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Measuring the haemodynamic responses elicited in the visual cortex from various spatial and temporal frequencies using NIRS

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Abstract

Previous research has found the optimum spatial and temporal frequency of a stimulus can elicit peak activation in the visual cortex. In this study eight participants looked at bullseye gratings with low and high spatial frequencies (0.5 & 3 c/deg) at varying temporal frequencies (1, 4, 8 & 30Hz). Their haemoglobin response in the visual cortex was recorded using Near Infra-red Spectroscopy (NIRS). Insignificant results were found in all measures, including oxygenated haemoglobin which reported $F(1, 8, 5.63) = .75, p < .44$. Trigonometric regression did illustrate increased visual activation when the bullseye grating was presented. It appears different frequencies can cause different haemodynamic response, but a larger sample and the elimination of disadvantages in NIRS is required to obtain significance.

Statement

This research project was carried out in accordance with ethical guidelines set out by the British Psychology Society in the University of Plymouth Stage 4 Psychology course handbook 2010-2011. Before the study was conducted it was approved by the University of Plymouth Faculty of Science and Technology ethics committee. No deception was used in the study. Flickering stimuli were used so it was necessary that participants who took part in the study did not suffer from flicker induced epilepsy.

I confirm that all the data in the project was collected by me and members of my research group.

Acknowledgements

Thank you to all the members of my research project group: Luke Perkins, Theodora Tilney, Sam White, Daniel Edgcumbe and Wendy Read for all their help and support during the data collection and write up of this project.

I would also like to thank my supervisor Bill Simpson for his invaluable guidance throughout the project. My interest in neuroimaging has increased immensely since being taught by Bill at the beginning of this research project.

Introduction

Vision is an intricate and complex system that enables us to recognise and interact with our external world. Despite its complexity, visual perception can process details effortlessly and respond instantaneously to the external world, making vision a fascinating area of research (Thorpe, Fize & Marlot, 1996). In order to process a visual display it needs to be clearly seen. In today's society individuals are constantly using their visual systems to process a constantly changing environment of visual displays and screens such as: televisions, computers, mobile phones and cinema in many different environments, in order to extract important information. To see visual displays clearly, the visual system must be able to appropriately process the spatial frequencies and temporal frequencies of the target. Research into spatial and temporal frequencies has been conducted for many decades to fathom the biological machinery of the eye, the cortex and the psychophysical mechanisms involved in visual perception (Blakemore & Campbell, 1969; Cue Bray, Bryant, Glover & Reiss, 2011; Patel, 1966). Increasing availability and technological advances in neuroimaging equipment, has facilitated an increased understanding of how the visual system process frequencies and visual displays.

Frequencies

The spatial frequency of an image is, the amount of space that brightness modulates across an image (cycles per degree) from light to dark (Graham & Nachmias, 1971). The temporal frequency of a stimulus is measured by the number of times a display is shown in duration (cycles per second). One hertz (Hz) is one cycle of on and off lasting one second so 2Hz would be 0.5 cycles a second and so on. It has been found that the spatial and temporal frequencies of a visual stimulus can manipulate levels of activation in the brain (Campbell & Robson, 1967). Frequencies are detected by the visual system to allow individuals to detect contrast, thus enabling

them to see things that do not clearly stand out from the background, such as dissociating an object in a dimly lit room from the background.

The contrast sensitivity produced by the spatial and temporal frequencies of stimuli has been under investigation for many decades now. Researchers aim to find the optimum frequency for activation (Campbell & Robson, 1967; Kulikoski and Tolhurst, 1973; Robson, 1966). Robson (1966) found a high spatial frequency, e.g. 4 cycles per degree (c/deg), creates fine bands of light and dark in a stimulus which could be easily processed as the bands are separable by contrast. A low spatial frequency (0.5 c/deg) consists of thick coarse bands and shapes. Each spatial frequency is acted on differently in visual processing. Research has found that the global features of an object are quickly processed, as they only require the detection of low spatial frequencies and contrast (Hughes, Nozawa & Kitterle, 1996). High spatial frequencies are used to process the local details of an object. This takes longer as local features are more detailed and specific (Vassilev, Mihaylova & Bonnet, 2002).

Early Research

A neuron's success in detecting an object relies on the object possessing an optimal spatial frequency. Movshon, Thompson and Tolhurst (1978) found, whilst conducting single cell recordings of cat's visual cortices, that the tuning threshold showed a preference for spatial frequencies between 0.3 and 3 c/deg. Additional studies measured the ganglion cells in cat and humans, with single cell recordings, using a range of spatial frequencies from 0.1 to 2c/deg and temporal frequencies from 1 to 16Hz. They found neuronal activation had an optimal sensitivity to temporal frequencies of 3-6 Hz. (Foster, Gaska, Nagler and Pollen, 1995; Tolhurst and Movshan, 1975). Research by Kulikoski and Tolhurst (1973) reported that cells in the visual cortex, known to detect temporal frequencies and flicker in the brain, could easily process low and medium rather than high spatial frequencies. When they increased the temporal frequency of an image, it reached a threshold that caused the viewer to perceive the transient flickering stimulus as sustained.

Nakayama and Mackeben (1989) researched the difference between sustained (constantly present) and transient stimuli (appearing and disappearing interchangeably). Nakayama *et al* (1989) indicated that transient stimuli are active at an earlier stage of visual processing. This could be because transient stimuli are constantly moving so the deeper visual areas do not have enough time to process the localised details, and the earlier stages, due to rod photoreceptors in the eye, respond faster to the global movement of the object. Additionally they found that when a stimulus kept increasing its temporal frequency, it eventually reached a threshold that caused the viewer to perceive the stimulus as sustained.

There are numerous implications drawn from spatial and temporal frequency research. The findings of research in this field can contribute to the diagnosis and understanding of many visual disorders and diseases with indirect visual disorders such as multiple sclerosis (Regan, Silver and Murray, 1977). Additionally temporal frequency research has aided the understanding of flicker rates in visual displays. Without temporal frequency and flicker research, it would not be known that filming a movie at 24 frames per second (24Hz) would produce realistic steady motion (Simpson, Shahani and Manahilov, 2005). Furthermore it is now understood that flickering light, within our threshold of detection, can cause epileptic seizures in a

small percentage of the population. As a result of these findings light bulbs are required to flash at over 50Hz, in order to lower potential side effects and produce steady coherent light for individuals. (Takuno, Komatsu, Hata, Nakajima and Kansaku, 2009). The implications drawn from spatial and temporal frequency research appears to be beneficial for the scientific, medical and the general community.

Robson (1966) conducted a pioneering psychophysical study on spatial and temporal frequencies. It measured psychophysical recordings from participants as they viewed a black and white visual stimulus, displayed at different spatial and temporal frequencies. This was to investigate whether differing frequencies caused varying contrast sensitivity responses in participants. Robson reported that contrast sensitivity and the ability to see the stimulus clearly, peaked at spatial frequencies between 2 and 10 c/deg. In temporal frequencies, contrast sensitivity peaked between 4 and 10 c/sec for both low spatial frequencies of 0.5 c/deg and high spatial frequencies of 4 c/deg. The study demonstrated how high spatial frequencies can maintain contrast sensitivity up to 10Hz, beyond this point contrast sensitivity decreases in both low and high spatial frequencies.

The conclusion of Robson's study was that an optimum spatial and temporal frequency can cause high contrast sensitivity. Robson's psychophysical study did demonstrate that neurons are finely tuned to spatial and temporal frequencies however; he lacked biological evidence to support this. A complete measurement of visual activation is required to validate this assumption and define the thresholds of spatial and temporal sensitivity to gratings in the visual system. Neuroimaging techniques allow researchers to work beyond a single neuron and measure both global and localised activation.

Fast Optical Signals (Electroencephalography & Magnetoencephalography)

Amongst the earliest neuroimaging techniques to study spatial and temporal frequencies was Electroencephalography (EEG). It had an advantage over the early psychophysical studies as it could record fast optical signals, including the scattering and changes in the neuronal activity of the associated area, milliseconds after the onset of a stimulus. Morrone, Fiorentini and Burr (1996) found when using EEG that the temporal frequency of visual evoked potentials (VEP) in infant's peaked at 10Hz. EEG does have its limitations; skull thickness and the scalp can distort the electric fields.

Neuroimaging techniques such as Magnetoencephalography (MEG) are less likely to distort as they use magnetic fields (Cohen and Cuffin, 1983). Fawcett, Barnes, Hillebrand and Singh (2003) recorded the fast optical signal of activation using MEG. Results found peak activation was at 4Hz in the medial visual cortex. V5/MT had a tuning from 1-30Hz (Anderson, Holliday, Singh & Harding, 1996) and V1's peak activation was between 8-10Hz (Fylan, Holliday, Singh, Anderson, Harding, 1997). Limiting factors of MEG are: the signal can be weak and it can be disturbed by external magnetic fields. The measurement of fast optical signals explains neural activity but not haemodynamic activity in the visual cortex. Different techniques are required to examine haemodynamic activity.

Slow Optical Signals (Positron Emission Topography & Functional Magnetic Resonance Imaging)

The majority of neuroimaging techniques and current research measure slow optical signals. This signal measures haemodynamic changes and neurovascular coupling which provide information about activated visual areas. Changes in blood flow are not instantaneous, so the slow signal takes a few seconds to be detected. (Meek, Elwell and Khan, 1995; Lloyd-Fox, Blasi and Elwell, 2010; Ruben, Obrig, Villringer, Bernarding, Hirth, Heekeren, Dirnagl, and Villringer, 1997; Takahashi, Ogata and Atsumi, 2000). This is not to say the current equipment - Near Infra-red Spectroscopy (NIRS) - cannot record fast signals; it can, it is just harder to imply haemodynamic results from it. The fast signal in NIRS is a more recent method of research and requires greater validation (Franceschini & Boas 2004). Vafaei, Meyer, Marrett, Evans and Gjedde (1998) used positron emission topography (PET) with temporal frequencies to study peak metabolic activation in the visual cortex using a reversing checkerboard ranging from 0-50Hz. The cerebral metabolic rate of oxygen corresponded to peak activation, found at 4Hz in the striate cortex. Other PET studies have found peak activation at 8Hz with the temporal frequency tuning depending on the spatial frequency of the stimulus (Fox & Raichle, 1984). PET has the disadvantage of a radioactive trace being injected into the body to record measurements. It appears that different neuroimaging methods produce peak visual activation at separate spatial and temporal frequencies.

Functional magnetic resonance imaging (fMRI) was created nearly four decades after PET and has the advantage of being completely non-invasive (Ogawa, Lee, Nayak and Glynn, 1990). Its use in temporal and spatial frequency research has been valuable. The majority of studies found peak temporal frequency activation at 6-11Hz (Ozuz, Liu, Chen, Iyer, Fox and Gao, 2001; Singh, Smith & Greenlee, 2000; Kwong, Belliveau, Chesler, Goldberg, Weisskoff, Poncelet, Kennedy, Hoppel, Cohen and Turner, 1992). Kaufmann, Elbel, Goessl, Puetz and Auer (2001) tested temporal frequencies between 0 and 22Hz using fMRI. The results found a linear increase in BOLD activation in the visual cortex up to 8Hz, and an activation plateau for frequencies any higher. The plateau of activation after 8 Hz is due to the flicker-fusion threshold. It states that after 8Hz it is difficult for the visual system to differentiate between new and previous cycles because a transient stimulus that is reversing so much that it begins to form a sustained pattern (Macknik, 2006). Toronov, Zhang and Webb (2007) displayed different frequencies whilst measuring participants with fMRI and NIRS. Participants were shown the black and white reversing checkerboard pattern stimulus every 20 seconds, at one of three frequencies (1, 2 and 6Hz). The results found that the largest BOLD activation was at 2Hz and 6Hz. The HbO₂ response from NIRS was similar to BOLD with 1Hz having the lowest and 6Hz the highest level of activation. A limitation of fMRI is that it only measures the BOLD signal which does not contain information about HbO₂; instead it just measures Hb (Lloyd-Fox, Blasi and Elwell, 2010; Obrin and Villringer, 1999).

Near Infra-red Spectroscopy (NIRS)

There have been many ways of recording and experimenting with various spatial and temporal frequencies. It is clear from the findings that different spatial and temporal frequencies cause peak activation, however each type of equipment has recorded differing levels at the point of occurrence. This may be due to individual differences in recording the visual cortex. The current study investigated the haemodynamic

activation, that differing spatial and temporal frequency produced. A relatively new piece of equipment known as Near Infrared Spectroscopy (NIRS) was utilised in this study. NIRS has the potential to aid our understanding of how the visual system processes haemodynamic responses from spatial and temporal frequencies. Its application for neuroimaging was only recognised a few decades ago by Jobsis (1977), who found that infra-red light could measure haemoglobin levels in biological tissue non-invasively. NIRS has not been widely recognised as a mainstream neuroimaging device; however its advantages over other neuroimaging devices, in cost and mobility, are making it increasingly popular (Lloyd-Fox, Blasi & Elwell, 2010).

How NIRS operates

NIRS uses infrared light to measure blood concentration and the oxygenation of haemoglobin in specific areas of the body especially vital organs such as the brain (Jobsis, 1977). NIRS has been used to study several areas in the brain, as well as the visual cortex, such as: the motor cortex (Obrig, Hirth and Junge-Hulsing, 1996), auditory activity (Hoshi & Tamura, 1993), sensorimotor functions (Franceschini and Boas 2004) and cognitive tasks (Chance Zhuang, Unah, Alter and Lipton, 1993). Infra-red light is ideal for non-invasive measurements of brain tissue because all others wavelength of light are absorbed by haemoglobin and water in the body (Jobsis, 1977, Villringer & Chance, 1998). The biological tissue of the brain is quite transparent to IR-light. When illuminated by IR-light between 650 and 1000 nanometres (nm) the targeted tissue allows measurements of haemoglobin parameters to be taken. The IR-light emitted from the NIRS probe passes through the skin, skull and brain tissue arcing back to the detector in a banana shaped curve. The detector is placed, on average, 30mm away from its original source (Jobsis, 1977; Lloyd-Fox, Blasi & Elwell, 2010; Villringer & Chance, 1998). The NIRS source and detector function as a pair on the probe, which is identified as a channel. Wolf, Wolf, Choi, Toronov, Paunescu, Michalos, & Gratton (2003) found that, the more channels an NIRS has, the higher the spatial resolution of the data would be.

The reason NIRS is an excellent tool for measuring the visual cortex is because visual stimuli cause haemodynamic activation in the visual cortex. When oxygen is transported to the cortex by haemoglobin, NIRS can measure it. The rush of HbO₂ into the area alters the absorption rate of I-R light passing through the targeted tissue, detectors on the NIRS probe receive differing amounts of light, depending on activation; allowing measurement.

Advantages and Disadvantages of NIRS

NIRS is an attractive experimental tool because it can measure haemodynamic activity non-invasively (Hoshi, 2003, Lloyd-Fox et al, 2010). The NIRS probe is small and allows free motion in the participant's head. As a result individuals excluded from fMRI: infants, the elderly and patients with mental or physical disabilities can be monitored by NIRS (Hoshi, 2003). NIRS is inexpensive and portable and can therefore be used in a daily life environment (Lloyd-Fox et al, 2010).

NIRS nevertheless has from some notable disadvantages. Individuals need to have blonde or light hair for the oximeter to work effectively. Dark hair attenuates infra-red light due to its high melanin content (Altshuler, Ilyasovand and Prikhodko, 1995). Poor spatial resolution reduces the effectiveness of NIRS in comparison to fMRI, it

has fewer channels in comparison to the voxels used in fMRI (Friston, Frith, Liddle, & Frackowiak, 1991). NIRS's ease of use and manoeuvrability makes it a valuable piece of equipment for measuring haemoglobin responses.

Spatial and Temporal Frequency Research using NIRS

NIRS has the advantage of measuring both HbO₂ and Hb concentrations in the brain. Since Jobsis numerous studies on visual responses to spatial and temporal frequencies have been carried out using NIRS (Heeger, Boynton, Demb, Seidemann & Newsome, 1997). Meek *et al* (1995) presented participants with 30 seconds of visual stimulus and then 30 seconds of a blank control screen. They reported oxygenated haemoglobin (HbO₂) concentrations increasing during stimulus presentation whilst deoxygenated haemoglobin (Hb) simultaneously decreased in the visual cortex. Meek, Firbank, Elwell, Atkinson, Braddick, & Wyatt (1998) tested infants and found peak haemodynamic response were at a temporal frequency of 5 Hz. McIntosh, Shahani, Boulton and McCulloch (2010) performed an NIRS study very similar to the present study. It examined changes in haemoglobin in the visual cortex. Participants viewed a reversal checkerboard stimulus with a temporal frequency of 7.5Hz. McIntosh *et al* (2010) found HbO₂ concentrations increased whilst Hb decreased when the participant viewed the reversing checkerboard in comparison to viewing a control screen.

Present Study

From previous research it can be shown that NIRS has many advantages over other types of neuroimaging equipment. It is reliable in its ability to detect the haemodynamic changes made by spatial and temporal frequencies in visual stimuli.

The current study used bullseye grating patterns with varying spatial and temporal frequencies similar to the stimuli in Robson's (1966) study. It was assumed that, by manipulating the spatial and temporal frequencies of a visual stimulus, different levels of haemodynamic activation could be produced in the visual cortex. The purpose of this study was to test whether a stimulus, with a certain spatial frequency and temporal frequency, could cause peak activation in the visual cortex. Peak activation was to be identified by using NIRS to measure the elicited haemodynamic response.

Method

Participants

Eight participants (3 male, 5 female aged 18-23) took part in the study, all were psychology undergraduates studying at the University of Plymouth. Participants volunteered for the study through a participant pool to obtain course credits. All participants were required to have blonde hair or very light coloured hair. They were required to have normal, or corrected to normal, vision and, most importantly, they must not suffer from flicker induced epileptic seizures. The study took place in the Psychology Vision Lab at the University of Plymouth and was approved by the University of Plymouth Faculty of Science and Technology ethics committee.

Materials

Instructions and consent for the task was given in a printed handout. A debriefing form was given at the end of the experiment.

The hair of the participant was parted using a comb, and hair clips were used to hold the parting in place. A water soluble green pen was used to mark 02 on the participant's scalp, so experimenters knew where to place the probe. The NIRS probe was kept in place using an elasticated headband placed around the circumference of the head.

Instrumentation

An Oxiplex TS (ISS Inc., Champaign, Illinois) dual wavelength (690nm and 830nm) frequency domain oximeter was used to record haemoglobin signals. The four source-detector distances of the probe ranged from 1.98 to 4.08 cm. The oximeter was modulated at a radio frequency of 110MHz with a cross correlation frequency of 4kHz (Franchesquini, Gratton, and Fantini, 2000; McIntosh, Shahani, Boulton and McCulloch, 2010). The oximeter recorded four haemodynamic responses from the participants; the ratio of oxygenated haemoglobin saturation to total haemoglobin concentration as a percent (Oxpc), total haemoglobin concentration (THC) which is equal to the total blood volume fraction localised in tissue, oxygenated haemoglobin concentration (HbO₂) and deoxygenated haemoglobin concentration (Hb) in the visual cortex.

Visual stimulus

The visual stimuli consisted of black and white concentric circles flickering in bullseye pattern. The stimuli was 12 degrees wide and had a field size of 27deg x 21deg, there was a red fixation mark at the centre of the stimuli. The spatial frequency of the stimulus was 0.17 c/deg in the coarse condition and 4 c/deg in the fine condition. In the rest periods between trials a uniform grey screen was presented, the grey was achieved through bit-stealing (Tyler, Chan, Liu, McBride & Kontsevich, 1992). The contrast for all trials was set at 0.97 and the mean luminance was 52 cd/m². The visual stimulation was shown to participants on a desktop computer which had a CRT monitor refresh rate of 100Hz.

Design

The experiment used a repeated measures design to record haemodynamic activation in the visual cortex. The spatial frequency patterns were tested over two days. One day only visual grating with a coarse spatial frequency was used. The subsequent day only fine spatial frequency visual gratings were used. To eliminate order effects, the days the spatial frequency ran were randomised. Contained within each spatial frequency condition were a set of randomised temporal frequencies. The temporal frequencies used, depended on the method the participant was subjected to. Three methods of temporal frequency presentation were used. Table 1 displays the participants in each method. Each run used a visual stimulus with a fixed spatial and temporal frequency lasting six minute. The order of the temporary frequencies was randomised to eliminate order effects.

Method 1 ran six trials using visual stimuli at .94, 1.88, 3.77, 7.55, 15, 30 Hz. Each trial consisted of 63, 5 second blocks. Each block was randomly assigned to display either the grey control screen or the flickering visual pattern while the oximeter recorded the haemodynamic responses.

Method 2 ran 4 temporal frequencies twice (.94, 3.77, 7.55, 30 Hz), so 8 runs were recorded for each spatial frequency. Each run was fixed to show the visual stimulus for 20 seconds then the control screen for 20 seconds. The total 40 seconds formed one cycle, and ten cycles were used in every run.

Method 3 ran 4 temporal frequencies twice (.94, 3.77, 7.55, 30 Hz. In each run 20 fixed pattern cycles consisting of 10 seconds displaying the control screen and 10 seconds presenting the bullseye grating were produced. The 10 second periods had a normal random variation of SD= .05 so the time could have been 9.5 seconds or 10.5 seconds. In this method participants were asked to press a telegraph key after each transition from stimulus to control and vice versa to help maintain attention and visual response throughout the trials.

Table 1: Methods of running the temporal frequency trials

Method	Participants	Temporal Frequencies Used
Method 1	1	.94, 1.88, 3.77, 7.55, 15, 30 Hz.
Method 2	2-5, 8	.94, 3.77, 7.55, 30 Hz each used twice
Method 3	6 & 7	.94, 3.77, 7.55, 30 Hz each used twice

Procedure

When the participant entered the lab the researcher explained the details of the experiment and then they signed a consent form. The participant was asked to look at a red fixation point in the middle of the screen throughout all the trials, and told they could blink naturally throughout. Using the 10-20 Standardised System (Odom, Bach, Brigell, Holder, McCulloch, Tormene & Vaegan, 2010), O2 was located over the visual cortex of the participant using a tape measure. Once O2 had been marked out on the scalp a vertical parting was made in the hair. The NIRS probe was then placed over the parting and an elastic headband was wrapped around the top of the participant's head to secure the probe and shield it from external light. During the experimental runs the participant looked at the screen while it displayed either a blank control screen or one of the reversing bullseye gratings. Concurrently the NIRS oximeter took the four haemoglobin recordings. Once the trials were completed, the headband, probe and clips were removed from the head. The participant was then given a debriefing form.

Analysis

Analysis was conducted to see if there were differences within temporal and spatial frequencies. Trigonometric regression displayed graphs of an average 40 second cycle haemodynamic response, 20 seconds of the stimuli present and 20 seconds replaced by the control screen. The total power of the graphs was analysed using a repeated measures ANOVA to see if changes in haemoglobin activation occurred between conditions. Statistical analysis was carried out in SPSS 17 and Excel.

Results

The results reported no significant differences in haemoglobin changes between the temporal frequencies and no significant change between the coarse and fine spatial frequencies, from the four NIRS output measures ($p > .05$). Participants 1 and 5-8 were excluded from analysis due to calibration errors, so only the data from participants 2, 3 and 4 ($n = 3$) was analysed. There were missing temporal frequencies in some of the participant's data, only .94Hz, 7.55Hz and 30Hz were used and 3.77Hz was omitted.

The participant's responses were averaged together for each measure (Oxpc, THC, HbO₂ and Hb). The descriptive statistics from the averaged responses are reported below in Table 2. From a quick inspection there are clear mean differences between spatial and between temporal frequencies. At 7.55 Hz in the oxygenated haemoglobin (HbO₂) measurement, the fine spatial frequency has a total power of .12 which is double the coarse condition's mean, therefore double the activation occurred in the trial. Deoxygenated haemoglobin (Hb) at .94 Hz had a total power of .02 for fine and .09 for the coarse spatial frequency, this shows less activation

Table 2: The Total f Powers Means and Standard Deviations Over All Temporary Frequencies in Both Spatial Frequency Condition ($n=3$ for each combination)

Temporal Frequency (Hz)	Fine			Coarse		
	.94Hz	7.55 Hz	30 Hz	.94 Hz	7.55 Hz	30 Hz
Oxpc	22 ±.18	.20 ±.12	20 ±.14	.20± .19	.12 ± .06	.20 ± .14
THC	.04 ±.02	.05 ±.04	.05 ±.04	.05 ± .06	.02 ± .02	.06 ± .05
HbO ₂	.11 ±.08	.12 ±.07	.11 ±.07	.10 ± .12	.06 ± .05	.12 ± .10
Hb	.02 ±.01	.02 ±.01	.02 ±.01	.09 ± .10	.04 ± .03	.07 ± .06

Note. The Means and Standard Deviations are set out as ($M \pm SD$)

occurred during the coarse trial as there was more Hb in the visual area. The mean total powers are also displayed in Figure 1, differences between spatial frequencies at 8 Hz are noticeable in all of the measures. Total power from each condition was obtained from the first and third harmonic components of the control screens square wave gratings (Campbell and Robson, 1967). These were then compared to sine wave responses from the bullseye gratings. The first and third harmonics for each measure was obtained from trigonometric regression of the raw data. Figures 2a to 5f illustrate participant 3's trigonometric graphs for conditions in the four measures. The trigonometric regression produced non-linear lines of best fit for haemodynamic activation in each spatial and temporal frequency condition. Figures 2 to 5 show how activation differs in the visual cortex when the visual stimulus is presented and when the control screen is shown. They also show how long it takes haemodynamic changes to occur in the visual cortex depending on what is present on the screen. The first 20 seconds of the trigonometric graphs is when the control screen is displayed and the last 20 seconds is when the bullseye stimulus is shown.

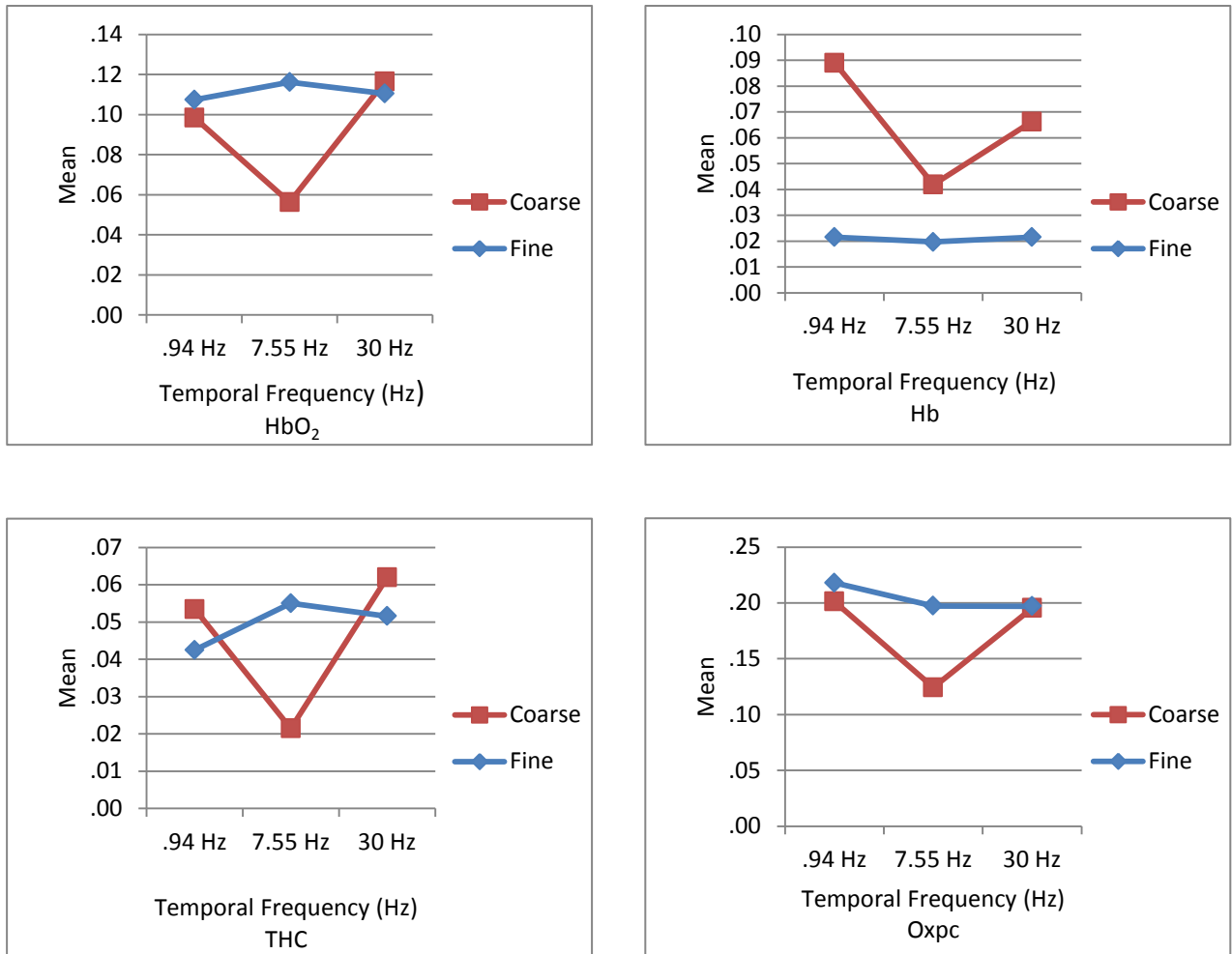


Figure 1: Displays a Graph for the Mean Total Power in each NIRS Measure. The Graphs Compare the Spatial and Temporal Frequency condition.

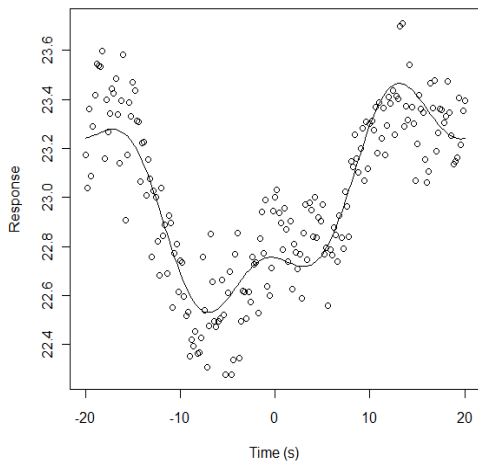


Figure 2a. Fine .94Hz

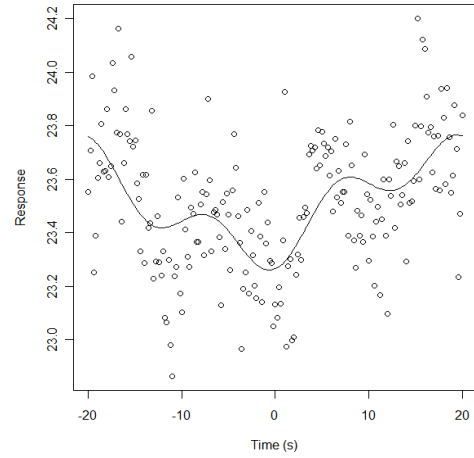


Figure 2d. Coarse .94Hz

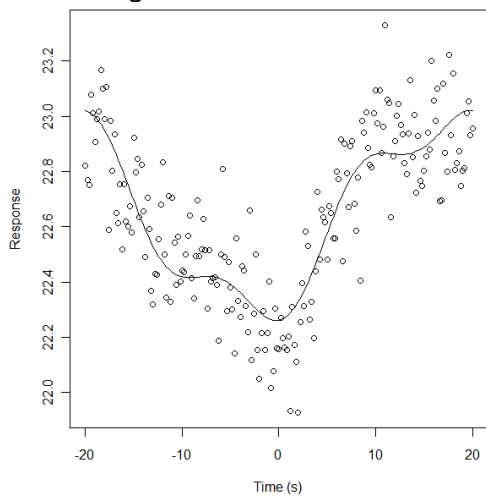


Figure 2b. Fine 7.55Hz

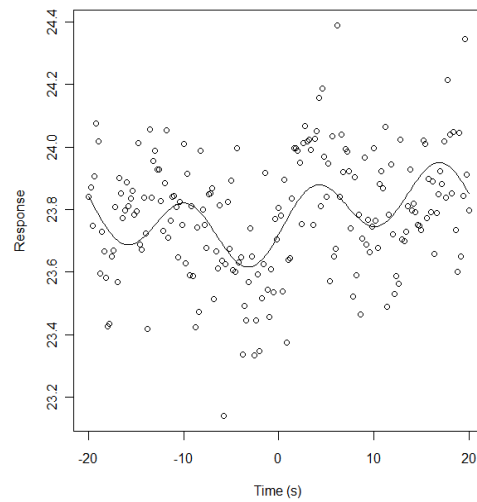


Figure 2e. Coarse 7.55Hz

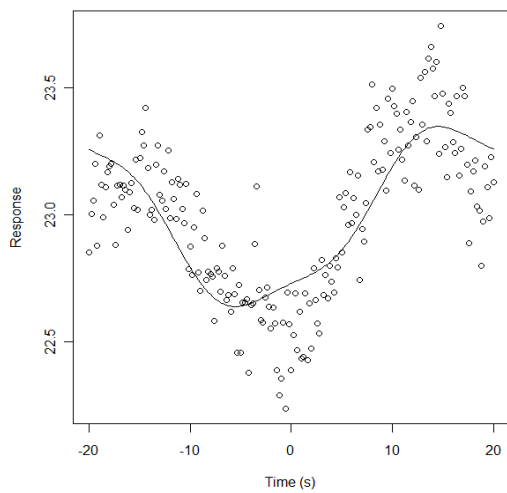


Figure 2c. Fine 30Hz

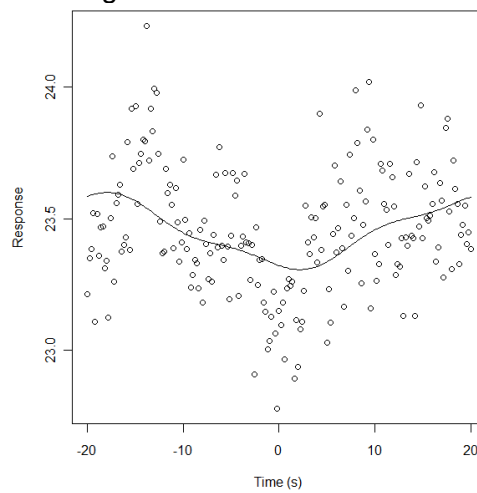


Figure 2f. Coarse 30Hz

Figure 2 a-f: Graphs showing the Non-linear line of best fit for Oxygenated haemoglobin (HbO₂) NIRS measurements for Participant 3.

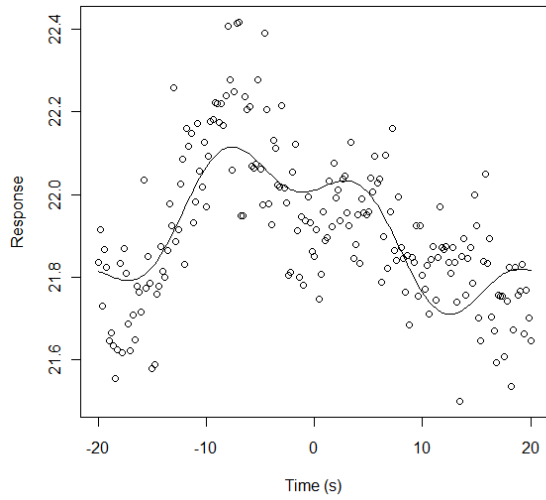


Figure 3a. Fine .94Hz

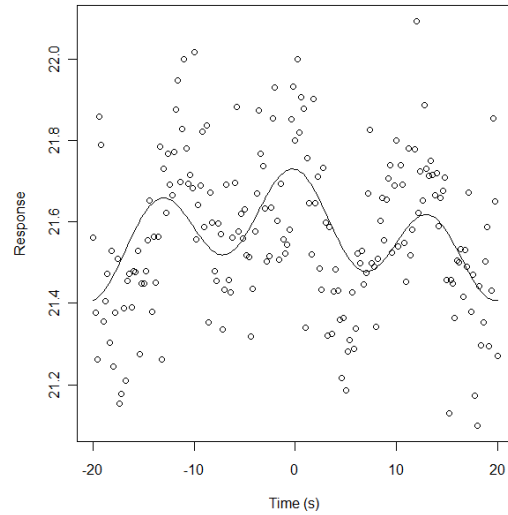


Figure 3d. Coarse .94Hz

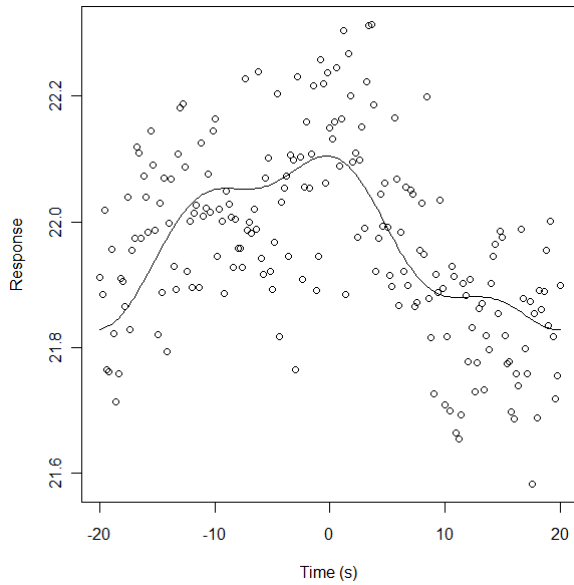


Figure 3b. Fine 7.55Hz

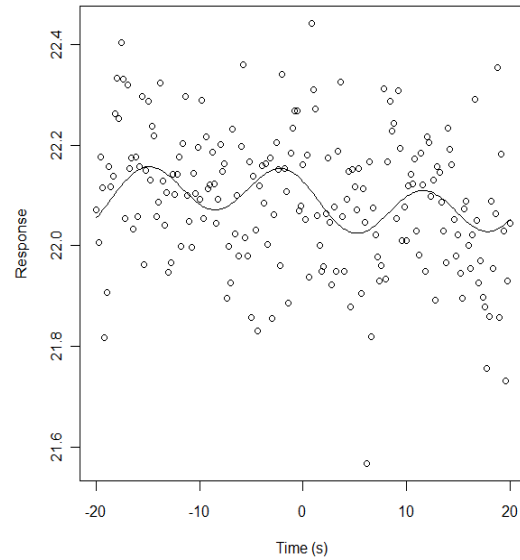


Figure 3e. Coarse 7.55Hz

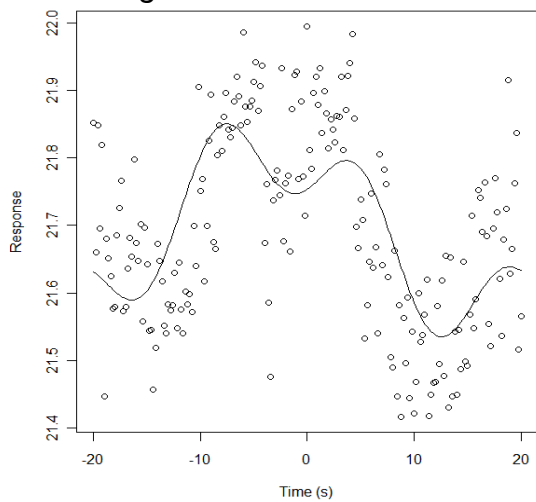


Figure 3c. Fine 30Hz

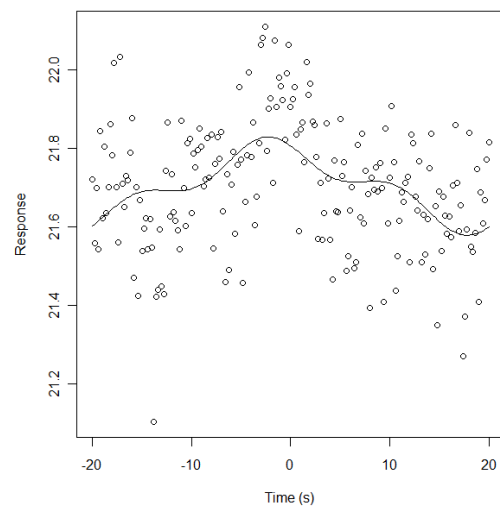


Figure 3f. Coarse 30Hz

Figure 3 a-f: Graphs showing the Non-linear line of best fit for Deoxygenated haemoglobin (Hb) NIRS measurements for Participant 3.

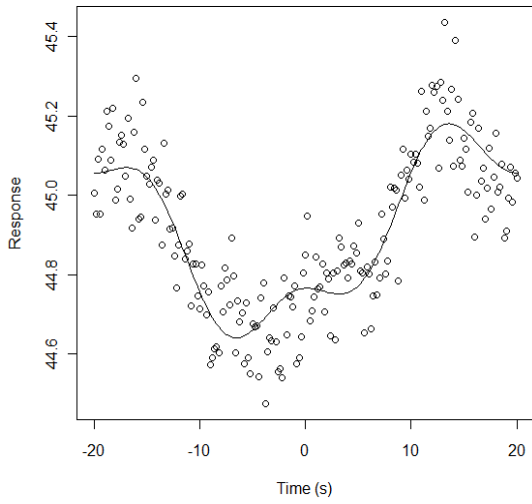


Figure 4a. Fine .94Hz

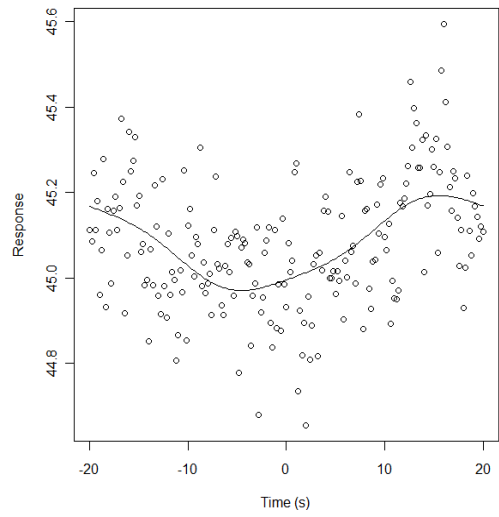


Figure 4d. Coarse .94Hz

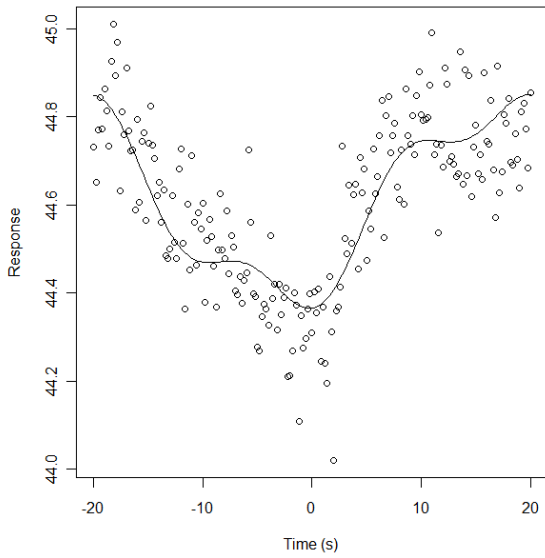


Figure 4b. Fine 7.55Hz

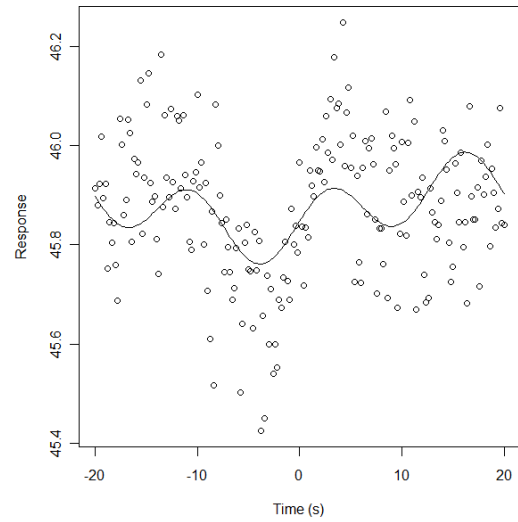


Figure 4e. Coarse 7.55Hz

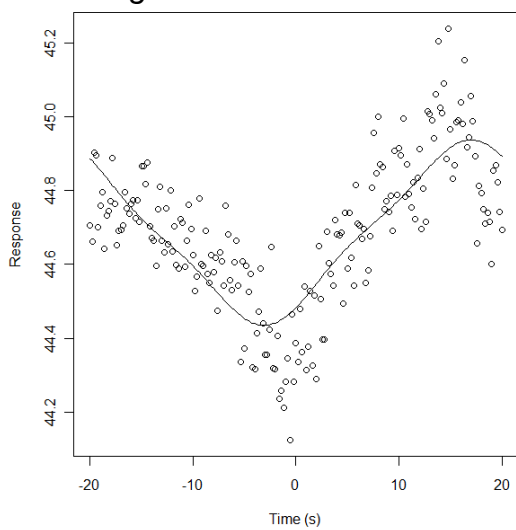


Figure 4c. Fine 30Hz

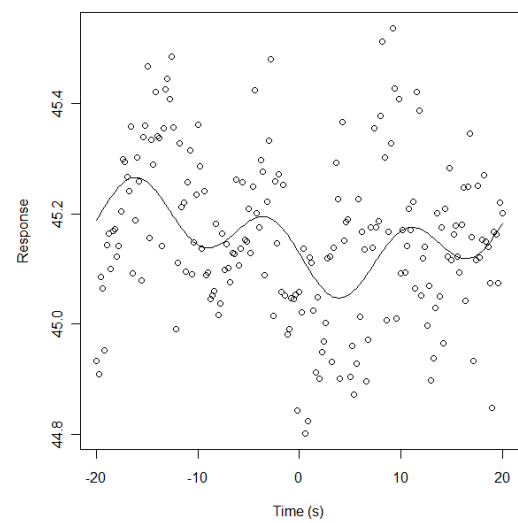


Figure 4f. Coarse 30Hz

Figure 4 a-f: Graphs showing the Non-linear line of best fit for Total haemoglobin concentration (THC) NIRS measurements for Participant 3.

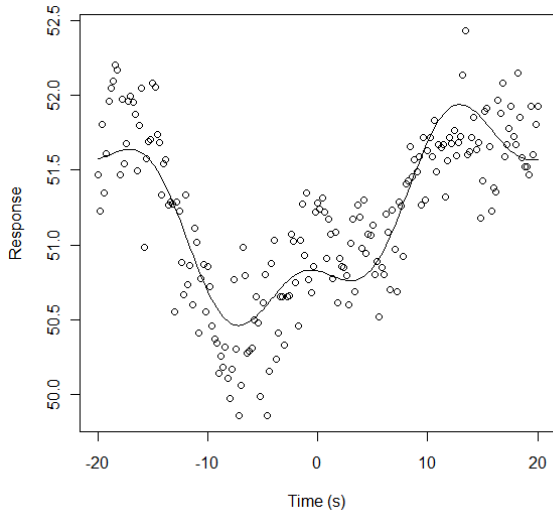


Figure 5a. Fine .94Hz

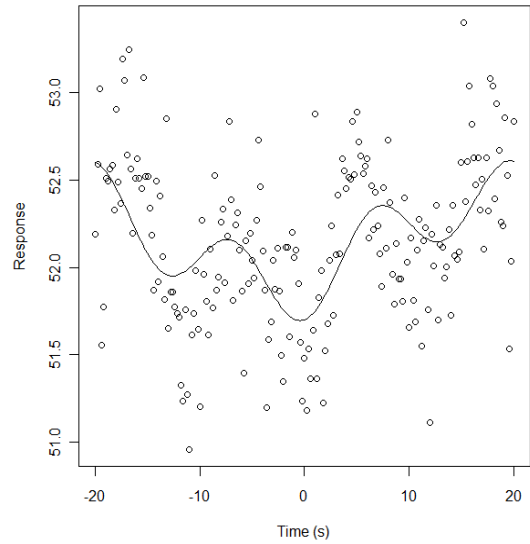


Figure 5d. Coarse .94Hz

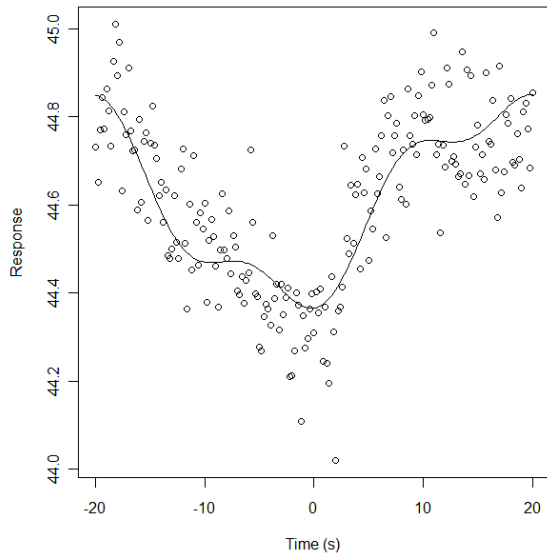


Figure 5b. Fine 7.55Hz

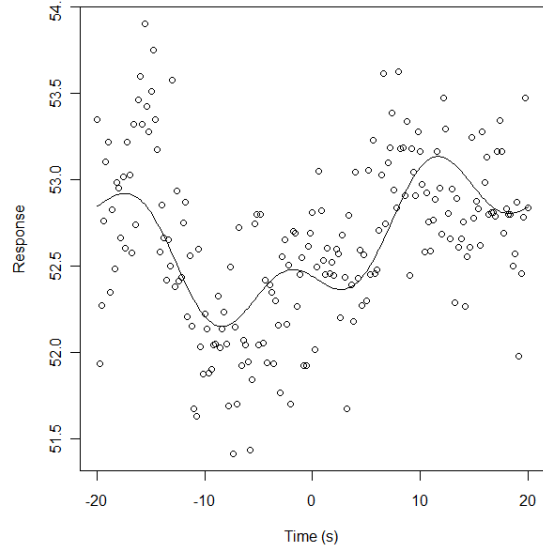


Figure 5e. Coarse 7.55Hz

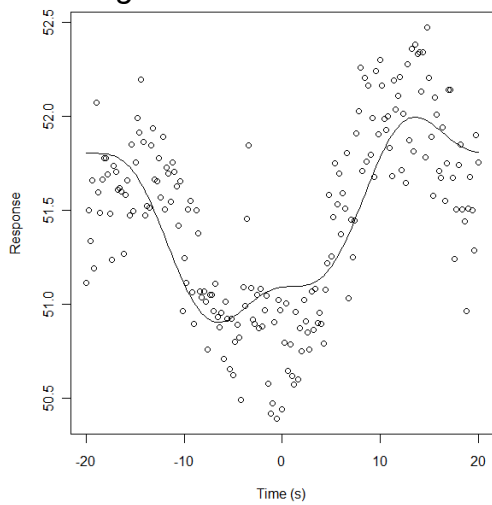


Figure 5c. Fine 30Hz

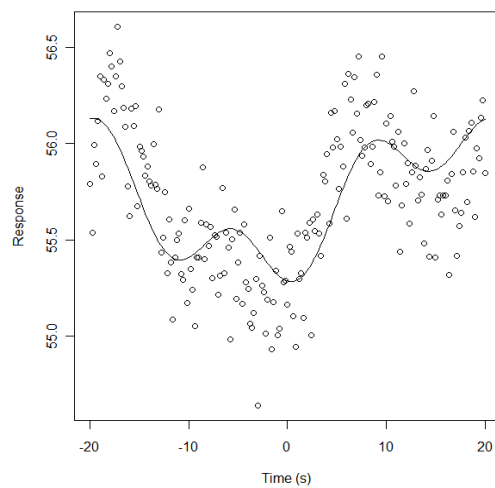


Figure 5f. Coarse 30Hz

Figure 5 a-f: Graphs showing the Non-linear line of best fit for oxygen saturation as percent (Oxpc) NIRS measurements for Participant 3.

If the graphs in Figures 2-5 displayed a horizontal line this would represent no difference in haemodynamic activation from when the stimulus was on or off. However there is a clear increase in HbO₂, THC and O_{xpc} with a decrease in Hb under all conditions when the bullseye stimulus is present on the screen and the opposite when the control screen is viewed. This shows that during the experimental trials activation continually rose at a steady rate until the stimulus was replaced with the control screen and at that time activation decreased, thus forming a wave.

When the graphs are studied it appears that the fine spatial frequency conditions produce smoother curves of activation compared to the coarse condition. Whereas in the coarse conditions activation seems to plateau then rise (vice versa for Hb) when the stimulus is presented, an example of this is shown in Figure 2d. This suggests that the fine spatial frequency causes more haemodynamic activation than coarse spatial frequencies in the visual cortex.

Although the descriptive statistics show differences between the mean total power, it cannot be certain that the differences are significant or not until an ANOVA is conducted. The total power for each measurement was subject to a repeated-measures ANOVA, to demonstrate whether or not the different frequencies, within the spatial and temporal frequencies, were significantly different in activation response.

Sphericity was assumed for the oxygenated haemoglobin (HbO₂) in the temporal frequencies however, for the spatial and temporal frequency interaction sphericity was violated, as it was $p = .05$ so the f -value was corrected using Greenhouse Geisser. The spatial frequency cannot have a test of sphericity as it only has two levels. No significance was reported for differences with spatial frequencies $F(1, 5) = .30, p < .60$, or differences within temporal frequency $F(2, 10) = .97, p < .41$. No significant interaction between spatial and temporal frequency $F(1, 8, 5.63) = .75, p < .44$ was found. The lack of significance meant the oxygenated haemoglobin in the participants responses did not change significantly between the temporal and spatial frequency conditions.

The deoxygenated haemoglobin (Hb) measure was similar with Mauchly's test of sphericity, assuming sphericity for temporal frequencies and the interaction between temporal and spatial frequencies. The main effects for differences between spatial frequencies reported no significance at $F(1, 5) = 3.72, p < .11$, so did the difference between temporal frequency in the Hb measure $F(2, 10) = 2.22, p < .20$. The interaction between spatial and temporal frequencies $F(1, 8, 5.34) = 1.28, p < .31$ demonstrated no significance. No significance means the deoxygenated haemoglobin responses from the participants did not change significantly between the different temporal and spatial frequencies.

The third measure, total haemoglobin concentration (THC), which is equal to the total blood volume fraction localised in tissue, had its sphericity met for the temporal frequencies and the interaction between temporal and spatial. The main effects reported no significance between spatial frequencies $F(1, 5) = 1.10, p < .77$, or temporal frequency $F(2, 10) = 1.38, p < .30$ and no significant interactions between spatial and temporal frequencies $F(1, 8, 7.12) = 2.72, p < .11$. No significance means the THC

responses in the participants did not change significantly between temporal and spatial frequencies.

The final measurement of percentage of oxygen saturation (Oxpc) reported a temporal frequency of $p = .20$ in Mauchly's test and the interaction between temporal and spatial reported no significance $p = .83$ therefore, sphericity was met. The main effect reported no significance for spatial frequency $F(1, 5) = .40$, $p < .68$, temporal frequency $F(2, 10) = .09$, $p < .77$ and no interaction between spatial and temporal frequency $F(1, 8) = .75$, $p < .50$. No significance means that the percentage of oxygen saturation in the participants did not change significantly between temporal and spatial frequencies. The non significance of the ANOVA and the trigonometric regression graphs will be assessed in the discussion.

Discussion

The current study investigated the haemodynamic responses produced from visual bullseye gratings varying in spatial and temporal frequencies in order to ascertain if different frequencies altered haemodynamic activation in the visual cortex. The main statistical analysis reported no significant differences in activation between the fine/high spatial frequency of 4 c/deg and the coarse/low spatial frequency at 0.17 c/deg. In addition there were no significant differences between the three temporal frequencies (.94, 7.55 & 30Hz). Despite this, trigonometric regression graphs of the data managed to illustrate increased activation in the visual cortex when the visual stimulus was present, when compared to the control screen.

Findings and Analysis

The ANOVA reported no significant increases in: oxygenated haemoglobin, total haemoglobin concentration, oxygen saturation as a percentage. No significant decreases in deoxygenated haemoglobin from when the visual stimulus was shown compared to the grey control screen ($p > .05$) was reported. No significance from the ANOVA could have been due to the small sample size during analysis ($n=3$). In future studies a larger subject sample would be of greater advantage as a larger sample causes the amount of bias to get smaller. Furthermore a larger sample size would reduce the influence individuals anatomical differences would have on the experimental results: such as skull thickness and hair colour etc. (Steedman, 2008). McIntosh *et al's* study (2010) found significant increases in HbO₂ concentrations and Hb decreases when viewing the visual stimulus and only used 7 participants, it would appear that having a large sample is not essential for significant, so long as the participants and oximeter produces reliable responses.

The descriptive statistics reported the mean total power from the spatial and temporal frequencies. They were compared to one another to see if different frequencies produced different amounts of haemodynamic activations. Reliable data would reinforce the notion that neurons have thresholds of activation to specific spatial and temporal frequencies seen in the visual field. It seems the findings were not consistent with this theory or with Robson's (1966) study and previous research into visual activation and spatial and temporal frequencies. The current study found fine and coarse spatial frequencies had similar HbO₂ activation at .94Hz and 30Hz and different responses at 7.55Hz (Figure 1). Robson's study found contrast sensitivity was similar in fine and coarse spatial frequencies at 7.55Hz and 30 Hz, with

activation at .94Hz being dissociable. When looking at the current experimental mean total power, the fine spatial frequency condition in HbO₂ slightly follows Robson's findings of activation. With 7.55 Hz having the most activation and .94Hz and 30Hz having slightly less either side (Figure 1) although the difference in this experiment is not as defined as Robson's.

The deoxygenated haemoglobin (Hb) response was recorded because its increase occurs when there is no activation in the visual cortex or when the participant cannot see the visual stimulus clearly enough to process it. Hb recordings from participants reported a high total frequency mean of .09 at .94Hz in the coarse condition, which was the highest Hb total power. The finding coincides with Robson's study because, at .94Hz, a stimulus with a low spatial frequency elicited low contrast sensitivity to the visual stimulus. Therefore in the current study, the coarse spatial frequency bullseye grating set to .94Hz produced the least activation in the visual cortex. This could explain why Hb had such a high total frequency at .94Hz because participants had struggled to see the image due to its low contrast and low temporal frequency. Accordingly, the haemodynamic response was not as high in the coarse condition because participants did not attend to the stimulus as much. When a visual stimulus is unattended to, the cerebral blood flow decreases as the localised region of the brain is not activated (Takahashi, Ogata & Atsumi, 2000). Participant 3's trigonometric graph of the coarse Hb response for .94Hz supports this assumption (Figure 3d). The activation levels fluctuate continuously when the stimulus is presented and when the control screen replaces it, there are no clear sign of Hb decreasing during the visual display.

The trigonometric graphs for participant 3 illustrate smoother activation in the fine spatial frequency condition's curves compared to the coarse condition. An explanation for this could be that the coarse bullseye gratings having a low spatial frequency which produced thicker black and white bands. These bands are harder to see and as a result, they elicit less activation. This finding is not in conjunction with Robson's study which found activation for fine and coarse spatial frequencies at 7.55 and 30Hz were similar. Contrast sensitivity was only dissociable when a stimulus with a low spatial and temporal frequency was presented compared to a high spatial frequency stimulus at the same temporal frequency.

Despite no significant findings, the current study has shown that, when a visual stimulus is presented haemodynamic activation increases even if it's not significant. This can clearly be seen in the trigonometric graphs (Figures 2-5). The activation response from HbO₂, THC & O₂ increases when the visual presentation was shown in all of the spatial and temporal frequency conditions. When the control screen appeared activation gradually decreased, returning to baseline recordings. Increases in HbO₂ were due to increased activation in the visual cortex which required more oxygen to process the complex and moving stimulus. There are similarities in haemodynamic activation especially in HbO₂ at .94Hz and 30Hz. The differences between spatial frequencies at 7.55Hz could imply that differences in haemodynamic activation only depend on spatial frequencies when an image is at the optimum temporal frequency, e.g.; 7.55Hz.

An alternative explanation for the increase in activation when the bullseye stimulus is presented could be because it is different to the control screen. The fact it varies in

spatial and temporary frequency may not make a difference. Future studies could examine a dissociation between the experimental and control screen, by varying the control screen. It could contain various objects or the bullseye could be presented in the control screen statically without moving or flickering to illustrate how activation is different from obvious sustained and transient images.

The four measures NIRS recorded were helpful in displaying differences in activation. In Lloyd-Fox's review (2010) 94% of published fNIRS infant studies use HbO₂ as the preferred measure for cortical activation. This was due to the measure typically having the highest signal to noise ratio (SNR). Additionally the direction of the HbO₂ change was the most consistent in infants and adults studies, an increase in HbO₂ was reported in 93% of the 94% of published studies. However the other 7% of studies, reported a decrease in HbO₂ when the stimulus was presented, implying that oximeters recordings are not always correct. Csibra, Henty, Volein, Elwell, Tucker, Meek & Johnson (2004) found this when testing participants however, unlike the current study a static face was the stimulus in the experimental condition of their study. The NIRS sensor may not have been placed over the fusiform face area of the brain which may have resulted in a negative HbO₂ response (Sergent, Ohta & MacDonald, 1992).

Limitations during the experiment

The calibration errors which resulted in five participants being discarded were due to the NIRS oximeter and its capability to record the response from the visual cortex. Before the trials began the NIRS oximeter on occasion would produce no reading because the hair was too dark and the light attenuated (Altshuler *et al*, 1995). Also the NIRS probe was not always positioned correctly over the target area or the probe moved out of place when securing the headband. These complications occurred a few times and as a result they slowed the experiment down. As with most NIRS studies the current study found hair reduced the probes grip on the head, causing it to slide out of place, creating anomalies and noise in the data (Lloyd-Fox *et al*, 2010). Excluding the noise and anomalies produced due to random variations and practical factors from the analysis, was time consuming and precautions should be taken in future studies to prevent it.

During experimentation the participants appeared bored and uninterested, especially when taking part in the second spatial frequency condition. This ran a few days after the first and could be due to 'carry over' effects from one trial to the next. It seems plausible that the participant's attention to the experiment was decreasing after every trial because they were passively watching a visual stimulus. Resultantly the haemodynamic response produced was not as strong as the first spatial frequency. Participant 2 ran her fine spatial frequency condition first and coarse condition second. This could explain why she produced a poor trigonometric response in the coarse spatial frequency condition; because she no longer paid attention to the passive visual stimulus due to loss of attention.

Kojima and Suzuki (2009) conducted research on attention using NIRS, the results suggested attention and HbO₂ increases are positively correlated. When paying active attention to a visual scene it enhanced HbO₂ responses much more than passively watching a stimulus. The present study found that gradually through the trials in the second spatial frequency condition, participants responses were not

changing as much between the stimuli being present and the control replacing it. This was due to the mundane visual stimuli causing boredom and a loss of attention. To try and combat this, Method 3 was designed. The experimenter instructed participants to press a telegraph button every time the stimulus and control screen came on screen. The theory being, that it would make the participant actively attend to the visual stimulus. Unfortunately participants in Method 3 were excluded due to calibration and recording errors. Future research is required on active and passive attention to visual stimuli. Moran and Desimone (1985) noted that area V4 of the visual cortex had been shown to have strong attentional modulation. The V4 response to an unattended stimulus is very low compared to attended stimuli. They reported selective attention altering the firing of V4 cells by 20%.

Haemodynamic activation corresponds to the evidence that humans have neurons that are finely tuned and directionally selective and sensitive to spatial frequency and size, in the visual cortex (Blakemore & Campbell, 1969). Cells in the visual cortex are finely tuned to activate to a certain range of frequencies. This study has contributed to finding the threshold of frequencies which cells in the visual cortex can selectively respond to. The reason the study used bullseye grating in the experiment was because a contracting sine wave bullseye has the same linear speed at all eccentricities, unlike a rotating pattern where the edges are faster than the centre. As a result, more activation is produced in the visual cortex compared to other stimuli. An additional advantage in using a bullseye is that it has orientations in all directions causing more firing in the visual cortex (Simpson, Shahani & Manahilov, 2005).

Advantages of using NIRS

Using NIRS to record the haemoglobin activation in this study has been helpful due to its capability to measure brain activity through intact skulls (Hoshi, 2003; McIntosh et al, 2010; Villringer & Chance, 1998). Its small probe permitted free motion allowing the participant to move whilst during testing. Its application means NIRS can be used on a variety of individuals which fMRI and PET studies exclude such as: the elderly and patients with mental or physical disabilities can now have non-invasive haemoglobin recordings. NIRS has been used to measure haemodynamic responses in a range of brain functions. It has looked at the effects of alcohol on haemodynamic changes (Obata, Morimoto, Sato, Maki & Koizumi, 2003), also it has helped individuals with visual disturbances such as optic neuritis, by detecting visual dysfunctions noninvasively at patients' bedsides (Miki, Nakajima, Takagi, Usui, Abe, Liu & Liu, 2005). One of its clear advantages is its applications for infants (Kusaka Kawada, Okubo, Nagano, Namba, Okada, Imai, Isobe, & Itoh, 2004; Meek et al, 1998; Taga, Asakawa, Hirasawa, & Konishi, 2004), as infants struggle to keep still in fMRI machines. By running visual tasks on infants and recording the haemodynamic response, researchers are able to see how the visual cortex develops and matures over time. Compared to fMRI research recording visual responses to spatial and temporal frequencies NIRS have a couple of key advantages. fMRI studies have to scrap scans if the participant moves their head in the scanner because it produces unreliable results which cannot be used, the scraping of data can waste time and money (Jezzard & Clare, 1999). Also NIRS oximeters measure HbO₂ and Hb concentrations in the brain, whereas fMRI only measures the BOLD signal which does not contain information on HbO₂ (Cui, Bray, Bryant, Glover, & Reiss, 2011; Lloyd-Fox, Blasi & Elwell, 2010).

NIRS is an inexpensive neuroimaging device and its small and mobile design allows it to be used in daily life environment such as bedsides. In contrast, other neuroimaging tools require large shielded room and cost a lot to not only purchase but also to maintain, for example MEG uses expensive liquid helium to keep the magnets and instruments constantly cool (Vrba & Robinson, 2001). NIRS has the further advantage of being able to be used simultaneously with other neuroimaging equipment because it uses no metal, only glass and plastic in its probe. Therefore it can be used whilst a participant is in an fMRI machine (Kleinschmidt, 1996; Toronov et al, 2007; Villringer & Chance, 1998) as well as other neuroimaging devices.

Disadvantages of using NIRS

The accuracy and reliability of NIRS as a neuroimaging device is still not widely accepted in the medical or scientific community, this is due to incomplete knowledge of which region in the brain is sampled by NIRS light. NIRS findings can be hard to generalise to the population due to individual differences in skull thickness, hair colour, brain size and probe placement (Okada and Delpy, 2003; Wolf *et al*, 2003). The current study ran into a number of these problems as it used men and women who have various skull thicknesses, also the hair colour and length was different in all individuals. As mentioned NIRS can only reach a depth of around 2cms meaning deep brain structures such as diencephalon are excluded from NIRS measurement (Hoshi, 2003). Finally the spatial resolution of NIRS is poor in comparison to fMRI, because there are fewer channels in comparison to the voxels used in fMRI (Friston, *et al*, 1991).

Conclusion

To conclude, despite the results having no significant in the ANOVA on all four measures, it is clear that bullseye stimuli can manipulate and increase the haemodynamic activation in the visual cortex. The mundane bullseye stimulus may not have produced as much activation compared to a real world object, but the ability to easily manipulate the visual stimuli's spatial and frequency has been beneficial to the study. Research into visual stimuli's spatial and temporal frequency is helpful in identifying a stimulus with spatial and temporal frequencies able to cause high levels of activation in the visual cortex. This can assist in the understanding of how haemoglobin is involved in the processing of visual stimuli and how the visual cortex operates in the human body.

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