

**Gamma-frequency entrainment using audiovisual 40 Hz flicker**

A Thesis  
Presented to  
The Academic Faculty of the Georgia Institute of Technology

by

Rahulkrishna Gurram Thimmugari

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## Abstract

Neural oscillations, or brain waves, are endogenous rhythms of synchronized electrical activity that are the result of communication between large groups of cortical and/or subcortical neurons. Using entrainment methodologies, neural oscillations can be exogenously modulated in a non-invasive manner. One such methodology is gamma-frequency audiovisual stimulation, referred to here as “flicker”. Building on previous work which has shown that flicker can significantly improve Alzheimer’s Disease pathology, the present work tests the effects of flicker on memory and attention in healthy adults. Using the Rapid Series Visual Presentation (RSVP) behavioral task, we found statistically significant improvements in response time as a result of 40 Hz stimulation, with an effect size (using Cohen’s  $d$ ) of 0.7026 when compared to No Stimulation and 0.5233 when compared to Random stimulation. We also found that Random stimulation, which delivers the same amount of stimulation as 40 Hz but on the minute timescale (while being asynchronous at the millisecond scale), increases False Alarm Rate (FAR), which is the rate at which subjects answer “Yes” on the RSVP task when the correct answer is “No”. Using the Signal Detection Model, these results were linked to the stimulation conditions affecting either the sensory and/or decision processes through either synchronicity/asynchronicity or through a power effect, i.e., sheer amount of stimulation. However, to support these speculations about the neural processes belying the behavioral results will require neuroimaging data. The key direction to take this study in the future would be to gather neuroimaging data, likely EEG.

## Introduction

Neural oscillations, or brain waves, are manifestations of synchronized electrical activity resulting from the communication of large groups of neurons in the cerebral cortex and subcortical structures. Detected by electroencephalogram (EEG), the shape/frequency of these waves are associated with a particular mental state. There are five widely recognized categories of waves with broad associations with consciousness states: gamma (30-120 Hz, problem solving/attention/concentration), beta (12-30 Hz, busy/active), alpha (8-12 Hz, relaxed/reflective), theta (4-8 Hz, deeply relaxed/drowsy), and delta (0.5-4 Hz, sleep) [14]. These associated mental states result from brief periods of network synchrony. Individual brain regions exhibit their own rhythms, and these rhythms are not necessarily associated with the above mental states. For example, the hippocampus, a brain structure responsible for long-term memory processing, exhibits distinctive activity associated with the theta band; this theta activity does not act as a marker for drowsiness but instead plays a significant role in both working and long-term memory processing [3].

Besides acting as markers for neural activity, brain waves can be entrained in a target to produce a change in that target's brain function. Broadly, entrainment is the coupling of any two oscillating systems such that they are phase-locked [7]. Knoblich et. al gives the example that two people sitting in rocking chairs side-by-side will involuntarily synchronize their rocking such that they are rocking at the same rhythm in temporal alignment; these two people are entrained to one another. Similarly, in neuroscience, entrainment is the use of rhythmic, exogenous stimuli to align with endogenous neural oscillations at the same frequency; these endogenous oscillations can either be in a particular brain region (local-circuit level) or can be summated across the whole brain (network level). The synchrony of exogenous and endogenous oscillations can serve as a method of amplifying the endogenous neural ones, thus enhancing brain function. Common neuroscientific techniques which can be used for this purpose include transcranial direct current stimulation (tDCS), theta-burst magnetic stimulation (TBS), and optogenetics [1,2,3,4,8,9].

Audiovisual gamma-frequency entrainment, referred to in my thesis as simply “flicker”, is a novel, non-invasive entrainment methodology and is the focus of this research. This methodology involves light and sound stimulation at 40 Hz. Previous studies have used visual flicker and optogenetic variants of these methods in the context of studying treatments for Alzheimer's Disease (AD) in both mouse models and humans [2,5,10]. Two of these three studies found significant effects in alleviating neurobiological pathologies of AD, namely amyloid beta plaques, but also in alleviating cognitive symptoms—such as the restoration of spatial memories [2,10]. It is unclear whether these benefits would be directly linked in humans, and what cognitive processes underlying spatial navigation behavior would be influenced by flicker—depending on the mechanism of action, it could even influence healthy brains. The current thesis research seeks to address this question, building on these previous studies by using an audiovisual flicker paradigm in cognitively healthy human adult subjects to test for whether and how it affects core memory and attention processes that are involved in navigation.

## Literature Review

Brain waves are important manifestations of neural activity. Many studies, and through a variety of methodologies including optogenetics, transcranial direct current stimulation (tDCS), and theta-burst magnetic stimulation (TBS), have shown that the targeted entrainment of specific brain regions to specific frequencies can significantly modulate attention and memory [1,2,3,4,8,9]. A novel, alternative methodology to the aforementioned three methodologies has recently emerged in the context of Alzheimer's Disease (AD), a neurodegenerative disease marked by progressive memory impairments. This alternative is noninvasive 40 Hz white light stimulation, henceforth referred to as "flicker". This methodology has been tested in both AD mouse models and AD human patients, with mixed results: the mouse model tests found reduced AD pathologies (namely in amyloid beta load) while the human trials found no effect on AD pathologies [5,10]. It is important to note that the human trials study was too small to be conclusive. The published literature on the effects of flicker in human subjects is extraordinarily limited. Importantly, it is yet unknown how the effects of flicker manifest in the cognition and neural function of healthy, human subjects. My thesis aims to test these effects. I will focus on measures of attention, but juxtapose with working memory as well, to get a better understanding of which active memory-relevant processes are influenced by short-term gamma stimulation. The data from this study will provide a platform for later studies of long-term memory and stimulation effects in samples with other population characteristics (e.g., non-age-related memory disorders).

In order to form a theory of how flicker may affect healthy, human subjects, it is useful to first review the studies which have used more common methods of entrainment. Hermiller et al. used TBS concurrent with fMRI to test for the immediate enhancement of hippocampal memory encoding [3]. TBS is a non-invasive technique that mimics the endogenous theta rhythms in the hippocampus (~4-8 Hz), which are thought to subservise mechanisms of episodic memory formation. The authors used a two-way design, comparing the effects of stimulation cast as theta-burst (50 Hz triplet pulses delivered at theta frequency - 5 Hz) and beta (single pulses delivered at 12.5 Hz, this is the control), and comparing the effects of stimulation on a hippocampal-network-targeted (HNT) area and the out-of-network supplementary motor area (SMA, this is the second control). They delivered stimulation for 2 seconds immediately before the onset of a subset of trials, scanned during all trials using custom fMRI parameters, and after completion of all trials, waited 15 minutes before administering a memory test. Hermiller et al. found that in the TBS x HNT combination, hippocampal fMRI activity was significantly increased during scene encoding. This increase was not seen in other condition combinations. The authors interpreted their results as support for both the beneficial influence of TBS on memory formation as well as for the role of theta-band oscillations in episodic memory. While the authors were limited in their ability to confirm a role of theta entrainment underlying these results (as fMRI cannot directly measure theta), this study does suggest that targeted entrainment can modulate memory function. For the purposes of this review, this finding is key—suggesting cortical oscillatory stimulation in humans can propagate and affect neural function downstream.

Optogenetics is another method that can be used for targeted entrainment. The major limitation of this method is that it is highly invasive: stimulation requires not only genetic manipulation of the target, but also the surgical insertion of a fiber optic cord to the specified brain region. For this reason, optogenetic methods are unlikely to be tested on humans in the near term. However, it is a highly precise technique used extensively in animal models. Etter et al. used optogenetics to stimulate the hippocampus of AD mouse models with both slow (30-60 Hz)

and fast (60-120 Hz) gamma oscillations [2]. Without stimulation, the AD mouse models displayed significant spatial memory impairment as well as reduced slow gamma oscillation amplitude. Using the Barnes Maze Task, the authors demonstrated that the slow gamma oscillation stimulation condition (at 40 Hz) not only restored slow gamma oscillation amplitude but also rescued the spatial memory of the mice. The fast gamma oscillation condition (at 80 Hz) did not accomplish either. This suggests that slow gamma stimulation, over fast gamma stimulation, can benefit endogenous oscillatory behavior and associated navigation function (which can depend on long-term memory and attention functions, among others).

A comparison of the above two studies raises the question of which plays a greater role in memory, gamma oscillations or theta oscillations? In a review of gamma frequency oscillations and their association with memory and attention, Jensen et al. advance several strong arguments for why gamma frequency synchronization is highly important for neuronal communication [6]. These arguments include the gamma frequency range providing the optimal timeframe for EPSP integration in target neurons and gamma synchronization providing a mechanism for gain control. While this may be true, theta activity appears to have an important role in both working and long-term memory tasks, and it is certainly an endogenous hippocampal rhythm [6,3]. It is more likely that neither is more important than the other, but gamma and theta rhythms are both very important to proper memory function, consistent with data that emphasize both short-term and long-term memory draw on theta-gamma coupling [11].

Cognitive domains outside of long-term memory and neural function outside of the hippocampus have also been tested in entrainment methodologies. For example, the dorsolateral prefrontal cortex (DLPFC) is a popular and relevant target for entrainment. Applying tDCS to the DLPFC, Boudewyn et al. tested for influences of stimulation on markers of proactive cognitive control [1]. The authors define proactive cognitive control as "...goal and context maintenance in order to anticipate upcoming cognitive demands...". In other words, it is the biasing of attention, based on task-relevant materials (e.g. the rules of the task), to the set of responses which are most likely to be correct. Using the Dot Pattern Expectancy (DPX) task to measure proactive control and EEG to monitor neural correlates of control, the authors found that tDCS increased gamma activity in the DLPFC which in turn was correlated with increased proactive control on the DPX. A separate study also targeted the DLPFC but the authors of that study, Hoy et. al, used TBS instead of tDCS [4]. Using n-back tasks, the authors assessed the persistent effects of TBS on working memory, administering the tasks three times: immediately after, 20 minutes after, and 40 minutes after stimulation. These tasks were administered concurrently with EEG. The behavioral results found improved performance in the TBS group and the EEG results suggested that this was linked to increased fronto-parietal theta synchronization as well as parietal gamma power. These two studies make an interesting comparison: both studies target the DLPFC but in different contexts and using different methodology. The former, using tDCS, finds support for the modulation of proactive control by gamma oscillations in the DLPFC, while the latter, using TBS, finds support for the modulation of working memory by diffuse gamma and theta oscillations. Both cognitive control and working memory are important antecedents of long-term memory and influence memory outcomes in the hippocampus [12]. While both studies suffer from several limitations (namely small sample sizes and poor EEG spatial resolution), it is evident that the DLPFC is an important brain region at the crossroads of memory function, attentional modulation, and entrainment.

Importantly for my thesis, audiovisual 40 Hz flicker appears to be capable of replicating such effects. Roberts et al. found very similar results to Hermiller et al.: in theta rhythm

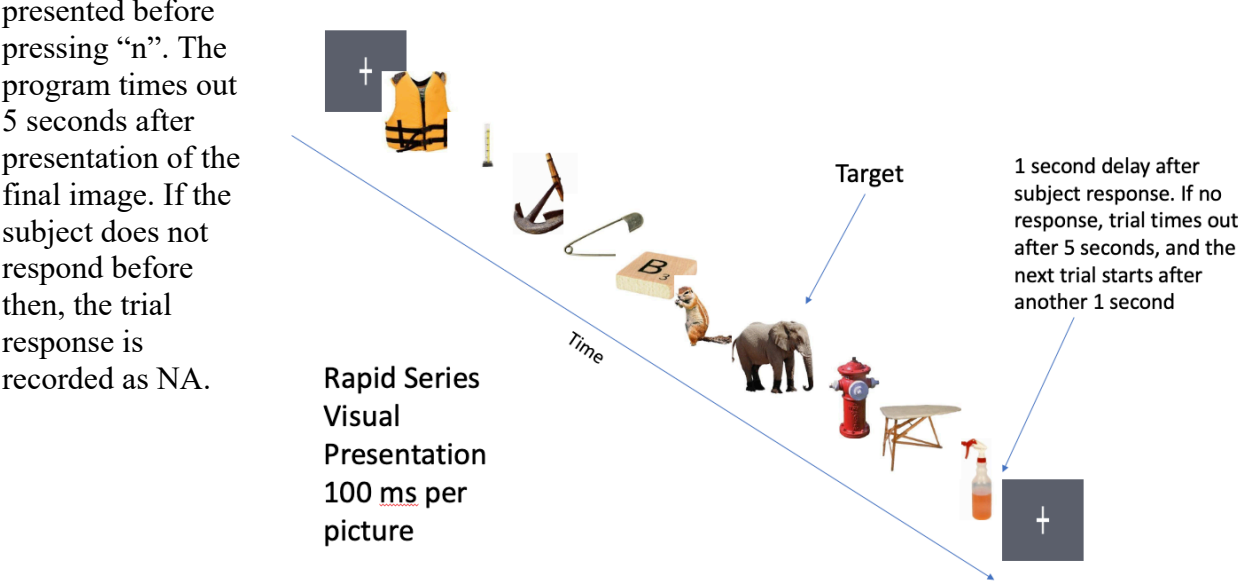
entrainment designs, both studies saw significant improvements in episodic memory [3,9]. The major difference is that Hermiller et al. used TBS while Roberts et al. used an audiovisual flicker paradigm in the theta range. It is important to note that the two papers finding similar results lends much credence to the flicker methodology. Out of all the studies reviewed herein, that of Roberts et al. is the only one which has tested the effects of flicker on healthy human subjects; and even this study only tested effects of theta range flicker—leaving open the question of how slow gamma stimulation (the target of AD-focused stimulation methods, as reviewed above) affects normal cognition. My study seeks to address this gap in the literature. I will examine the effects of 40 Hz gamma range audiovisual flicker on working memory and attention in healthy human subjects. Given its strong mechanistic association with regulating attention to stimuli at the circuit level, I theorize one means by which slow gamma stimulation in humans may influence memory outcomes is through attention [13]. I will use an RSVP task, as described in Krancioch et al., to test for attention effects as well as a modified n-back task to test for working memory effects [4,7].

# Methodology

*Participants.* 75 subjects participated in this study. All subjects were recruited from the Sona database and were provided informed consent for the study protocol. Age range varied from 18-28, although the majority of subjects were college-aged (18-22). Given the nature of the flicker methodology, any subjects with history of epilepsy were excluded. Subjects were paid at a rate of \$15/hour for their time.

*Setup.* The setup consists of three computer monitors, arranged so that one is in the center and two are at 45° angles in the periphery, and one set of over-the-ear wired headphones. The task is completed on the center monitor while the visual flicker plays on the peripheral monitors and the audio flicker plays over the headphones. Visual and audio flicker are synced so that they run at the same rate. The stimulation is run under three conditions. One, 40 Hz, where stimulation flickers at a rate of 40 times per second. Two, random, where stimulation flickers at a pseudorandom, non-epileptic frequency. In this condition, an average of 40 pulses per second are delivered over the course of a minute. Three, none, where no stimulation is delivered at all. In all figures, tables, and discussion, the terms “rand” and “random”, as well as “none” and “no stimulation”, are used interchangeably.

*Task.* While two individual tasks were planned for this study, pilot data suggested that the modified n-back working memory task does not show significant results under this paradigm; hence, only the RSVP task was used. This is a type of discrimination task. Ten images are presented in rapid series (1 image/100 ms) and subjects are asked to match a word describing a particular image to an image presented in the series. If they see the image, they are instructed to indicate so by pressing the “y” key on the keyboard. If they do not see the image, they are to indicate so by pressing the “n” key on the keyboard. The subjects are asked to respond as quickly as possible. If the subject sees the image halfway through the sequence, they are instructed to not wait until the end of the sequence to press “y”, but to rather press it as soon as they see it. Naturally, if the subject does not see the image at all, he would wait until all images are presented before pressing “n”. The program times out 5 seconds after presentation of the final image. If the subject does not respond before then, the trial response is recorded as NA.



**Figure 1.** RSVP task.

This design is an application of the signal detection model. This model describes the four possible outcomes for detecting a signal (Table 1). One, the signal is present, and the subject decided that it is present (Hit). Two, the signal is not present, but the subject decided that it is present (False Alarm). Three, the signal is present, but the subject decides that it is not present (Miss). And finally, four, the signal is not present, and the subject decides that it is not present (Correct Rejection). In relation to

		Signal:	
		Present (Y)	Absent (N)
Decision:	Present (Y)	Hit	False Alarm
	Absent (N)	Miss	Correct Rejection

**Table 1.** Signal Detection Model.

the present study, a Hit is when the subject presses “y” and the image was indeed presented in that trial; a False Alarm is when the subject presses “n”, but the image was presented in that trial; a Miss is when the subject presses “y”, but the image was not presented in that trial; and a Correct Rejection is when the subject presses “n” and the image was indeed not presented during the trial. In addition to these four outcomes, this study adds the fifth “No Response”, where the subject fails to respond at all to the trial. Here, Hits and Correct Rejections are examples of “Correct Responses”, Misses and False Alarms are examples of “Incorrect Responses”, and No Response is a category all on its own.

Response time (RT) data is also recorded. For “Yes” trials (trials where the image is presented), RT is defined as the time difference between the presentation of the image (at whatever position it is in the image sequence) and the subject’s response. For “No” trials, it is the time difference between presentation of the final image of the sequence and the subject’s response. Importantly, trials in which the subject did not respond (No Response trials) are not thrown out when analyzing RT but instead assigned an RT of 5 seconds. The justification for this is that the subjects did not choose to not respond to the trial, but rather they ran out of time to respond to the trial. If the program did not time out at 5 seconds after the final presentation, but instead at 10 seconds, it is possible the subjects would have recorded a response. Hence it is reasonable to record No Response trials as having a 5 second RT.

A total of 38 trials with 10 images per trial were presented to the subjects. All images were of easily recognized objects, animals, fruits, and/or vegetables. Partway through the study, a 76-trial version of the task was used. For the subjects who took the 76-trial version, only the first 38 trials of their data were used in the final analysis.

*Analysis.* First, Matlab was used to compile raw data into .xlsx files, after which R was used to perform the relevant statistical analyses.

Each subject’s Score is calculated from the data. This Score includes Percent Correct (number of correct trials divided by the total number of trials); Percent Incorrect (number of incorrect trials divided by the total number of trials); and Percent Missing (number of no response trials divided by the total number of trials). Hit Rate, Miss Rate, Correct Rejection Rate, False Alarm Rate, E1 Rate, and E2 Rate are also calculated according to the following equations:

$$\#Hit + \#Miss + \#E1 = \#y \tag{1}$$

$$\#CorrectRejection + \#FalseAlarm + \#E2 = \#n \tag{2}$$

E1 here refers to the number of No Responses recorded for trials in which the image was presented and E2 refers to the number of No Responses recorded for trials in which the image was not presented. Hit Rate is defined as the number of Hits divided by the number of trials in which the image was presented. This same logic applies to Miss and E1, and the equivalent is given for Correct Rejection, False Alarm, and E2. Hence, every term in Equation 1 is divided by #y and every term in Equation 2 is divided by #n to get the relationships between each rate:

$$\frac{\#Hit}{\#y} + \frac{\#Miss}{\#y} + \frac{\#E1}{\#y} = 1 \quad (3)$$

$$HitRate + MissRate + E1Rate = 1 \quad (4)$$

$$\frac{\#CorrectRejection}{\#n} + \frac{\#FalseAlarm}{\#n} + \frac{\#E2}{\#n} = 1 \quad (5)$$

$$CorrectRejectionRate + FalseAlarmRate + E2Rate = 1 \quad (6)$$

Each subject's average RT was also calculated from the data. This includes Correct RT, which is the subject's RT averaged over his correct trials; Incorrect RT, which is the subject's RT averaged over his incorrect trials; and Per Participant RT, which is the subject's overall RT, averaged over all of his trials. In addition, to investigate possible learning curve effects, Per Trial RT was also calculated. First, all RT data was listed as a 38x75 matrix, with trials as rows and subjects as columns. Then, the columns were sorted by condition, to make three separate 38x25 matrices, with each matrix corresponding to each of the three conditions (40 Hz, No Stimulation, and Random). Lastly, each matrix was averaged across rows (trials), between subjects, to calculate Per Trial RT.

Complete sets of orthogonal contrast codes were built to analyze differences between experimental conditions in both Score and RT. These sets are shown in Tables 2, 3, and 4.

Contrast codes are a way of assigning numbers to categorical data to analyze differences between the categories [15]. For example, L1 in Table 2 analyzes the difference between the No Stimulation condition versus the pooled matrix of the 40 Hz and Random conditions. Likewise, L2 analyzes the difference between 40 Hz and the Random condition, while ignoring the No Stimulation condition. To be a contrast code, the assigned numbers must sum to zero. To be orthogonal contrast codes, the multiplication of each code must sum to zero (this is the L1\*L2 column in Table 2). To form a complete

	L1	L2	L1*L2
40Hz	-1	1	-1
None	2	0	0
Rand	-1	-1	1
Sum	0	0	0

**Table 2.** First complete set of orthogonal contrast codes.

set of orthogonal contrast codes, there must be  $m-1$  codes, where  $m$  is the number of categories, in this case three. Thus, each of these three tables comprise a complete set of orthogonal contrast codes [15]. This methodology allows for the identification of all between group variability, leaving only within group variability for the error term when running an ANOVA test. This elimination of between group variability from the error term makes for a more powerful statistical test: it increases the size of the F-ratio (compared to an omnibus ANOVA which includes between group variability in the error term) while also allowing the researchers to pose very specific questions of the data [15].

A model comparison approach is required in order to use the contrast codes. This means the use of Compact and Augmented models, where the Compact model corresponds to the null hypothesis and the Augmented model corresponds to the alternate hypothesis. When analyzing Score, Correct RT, Incorrect RT, and Per Participant RT, each contrast code is listed as a 75x1 vector in a data frame. This is because each individual code (for example, -1 in L1 for 40 Hz) is repeated 25 times, once for each subject; including each condition, this makes for a vector of length 75 for each contrast code. When analyzing Per Trial RT, this vector is of length 114; this is because there are three conditions of 38 trials each, with an individual code attached to each trial. Each contrast is then used to predict an outcome variable. For example, running this analysis using R:

$$cMod <- lm(df$percentIncorrect ~ df$L1)$$

$$aMod <- lm(df$percentIncorrect ~ df$L1 + df$L2)$$

$$anova(cMod, aMod)$$

Here, the compact model is built by using L1 to predict for Percent Incorrect. The augmented model is built by using both L1 and L2 to predict for Percent Incorrect. Then, an ANOVA test is used to compare the models. This comparison is an example of hierarchical regression as L1 is present in both models; the specific effect of L2 on Percent Incorrect is therefore analyzed while controlling for any variance from L1. Thus, the specific question of L2 (is there a significant difference between the 40 Hz and Random conditions, ignoring No Stimulation) is asked of the Percent Incorrect data. If the ANOVA returns  $p < 0.05$ , then the null hypothesis would be rejected and there would be evidence suggesting a significant difference between the two conditions. If the ANOVA returns  $p > 0.05$ , then the null hypothesis would fail to be rejected and there would

	L3	L4	L3*L4
40Hz	2	0	0
None	-1	1	-1
Rand	-1	-1	1
Sum	0	0	0

**Table 3.** Second complete set of orthogonal contrast codes.

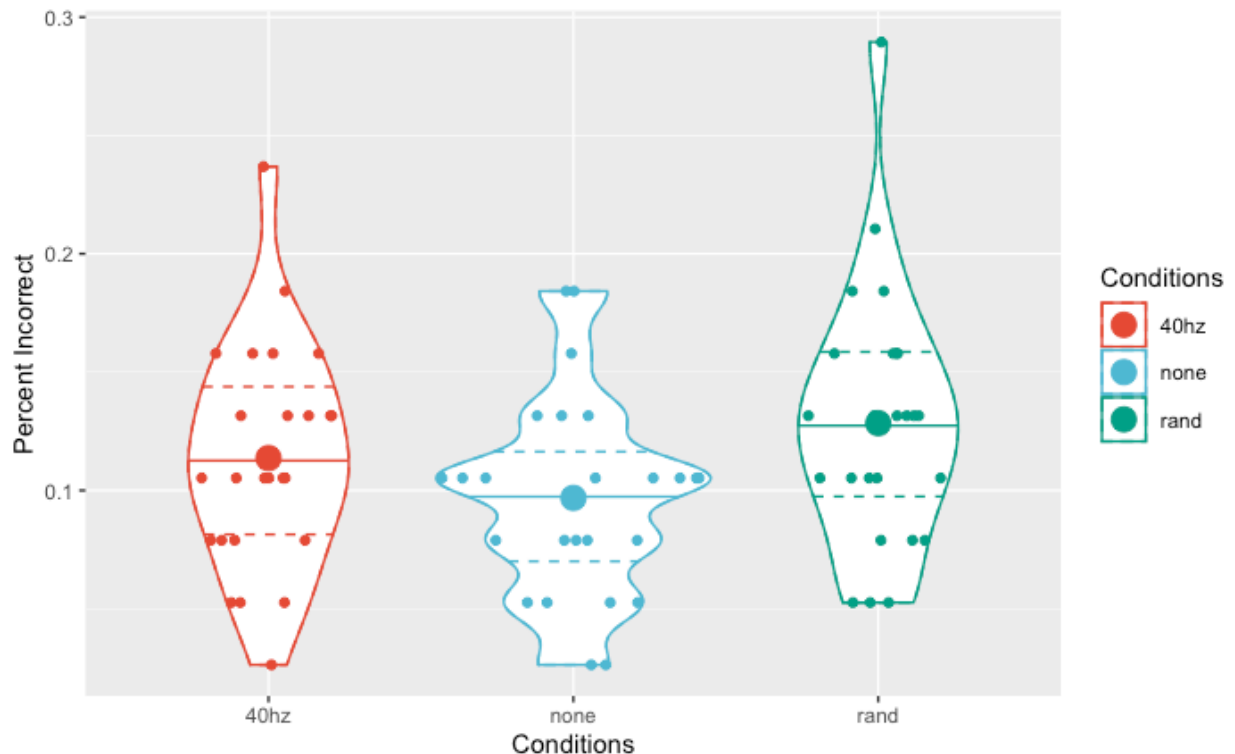
	L5	L6	L5*L6
40Hz	-1	-1	1
None	-1	1	-1
Rand	2	0	0
Sum	0	0	0

**Table 4.** Third complete set of orthogonal contrast codes.

be no evidence suggest significant difference. In this study,  $p < 0.15$  is labeled as trending,  $p < 0.05$  is labeled as significant, and  $p < 0.01$  is labeled as highly significant. This model comparison analysis was run on all outcome variables using all six contrast codes to predict each outcome.

## Results

The following tables and figures depict the results of this study. All violin plots show jittered data, with the large dot symbolizing the mean; the solid line, the median; and the dashed lines, the 1<sup>st</sup> and 3<sup>rd</sup> quartiles. Any effect sizes given are calculated using Cohen's d. Tables show p-values calculated using the orthogonal contrast code methodology described in the Methods section.



**Figure 2.** Statistical significance in the Score sub-group Percent Incorrect. This shows the percentage of incorrect trials out of total number of trials per participant. Mean Percent Incorrect for 40 Hz: 0.1134; Mean Percent Incorrect for No Stimulation: 0.0968; Mean Percent Incorrect for Random: 0.1284. This figure shows that Percent Incorrect is, at the 0.05 alpha level, significantly lower in the No Stimulation condition compared to the Random condition, with an effect size of 0.6436.

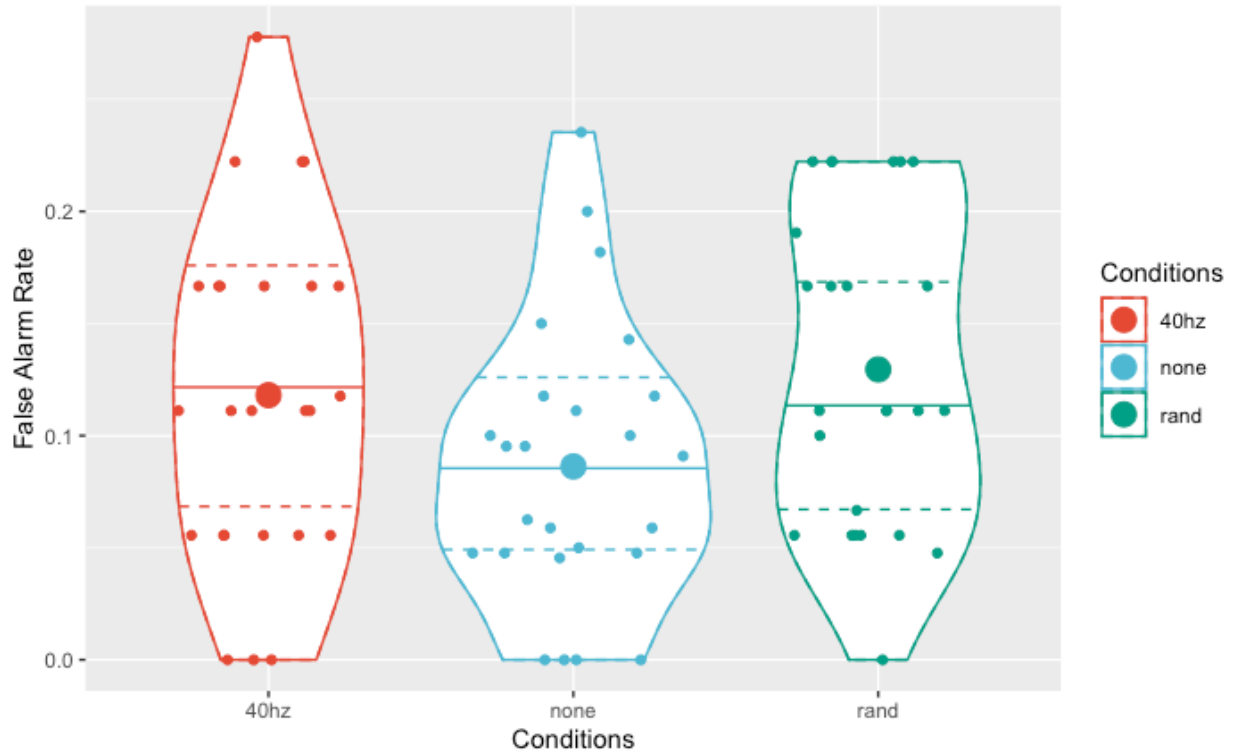
The main result shown in this figure is that subjects in the No Stimulation condition showed a significantly lower percentage of incorrect responses when compared to subjects in the Random group. This implies that Random stimulation causes an increase in incorrect responses, with this effect size being moderate at 0.6436. A secondary result is that pooled mean of 40 Hz and Rand is significantly higher than the mean of None, with this effect size being also moderate but lower at 0.4939. This seems to imply that synchronous 40 Hz stimulation may have the effect of increasing Percent Incorrect if used in tandem with Random stimulation. By itself, 40 Hz stimulation does not show a significant or trending effect on increasing Percent Incorrect (see

Table 5 below for the corresponding p-value). Another significant effect was found when comparing the pool of 40 Hz and None versus Rand, but this has no practical significance.

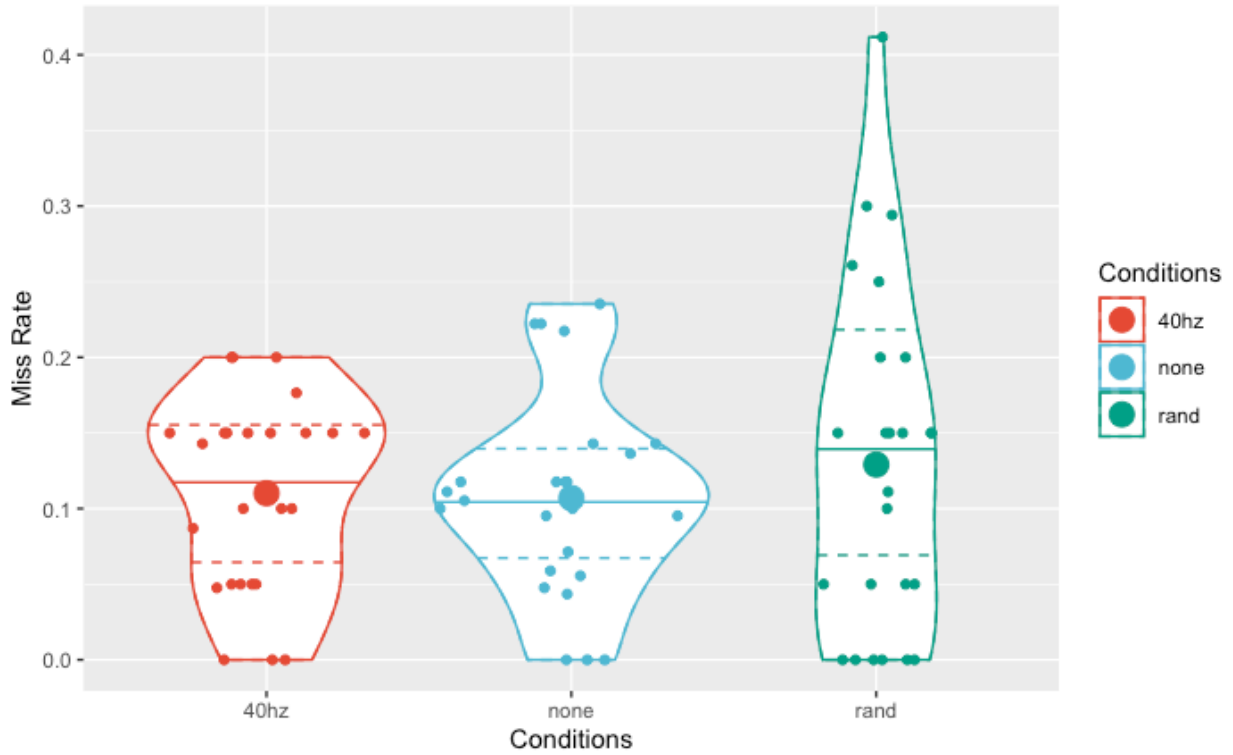
<b>P-values (Score)</b>			
<b>Comparisons</b>	<b>Percent Correct</b>	<b>Percent Incorrect</b>	<b>Percent Missing</b>
<b>40Hz + Rand vs None</b>	0.797	0.043	0.374
<b>40Hz vs Rand</b>	0.273	0.281	0.434
<b>Rand + None vs 40Hz</b>	0.281	0.929	0.263
<b>Rand vs None</b>	0.743	0.023	0.704
<b>40Hz + None vs Rand</b>	0.410	0.053	0.816
<b>40Hz vs None</b>	0.440	0.219	0.247

**Table 5.** P-values from model comparisons for Score outcomes. Medium orange shading indicates significance ( $p < 0.05$ ). Among Score outcomes, only Percent Incorrect shows significant differences between experimental conditions. The pooled mean of 40 Hz and Rand is significantly higher than mean of None; the pooled mean of 40 Hz and None is significantly lower than the mean of Rand; and the mean of Rand is significantly than the mean of None.

Incorrect responses can be split into false alarms and misses. In order to further investigate the significance of Percent Incorrect, the model comparison methodology was used to predict False Alarm Rate (FAR) and Miss Rate (MR). As shown in Figures 3 and 4 below, FAR largely replicates the Percent Incorrect results, while MR shows no significance or trends with any comparison: FAR is significantly higher under Random stimulation compared to No Stimulation, and the pooled mean of 40 Hz and Rand is significantly higher than the mean of None. This implies that it is the false alarms which are driving the Percent Incorrect results, and not the misses. FAR also shows a trend for 40 Hz being higher than None, which is not seen in the Percent Incorrect data. This seems to support the idea that synchronous 40 Hz stimulation may also increase the rate of incorrect responses, although this effect is much weaker than that of asynchronous Random stimulation and can only be brought out to a significant level if synchronous and asynchronous stimulation are used in tandem with each other.



**Figure 3.** Statistical significance in False Alarm Rate (FAR). As stated in the Methods section, FAR describes the rate at which participants answer “Yes” for trials where the correct answer is “No”. Mean FAR for 40 Hz: 0.1180; Mean FAR for No Stimulation: 0.0862; Mean FAR for Random: 0.1295. This figure shows that FAR is, at the 0.05 alpha level, significantly lower in the No Stimulation condition compared to the Random condition, with an effect size of 0.6081. This figure also shows that FAR is, at the 0.15 alpha level, trending when comparing the No Stimulation condition to the 40 Hz condition.

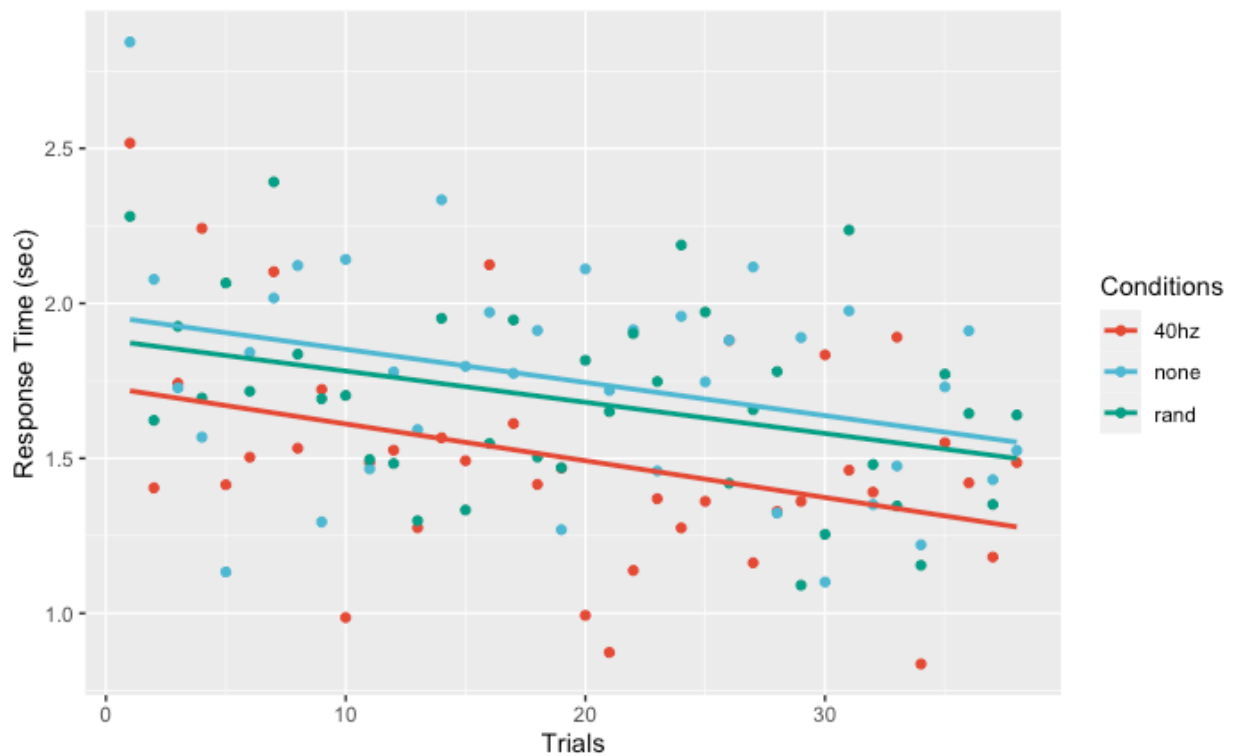


**Figure 4.** No statistical significance in Miss Rate (MR). As stated in the Methods section, MR describes the rate at which participants answer “No” for trials where the correct answer is “Yes”. Mean MR for 40 Hz: 0.1102; Mean MR for No Stimulation: 0.1069; Mean MR for Random: 0.1291. This figure shows that MR is not significant or trending for any comparison between conditions.

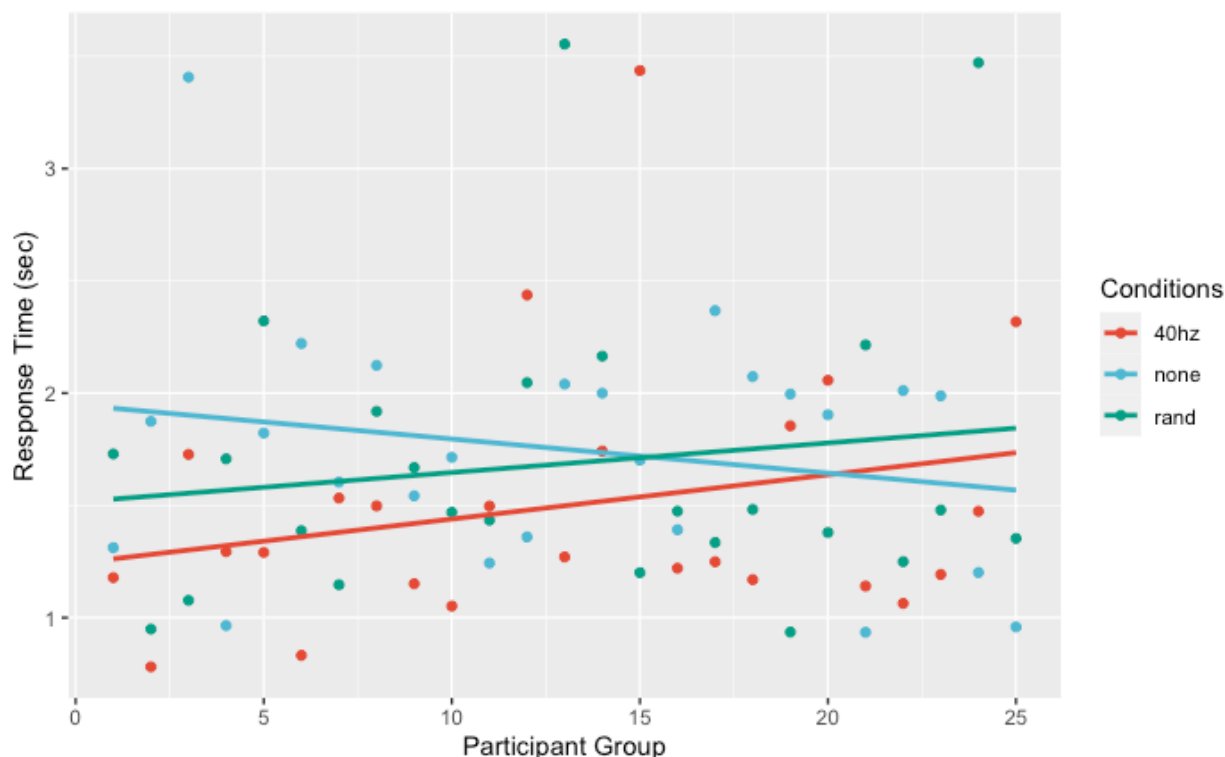
P-values (False Alarm and Miss Rates)		
Comparisons	False Alarm Rate	Miss Rate
<b>40Hz + Rand vs None</b>	0.031	0.541
<b>40Hz vs Rand</b>	0.562	0.429
<b>Rand + None vs 40Hz</b>	0.554	0.704
<b>Rand vs None</b>	0.031	0.356
<b>40Hz + None vs Rand</b>	0.114	0.323
<b>40Hz vs None</b>	0.111	0.893

**Table 6.** P-values from model comparisons for False Alarm and Miss Rates. The lighter orange shade indicates not significant but trending ( $p < 0.15$ ). The significance seen in Table 2 with the Percent Incorrect outcome seems to be entirely carried by False Alarms. In addition, the 40 Hz vs None comparison is trending in FAR, whereas there is neither significance nor trend in Percent Incorrect for this comparison.

The following two figures, Figures 5 and 6, show Per Trial RT and Per Participant RT respectively. Per Trial RT is firstly a learning curve investigation; it is evident from the negative slopes on the regression lines that all three conditions show a learning curve, i.e., response times decrease as subjects see more trials, and hence learn to respond faster. Secondly, there is a very clear visual separation of the 40 Hz data from the No Stimulation and Random data for Per Trial RT: the response times of subjects in the 40 Hz condition are on average lower across all trials when compared to the response times of subjects in both No Stimulation and Random conditions. Model comparison results statistically support this visual difference:  $p < 0.01$  for the 40 Hz versus None comparison, with effect size on the high end of moderate at 0.7026; and  $p < 0.05$  for the 40 Hz versus Random comparison, with effect size on the low end of moderate at 0.5233. Per Participant RT, on the other hand, only shows a trending effect between the 40 Hz and No Stimulation conditions. This difference seems to imply that synchronized 40 Hz stimulation only affects subjects on a trial-by-trial basis; when response times are averaged within subjects, differences between types of stimulation disappear.



**Figure 5.** Statistical significance when predicting Per Trial RT. As stated in the Methods section, Per Trial RT describes response times averaged across trial number, between subjects separated into their respective condition. Therefore, in this figure, each trial is labelled with three data points, one for each condition, and these data points describe the average response time per trial for that condition. It is important to note that No Response trials, i.e. trials the subject timed out from not responding, were set to a response time of 5 seconds and included in the analysis. Mean Per Trial RT for 40 Hz: 1.4983; Mean Per Trial RT for No Stimulation: 1.7502; Mean Per Trial RT for Random: 1.6860. This figure shows that Per Trial RT is, at the 0.01 significance level, is significantly lower in the 40 Hz condition compared to the No Stimulation condition, with an effect size of 0.7026. This figure also shows that Per Trial RT is, at the 0.05 significance level, significantly lower in the 40 Hz condition compared to the Random condition, with an effect size of 0.5233.

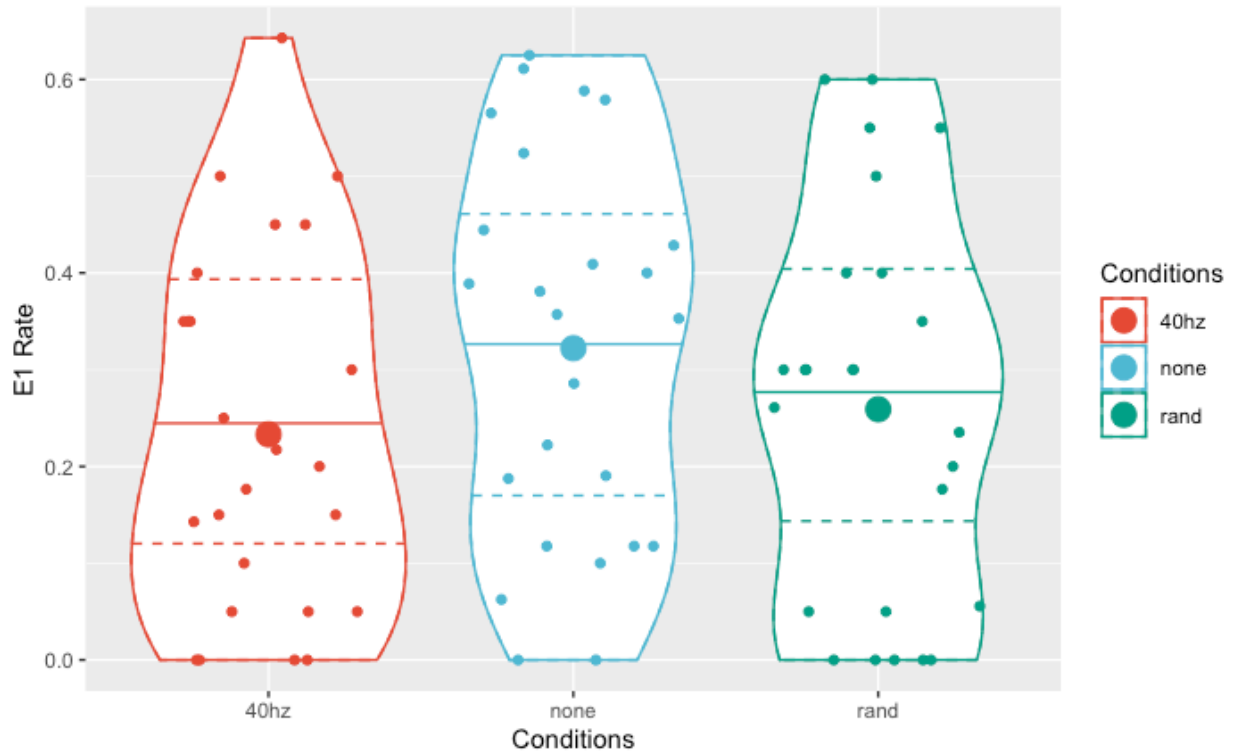


**Figure 6.** Trending when predicting Per Participant RT. As stated in the Methods section, Per Participant RT describes response times averaged within subjects, across all trials each subject sees. Therefore, in this figure, there are 75 data points, each describing a subject's response time. The data are grouped by Participant Group, with three data points per group (one for each condition) for a total of 25 groups. The data are displayed this way, in an overlaid fashion, in order to more directly compare each condition visually. Just as with Per Trial RT, the No Response trials of Per Participant RT were set to a response time of 5 seconds and included in the analysis. Mean Per Participant RT for 40 Hz: 1.4983; Mean Per Participant RT for No Stimulation: 1.7502; Mean Per Participant RT for Random: 1.6860. This figure shows that there is no significance for any comparison when predicting Per Participant RT. There is however a trending effect for the 40 Hz versus None comparison, with 40 Hz trending lower.

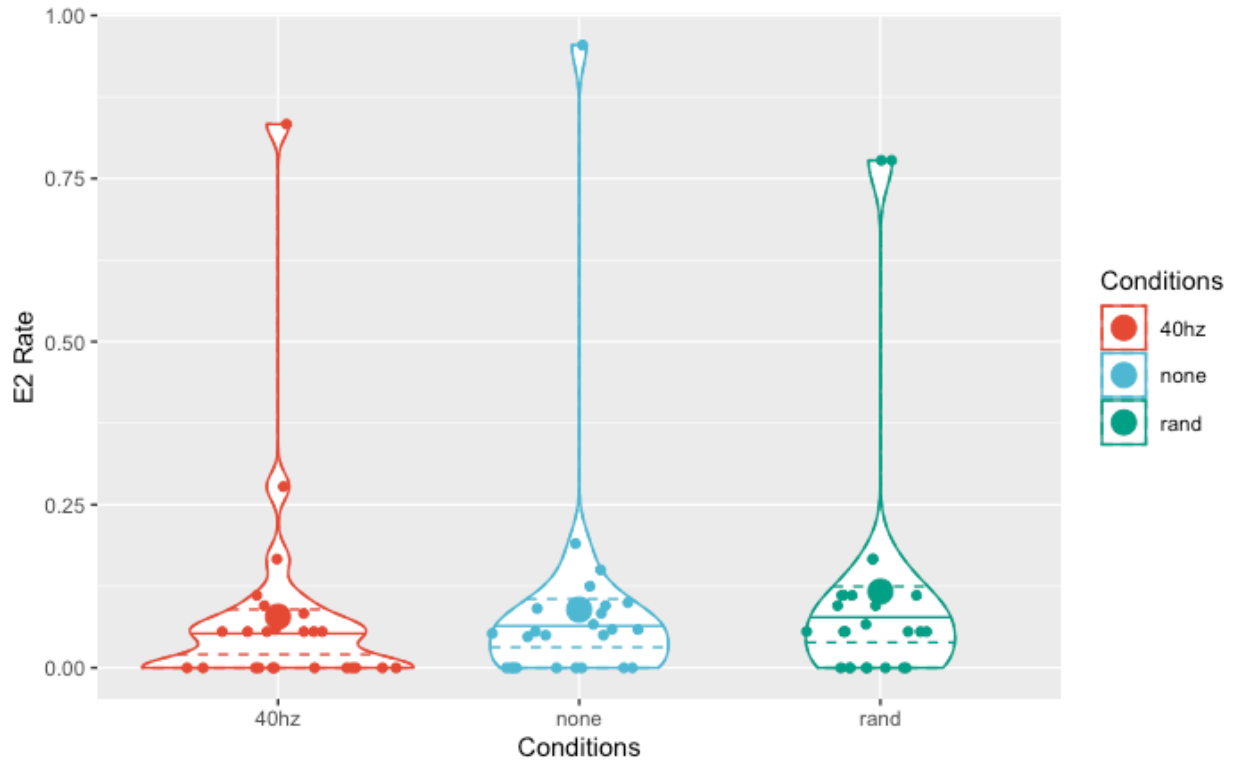
<b>P-values (RT)</b>				
<b>Comparisons</b>	<b>Correct RT</b>	<b>Incorrect RT</b>	<b>Per Participant RT</b>	<b>Per Trial RT</b>
<b>40Hz + Rand vs None</b>	0.591	0.246	0.283	0.023
<b>40Hz vs Rand</b>	0.895	0.907	0.270	0.020
<b>Rand + None vs 40Hz</b>	0.702	0.495	0.137	0.002
<b>Rand vs None</b>	0.689	0.343	0.705	0.419
<b>40Hz + None vs Rand</b>	0.877	0.630	0.674	0.370
<b>40Hz vs None</b>	0.595	0.288	0.140	0.002

**Table 7.** P-values from model comparisons for RT outcomes. The dark orange shading indicates high significance ( $p < 0.01$ ), the medium and light the same as in Tables 2 and 3. Here, Per Trial RT is highly significant in the Rand + None vs 40 Hz and the 40 Hz vs None comparisons. For the same comparisons, Per Participant RT is trending. The only difference between these two datasets is that RT is averaged within participants for Per Participant RT and RT is averaged between participants, per trial, for Per Trial RT.

The above table, Table 7, shows the p-values from each model comparison for all RT outcomes. It is important to note that neither Correct RT nor Incorrect RT show significance or trending for any comparison. Significance and trending effects only start to appear when including No Response trials, the response times of which are set at 5 seconds, in the analysis (this would be in Per Participant RT and Per Trial RT). Since Per Trial RT is lower in the 40 Hz condition compared to both the No Stimulation and Random conditions, it would be reasonable to hypothesize that this difference is the result of a greater number of No Responses in the No Stimulation and Random conditions. In other words, E1 and E2 rates, defined in the Methods section, would be significantly affected by No Stimulation and/or Random stimulation. The following figures and table show the results of this investigation.



**Figure 7.** Trending in E1 rate. As stated in the Methods section, E1 rate describes the rate at which subjects time out of trials where the correct response is “Yes”. Mean E1 rate for 40 Hz: 0.2332; Mean E1 rate for No Stimulation: 0.3222; Mean E1 rate for Random: 0.2591. This figure shows that E1 rate is only trending for the 40 Hz versus None comparison, with 40 Hz trending lower.



**Figure 8.** No significance or trending in E2 rate. As stated in the Methods section, E2 rate describes the rate at which subjects time out of trials where the correct response is “No”. Mean E2 rate for 40 Hz: 0.0784; Mean E2 rate for No Stimulation: 0.0892; Mean E2 rate for Random: 0.1170. This figure shows that E2 rate is not significant or trending for any comparison between conditions.

P-values (E1 and E2 Rates )		
Comparisons	E1 Rate	E2 Rate
<b>40Hz + Rand vs None</b>	0.119	0.855
<b>40Hz vs Rand</b>	0.643	0.472
<b>Rand + None vs 40Hz</b>	0.237	0.595
<b>Rand vs None</b>	0.261	0.604
<b>40Hz + None vs Rand</b>	0.701	0.475
<b>40Hz vs None</b>	0.114	0.840

**Table 8.** P-values from model comparisons for E1 and E2 rates. Only trending effects ( $p < 0.15$ ) are shown for E1 rate, while neither trending nor significance is shown for E2 rate.

The E1 rate is at most trending for the 40 Hz versus None comparison, while the E2 rate shows neither trends nor significance for any of the comparisons. This would imply that the rate of No Responses is not significantly higher among the No Stimulation or Random conditions, which does not support the earlier hypothesis. There must be some deeper combination of factors, then, working in concert to produce this situation of a per-trial group difference.

## Discussion

In summary, two main results were found in this study. The first is that False Alarm Rate (FAR) was found to be significantly higher under the Random stimulation condition compared to the No Stimulation condition. The second is that Per Trial RT was found to be significantly lower under the 40 Hz stimulation condition compared to both the No Stimulation and Random stimulation conditions.

The first main result is analyzed through the lens of Signal Detection Theory (SDT). According to SDT, when responding to a stimulus, there is first a sensory process and then a decision process that occurs before the response. SDT models the sensory process as a continuous output of random Gaussian noise. Then, when a signal arrives, it combines with the already-present noise and creates a perturbation in the distribution. The decision process looks at this new signal plus noise distribution and compares it to the old noise distribution; if there is enough of a difference, it decides that the signal is real. If there is not, then it decides that the signal is false [16]. The decision process can therefore be described by a simple mathematical equation:

$$\frac{N + S}{N} \geq D,$$

where N equals the mean of the noise distribution, S equals the mean of the signal distribution, and D equals the decision criterion. If the ratio of N + S to N is greater than or equal to the decision criterion, the decision process decides that the signal is real.

This ratio can be larger than the decision criterion in one of three ways. One, the signal can be strong enough to increase the ratio above the decision criterion. Two, the noise output of the sensory process can be artificially weakened such that the ratio increases above the decision criterion. And three, the decision criterion itself is lowered to below the ratio. These latter two ways provide possible explanations for why FAR may be increased under the Random stimulation condition

False alarms are the result of a potent lure—a false signal is decided by the decision process to be the real signal. This false signal is likely of average strength; under normal conditions, it would not be able to pass the decision criterion. If, however, the continuous noise output of the sensory process is artificially decreased, then even this signal of average strength would be able to pass the decision criterion. In other words, the sensory process would become less sensitive to the target. Likewise for the third method: if the decision criterion was artificially lowered, an average strength signal would also be able to pass through the decision process and thus cause a false alarm. Random stimulation could therefore cause increased FAR through either the sensory process, the decision process, or both. The sensory process is linked to the subcortical attention modules while the decision process is linked to the cortical decision-making network. To affect either process, the asynchronous Random stimulation likely interferes with the endogenous rhythms of the relevant neural circuits—exactly how this interference works is unclear from the behavioral data presented here. It is possible that the asynchronicity of Random stimulation is disrupting endogenous neural oscillations, but it is also possible that the interference is working through a power effect, i.e., the sheer amount of stimulation is what is disrupting the oscillations. Perhaps it is a combination of both asynchronicity and power effect that is causing the interference. This combination may explain the weak, trending link seen between 40 Hz stimulation and FAR: 40 Hz stimulation and Random stimulation share the same

amount of stimulation, but 40 Hz is synchronous while Random is asynchronous. Both conditions might be increasing FAR through the power effect, but the asynchronicity of Random further increases FAR, to make the relationship significant, but the synchronicity of 40 Hz does not further increase FAR and keeps the relationship at only a trending level. While these hypotheses and speculations are based on the solid ground of behavioral data, to properly support or deny them would require evidence from neuroimaging data.

While audiovisual stimulation, particularly Random stimulation, was associated with a particular type of perceptual-detection error (FAR), the RT results indicate a significant 40 Hz benefit. Per Trial RT, response time averaged between subjects per trial, was found to be significantly lower in the 40 Hz condition compared to both the Random and No stimulation conditions. This means that on a per trial basis, RT is lower across all trials in the 40 Hz condition. However, these same differences are not present, or at most trending, in Per Participant RT, which is the same data but averaged within subjects across trials. Moreover, neither Correct RT nor Incorrect RT show any group differences. These differences only start to appear when including all types of trials in the calculation of the averages—this would contain No Response trials as well, the RTs of which are set at 5 seconds. These points taken together suggest that No Responses (E1 rate and E2 rate) are disproportionately affected in the Random and No stimulation conditions. However, model comparison results suggest at most trending effects for E1 when comparing 40 Hz and No stimulation, and no significance at all for E2. While E1 Rate may play some role in producing this differential per trial effect, there is likely some combination of many factors that create this situation of per trial group difference—including a confluence of subthreshold RT differences and the subthreshold greater tendency to have No Response (very long RT) trials.

It is at least clear from the per trial data that 40 Hz stimulation improves response times compared to Random and No stimulation. This appears to support our initial hypothesis that slow gamma stimulation should provide some cognitive benefit to short-term memory and/or attention through hippocampal synchronization. One interesting consideration is that this benefit may not be wholly unrelated to the elevated FARs under stimulation conditions—while stimulation may lower sensitivity in the perceptual cortex, for example, which may lend itself to more FARs, synchronous stimulation may alter decision process thresholds “downstream”—lending itself to faster responses to whatever the output of the sensory process is. Without neuroimaging data, however, it is impossible to tell if synchrony did happen, to what extent it happened, and which brain regions were affected. With this task specifically, it is possible that RT in 40 Hz decreased as a result of faster decision making, not as a result of being more attuned to the task. If that is the case, then we would expect to see cortical synchronization, such as in the ventrolateral prefrontal cortex [17].

## Future Directions & Conclusions

In sum, these data suggest that Random stimulation increases the probability of false alarms while 40 Hz stimulation decreases response time. By extension, the data make certain predictions about the neural processes belying the behavioral outcomes. The most important future direction to take the study then, would be gathering neuroimaging data, likely EEG. Specifically, this future EEG study would test for the specific brain regions activated/synchronized and it would also test synchronicity/asynchronicity and for the level of power effect when analyzing FAR.

## References

1. Boudewyn, M., Roberts, B. M., Mizrak, E., Ranganath, C., & Carter, C. S. "Prefrontal transcranial direct current stimulation (tDCS) enhances behavioral and EEG markers of proactive control." *Cognitive Neuroscience* 10, no. 2 (2019): 57-65.
2. Etter, G. et al. "Optogenetic gamma stimulation rescues memory impairments in an Alzheimer's disease mouse model." *Nature Communications* 10 (2019): 1-11.
3. Hermiller, M. S., Chen, Y., Parrish, T. B., & Voss, J. L. "Evidence for immediate enhancement of hippocampal memory encoding by network-targeted theta-burst stimulation during concurrent fMRI." *Journal of Neuroscience* 40, no. 37 (2020): 7155-7168.
4. Hoy, K. E. et al. "Enhancement of working memory and task-related oscillatory activity following intermittent theta burst stimulation in healthy controls." *Cerebral Cortex* 26, (2016): 4563-4573.
5. Ismail, R. et al. "The effect of 40-hz light therapy on amyloid load in patients with prodromal and clinical Alzheimer's Disease." *International Journal of Alzheimer's Disease* (2018): 1-5.
6. Jensen, O., Kaiser, J., & Lachaux, J. "Human gamma frequency oscillations associated with attention and memory." *Trends in Neuroscience* 30, no. 7 (2007): 317-324.
7. Kranczioch, C., Debener, S., Herrmann, C. S., & Engel, A. K. "EEG gamma-band activity in rapid serial visual presentation." *Experimental Brain Research* 169 (2006): 246-254.
8. Pellegrino, G. et al. "Transcranial direct current stimulation over the sensory-motor regions inhibits gamma synchrony." *Human Brain Mapping* 40 (2019): 2736-2746.
9. Roberts, B. M., Clarke, A., Addante, R. J., & Ranganath, C. "Entrainment enhances theta oscillations and improves episodic memory." *Cognitive Neuroscience* 9, no. 3-4 (2018): 181-193.
10. Singer, A. C. et al. "Noninvasive 40-hz light flicker to recruit microglia and reduce amyloid beta load." *Nature Protocols* 13, no. 8 (2018): 1850-1868.
11. Tamura, M. et al. "Hippocampal-prefrontal theta-gamma coupling during performance of a spatial working memory task." *Nature Communication* 8 (2017).
12. Brown, T. I. et al. "Cognitive control, attention, and the other race effect in memory." *PLoS One* 12, no. 3 (2017).
13. Börgers, C., Epstein, S., & Kopell, N. J. "Background gamma rhythmicity and attention in cortical local circuits: a computational study." *PNAS* 102, no. 19 (2005): 7002-7007.
14. Abhang, P. A., Gawali, B. W., & Mehrotra, S. C. "Technological basics of EEG recording and operation of apparatus." *Introduction to EEG and Speech-Based Emotion Recognition*, (2016): 19-50.
15. Judd, C. M., McClelland, C. H., & Ryan, C. S. "Data analysis: A model comparison approach to regression, ANOVA, and beyond." Third Edition (2017): 179-196.
16. Harvey, L. O. "Detection sensitivity and response bias." (2003)
17. Badre, D. & Wagner, A. D. "Left ventrolateral prefrontal cortex and the cognitive control of memory." *Neuropsychologia* 45, no. 13 (2007): 2883-2901.