

# Influence of continuous flash suppression mask frequency on stimulus visibility

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## **Abstract**

The continuous flash suppression (CFS) paradigm has been increasingly used in consciousness research, but its mechanisms are still not fully understood. To better understand its temporal properties, we presented the CFS masks at 9 frequencies, and examined their influence on stimuli visibility, while taking into account the inter-individual variability and the change of CFS suppression as the experiment progressed. The frequencies consisted of fundamental frequencies of 3, 4 and 5 Hz, and their 2<sup>nd</sup> and 3<sup>rd</sup> harmonics, which included the 10 Hz frequency typically used in most of the CFS studies. We found that the suppression of stimulus awareness was stronger under 4, 6 and 8 Hz than 10 Hz. After controlling the inter-individual variability by mixed-effects analysis, we found that the numbers of seen trials was lower for the 4 Hz-basis frequencies than the 5 Hz ones, and was lower for the 2<sup>nd</sup> than 3<sup>rd</sup> harmonic. We propose that this may be caused by an interaction between the CFS masks and the ongoing sampling of the attentional mechanism. Examining individual data, we also found a time effect that the participants saw significantly more stimuli as the experiment progressed. Our results suggest that these factors need to be taken care of in future CFS studies, to achieve optimal visual awareness suppression and ensure the generalizability of results.

## **Keywords**

Continuous flash suppression, frequency, face perception, body perception, time effect, visual awareness

## Introduction

The study of perception outside awareness has advanced our understandings of brain functions. Studies of brain lesioned-patients uncovered phenomena such as blindsight (Celeghein, de Gelder, & Tamietto, 2015; de Gelder, Vroomen, Pourtois, & Weiskrantz, 1999; Van den Stock, Tamietto, Hervais-Adelman, Pegna, & de Gelder, 2013; Weiskrantz, 1986), agnosia (Farah, 2004), optic ataxia (Jakobson, Archibald, Carey, & Goodale, 1991), leading to the establishment of the two-stream visual processing model (Milner & Goodale, 2006). The blindsight phenomenon was of particular interest, where patients with V1 lesion could still report and react above chance to visual stimuli, without being conscious of the visual stimuli being present. Given the theoretical importance of vision without consciousness and because blindsight patients are rare, efforts have been made to establish and study similar phenomena in neurologically intact participants.

Continuous flash suppression (CFS) has been a prime candidate paradigm for such purposes (Tsuchiya & Koch, 2005). CFS utilizes dichoptic presentation of stimuli, and can successfully suppress visual awareness of a static lower-contrast target stimulus in one eye for up to several seconds, by presenting a dynamic and high-contrast flashing mask in the other eye (Tsuchiya, Koch, Gilroy, & Blake, 2006). Despite the potent suppression effect, some information of the stimuli could still transpire to higher visual areas. Because of this blindsight-like property, CFS has been increasingly used as a tool for consciousness research, to study both the non-conscious processing of simple stimuli like checkerboards and Gabor patches, as well as more complex stimuli like faces and words, which could also contain emotional or semantic contents in addition to the visual form (e.g. (Costello, Jiang, Baartman, McGlennen, & He, 2009; Jiang & He, 2006; Yang, Zald, & Blake, 2007).

Despite increasingly wide applications of the CFS paradigm in consciousness research, there are concerns regarding the generalizability of the results obtained from this paradigm. One concern relates to the fact that its suppression mechanisms are still not fully understood. Investigations on the spatial domain of both the stimuli and the mask pattern have demonstrated that low-level properties such as contrast can influence whether a stimulus is perceived by the participant (Gray, Adams, Hedger, Newton, & Garner, 2013; Yang, et al., 2007). At the same time, the strength of suppression is related to the spatial frequencies of the dynamic mask pattern as well as that of the stimulus (Stein, Seymour, Hebart, & Sterzer, 2014; Yang & Blake, 2012). However, so far investigations on the temporal dynamics are still scarce, especially concerning the temporal frequencies of the dynamic mask pattern.

In the influential article that established the CFS paradigm (Tsuchiya & Koch, 2005), the authors used a 10 Hz-flash frequency of the dynamic mask pattern. Their choice was based on the observation in a separate test with 4 participants naïve to the paradigm, that the optimal suppression length was obtained with a flash frequency of ~3-12 Hz (the 10 tested frequencies ranged from 0.78 to 100 Hz). So far most published CFS studies have used this flash frequency, following their example.

Three recent studies investigated the influence of flash frequency on the visual awareness of stimuli, spanning different frequency ranges and measuring different dependent variables (Han, Lunghi, & Alais, 2016; Kaunitz, Fracasso, Skujevskis, & Melcher, 2014; Zhu, Drewes, & Melcher, 2016). Kaunitz et al. tested 5 frequency levels (5.3, 8.5, 10.6, 16.6 and 28.5 Hz) on the visibility of transiently presented checkerboard targets, and found a general decrease of seen trials as the frequency increased (Kaunitz, et al., 2014). Zhu et al. used more complex stimuli of faces and houses, in addition to simple symbols, and measured the break-through contrasts of the stimuli at 10 frequency levels of the mask (0, 1, 3, 5, 7, 10, 13, 16, 20, 32Hz). They found that the stimuli contrast showed a skewed normal curve across frequencies, peaking around 6 Hz (higher suppression effect there) (Zhu, et al., 2016). Han et al. did not examine individual flash frequencies directly; instead they used a temporal filter on the noise masks (0.375, 0.75, 1.5, 3, 6.25, 12.5, 25 Hz) and examined the suppression duration for 4 images of natural stimuli. They found the suppression duration peaked at very low frequencies around 1 Hz, although the contrast sensitivity curve across frequencies did not show the same pattern, which peaked at 6.25 Hz (Han, et al., 2016). These studies did not optimally sample the frequency range found by Tsuchiya & Koch (2005), and did not reach conclusions about a consistent frequency range needed for strong suppression.

Another concern about the generalizability of results obtained with the CFS paradigm relates to the substantial inter-individual variability in suppression time observed by recent studies, not only for simple stimuli (Yamashiro, et al., 2014), but also for complex stimuli varying on social dimensions (Getov, Kanai, Bahrami, & Rees, 2015).

To better understand the properties of the CFS paradigm, in the current study, we examined the effect of flash frequencies on the visibility of stimuli, while taking into account the possible inter-individual variability. We chose 9 frequency levels from 3-15 Hz on two objectives. This allowed us to better sample the optimal frequency range around 3-12 Hz found by Tsuchiya & Koch (2005), also including the routinely used 10 Hz frequency. It also allowed us to test hypotheses on the relationships between stimuli visibility and the frequencies. We hypothesized 3 possible relationships: 1) monotonic, meaning that the stimulus visibility may increase (or decrease) while the flash frequency increases; 2) quadratic, meaning that the stimulus visibility may peak in the mid-range frequencies, while being low at both very low and very high frequencies (or the other way around); 3) stimulus visibility may be related to the fundamental frequencies of the flash masks, and their 2<sup>nd</sup> and 3<sup>rd</sup> harmonics. To be able to test all 3 hypotheses, we chose fundamental frequencies of 3, 4, and 5 Hz, and their 2<sup>nd</sup> and 3<sup>rd</sup> harmonics (6, 8, 10 Hz and 9.23, 12, 15 Hz respectively). The 9.23 Hz (approximation of 9 Hz) was due to LCD monitor refresh-rate limits. For the target stimuli, we used 10 whole body images displaying neutral actions to maximize stimuli relevance, while avoiding ceiling/floor effects. We performed mixed-effects analysis to examine and control inter-individual variability, and we performed correlation analysis on individual data to examine the confounding time effect of experiment progression on stimulus visibility.

## **Materials and methods**

### **Participants**

Fifty-five female participants (age range 17-28) were recruited from the campus of Maastricht University and took part in the study. Most of them were naïve to the CFS paradigm. We tested female participants only, because this whole session of the current study served as a screening test for another experiment not reported here, for which previous research has reported gender differences. Participants all had normal or corrected-to-normal visual acuity, normal stereo color vision, and no history of neurological disorders. They gave written consent before participation, and received either monetary rewards or course credits after participation. The experiment was approved by the ethical committee of Maastricht University, and was carried out in accordance to the Declaration of Helsinki.

### **Stimuli**

Gray-scale images of neutral faces and bodies were used. For the face stimuli, 10 identities (5 females) were chosen from the Radboud Face Database (Langner, et al., 2010), aligned at the eye level. For the body stimuli, 10 identities (males only) displaying an action of talking on the phone were chosen from the set developed by Stienen and de Gelder (2011), aligned at the feet level, with facial information removed. The face and body stimuli spanned visual angles of  $2.83^\circ \times 2.16^\circ$  and  $4.43^\circ \times 1.88^\circ$  respectively, and were embedded in a gray rectangle background (240 × 160 pixels, visual angle  $6.73^\circ \times 4.48^\circ$ , RGB value 128,128,128). These stimuli were a subset of the stimuli used in a previous CFS study, where we found that the suppression time for the face stimuli were shorter than the body stimuli (Zhan, Hortensius, & de Gelder, 2015). In the current study, the face stimuli were used for determining the eye dominance for each participant, to facilitate break from suppression and to have an adequate number of seen trials in a relatively short test. We then used the body stimuli in the main experiment of flash frequencies, to diminish possible ceiling effects of “seen”.

Six-hundred unique colored mask patterns were constructed, by randomly drawing small rectangles of different colors (the heights and widths were within  $2^\circ$  visual angles) in the area of 240×160 pixels. In each trial of the experiments, the dynamic mask patterns were randomly drawn from this pool without replacement.

### **Dichoptic presentation**

The stimuli and the dynamic mask patterns were presented in Matlab R2013b (the MathWorks, Natick, MA, USA) with Psychtoolbox (Brainard, 1997; Pelli, 1997), on an LCD screen (Acer VG248, resolution = 1920×1080, refresh rate=120 Hz). To aid the free-fuse of the dichoptic views for the participants, two black rectangle frames (240×160 pixels, 10 pixels thick) were projected side by side in the center of the screen, 254 pixels apart from each other. A white fixation cross was

presented at the center of each rectangle frame. In each trial, the dynamic mask pattern and the stimulus were projected separately into one rectangle frame. Participants viewed the stimuli on the screen through a pair of prism glasses (diopter=7) (Schurger, 2009) while resting their chin in a chinrest, with a viewing distance of about 57 cm. A cardboard was placed between the screen and the participant, dividing the screen into two equal halves, so that each eye of the participant saw one half of the screen without crosstalk. Participants were asked to free-fuse the two views in one stable rectangle box, without drifting apart. For participants who could not free-fuse the views with the glasses of diopter=7, glasses of diopter=5 were used instead.

## **Procedure**

The experimental session consisted of an eye-dominance test of 6 min, and the main experiment lasting 49 to 58 min (depending on response times of the participant, and the self-paced resting periods between experimental blocks). Both tests started after a stable free-fusing of two views was established. Participants were instructed to keep fixation on the fixation cross throughout the whole experimental session, keeping their head as still as possible, and not to blink during stimulus presentation if possible. They reported their subjective awareness of the stimuli in both tests by pressing the 1 (seen) and 2 (unseen) keys on the keyboard with the left hand. They were instructed beforehand that they should respond “seen” as long as they saw some part of the stimulus during the presentation of the mask patterns. For trials in both tests, the response window was 2 s, and the inter-trial-interval was 1 s.

In the eye dominance test, neutral faces of 10 identities (half female) were presented to the participants under CFS, with the dynamic mask pattern flashing at 10 Hz. Each stimulus image was presented to each eye 3 times, resulting in a total of 60 trials. The order of the stimuli presentation and the eye the stimuli were projected to were both randomized. For each trial, the face stimulus was faded in from 0% contrast to full contrast in 1.5 s, maintained at full contrast for 1 s, and then faded out to 0% contrast in 0.5 s. The full contrasts of the faces were to facilitate the breaking from suppression during stimuli presentation, in order to have an adequate number of seen trials for each eye. The numbers of seen trials per eye were counted, and the eye with the higher number of seen trials was assigned as the dominant eye for that participant. When the numbers of seen trials were equal between both eyes, the right eye was assigned as the dominant eye (this was the case for 6 participants, 3 of whom were included in further analysis).

In the main experiment, neutral body stimuli of 10 males were presented to the participants' non-dominant eye under CFS (19 into the left eye and 18 into the right eye), while the dynamic mask varied in 9 different frequencies: 3, 4, 5, 6, 8, 9.23, 10, 12, 15 Hz respectively. The 9.23 Hz condition was limited by the LCD screen's refresh rate, during which each dynamic mask pattern stayed on the screen for 13 frames. Each stimulus was presented 4 times per flash frequency, resulting in a total of 360 trials. The order for both the body stimuli and the frequencies was randomized across the whole experiment, and the trials were then split in to 6 blocks after randomization. To avoid a ceiling effect of “seen” responses, for each trial, the body stimulus was faded in from 0% to 50% contrast in 1.5 s, stayed at 50% contrast for 1 s, and faded out to 0%

in 1.5s. The dynamic mask was presented for another 1 s after stimuli offset, to avoid perception of stimulus afterimages. In total the dynamic mask was kept on screen for 5 s per trial.

## Data analyses

The data of 37 participants (mean age=20.16, SD=1.91, range 17-28) were included in the subsequent analyses. For the participants whose data were excluded from analysis, 2 participants did not complete the main experiment due to not being able to maintain the merging of the two boxes. The data of the other 16 participants were excluded for ceiling or floor effects based on these criteria: 2 participants missed responses for more than 10% of all trials (36 trials), 12 participants responded “seen” for more than 90% of the trials (324 trials), and 2 participants responded “unseen” for more than 90% of the trials (324 trials).

For the data of the main experiment, the numbers of seen trials per flash frequency were counted, and then centered by the average number seen trials across frequencies within each participant to obtain normal distributions. We performed two repeated-measures ANOVAs in SPSS with the centered number of seen trials. The first ANOVA had one factor “flash frequency” with 9 levels; the second ANOVA had the factor “fundamental frequency” (3, 4, 5 Hz), and the factor “harmonic levels” (fundamental frequency, their 2<sup>nd</sup> and 3<sup>rd</sup> harmonics). The Greenhouse-Geisser correction was used when sphericity was violated. For both ANOVAs, putting the “dominant eye” (the eye that the stimuli were projected into) as a between-subject factor was not significant,  $F(1,35)=3.014$ ,  $p=.091$ ,  $\eta_p^2=.079$ , neither did it show an interaction with the fixed effects (ANOVA 1:  $F(5.086,178.024)=0.634$ ,  $p=.676$ ,  $\eta_p^2=.018$ ; ANOVA 2, fundamental frequency  $\times$  dominant eye:  $F(1.653, 57.841)=0.367$ ,  $p=.694$ ,  $\eta_p^2=.010$ , frequency level  $\times$  dominant eye:  $F(2,70)=1.715$ ,  $p=.188$ ,  $\eta_p^2=.047$ ), thus the factor “dominant eye” was removed from subsequent analyses.

Because we observed a considerable amount of inter-individual variability across the frequencies, we then performed mixed effects analyses, in order to examine 1) the influence of the variation between participants (random effect factor: *subj*) on the centered count of seen trials (dependent variable *count*), and 2) whether the fundamental frequency plus harmonics model (fixed effect factors: *freqfund*, *freqhar*) better described the data, comparing to simply grouping 9 frequencies into low (3, 4, 5 Hz), middle (6, 8, 9.23 Hz), and high (10, 12, 15 Hz) frequencies without considering the fundamental frequency (fixed effect factor: *freqlv*). The grouping of frequencies into low/middle/high levels was done to enable the estimation of the random effect. The analysis was performed in R, with the packages **lme4**, **lsmeans**, **pbkrtest**. The models were fitted with R’s default function **lm**, and the function **lmer** in **lme4**. The comparisons between models were performed as likelihood ratio tests between a full model and a reduced model removing the factor in question, with the likelihood ratio tests performed by function **anova** in **lme4**. The pairwise comparisons between levels of fixed effects after finding the best model justified by the data were performed with the function **lsmeans** and **pbkrtest**, with the Tukey method for multiple comparison adjustments.

Apart from the inter-individual differences, the stimulus visibility may also change as the experiment progressed, and may also result from different responses across different stimuli. To further understand the role of these factors, we examined the Kendall's tau-b correlations between the outcome visibility, with the trial orders (from 1 to 360) to represent the time effect, the flash frequencies, and the 10 stimuli identities. The correlation analyses were performed in MATLAB R2016a (the MathWorks, Natick, MA, USA), with the function `corr`. Within the data of each individual participant, the no-response trials were removed before performing the correlation. To validate the time effect, we compared the number of seen trials between the first block and the last (6<sup>th</sup>) block, with the Friedman test. We also performed the same correlation analysis in the eye-dominance test data, correlating the visibility of each trial with the trial order (from 1 to 60), the face stimuli identities, and the eyes the stimuli were projected to. The resulting tau-b coefficients across all participants were then compared to 0 with one-sample t test (two-tailed, FDR corrected) to determine whether the correlations were significant for the group, and the comparisons of correlations between each other were performed by paired-samples t tests (two-tailed). In addition, we computed the eye dominance bias scores for individual participants, by dividing the absolute difference of seen trials between left and right eyes with their sum ( $|L-R|/(L+R)$ ). The score would be 0 for perfectly balanced dominance (30 seen trials for each eye), and would be 1 for the most unbalanced dominance (30 seen trials for one eye and 0 seen trials for the other).

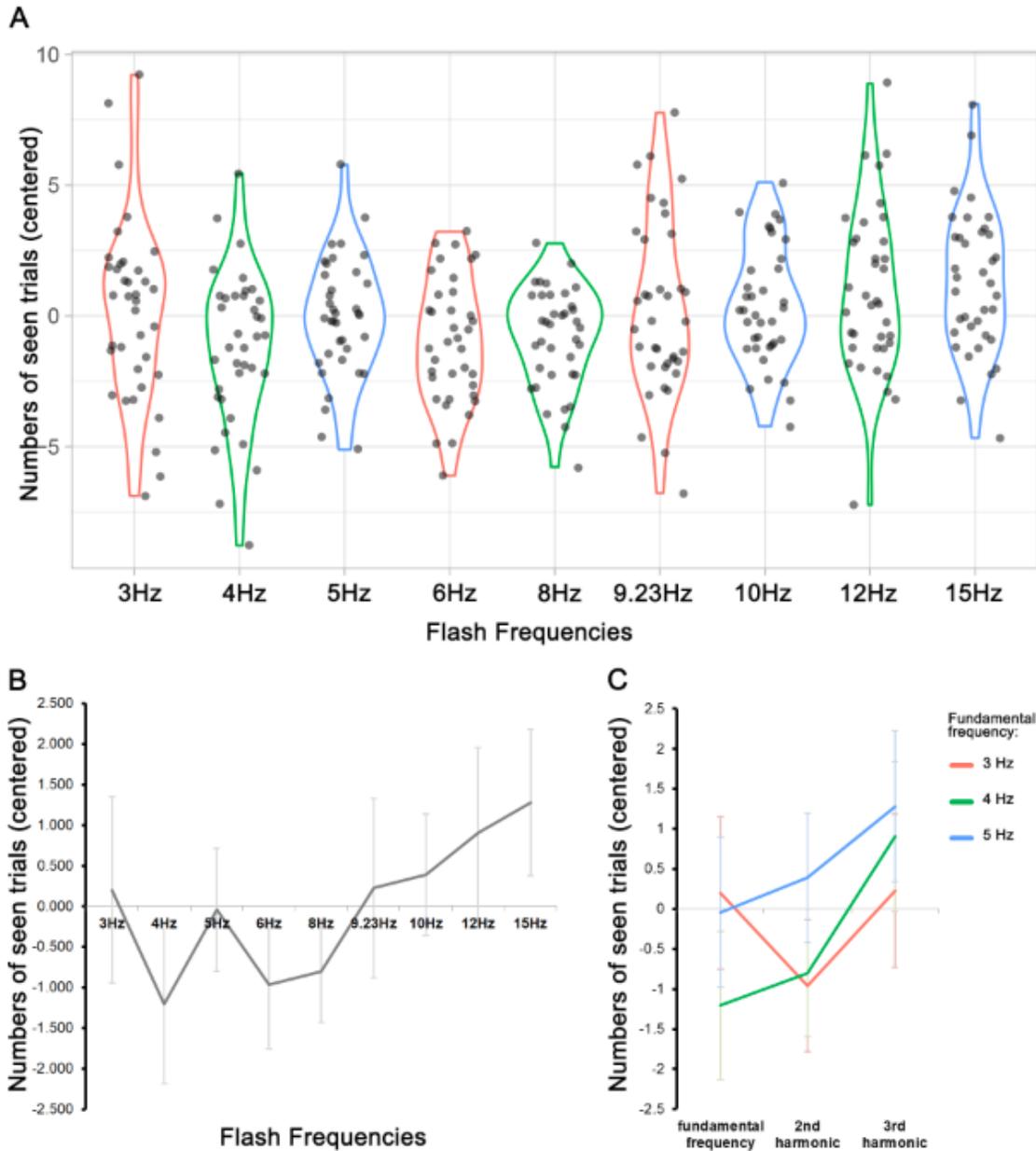
## Results

### The effect of flash frequencies

#### Repeated-measures ANOVAs

The repeated-measures ANOVA of 9 flash frequencies showed a significant main effect of frequency,  $F(5.164, 185.914) = 3.095$ ,  $p = .010$ ,  $\eta_p^2 = .079$ , indicating that the average number of seen trials was different across frequencies. The test of polynomial contrasts across the 9 frequencies showed a significant linear trend,  $F(1, 36) = 4.725$ ,  $p = .036$ ,  $\eta_p^2 = .116$ , a significant quadratic trend,  $F(1, 36) = 5.902$ ,  $p = .020$ ,  $\eta_p^2 = .141$ , and a significant 6<sup>th</sup> order trend,  $F(1, 36) = 4.427$ ,  $p = .042$ ,  $\eta_p^2 = .110$ . See **Figure 1B**. Contrasting the 8 frequency levels to the routinely used frequency of 10 Hz, the numbers of seen trials under 4, 6 and 8 Hz were significantly fewer than that of 10 Hz (4Hz:  $F(1, 36) = 6.148$ ,  $p = .018$ ,  $\eta_p^2 = .146$ ; 6Hz:  $F(1, 36) = 6.262$ ,  $p = .017$ ,  $\eta_p^2 = .148$ ; 8Hz:  $F(1, 36) = 7.426$ ,  $p = .010$ ,  $\eta_p^2 = .171$ , FDR corrected), indicating that 10 Hz was not the optimal frequency to induce stronger suppression.

When sorting the flash frequencies by the fundamental frequencies (3, 4, 5 Hz) and their 2<sup>nd</sup> and 3<sup>rd</sup> harmonics, the repeated-measures ANOVA showed a significant main effect of fundamental frequency,  $F(1.663, 59.878) = 3.812$ ,  $p = .035$ ,  $\eta_p^2 = .096$ , and a significant main effect of harmonic levels,  $F(2, 72) = 4.077$ ,  $p = .021$ ,  $\eta_p^2 = .102$ . Their interaction was not significant,  $F(3.337, 120.127) = 1.899$ ,  $p = .127$ ,  $\eta_p^2 = .050$ . Both main effects showed quadratic trends,  $F(1, 36) = 4.427$ ,  $p = .042$ ,  $\eta_p^2 = .110$ ,  $F(1, 36) = 4.310$ ,  $p = .045$ ,  $\eta_p^2 = .107$ .



**Figure 1** Numbers of seen trials per flash frequency, centered within each participant. **A.** Raw data. The individual dots in each frequency represent numbers of seen trials of individual participants, jittered on the x axis for visualization purpose. **B.** The average number of seen trials per frequency. Error bars denote 95% confidence intervals. **C.** The average number of seen trials plotted by harmonic levels for each fundamental frequency. Error bars denote 95% confidence intervals estimated with mixed-effects analysis, after accounting for random effects of participants. See **C** for color codes of the harmonic levels.

## Mixed effects analysis

In the mixed effects analysis, we constructed and compared models with different fixed and random effects. The fixed effects were to compare the fundamental flash frequencies, their harmonics and the interaction (fixed effect factors: *freqfund*, *freqhar*) with the frequencies grouped into low/middle/high frequency levels (fixed effect factor: *freqlv*). The random effects were to examine whether there were significant interactions of fixed effects of flash frequencies with the individual participants (random effect factor: *subj*). The interaction terms included simple scalar terms ( $1|freqlv:subj$ ) and ( $1|freqfund:subj$ ), ( $1|freqhar:subj$ ), and more complex terms that included a random slope for every participant: ( $0+freqlv|subj$ ), ( $0+freqfund|subj$ ), ( $0+freqhar|subj$ ). The count of seen trials centered within each participant served as the data input. Because of the centering, we did not include a random intercept for each participant (the term  $1|subj$ ) in our models. See supplementary material for all the models examined, and the likelihood tests between them.

The final model justified by the data consisted of the fixed effects of the fundamental frequencies, their harmonics and the interaction between them, and random slopes of each participant for both fixed effects (m17 in supplementary material). Comparing the full model to the one with fixed effect only showed significant random effects (m17 compared to m03),  $\chi^2(12)=38.394$ ,  $p=.0001324$ , which was the inter-individual variability. Leaving out the fixed effects one by one from the full model showed that both the main effects of fundamental frequencies ( $\chi^2(2)=8.9156$ ,  $p=.01159$ ), their harmonics ( $\chi^2(2)=7.8124$ ,  $p=.02012$ ) and the interaction between them ( $\chi^2(4)=9.6626$ ,  $p=.04651$ ) were all significant. See **Figure 1C**.

Pairwise comparisons between the fundamental frequencies showed that the 4Hz frequencies had lower numbers of seen trials than the 5Hz frequencies,  $t(36)=-2.909$ ,  $p=.0166$ . Pairwise comparisons between the frequency harmonics showed a lower number of seen trials at the 2<sup>nd</sup> harmonic than the 3<sup>rd</sup>,  $t(36)=-2.794$ ,  $p=.0220$ .

## The time effect

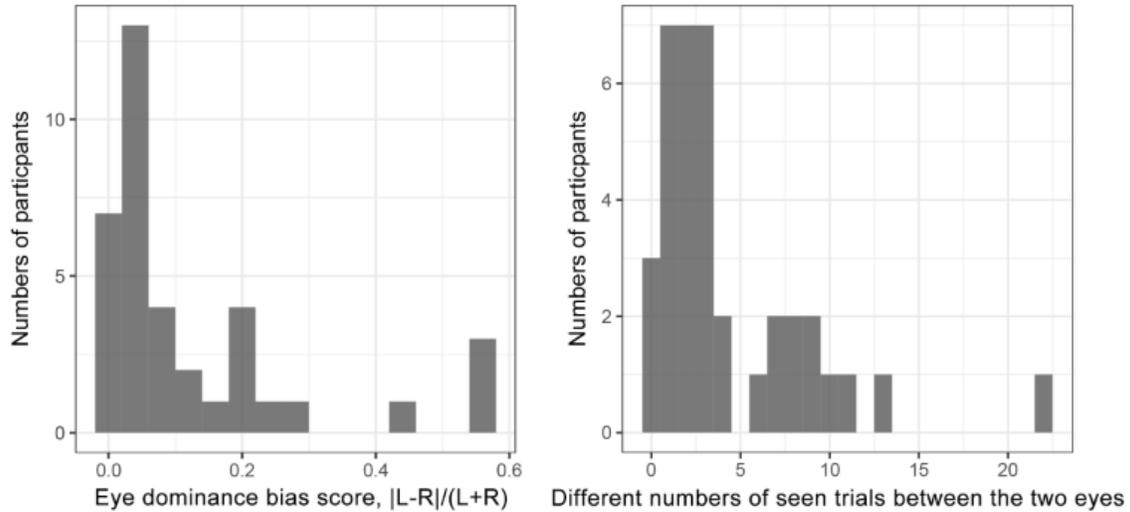
To examine the time effect that the stimulus visibility change during the progression of the experiment, a possible influence on individual differences of stimulus visibility, we performed the Kendall's tau-b correlations between visibility per trial, the flash frequencies, and the body stimuli identities in individual datasets of the main experiment. At the group level, the trial order was significantly correlated with stimulus visibility, mean tau-b coefficient=0.211,  $t(36)=9.765$ ,  $p=1.17\times 10^{-11}$ . The flash frequency was also significantly correlated with stimulus visibility, mean tau-b coefficient=0.027,  $t(36)=2.189$ ,  $p=.035$ , although the coefficients were one order of magnitude smaller than that of the trial order effect,  $t(36)=8.679$ ,  $p=2.37\times 10^{-10}$ . The correlation of stimulus ID with the stimulus visibility showed a trend to significance with small coefficients, mean tau-b coefficient=0.015,  $t(36)=1.866$ ,  $p=.070$ .

As a reference for the magnitude of correlation, we additionally examined the correlation of trial order to the response time (RT) for each trial. Although participants were not required to respond as fast as possible, the RT was negatively correlated with the trial order, mean tau-b coefficient=-0.142,  $t(36)=-9.843$ ,  $p=9.48 \times 10^{-12}$ , reflecting increasingly faster RT as the experiment progressed. This effect was not correlated with the trial order effect on visibility though, as the second-level correlation of these two sets of tau-b coefficients was not significant, second-level tau-b coefficient=-0.009,  $p=.9482$ , showing that responding more “seen” trials was not directly related to responding faster.

To confirm the time effect on visibility, we compared the numbers of seen trials between the first and the last (6<sup>th</sup>) block of the main experiment. The Friedman test showed that the numbers of seen trials was significantly higher for the last block than the first block,  $\chi^2(1)=25.00$ ,  $p=5.73 \times 10^{-7}$ . Out of 37 participants that were included in the analysis, 33 saw more trials in the last block compared to the first one.

To check whether this time effect was already present in the eye dominance test before the main experiment, we performed the Kendall’s tau-b correlation on the eye dominance test data. The trial order was again significantly correlated with the stimulus visibility, mean tau-b coefficient=0.122,  $t(36)=4.152$ ,  $p=1.93 \times 10^{-4}$ . The face stimuli identities were also significantly correlated with the stimulus visibility, mean tau-b coefficient=0.057,  $t(36)=3.203$ ,  $p=.003$ , not significantly smaller than the trial order effect,  $t(36)=1.762$ ,  $p=.087$ . The left or right eye that the stimuli were presented into were not significantly correlated with the stimulus visibility, mean tau-b coefficient=0.014,  $t(36)=0.360$ ,  $p=.72$ .

We additionally computed the bias of the eye dominance for individual participants, which would range from 0 for perfectly balanced eye dominance, to 1 for completely unbalanced eye dominance. The mean bias score for the 37 participants was 0.130 (SD=0.164), but showed substantial variability, ranging from 0 to 0.578, with the majority having low bias scores. See **Figure 2**.



**Figure 2 A.** Distribution of the eye dominance bias scores across individual participants. **B.** Distribution of the numbers of seen trials difference between eyes.

## Discussion

In this study we examined the effect of CFS mask flash frequency on stimulus visibility, using 9 different frequencies ranging from 3 to 15 Hz, including 10 Hz, which is currently the most commonly used frequency in CFS experiments. We found the number of seen trials differed across the frequencies. Additionally, we observed considerable inter-individual variability across frequencies. Controlling for this inter-individual variability using mixed-effects analysis, we found that the data were better described by a model of fundamental frequencies (3, 4, 5 Hz) and their harmonics. Both the fundamental frequencies, their harmonics and their interaction were significant, showing that the number of seen trials was lower for the 4Hz frequencies compared to the 5 Hz frequencies, and lower for the 2<sup>nd</sup> than the 3<sup>rd</sup> harmonic. Examining correlations in individual data revealed that a time effect of the experiment had considerable influence on stimulus visibility: most of the participants progressively saw more trials during the course of the experiment. This time effect was already present in the short eye-dominance test before the main experiment.

### The effect of flash frequencies on stimulus visibility

We found that frequencies of 4, 6 and 8 Hz showed stronger suppression than 10 Hz in the current sample of participants. We set out to better sample the optimal frequency range ~3-12 Hz suggested by Tsuchiya & Koch (2005), which corresponded to 3.125, 6.25 and 12.5 Hz in their data. Comparing to the other studies on flash frequencies, our result is in agreement with Zhu et al. (2016), where they found the stimulus contrast to break from suppression peaked at around 6 Hz (5 & 7 Hz in their data).

The 3 frequencies found as optimal in the current study might not be trivial, and could have functional importance for visual processing. Accumulating evidence from recent studies suggests that spatial attention is employed in the frequency range of ~7 Hz, and this attentional resource may be divided into lower frequencies if multiple targets are present (for reviews see (Fries, 2015; VanRullen, 2016; Vanrullen & Dubois, 2011). Two studies with covert spatial attention were of special interest. One study examined the visual target detection with varying target onset to the visual cue, on two objects with 3 possible locations: one was the cued location, another was on the same object of the cued location, a third was on the non-cued object. They found periodicity in the detection performance at 8 Hz on the cued and same-object location, and at 4 Hz for the different-object location. The performance of the same and different object followed an anti-phase relationship (Fiebelkorn, Saalman, & Kastner, 2013). The other study examined the phase differences of visually induced gamma-band activity for two targets, between hits and misses. They found that this measure was modulated at 4 Hz but not at other frequencies from 2 up to 20 Hz, indicative of continuous attentional sampling at 8 Hz. They also verified that this 4 Hz modulation was not caused by eye movements (Landau, Schreyer, van Pelt, & Fries, 2015). The 4 and 8 Hz in these two studies were of the fundamental frequency of 4 Hz.

Our results of the mixed-effect analysis found that the fundamental frequency and harmonics model better described the data than a simple model of low/middle/high frequency levels, thus was consistent to the observation that the attention samples the visual scene at specific fundamental frequencies (specifically in the fundamental frequency of 4 Hz). Relating to the research of rhythmic attention, the stronger suppression effect we found at 4 and 8 Hz may thus reflect an interaction of the CFS mask to the attentional mechanism, with the multiple colourful rectangles optimally occupying the attentional resources at those sampling frequencies. Further research with higher temporal resolution may help testing this hypothesis. For the stimulus, we always presented one stimulus at the center of the visual field in each trial. However, it is also worth testing whether the flash frequency would interact when multiple stimuli or multiple locations were used, as indicated by previous spatial attention findings.

For the frequencies found by Fiebelkorn, et al. (2013), variability was found across participants but was relatively stable for each participant (personal communication to the authors). In our experiment, we controlled the inter-individual variability by mixed-effects analysis, but did not thoroughly examine it. Future experiments could shed more light on the variability, by linking the optimal flash frequency of each participant under CFS to other behavioural measures.

### **The increase of stimulus visibility as the experiment progressed**

Our data showed a time effect: an increase of seen trials over the course of the experiment (length ~50 min), which could have been overlooked without examining individual data. For participants who were mostly naïve to the CFS paradigm, this effect was already present in the much shorter eye dominance test (6 min) before the start of the main experiment. This

important finding indicates that the time effect could possibly affect all CFS experiments, especially those utilizing naïve participants and stimuli that are more ecologically valid, such as faces and bodies.

Although not systematically studied before, this effect has been reported in a few CFS studies. One study mentioned it as a “learning to see” effect, showing that the hit rate under CFS grew from 36% in the first 40 trials to 64% in the last 40 trials. This effect was found together with their main research question, that hearing a valid verbal cue could facilitate the suppressed stimuli into awareness (Lupyan & Ward, 2013). Another study adjusted the target stimuli’s contrasts during the course of experiment for individual participants, and they found the contrast threshold for detecting the stimuli lowered progressively (stimuli increasingly visible) across experimental sessions (Ludwig, Sterzer, Kathmann, Franz, & Hesselmann, 2013). A third study attributed the improvement of stimulus visibility as a training effect, and found it was generalized to stimuli of a different orientation, but not when the stimuli and masks were swapped between eyes (Mastropasqua, Tse, & Turatto, 2015).

The increase of stimulus visibility during the experiment may relate to several causes. One possibility is that the participants may have consciously changed their decision criteria of “seeing a stimulus” as the experiment progressed. However, considering that the stimulus visibility increased in both the short and long test of our experiment, and the presence of invisible trials, it is not likely the main cause. A more likely cause may indeed be the accumulation of categorical information and the resulting expectation. Following Lupyan & Ward (2013), a recent CFS study found the cueing effect with written words for both complex and simple visual targets, and extended this effect to the sandwich masking paradigm (Stein & Peelen, 2015). In our experiment, seeing faces/bodies would likely act as cues themselves, to boost the stimulus visibility of subsequent trials. Other causes may be related to the fatigue and eye movements during the course of the experiment, but it needs further experimental investigations by combining CFS with eye tracking to confirm. A question also remains whether the increase of visibility follow the same slope for different stimulus categories.

## **The eye dominance test**

To achieve more stable suppression of visual awareness, some previous CFS studies had presented target stimuli into the non-dominant eye, which was established by an eye dominance tests before the main experiment. The methods to determine eye dominance was not reported in all studies, and varied across those studies that did report, including the hole-in-the-card test relying on monocular viewing (e.g. (Hesselmann, Hebart, & Malach, 2011), and tests based on binocular rivalry (e.g.(Yang, Blake, & McDonald, 2010). However, these two measures showed low consistency with each other (Mapp, Ono, & Barbeito, 2003; Yang, et al., 2010). In our experiment, we opted for the binocular method in order to have the same measure (number of seen trials) as the main experiment. We found weak biases between eyes for most of our participants, consistent with Yang, et al. (2010). The eye dominance of our

participants could have been changed into the opposite by only a few trials' difference, indicating our measure was not a stable one. More stable measure could be the time for each eye that a stimulus breaks from suppression (Yang, et al., 2010), although a recent study found that the imbalance of the eye dominance could be introduced by CFS presentation into one of the eyes (Kim, Kim, & Blake, 2017), which complicates the story.

## **Conclusion**

In summary, the current experiment found that the flash frequency of the CFS masks influenced stimuli visibility, with higher suppression strength at 4, 6, and 8 Hz, instead of the routinely used 10 Hz. These frequencies corresponded to the sampling frequency of spatial attention. In addition, we also observed significant inter-individual variability and an increase of visible trials as the experiment progressed. Future CFS studies need to take these factors into consideration to ensure maximal generalizability of results obtained with the CFS paradigm, and may benefit from using a flash frequency of 8 Hz.

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