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Evaluating the feasibility of the steady-state visual evoked potential (SSVEP) to study temporal attention

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Abstract

Improvements in perceptual performance can be obtained when events in the environment are temporally predictable—and temporal predictability improves attention and sensory processing. The amplitude of the steady-state visual evoked potential (SSVEP) has been shown to correlate with attention paid to a flickering stimulus even if the flickering stimulus is irrelevant for the task. However, to our knowledge, the validity of the SSVEP to study temporal attention has not been established. Therefore, we designed an SSVEP temporal attention task to evaluate whether the SSVEP and its temporal dynamics can be used to study temporal attention. We used a forced-choice perceptual detection task while presenting task-irrelevant visual flicker at alpha (10 Hz) and two surrounding frequencies (6 or 15 Hz). Temporal predictability was manipulated by having the interstimulus intervals (ISI) be constant or variable. Behavioral results replicated previous studies confirming the benefits of temporal expectations on performance for trials with constant ISI. EEG analyses revealed robust SSVEP amplitudes for all flicker frequencies, although a main effect of temporal expectations on SSVEP amplitude was not significant. Additional analyses revealed temporal predictability-related modulations of SSVEP amplitude at 10 Hz and its second harmonic (20 Hz). The effect of temporal predictability was also observed for the 6 Hz flicker, but not for 15 Hz for any ISI condition. These results provide some evidence for the feasibility of the SSVEP technique to study temporal attention for stimuli with flicker frequencies around the alpha band.

KEYWORDS

attention, EEG, methods, SSVEP

1 | INTRODUCTION

Time is crucial for brain and cognitive function. Our ability to perceive and interpret the world is largely determined by our ability to predict, perceive, and attend to changes over time. The ability to allocate our brain resources to perceive and attend to the predicted onset of a particular event while ignoring nonrelevant stimuli has been named temporal attention (Babiloni et al., 2004). Temporal attention can be maximized when events in the environment are temporally predictable (Coull & Nobre, 1998; Miniussi, Wilding, Coull, & Nobre, 1999; Rohenkohl, Coull, & Nobre, 2011).

Temporal expectancies have been extensively studied in the laboratory using an adaptation of Posner's spatial orienting task (Posner, 1980; Posner, Snyder, & Davidson, 1980). The manipulation of cue timing can induce temporal expectations, and participants are able to use such cues to predict the time onset of the upcoming stimulus. Temporal expectations induced by the cue can be generated by using predictive (Davranche, Nazarian, Vidal, & Coull, 2011), instructive (Coull & Nobre, 1998), or rhythmic stimulation (Rohenkohl et al., 2011; Rohenkohl & Nobre, 2011). Also, manipulating hazard rates (Vangkilde, Petersen, & Bundesen, 2013) or the regularity or interstimulus interval (ISI) between presentations (Jepma, Wagenmakers, & Nieuwenhuis, 2012) could generate temporal expectations.

Temporal expectations have been shown to improve attention and sensory processing across different domains (Woodrow, 1914). For example, in a predictive visual temporal attention task using different time intervals for central cues that would predict next target presentation, Miniussi et al. (1999) reported that participants showed faster reaction times (RTs) for trials predicted by shorter time cues (e.g., 600 ms) as compared with longer time cues (e.g., 1,400 ms) or trials where the target was unpredictable (e.g., cue for 600 ms and target presented at 1,400 ms). Similarly, Doherty, Rao, Mesulam, & Nobre (2005) studied the effects of spatial and temporal expectations on visual attention processing. By using rhythmic cues, the authors manipulated expectations regarding location and/or time of target appearance after being briefly occluded by a barrier presented on the screen. The authors reported faster RTs for all the expectation conditions (e.g., spatial, temporal, or combined spatial and temporal expectation) as compared with conditions that lacked such expectations.

This general phenomenon of improved performance has been replicated several times showing the effects on perceptual sensitivity (d') during a rapid-serial-visual-presentation task (RSVP; Correa, Lupiáñez, & Tudela, 2005), on RTs when using symbolic (Griffin, Miniussi, & Nobre, 2001) or rhythmic cues (Doherty et al., 2005; Mathewson et al., 2012; Mathewson, Fabiani, Gratton, Beck, & Lleras, 2010), or the enhancement of temporal expectations when combined with spatial attention-orienting tasks (Doherty et al., 2005; Rohenkohl, Gould, Pessoa, & Nobre, 2014). Regarding the neurophysiology of temporal attention, several studies have described the left inferior parietal cortex (Coull & Nobre, 1998), intraparietal sulcus (Davranche et al., 2011), premotor and supplementary motor areas, and a network of bilateral frontoparietal areas (Coull, Frith, Büchel, & Nobre, 2000; Coull & Nobre, 2008) as key neuroanatomical regions responsible for temporal expectations. Modulatory effects of temporal expectancies on the ERPs N1, N2, N2pc, and P300 (Griffin, Miniussi, & Nobre, 2002; Miniussi et al., 1999; Rolke, Festl, & Seibold, 2016; Seibold, Fiedler, & Rolke, 2011; Seibold & Rolke, 2014; see Correa, Lupiáñez, Madrid, & Tudela, 2006, for details), and event-related synchronization/desynchronization of the ongoing EEG (Babiloni et al., 2004; Rohenkohl & Nobre, 2011) components have previously been described in several electrophysiological studies.

Although these approaches have helped to better understand the neural correlates of temporal attention and its role on perceptual processing, an alternative technique—the steady-state visual evoked potential (SSVEP)—has, to our knowledge, never been used to study temporal attention. The SSVEP is a rhythmic neural response that tracks a flickering sensory stimulus (a.k.a. photic drive; Luck, 2005; Regan, 1989) and can be induced with a flickering stimulation in the range from 1 to 100 Hz (Herrmann, 2001).

The SSVEP is a robust method to study visual perception (Ales, Farzin, Rossion, & Norcia, 2012), spatial (Müller & Hillyard, 2000) and selective attention (Hillyard et al., 1997; Silberstein et al., 1990), cognitive fatigue (Mun, Park, Park, & Whang, 2012), and working memory (Gulbinaite, Johnson, de Jong, Morey, & van Rijn, 2014). Similarly, the attentional effects on SSVEP have also been evaluated in performance for brain-computer interfaces (Mora-Cortes, Manyakov, Chumerin, & Van Hulle, 2014) and evaluated in clinical studies in patients with schizophrenia (Silberstein, Line, Pipingas, Copolov, & Harris, 2000).

The SSVEP has been extensively used to study visual attention because the amplitude of the SSVEP correlates with attention to each flickering stimulus (Andersen & Müller, 2010; Ding, Sperling, & Srinivasan, 2006; Kashiwase, Matsumiya, Kuriki, & Shioiri, 2012; Kim, Grabowecy, Paller, Muthu, & Suzuki, 2007; Morgan, Hansen, & Hillyard, 1996; Müller, Teder-Sälejärvi, & Hillyard, 1998; Saupe, Schröger, Andersen, & Müller, 2009; Senkowski, Saint-Amour, Gruber, & Foxe, 2008; Vialatte, Maurice, Dauwels, & Cichocki, 2010; Walter, Quigley, Andersen, & Müller, 2012). Additionally, the SSVEP provides a high signal-to-noise ratio (SNR) measurement of brain activity and can be readily associated with specific stimuli, even if multiple stimuli are displayed at the same time (Norcia, Appelbaum, Ales, Cottureau, & Rossion, 2015). SSVEPs can also be used to study time-varying fluctuations in attention (Jamison, Roy, He, Engel, & He, 2015; Keil & Heim, 2009; Talsma, Doty, Strowd, & Woldorff, 2006; Wieser & Keil, 2011).

Although changes in SSVEP amplitude are generally related to attention, there is some evidence that this effect may depend on the stimulation frequency (Bridwell & Srinivasan, 2012; Ding et al., 2006; Srinivasan, Bibi, & Nunez, 2006). In other words, the effects of attention on the SSVEP may depend in part on the stimulus flicker frequency, even if the flickering stimulus is irrelevant for the task. Furthermore, the alpha band (~ 10 Hz) has been described to be part of the substrate of temporal attention (Busch & VanRullen, 2010; Mathewson et al., 2012; Rohenkohl & Nobre, 2011), and ongoing endogenous alpha activity can influence awareness of visual targets, suggesting a pulsed inhibition of the sensory processing generated by alpha oscillations (Mathewson, Gratton, Fabiani, Beck, & Ro, 2009; Mathewson et al., 2011) which may interfere with ongoing visual/attentional processing (Mathewson et al., 2012; Spaak, de Lange, & Jensen, 2014). Therefore, the alpha band might have a special place in SSVEP correlates of sensory perception.

Despite the robustness of the SSVEP, to our knowledge it has not been evaluated in the study of temporal attention. This seems important to demonstrate, in part for use in fundamental science, and in part because temporal attention and the use of temporal cues to guide behavior is impaired in

older adults (Zanto et al., 2011) and in clinical disorders including Parkinson's disease (Rochester et al., 2005; Tagliati, Bodis-Wollner, & Yahr, 1996) and schizophrenia (Liotti, Dazzi, & Umiltà, 1993; Nestor et al., 1992; Silberstein et al., 2000).

Thus, there were two goals to our study: first, to evaluate whether the SSVEP and its temporal dynamics can be used to study temporal attention; second, to evaluate whether attention-modulation of the SSVEP is similar for three different visual flicker frequencies (6 Hz, 10 Hz, and 15 Hz). We selected 10 Hz flicker because it is the center of the alpha band and because, as described above, alpha oscillations seem to play a prominent role in temporal attention (Rohenkohl & Nobre, 2011). We then used two additional frequencies outside the alpha band range partly as control conditions for the alpha flicker, and partly to explore whether SSVEP amplitude would correlate with temporal attention for different stimulus frequencies. Therefore, we selected the 6 Hz and 15 Hz.

To this end, we implemented a modified version of a task design by Mathewson et al. (2009), in which subjects reported whether they perceived a dim gray stimulus (target) that was briefly flashed on a computer monitor. We manipulated the ISI in different blocks to be either constant (ISIC—temporally predictable stimulus onsets) or variable (ISIV—temporally unpredictable stimulus onsets; Woodrow, 1914). We predicted that, in the ISIC compared to ISIV blocks, perceptual task performance would be improved and that EEG data would show increased SSVEP amplitude.

2 | METHOD

2.1 | Participants

Thirty subjects participated in the data collection. Data from two subjects were rejected due to poor behavioral performance. During EEG preprocessing, the data of one subject were rejected due to excessive eyeblink artifacts in more than 30% of the trials and excessive lateral eye movements (i.e., left–right) at the time of target presentation. Finally, during the SSVEP analysis, data from two subjects were rejected due to an absence of visible SSVEP response in the power spectral plots. Thus, data from 25 participants (11 male, mean age 25.04, one left-handed) were included in the final analysis. All subjects had normal or corrected-to-normal vision. The study was conducted in accordance with the Declaration of Helsinki, relevant laws, and institutional guidelines, and was approved by the local ethics committee at the Psychology Department at the University of Amsterdam. Participants signed an informed consent document prior to the beginning of the experiment, and they were paid for their participation.

2.2 | Task

Our task was designed to assess visual target detection performance with ISIC or ISIV. Participants were seated at a distance of approximately 90 cm from and at eye level of a 24" BenQ monitor (1,920 × 1,080 pixels) with a refresh rate of 120 Hz. Stimulus presentation and behavioral response collection were controlled by custom-written scripts in MATLAB (The Mathworks, Natick, MA) using the Psychophysics Toolbox (Brainard, 1997; Kleiner, Brainard, & Pelli, 2007; Pelli, 1997).

Participants were requested to fixate the center of the screen. At the start of each trial, a circle (approximately 1.87° of visual angle; note that all measurements here are approximate because subjects were not restrained, e.g., by a chin rest) was presented in the position of the fixation cross (0.47°) for 100 ms. Subjects were asked to press the left mouse button if they saw a complete black circle or the right mouse button if they saw a smaller gray circle (0.93° of visual angle; a target) in the center of the black one. We call trials "target present" when the small circle was presented. This response mapping was not counterbalanced across subjects, although our primary analyses were not based on motor response hand. Subjects were instructed to respond as quickly and accurately as possible after stimulus presentation, and they did not receive feedback regarding their performance. Figure 1 shows the stimulus features and the time line of a trial sequence presented to each participant.

Surrounding the fixation and target stimulus was a black task-irrelevant rectangle (22.50° × 17.40° of visual angle) placed at 11.43° of visual angle from the top of the screen (Figure 1a,b). The rectangle was presented asymmetrically because this arrangement increases SSVEP amplitude by decreasing the possibility of electrical field cancellation across the calcarine sulcus (Vanegas, Blangero, & Kelly, 2013). Both the rectangle and the fixation cross were presented continuously throughout each block. In each block, the rectangle flickered in a specific frequency (6, 10, or 15 Hz). Square-wave time series were created for each frequency (50% on per frequency). Furthermore, in different blocks, the ISI was either constant at 1,200 ms or variable, between 800 and 1,600 ms with a mean of 1,200 ms (ISI on each trial was drawn from a uniform distribution, and participants were not informed about the different ISIs used in the task). These two independent manipulations produced six distinct block types, and each block type was presented twice. Thus, each subject completed 12 blocks, each comprising 124 trials and lasting approximately 2.5 min. Self-paced rest breaks were given between blocks.

The flickering frequency of the rectangle and the ISI were presented in pseudorandomized order with the restriction that two consecutive blocks could not have the same flicker frequency. Targets were presented in 50% of the trials

FIGURE 1 Stimulus features and time line of a trial sequence implemented to evaluate temporal attention. (a) Individual features (e.g., spatial dimensions and flickering rate) of the stimuli presented on the screen. (b) Individual time line presentation of one trial. Note that flickering rate of the frame, ISI, and presentation of the target and nontarget were counterbalanced. (c) Topographical maps and power spectra from Participant 17 as illustration. Top: RESS topographical maps for the frequencies used in our experiment showing higher SNR. Bottom: corresponding power spectrum at the corresponding fundamental frequency (6, 10, 15 Hz) and the second harmonic (12, 20, 30 Hz)

on each block. Subjects were verbally and visually instructed and performed a practice block of 124 trials before starting the actual experimental session.

The color-changing titration procedure was calculated based on target-present trials performance.

2.3 | Behavioral data analysis

Trials with RTs faster than 150 ms or slower than 2.5 standard deviations of the mean per condition and all error trials were excluded from the behavioral analysis. The statistical analyses of the 12 obtained conditions were performed using the statistical software SPSS 22.0 (SPSS, Chicago, IL). The analyses for differences between conditions were tested using repeated measures analyses of variance (ANOVA) with factors frequency, ISI, and target, with the corresponding values 6, 10, and 15 Hz, constant and variable, and target present and target absent, respectively. Behavioral responses for accuracy were classified as hits, misses, or false alarms. Hit responses were defined as the response within the defined time window for RTs and when the target was displayed. Responses given within the time window and reported as a target when it was not displayed were defined as false alarms. We primarily analyzed d' , a signal-detection theory measure of discriminability. d' was quantified as $z(p_h) - z(p_f)$, where p_h and p_f are the probabilities of hits and false alarms, and $z()$ indicates the conversion from probability to standard deviation unit.

When appropriate, to compensate in case of violation of sphericity, significance levels were adjusted using the Greenhouse-Geisser correction when performing repeated

measures ANOVA, and all the effect sizes are reported as partial eta-squared (η_p^2) or Cohen's d .

2.4 | EEG recordings and preprocessing

The EEG data were recorded with a sampling rate of 1024 Hz from 64 scalp electrodes. Two additional electrodes were placed in the outer canthi of each eye to measure horizontal eye movements. All data were collected using a BioSemi ActiveTwo system (see www.biosemi.com for details). Data were preprocessed with the EEGLAB toolbox for MATLAB (Delorme & Makeig, 2004).

The data were high-pass filtered offline at 0.5 Hz and rereferenced offline to the scalp average. After baseline subtraction (-200 to 0 ms prestimulus baseline), trials with muscle and blinking artifacts during the stimulus were visually identified and manually removed (average of 9%). Then, additional artifacts or any other kind of noise not related to brain activity were removed using independent component analysis in EEGLAB (Delorme & Makeig, 2004). Additionally, although the EEG analysis was performed using the RESS filter, we initially applied the Laplacian transform in all the data in order to improve the spatial selectivity of the data (Cohen, 2014). However, the Laplacian transform has minimal effect in the SSVEP SNR (Cohen & Gulbinaite, 2017), and the results did not qualitatively differ with or without the Laplacian. All EEG analyses involved only correct trials.

2.5 | SSVEP analyses

We performed two sets of analyses on the SSVEP data for both the fundamental frequency and the second harmonic

response: static SSVEP, in which the Fourier transform was computed over the time window of interest from -600 to 600 ms, and dynamic SSVEP, in which a narrow band-pass Gaussian filter was applied to inspect time-varying changes in SSVEP amplitude. Whereas the former method provides higher frequency resolution and increases SNR characteristics, the latter method provides higher temporal precision and thus the ability to detect time-varying changes in SSVEP amplitude resulting from phasic task events. The time windows for analysis were selected after visual inspection of pilot data, and were applied equally to all conditions.

SSVEP was extracted using a spatial filtering method termed rhythmic entrainment source separation (RESS; Cohen & Gulbinaite, 2017). The basic assumption is to create linear spatial filters that then multiply the EEG electrode time series data to produce a single RESS component per frequency. These components are then analyzed instead of analyzing the data from each electrode. The RESS method involves using generalized eigendecomposition to construct an optimal spatial filter that maximizes the SSVEP effect. The eigenvector with the largest eigenvalue maximally differentiates two covariance matrices (a “signal” and a “reference” covariance matrix), and that vector is used as channel weights to combine data from all electrodes. The signal covariance matrix was obtained from the narrowband filtered data at each stimulus frequency and its second harmonics. The reference covariance matrix was obtained from data filtered at the closely spaced up- and down-neighbor frequencies. Constructing the reference matrix in this way provides accurate SSVEP time course reconstruction. Similarly, because neural responses are topographically and anatomically different compared to responses at the fundamental frequency, implementing RESS to separate SSVEP harmonics reduces possible confounding effects induced due to muscle artifacts, particularly at temporal and frontal electrodes. More details about temporal filtering and the RESS method are presented in Cohen and Gulbinaite (2017). Importantly, the spatial filters were computed per frequency pooling data across all conditions separately for each participant; thus, although the spatial filter maximizes the overall SSVEP amplitude, there is no potential for biasing any condition comparisons. The power spectrum was computed via the fast Fourier transform (FFT) for each flicker condition (6, 10, and 15 Hz) pooling over ISI and target conditions, in the time window from -600 ms to 600 ms peristimulus onset. First, we computed the FFT separately for each trial, and then averaged the power spectra over trials per condition. The static SSVEP was computed as SNR as the ratio of the power at the flickered frequency to the average of neighboring frequencies surrounding each frequency (± 2 Hz, excluding a 0.5 Hz window around each center frequency) allowing us to convert power spectrum into SNR units to facilitate comparability across groups (Figure 1c). Additionally, we computed

the SNR per condition to look for differences between the ISIc and ISIV conditions. The SNR was computed for correct trials pooled over target conditions and defined by frequency and ISI with the corresponding values of 6, 10, and 15 Hz and constant and variable ISI, respectively, using a sliding trial window that included 18 trials on either side of the current trials, thus having a running average of 37 trials. This trial series was then interpolated to 200 units in order to average across subjects with different trial counts.

For the computation of the dynamic SSVEP amplitudes, a narrow-band Gaussian filter was implemented for each of the flicker conditions (6, 10, and 15 Hz) with a full width at half-maximum (FWHM) of 3 Hz (the standard deviation of the filter). This Gaussian was pointwise multiplied by the power spectrum of the EEG data, and the inverse Fourier transform was applied to reconstruct the time course of the activity (i.e., convolution). After filtering the data, the amplitude of the frequencies of interest was extracted as the squared magnitude of the result of the Hilbert transform. We call this time series the “SSVEP time series.” To facilitate comparisons across subjects, the SSVEP time series was baseline-normalized to a prestimulus period of -600 to -200 ms, separately for each frequency. Because in our task the flicker stimulus was present all the time, including the peristimulus period, the baseline was computed based on the average of all conditions within each frequency, thus allowing for detection of possible condition differences in prestimulus activity. Baseline normalization allows rescaling the activity relative to the selected baseline, which facilitates comparison between frequencies (Cohen, 2014).

Our analyses were focused on the static (e.g., SNR) and dynamic (e.g., time-varying changes in amplitude) components of the SSVEP. Because the target onset was nonphase-locked with respect to the flicker stimulus onsets, it was not possible to compute intertrial phase coherence (ITPC; Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1996). ITPC provides an amplitude-independent measure on how the stimulus EEG response is phase-locked to the stimulus dynamics (Kim et al., 2007). For example, Kim et al. concluded that ITPC showed attentional effects of the SSVEP phase-locking in response to changes in attended stimulus suggesting an increased synchronization of neural population.

2.6 | SSVEP statistical analyses

The analyses for the SNR differences between conditions were tested using repeated measures ANOVA with factors frequency (6 Hz, 10 Hz, 15 Hz), ISI (constant, variable), and target (present, absent). In order to detect time-varying changes in SSVEP amplitude resulting from phasic task events, statistical analyses of the EEG SSVEP time series were implemented for the 12 experimental conditions (flicker, ISI, target) and for the 6 reorganized conditions pooled over target conditions and defined by frequency and

ISI with the corresponding values of 6, 10, and 15 Hz and constant and variable ISI, respectively. Nonparametric permutation testing was used in combination with cluster correction to evaluate condition differences in dynamic SSVEP over time (Maris & Oostenveld, 2007). To create a distribution of null-hypothesis time courses, we computed the difference of ISIC – ISIV for each subject, and then multiplied this condition difference for a random subset of subjects by -1 (which is equivalent to randomizing ISIV – ISIC vs. ISIC – ISIV). This was repeated for 1,000 iterations. Finally, cluster correction was implemented to correct for multiple comparisons over the all-corresponding time points of the selected time window with a threshold value for pixel and cluster levels of $p = .05$.

Post hoc analyses for the second harmonics (12, 20, and 30 Hz) of the flickering stimulation were performed for the SNR and the dynamic SSVEP following the same parameters implemented for the fundamental frequencies.

3 | RESULTS

3.1 | Behavioral results

Table 1 lists all mean RTs and d' values; here, we report only significant ANOVA effects. For RTs, there were significant main effects for the ISI and target factors, such that subjects were faster to respond in blocks with ISIC compared to ISIV ($M = 403.65$ ms and $M = 425.92$ ms; $F(1, 24) = 28.49$, $p < .001$, $\eta_p^2 = .543$), and target-present trials compared with target-absent trials ($M = 406.02$ ms and $M = 423.54$ ms; $F(1, 24) = 14.63$, $p = .001$, $\eta_p^2 = .379$) (Figure 2a). There was no significant main effect of flicker frequency, nor any interactions involving this factor (all $ps > .1$). Furthermore, interactions analysis between ISI and target factors did not reveal significant effects (all $ps > .1$).

Although there was no statistically significant interaction effect involving flicker frequency, we performed an additional post hoc analysis in which we compared RTs on target-present versus target-absent trials for the 10 Hz condition compared to the average of 6 Hz and 15 Hz conditions. Paired samples t test showed no significant effects (all $ps > .1$) between conditions. Because the difference between ISIC and ISIV for the target absent conditions at 15 Hz is smaller than the difference between the 6 and 10 Hz for the ISIC versus ISIV (Figure 2a), we evaluated whether the effect of temporal attention for the 15 Hz conditions was as strong as for the 6 and 10 Hz. Therefore, we compared RTs on target-present versus target-absent trials for the average of the 6 and 10 Hz conditions compared to the 15 Hz condition. Paired samples t test showed no significant effects (all $ps > .1$) between conditions (see Table 1).

For all conditions, accuracy was higher than 80%. Our analyses focused on d' because it is more sensitive for measuring discrimination performance compared to accuracy (statistics for

accuracy are shown in Table 1). For all the flicker frequencies, trials with ISIC showed higher d' as compared with trials with ISIV (Figure 2b). A significant effect was found for ISI, meaning that participants were more accurate at detecting targets in trials with ISIC as compared with ISIV trials ($M = 2.26$ and $M = 2.08$; $F(1, 24) = 5.99$, $p = .022$, $\eta_p^2 = .200$). Results for frequency flicker effect and the interaction between frequency and ISI did not reveal significant effects ($p > .1$).

Follow-up t tests (Table 1, bottom cell) revealed higher d' on ISIC and ISIV trials for the 10 Hz condition ($M = .3156$, $SD = .40344$) compared to the average of 6 Hz and 15 Hz conditions (6 and 15 Hz: $M = .1113$, $SD = .42962$; $t(24) = 2.31$, $p = .029$, $d = 0.46$). However, the ISI $\times$ Frequency interaction term in the ANOVA was not significant, so this t test result should be interpreted cautiously.

3.2 | EEG results

3.2.1 | SNR for fundamental frequencies

For all the experimental conditions and all the flicker frequencies, the SNR spectrum exhibited peaks at the SSVEP frequencies as well as several higher harmonics (Jackson & Bolger, 2014), indicating that our experiment was sufficient to elicit robust SSVEP effects. Repeated measures ANOVA (with factors frequency, ISI, and target—see Method) for each of the experimental conditions showed a significant main effect for frequency, $F(2, 48) = 12.06$, $p < .001$, $\eta_p^2 = .335$, and interaction effects for ISI $\times$ Target, $F(1, 24) = 7.24$, $p = .013$, $\eta_p^2 = .232$, and Target $\times$ Frequency, $F(2, 48) = 3.69$, $p = .032$, $\eta_p^2 = .133$. The Frequency $\times$ ISI and ISI $\times$ Target $\times$ Frequency interactions showed significant effects but reported violation of sphericity in the Mauchly's test: $\chi^2(2) = 6.99$, $p = .030$ and $\chi^2(2) = 14.45$, $p = .001$, respectively. Therefore, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity for the ISI $\times$ Frequency ($\epsilon = .792$, $F(1.58, 38.02) = 4.52$, $p = .024$, $\eta_p^2 = .159$) and the ISI $\times$ Target $\times$ Frequency ($\epsilon = .684$, $F(1.38, 32.84) = 3.32$, $p = .045$, $\eta_p^2 = .121$). However, the main effect of ISI was not statistically significant, $F(1, 24) = 0.40$, $p = .533$, $\eta_p^2 = .016$, indicating that there were no generic effects of temporal expectations across all SSVEP frequencies. Figure 3a shows the SNR for the experimental conditions at the fundamental frequency. The bar plots show, first, higher SNRs for 10 Hz and 15 Hz compared to 6 Hz, and second, the lack of a main effect of ISI condition. Figure 3c (left) shows the topographical distribution of the forward model of the RESS spatial filter averaged over all participants, targets, and ISI conditions with maximum response at posterior central electrodes. Online supporting information Table S1 contains all the ANOVA results for the fundamental frequencies.

TABLE 1 Results from all experimental conditions

Frequency (Hz)	ISI ^a	Target ^b	Reaction times (ms)		Accuracy (%)		
			Mean	SD	Mean	SD	
Descriptive statistics							
6	C	P	397.52	71.48	85.18	3.18	
	C	Ab	411.43	65.18	84.68	10.24	
	V	P	414.90	67.44	84.75	3.31	
	V	Ab	436.63	52.01	83.39	11.19	
10	C	P	393.01	64.37	86.78	3.87	
	C	Ab	407.30	57.61	86.41	11.48	
	V	P	420.08	64.05	83.80	3.26	
	V	Ab	441.34	61.00	83.11	10.27	
15	C	P	398.21	68.20	85.35	4.34	
	C	Ab	414.37	53.61	86.23	8.83	
	V	P	412.39	70.62	84.95	3.47	
	V	Ab	430.15	67.13	82.50	11.78	
Variable		<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i> *	
Repeated measures ANOVA for reaction times							
Frequencies		2	154.57	77.28	0.130	.878	
ISI		1	37210.39	37210.39	28.486	.000	
Target		1	23012.00	23012.00	14.63	.001	
Frequencies × ISI		2	3067.69	1533.85	2.367	.105	
Frequencies × Target		2	11.68	5.841	0.015	.985	
ISI × Target		1	559.03	559.03	1.756	.198	
Frequencies × ISI × Target		2	142.40	71.199	0.219	.804	
Repeated measures ANOVA for <i>d'</i>							
Frequencies		2	0.047	0.024	0.307	.737	
ISI		1	1.207	1.207	5.998	.022	
Frequencies × ISI		2	0.388	0.194	2.52	.091	
Pair	Mean	SD	95% CI of the difference	<i>r</i>	<i>t</i>	<i>df</i>	Sig. (2-tailed)
Results of the <i>t</i> test and descriptive post hoc statistics for the reaction times							
All conditions 6 + 15 Hz	.77618	44.23	−17.48, 19.03	.60*	.088*	24	.931
All conditions 10 Hz							
All conditions 6 + 10 Hz	−1.6729	49.21	−21.97 18.64	.60*	-.170*	24	.866
All conditions 15 Hz							
Results of the <i>t</i> test and descriptive post hoc statistics for <i>d'</i>							
All conditions 6 + 15 Hz	.20429	.44045	.022, .386	.44*	2.31*	24	.029
All conditions 10 Hz							

Note. Upper table contains the descriptive statistics for RTs and accuracy for all conditions. Middle tables contain the results of the ANOVA analysis performed for RTs and *d'*, respectively. Bottom tables contain the results of the post hoc *t* test analysis performed for RTs and for *d'*. *SS* = sum of squares; *MS* = mean square.

^aC = constant; V = variable.

^bP = present; Ab = absent.

**p* < .05.

3.2.2 | SNR for the second harmonic

Given the robust harmonic SSVEP power in our data (e.g., Figure 1c), we conducted additional exploratory (post hoc) tests to determine whether the second SSVEP harmonic response might be a better marker of temporal expectations. Results of repeated measures ANOVA (with factors

frequency, ISI, and target—see Method) for the second harmonics (12 Hz, 20 Hz, 30 Hz) showed significant effects for the flicker-frequency factor, $F(2, 48) = 10.1$, $p < .001$, $\eta_p^2 = .296$, and for the interaction between ISI and target factor, $F(1, 24) = 4.93$, $p = .036$, $\eta_p^2 = .170$. We also obtained a marginally significant interaction between ISI and frequency

FIGURE 2 Behavioral results for the reaction times and d' . Error bars represent standard error of the mean. (a) Participants showed faster reaction times for conditions with ISI constant for both target-present and target-absent trials. This effect was similar for conditions at 10 Hz as compared with conditions where the flickering frequencies were either 6 or 15 Hz. (b) d' represents the probability of hits and false alarms converted to standard deviation units

but the Mauchly's test showed violation of sphericity: $\chi^2(2) = .708$, $p = .002$. After correcting the degrees of freedom using Greenhouse-Geisser estimates of sphericity, the effect was not significant ($\epsilon = .708$, $F(1.41, 33.97) = 2.71$,

$p = .097$, $\eta_p^2 = .101$). Again, there was no main effect of ISI condition, $F(1, 24) = 1.47$, $p = .199$, $\eta_p^2 = .068$. Figure 3b shows bar plots of the SNRs for the second harmonic. The bar plots show similar SNR differences between 20 Hz and

FIGURE 3 Average SNRs per experimental condition. (a) SNR values for the fundamental frequencies (6 Hz, 10 Hz, 15 Hz) for target-present (left) and target-absent conditions (right) between ISlc versus ISlv. (b) SNRs obtained from the second harmonics (12 Hz, 20 Hz, 30 Hz) of the flicker stimuli. Arrangement of target and ISI factors in (b) is the same as in (a). (c) Topographical maps of subject average for the SNRs for the fundamental frequencies (left) and the second harmonics (right). Values in the y axis correspond to converting power spectrum to SNR units. Error bars represent standard error of the mean. ** $p < .01$

SNR across Correct trials: sliding window of 37 trials

FIGURE 4 Differences between the ISIC versus ISIV conditions for the SNR values across trials for the 6, 10, and 15 Hz. Values in the y axis correspond to converting power spectrum to SNR units

30 Hz compared to 12 Hz, the lack of a main effect of the ISI condition as observed in Panel A, and higher SNR for the 20 Hz compared to the 12 Hz and 30 Hz. Figure 3c shows the topographical distribution of the forward model of the RESS spatial filter at the second harmonics (right) averaged over all participants, targets, and ISI conditions with maximum response at posterior central electrodes. Supporting information Table S1 contains all the ANOVA results for the second harmonic.

3.2.3 | SNR across trials per condition

To determine whether attention-modulated SSVEP amplitude might have been present only later during the experimental blocks, we computed SNR over sliding trial windows per condition, and averaged early (first third) versus late (last third) of trials in each block. We performed repeated measures ANOVA with factors trial position (early vs. late), ISI (constant vs. variable), and frequency (6, 10, and 15 Hz). Significant main effects were obtained only for the frequency factor, $F(2, 48) = 11.35$, $p < .001$, $\eta_p^2 = .000$. The interaction between Frequency $\times$ ISI showed significant effects but reported violation of sphericity in the Mauchly's test: $\chi^2(2) = 10.82$, $p = .004$; therefore, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = .727$, $F(1.45, 34.90) = 3.45$, $p = .056$, $\eta_p^2 = .126$), producing a nonsignificant effect. However, the main effect of ISI, $F(1, 24) = 0.92$, $p = .764$, trial position, $F(1, 24) = 0.002$, $p = .967$, and Trial Position $\times$ ISI interaction, $F(1, 24) = 1.28$, $p = .268$, were not significant. Figure 4 shows the SNR across trials for each of the flickering conditions for the two ISI factors. For the 6 Hz ISIC condition, the SNR was constantly higher as compared to the ISIV conditions. Similar SNR effects can be observed for the 10 Hz ISIC condition as

compared to the ISIV condition. However, it can be observed that for the 10 Hz flicker there is an inverse effect between the ISIC and ISIV for trials around midblock. And for the 15 Hz, the changes in the SNR are higher for the ISIV as compared with the ISIC.

3.2.4 | Dynamic SSVEP results for fundamental frequencies

To analyze SSVEP changes in amplitude across time, all correct trials were pooled over target condition and separated according to frequency and ISI factors. For the 6 Hz and 10 Hz flickering conditions, a significant change in amplitude was observed for conditions with ISIC as compared with ISIV conditions (all p values $< .05$, cluster-corrected for multiple correlated tests over time points). These effects were observed for the time interval between 68 ms and 205 ms for the 6 Hz and 123 ms to 301 ms after target presentation for the 10 Hz flicker condition. An opposite effect was observed for the 15 Hz flicker condition where trials with ISIV showed positive significant changes in amplitude relative to the baseline as compared with the ISIC trials. Also, this difference in amplitude was significant for the complete analyzed time window ($p < .05$). Figure 5a shows the results of the non-parametric permutation testing that was used in combination with cluster correction to evaluate condition differences in dynamic SSVEP over time for correct trials.

3.2.5 | Dynamic SSVEP results for the second harmonic

As with the static SSVEP, we conducted a post hoc analysis to determine whether the second harmonic might be a more robust indicator of temporal attention. The second harmonic

FIGURE 5 SSVEP time course amplitudes for correct trials pooled over target conditions for correct ISIC versus correct ISIV trials. SSVEP time series were baseline-normalized to a prestimulus period of -600 to -200 ms. SSVEP percentage change amplitudes for (a) the fundamental frequencies (6 Hz, 10 Hz, 15 Hz), and (b) the second harmonic (12 Hz, 20 Hz, 30 Hz). Dotted line at time 0 represents stimulus presentation. Gray areas represent statistically significant differences between ISI-constant and ISI-variable conditions ($p < .05$)

of the 6 Hz flicker (12 Hz) showed increase in the SSVEP amplitude for trials with ISIV as compared with conditions with ISIC. This reversed effect was significant for the time windows between -550 ms and -326 ms before target presentation, and between 226 ms and 547 ms after target presentation ($p < .05$). For the harmonics at 20 Hz and 30 Hz, the change in amplitude was larger for ISIC trials versus ISIV trials. The 20 Hz showed a significant difference for the time window between -142 ms and 390 ms. And for the harmonic at 30 Hz, the difference was significant for the entire time window as observed for the fundamental frequency (15 Hz). However, this effect in the second harmonic (30 Hz) was opposite to the fundamental frequency being higher for conditions with ISIC. Figure 5b shows the results of the dynamic analysis for the second harmonics.

4 | DISCUSSION

4.1 | SSVEP and temporal attention

In this study, we evaluated whether the SSVEP and its temporal dynamics could be used to study temporal attention, and whether the effects of attention modulation were similar for the 6 Hz, 10 Hz, and 15 Hz SSVEP stimuli. We designed an SSVEP-temporal attention task where temporal expectations

were induced by manipulating duration of intervals between ISIs while participants differentially responded between a target and nontarget visual stimulus. We found that the amplitude of the SSVEP was significantly increased for the fundamental frequencies at 6 Hz and 10 Hz in blocks with ISIC compared to ISIV blocks. Similar effects were observed for the second harmonic of the alpha band stimulus (20 Hz) for blocks with ISIC.

To our knowledge, this is the first study evaluating the applicability of SSVEP in temporal attention. The behavioral evidence showed significant improvement for the ISIC versus ISIV condition, which confirmed strong temporal expectations effects in our experiment. The SSVEP effects, however, were not as strong as expected, and we cannot conclude that SSVEP is universally appropriate for EEG studies of temporal attention. On the other hand, several of our results support the use of SSVEP in some cases, particularly in the alpha band range for the fundamental and the second harmonic. Hence, the SSVEP may provide a feasible tool to study temporal attention if used carefully.

It is possible that the effects would have been stronger if the flicker had been presented foveally or were task relevant. On the other hand, the overall SSVEPs had high SNR (e.g., Figure 1, 3), and task relevance is not a prerequisite for attention modulations in spatial and object-based attention tasks

(Müller et al., 2006; Senkowski et al., 2008). Similarly, in our experimental design, we use rhythmic cues to induce temporal expectations. An important avenue for future research is to determine whether more robust effects would be obtained with different methods of inducing temporal expectations (e.g., instructive/predictive cues). Given that different ways of inducing temporal expectations could activate different neurophysiological mechanisms (Rohenkohl et al., 2014), using alternative ways to induce temporal expectancies (e.g., Coull & Nobre, 1998; Cravo, Rohenkohl, Wyart, & Nobre, 2011; de Graaf et al., 2013) in future SSVEP-temporal attention paradigms might improve the effects on SSVEP. Additionally, it is possible that a different design that allows for ITPC analysis may provide additional insights into whether and how SSVEP could be useful for studying temporal attention. Again, although we would not rule out the potential utility of the SSVEP technique for studying temporal attention, such studies may require carefully selected stimulus parameters and experiment design choices.

4.2 | SSVEP and attention

The SSVEP has extensively been used to study sensory (Regan, 1989) and visual processes (Norcia et al., 2015), and particularly it has been used to study attentional processing (e.g., Morgan et al., 1996; Pei, Pettet, & Norcia, 2002). Also, the increased amplitude of the SSVEP for the attended as compared to the unattended conditions has been replicated many times (Andersen & Müller, 2010; Kashiwase et al., 2012; Kim et al., 2007; Morgan et al., 1996; Müller et al., 2006; Saupe et al., 2009). However, these studies have focused on spatial or object attention, and it has thus remained unclear whether SSVEP would also be useful for studying the dynamics of temporal attention. Our results are in line with previous reports in the sense that we found possible temporal attention modulation on SSVEP. Nonetheless, it is important to stress that in our temporal expectations paradigm this effect on SSVEP was not observed for 15 Hz flicker.

Our results also showed changes in the amplitude of the second harmonic for the 20 Hz and 30 Hz, which could be related to the effects of temporal expectancy induced by the ISIc. Nonetheless, changes in amplitude of the second harmonic at 12 Hz were for the ISIV condition. Again, because our study is the first to evaluate the use of the SSVEP to study temporal attention, the inverse effect on the 12 Hz second harmonic should be confirmed in future studies before giving potential explanations related with neural mechanisms of temporal attention. One approach to further evaluate our results would be to examine the ITPC. It has been previously shown that ITPC provides a measure of the phase consistency of EEG responses to stimulus dynamics (Kim et al.,

2007). However, our results for the second harmonic are consistent with previous reports describing the effects of attention modulation of the SSVEP second harmonic (Belmonte, 1998; Müller, Picton et al., 1998; Pei et al., 2002; see Norcia et al., 2015, for a review).

4.3 | Relation to motor processing

Some studies have suggested that behavioral effects could be better explained by motor effects in a temporal attention task (Correa & Nobre, 2008; Davranche et al., 2011). In order to isolate the motor-related activity including any possible effects induced by the response mapping we implemented, we designed a forced-choice perceptual task where the motor response was not requested until stimulus presentation. In this way, the motor effect could be attributed to separate effects presented at the level of the motor cortex (Rohenkohl & Nobre, 2011), confirming the improvement in behavioral performance as the result of temporal expectations induced by rhythmic intervals. This seems relevant because temporal attention involves more processes than motor preparation or holding motor responses (Coull et al., 2000).

4.4 | Conclusions

Part of the interest of determining whether SSVEPs can be useful for studying temporal attention is for potential applications in clinical research, in particular in conditions associated with impairments in orienting attention in time or the use of temporal cues to improve performance, such as Parkinson's disease (Rochester et al., 2005; Tagliati et al., 1996) and schizophrenia (Liotti et al., 1993; Nestor et al., 1992; Silberstein et al., 2000). SSVEPs for temporal attention could also be relevant for studying changes in healthy aging, because changes in temporal attention have also been reported in this population (Zanto et al., 2011; Zanto, Hennisgan, Mattias, Clapp, & Gazzaley, 2010), and SSVEPs for spatial/object attention have already been employed to study changes in attention in aging (see Vialatte et al., 2010, for a review).

Despite the theoretical and practical relevance of using SSVEP to study temporal attention, our results provide some behavioral and EEG evidence to show the feasibility of the SSVEP to study temporal attention and the effects of temporal predictability on SSVEP. However, we must highlight that this was observed in our experiment mainly for the 6 Hz and 10 Hz and not for the 15 Hz flicker frequency. Further research will be required to determine how to optimize stimulus and experiment design parameters for using SSVEP to study temporal attention.

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SUPPORTING INFORMATION

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Table S1

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