkent.edu.tr and ider@ee.bilkent.edu.tr

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Abstract

Objective. Previous human steady state visual evoked potential (SSVEP) experiments have yielded different results regarding the range of stimulus frequencies in which period doubling (PD) behavior is observed. This study aims at obtaining experimental and statistical data regarding the frequency range of PD generation and also investigates other characteristics of PD.

Approach. In two sets of experiments, seven subjects were presented a sinusoidal flickering light stimulus with frequencies varying from 15 to 42 Hz. To observe the short term variations in PD generation, another set of 5 successive experiments were performed on five subjects with 10 min breaks in between. To obtain the SSVEP responses, filtering, signal averaging and power spectral density (PSD) estimation were applied to the recorded electroencephalogram. From the PSD estimates, subharmonic occurrence rates were calculated for each experiment and were used along with ANOVA for interpreting the outcomes of the short term repeatability experiments.

Main results. Although fundamental (excitation frequency) and second harmonic components appear in almost all SSVEP spectra, there is considerable inter-subject and intra-subject variability regarding PD occurrence. PD occurs for all stimulus frequencies from 15 to 42 Hz when all subjects are considered together. Furthermore, the statistical analyses of short term repeatability experiments suggest that in the short term, PD generation is consistent when all frequencies are considered together but for a single frequency significant short term differences occur. There also is considerable variation in the ratio of subharmonic amplitude to fundamental amplitude across different frequencies for a given subject.

Significance. Important results and statistical data are obtained regarding PD generation. Our results indicate that modeling studies should attempt to generate PD for a broader range of stimulus frequencies. It is argued that SSVEP based brain–computer interface applications would likely benefit from the utilization of subharmonics in classification.

1. Introduction

Steady state visual evoked potentials (SSVEPs) are oscillatory potentials elicited in the electroencephalogram (EEG) by flickering light stimulation [1, 2]. SSVEPs have been widely used in engineering applications such as brain–computer interfaces (BCIs) [3] and cognitive and clinical research [4, 5], however, there is not a precise explanation about their mechanism of generation. In particular, some nonlinear resonance phenomena of SSVEPs, which have been observed by several researchers [6–9], need to be better understood. These phenomena are characterized by brain responses at harmonic and subharmonic frequencies (period doubling (PD) behavior) as well as the response to the stimulus frequency. (Throughout this manuscript, ‘subharmonic frequency’ is used to refer to half of the stimulus frequency, and ‘period doubling’ is used to refer to the generation of subharmonic frequency.)

Crevier and Meister have investigated the PD phenomena under bright full field flickering square wave light stimulus in salamander and human [6]. For humans, both in electroretinogram (ERG) and EEG, they reported PD regime in response to stimuli between 30 and 70 Hz [6].
Herrmann conducted an extensive study in which he used a square wave light source as the flickering stimulus to induce SSVEP responses in EEG [7]. He varied the stimulus frequency from 1 to 100 Hz with 1 Hz steps and reported that subharmonic oscillations occur near alpha band. He did not state any specific range of stimulation frequencies that yields PD behavior. From the data and graphs presented in his paper, it is observed that PD occurs in response to roughly half of the stimulation frequencies in the range of 15–30 Hz.

Tsoneva et al have used repetitive visual stimuli in 40–60 Hz (gamma band) to study temporal and spatial properties of SSVEP and analyze the interaction between SSVEP and ongoing brain rhythms. They have used square wave light source with 2 Hz steps, excluding 50 Hz. They reported subharmonic responses throughout this stimulus frequency range along with valuable spatiotemporal information regarding SSVEPs [8].

Roberts and Robinson used Robinson’s Thalamocortical Model [10] to simulate Herrmann’s experimental procedure and the model predicted similar results [11] to Herrmann’s experimental results. They reported PD behavior for stimulus frequencies 15–24 Hz. They have also pointed out that square wave contains harmonic components by itself and is not the best stimulus for obtaining the most accurate results. So they proceeded to apply sinusoidal flicker instead of square wave flicker to the model and came up with similar predictions [11]. They also stated that these predictions regarding sinusoidal excitation should be experimentally verified.

Labecki et al undertook both experimental and simulation studies. They conducted an SSVEP experiment in which they used both square wave and sinusoidal light sources as the flickering stimuli to induce SSVEP responses in EEG [9]. Their experiment was rather limited in scope; they used 2 distinct frequency values for both modulation types (namely 5 and 15 Hz). They stated that in the spectrum of EEG response to 15 Hz stimulus frequency, 7.5 Hz peak was statistically significant; however it is not clearly observable by the eye. They also applied their experimental procedure to Lopes da Silva’s simple cortex model [12] to reproduce their experimental findings [9]. They reported that subharmonic responses occur in response to stimulus frequency range of 17–21.5 Hz when the input was square wave and 15–22 Hz when the input was sinusoidal wave.

There is considerable ambiguity on the subject of PD in SSVEP experiments in the literature regarding at which stimulation frequencies PD occurs and how consistent this occurrence is. This ambiguity also holds back the investigators who aim at developing realistic cortex simulation models. Besides, an inclusive experiment that investigates the nonlinear behaviors of SSVEPs induced by a purely sinusoidal stimulus is absent. Therefore, there is need for additional experimental data to clarify the various issues related to subharmonic generation. In this study, we have conducted SSVEP experiments with such focus and have compared our findings with the findings of other researchers and with the predictions of the abovementioned simulation studies. We have also identified and explained various characteristics of PD behavior.

2. Materials and methods

2.1. Experimental procedure

A total of 9 healthy (no neurological or psychiatric disorders) subjects with a mean age of 22 (6 males, 3 females) participated in the experiments. All subjects have normal or corrected-to-normal vision. All subjects signed an informed consent form which explains the objectives of the study and that flicker stimulation may cause epileptic seizures.

A simple Do-It-Yourself (DIY) cardboard VR headset with two, white, high gloss, 5 mm light-emitting diodes (LEDs) inside, where these two LEDs stay 2–3 cm in front of subjects’ eyes, was built for the purpose of full visual field illumination (figure 1). The stimulus is modulated with a sinusoidal waveform at 100% modulation depth. The peak illuminance at 2 cm away from one of the LEDs is 410 lux as measured by Trotec BF06 luxmeter.

An FPGA board (Nexys 2™ Spartan-3E FPGA Trainer Board (Digilent Inc., United States) with a 50 MHz crystal oscillator (100 ppm tolerance)) is used to generate a very accurate sinusoidal waveform (with less than 1mHz error). This digital sinusoidal waveform was converted to a sinusoidal voltage waveform by a 12 bit D/A converter (MCP4921-E/P). To convert the voltage waveform generated by the FPGA to light modulation, the circuit in figure 2 is used. The input to this circuit has 3.3 V peak-to-peak sinusoidal wave with 1.65 V DC offset voltage. The max. current in this configuration is 3.3 V/1.2 kΩ = 2.75 mA. This circuit is linear as long as the transistor is used in its
non-sat region. The LEDs are driven at their linear region. To make sure that the generated sinusoidal light waveform is free of harmonics and is a ‘pure’ sinusoid, the measurement circuit in figure 3 is used. This circuit utilizes a PIN photodiode (BPW24), whose response is very linear when reverse biased [13], to convert the light intensity into voltage waveform. The reverse current through the diode generates a voltage at the OPAMP output. The generated light had a ∼40 dB difference between the first and second harmonic.

Seven out of nine subjects participated in two different sets of experiments. They were first presented a frequency range of 15–32 Hz with 1 Hz steps (experiment E1). On another day (within 2 weeks), they were presented a frequency range of 28–42 Hz with 1 Hz steps (experiment E2). We divided the stimulation frequencies into two experiments in order not to exhaust the participants and also to observe long-term differences regarding PD generation. Additionally, to test the short-term repeatability of our experimental procedure and also to observe the short-term variability of our findings, five out of nine subjects participated in 5 experiments in a row, in which they were presented a frequency range of 25–35 Hz with 1 Hz steps, keeping the same cap and electrodes in position. There was a 10 min rest period between each successive experiment.

For all experiments, at each frequency, sinusoidal light was presented for 30 s with 10 s rest in between during which constant light at a level of half of the maximum brightness (LED circuit is driven by the DC offset voltage) was applied. An experiment was over when all the frequencies were presented once to the subject. For example, for 15–32 Hz experiment, there were 18 30 s recordings during which the stimulus was presented. In the beginning of each period of the sine wave, a marker pulse was also transmitted from FPGA to the EEG amplifier. For instance, for a 30 Hz sine wave, 900 markers were sent to the EEG amplifier during the 30 s stimulus presentation.

2.2. Data acquisition
The EEG was recorded with Brain Products V-Amp 16 channel EEG amplifier along with actiCAP, a standard 10–20 EEG cap with 32 electrode sites (Brain Products, Gilching, Germany). EEG was recorded from electrodes ‘O1, Oz, O2, Pz, P3, P4’ and they were referenced to FCz electrode. The ground electrode was placed over nasion, on the forehead. BrainVision Recorder was used to record the EEG and marker pulses simultaneously. The electrodes are active and ImpBox (Brain Products, Gilching, Germany) was used to measure the impedance values of electrodes. Electrode impedances were kept below 10 kΩ. The sampling rate was 1 kS s⁻¹. Data were filtered with a 50 Hz Notch filter during recording.

2.3. Data analysis
The EEG recordings were first exported to MATLAB using BrainVision Analyzer software and were analyzed in MATLAB (The MathWorks, Inc., Natick, MA, USA). For each experiment, 5–100 Hz bandpass filter was applied to each 30 s recording since fundamental frequency, its harmonics and subharmonics lie within this range and also to eliminate DC offset and slow components due to head movements. For each channel, from the 30 s recording of each frequency stimulation, fifteen non-overlapping 2 s long epochs were extracted. Epochs are chosen to be 2 s long to ensure that the subharmonic frequencies of odd stimulus frequencies have an integer number of cycles in an epoch.

SSVEPs are typically observed from frequency spectrums of EEG recordings. For each stimulation frequency and for each channel, three different types of power spectrum density (PSD) estimates were calculated:

(i) PSD estimate of the 30 s long data,
(ii) average of PSD estimates of each epoch,
(iii) and PSD estimate of the average of fifteen epochs.

For PSD estimate calculations, Welch’s method was used. In addition to the standard PSD plots, a two dimensional plot was also generated for the spectrums of the averaged epochs. In this plot, the stimulation frequencies are placed on the horizontal axis and the PSD estimate of the response to each stimulus frequency are grayscale coded on the vertical axis (such as in figures 6 and 7). This plot is called PSD versus stimulus frequency (PSD-SF) plot in the rest of this document for easy referral.

To determine if a subharmonic component (corresponding to period doubling) occurs or not, the PSD of half frequency component should be clearly distinguishable in amplitude (an outlier) from surrounding frequency components. The PSD estimate of 30 s long data was calculated with a frequency resolution of 0.1 Hz. From this data, a 21-point array that holds the PSD estimates in the ‘subharmonic frequency ± 1 Hz’ interval was extracted. For example, if the stimulation frequency was 30 Hz, this array would be composed of PSD estimates of 14–16 Hz with 0.1 Hz steps. To detect the occurrences of period doublings, MATLAB’s ‘soutlier’ function was used on the extracted array. Outliers, which were more than three times the scaled median absolute deviation away from the median, were detected from this array. If the detected outlier was found to be at the half fundamental frequency, then this outlier was regarded as a subharmonic occurrence and subharmonic occurrence value was assigned the value of ‘1’ (else as ‘0’). For each experiment, subharmonic occurrences were identified by using the above method for each stimulus frequency in all channels.

Subharmonic occurrence rate across channels (SORc) for any given frequency and experiment was defined as average occurrence in all the six channels that were recorded. For the case of short term repeatability experiments, in addition to SORc values, subharmonic occurrence rate across experiments (SORe) for any given frequency and channel was defined as average occurrence in all the 5 consecutive experiments. ANOVA was done on the SORc and SORe values of short term repeatability experiments to obtain statistical results. For both ANOVA tests, the independent variable was the stimulation frequency and the dependent variable was SOR. We have chosen to base our statistical decisions on a significance level of $p = 0.05$.

### 3. Results

To illustrate the benefit of averaging in data analysis, spectrum of a raw epoch and the spectrum of the average of the fifteen epochs within a 30 s recording are compared in figure 4. It can be seen that averaging highly attenuates the alpha power (8–12 Hz) which exists in the raw recording. This happens because alpha components are not synchronous with the applied stimulus frequency and therefore they diminish with averaging. In the spectrum of the averaged epoch (figure 4(d)), the fundamental (32 Hz), harmonic (64 Hz) and subharmonic (16 Hz) peaks are more easily observable than in the spectrum of the raw epoch (figure 4(b)).

Figure 5 compares the three different PSD estimates that are explained in data analysis section. With a stimulation frequency of 40 Hz, in the first type of PSD plot (figure 5(a)), there is a clear high amplitude 20 Hz signal component along with the expected 40 Hz signal component. In the second type of PSD plot (figure 5(c)), the subharmonic (20 Hz) component is not as distinguishable from the surrounding spectral components. In the third type of PSD plot (figure 5(d)), the 20 Hz component is again more clearly evident. This figure again clearly demonstrates that signal averaging helps to suppress the alpha components and thereby enhances the appearance of the subharmonic component. Among the three types of spectrums presented in figure 5, although subharmonic components are observable in all three, type 3 spectrums are especially preferable to obtain PSD-SF plots because with the other two types of spectrums, the stronger alpha range and nearby spectral components preclude the clear demonstration of PD on a PSD-SF plot.

PSD-SF plot averaged across all subjects (for channel Oz) for experiment E1 (with 15–32 Hz stimulation range) is given in figure 6. Components on the 1:1 line, on which the fundamental frequency components line up, are clearly visible. In a similar manner, the 2:1 line, on which the second harmonic components line up, is observable. The subharmonic components, which are to be on the 1:2 line, are also visible throughout this frequency range. Figure 7 presents the PSD-SF plot averaged across all subjects (for channel Oz) (data from subject S6 was discarded due to EEG artifacts for this recording) for experiment E2 (with 28–42 Hz stimulation range). As expected, the 1:1 and 2:1 lines (fundamental and second harmonics) are clearly visible. As the excitation frequency (stimulus) is increased from 28 to 42 Hz, a distinct and significant half frequency component follows (1:2 line). Subharmonic responses are more clearly discernable in figure 7 than in figure 6 because the subharmonic frequencies in figure 7 do not fall in the alpha band. Subharmonic responses in figure 6 are still visible within the alpha band due to the existence of a frequency dependent trend. If signal averaging is not used before PSD estimation, this trend is not visible.

Table 1 presents at which frequencies PD is observed for each subject in the 15–32 Hz and 28–42 Hz experiments. Considering the 15–32 Hz experiments (E1), in three subjects (subjects S1, S5 and S9), PD is observed throughout nearly the whole
frequency range (for subjects S1 and S9, the observed frequencies are contiguous and for subject S5 the frequencies that depict PD are separate (spaced)). For subjects S2, S3 and S6, PD is observed in the second half of the stimulus frequency range (from 25 Hz and onward). For subject S4 on the other hand, PD is not observed at all. In the 28–42 Hz experiments (E2), out of the 15 stimulation frequencies, subjects S2, S9, S3, S5, S1, S4 and S6 generate subharmonic responses at 10, 10, 9, 8, 7, 5 and 5 frequencies respectively. S2, S3, S4 and S9 depict contiguous responses but the others have spaced-out responses. Therefore, there is substantial subject to subject variation regarding the frequencies of PD phenomena. Furthermore, for a given subject, considering the overlapping stimulation frequencies in both experiments (28–32 Hz), experiments performed in different days also may yield different results.

Table 2 shows the subharmonic occurrence rates across channels (SORc) for subject S3 within the 5 consecutive experiments which were performed for observing the short term repeatability of the
experimental procedure. From table 2, SORc value, averaged across all frequencies (bottommost row) for a single experiment is observed to vary from a minimum of 24% to a maximum of 52%. The change in this rate does not follow a trend in time (experiment number). One-way repeated measures ANOVA test [14] (which is an extension of the paired t-test) concludes that, when all frequencies are considered together, the experiments do not differ significantly ($F(4, 40) = 1.322, p = 0.2783$). Similar results are obtained for the other subjects except for subject S2 (see table 4 for statistical details). In S2 experiments are found to be different and however, this is only due to the difference between experiment 1 and experiment 4 revealed by a post hoc test which analyzes experiments in pairs ($p = 0.035$). On the other hand, even between experiments which are not found to be different statistically when all frequencies are considered together, there are substantial individual variations in SORc values when a single frequency is considered. For example, for 31 Hz SORc value varies from 100% to 0% across experiments in table 2.

For a reason explained later in the conclusions and discussion section, we have also investigated if there is significant difference between occipital channels (O1, Oz, O2) and parietal channels (P3, Pz, P4). Table 3

**Figure 6.** Averaged PSD-SF plot across all subjects (channel Oz) for the experiment that covers the stimulus frequencies from 15 to 32 Hz. Horizontal dashed red lines visualize the alpha band (8–12 Hz interval). The dotted red lines are put for better demonstration of the trends in data. The lower limit of the colorbar is set to 0.1 to eliminate confusion due to background noise in spectrums. The upper limit is saturated at 0.5 for clear observation of subharmonic peaks.

**Figure 7.** Averaged PSD-SF plot across all subjects (channel Oz) (data from subject S6 was discarded due to EEG artifacts for this recording) for the experiment that covers the stimulus frequencies from 28 to 42 Hz. Horizontal dashed red lines visualize the alpha band (8–12 Hz interval). The dotted red lines are put for better demonstration of the trends in data. The lower limit of the colorbar is set to 0.1 to eliminate confusion due to background noise in spectrums. The upper limit is saturated at 0.5 for clear observation of subharmonic peaks.
shows the average of subharmonic occurrence rates across experiments (SORc) of O channels and P channels for subject S3. One-way ANOVA test shows that there is no significant difference between O channels and P channels (F(1, 20) = 2.61, p = 0.122) for S3. In fact, as shown in the second column of table 4, one-way ANOVA concludes that O channels and P channels do not differ significantly in all subjects (F and p values are given in table 4).

We have also studied the variations of the subharmonic peak relative to the fundamental peak. Indeed, as shown in figure 8 as an example, the ratio of the amplitude at the subharmonic frequency to the fundamental component is less than one in 27–30 Hz region and larger than one at 31 and 32 Hz for subject S2 (channel Oz). In general, considering the observations in all experiments of the study, the amplitude of the subharmonic frequency relative to the amplitude of the fundamental frequency does not follow a trend and is variable with respect to frequency. Figure 9 shows the change in relative amplitude for subject S2’s repeatability experiments. In this figure, it can be observed that relative amplitudes vary with respect to frequency for an experiment and vary with respect to experiment for a frequency. It can also be seen that although relative amplitude generally resides below 1,
there are cases when subharmonic component is much larger than the fundamental component.

4. Conclusions and discussion

Our experimental results indicate that PD may occur in all stimulation frequencies (15–42 Hz) that our experiments have covered. In earlier studies, which covered a broad range of stimulation frequencies (1–100 Hz), Herrmann’s findings indicated subharmonic generation for the stimulus range of 15–30 Hz [7], and Crevier and Meister reported subharmonic occurrence range for stimulus frequencies between 30 and 70 Hz [6]. Additionally, Tsonoeva et al have studied the stimulus frequency range of 40–60 Hz and have observed subharmonic generation in this frequency range [8]. Therefore, considering the results of all these studies and the current study, it is likely that subharmonic generation occurs in a broad range of frequencies, and as of now an exact frequency range of subharmonic generation cannot be stated. The differences between the results of different studies may perhaps be explained by factors specific to the subjects included in the experiments and also specific to the experimental conditions, which however need to be identified in further studies.

The mechanisms of subharmonic frequency generation is still an unresolved issue. In the 2012 study of Roberts and Robinson [11], Robinson’s Thalamocortical model [10] was tuned to reproduce Herrmann’s findings [7]. There, the model showed PD behavior only in 15–24 Hz stimulus interval. Similarly, Labecki et al used Lopes da Silva’s cortical column model [12] to reproduce PD behavior in 15–22 Hz stimulus interval [9]. However, our study indicates that a model should include a broader frequency region in its PD regime. Future models should be able to provide means for tuning the subharmonic frequency range and preferably be able to relate these to physiological and environmental conditions (such as those mentioned in the next paragraph). It is vital to adjust these models according to such factual experimental information so that model predictions, regarding other nonlinear phenomena and dynamics and physiological mechanisms, could be basis for further research.

The summarized results in table 1 suggest that there is substantial inter-subject variability and also substantial intra-subject variability among experiments that are conducted in different days. We have also conducted on five subjects the so called short-term repeatability experiments in order to observe the short term effects on subharmonic generation. Although, PD generation is mostly consistently observed in consecutive experiments when all frequencies are considered together, there is considerable variation in the SOR values of a frequency across experiments. It may be conjectured that both long term effects (electrode placement, physiological state, psychological state, illness, hunger, etc) and short term effects (changes in gaze, attention level, fatigue, etc) may be factors that cause these variations.

The effect of attention on SSVEP fundamental peak amplitude is investigated in [15]. Mutual influence between fatigue level and SSVEP parameters have also been reported in [16]. Level of attention and fatigue may also be altering PD generation. In any case, it may be wise to conduct PD experiments, which aim at investigating subharmonic frequency generation, with random stimulus frequency order to eliminate the doubt related to fatigue and attention. Random stimulus frequency order may also be important to circumvent possible biases due to hysteresis effects predicted by Roberts and Robinson in their simulation study [11].

From the ERG recordings of both humans and animals, PD behavior was reported [6, 17, 18] which indicates that subharmonic generation can occur at the retina. Lateral geniculate nucleus takes inputs from retinal ganglion cells and projects most of its output to primary visual cortex which are then projected to other visual cortex areas [19]. The electrode sites, O1, Oz and O2 that were used in our experiments, correspond to primary (Oz) and secondary visual (O1, O2) cortices [20]. Accordingly, in our repeatability experiments, we argued that subharmonic occurrence rates may be consistently different between O and P channels. However, the statistical analysis we have
performed on the repeatability experiments yielded no difference between channels (see table 4).

As shown in figure 9, the ratio of subharmonic to fundamental component amplitudes varies considerably. In fact, there are cases where a fundamental peak may not occur at all while a subharmonic component occurs (such cases are saturated with a ratio of 2 in figure 9). The amplitudes of subharmonic and fundamental components also change considerably from channel to channel. There may even be some extreme cases such as shown in figures 10(a) and (b) where fundamental component is almost non-existent in channel O1 whereas it is of the same order of magnitude with subharmonic component in channel Oz. These channel to channel variations may be the result of phase differences/delays between areas in the cortex. Figure 10(c) shows an example of another nonlinear interaction that is not as commonly visible as other observed nonlinear phenomena. There is a peak at third harmonic of the subharmonic frequency \((f + f/2)\) in SSVEP spectrum. It is not clear whether such behavior is a result of nonlinear interactions of different cortical areas or maybe an inherent property of the cortex in general.

SSVEP is widely used in BCI applications due to its robustness and speed [5, 21, 22]. The reported phenomena in the current study includes cases in which subharmonic peaks are greater in amplitude than fundamental peaks or in which fundamental peaks are non-existent while subharmonic peaks are clearly

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**Figure 8.** The power spectrums of averaged epochs for 27–32 Hz stimulus frequencies. Data belongs to S2 (channel Oz). The peaks on different spectrums are marked with data tips. The ratio of the amplitude at the subharmonic frequency to the fundamental component is varying. Note that some peaks are outside the vertical range, their peak values can be seen on the corresponding data tip.
observable. In a BCI application using the SSVEP paradigm, total lack of fundamental component would likely degrade classification accuracy. Thus, it can be claimed that utilizing subharmonic peaks in addition to fundamental peaks in SSVEP responses would improve BCI performance. This requires target frequencies to be chosen for a particular subject within the frequency range where PD behavior is observed. Similarly, since the second harmonic components are consistently observed in all of our experiments, additional use of this harmonic in SSVEP BCI applications is also justified.

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