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Abstract

The effects of spatial and temporal frequencies of a visual, sinusoidal bullseye stimulus on haemodynamic responses in V1 were investigated using Near-Infrared Spectroscopy. Eight psychology undergraduates viewed a reversing bullseye pattern with differing spatial and temporal frequencies and haemodynamic responses in V1 were measured. It was found that optimum responses were recorded when the spatial frequency was 4.00 c/deg and when temporal frequency was 7.55 Hz; however no significant differences between frequencies were found. The mixed findings provided some support for past findings, implying that cells are finely tuned to detect certain stimulus properties, and demonstrated NIRS as a valid measuring tool in vision research. Future research needs to be conducted in order to improve the quality of the data.

Contents

Statement of Ethical Clearance
Ethical clearance was granted by the University's ethics committee and, throughout the course of the project, all ethical guidelines were adhered to in order to ensure the participants were not subjected to any kind of harm. Before each experiment, participants were given a consent form and a briefing explaining that they will not be deceived, all data will remain anonymous and that they have the right to withdraw at any time. After the experiment, participants were given a debriefing, a sheet reiterating what was said in the brief and also details of the researcher and supervisor so they can be contacted if the participant wished to do so.

Statement of Data Collection
Participants were recruited using the university's psychology participation pool. Data collection was shared between myself, Thomas Bridge, Sam White, Theodora Tilney, Daniel Edgcumbe and Wendy Read. Altogether, eight participants were recruited via the participation pool.

Introduction
The rise of neuroimaging techniques over the last half a decade has lead to a great focus on mapping the functional properties of the brain. One area that has seen a vast deal of research is the psychophysical properties of vision. Many studies have looked at various stimulus properties and how they affect detection of stimuli. In particular, many studies have investigated the effects of stimulus spatial frequencies (SF) and temporal frequencies (TF) on the ability of our visual systems to detect that stimulus. Both SF and TF can be described in terms of the types of detectors they represent in the visual system (Kulikowski & Tolhurst, 1973). The detectors specific for SF properties work by analysing the shape and size of the stimulus and respond to both stationary and moving patterns. The detectors specific for TF are sensitive to temporally modulated patterns and when they become excited, it allows the visual system to perceive movement or flicker.

One classic study that investigated this before neuroimaging techniques were available was by Robson (1966). This study investigated SFs and TFs of gratings on contrast sensitivity of the visual system, that is, the ability of the visual system to detect differences in shading. It was found that when both SFs and TFs were high, contrast sensitivity was decreased. However, it also showed that when the effects of SF and TF were combined together, very low SFs and TFs also led to low contrast sensitivity. In other words, when SF and TF were either very high or very low, the human visual system had difficulty in the detection of that stimulus. Cells which analyse spatial properties give a continuous, sustained response as spatial features of the stimulus remain constant and so produce a steady level of neuronal firing until the stimulus is removed. Cells which analyse temporal properties give varying, transient responses as the neurons only fire when there is a temporal change in the stimulus such as it turning off or on. These cells are unresponsive whilst the stimulus remains stationary (Kulikowski & Tolhurst, 1973). It appears that responses in the visual system are at their optimum when SF is around 4.00 c/deg and when TF is

around 6.00 – 8.00 Hz. These findings have prompted a great body of research looking into why this might be the case.

One possible explanation for this effect is that neurons within the visual cortex are finely tuned so that they only detect certain frequencies. That is, cells in the visual cortex will only become activated when the observed stimulus has SFs and TFs that those cells are tuned to detect (Foster, Gaska, Nagler & Pollen, 1985). However, the results found in Robson's (1966) study were based on self-reported psychophysical data and, therefore, cannot shed any insight into whether this explanation is feasible. Fortunately, development of technology and the increased use of neuroimaging techniques in scientific investigation have opened up a scope to investigate this. One of the first methods to be developed was the direct measurement of evoked potentials by placing electrodes over the relevant places of the scalp.

A study by Campbell and Maffei (1970) used this method in order to investigate evoked potentials in response to gratings varying in SFs. Electrodes were placed on the scalp on and around the visual areas of the brain and the participants psychophysical contrast threshold was established. Contrast was then set at the threshold and evoked potentials were recorded at uniform increments of contrast levels. It was found that, for all SFs used, the amplitude of evoked potentials increased as contrast level increases. It was also found that lower SFs started producing evoked potentials at lower contrasts and that little, if not none, evoked potentials were recorded when SF went below 3.50 c/deg. These results provide some support for Robson's (1966) study and for the idea that cells only respond to certain frequencies as it appears that SFs are only processed from around 3.00 c/deg upwards.

Many other studies have found similar results to Campbell and Maffei (1970) but the use of electrophysiological recordings is only a basic method for providing neurological evidence for visual processes and presents a number of problems. One such problem is that, even though evoked potentials have a great temporal resolution, the spatial resolution of such recordings is poor (Russo, Martinez, Sereno, Pitzalis & Hillyard, 2001). As only a relatively small number of electrodes are usually used in studies, it means that finding the precise location of the cortex responsible for the neuronal activity can be difficult. Even a single electrode on its own can cover quite a number of cortical areas. In order to address this problem, other methods that utilise electrical signals have been developed. One major development has been that of Magnetoencephalography (MEG). MEG works by measuring the magnetic fields that are created during electrophysiological activity in firing neurons (Hämäläinen, Hari, Ilmoniemi, Knuutila & Lounasmaa, 1993).

One study that used MEG to investigate the TF tuning of cells in the visual cortex was conducted by Fawcett, Barnes, Hillebrand and Singh (2004). Specifically, they focused on the frequency-specific oscillatory power occurring during neuronal activity whenever a task is performed which is measured using event-related desynchronisation (ERD) measures. Six participants were tested using a checkerboard stimulus with a 15 second on/off period and varying TFs presented in a random order. Difference in MEG data between active and passive states showed that the amplitude of oscillatory power varied as a function of temporal frequency with response peaking at 8.00 Hz and decreasing either way. It also showed that the majority of neuronal activity occurred within primary visual cortex (V1). This provides

support for previous findings whilst providing more accurate information regarding location of activity.

Even though the use of MEG can provide much more accurate data than measuring evoked potentials, this method still presents a few disadvantages. One of these disadvantages is that the magnetic signal measured in MEG comes from a fairly weak source and so may be subject to interference from external electrical fields (Leuchter & Cook, 1997). This problem can be overcome by using methods that don't rely in the electrical signals in cells. This includes the method of Positron Emission Tomography (PET). In this method, the individual is administered with a radioactive isotope attached to a chemical. When it decays, it releases single photons that can be detected by a gamma camera, which gives information about the distribution of isotopes in the organ (Ollinger & Fessler, 1997).

A study by Vafaee and Gjedde (2000) used PET in order to study the coupling between energy requirements of neurons and the brain's supply of glucose and oxygen. They tested the hypothesis that cerebral blood flow (CBF) increases as oxygen consumption, resulting from neuronal activity, increases. Participants were required to view a yellow-blue, reversing checkerboard with varying TFs of 1.00, 4.00 and 8.00 Hz. It was found that CBF in V1 increased as a response to stimulus presentation and there was little difference between the various TFs, although it did peak at 8.00 Hz which supports previous findings.

PET has become a very widely used neuroimaging technique used in both clinical and experimental settings. However, one problem with its use is that it has relatively poor spatial resolution compared with other methods (Imbesi, 2009). This can be overcome using neuroimaging techniques that involve the measurement of oxygen levels in the brain. In particular, an extensively used technique is that of functional magnetic resonance imaging (fMRI). This method doesn't measure neuronal activity directly but, rather, processes that are associated with it. These include cerebral blood flow (CBF), cerebral blood volume (CBV) and blood oxygenation. In most cases, the blood oxygenation is measured using the blood oxygenation dependent level (BOLD) response which measures the local susceptibility changes that come from changing levels of deoxyhaemoglobin (Ogawa, Menon, Tank, Kim, Merkle, Ellermann & Ugurbil, 1993).

As haemodynamic measurements are not a direct measurement of the brain's response to stimuli, it is questionable whether fMRI can be used as a valid technique. One study that set to investigate this was conducted by Gratton, Fabiani, Corballis, Hood, Goodman-Wood, Hirsch, Kim, Friedman and Gratton (1997) who set to compare event-related optical signals (EROS), visual evoked potentials (VEP) and fMRI in the measurement of neuronal activity in response to visual stimulation. Participants were tested using reversing black and white grids with a set TF and SF and were measured using the three different techniques. Spatial correspondence was found between EROS and fMRI and temporal correspondence was found between EROS and VEP data. This provides a validation for the use of haemodynamic measurements in investigating neuronal activity as a response to visual stimuli.

One study by Singh, Smith and Greenlee (2000) used fMRI measurements to measure specifically the effect of TF and SF on the haemodynamic response of neurons in the visual cortex. The stimulus used consisted of horizontally oriented, sinusoidal luminance gratings drifting vertically upwards which participants viewed using a mirror. Each of the gratings consisted of one SF moving at a fixed velocity and one TF. Both SF and TFs were varied with SFs either being .40, 1.00, 2.00, 4.00 or 7.00 c/deg and TFs being .00, 2.00, 5.00, 9.00 or 18.00 Hz. The stimulus was presented with an on/off cycle of 27 seconds each. The results showed that all participants had a stimulus correlated haemodynamic response with varied responses being a function of both SF and TF. All visual areas demonstrated a dip at SFs of 2.00 c/deg and in V1 there was a peak between 1.00 and 4.00 c/deg with a rapid decrease in response after 7.00 c/deg. All areas also showed an increase of response from TFs that were .00 to 8.00 Hz and then a decrease thereafter. These results support previous findings and shows that measuring haemodynamic response is a valid method of indirectly measuring neuronal activity.

Although using a combination of all of these allows a reliable and valid approach to measuring the brain's response to visual stimuli, they all share a common problem. As they all require using large sets of equipment, it can be difficult for clinical use such as testing at the bedside. It also means that these methods are difficult to use with infants (Owen-Reece, Smith, Elwell & Goldstone, 1999). One relatively recent neuroimaging technique that has been developed which overcomes these problems is Near Infrared Spectroscopy (NIRS). The use of NIRS in measuring haemodynamic activity in the brain was first described by Jobsis (1977) who was investigating non-invasive neuroimaging techniques. Two types of changes have been found to occur in brain tissues which are associated with neuron activation, fast effects (with a latency of 50 – 300ms) and slow effects (with a latency of 2 – 7s) (Gratton, Toronov, Wolf, Wolf & Webb, 2005). Fast effects come directly from neuronal activity itself but slow changes have a haemodynamic basis. NIRS works in a similar way to fMRI in that it allows the recording of haemodynamic response in the brain associated with slow effects, by utilising optical signals.

Visible light (450 – 700 nm) can only penetrate biological tissue by up to 1cm as the medium either scatters or absorbs it, therefore it cannot be used for readings of internal organs. However, light that is near infrared (650 and 900 nm) is able to go further and so can reach tissues that are deeper such as cortical structures. NIR light is scattered, absorbed or reflected when passing through tissue. In order for a signal in NIRS to be picked up, there must be more light scattered than absorbed (McIntosh, Shahani, Boulton & McCulloch, 2010). Both oxyhaemoglobin (OXY) and deoxyhaemoglobin (DEOXY) have absorption spectra that fall within the infrared range. This allows real-time measurement of DEOXY and OXY concentrations in the brain and so provides a valid recording of neuronal related, haemodynamic activity (Owen-Reece et al, 1999).

NIRS can be measured by a number of commercially available equipment designs which all work in a similar fashion (Owen-Reece et al, 1999). As an adult's head is too large for NIR light to pass all of the way through, both the transmitting and receiving optodes are placed linearly, a few centimetres apart on the same side of the head. The photodiodes create specific wavelengths of light which are then transmitted in an arc shape and detected by a silicon photodiode. The light

attenuation is then converted into concentration data by a computer in order to give readable data regarding haemodynamic activity. This includes information on DEOXY concentration, OXY concentration, total haemoglobin concentration (THC) and percentage of oxygen saturation of the blood (OXPC). As the transmitters are positioned at different distances from the receiver, it means that each arc is a different height and therefore allows measurement of activity at different cerebral depths (McIntosh et al 2010).

Most literature of NIRS and visual stimulation using stimuli with gratings or checkerboards has focused on observing haemodynamics in animals or infants; little has been done in terms of human adults. One recent study by McIntosh et al (2010) that has focused on this used NIRS to investigate the effects of visual stimulation from a checkerboard stimulus on haemodynamic response of the visual cortex. Participants with lightly pigmented or no hair were asked to view a grey screen and to concentrate on a fixation point in the centre whilst the stimulus was presented. The stimulus consisted of a reversing checkerboard with a fixed TF of 7.50 Hz and had an on/off period of 30 seconds each. Consistent with previous studies, the results showed that visual stimulation caused an increase in OXY and a decrease in DEOXY. These findings provide support to the use of NIRS as a valid method for measuring haemodynamic response to a visual stimulus with both spatial and temporal properties.

In the previous study, both TF and SF were fixed at a constant level. NIRS can be used in order to investigate the effects of TF and SF on the amplitude of haemodynamic response to visual stimuli. One study that touched on this was conducted by Toronov, Zhang and Webb (2007). They set to investigate the spatial and temporal characteristics of functional NIRS (fNIRS) in response to visual stimulation and compare it to fMRI. Participants were asked to view a reversing checkerboard with varying TFs at 1.00, 2.00 or 6.00 Hz with a 20 second on/off period. It was found that there was a linear increase in response as the TF was increased which provided evidential support for previous findings. Also, it was found that there was a correlation between fMRI recordings and fNIRS recordings. This provides extra validation for the use of NIRS on measuring haemodynamic activity in response to visual stimulation and in measuring response to changes in TF.

As well as neuroimaging methods, there are several other ways of investigating the effects of SF and TF on visual detection. These provide further evidence and can act as a strong support if coupled with different imaging techniques. One such approach is to investigate reaction times to gratings with different SFs. One such study was conducted by Vassilev, Mihaylova and Bonnet (2002) who measured reaction times as well as VEPs as a response to visual stimulation. Participants were asked to view sinusoidal gratings with different SFs whilst VEPs were recorded. They began each trial by pressing a button which produced a click. A grating might then appear after 800ms with a probability of 0.5, if it did then the participant had to click another button as fast as possible. The results showed that both reaction times and VEP responses are faster as SF increases. This is consistent with previous findings and suggests that using reaction times with an imaging technique may provide more supportive evidence. It may also be useful as it can act as a way to keep the participant attended on the task.

Present Study
The present study will investigate the relationship between SF and TF of a visual stimulus on the haemodynamic response of cells in V1. It will attempt to replicate the findings that were found in the Robson (1966) study by using NIRS instead of psychophysical measures. A similar method to the one used in the McIntosh et al (2010) study will be used except that the SF and TF will be varied. A circular bullseye grating will be used as it will give an optimal neuronal response. This is shown in a study by Mahon and De Valois (2001) where they measured response in the lateral geniculate nucleus (LGN), V1 and V2 cells to Cartesian (parallel) and non-Cartesian (concentric, radial and hyperbolic) gratings in monkeys. They found that cells were more responsive to concentric patterns rather than parallel patterns and suggests that non-Cartesian gratings should be used in order to gain an optimal neuronal response.

Hypotheses
The hypotheses are based on the findings from Robson's (1966) study and are as follows:

(1) There will be a difference in haemodynamic responses between three different TFs. Responses should show a reversed U-shaped curve, increasing up to and peaking at 7.55 Hz and then decreasing thereafter. (2) There will be a difference in haemodynamic response between two different SFs. Responses should be greater for an SF of 4.00 c/deg than for an SF of 0.17 c/deg. (3) There will be a relationship between SF and TF. Responses should be at an optimum level when SF is at 4.00 c/deg and TF is at 7.55 Hz.

Method

Participants
Eight psychology undergraduates participated in the study and were all above the age of eighteen. Participants were recruited using the participation pool system at the University of Plymouth. All participants had naturally light coloured hair or bald over the area of the head where the probe was placed. This was because the experiment relied on optical signals being passed through the scalp and the pigments in dark hair would absorb the light at the wavelengths used, therefore participants needed to have light hair. Also, it was checked that none of the participants had flicker induced epilepsy as the stimulus may trigger an episode off in a person who is susceptible to it.

Materials and Design
This experiment used a within-subjects design. The test stimulus was made by creating a programme on an MS-DOS operating system. For the final two participants, a button was made by one of the experimenters by connecting an old telegraph key to wooden board in order to maintain the participant's attention on the task. Participants were measured using the OxiplexTS Near-Infrared tissue oximeter which is a frequency domain oximeter that measures haemoglobin concentrations in the brain by emitting near infrared light through the skull at two different wavelengths, 690 nm and 830 nm (ISS Inc, 2001). The probe is made up of four light emitters and one detector. This is positioned so that the distance between the

detector and each of the emitters ranges from 1.93 cm and 3.51cm and the light is emitted in a curve from each emitter to the detector (McIntosh et al, 2010).

Procedures
Before the experiment could begin, preparations had to be taken in order to set up the equipment with the participant. The participant was fully briefed (Appendix A) and asked for consent. Measurements were taken of the participant's head using the international 10/20 system in order to find area 0_2, which is where the probe was placed (Odom, Bach, Brigell, Holder, McCulloch, Tormene & Vaegan, 2010). Once area 0_2 was found, a mark was placed using a non-permanent marker and then the participant's hair was parted using a comb and hair clips so that the hair part was vertical and the mark was in the centre. The probe was then placed over the hair part so that the centre of the probe was placed over the mark. The probe was then attached to the head using 2 headbands which held it in place and also completely covered the probe so that no external lights could reach the emitters. Recordings were taken with a sample rate of 5.00 Hz (recordings taken every 0.2s).

Once the participant was ready, the stimulus was set up so that the experiment was ready to begin. The stimulus used was a bullseye pattern set against a grey background. The central bullseye patch was 12.00 deg wide and was viewed from 80.00 cm. The field size was 27.00 deg x 21.00 deg and the grey levels were achieved through bit-stealing, which involves 'stealing' adjacent bits of colour variation in order to create a more precise grey luminance (Tyler, Chan, Liu, McBride & Kontsevich, 1992). The stimulus contrast was set at 0.97, the mean luminance was 52.00 cd/m^2 and the CRT monitor refresh rate was set at 100.00 Hz.

In each run, both the SF and the TF was fixed. Two SFs were used, in the coarse pattern condition the SF was set at 0.17 c/deg and in the fine pattern condition the SF was set at 4.00 c/deg. Each set of runs was fixed at one of these frequencies. Participants were tested within both conditions but the order in which they were tested was chosen randomly. Within each set of runs, a number of different-pattern TFs were used. These were .94, 1.88, 3.77, 7.55, 15.00 or 30.00 Hz which were chosen in a random order within each session (For the order of frequencies given to each participant, see Appendix B). Throughout each trial, participants were asked to fixate on a red square in the centre of the bullseye. At the end of each experiment, participants were debriefed in the appropriate manner (Appendix C).

Three different procedures were used throughout the entire experiment in accordance with the development of the experimental procedure. All three methods contained runs that lasted 6.66 minutes each. Method one involved stimulus on/off presentation in blocks of 5 seconds each. Each run was made up of 63 blocks of 5 seconds with each block having a 0.5 chance of being either static grey or having a flickering pattern. Method two involved a pattern alternation of 20 seconds on and 20 seconds off with each run consisting of 10 on/off cycles. In method three, pattern on/off alternation was 10 seconds plus a normal random variation with a standard deviation of 0.5 seconds. This method also included an attention element whereby participants pressed a telegraph key after each transition from on to off and off to on. This was in order to maintain attention and therefore maintain responsiveness of V1. Each run consisted of 20 cycles. Below is a table listing each participant and which method was used with them (see Table 1).

Table 1: Table to show participant allocation to method type

Method Type	Participants
1	1
2	2, 3, 4, 5 and 6
3	7 and 8

Data Analysis
For the analysis, all raw data was first converted into .txt files so it could be computed by the statistical software R. Using R., graphs to show total responses over each run were produced in order to find and remove any outliers. After this, graphs to show the average response over an on/off cycle were produced along with descriptive statistics and information on the power at f and 3f. The on/off cycle makes up a square wave which consists of many sine-waves with frequencies that are odd multiples of the fundamental frequency (Campbell & Robson, 1968). The first two fundamental frequencies were used for analysis (f and 3f) and were added together the make the total power (F). In these graphs, trigonometric regression was used in order to fit a nonlinear line of best fit. This was so that real-time changes in relation to stimulus turning on or off could be shown (Eubank & Speckman, 1990). From these graphs, it was decided that only three out of eight of the participant's datasets could be used in the analysis due to poor output of response. As some of the participants data for the TFs of 1.88, 3.77 and 15.00 Hz were missing, only response from trials using .94, 7.55 and 30.00 Hz were analysed. From the three datasets that were used, the total power of F was analysed using a repeated measures, 2-way ANOVA.

Results

Table 2. and Table 3. below show the means and standard deviations for each SF and TF in all four variables. The tables show that, for OXPC, THC and OXY, there is little difference in the mean responses between the fine and coarse conditions at TFs of .94 and 30.00 Hz. However, at 7.55 Hz there is a clear difference in the means with mean power being larger in the fine condition than in the coarse condition (OXPC – Fine = .20 (SD =.11) and Coarse = .13 (SD =.06), THC – Fine = .06 (SD =.05) and Coarse = .02 (SD =.02), OXY – Fine = .12 (SD =.07) and Coarse = .07 (SD =.05)). This shows that, at 7.55 Hz, a visual bullseye stimulus with a higher SF gives a larger haemodynamic response than one with a lower SF. For the DEOXY, there is a clear difference across all 3 TFs with the means being larger for the coarse condition (.94 Hz = .09 (SD =.10), 7.55 Hz = .04 (SD =.03), 30.00 Hz = .06 (SD =.05)) then the fine condition (.94 Hz = .02 (SD =.01), 7.55 Hz = .02 (SD =.01), 30.00 Hz = .02 (SD =.01)). Within the fine condition alone, there is little difference between the powers at all 3 TF conditions. However, in the coarse condition, there is a decrease in DEOXY response from .94 Hz (.09 (SD =.10)) to 7.55 Hz (.04 (SD =.03)) and then an increase from 7.55 Hz to 30.00 Hz (.06 (SD =.09). As an increase in DEOXY concentration suggests little haemodynamic activity, these results show that a visual bullseye stimulus with a higher SF gives a larger haemodynamic response than one with a lower SF and that the response is at its largest when TF is 7.55 Hz.

Table 2: Table to show the means of the total powers for all measures

	Fine			Coarse		
	.94	**7.55**	**30.00**	**.94**	**7.55**	**30.00**
OXPC	.21	.20	.20	.20	.13	.19
THC	.04	.06	.05	.05	.02	.06
OXY	.11	.12	.11	.11	.07	.12
DEOXY	.02	.02	.02	.09	.04	.06

Table 3: Table to show the standard deviations of the total powers for all measures
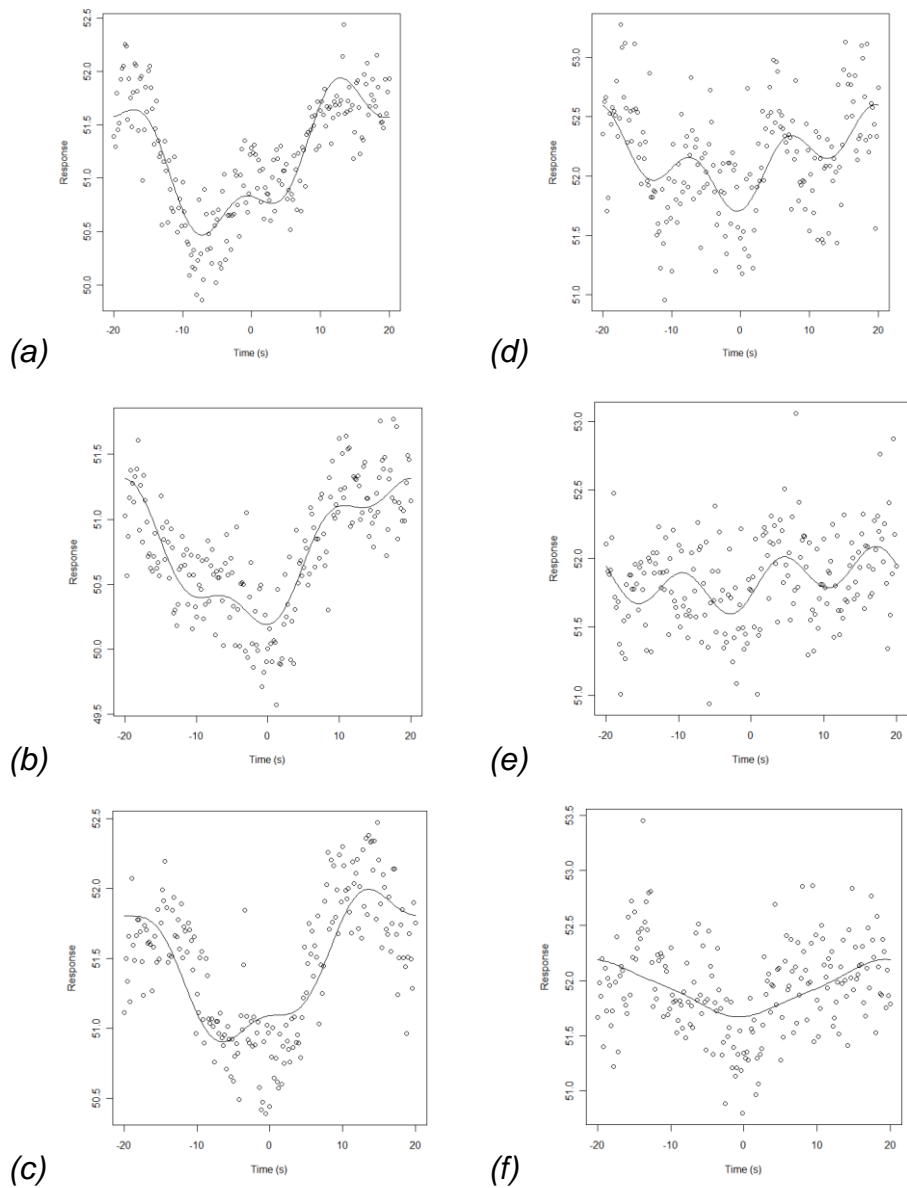
	Fine			Coarse		
	.94	**7.55**	**30.00**	**.94**	**7.55**	**30.00**
OXPC	.18	.11	.14	.20	.06	.14
THC	.02	.05	.04	.06	.02	.05
OXY	.08	.07	.07	.11	.05	.09
DEOXY	.01	.01	.01	.10	.03	.05

These results are plotted in the graphs below and allow the differences in the data to be viewed more clearly (Figure 1.).
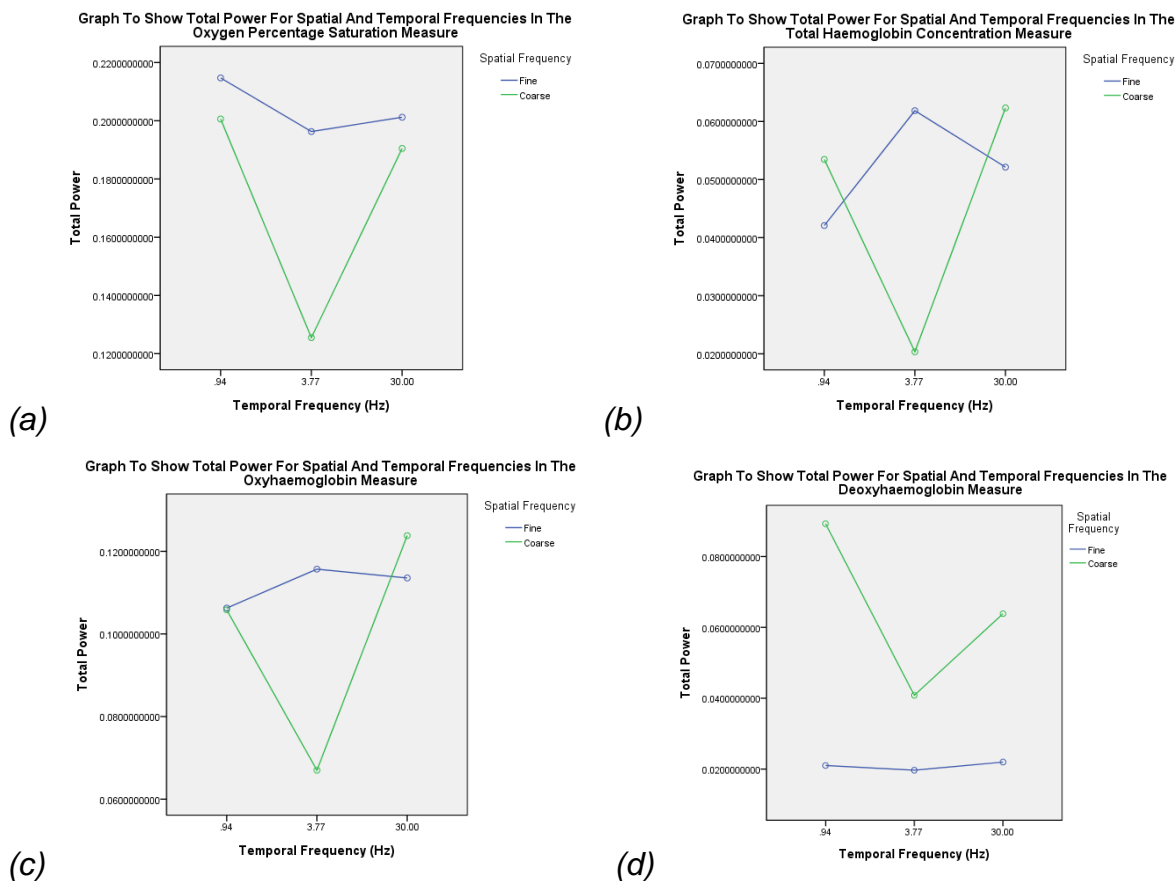
Figure 1: Graphs to show total power for spatial and temporal frequencies in the (a) oxygen percentage saturation, (b) total haemoglobin concentration, (c) oxyhaemoglobin and (d) deoxyhaemoglobin measures

By looking at trigonometric graphs that show the average response over one on/off cycle, the optimal response to the stimulus can be seen. Figures 2, 3, 4, and 5 below, show the average responses for OXPC, THC, OXY and DEOXY, respectfully, from one participant's data. It can be seen that the haemodynamic response was at its largest in the fine condition when TF was either .94 or 7.55 Hz, with it showing a clear increase in response from about 10 – 20 seconds after the stimulus was first presented. It also shows that, overall, the fine condition gave more clearly defined responses than the coarse condition.

Figure 2: Graphs to show the average on/off response from OXPC in Participant 5 for (a) Fine .94 Hz, (b) Fine 7.55 Hz, (c) Fine 30.00 Hz, (d) Coarse .94 Hz, (e) Coarse 7.55 Hz and (f) Coarse 30.00 Hz.
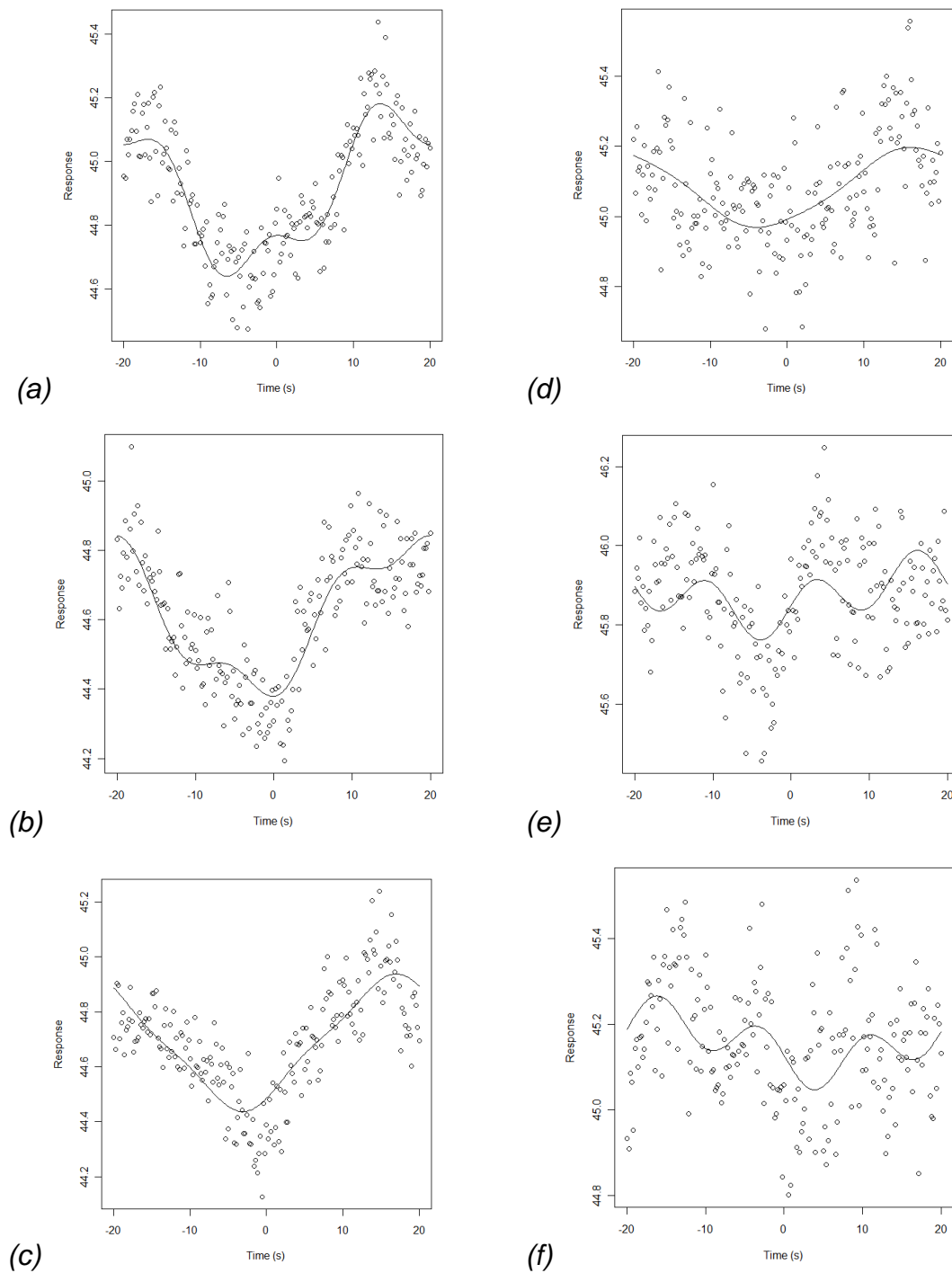
Figure 3: Graphs to show the average on/off response from THC in Participant 5 for (a) Fine .94 Hz, (b) Fine 7.55 Hz, (c) Fine 30.00 Hz, (d) Coarse .94 Hz, (e) Coarse 7.55 Hz and (f) Coarse 30.00 Hz
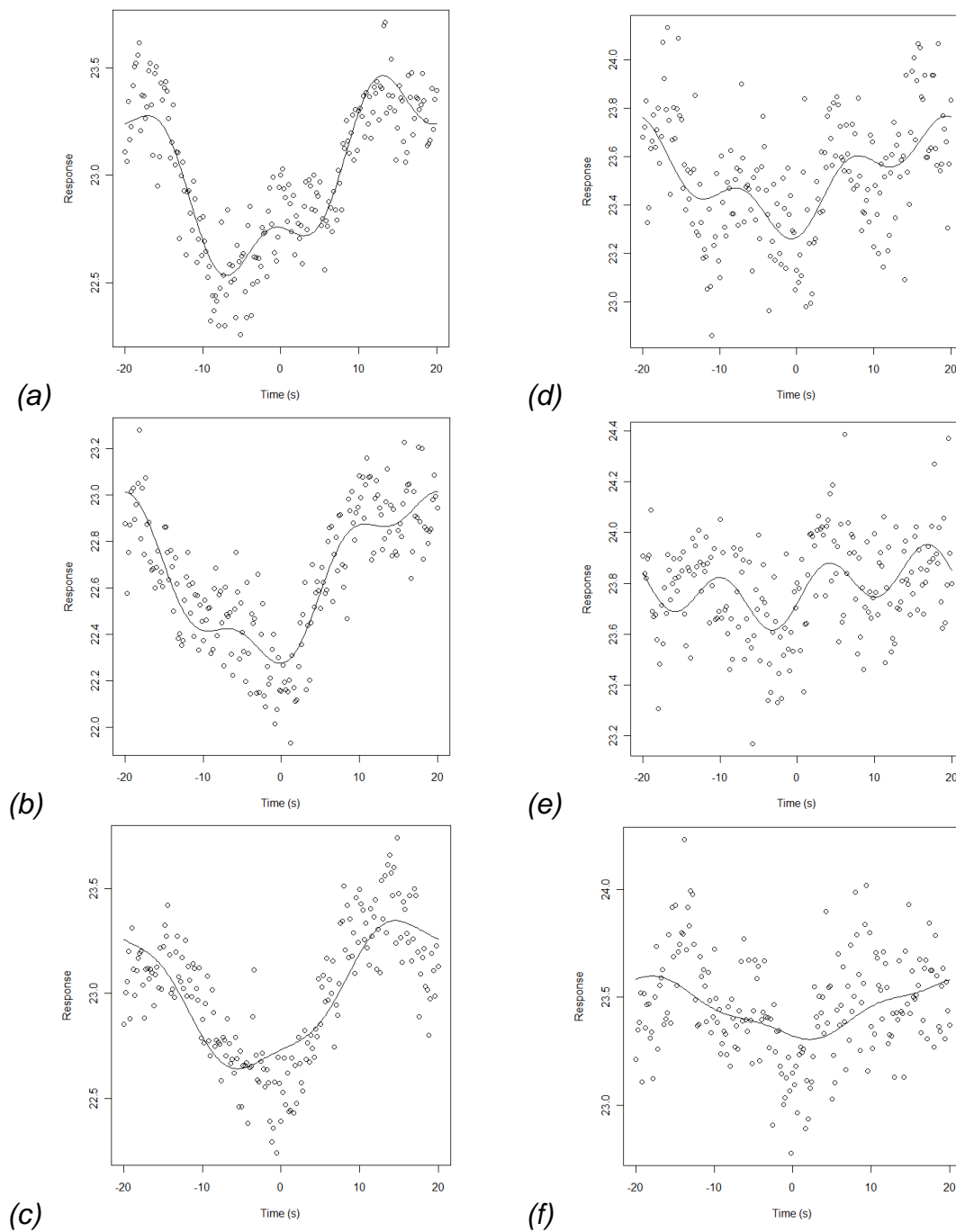
Figure 4: Graphs to show the average on/off response from OXY in Participant 5 for (a) Fine .94 Hz, (b) Fine 7.55 Hz, (c) Fine 30.00 Hz, (d) Coarse .94 Hz, (e) Coarse 7.55 Hz and (f) Coarse 30.00 Hz

Figure 5: Graphs to show the average on/off response from DEOXY in Participant 5 for (a) Fine .94 Hz, (b) Fine 7.55 Hz, (c) Fine 30.00 Hz, (d) Coarse .94 Hz, (e) Coarse 7.55 Hz and (f) Coarse 30.00 Hz.
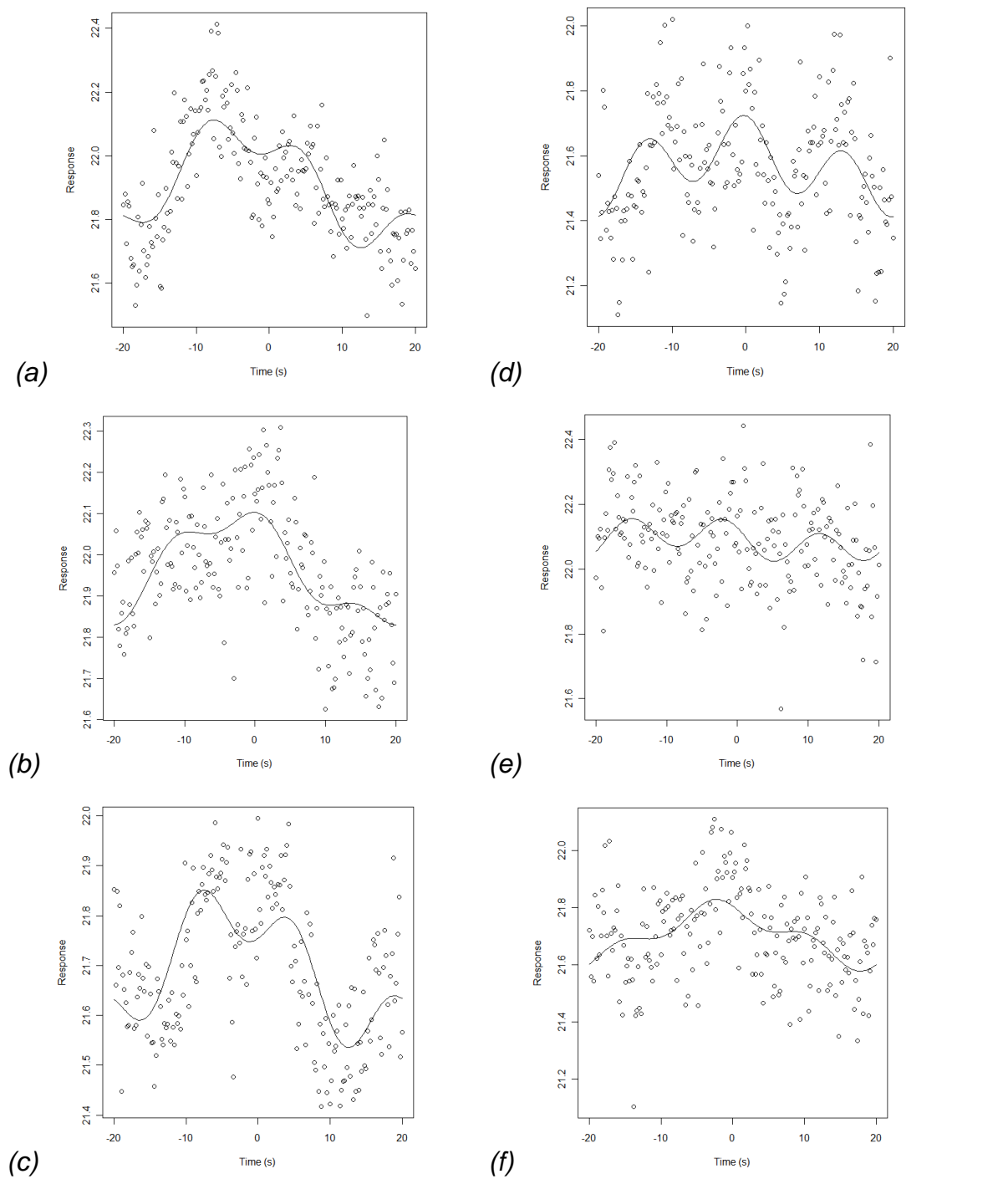
In order to investigate whether these differences were significant, the data were subjected to repeated-measures 2-way ANOVA for each of the four measures. In OXPC, Mauchly's test of Sphericity suggests that the sphericity can be assumed for TF (p =.85) but there is a problem with the assumption for the interaction of TF with SF (p =.01), so the greenhouse-geisser was used for this. From the ANOVA table, it can be seen that none of the variables or interactions were significant (SF, *F (1, 5) =.18, p=.69, TF, F (2, 10) =.98, p=.41, SF*TF, F (1.06, 5.29) =.32, p=.61).* This suggests that there is no significant difference in SFs and TFs and also no significant interaction between them for the OXPC measure. In THC, Mauchly's test of Sphericity suggests that the sphericity can be assumed for TF (p =.51) and the interaction of TF and SF (p=.48). From the ANOVA table, it can be seen that none of the variables or interactions were significant (SF, *F (1, 5) =.22, p=.67, TF, F (2, 10) =.93, p=.43, SF*TF, F (2, 10) =3.72, p=.06).* This suggests that there is no significant difference in SFs and TFs and also no significant interaction between them for the THC measure.

In OXY, Mauchly's test of Sphericity suggests that the sphericity can be assumed for TF (p =.39) but there is a problem with the assumption for the interaction of TF with SF (p =.05), so the greenhouse-geisser was used for this. From the ANOVA table, it can be seen that none of the variables or interactions were significant (SF, *F (1, 5) =.16, p=.71, TF, F (2, 10) =.92, p=.43, SF*TF, F (1.12, 5.60) =.61, p=.49).* This suggests that there is no significant difference in SFs and TFs and also no significant interaction between them for the OXY measure. In DEOXY, Mauchly's test of Sphericity suggests there is a problem with the assumption for both TF (p=.00) and the interaction of TF with SF (p =.01), so the greenhouse-geisser was used for this. From the ANOVA table, it can be seen that none of the variables or interactions were significant (SF, *F (1, 5) =3.72, p=.11, TF, F (1.03, 5.14) =2.13, p=.20, SF*TF, F (1.05, 5.26) =1.30, p=.31).* This suggests that there is no significant difference in SFs and TFs and also no significant interaction between them for the DEOXY measure.

Discussion

The aim of this study was to use NIRS in order to replicate Robson's (1966) findings and investigate the effects of SF and TF of a visual bullseye stimulus on the haemodynamic response in V1. The results from the current study show that, for OXPC, THC and OXY, there was very little difference in haemodynamic responses between the fine and coarse conditions at TFs of .94 and 30.00 Hz. However, at 7.55 Hz, a visual bullseye stimuli with a higher SF gave a larger haemodynamic response than one with a lower SF. In the DEOXY measure, there is a difference between the fine and coarse conditions in all TFs with a higher concentrations of DEOXY in the coarse condition than the fine and, hence, larger haemodynamic responses for higher SFs. Looking at each SF individually, there was almost no difference in response between all TFs in the fine condition but in the coarse condition, there was a decrease in DEOXY concentration from the TF at .94 Hz to the TF at 7.55 Hz and then an increase from the TF at 7.55 Hz to the TF at 30.00 Hz. A repeated-measures ANOVA showed that none of these differences were significant.

The results of the ANOVA suggest that the null hypotheses can be accepted. That is, there will be no difference in haemodynamic response between visual bullseye stimuli with various TFs and SFs and that there will be no relationship between the two. However, this may be because such a small sample size of only three participants was used for statistical analysis which makes it difficult to detect any

actual statistically significant differences in the means (Lunney, 1970). By looking at graphs showing average responses over one on/off cycle, it can be seen that there is some difference. The results do provide some evidence for a difference that is consistent with Robson's (1966) findings. That is, both the current study and Robson's (1966) study found that there was an optimal response to visual stimuli with a SF of around 4.00 c/deg and a TF of about 6.00 – 8.00 Hz. Both studies also show that, overall, responses were larger for stimuli with higher TFs than lower TFs. This can be explained by Foster et al's (1985) study on the SF and TF selectivity of V1 cells in macaques. They suggested that cells in V1 are selectively tuned to respond to certain SFs and TFs of a visual stimulus. Neurons responded best to SFs of between 0.50 – 8.00 c/deg and TFs between 5.60 – 8.00 Hz, anything out of these ranges elicited very minimal, if not zero, response as cells are not tuned to detect these frequencies.

The current study also provides support for previous studies on the use of NIRS as a way of measuring neuronal activity. The findings support those found by the McIntosh (2010) study who found that NIRS can be used as way of measuring absolute rather than relative OXY and DEOXY changes in V1 as a response to visual stimuli. Likewise, it also supports findings from the Toronov et al (2007) study who investigated TF properties of a reversing checkerboard using NIRS and fMRI. They found that as TF increased from 1 Hz to 6 Hz, there was an increase in haemodynamic response. This was supported by some of the findings in the current study as, in the fine condition, there was an increase in haemodynamic response as TF increased from 0.94 Hz to 7.55 Hz. This supports the validity of using NIRS to measure haemodynamic response to varying properties of visual stimuli. Furthermore, it provides support to the idea that haemodynamic responses are closely linked to direct neuronal activity as it had similar findings to studies using other neuroimaging techniques such as Vafaee and Gjedde's (2000) study that used PET and Fawcett et al's (2004) study that used MEG to investigate this activity in similar experimental conditions.

Not all of the findings from the current study provided supporting evidence for previous research. The findings from Robson's (1966) and other studies suggest that, apart from when TF is very low, responses to visual stimuli should gradually decrease as SF increases past approximately 3.00 c/deg. Also, apart from when SF is very low, responses should also decrease as TF increases past 6.00 – 8.00 Hz. However, some of the results from the current study suggest that, at an SF of 0.17 c/deg, response actually decreases from when TF is .94 Hz to 7.55 Hz and then increases from 7.55 Hz to 30.00 Hz. This is obviously inconsistent with previous findings and there may be a number of reasons why this occurred.

One possible reason for these inconsistencies could be down to gender differences. Gender differences in BOLD amplitude were investigated in a study by Kauffmann, Elbel, Gössl, Pütz and Auer (2001). In this study, participants were asked to view a high contrast dartboard stimulus with varying TFs in a mirror whilst recordings were taken using fMRI. Similar findings were found to the current study in that the TF that gave the optimum response was at 8.00 Hz. However, it was also found that men tended to have a higher BOLD amplitude in V1 in the right hemisphere than women. It was thought that this is because there are some structural brain differences between men and women. Women tend to have a higher percentage of grey matter than men, but men have more white matter and cerebrospinal fluid. These

differences mean than men have a higher metabolic rate which would explain the higher BOLD amplitude. These results have great implications on the current study as it suggests gender differences in V1 which may lead to a difference in haemodynamics and therefore, non-reliable data.

Another problem which may have led to inaccurate results may come from the reliability of the international10/20 system as an accurate measure. The 10/20 system works on the basis that everybody's brain structure is exactly the same; however this is obviously not the case. Homan, Herman and Purdy (1987) investigated this by using computerised tomography (CT) to correlate markers stated in the 10/20 system with underlying cortical structures. It was found that, although the 10/20 system correlated strongly with the underlying cortical structures, there was a great variability of these structures and also the structure of the skull. In particular, it was found that a great majority of subjects had cortical and skull asymmetries which could lead to the inaccurate locating of underlying structures. Even when the skull was symmetrical, there was a strong deviation of markers from the actual cortical structures. In relation to the current study, the use of the 10/20 system may have led to signals from the wrong cortical structures or very weak signals from the correct ones.

Another way in which individual differences may have played a part in creating inconsistencies in the result is through variations in hair densities. McIntosh et al (2010) found differences in signal strength between people with different densities of hair, as measured by the width of participant's hair parting. They found that participants, who were matched for age and hair colour, gave a clearer signal when they had a lower hair density than a higher hair density. Those who had a higher hair density tended to produce signals that were outside acceptable limits. This has implications for the current study as it could be that those who produced poor data may have had a higher hair density than those who produced good data. This could be solved in the future by using only participants who have a low higher density.

Problems of the haemodynamic response may have also arisen due to the length of time participants were asked to concentrate on the stimulus for. Having to concentrate passively on a visual stimulus for an extended period of time could lead to several problems including attentional deficits and habituation of the neuronal response to the prolonged exposure of the test stimulus. The results of a study by Afra, Cecchini, Pasqua, Albert and Scoenen (1998) found effects of habituation in normal, healthy subjects. Participants were asked to view a reversing black and white checkerboard with a reversal rate of 3.10 Hz whilst VEPs were recorded. It was found that, in normal, healthy subjects, responses gradually declined over time during continuous exposure to the stimulus. It was thought that this may be due to difficulties in sustaining attention over long periods of time. However, even when arousal levels were controlled for in reaction time tasks, a decrease in response was still recorded (Skuse & Burke, 1992). This means that a prolonged exposure to a visual stimulus may lead to habituation of cell response. In relation to the current study, this could mean that runs at the end of the test period could give overall smaller responses to the stimulus and, therefore, unreliable data.

For possible future research, several improvements could be made in order to gain more reliable results. Implications from the Kauffmann et al (2001) study suggest that gender should be controlled for. Future research could use purely male or

female participants or use both genders but analyse the data separately and compare them. In order to solve accuracy problems in locating V1, an alternate neuroimaging technique could be used in conjunction with NIRS in order to provide validation of any findings. Although using another measurement will not directly improve the locating of V1, it means that if the results from NIRS recordings are strongly correlated with a technique that has a high spatial resolution such as fMRI, then it is likely that the NIRS probe was placed over the correct area. The use of multiple techniques has been demonstrated to provide useful validations in past research such as the Toronov et al (2007) study. Finally, in order to counteract any habituation to the stimulus, each test period could be broken down into smaller segments or a different stimulus could be presented in between trials in order to 'reset' the neuron. The study could also have a reaction time element to it in order to control for attention (Vassilev et al 2002).

There are many advantages and limitations in using NIRS in experimental procedures rather than other neuroimaging techniques. One advantage is the biochemical specificity to substances such as DEOXY and OXY that NIRS recordings rely on (Gratton et al, 2005). It provides details about the workings of metabolic processes in areas of the brain as a response to tasks that are tailored to activate neurons in these locations. Not only that but other techniques for neuroimaging, such as PET or MEG, measure either direct neuronal activity or vascular components whereas NIRS can measure both of these in the form of vascular responses of DEOXY and OXY and also intracellular events that are directly related to neuronal activity (Villringer & Chance, 1997). This means that using NIRS as a neuroimaging technique should be more reliable than using methods such as PET.

One very important advantage of using NIRS over other neuroimaging methods is its ability to investigate functional changes in the brain non-invasively (Owen-Reece et al, 1999). Methods that are usually used to investigate CBV and CBF, such as PET or xenon washout, see subjects being administered with radioactive tracers and need intra-venous or intra-arterial access. As NIRS works by shining light through the scalp from an external emitter, it is completely non-invasive. Another advantage of NIRS is its ability to record real-time changes (Owen-Reece et al, 1999). Unlike some other neuroimaging methods, NIRS allows the absolute, rather than relative, quantification of CBV. As NIRS has good temporal resolution, this absolute quantification means that investigators are able to observe accurate real-time measurements of haemodynamic activity

As mentioned previously, one limitation to using NIRS is its poor spatial resolution. Once placed over the scalp, it is uncertain exactly what cortical structure is directly beneath (Hoshi, 2003). All that is known is that a signal arises from both cerebral and extracerebral tissues which make the signal even more inaccurate. NIR light does not completely penetrate the extracerebral layers but is scattered so that only around 30% of emitted light actually reaches the cerebral cortex (Owen-Reece et al., 1999). This issue could possibly be solved by using a method with good spatial resolution; however it would be very difficult to run the two methods in parallel so it will be impossible to identify the exact structures beneath the diodes. Also, as NIR light can only penetrate the head by a few centimetres, deep cortical structures such as the thalamus are unable to be investigated non-invasively (Hoshi, 2003).

One other limitation comes from the interpretation of what contributes to the NIRS signal itself. Although the fluctuations in the signal clearly represent haemodynamic activity in response to external stimulation, there are other, internal factors that contribute as well (Hoshi, 2003). One internal factor that can contribute comes from basic, systemic physiological processes that include arterial pulse oscillations and respiration which cause a patterned change in CBV and CBF. Another factor comes from haemoglobin oscillations which have been suggested   to be a result of vasomotor responses to spontaneous neuronal activity. Both of these factors occasionally have similar amplitudes to functional signals and, therefore, caution should be taken when interpreting NIRS measurements (Hoshi, 2003).

The use of NIRS has many implications as a useful tool in both the experimental and the clinical setting. In the past, it has been difficult to perform neurological examinations on infants and young children as methods such as fMRI use large, loud equipment. These are deemed as unsuitable to use on children without sedation, which would be unhelpful in investigating effects of external stimulation on neuronal processes. NIRS makes it possible to carry out experimental procedures on awake infants. This is demonstrated in a study by Meek, Firbank, Elwell, Atkinson, Braddick and Wyatt (1998) who used NIRS in investigating the effects of a reversing checkerboard with a TF of 5.00 Hz on awake infants aged 0 – 14 weeks. They found that infants had an increased CBF in the occipital lobe due to stimulation. They also found, most importantly, that there was an increase in cerebral blood utilization that outdid this haemodynamic effect, which does not occur in adults. The fact that NIRS was able to show a difference between a child and an adults brain, demonstrates just how useful the method can be in experimental procedure.

As well as use in experimental research, NIRS has many clinical applications. First of all, NIRS equipment is portable and does not require large, heavy pieces of equipment. Therefore, it is readily available to use at the bedside and so may be more suitable to some patients who, for some reason, are unable to be tested with methods such as MRI (Villringer & Chance, 1997). Also, NIRS equipment generally costs a lot less than other neuroimaging equipment which means that, by investing in NIRS machines, clinical institutions are able to run more cost-effective diagnostic tests (Villringer & Chance, 1997). So far, NIRS has proven a useful tool in providing a non-invasive method for monitoring and detecting many anomalous cerebral events such as strokes, haematomas and brain injuries (Hillman, 2007). Hopefully, the scope for NIRS use in clinical settings will become greater as technology develops and its applications increase.

Conclusion
Although the current study has produced mixed findings, it has provided some support for past findings on the effects of various spatial and temporal frequencies of visual bullseye gratings on the haemodynamic response. It has shown that the use of NIRS as an experimental, neuroimaging technique may be a useful tool when investigating these effects. However, in order to improve any future investigations, current methodologies need to be reassessed. This includes looking at controlling for gender, individual differences and hair density as well as ways to solve the problem of neuronal habituation to the stimulus. Over the last couple of decades, NIRS has increasingly become a useful instrument in both experimental and clinical applications and will continue to do so in future development.

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