EEG Signal Dynamics in Unrestricted Natural

Visual Search

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by

Alexander Varatharajah BEng

Department of Engineering

University of Leicester

Abstract

EEG signal dynamics in unrestricted natural visual search: By Alexander Varatharajah. Visual search is a skill that if not mastered, would leave you considerably unfit for Darwin's idea of survival. Prior knowledge of the cognitive processes involved in visual search stemmed from modelling and behavioural studies collecting subjects' reaction times, their eye movements, or EEG target signatures under fixed-gaze. To further understand these concepts, progression has to be made with the collection of data. Recent co-registration of EEG and Eye-Tracking has further enhanced this understanding by allowing brain responses to be seen in the form of fixation-Related Potentials (fRPs). To date, only a handful of studies have investigated brain correlates of visual search paradigms involving eye-movements. In this research, subjects search for targets in natural images, resembling the children's game; "Where's Waldo?" Results show early and late target detection effects. Also presented is a novel full trial analysis, that has allowed investigation into local and global fRPs driving characteristics, which were related to classical concepts of expectancy and surprise. Additionally, potential pitfalls from commonly used techniques are highlighted.

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Abbreviations

Abbreviation	Meaning
EEG	Electroencephalogram
ERP	Event-Related Potential
VEP	Visually Evoked Potential
VPP	Vertex Positive Potential
ISPV	Interstimulus Perceptual Variability
ET	Eye Tracker
AOI	Area Of Interest
fRP	Fixation-Related Potential
sRP	Saccade-Related Potential
ICA	Independent Component Analysis
РСА	Principle Component Analysis
ANOVA	Analysis Of Variance
MANOVA	Multivariate Analysis Of Variance
МСР	Multiple Comparisons Problem
BDF	Brain Data File
EDF	Eye Data File
CRD	Corneo-Retinal Dipole
SP	Spike Potential
EOG	Electrooculogram
RC	Resistor-Capacitor
FDR	False Detection Rate
S	Seconds
NaN	Not A Number
ISI	Interstimulus Interval
SSVEP	Steady-State Visually Evoked Potential

Chapter 1 Background of Electroencephalography and Eye-Tracking

1.1 Brief History of Electroencephalogram, Eye Tracking and the Co-Registration of the two Technologies

1.1.1 Electroencephalography

Before the electroencephalogram (EEG) was created, electrical activity had already been seen in the mammalian brain (Caton 1875; Cajal 1894; Beck 1890; Beck & Cybulski 1891). William James may have been very "emotional" (James 1884) that he had not been part developing one of today's most popular non-invasive techniques to collect neural activity. Though, this was some 40 years before Hans Berger had made his first recordings in 1924. Berger reported that although the activity could be seen, the potentials were not large enough to be recorded and classified. Berger spent years adapting his methods for recording, and in 1929 reported his first recordings on an EEG on a 40 year old male. However, this was using pins directly inserted into the scalp. Even though he was careful, his research still met widespread criticism and disbelief; this was probably due to him primarily being a clinician, as well as his lack of a physiological background (Gloor 1969). However, in the late 1930's Lord E.D. Adrian collaborated with an electrical engineer called Brian Matthews to confirm Berger's findings. They created a multichannel EEG system where each channel had a differential amplifier. This system enabled them to localise areas of interest to see more thoroughly the underlying potentials of EEG; both temporally and spatially. Computerised telemetry of EEG has been used from as early as 1973 but the specifications of voltage and frequency has given rising concern for data storage. Advancements in technology led to a greater need for system software design (Collura 1993).

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Figure 1.1 Non-eye movement related EEG artefacts: Top Panel: Muscular artefacts. **Middle Panel:** Skin potentials, artefacts from sweat. **Bottom Panel:** Cardiac artefacts. Images taken from (Benbadis 2015). **The artefacts are highlighted by the red boxes.**

EEG, in its present state, is a non-invasive technology involving a scalp hat that connects electrodes to localised sites. EEG is a great tool for looking at the dynamics of cognitive processes, as it is cheap to run and has a very good temporal resolution for brain potentials. Sampling rate frequencies are typically 1000 Hertz (Hz) for brain potentials. It allows for many

applications from understanding neurological processes, to creating assistive technologies, but it has a major problem; it is very noisy. Luck (2005), describes in great detail, the process for the collection and analysis of EEG experimental data, and many reasons behind the different techniques used in EEG studies.

Potentials are voltage signals produced by the brain in response to a stimulus. Brain potentials are very small in amplitude and the noise from the EEG "drowns" the signal out. Often referred to as artefacts on the signal (artefacts are signals not from the brain in response to a stimulus. They are from external sources and are not useful to the study), there are many forms of electrical activity that can infringe on the EEG trace. Ambient noise from external electrical devices causes a constant 50Hz to be picked up on the EEG. This is removed using a notch filter post recording; though it is better to minimize the amount of electrical devices during data collection. There are also forms of physiological noise produced by the subject being recorded. Figure 1.1 shows examples of different types of EEG signal artefacts (The figures are made from bipolar EEG, although the main body of work lies in referential; the artefacts that appear are identical in behaviour). Skin has potentials that can imprint of EEG as slow drifting voltage (see middle panel of Figure 1.1). However, the most common noise comes from muscular contractions. Cardiac contraction signals cannot be avoided (see bottom panel of Figure 1.1), but the other muscular activity can be circumvented by asking subjects to keep all movements to a minimum. The electrical activity from eye movements, in the context of the current thesis, is the most challenging to overcome.



Figure 1.2 Segment of all EEG channels for one subject showing eye movements: On the left are the labels for each channel. And the raw signal from the EEG (top section) is shown in navy blue. There are also the eye tracker signals shown (at the bottom section), which also have traces in navy blue. The two red squares surround an eye movement called a saccade and the green box surrounds an eye movement called a fixation.



Figure 1.3 Segment of all EEG channels for one subject showing eye movements: On the left are the labels for each channel. And the raw signal from the EEG (top section) is shown in navy blue. There are also the eye tracker signals shown (at the bottom section), which also have traces in navy blue. The green box surrounds an eye movement called a blink.

In Figure 1.2, highlighted is electrical activity produced eye behaviour that can be seen on the traces of the EEG. The red boxes in Figure 1.2 are highlighting an eye movement called a saccade (a ballistic eye movement between two focal points), this eye movement may not have been seen directly from the EEG traces, considering how noisy the signal is. However, eye tracker traces that have been sent to the EEG (at the bottom of Figure 1.2), which show the eye movements as they happen (a benefit that will also be discussed later in the chapter as well as the rest of the thesis). Figure 1.2 also has a green box that highlights an eye behaviour called a fixation (a period of steady gaze, where the eyes do not move). Highlighted in the green box of Figure 1.3 is an eye activity that can be seen on the EEG traces called a blink (a period where the eye lids close and no visual information can be gathered).



Figure 1.4 ERP averaging from EEG epochs: The figure which can be found in the textbook *Introduction to Event Related Potentials* (Luck 2005), gives a small-scale example of the averaging process. There is a raw EEG signal (left side) where a particular channel has been segmented (epoched) to an appropriate time interval at a given onset of stimulus (time-locked event). These signals are averaged to produce the ERP (right side) the voltage on the y-axis is negative up as this is how ERPs were presented in the past.

Figure 1.2 and 1.3 highlight not only how noisy the EEG signal is, but also some of the artefacts that have to be dealt with. This is one of the main reasons that the vast majority of EEG studies have involved fixed-gaze tasks (a task where the eyes do not move for the duration); the eyes fixate on a point on the screen (normally a cross in the centre), and stimuli is flashed at this point. The response produced is a potential referred to as an Event-Related potential (ERP). EEG tasks are designed in such a way that the same response should be elicited each time. The response is time locked to the onset of the stimulus that is presented, the ERP produced, is desired to be studied; however noise will almost always be larger than the potential in a single trial. Fortunately this can be overcome, as an averaging technique can be used to visualise and analyse ERPs.

A=X(t) + R (1.1)
R x (1/
$$\sqrt{N}$$
) (1.2)

Brain potentials that exist in an epoch of an EEG trace (a segment of EEG signal of interest represented by A in Equation 1.1); are time-locked to the onset of the given stimulus. Within the epoched segment, an ERP waveform will be contained (X(t) in Equation 1.1), plus random background noise (represented by R in Equation 1.1). Examples of EEG epochs are shown in the left hand side of Figure 1.4. It is almost impossible to determine from the naked eye the onset or the amplitudes of the potentials in a single trial ERP epoch. However, if many trials

that contain the same ERP are collated and averaged, then over this "grand average", an ERP waveform should be seen (Right hand side of Figure 1.4). If R along with the number of trials (represented by N in Equation 1.2); then the noise reduction is represented by Equation 1.2. For example, if there are 4 trials the signal to noise ratio (SNR) will double because $(1/\sqrt{N}) = 1/2$. The theory is more trials that are averaged, the more the noise will reduce. However, as N increases the effect of the reduction decreases; therefore a trade-off has to be made in terms the time to run as many trials to get a desired SNR. In (Luck 2005) it is advised to prioritise the quality of the data collection process over the number of trials made in an experiment.

For classical ERPs the onset of the stimulus is used. Normally tasks such as the Oddball paradigm (Sutton et al. 1965), which is a famous fixed-gaze paradigm that induces very clean potentials; are also used. In the oddball paradigm stimuli are flashed according to the control set by the experimenter; therefore the onset of target stimulus is known. The progression of the oddball paradigm would normally run as following: there would be several distractor stimuli (for example a circle) flashed in a steady time, then at a time devised by the experimenter a target stimulus (for example a square) would be flashed. The subject involved in the task would also be given instructions for the task (for example, to count the number of target stimuli presented). Each stimulus would elicit a response in the form of a brain potential. These types of potentials produced due to visual stimuli are often also referred to as a visually evoked potential (VEP).

ERPs are a powerful tool for neuroscientists to see motor responses. If the processing involved with a response to specific stimuli in an experiment is to be understood; then ERPs provide a measure of continuous processing between said response and stimuli. At this point in the thesis, the major ERP components involved with visual processing that are focussed on during the course of the thesis will be introduced. They are described as follows; "P" represents a positive potential and "N" negative, the number following is the time-frame (milliseconds) and in parenthesis shortened aliases the potentials may also take:



Figure 1.5 ERP from a processing of a word in reading: shows a figure from works by Sereno and Rayner (2003). There are clear labels for the ERP components previous spoken about such as P1, N1, P2, N2, P3 (P300) and there is another component which is called the N400 which has been known for the detection of non-congruent words or sentence structure in reading (for a full understanding of the origins of the N400 and the research to current status see (Federmeier 2014)).

The P100 is a positive potential that onsets at around 60-90ms post-stimulus and normally peaks between 100-130ms (shown as the peak at ~100ms in Figure 1.5 and in the bottom right panel in Figure 1.6). It exhibits largest at the lateral occipital electrode sites; which is very consistent. Therefore, when checking EEG data this is normally the first potential to be investigated as a check for data accuracy. It can be fairly sensitive to parameters of the stimulus such as luminance, where increased luminance has been shown to increase the amplitude of the response, but not having a significant effect on the peak latency (Johannes et al. 1995). But attention has been known to increase the amplitude of the P100 (Hillyard et al. 1973; H J Heinze et al. 1990). This potential although not the main focus of the current thesis, does have an important role to the understanding of aspects discussed throughout. For clarity it is also worth mentioning the C1 a VEP, which can elicit from 60-80ms and can also be positive for stimuli in the lower visual fields (Jeffreys & Axford 1972; Clark & Hillyard 1996).

N100(N1): The P100 is followed by the N100 and can contain many subcomponents; the earliest of which can peak at around 100ms-150ms post-stimulus (shown as the negative deflection at \sim 150ms in Figure 1.5 and in the bottom right panel in Figure 1.6). It arises around the parietal-occipital electrode sites. Like the P100, spatial attention can affect the magnitude of the potential; with an increase in amplitude as a response to an increase in attention (H. J.

Heinze et al. 1990). However, unlike the P100 the latency of the peak amplitude is affected by luminance, with low luminance resulting in significantly longer peak latencies (Johannes et al. 1995).

P200/P2: The P200 potential follows the N100 and appears around the central scalp sites. Although not a significant potential in regards to the current thesis, it has been known to be used in auditory studies in particular recently to study the sensitivity of the potential to complex sounds (Shahin et al. 2005; Shahin et al. 2007).

N170/Vertex Positive Potential (VPP):



Figure 1.6 ERPs for face recognition: shows the other ERP component mentioned. It is from the works of Rousselet. et al. (2004). Clearly the N170 can be seen for face recognition for both human and animal but not for neutral images (lower 2 figures, the yellow line represents neutral targets presented, the green for animal faces and the blue for human faces). These are grand average ERPs.

In the 70-80's there was a significant effort to match the face-perception cells found in monkeys (Desimone & Gross 1979; Jeffreys 1989; Gross et al. 1972; Perrett et al. 1982). Jeffreys had an idea that because humans share a similar cortex, the same face-perception cells must be present in humans. Using scrambled pictures of faces, animals and objects, Jeffreys was able to collect a VEP's at the centro-parietal electrode sites, coining the term "Vertex Positive Potential". The difference in the potential manifests between 150-200ms; showing greater response in terms of amplitude to a picture of a face, while non-faces elicit lower amplitudes. This work was the first in the understanding of the VPP. Furthermore, the VPP was also found to contain face-perception properties, where larger amplitudes were elicited for faces than to other objects (Bentin et al. 1996). This amplitude for faces has been found to be the same for inverted faces, although the latency of the peaks differ (Itier & Taylor 2004). In the recent past, it was found

that VPP and the N170 are actually manifests of the same potential; although that it depended solely on the reference used in the analysis (Joyce & Rossion 2005). The potential is the same across average, earlobes, nose, and mastoid references; and both components can be accounted for by the configuration of the dipoles, shown in Joyce et al. 2005 to be unique; modelled by equivalent dipolar in the occipital-temporal region. The discovery that faces elicit higher amplitudes has come to some scrutiny; with those who doubt the legitimacy of the faceperceptive potential to have great strength, in regards to its categorisation, but also to its existence entirely (Rousselet et al. 2004). In this study it was found that the same potentials for both human faces and animal faces, showed no significant differences; although there was a difference of peak latency (some results shown in Figure 1.6). There has also been a study that suggested because faces are often seen directly square on, an uncontrollable interstimulus perceptual variability (ISPV) is created (Thierry et al. 2007). The study found results that would argue against previous work to show that the N170 is actually modulated for ISVP, and that there is only a latency difference between the amplitudes of the N170 for faces and objects. Therefore, a methodological artefact was causing the effect. This led to an interesting review article (Rossion & Jacques 2008) defending the field of N170 face perception research. In this review, the work of Thierry et al. 2007 is broken down, and a ten point "do's and don'ts" of ERP analysis is explained. There has also been single trial analysis of the N170, to the extent that at the level of a single trial it has been found that there is a modulating property due to conscious recognition (Navajas et al. 2013). Finally, the N170 has not shown any modulation for target recognition tasks (Carmel & Bentin 2002), which in the context of the current thesis is fairly important. The N170/VPP, although in some respects quite controversial, still seems to be the main focus for understanding face processing. It is also an interesting potential for the current thesis, as the main body of the work concerns faces and in particular the recognition of target faces.

P300/P3:



Figure 1.7 Classic P300 responses: A: shows the P300 eliciting for the detection of a target in a single stimulus task. **B:** shows the P300 elicited for an oddball task where the infrequent stimulus T elicits larger P300 (thick line) than the more frequent S stimulus (thin line). **C:** shows a three stimulus task where T is the target but D is an irregular distractor and S is a frequent stimulus that is not the target. The D stimulus evokes a P3a (dashed line) and the Target evokes a P3b (solid line) for target detection. A-C are taken from a P300 review (Polich 2007). **D:** shows how the amplitude of the P300 changes with respect to discriminate target to target interval (Gonsalvez & Polich 2002).

Normally the onset latency elicits at ~300ms and is typically long and with low frequency. There had long been an interest in linking the behavioural aspect of psychological concepts such as attention, with the ongoing brain activity in response. With the continued progress of EEG technology, the electrical activity of the scalp is able to be recorded, and tasks that evoke responses intriguing to behaviour can therefore be studied. The P300 was discovered as a product of the anticipation of the stimulus (Sutton et al. 1965). This work however used auditory stimuli as well as a flash of light. Uncertainty was created by first keeping one stimulus constant but varying the following stimulus randomly, this evoked a positive potential at approximately 300ms post-stimulus, thus the "odd-ball" task and the term "P300" were created. The P300 has shown a discriminating characteristic, which modulates the potential based on the detected stimuli. Following the finding by Sutton et al. 1965, a study found, using auditory stimuli; sounds detected correctly elicited a higher amplitude response of the P300 than that of the undetected, falsely detected or correctly detected non stimuli (Hillyard et al. 1971). An example of the P300 potential can be seen in Figure 1.7A-C taken from (Gonsalvez & Polich 2002). In Figure 1.7A, a P300 is elicited when an infrequent target stimulus is presented. In contrast the P300 is much smaller for the frequent stimulus (see Figure 1.7B). The P300 has also been shown to increase in amplitude with increasing inter-stimulus-interval (ISI) (see panel Figure 1.7D (Gonsalvez & Polich 2002)). It seems that the P300 has certain characteristics that can be modulated, and therefore is an interesting potential to understand. The potential shows cognitive processes such as the recognition to rare or novel stimuli. Perhaps the hardest task is to understand how the different characteristics of response affect the total amplitude of the potential. It has been found that there are two major subcomponents of the P300, the P3a and the P3b (Squires et al. 1977). The P3a elicits at the more frontal electrode sites and is larger for novel stimuli, whereas the P3b elicits more in central and parietal regions, and larger for the detection of a target. These sub-components are shown in Figure 1.7C where a three-stimulus task is presented; D is a very infrequent distractor that elicits a P3a (this could be associated with surprise of a novel stimuli). Also in Figure 1.7C T is a target stimulus, which elicits a P3b associated to target detection.

With the potential being so large and having such a range of characteristics to investigate it is one of the most studied in EEG research. The P300 has not just cognitive context, but also environmental factors have been shown to alter the response, therefore many methodological strategies have been implemented to test the features of the potential (see (Polich & Kok 1995), for a full review on the investigations of the P300 from 1965-1995).

Another use for the P300 is as a tool for brain computer interfaces (BCI), (BCI is the use of an external device controlled via cerebral activity) as it is classified with much more ease than the other potentials mentioned previously. In assistive technologies, patients who suffer from diseases such as 'locked in syndrome' could utilise a non-invasive tool to help them

communicate. There have been many studies utilising the P300 for this very task, a P300 speller is something that was fashioned. This can be used essentially as a hands-free keyboard (Farwell & Donchin 1988). It is an alphabetic matrix presented line by line, the subject focusses attention on the line that the letter they want to use is in, then each letter is highlighted until the letter that the subject wants is highlighted and an attentive switch is made. The P300 from the subject is collected and classified by a computer so to recognize the selection of the letter. Due to its ease of classification many researchers have looked into the features that can be manipulated by experiment and used as classifiers; the focus has now switched onto to the efficiency of the classification to make faster responding BCI systems (Blankertz et al. 2001; Treder & Blankertz 2010; Arboleda et al. 2009). There are a few different classification techniques, but the best performers are step-wise linear discriminant analysis and Fisher's linear discriminant, each having an average accuracy of approximately 70-80% in between 5-8 sequences (Krusienski et al. 2006). This potential in the context of the current thesis is probably the most significant, as the majority of the work is based on the P300 produced for the detection of target faces.

For averaged ERPs the resolutions of the temporal and magnitude characteristics mean that the onsets of potentials are not the actual latency that the potential elicits. However, the fundamental point is that ERP can be classified by using the averaging technique discussed earlier. If the classification of these components in a single trial is of interest; the averaging process cannot be used. Because each trial may have similar (but not identical) cognitive processes invoking potentials, therefore grand average ERPs cannot be taken into this consideration. Nonetheless, because the SNR of single trials is so low, different techniques have to be implemented in order to characterise what is seen in a single-trial. There have been many works from sophisticated wavelet de-noising (Quiroga 2000) and various other types of filters (laplace, bandpass, etc).

A lot of insightful findings have been made from ERP research, but a lot of what is known could be considered "unnatural". This is because of the control element of the tasks, forcing restraints on eye-movements. Electrooculography (EOG) is a series of electrodes that collect the eye movement voltage changes. There have been efforts in performing paradigms with EOG in order to correct for artefacts. A consistent method has seen the use of regression analysis to correct eye movement artefacts (Verleger et al. 1982; Schlögl et al. 2007), others have used a prorogation factor from EOG to EEG (Gratton et al. 1983), and more comprehensive correction using multiple sources (Berg & Scherg 1994). There are different advantages to each correction method, and those that additionally take account of phase differences and frequencies from EOG (Croft & Barry 2000). Though, even with the research using EOG there still seems to be a reluctance to relinquish control of eye-movements during paradigms.

The majority of studies mentioned in this section have presented results from fixed-gaze paradigms. This comes back to what was discussed earlier, in the sense that eye movements

infringe on the information of the ongoing brain activity collected by the EEG. Although we owe a lot of what is known to the strategy of fixed-gaze tasks, the progression of understanding neural processing of visual information lies in understanding natural responses; and this comes with the development of the collection of brain potentials without restricting eye movements (more to be discussed later in the chapter).

1.1.2 Eye Tracking

Before getting into a brief history of eye tracking, although some eye movements were mentioned earlier in the EEG section 1.1.1; it may be good to know what some of our eye movements are called:



Figure 1.8 Scan path for a different tasks: This figure was taken from some very famous work (Yarbus 1967). It shows typical trial behaviour of a subject, when they are asked to find some information about the scene. For example the bottom right figure shows the eye movements when subject were asked how old the people in the scene were. The cluster of black show fixations to faces being made, while the single lines show the saccades passing between the fixations.

Figure 1.8 gives an example of very early recordings of eye movements. Fixations are an elongated gaze at a single location; where visual information is gathered, they alternate with saccades. Saccades are fast ballistic movements of the eye, it is understood that the eye is effectively "blind" during this eye movement. We make on average around 4 per second and they are vital for our understanding of our environment visually (for a better understanding of saccades see (Liversedge et al. 2011)). Micro-saccades are another of the fixational eye movements; they are the shortest of all eye movements, and are produced during a fixation to reduce the neural adaption; which prevents the image from fading. In Figure 1.8. micro-saccades can be seen as very short movements around the location of a fixation. They are the conjoined cluster of red lines within the coloured circles, as they are very rapid eye movements,

and have been shown to assist in visual acuity and focus during a fixation (See (Martinez-Conde et al. 2004) for a good understanding of micro-saccades).

In past recorded events, there was the theory that the eyes simply passed over scenes smoothly, and singled out areas of interest (AOI), then fixating in a still unmoved way, which is not what we know today. At the turn of the 19th century the progress of eye tracking devices came at the same time as the evolution of ideas about the link between fixations, saccades, perception and cognition. Soon after the discovery of saccades and fixations, a scientist called Judd created a 'kinetoscopic' eye tracker in which a small fleck of Chinese white was attached to the cornea. From this technique they quickly observed that fixation locations were not entirely selected by the stimulus, but could possibly be from instructions of the tasks. This drove the question; what drives the selection for a fixation? This inspired work in the later 20th century and in Guy Buswell's work "how people look at pictures". His research was the first to look at overall fixation distribution, fixation locations, comparing early fixations and later latency fixations. He also looked at how fixation duration changed over time, and looked at the consistency of observers viewing the same image. Then, quite intuitively, investigated at how the instructions of the task affected the results. This was in fact one of his most overlooked aspects of work. Subsequently the research into eye movements has taken two forms: one approach with experimental control, and the other to research into more 'naturalistic' and 'real' scenes. In 1950 Yarbus confirmed Buswell's work and their work in psychophysics as well asvisual search has led to findings that features such as orientation, colour and luminance can affect attention (Wade & Tatler 2005).

Eye trackers (ET) are a non-invasive technology with a good temporal resolution (depending on the eye tracker used) with an excellence response time. In the modern sense now they employ high-speed cameras to detect eye movement using algorithms with mostly a reflective technique using infra-red leds and a 'glint' on the pupil for positioning. The main advantage of present ET is their ability to acquire mass amounts of eye movement data from visual experiments. So for eye movement experiments we can look very closely at the types of eye movements made. The main disadvantage is that it cannot be known what cognitive processes and brain potentials that are induced, underlie the behaviour exhibited behind eye movements with eye tracking alone. For instance, saccades can be detected with an ET, however from EEG studies they are understood to be the cause for an occipital potential called the lambda wave; the occipital potential mentioned briefly earlier (Thickbroom et al. 1991). The point is that the eye tracker in this instance can detect the eye movement, but cannot give information regarding how it affects visual processing.

In order to study eye movements, first they must be detected. A well-known, tried and tested algorithm for detection has been developed (Engbert & Kliegl 2003). Time series eye positions are converted to velocities and the algorithm detects a threshold set for the velocities (i.e. above

a certain threshold is classified as an eye movement). This has been tested for robustness against random noise. The algorithm has three parameters that need to be defined. Firstly, there needs to be a minimum threshold for saccade duration, this is typical very small (3-6ms) (Engbert & Mergenthaler 2006; Kamienkowski et al. 2012). Secondly, the velocity threshold of the saccade is required and is normally a factor of the mean or median velocity. It also takes into consideration the noise level. Lastly, the inter-saccadic-interval (ISI) has to be defined. This is the threshold of the period between two consecutive saccades; in order to avoid a corrective saccade being registered as a new one.

Most research using ET solely have been to gain an understanding of cognitive processes such as visual perception. Some early computerised research used a double Purkinje image eye tracker that plotted a voltage traces for vertical and horizontal eye movements at a rate of 500Hz, while researching visual perception in smooth pursuit (Festinger et al. 1976). There have been many studies investigating scene perception, from famous early works by Buswell and Yarbus (discussed briefly earlier) in which different instructions changed the eye movement behaviour and the scene perception; to work researching effects of eye movement parameters on scene perception (Henderson & Hollingworth 1999; Henderson et al. 2013). There is also research into how cultural backgrounds effect scene perception (Chua et al. 2005). Some of the questions asked in 1935-1967 are still being asked in recent research, showing how vital the understanding of visual perception still is (for an updated review on many studies involving perception see (Schütz et al. 2011)).

Another cognitive process, which still interests neuroscientists, is attention. Many researchers are still using solely ET in order to gather a further understanding. A question that seems to be asked is: what neural mechanism decides where the focus of the next fixated region of a scene is? The underlying processes that govern attention are highly sort after. Some studies have focused on looking at the eye movements themselves as a tool for investigation (Hoffman & Subramaniam 1995; Fischer & Breitmeyer 1987). Whilst others have looked to a hierarchical system either bottom up in terms of the immediate eye movements, or a top down task relevant, attentive effect (Desimone & Duncan 1995; Parkhurst et al. 2002).

For simple visual tasks, where only the eye movement behaviour is of interest to the researcher, there will always be a use for ET. Comparatively to EEG with its non-invasive environment an ET could also be used for assistive technologies. There are already "eye typing" programs, and researchers are trying to vastly improve performance using different techniques; such as prefix highlighting (Diaz-Tula & Morimoto 2016) and gaze free eye swiping (Kurauchi et al. 2016), and unlike the EEG P300 spellers, need no time to classify, but a short period to calibrate the eyes at the start. Challenges that would occur for ET solutions would only arise when subjects have a lack of eye movement control; such as nystagmus.

1.2 Visual Search

Visual search is a skill that if not mastered, would leave you considerably unfit for Darwin's idea of survival. The majority of people are not aware, but will perform visual search many times throughout the day. During the reading of this thesis, the viewer has probably already performed a visual search if attention was shifted to any figures that were directed to. This constant activity may go unnoticed, as the cognitive process of conducting a search is instinctual, but it is still vital to everyday life. Visual search is a task in which the goal is to find a target. The target is normally an object of some description that is distinct from other objects that are not the target (known as distractors) in the general scene the subject is viewing. This has been a field of interest for over 40 years, initial interests were on the attention driven effects of search. More recent studies have focused more on the eye movements that drive cognitive processes and even strategy in a visual search (Najemnik & Geisler 2005; Findlay et al. 2001; Findlay 1997; Deubel & Schneider 1996; Ludwig & Gilchrist 2002). Foveal and parafoveal processing have been investigated to see what drives a target selection (Ludwig et al. 2014). Prior knowledge of the cognitive processes involved in visual search stemmed from behavioural studies; collecting subjects' reaction times, eye movements, or even EEG target signatures under fixed-gaze (Gonsalvez & Polich 2002). There is also an interest in the scan path to analyse strategy; whether there is a systematic approach or any memory effects that can invoke the progression of fixations within a search (Gilchrist & Harvey 2000; Gilchrist & Harvey 2006).

Modelling of visual search has been an area of concern for researchers; it has been an interest to break down the effects of attention. Processes such as perceptual saliency and creating saliency maps of task scenes as a strategy; as well as processes like inhibition of return and object recognition, have been used as the foundations of models for visual search (Klein 1988; Itti & Koch 2001; Heinke et al. 2002; Ludwig 2009). Though as it stands, modelling of visual search is still a huge problem for many researches, and has been for decades (see (Eckstein 2011)). It is now well-known that certain contexts and manipulations of the task may assist a visual search (Henderson 2003). However, much of what is known in the field comes from an 'artificial' background. Consequently, there has been a rise in interest in visual search of natural scenes as the complexity of natural scenes may provide a greater depth of behaviour for investigation (Mack & Eckstein 2011) and also in free-viewing visual search (Otero-Millan et al. 2008). With respect to the current thesis, where faces are the natural stimuli focussed. There has been development in visual search to investigate how different parameters involved in face processing are affected; such as rapid saccades made towards faces (Crouzet et al. 2010; Crouzet & Thorpe 2011) and how faces "pop out" in visual search tasks(Hershler & Hochstein 2005). Although the behaviour in the studies mentioned can be evaluated and associated to cognitive processes, they cannot confirm the underlying mechanisms for any physiological

response. With the basis of the current thesis investigating free-viewing visual search, the main aim for the future would be to understand responses made; and this would have to utilise the coregistration of EEG and ET.

1.3 Co-Registration

In sections 1.1.1 and 1.1.2 the limitations of the two non-invasive technologies of EEG and ET were discussed. The main limitation for EEG is the restrictive constraint on eye movements during the majority of experiments. For ET the main limitation is the lack of information regarding the cognitive processing of the visual stimulus being presented. So it stands to reason that the combination of EEG and ET can be very informative in understanding the underlying cognitive processes of the visual pathway involving eye movements. Neuroscientists are trying to link the psychological behaviour with the physiological events during visual processing. Processes such as perception, which is the way the brain regards, interprets or understands the information it receives. As well as attention, which is how the brain notices and specifies what it wants to process; are just some of the mechanisms neuroscientists want to understand. In the main body of the current thesis, in which visual processing is the particular field being investigated; these processes such as attention and perception have been investigated with both technologies; however each field has limitations for these types of investigations.

Up to the last decade EEG and ET had been two separate research areas, while each technology gives data to further the knowledge of cognitive processes; not a lot of research has been made into combining both in order to confirm some of the understanding. For example, an ET can inform a researcher where a subject fixated during an experiment and how long they fixated for. From this information fair assumptions can be made of whether or not the fixated area was attended, though it cannot be known what brain response was made to the fixated area. Likewise for EEG, various methods (discussed earlier) show the neuronal activity in humans. However, this cannot provide us with the fixation position or any eye movement related behaviour for that matter (scan paths etc.). Furthermore, what AOI the subject fixated to cause the activity. Although, assumptions can be made from the stimuli and paradigms used. However, considering the overlapping research concepts in both fields; it would be a fair transition to manipulate a paradigm to involve both concurrent recording of EEG and ET.

The earliest work involving the co-registration of EEG and ET involved the investigation of the distractor effect, where there is a suppression of saccades directly after a visual stimulus (Graupner et al. 2007). With the foundations set for the concurrent recording of EEG and ET, the projected pathway of the field is continued growth. Nevertheless, when considering an experiment involving the co-registration of EEG and ET, various challenges can arise. There is a difficulty in how to analyse the data; as eye movements create artefacts in the EEG trace.

Careful planning has to be made in regarding the methodology used; the paradigm has to be designed with eye movements in mind. A problem that artefacts can cause is obscuring EEG data; making it uninterpretable, or lead to false conclusions due to them being able to mimic almost any kind of cerebral activity. Another methodological issue is how to align the signals appropriately. In the past research of EEG, neuroscientists have extensively relied on VEPs or ERPs to envisage the potentials produced in visual processing. When there are no eye movements these techniques are fairly safe for interpreting responses. However, in a freeviewing task a different approach has to be made. By knowing the timing of each fixation as it is made, with the signals of the two technologies properly aligned; a new analysis can be formed. This analysis involves the averaged potentials, which neuroscientists can study, to be aligned to the onset of the fixational or saccadic eye movement. These potentials are called fixation-related potentials (fRPs) or saccade-related potentials (sRPs), and are the part of the major work involved in the current thesis. fRPs can be used for a better understanding of neural processes involved in free-viewing. As they are isolated fixations (a period of which there is steady gaze i.e. no eye movement), there should theoretically be no artefact from eye movements imprinting on the potential produced. This is a pivotal step in the progression of understanding the underlying cognitive processes involved in natural eye movement tasks. Furthermore, in the framework of the current thesis, where visual search is investigated; this could not be more essential.

Improvements are constantly being made to the methods in which EEG and ET data is collected. Whether this is in improved displays of stimuli (Richlan et al. 2014), or in the cleaning of data acquired. There are many artefact removal methods that can be used to remove them, ranging from removing bad trial by eye to complex independent component analysis (ICA) such as EyeCatch, which contains over half a million eye movements from EEG data (Bigdely-shamlo et al. 2013). There are continuing to be new methods of removal or even correction of artefact using regression or ICA techniques (Plöchl et al. 2012; Henderson et al. 2013). The improvements in current methods allow fields that were more heavily based on control, to avoid artefacts; to be more adventurous while also salvaging many trials that may have previously been lost to artefacts. However, there are still many challenges involved in artefact correction. Artefact correction based on regression models requires estimates on sources of activity, and if they are not adequate this can affect the efficiency. ICA correction methods sometimes have an issue with objectively differentiating the independent components of artefacts from independent components of the neural activity that is of interest. Therefore, the potential to remove data that is of value is a possibility. Furthermore, successful ICA also depends heavily on how well the signal decomposition is.

Each field has its own direction in how it utilises co-registration. Whether the area involves investigation into the processing of eye movements (Kovalenko & Busch 2016), or the

underlying mechanisms of reading; potentials such as the N400 (elicits to meaningful words and incongruity) are explored (Dimigen et al. 2011). In a world where reading has also become more digitalised, a curiosity into understanding behavioural differences between digitised reading and paperback (Kretzschmar et al. 2013), as well as natural word recognition processing (Kornrumpf et al. 2016); are just a few reading investigations that are capitalising on the technology. There are also those wishing to research into "real world" uses, for instance understanding information systems (Léger et al. 2014) or the hazard perception driving test (Savage et al. 2013). Other fields have looked more into the underlying mechanisms of visual processing through artificial stimuli (Kovalenko & Busch 2016; Ehinger et al. 2015). Additionally, there are those running investigations using natural scenes (Dominguez-Martinez et al. 2015; Simola et al. 2015).

The visual search community is among the fields that have benefitted from co-registration of EEG and ET; especially those involving uncontrolled eye movements. There are now multiple areas that are able to look at the processes involved in target acquisition on a physiological level. From research that involves the processing of artificial stimuli (Kamienkowski et al. 2012; Brouwer et al. 2013; Ušćumlić & Blankertz 2016), to learning about underlying mechanisms of the processing of natural scenes (Ossandón et al. 2010; Nikolaev et al. 2011; Kaunitz et al. 2014; Devillez et al. 2015). Even research adding to BCI systems used for assistive technologies (Wenzel et al. 2016). The visual search field involving co-registration of EEG and ET is the background to investigation that the current thesis centres on.

Co-registration is vital to discovering what underlies responses to visual stimuli, in the past where EEG and eye tracking have been separate there were hypotheses made solely using EEG. It has highlighted confounds, and potential pitfalls in previous research. For example, it was thought that object coding was made by synchronous gamma-band (30-70Hz) activity in visual processing. Populations of neurons encoded different sections of the object (Fries et al. 2007). But recent co-registration of eye tracking and EEG found that gamma-band activity can be attributed by micro-saccades (Yuval-Greenberg et al. 2008). If our understanding of visual processing is to increase, then progress must continue in co-registration. Considering that a few years ago, there were only a handful of published research papers, the field has seen popular growth. With the benefits of co-registration of EEG and ET clear from the content acknowledged in this chapter, the forecast can only see more exploration in the area.

1.4 Comparison between Targets and Distractors Statistical Analysis

One final section, which will be important to some of the main findings of the thesis, describes some concerns that will be addressed in regards to the statistical robustness of the analysis. The paradigms used in the present thesis (as will be explained in later chapters) exhibit multiple fRP components. The main component of concern is the P300, as explained earlier, the P300 should

exhibit when the subject recognises the target. As a result, this particular potential should not be elicited by distractors. Therefore a comparison can be made to make between targets and distractors, the distractors being the control.

Different statistical analysis methods have been implemented since the P300 was first found to disentangle the effects from multiple components. Early work used principle component analysis (PCA) that defined components by identifying the latency ranges over which distinct sources of variance occur. From each source of variance PCA identifies linear combinations of amplitudes that could be sources. Then to test for similarities, analysis of variance (ANOVA) comparing the means of two or more independent samples, can be used (Squires et al. 1977). Even more recent studies have used multivariate analysis of variance (MANOVA) (Gonsalvez & Polich 2002), this is simply an ANOVA that test two or more dependent variables. However, due to the assumptions that have to be made when applying these statistical tests, they may not be the most robust for this type of analysis.

Due to the investigation being an EEG experiment, that samples at 1024hz with 64 channels, there are multiple time-pairs that need to be compared. There is a concern raised called the multiple comparisons problem (MCP) and there is a procedure to try and overcome it (Maris & Oostenveld 2007). For most experiments that compare time-samples to find significance, a conditional probability distribution can be built. A statistical test that samples using this distribution is a permutation test; this test controls the false positives, with or without a conditional distribution. This is a great advantage of non-parametric statistical analysis over parametric. The non-parametric analysis allows the option and the freedom to choose test statistics that is the right fit for the distribution being looked at. It provides a simple solution for the MCP problems; by allowing the use of prior knowledge with respect to the spatial and temporal regions; where a known effect is likely to exhibit. Instead of evaluating the difference between of the conditions for each sample separately, it evaluates for the entire spatiotemporal region a single test statistic; eliminating MCP. A comparison can be made between targets and distractors of the averaged fRP, a non-parametric Wilcoxon Rank-Sum test (Wilcoxon 1945) can be applied to each channel time-pair across all subjects. In order to correct for MCP the results have to be filtered across time samples and recording sites with the following criteria: (1) Keep only the samples that p < 0.05 (2) For each channel, a given time point is considered significant if it is part of a cluster of 8 or more consecutive significant time points for a 30ms window (Dehaene & Naccache 2001; Kamienkowski et al. 2012). (3) Each sample is considered significant if for the same time point at least one neighbouring channel fulfilled (1) and (2).

Fieldtrip is a Matlab toolbox that contains algorithms for simple to advanced EEG analysis. It uses non-parametric cluster based permutation tests. It is a tried and tested method and is well known for robust analysis. For the present study the method of choice for analysis was the Monte Carlo method for calculating the significance probability. The t-value is the calculated

difference (between the two samples being compared) in units of standard error. It is calculated using independent samples T-test with a cluster alpha level of 0.05. Every sample tested will have its t-value compared to this value. Then if two clusters are connected via a small bridge of samples with a statistic threshold then they are considered one cluster. This is considered a tried and tested method for eliminating MCP.

1.5 Thesis Overview

The central focus of the current thesis is to progress the understanding of visual processing within the topic of free-viewing visual search; utilising the co-registration of EEG and ET. The chapters presented will give a detailed account of the progression of the current study. They will describe the combination of EEG and ET, as well as published work that utilised the technologies to collect data from a visual search paradigm with trained eye movements. Then the thesis will discuss an updated version of the visual search paradigm that removes the training. The aim was to try and understand aspects of the neural activity involved in visual search with unrestricted, natural eye movement. Finally, the thesis will discuss results in the context of a wider view; possible uses and future investigations.

Chapter 1 has given a background and history of the technologies. The limitations of the ET and EEG were also described. In sections involving EEG an overview of classically studied brain potentials, such as P100, N170 and P300 were discussed. A background of EEG and ET involvement in visual search, which is one of the main topics involved in the current thesis, was described. The current status of co-registration of EEG and ET was discussed, displaying how few studies focus on visual search involving unrestricted eye movements. Chapter 1 has highlighted areas of interest in methodology and analysis, which will be explored further throughout the current the thesis.

Chapter 2 will give a methodological overview of combining EEG and ET, while also highlighting challenges faced when studying this field. The hardware and software setup will be shown and the signal processing procedures explained. The method of aligning signals from the two different technologies will be discussed, and how potential pitfalls in signal processing can be avoided.

Chapter 3 presents a part of the published work in the visual search field, which utilised the efforts shown in Chapter 2, for its data acquisition and processing. Results show fRPs produced for a "Where's Wally?" paradigm. fRPs are also compared to that of their fixed-gaze counterparts and an effect of saccade amplitude on the P100 potential is shown. Finally, limitations of the paradigm are discussed.

Chapter 4 will detail the main body of the current thesis. Exploring new methods and analysis, taking lessons learned in Chapter 2 and Chapter 3 to further knowledge of visual processing of

natural visual search. Robust fRPs are produced, and common practices such as baseline correction and reference choice are investigated. The chapter will highlight potential pitfalls that can occur in ERP, sRP and fRP studies; and present a novel full trial analysis method that can be advantageous to those investigating similar areas. The chapter will also show how local and global dynamics affect potentials; and to the best of knowledge, demonstrates the first free-viewing modulation of the P300, that mirrors classical concepts found in fixed-gaze EEG research.

Chapter 5 will be the concluding chapter emphasising the main contributions that the current thesis had to the visual processing community. Discussions on areas of the current thesis that would be worthy of publishing will be made. Furthermore, the future direction will be discussed and further exploration into the research area will be encouraged; by acknowledging regions that can be modified and improved, as well as fields that could find the results useful.

Chapter 2 Methodological Challenges

2.1 Combining EEG and ET signals

2.1.1 EEG equipment and software

In the research that follows in the current thesis, the EEG used was a 64-channel Biosemi Active-Two recorded with a sample rate of 1024Hz.



Figure 2.1 Biosemi Active-Two 64-Channel EEG setup: The top picture is a subject sitting the setup for the experiments. He is wearing the 64-channel EEG scalp cap with the electrodes connected. Four external electrodes are secured around the eyes to take a reading for the electrooculogram (EOG) and two other electrodes secured to the mastoid bone for a linked mastoid reference. In front of the subject is a head rest so that there is minimal movement avoiding unnecessary artefacts. The bottom picture is the Biosemi Active-Two AD-box that connects the electrodes converting the analogue signal to digital to be seen in the Biosemi software. The analogue electrodes from the scalp and external electrodes are connected at the top of the AD-box. The orange wire that is connected to the front of the box is the fibre optic connection to the host PC to receive the triggers (seen later), and in the current case it also receives the eye tracker digital channels.



Figure 2.2 Biosemi Actiview Software: Records the EEG data at the settings set by the user. The right hand side bar shows the settings of the recording. The left hand side of the figure shows the various channels and the variable views. And in the centre are the digitized EEG traces.



Figure 2.3 Biosemi Active-Two 64-Channel EEG: The layout of the 64 channel electrodes on the scalp. The nose and the front of the head are at the top of the figure.

The Biosemi Active-Two has a large number of channels with high temporal resolution and sample rate. It has active electrodes, which improves the low frequency noise and input impedance. This was used to record the scalp activity during the experiments conducted, the data is then stored in a brain data file (BDF). The setup can be seen in Figure 2.1 and the software used to record the data can be seen in Figure 2.2. Just for reference Figure 2.3 shows the layout of the electrodes on the scalp.

2.1.2 ET equipment and software

The eye tracker that is used for the current thesis was the Eyelink 1000 system (SR Research, Ontario, Canada). The equipment is a high speed camera, constructed for eye movement recording. It has a high sample rate (up to 1000Hz), very low temporal and spatial noise. It is also very accurate in eye movement collection (typically around 0.25-0.5°).



Figure 2.4 Eyelink 100 Eye-Tracker: The eye tracking hardware, on the left is a light board made of infrared LEDs and on the right is the high resolution camera.


Figure 2.5 Eyelink 100 software: The software used to collect the eye movements. On the screen is an example of the eye tracker software. On the right there are the different options to use and set the parameters of the recording. In the centre, shows the pupil of the eye that is being tracked. On the right are the settings for viewing preferences.

Figure 2.4 shows the eye tracking hardware that is used in the experiments, and Figure 2.5 shows the software from Eyelink used to perform the recording. The host PC is dedicated to processing the camera data. The data is stored as lines of messages saved into an eye data file (EDF). Data collected is usable in real time with very little delay, for example; in the current studies, accessing the data in the middle of a trial in order to end the trial using a fixation made in a certain location. The EDF has to be then converted into an ASCII file, so that using Matlab; the data can be read in and stored in user defined structures.

2.1.3 ET messages and EEG triggers

In order to combine the two signals for analysis, they have to be aligned. There are two clocks involved; one from the EEG and the other from the Eye Tracker. The usefulness of this combination depends on the synchrony to a millisecond precision. In EEG studies, triggers are sent from the host PC to the PC recording the EEG. The triggers sent are timestamps of the events occurring in the experiment (often the start and end of a trial), with different paradigms having other specific triggers tailored for that experiment. A delay will be incurred from the time it takes for the signal to be received but this will be a known constant.

129564	503.3	362.2	1057.0	496.9	337.1	1108.0	
129566	503.5	362.3	1055.0	496.9	337.0	1109.0	
129568	503.7	362.4	1053.0	497.0	337.0	1109.0	
129570	503.7	362.5	1052.0	497.0	337.2	1109.0	
129572	503.5	362.6	1053.0	496.9	337.3	1109.0	
129574	503.4	362.7	1054.0	496.9	337.4	1109.0	
MSG 129576	startTrial						
129576	503.3	362.8	1055.0	497.2	337.4	1109.0	
129578	503.3	362.6	1054.0	497.4	336.8	1109.0	
129580	503.6	362.4	1052.0	497.5	336.4	1108.0	
129582	504.3	362.4	1051.0	497.2	335.9	1107.0	
129584	504.9	362.6	1051.0	497.0	335.7	1106.0	
129586	505 2	362 6	1051 0	497 0	225 5	1107 0	
120000	303.2	302.0	1031.0	407.0	333.5	110/.0	

Figure 2.6 Example of ET message: A message to show the start of a trial has been inserted to the eye data file (Highlighted in blue); each line is a sample containing data from the eye positions and pupil sizes.



Figure 2.7 Example of triggers being sent to the EEG trace shown: Different channel traces are shown in the navy blue each labelled in the y-axis. The vertical coloured lines are different triggers that can be sent to the EEG to show events that occurred at a point in time during an EEG experiment.



Figure 2.8 Example of ET traces being sent to the EEG: Different channel traces are shown in the navy blue each labelled in the y-axis. The ET traces are the step shaped traces. Flat lines indicate a fixation while an increase or decreases in amplitude signify a saccadic eye movement. Different artificial triggers (coloured vertical lines) have been added to the data.

There are three main ways of aligning this signal: Method 1 requires the stimulus presenting computer to send messages and triggers. Messages are short text strings that can be inserted into the EDF by the ET computer (see Figure 2.6). The corresponding event triggers are still sent to the EEG (see Figure 2.7), and the messages act like the corresponding triggers for the ET. The commands for the messages and triggers need to be sent in immediate succession. This needs both signals to have the same sampling rates (SR), if the two systems have different SR then some post-processing will be needed to re-sample the data; for the message and triggers to align. Method 2 requires the ET traces to be fed directly to the EEG as separate channels (see figure 2.8). Using a digital-to-analogue converter (DAC) the ET signals can be fed into the ADbox of the Biosemi Active-Two. This aligns the signals appropriately with very little delay, but the pixels are converted to voltages and need to be scaled. A consideration has to be made because the DAC will increase noise. In this method the SR are the same as both signals are read in the EEG. Method 3 sends simultaneous common triggers to both the ET and EEG using a Y-shaped cable attached to the parallel port of the computer presenting the stimulus. The triggers are sent at the same time to both ET and EEG, though that does not mean that they are fully without delay. However, this requires extra cables and ports; hence for some setups that are without these extra features, the method will not work.



Figure 2.9 EEG and ET setup: An example of how the EEG and ET setup looks like.

In the setup of Figure 2.9, the first two methods are used in the current thesis, as they both have their roles to play. It is also advantageous to record as much data as possible if the resources are available; as the methods used act like a back-up for one another.

Using a combination of methods 1 and 2 makes synchronisation easier. To align the ET traces sent to the EEG with those produced from the ET data. The data will have to be re-sampled to have the same SR. Then the two clocks can be aligned; as the triggers and messages are sent in immediate succession. The combination of method 1 and 2 was used as the basis of the data collection for Chapter 3 and 4. It was utilised as insurance in case one signal failed, and as will be seen later in the chapter this was used to test the signal alignment.

2.1.4 Signal Alignment Concern

With all innovation, there are teething problems that have to be addressed. An initial concern before (Kaunitz et al. 2014) was published, was that a signal problem was found as a result of trying to observe any properties that modulate the fRPs produced (in this instance the concern was to find properties locked within the P100 potential). This led to further investigation to possible reasons for the signal problem found.



Figure 2.10 Noticing a signal processing issue: Two different alignments of the P100 potential at the Oz electrode are shown; fixation-aligned (left hand side) and saccade-aligned (right hand side). Each trace are the grand average potentials isolated for saccade size. Saccades of sizes 1- 2° (green), 2- 3° (orange) and > 3° .

Figure 2.10 was produced before the submission of the details of the data collection and processing can be found in (Kaunitz et al. 2014). This study will also be discussed in Chapter 3. In sRPs there is a small peak at the onset of the saccade (see right panel of Figure 2.10), it has been found to contain the corneo-retinal dipole (CRD) and also the spike potential (SP) (Plöchl et al. 2012). The miss-alignment in Figure 2.10 is not so noticeable in the fRP (left panel of Figure 2.10), however is very prominent in the sRPs (right panel of Figure 2.10). The small peak occurring just before t=0 in the saccade aligned ERP was assumed to be a product of a saccadic artefact (the SP)though as it was occurring before the onset of the saccade; this drew some questions on its origin.



Figure 2.11 Single Trial sRPs with a highlighted issue: The top section of the figure contains the single trial epochs with the onset aligned to the saccade. The Bottom of the figure is the

grand average sRP (blue). The red circle is highlighting an initial peak before the onset of eye movement.

The issue raised in Figure 2.10 is that there is a peak of activity related to eye movement before the onset of an eye movement at t=0. On closer inspection of the single trials in Figure 2.11 it is clear there is a peak of activity occurring before t=0, clarifying there is a miss-alignment.

The CRD has been found to contain frequencies below 30 Hz and the SP has been found to contain frequencies above 30 Hz. Therefore if the artefact that is present in this data is the SP, by filtering the signal between 30-100Hz; the CRD can be eliminated and the SP can be preserved. If preserved the SP should be a biphasic shape. The next step was to clarify whether the small peak of activity occurring before t=0 was the SP (which should be occurring after t=0 in the saccade-aligned ERP). If this was an eye movement, then explanations for why the missalignment could be occurring needed to be found. The EEG data was recorded with a 64-channel 10-20 montage using Active-Two System (Biosemi, Amsterdam, Holland) at 1024 Hz and down sampled to 256Hz. The Data was imported into Matlab through EEGLAB toolbox (Delorme & Makeig 2004) using linked mastoids as the reference. Filtering was applied using Matlab's signal processing toolbox with a 6-order Elliptic filter with a 0.1dB ripple in the passband and 60dB attenuation in the stopbands. Forward and reverse filtering was used to correct for distortion in the phase that occurs from a one-pass filter.



Figure 2.12 Fixation aligned fRP filtered between 30-100Hz: Here are all the channels across the scalp with FPz highlighted in green and the Oz electrode highlighted in red. The black vertical line shows t=0.

From Figure 2.12 it is not very clear if there is a biphasic shape. The onset of the SP, if it is eliciting could be lost in the alignment. However, the SP is known to be a saccadic artefact would be prominent in saccade aligned version.



Figure 2.13 Saccade aligned sRP filtered between 30-100Hz: Here are all the channels across the scalp with FPz highlighted in green and the Oz electrode highlighted in red. The black vertical line shows t=0.

In Figure 2.13, with the saccade alignment, the biphasic shape can be seen clearly across all channels, therefore it can be safely assumed to be the SP. The issue at this point was that it was on-setting \sim 23ms before the onset of the saccade, which for a saccadic artefact cannot be possible. After clarifying that the artefact was the SP (Plöchl et al. 2012), a few ideas to find a solution to the miss-alignment were explored. In order to find the cause of the problem a few avenues had to be explored. Firstly, it was investigated whether there was a filtering effect on the signal.



Figure 2.14 Saccade aligned sRP Oz channel with original filtering between 0.1-40Hz with no artificial delay: The trace is the averaged sRP (red). The black vertical line is t=0, by not adding any artificial delay the saccade onsets at t=0; for visualisation purposes to see any filtering shift effect.



Figure 2.15 Saccade aligned sRP Oz channel filtered between between 30-100Hz with no artificial delay: The trace is the averaged sRP (red). The black vertical line is t=0, by not adding any artificial delay the saccade onsets at t=0; for visualisation purposes to see any filtering shift effect.

By comparing the peaks immediately after t=0 in Figure 2.14 and 2.15 it can be seen that no shift effect occurs for the different filter band pass conditions. Hence, the miss-alignment was not a direct result of filtering. With this information to hand, another possibility was that the saccade detection algorithm being used was perhaps giving miss-timed results.

Eye movements were collected using the EYELINK 1000 system (SR Research, Ontario, Canada). The ET was used in binocular mode with stabilised-head and sampling rate of 500Hz in each eye. Saccades were detected using an adapted version of velocity-based Engbert and Kliegl's algorithm (Engbert & Kliegl 2003); using the parameters described in (Kamienkowski et al. 2012)



Figure 2.16 Kliegl saccade detection algorithm: The blue trace is the x-axis positon of the eye from the EEG eye trace. The vertical red lines are the times the Kliegl algorithm detected a saccade.

It seems from Figure 2.16 that the algorithm seems to be working correctly as the red vertical lines seem to align to the eye movement.



Figure 2.17 Closer inspection of Kliegl saccade detection algorithm: The blue trace is the x-axis positon of the eye from the EEG eye trace. The vertical red lines are the times the Kliegl algorithm detected a saccade.

From a closer inspection in Figure 2.17, with eye position plotted, the algorithm has no delay in the detection; therefore this is not causing any delay. If there is no delay with the processing of the data, another possibility is that the delay could be related to the digital-to-analogue converter. These can come from two sources: the analogue card in the host PC and the resistor-capacitor (RC) filter that was added before sending it to the EEG. The time of decay of the RC filter is ~220 microseconds so this cannot be the source of the problem. This was also compounded as the reconstructed EEG trace shows saccades well synchronised with the EEG channels.

Another possibility was to investigate whether the assumption that the delay between the ET and EEG was constant. This was a key concern, as in order to check the consistency; all the subjects tested had to have received both the triggers as well as the ET traces to the EEG. Thankfully, many subjects had received both types of signal before the hardware issue.



Figure 2.18 EEG and ET traces: The EEG traces (dashed red) are the ET traces sent to the EEG during the experiment, and the ET (solid blue) is the original signal, the signals are resampled (EEG:256Hz and ET:250Hz) in order to minimize SR diffrences without losing too much resolution. The 1^{st} and 3^{rd} plots represent the x-axis and y-axis position of the eye respectively. The 2^{nd} and 4^{th} plots represent the x-axis and y-axis velocity of the eye respectively.

It is very clear to see from Figure 2.18, that the ET trace leads the EEG trace by a certain number of samples. This number was expected to be quite constant across all subjects as the delay only really depends on the time it takes to send the ET to the EEG.

$$\chi^2$$
 (Sum of square differences) = $\sum_{i=1}^n (\beta x - y_i)^2$ (2.1)

The sample lag for each subject can be calculated by creating a lag vector that shifts the signal of the ET trace on sample at a time (see Equation 2.1); where y_i is the sample of interest. An advantage in this instance was the prior knowledge that there was roughly a 6 sample difference. Therefore, taking the sum of the squared differences of each sample lag in the lag vector βx (in this case [-10 10] samples), the sample lag to apply to the signal will be at the minimum difference.



Figure 2.19 Example of Sum of the squared differences between EEG and ET trace Vs. Sample Lag: For one subject and one trial (an average trial contained ~2000 samples), a lag vector was created which shifted the eye position of the eye tracker trace one sample at a time. Taking the sum of the squared difference of ET trace and the EEG eye trace at the same sample of interest; led to a certain number of samples lag where the difference between the two signals reduced to close to zero; aligning them. The minimum difference between the two signals, in this example; was approximately 5 samples.

In the setup, the EEG trigger pulses led the ET messages by a fixed delay from the trigger being sent. The initial assumption was that this lag was the same for each subject in the experiment. But one major problem that arose was that in some subject cases the ET traces were not sent to the EEG. This was later found to be a hardware issue and quickly fixed. Due to the initial assumption that there was a constant sample lag, artificial delay was added to the ET signal to align it to the EEG in this case that was 5 samples * 4ms (sample rate in milliseconds).



Figure 2.20 Subject 1 lag vector test results: The left part of the figure shows the sum of the squared difference between the two signals for all the EEG channels. On the right, is a histogram of the different sample lag, between the two signals, for all the channels.

The analysis for Figure 2.20 was carried out for a further 6 subjects, there was a range found of between 4-10 samples and a mean average of 6. With that in mind and 6 samples corresponding to 24ms of delay, this can be added or subtracted from the signal for all the channels. One point to bear in mind at this point is that there was already artificial delay (20ms) added to the signal (as there was an expected constant delay). What this implies is that the "lag" was artificial; this will be explained later in the Chapter.



Figure 2.21 Re-Aligned signal between EEG and ET trace: The EEG traces (dashed red) are the ET traces sent to the EEG during the experiment, and the ET (solid blue) is the original signal. The artificial delay has been added and the signal is now properly aligned.

The two clocks were now aligned (see Figure 2.21), which means that fRPs or sRPs could be created from the data. This could be done by segmenting the periods of time containing the fixation either aligning the onset of the epoch to the fixation or saccade. Firstly, using a modified version of Engbert and Kliegl's velocity-based algorithm (Engbert & Kliegl 2003) (discussed in Chapter 1), the fixations and saccades can be added to the data as artificial triggers in the EEG data (see Figure 2.8). Then using these triggers the EEG traces can be segmented into either "fixation-aligned" or "saccade-aligned". Following this; the segments can be averaged into fRPs and sRP respectively (as explained in Chapter 1).



Figure 2.22 sRPs for all the channels with 24ms delay added: All 64 channel sRPs are plotted and the vertical black line signifies t=0.

After the sRPs were created, seen in Figure 2.22, it could be seen that there is still the onset of the eye movement occurring before t=0. This was to be expected as the initial delay added was already 20ms. Therefore, the miss-alignment is still apparent. As mentioned earlier; the miss-alignment seems to be artificial; caused by pre-emptively adding delay that was expected. Hence, the next step would be to see what the result would be if no delay is added.



Figure 2.23 sRPs for all the channels without delay added: All 64 channel sRPs are plotted and the vertical black line signifies t=0.

What can be gathered from Figure 2.23 is that the miss-alignment seemed to have been eradicated and the onset of the eye movement was now exactly at t=0. This was not initially anticipated as there is a delay between the signals of the ET trace being sent to the EEG trace. The miss-alignment for this study was relinquished by removing all artificial delay. Although the fix seems simple, it was important to investigate the other factors involved; so that future studies are aware that any delays are not caused by the processing procedures investigated. The validity of the results found in the published work of Chapter 3 remains. This was an imperative finding, because if the artefact reported was later found to be a product of noise or a result of methodological procedure then the validity of the results would have been compromised. These results show there was a fix to the delay, and there was no harm to the strength of the results presented in Chapter 3 and Chapter 4.

2.2 Further Synchronisation Problems

The data up until this point was collected from a paradigm that had training to restrict eyemovements (to form the paper discussed in Chapter 3). The following two figures were obtained using data collected from completely free-viewing, visual search experiment (which formed part of the analysis discussed in Chapter 4). During the analysis of this data, another alignment issue arose. A novel analysis, in which the full trial of an experimental paradigm was being investigated, was showing signs of a miss-alignment of data.



Figure 2.24 Single Trial fRPs sorted by trial duration: The top section of the figure contains the single trial epochs with the onset aligned to the fixation to the target in the experiment. The Bottom of the figure is the grand average fRP (red). The solid vertical black line signifies t=0 (onset of the fixation).

The clear point that stands out in the top panel of Figure 2.24; just after the onset of the fixation (the vertical black line), the activity was staggered, and in this instance it seemed like the effect occurred most prominently in the longer trials. Initially it was thought that this was a jitter, and with longer trial durations that jitter would increase. However, the onset of the potential, which was the P100 (which should peak at \sim 100ms); had a much earlier onset (in some cases at 20ms). This led to the possibility of a trigger timing issue.

After some exploratory analysis this was found to be a coding error. Trials in this experiment were due to end after 20s if no target was found (the experiment will be fully explained later in Chapter 4). But a criterion was for the subject to fixate to the target for 1 second when they found it. The experiment code was found not to have a pause feature to avoid ending the trial if the current fixation of interest (in real time) was on the target in the visual search. Hence, if the subject found the target at 19.5 seconds into the trial, the trial would end after 20 seconds regardless of the fixation on the target.

The data was structured in this case by aligning the end of the data to the trigger that signalled the end of the trial. This trigger that is sent after the final fixation to the target (which lasted 1 second), and was not expected to vary. But in order to avoid throwing away usable data, a solution was to re-align the data to the trigger that represented the final fixation to the target.



Figure 2.25 Single Trial fRPs sorted by trial duration: The top section of the figure contains the single trial epochs with the onset aligned to the fixation to the target in the experiment. The Bottom of the figure is the grand average fRP (red). The solid vertical black line signifies t=0 (onset of the fixation).

Immediately the difference is obvious in Figure 2.25, the P100 is aligned properly and all activity in the top single trial raster plots is aligned. This means two things. Firstly, there is a variance of time from the end of the final fixation to the target, to that of the trigger being sent to signify the end of the trial. Secondly, and probably most importantly; the alignment of the two signals can lead to false conclusions; if not applied correctly. In this example, if the comparison were made solely on the grand average fRPs, with no single trial analysis, then the averages would have very different shapes. In Figure 2.24, just after t=0 the grand average potential is much lower in amplitude and broader in time. This is expected when there is a missalignment of signals. Whereas, in Figure 2.25, the same peak was narrower and much larger in amplitude. Thus, had it not been for the single trial analysis sorted by trial duration; the timing issue may not have been noticed. Therefore, when attempting this type of experiment; it cannot be recommended more for a single trial plot of the results to be performed at some stage in the analysis.

Summary

The solutions to some of the synchronisation problems in this chapter, and the clarification of the SP are the main contributions in order to get the results for the publication (Kaunitz et al. 2014), discussed later in chapter 3. Fundamentally, the challenges exhibited in this chapter had to be overcome in order to produce further robust analysis and valid conclusions. The synchronisation results were laboured for and common signal altering techniques were

investigated, while the biggest challenge, combining the two signals; was accomplished. This chapter has provided a great foundation to press on with the new experiments. The results mean that it is possible to combine these two technologies in a constructive way, to draw deeper insights into processing of the visual pathway.

Chapter 3 Visual Search with Restricted Eye Movements

3.1 Paradigm: Where's Waldo?

One of the most complex challenges in EEG is designing a dynamic visual search that involves eye movements. To date only a handful of works have investigated this field using solely EEG. However with the recently developed co-registration, this is now a possibility for further investigation. For clarity, the basis of the data collection, processing of the signals and confirmation of the signal alignments were the main contributions to this study. This chapter describes the investigation that provided the foundation for the main work in the thesis in Chapter 4. The figures and analysis discussed in the current chapter were provided by the first author in (Kaunitz et al. 2014).

In this preliminary study an altered version of the well-known game "Where's Waldo?" was implemented. "Where's Waldo?" is a children's game. It is an image that contains Waldo, who wears red and white stripes and he is hidden amongst very similar looking people. The task is to find Waldo (the target) amongst all the similar looking people (the distractors).

Although the task investigated was a visual search containing many similar objects, there were some clear differences compared to the actual "Where's Waldo?" task. Firstly, the images used were of natural stimuli. They were scenes of crowds at a football stadium; therefore they contained many faces. Subjects were tasked to find one target face amongst the crowded scene. Each trial started at the press of the space bar, at which point the subject would be presented with the target face for the upcoming trial; for 3 seconds in order to memorise it. The target faces were also resized from the original as not to create a bias or strategy. A fixation dot was then presented at a random location on the screen. Subjects had to fixate on this dot for 1 second and then the visual search began. The subjects then searched through the crowded scene until they found the target face. Once found the subject had to fixate on the target for 1 second and the trial would end. The trial would automatically end after 20 seconds should the target not be found. To avoid any areas of saliency in the images, they were grey scaled and made isoluminat. There were 180 trials presented in pseudo random order, and split up into blocks of 60 trials with 5 minute breaks between for subjects to rest. There were 60 images containing between 23 -35 faces, from each image 3 target faces were chosen to make up the 180 trials. Before the experiment began each subject was trained to search for targets in an unrushed way. This was done using a 1 Hz clicking metronome. This was in order to create unrushed visual search trials. The subjects were also given feedback at the end of each trial; if they had made less than 2 fixations of 0.5 seconds then they were told to slow down their search. The rationale behind this control was to create elongated fixations in order to study late latency fRPs. The training images were not used in the actual experiment. Overall by training and giving some simple instruction

1561 targets were fixated and 4655 distractors were fixated across all subjects; the number of long fixations made to distractors increased. This was a very important factor for the initial study, as the longer fixations created the option to investigate clean evoked brain potentials for late latency responses.



Figure 3.1 Behavioural Trial with scan path and ET traces: The top part of the figure is a background image of a crowded scene used in the trials. The target is surrounded by a red square. The dots represent fixations made within the trial; the diameter size shows the duration of the fixation and the progression is shown by the colour, the key is in the top right of the figure. The bottom panel shows the ET traces for the horizontal (blue) and vertical (green) positions; the red vertical line is the onset of the target fixation.

Figure 3.1 gives a typical example of a trial progression for the visual search task. As discussed previously, each of these fixations can be isolated and epoched from the EEG trace to investigate the fRPs produced. Fixations to targets and distractors were made within rectangles that perimeter the whole face. This was in order for drift in calibrations of the eye-tracker during the course of the trial, to have a reduced effect and register a fixation. The faces were on average too small for the resolution of the eye-tracker to specify exact locations of the face the subject fixated to. This would require much larger faces and a different focus of study.

3.2 Behavioural and fRP results

One of the advantages of using an eye tracker in a co-registration study is that it records the subject behaviour during the entire trial. Properties of all the eye movements are accessible, and therefore they can be analysed.



Figure 3.2 Behavioural eye movement statistics: The top panel are the distributions for the amount of fixations made per trial, the middle panel are the distributions of fixation durations, and the bottom panel are the distributions for saccade amplitudes.

Subjects made on average a mean of 8.4 (SD: 1.84) fixations and took a mean time of 8.5 seconds to find the target. The distribution of fixation durations in figure 3.2 is skewed at ~0.22 seconds, with a tail to the longer durations. As discussed in previous chapters, the eyes naturally make saccades every 200-250ms. Therefore the later tail of the distributions is a direct result of the training given before the experiment began. The saccade amplitude distribution was also skewed with a mean of 3.7 (SD: 2.3) degrees. For each trial the targets (which had a duration of

1 second) and distractors (of which had a duration of over 0.5 seconds) were analysed. EEG segments aligned to the fixation onset were epoched between [-0.2 -0.5] seconds from the fixation time. A baseline correction was applied per epoch in the time window [-0.1 -0.2] seconds. These epochs for targets and distractors were averaged over all trials and subjects to create grand average fRPs. The main focus of analysis for the fRPs was across the midline electrode channels to compare differences between targets and distractors. A non-parametric Wilcoxon rank-sum test was applied to each (channel, time) pair between the target and distractor grand average fRP. To account for multiple comparisons a false discovery rate (FDR) procedure was also applied; samples were only considered statistically significant if the p-value of the rank-sum test was below the 5% threshold set for the falsely rejected null hypothesis.



Figure 3.3 Grand Average fRPs for targets and distractors: Here fixations onsets at 0ms and the fRPs were baseline corrected [-200 -100] and for the distractor condition the fixations were of \geq =0.5 second durations. Channels Fz, Cz, Pz and Oz are shown for the Target (red) and Distractor (blue) conditions. The mean shown by the solid lines and SEM is shown by the slightly opaque outlines. The grey bar across the top of each plot is the significance difference calculated using a FDR to account for the multiple comparisons problem. Figure was taken from (Kaunitz et al. 2014).

In the grand average fRP (Figure 3.3), there are some clear brain potentials that can be seen. In the Oz electrode there is a P100 for both target and distractors. In Fz, Cz, and Pz there is a VPP potential which is associated to the facial processing, therefore this was expected. Another expected potential that elicited in the target grand average fRP was the P300, this elicited to the detection of the target. This does not elicit in the distractor grand average fRP, and is shown to be significantly different.



Figure 3.4 Topographical plots of the grand average subject response: The top panel shows the topographies for the target responses. The middle panel shows the distractor response, and the bottom panel shows the difference between the two conditions.

In the early stages of response, between [0 200]ms after fixation onset, targets and distractors elicit almost identical visual potentials. This diverges after ~250ms post fixation and the target discriminating P300 begins to elicit, this can be seen spatially in the topography in Figure 3.4 in the centro-parietal regions.



Figure 3.5 Preceding saccades modulating P100 potential: The left figure is the grand average fRPs aligned to the onset of the fixation. There are three separate traces, the green is the grand average of all trials in which the saccade amplitude was between 1-2 degrees, the orange is the grey is the average of all trials in which the saccade amplitude was between 2-3 degrees, and the grey is the average of all trials in which the saccade amplitude was greater than 3 degrees. The right figure is the grand average sRPs aligned to the saccade onset; the layout is the same as

the right. At the top right of each figure is a scatter plot of the P100 peak amplitude as a function of saccade amplitude.

An observation from Figure 3.5 is the differences in onsets of the P100 potential for different saccade sizes in the left panel. However, this was to be expected due to saccade duration being directly proportional to saccade amplitude. As the left panel potentials are aligned to the onset of fixation, the onsets will be directly affected. This is further clarified in the right panel of Figure 3.5 the onsets of the P100 potential are very well synchronised when aligning the potential to the onset of the saccade. There was a significant trend when a regression was run to analyse P100 amplitudes as a function of preceding saccade amplitudes. There was a significant positive correlation found for fixation aligned (Pearson r = 0.17, $p = 10^{-10}$) and for saccade aligned (Pearson r = 0.15, $p = 10^{-9}$). The results presented in Figure 3.5, in contrast to Figure 2.10, were properly aligned after the investigations made in Chapter 2.

3.3 Main findings from study

The main objective of this work was to provide the best foundation for the future of coregistration studies involving ET and EEG with eye movements. In order to do that the results would have to contain robust cognitive potentials that relate to previous studies involving fixedgaze. A common paradigm used in fixed-gaze, is the Oddball paradigm (Sutton et al. 1965). The experiments involve flashing stimuli at a subject as they fixate a point on the screen. The results often give well known components. As a control, as well as a basis for comparison the authors used a visual oddball version of the visual search, which contained the same stimuli involved in the dynamic visual search.



Figure 3.6 Comparison of fRPs and oddball ERPs for targets and distractors: The right plot is the same as figure 3.3 for comparison purposes. The ERPs produced by the oddball paradigm are on the right.

Although there are a lot of similarities between the two plots in Figure 3.6 as in the target discrimination occurs in both experiments in the target ERP conditions, and the VPP also occurs for targets and distractors in both experiments. There are some very clear differences in the shapes of the potentials produced by both experiments. There are very clear P3a and P3b components in the oddball task not present in the dynamic counterpart. The P100 in the Oz electrode for both experiments shows differences; not only is the amplitude much larger in the dynamic version, it also onsets earlier. These differences were related to an increased effort to distinguish targets amongst a crowd of distractors, which is a higher attentional load. It could also be an influence of preceding saccade amplitude or perceptual differences in the two processes. The restrictions on the eye movements could also be an influential factor, as the increased motor control could have introduced other neural activity. There is also the fact that the two experiments are intrinsically different in the sense that fixed-gaze oddball task did not have influences on where to look next, and in the dynamic task subjects are also constantly updating the information on the scene they perceived.

Single trial classification of the stimulus identity was also investigated. Target and distractor faces were both distinguished at an above chance level for both the oddball and dynamic tasks (binomial test, p < 0.05 against the null hypothesis). The largest contribution to the classifier was in the centro-parietal channels at ~450ms. The spatial location and time window of the most informative electrode were consistent with the ERPs produced when a blind analysis was applied.

Furthermore, after discovering robust fRPs can be obtained from such visual tasks, and be classified; an important question was whether any properties could modulate the signals produced. Another important finding in this study was finding said properties. In Figure 3.5 a significant positive correlation was found between the amplitude of the P100 potentials in Oz and the saccade amplitude preceding the potential. This was a big finding because this sort of modulation would not have been found unless the task involved eye movements which were not removed from the signal. One additional finding was a non-cognitive, artefact-related and sensory potential. Near to the fixation onset a frontal component was found that was generated by the spike potential (SP). This was also part of the contribution towards the study as well as the signal processing issues presented in Chapter 2.

Understanding visual processes in EEG, in the presences of eye movements is a difficult task; there are both theoretical and practical implications. This investigation not only allowed the direct comparison of potentials produced in fixed-gaze tasks, and that of a new dynamic visual search, but also found direct influences from eye movements on fRPs and sRPs. It has shown that robust cognitive fRPs can be obtained from tasks involving eye movements, and has set the foundation for future studies.

3.4 Discussion on limitations of current study

The present study has shown that it is possible to extract brain potentials from EEG in paradigms that involve in free eye movement and natural scenes. Visual search is a very complex task, and subjects were submitted to high cognitive load processing. It was also a big challenge practically. EEG in the past had steered away from eye movements, due to the amount of artefactual activity produced. Hence, the present study is impressive considering trends in brain potentials produced were found as a direct correlation of a property of eye movements. With all the practical challenges and hurdles to overcome in this study, it has laid a great platform for further studies to investigate eye movement related tasks. Furthermore, the foundation has also been set for the investigation of brain potentials in visual search; with robust fRPs being found in this study.

The study made a lot progress; however, there is still a long way to go into understanding the processes involved in visual search and visual processes in general. One key argument some may have with this study was that because of the instructions and training given to elongate fixation duration. The study cannot be considered a completely natural visual search. The rationale was sound because late latency potentials were targeted, and because natural eye movements occur every ~200-250ms, any potentials that elicit after this period would contain artefacts. However, the main reason for the use of co-registration is to move closer and closer to understanding natural visual tasks. Therefore, the need to move away from constraining subjects in their natural processes has to be pursued. Free-viewing implies the freedom of eye movement from restrictions and training. Therefore, the future direction will involve removing training, while also diversifying the analysis to investigate more fixation duration types. One certain expectation, for any completely natural free-viewing task; is that the behaviour statistics should change significantly compared with the current study.

The next step in this investigation would be to move more towards the natural processing that was discussed earlier. By removing the constraints and using this study as an initial comparison, the future task can be viewed as a completely naturalistic free-viewing visual search. However, something that needs to be clarified will be how the brain potentials being produced will be affected. As seen in the current study, fRPs for dynamic tasks compared to the potentials produced in the fixed-gaze oddball contain similar activity, but have different shapes overall. Given the authors hypothesis that the differences could come from the training and instructions; it would be interesting to see whether the potentials produced would remain similar to that presented in this chapter, or move back towards that elicited in fixed-gaze processing. The next study could also further investigate different properties that could affect potentials. With the different dynamics of eye movements being unconstrained the possibilities are open to eye movement effects on fRPs and sRPs. It is these next steps using the current study as a base that will allow further investigation of brain potentials in completely free-viewing, naturalistic visual search.

Chapter 4 Free-Viewing Visual Search

Free-viewing visual search implies that eye movements made are solely controlled by the viewer; "free of restrictions". To get visual search paradigms to be as close to a natural visual search as possible; there should not be any instructions given to subjects except to search for a target. Paradigms that conform to this structure will be free of restrictions in how to move the eyes to perform the task. There were some key limitations presented in Chapter 3. The prolonged eye movements that were made were a product of training, instructions and cues before and within the experiment. Therefore the subjects participating were not in full control of their eye movement decisions. As will be seen in this chapter; eye movement properties, are very different when these parameters are removed. Consequently, the previous study cannot be considered a completely free-viewing task.

In order to get a better understanding of natural visual processing, in visual search; a subject must be able to perform the task in such a way, that the eye movement properties (such as the size of saccades made or fixation durations), will not be inhibited. The only way to ensure this behaviour from subjects is by giving the basic instructions for the task with no encouragement on how to perform eye movements for analytical gain.

4.1 Updated 'Where's Waldo?' experiment

The procedure for the paradigm is as follows; the trial began when the participant clicked the space bar, the subject was then presented with the target that they were expected to find for 3 seconds(s). After the presentation of the target the subject was required to fixate on a randomly placed black dot on the screen for 1s. Once they had fixated the black dot, the image of the crowd that the target face was taken from was presented and the exploration began. The task for the subject was to search for the target face amongst the crowd of faces and once they had found it, fixate on it for 1s (depicted in Figure 4.1 below). The trial would end when either the subject found the target, or if the elapsed time of the trial reached the maximum trial time of 20s. The 60 images were presented in pseudo random order and the 180 trials presented in 3 blocks of 60. Between each block the subjects were asked to rest. In each block the target faces were all different and the same stadium image was not used twice within the same block. The target faces, when presented before the trial started, varied in size by about 2-4 degrees of visual angle across trial. These guidelines were so that subjects could not forge any tactics or strategy into finding the target face.



Figure 4.1 Updated 'Where's Waldo?' paradigm : *From left to right:* **Target Presentation:** the first box shows the target that the subject has to find the presentation lasts for 3 seconds. **Fixation Dot:** the next box shows the random fixation point the subject has to fixate for 1 seconds to start the trial. **Crowd Presentation:** the subject is presented the crowds' image that the subject search for the target in, the subject has a maximum of 20 seconds (before the trial ends) to find the target. **Target Fixation:** once the subject finds the target face; they make a fixation to it for 1 seconds to end the trial.

The restrictions in Kaunitz et al., may lead some to think that task the subjects are participating in is not 'natural' or 'free-viewing'. What the current work does is take those restrictions away. Subjects were advised simply to search for the target face amongst the distractor faces at their leisure, and once the target is found fixate on it for 1 second, then the trial would end. Therefore, the data acquired from this experiment gives a closer look to how the brain 'naturally' responds and cognitively processes it makes to a completely free-viewing task.

The number of fixations to distractors that are greater than 500ms is expected to decrease dramatically, which is an obstacle that this new paradigm creates. The average fixation during scene viewing is around 200ms (Smith & Henderson 2011). This will be shown to be a challenge, and can affect the type of analysis run; as the previous work compared targets and Distractors that were above 500ms. There will also be a pressure to run a more robust statistical analysis for this kind of comparison. The previous work used a false detection rate (FDR) as a counter to the multiple comparisons problem (MCP). In the current study this type of analysis will be tested for robustness, as well as cluster based permutation testing (Maris & Oostenveld 2007; Oostenveld et al. 2010). With this increase of eye movements there will be a problem of more artefactual components affecting the EEG, which will have to be addressed.

As mentioned in Chapter 1, although the field of co-registration is growing, a note for consideration would be the amount of comparable work available for natural free-viewing visual search. A small amount of existing studies involve concurrent recording of EEG and eye-

tracking; and of those there are few co-registration studies involving conditions applied to eye movements. There are even fewer involving completely unrestricted eye movement. This can create two different dynamics. One, the challenge to overcome not only comparing the brain potentials produced in the experiment with those already well known, but also find strong conclusions for the differences as well. Two, this is a very novel field of work, which means the potential for new discoveries is high.

4.1.1 Participants

There were 17 participants performing the paradigm (13 male/4 female; aged between 21-31 years) all had normal or corrected to normal vision, they gave written consent and they were also ignorant to the tasks underlying questions. This experiment was performed more than 2 months after Kaunitz et al., was performed to ensure no memory effects were present 9 subjects that participated in both experiments. This was confirmed by a comparison made between the subjects that participated in the both and subjects that only participated in the second study. A Wilcoxon Rank-Sum test was applied to random subsets of the distributions of fixation durations. No significant differences were found (p=0.19).

4.1.2 Apparatus

Stimuli were presented in a cathode ray tube (CRT) monitor with a screen resolution of 1024 x 768 pixels and at a refresh rate of 75Hz. Subjects sat 60cm from the monitor in a chair, with their heads stabilised using a specially designed 'cheek rest' (to avoid EEG artefacts from muscular activity from the jaw) and responses were made on a standard 'qwerty' keyboard. All experiments explained were presented and operated from MATLAB (MathWorks 2000) using the Psychophysics Toolbox extensions (Brainard 1997; Pelli 1997).

4.1.3 Stimuli

In a database there were 60 gray-scale images. The images were of football crowds in stadiums, each image was 800x768 pixels and contained between 23-35 distractors (30.68 mean average). From each image 3 faces were chosen as targets and the luminance of the image was evenly distributed to avoid characteristics of the image that could be more attended.

4.1.4 EEG and eye data acquisition

The EEG data was recorded with a 64-channel 10-20 montage using Active-Two System (Biosemi, Amsterdam, Holland) at 1024 Hz. The Data was imported into Matlab through EEGLAB toolbox (Delorme & Makeig 2004) using linked mastoids as the reference.

Datasets that were created were down-sampled at 256 Hz and band-pass filtered at 0.1 - 40 Hz (six order elliptic filter). The start of the fixation on distractor or target face was taken as the onset of the trial. The responses for the target from each crowd image were analysed, as were the fixations to distractors. For fixations to distractors the durations were considered based on the previous analysis presented in Chapter 3. Therefore, for the initial analysis, the fixation to distractor duration chosen was 500ms and the EEG data was aligned to fixation onset and epoched between [-0.2 0.8] seconds from the start of the fixation.

Eye movements were registered with an EYELINK 1000 system (SR Research, Ontario, Canada). The ET was used in binocular mode with stabilised-head and sampling rate of 500Hz in each eye. Saccades were detected using an adapted version of velocity-based Engbert and Kliegl's algorithm (described in Chapter 1); using the parameters described in Kamienkowski et al., 2012. Only saccades larger than 1 degree were kept for the analyses of the data, as saccades below this threshold were considered microsaccades (Otero-Millan et al. 2008). For all the experiments a drift correction was made every 10 trials, and a recalibration of the ET every 60 trials (before the beginning of a new block).

4.2 Behavioural Results

A key reason, and one of the many bonuses, for using an ET; is its ability to gather information in terms of detailed eye movement behaviour. Some eye movements can be gathered from an EOG from the EEG, such as saccades or fixation durations. However, information such as the position of the fixation made on the screen cannot be known without an ET. There is a lot that can be learnt from the eye movement data gathered; in terms of the cognitive processes that occur during trials. It may also explain what may be seen in the physiological results, or at least help the understanding of what is taking place in the brain.

Depending on the task, information about a scene could be gathered with a fixation duration 45-75ms (Rayner 1998). In a study (Rayner 2009), a moving mask paradigm was used that appeared after every fixation for set period of time. This found that the gist of a scene could be perceived with fixation durations as little as 40ms. Other studies in scene perception use fixations >50ms (Tatler et al. 2006), and after developing a new algorithm for the detection of eye movements it was also suggested that for scene viewing a minimum fixation duration threshold of 40ms can be used (Nyström & Holmqvist 2010). In the current Chapter there were only 17 fixations from 17 subjects detected between 40 and 50ms; therefore fixations in this study fixations are only considered if the duration is >50ms. Due to the task requirement to fixate on the target for 1000ms all fixations >1000ms were also removed; this was to remove any fixation made that were mistaken identity or fixations slightly "off target" (i.e. target found but not within the detection area confirmed by the ET).

Table 4.1 Fixation to Target and Distractor properties: Listed for each subject are properties of the Targets and Distrators for all trials in terms of numbers. From left to right: Subject number, Fixations to distractors >500ms, Fixations to distractors >400ms, Fixations to distractors >200ms, Fixations to Target, Total Fixations (i.e >50ms). At the bottom it gives the grand averages (S.D.).

Subject	Fix2dist	Fix2dist	Fix2dist	Fix2tar	Total	Mean	Std	Median
	>500ms	>400ms	>200ms	>500ms	Fixations	(ms)	(ms)	(ms)
S1	49	148	1001	134	2445	288	281	212
S2	73	125	742	96	1675	333	343	240
S3	17	62	849	153	1611	336	313	246
S4	11	23	480	136	1730	260	261	190
S5	46	110	655	100	1566	323	294	236
S6	20	51	653	98	1767	263	243	202
S7	10	30	541	114	1658	272	278	198
S8	32	81	575	99	1286	291	250	228
S9	36	100	873	145	2223	294	269	210
S10	59	111	934	145	1947	335	354	242
S11	12	46	824	134	1739	306	268	232
S12	84	158	874	94	2092	337	403	228
S13	58	119	737	105	1828	295	270	216
S14	33	78	774	147	1930	306	339	216
S15	84	182	1145	122	2126	335	278	256
S16	31	72	513	68	1407	281	253	206
S17	19	45	742	123	1520	290	256	234
Total	674	1541	12912	2013	30550	-	-	-
G.A	39.6	90.6	759.5	118.4	1797	302.6	291.3	223
	(24.8)	(46.3)	(180.2)	(24)	(301.9)	(26.4)	(44.3)	(18.6)

The most prominent observation from Table 4.1 is the small amount of fixations that were made above 500ms per subject. When compared to fixations that are greater 400ms, the number almost doubles for 90% of the subjects, and in some cases even triples. Observing the total, the number of fixations greater than 400ms is more than double that of fixations greater than 500ms. In the previous study there were over 4000 fixations made to distractors >500ms. In the current work the number of fixations >500ms is just 14% of that of the previous work. This was hypothesised earlier, but already there seem to be some key differences appearing from the current study, as opposed to that discussed in Chapter 3 with the restricted version.

Histograms are plotted for fixation durations, number of fixations preceding a target, saccade amplitude and trial duration.



Figure 4.2 Subject behaviour: A: Fixation durations made to distractors; shows a clear peak at ~220ms, this is a typical fixation duration in natural unrestricted visual search. Mean fixation duration was 249ms (SD:16ms) and median of 216ms. **B:** Fixations preceding distractors; these are all the fixations preceding the target and not just the fixations to distractors i.e. the "True" Fixations preceding target. Mean number of fixations per trial was 12 (SD:12) and median of 8. **C:** Distractor saccade amplitudes; contains all the saccade amplitude in degrees from all the valid trials. Mean amplitude of 3.6 degrees (SD:3.15 degrees) and median of 2.7 degrees. **D:** Trial duration; the mean duration of a trial to find the target, per trial; was 5 (SD:3.9) seconds and median of 3.5 seconds.



Figure 4.3 Further Subject Behaviour: A: Distractor saccade durations; contains all the saccade durations from all the valid trials. Mean duration was 35.4ms (SD:13.4ms) and median of 34ms. B: Target saccade durations; Contains all the saccade durations from all targets valid targets. Mean duration was 33.4ms (SD:12.6ms) and median of 32ms. C: Fixations close but not directly on target; mean number of fixations made close to the target per trial was 0.81 (SD:1.1) and median of 0. D: Duration from previous trial to the start of current; this is the time that it takes for the subject from finishing the previous trial to the starting the current. The mean duration for this period was 6 (SD:1.7) seconds and a median of 5.5 seconds.

The number of fixations that are >200ms are 10 times more than fixations made >500ms. The median fixation duration was found to be 216ms. Both results show a progressively more natural response to the task. It is apparent from Table 4.1 and Figure 4.2A; that the fixation duration distribution conforms to what is commonly found in natural eye movement behaviour (Smith & Henderson 2011). The number of fixations made can be beneficial for understanding the saliency or complexity of a scene (Tatler et al. 2005). It may also have implications on the physiological response (Gonsalvez & Polich 2002). Given that the current thesis involves solely visual search, the number of fixations made prior to finding the target could give an indication of how difficult the target was to find (see Figure 4.2B). There is also another factor that could show the difficulty of the search, which would be the duration that it takes to find the target within one trial (see Figure 4.2D).

Discovering whether a target needs to be fixated in order to be detected is also something of interest, and one way to clarify this was to find how many fixations were made close to the targets position (see Figure 4.3C). There was also the hypothesis that the saccade durations, of the saccades made to the targets, should be shorter; if the target detection is made in the

periphery. Therefore if the target saccade durations are similar to the overall saccade durations (see figure 4.3A and Figure 4.3B), target detection is not very likely to occur totally from the periphery; but more from direct fixation. Figure 4.3C was created by doubling the area of detection of the target; the fixations were considered "close" if they fell within this section. The distribution in Figure 4.3B also shows a similarity to that in Figure 4.3A.



Figure 4.4 Target vs. distractor saccade properties: saccade amplitude and duration are compared for targets (red) and distractors (blue).

The distributions in Figure 4.4 seem to show differences in terms of the saccade properties. After running a t-test and a Wilcoxon ranksum test, it was found that the mean and medians of the distributions are significantly different (p-values for the tests were $p=2.2 \times 10^{-7}$ and $p=6.8 \times 10^{-8}$ respectively). As the median of the target saccade duration distribution is 32ms, this result would seem to suggest that saccades made to targets are shorter than the overall distribution. Therefore there is the possibility that saccades are made to targets from fixating close to the target, detecting, and then making a shorter saccade to it. The result suggests that in order to detect the target the subject would have to see it in the periphery before fixating directly on it. A previous finding showed that shorter saccades were made to targets if they were expected (Carpenter et al. 1995). Although the result cannot shed any light on the expectancy of targets, it is results such as this that can be utilised for further research into modelling (discussed later in Chapter 5).


Figure 4.5 Exemplary behavioural trial: Top Panel: shows the search from the first trial from the first subject and superimposed are the scan path (ET samples shown in red) and fixations made throughout the trial. The random fixation dot before the trial starts is plotted as a blue circle. Dots represent fixations made within the trial over 50ms (valid fixations); the diameter size shows the duration of the fixation and the progression is shown by the colour, the key is in the bottom left of the top panel. The darker the fixation, the lower the fixation rank; the lightest is the last fixation (to target). **Bottom Panel:** shows the eye traces of the trial in blue are the horizontal (x) positions of the eye and in green the vertical (y) positions. The vertical red line shows the end of the trial (fixation to the target, with duration of 1000ms).

An example of one of the benefits of using an eye tracker is shown in Figure 4.5, where a subject's scan path in the visual search can clearly be seen. As well as the fixation durations being visualised by dots with a diameter proportional to the duration. These types of results are very useful for studies involving the understanding of strategies for visual search.

4.3 Robust fRPs

The next step for the data collected in the current work was to follow the analysis format of that in Kaunitz et al. 2014. The analysis in the previous work, in particular for the fRPs, focused on

the midline electrode sites for the frontal, central, parietal and occipital (Fz, Cz, Pz and Oz). This study also selected fixations >500ms in order to have clean fRPs to analyse late potentials such as the P300 (the rationale discussed in chapter 3). One interest was in understanding the physiological differences between the responses from the target face that had been presented at the beginning of the trial, and the other faces that had been fixated in the search up to finding the target. Target and distractor faces were very similar in terms of shape, size and luminance; therefore the early processing involved for the stimuli was hypothesised to also be alike. Using the cluster based permutation for MCP correction (Maris & Oostenveld 2007); robust fRPs were produced. Previous studies have used baseline correction for fRP analysis (Ossandón et al. 2010; Dimigen et al. 2011; Plöchl et al. 2012; Kamienkowski et al. 2012; Kaunitz et al. 2014; Devillez et al. 2015; Dominguez-Martinez et al. 2015; Simola et al. 2015; Wenzel et al. 2016). As seen in the fixation duration histogram in Figure 4.2A the average duration was \sim 216ms (median) and looking at Figure 4.3A, the averaged saccade duration was ~34ms (median). The rationale behind the baseline, was that in the period [-200 -100] will be taken within a fixation; as the fixation prior to the one of interest will have started at -250ms and last \sim 216ms. Therefore, there should be very few artefacts from eye movements; such as saccades. Thus, the averaged fRPs in this part of the analysis were baseline corrected between [-200 -100] prefixation.



Figure 4.6 fRPs from midline electrodes (500ms): Here fixations onsets at 0ms and the fRPs were baseline corrected [-200 -100]. For the distractor condition the fixations were of \geq =500ms durations (as in Kaunitz et al.) and <1000ms. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The mean shown by the solid lines and standard error of the mean (SEM) is shown by the slightly opaque outlines. The colour bar at the top of each channel plot, shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010). The number of distractor epochs for the figure are n= 534.

From Figure 4.6, the early processing [0 170]ms shapes resemble previous findings for low level features (such as the P100 and N100 potentials), as well as face processing (VPP); for both the targets and distractors. There are significant differences within the P300 window [300 500]ms across the two conditions. The P300 potential for the target fRPs stands out, which was expected from the results of Chapter 3. However, something that is unexpected is a significant difference between the two conditions occurring ~170ms at the point the VPP presents itself. Although, in a recent studies (Caharel et al. 2015; Maratos et al. 2015) have found that face recognition for target, and emotive expression, as well as orientation of the face fixated; show an increased amplitude in the face sensitive potentials. Therefore, it is possible for the differences seen between the conditions, to be a result of the faces in the crowded scenes having many different orientations and emotive expressions (given the naturalistic setting of the scenes). Furthermore, this would also be compounded with the effect of increased amplitude for target faces. There was also a study (Jacques et al. 2007) that found differences in the N170

after the presentation of a face for 3 second followed by another face stimulus; which could be an influence as the target face was presented for 3 seconds prior to the onset of the exploration presentation. However, these studies were highly controlled, and given the free-viewing eye movement in the current task; there could be other ecological factors affecting the potentials. Eye movements have been shown in Kaunitz et al. 2014, in particular the saccade amplitude; can modulate the brain potentials produced in visual tasks.

Initially it was thought this may be an artefactual component caused by the small number of trials that make up this average in the distractor condition. Due to the natural behaviour of eye movements in the task, it can be seen in Figure 4.2A that very few fixations that occur in this of experiment last longer than 500ms. The rationale in using such long fixations was to avoid any artefacts from eye movements made within the P300 window in the distractors for the comparison to targets.

From Table 4.1 for fixation to distractors >400ms there were more than double the number of trials that make up the Distractors averaged fRP, than that of the >500ms fixations. By lowering the threshold and being less conservative with this property, a smoother potential with slightly less variation in the mean was created. The hypothesis was that the early difference between targets and distractors, at the level VPP; could possibly decrease.



Figure 4.7 fRPs from midline electrodes (400ms): Here fixations onsets at 0ms and the fRPs were baseline corrected [-200 -100] and for the distractor condition the fixations were of \geq =400ms durations. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The mean shown by the solid lines and SEM is shown by the slightly opaque outlines. The colour bar at the top of each channel plot, shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010). The figure is made up of fixations that were of \geq =400ms durations and <1000ms. The number of distractor epochs for the grand average fRP in the figure is n= 1418.

The result from the less conservative approach found lower variation about the mean of the grand average distractor fRPs (see Figure 4.7). However, the significant difference between the two conditions, at the level of the VPP; was still existent. The result was not entirely unexpected as the number of trials only doubled from a fairly low starting number, so may still contain some noise.

The difference, at this point; could still have been a product of the number of trials adding to the average of the distractors. One interesting detail, when observing the number of fixations to distractors >200ms in Table 4.1; there are a large number of fixations (n=12765) in this category. Another hypothesis was if the criteria for fixation selection was set to fixation durations >200ms, any differences would be very unlikely to be a product of a lack of statistics.



Figure 4.8 fRPs from midline electrodes (200ms): Here fixations onsets at 0ms and the fRPs were baseline corrected [-200 -100] and for the distractor condition the fixations were of \geq =200ms durations. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The mean shown by the solid lines and SEM is shown by the slightly opaque outlines. The colour bar at the top of each channel plot shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010). The figure is made up of fixations that were of \geq =200ms durations and <1000ms. The number of distractor epochs for the figure is n= 12765.

The most surprising result from the grand average fRPs in Figure 4.8, was the difference that was initially occurring at ~170ms (from Figures 4.6 and 4.7); seems to be occur earlier. From Figure 4.8, Fz and Cz have differences that are too early to be cognitive processing of the stimulus. Given the stimuli involved in the task, the difference is unlikely to be a low level feature modulation or target detection. One potential modification, would involve running a new experiment; free-exploration of the scenes in order to get completely clean distractors, free from higher level influences from the task. This method would give another way to compare target and distract signatures. Although, it may not explain full story as the targets would come from a visual search task, which would have a different cognitive load.

Initial analysis show fRPs that contain specific potentials expected; such as the P100, VPP/N170 and P300, which are comparable to previous findings (discussed in Chapter 1). The fRPs across the conditions are compared with the tried and tested method for MCP, and can be seen visually in Figure 4.6, 4.7 and 4.8. Areas of significant difference were clear to see at expected regions, such as at the level of the P300. However, some unexpected differences were raised at the level of the VPP/N170, and this difference was unlikely to be an effect of a small number of trials. Though, as discussed previously, fixations were accepted if >50ms; so perhaps there are artefacts in the baseline causing the difference between the two conditions (this will be investigated later in the chapter).

4.4 False Discovery Rate vs. Cluster Based Permutation

The multiple comparisons problem has been mentioned before in Chapter 1, and was an issue in the current experimental setup. There were 64 electrodes running over many time points, in many trials, over many subjects, as well as across two conditions (targets and distractors). The method used for the work in Chapter 3 was a FDR (using the method parameter set out in (Kaunitz et al. 2014)), a non-parametric Wilcoxon Rank-sum test to each (channel, time) sample between the two conditions of the average fRPs. To account for MCP, samples were considered statistically different between the two conditions, when the p-value of the Wilcoxon Rank-sum test fell below a threshold for the falsely rejected null hypothesis (that there were no statistical differences). The threshold was set to 5%. The method was theoretically sound, though it was not confirmed as a completely robust method for nullifying MCP by comparing it to a tried and tested method.

The tried and tested method for MCP originates from non-parametric cluster based permutation. In this method each (channel, time) sample, across the two conditions (targets and distractors), were compared by means of a t-value. All values that exceed the t-value criteria set (in current work set at 0.05), qualified for the clustering stage of the procedure. Each cluster was made from samples connected on the basis of spectral, temporal and spatial distance. Then using the Monte Carlo method for random permutations (large number of repetitions), and simultaneously calculating the test statistic each of these permutations; a histogram of the test statistics was be built. The permutations that had a test statistic larger than that of the observed one were taken (this proportion was the significance probability), and if it was lower than the critical alpha specified, the two experimental conditions were considered significantly different. The p-values could be calculated for the second largest cluster, third, fourth etc. Therefore the cluster could be considered significant if larger than the critical alpha level set.



Figure 4.9 Scalp Plots showing P-values produced from the MCP correction methods: Scalps maps representing the passage of time from 0-400ms with the significant differences between target and distractors, of the averaged fRP, the spatial areas of the scalp highlighted. On the scalp map for 0ms, the 64 electrode positions are shown. The darker the blue, as shown in the colour bar, the higher significant differences for the electrode that is highlighted. **Top Panel:** used FDR for MCP correction. **Bottom Panel:** used Fieldtrip for MCP correction.

Figure 4.9 used data taken from the previous and current chapters study. The topographies show spatial areas of significance (p<0.05) between the amplitudes of targets and distractors. Observing the topographies, there is very little difference between the two statistical analysis methods. The FDR method (top panel in Figure 4.9) is slightly more conservative. However, both methods confirm areas of significant differences, between two experimental conditions. The result confirms the reliability of the previous method for the MCP correction via comparison with a tried and tested method for MCP. Thus means two things; both methods are appropriate for this type of experiment, and that the results found are statistically robust.

4.5 VPP and N170 in Free-viewing

The N170, as discussed in Chapter 1, has been shown to elicit greater amplitude for faces as opposed to other categories such as cars. In the current work there are only face stimuli, therefore further investigation into the modulation of the potential with different categories was not possible. However, it is understood for fixed-gaze studies that the VPP is a manifestation of the N170 (Joyce & Rossion 2005). Therefore it was speculated that this should also exist within the free-viewing counterpart.

ERPs are manifestations of activity produced by neurons firing within the cortex. The activity is described as a dipole with positive and negative points. The potentials produced from the dipoles depend on the point of view of the reference. This is the difference between the active electrode of interest and the chosen reference electrode. In particular using an average reference can give the most optimal balance between positive and negative peaks. Therefore re-referencing the current data to common average, comparisons were made between the N170 and the VPP from a mastoid reference.



Figure 4.10 Target fRPs from mastoid and average references: fixations onsets at 0ms and the fRPs were baseline corrected [-200 -100] **A:** shows the topographies with a mastoid reference. **B:** Topographies with average reference. **C:** fRPs for Cz electrode with a mastoid reference (pink), the PO7 electrode (dashed blue) and PO8 electrode (dashed green). The vertical dashed red line shows the approximate time of the peak of the VPP and N170 (170ms). **D:** VPP/N170 –P100/N100 averages; each bar is the average amplitude difference of the VPP and the N100 (for Cz Mastoid reference) and average amplitude difference of N170 and the P100 (for PO7 and PO8 Average reference)

Figure 4.10A and B show the target topographies from the original mastoid and average reference respectfully. For both mastoid and average references, the first topographies (-33ms) shows the activity at the point that the average saccade is made to the target. The onset of the fixation (0ms) shows a frontal activity, most likely the result of the eye movement. Then at 81

100ms there is a strong P100 potential in occipital region from the processing of the low level features of the stimulus. Across the first three topographies (-33ms, 0ms and 100ms), the two references show similar activity, though this changes at 170ms. As expected, in the mastoid reference the VPP elicits in central areas very strongly. Conversely, the N170 in the average reference has less negative magnitude amplitude, and seems less lateralised in the areas it is expected to elicit. One possibility could be that in free-viewing the amplitude of the N170 is smaller than that of its fixed-gaze counterpart. There is also the possibility that the timing jitter of the N170 produced in free-viewing could affect the power, as more jitter in the peak would create less amplitude (after averaging, similar to the effect seen with the signal alignment issue Chapter 2). However, the result in Figure 4.10D shows that the average difference between the amplitude of the VPP and N100 for Cz with a mastoid reference, as well as the N170 and P100 for PO7 and PO8 with an average reference are similar. Despite the differences seen in the topographies, it does seem that the VPP and N170 can be shown to manifestations of the same potential in free-viewing; as they both have their strongest peaks at virtually the same time (see the potentials aligning to the dashed red line in Figure 4.10C).

4.6 Baseline Correction

During an EEG experiment, the voltage traces over the duration of the recording can be affected by many different environmental factors (discussed in Chapter 1). Baseline correction is a method used to eliminate voltage drifts, and other factors affecting voltage to give a "baseline" for a local comparison; removing any global effects.

If the early differences that were mentioned in section 4.3 were due to the baseline containing artefacts; then a reasonable strategy was to redefine what type of baseline chosen. An initial window was selected to be [-150 -80], as the saccade preceding the fixation of interest will onset \sim 34ms (according to the median of the distribution of saccade durations in Figure 4.3A) prior to the fixation; also from Figure 4.3A there were very few saccades >80ms long. Therefore the selected baseline period was expected to contain very few saccadic artefacts.

For this part of the investigation, fixations to distractors with durations >400ms were used, rather than those >500ms or >200ms, as it was seen as a fairer tactic. Although fixations to distractors >200ms had more trials, the number of trials making up the target averaged fRPs were \sim 2000 and the number of trials for fixation to distractors >400ms are \sim 1500. There was also the added benefit that the properties surrounding the fixations of interest (for targets and distractors) were more similar in these cases, especially in terms of the task required (Tatler et al. 2006).



Figure 4.11 fRPs from midline electrodes ([-150 80]ms baseline): Fixations onset at 0ms and fRPs were baseline corrected [-150 -80]ms and for the distractor condition the fixations were of >=400ms and <1000ms durations. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The mean is shown by the solid lines, and the SEM is shown by the slightly opaque outlines. The colour bar at the top of each channel plot shows the p-values for the significant differences; using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010).

There was still an early significant difference observed in Figure 4.11 at ~170ms with the new baseline of [-150 -80]. However, this could be due to the miss-match of many fixation durations and saccades preceding the fixation of interest. Many potential artefacts could be causing the early difference. Therefore, considering a baseline within the fixation of interest was thought to be better suited for this case. As the past eye movements would not be affecting the current fixation, which could add noise to the trial in terms of pre-fixation artefact influence. Another consideration made was regarding early potentials, produced within the current fixation of interest, which can influence the baseline correction. Early potentials such as the P100 or N100 can onset as early as 60ms reaching a peak between 80-100ms (Luck 2005). Therefore, a baseline in the period [0 50]ms was used to minimise the possibility of early potentials affecting the baseline correction.



Figure 4.12 fRPs from Midline electrodes (baseline:[0 50]ms): Here fixations onsets at 0ms and the fRPs were baseline corrected [0 50] and for the distractor condition the fixations were of >=400ms and <1000ms durations. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The mean shown by the solid lines and SEM is shown by the slightly opaque outlines. The colour bar at the top of each channel plot shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010).

The early difference did not seem to change for the baseline correction period within the current fixation of interest. Figure 4.12 showed a difference at the VPP, and considering the other figures (Figure 4.7 and 4.11) it seems that baseline correction using a window in the data does not directly the cause the difference.

The foundations for the baseline changes used in Figure 4.11 and 4.12 were thorough. If there were eye movement artefacts, early potentials or even noise in the baseline, it could affect the resultant signal significantly. Each baseline that was used for the tests had a valid justification for use, and the subsequent fRPs produced; show minimal differences. Finally, the regions of significant difference (between the two conditions) across the midline electrode sites found did not show a huge degree of change. While the early difference, between conditions, at the level of the VPP had significant differences across the baseline conditions considered.

Baseline correction is a technique used to bring all epoched trial potentials to the same level. Therefore, any drift over the duration of the experiment should be removed, and not infringe on overall averaged fRPs. The method of baseline correction preserves the local properties of fRPs for the analysis. However, there are losses in the global properties of fRPs. Any potential modulation caused in the build-up throughout the trial is lost with baseline correction. Thus, the only way to preserve global properties was not baseline correcting trials, then creating averaged fRPs.



Figure 4.13 fRPs from midline electrodes: Fixations onset at 0ms and fRPs were not baseline corrected. The distractor condition the fixations were of \geq =400ms and \leq 1000ms durations. Channels Fz, Cz, Pz and Oz are shown for the target (red) and tistractor (blue) conditions. The mean shown by the solid lines and the SEM is shown by the slightly opaque outlines. The colour bar at the top of each channel plot shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010).

The results illustrated in Figure 4.13 draws some very interesting observations. Firstly, the difference seen in the baseline corrected analysis between target and distractor fRPs at ~170ms disappeared. In the Cz and Pz electrode at ~300ms there begins a significant difference, which is the P300 potential; the target detection property in the potential (Sutton et al. 1965; Farwell & Donchin 1988; Polich & Kok 1995; Gonsalvez & Polich 2002). Though, there appeared to be a pre-fixation difference between targets and distractors. The method did not baseline correct in this instance. A possibility for this pre-fixation difference could be a modulation due to varying global properties (as will be discussed later in the chapter).

A confound arose from the baseline investigation, as conclusions drawn from fRPs with baseline correction can be drastically different from fRPs without baseline correction. Therefore

it is suggested to err on the side of caution; in regards to what method to apply, and the conclusions drawn from the results.

4.7 Matching Properties

In Kamienkowski et al. 2012 fixations between targets and distractors were matched based on the eye-movement properties of each fixation. The preceding saccade horizontal (dx) and vertical (dy) amplitudes as well as the duration (dt) were used as matching parameters. This was to avoid any baseline differences created from the eye movements so that the fixation-Event related Potentials for targets and distractors could be compared without artefactual components affecting the results. In this study there were the same amount of subjects used producing a similar amount of trials. Though, the instructions used resulted in increased fixation duration, converse to the current work; which had no instructions regarding eye movements.

In section 4.3 for Figure 4.7; early differences were found and needed to be verified that they were not a result or by-product of mixed artefactual components of the preceding eye movements. The pre-requisites for the matching procedure in Kamienkowski et al. 2012; was for n-parameters matched to have no significant differences. After applying their matching procedure it was found that there were significant differences in the 3 parameters used. Therefore another strategy had to be implemented for a robust matching method.

$$d_{st}^2 = (x_s - y_t)V^{-1}(x_s - y_t)'$$
(4.1)

K-nearest neighbours (KNN) is an algorithm that finds the "nearest neighbour" for a mx-by-n matrix X in each point of a my-by-n matix Y. The method is exhaustive and uses replacement; first calculating the distance of each point and then finding the smallest distance. Once matched the element is placed back in the pool to be matched for distance again. The method used a standardized euclidean distance metric seen in Equation 4.1 where x_s is a column vector from X that corresponds to y_t a column vector from Y. Where V is the n-by-n diagonal matrix whose jth diagonal element $s(j)^2$, where s is the vector contain the inverse standard deviations; this scales the difference between rows x_s and y_t by dividing the coresponding elements by the standard deviation.



Figure 4.14 Distributions and scatterplots of behaviour parameters for Targets vs. Distractors: Top Panel: Horizontal saccade amplitude. Middle Panel: Vertical saccade amplitude. Bottom Panel: Saccade duration. Each panel contains: a scatterplot (black circles) with a least squares line plotted (green) of the data in the top right. A histogram of the target property distribution in the top left, and a histogram of the distractor property distribution in the bottom right.

Table 4.2 Eye-movement Properties for matching procedure: each parameter of the eye movement properties for targets and distractors were compared. The structure of the columns: show the median ([Inter-Quartile Range]) fixations to distractors and targets. The p-values were a result of a Wilcoxon ranksum test. Properties were taken from n = 1895 matched target and distractor fixations based on the closest match based on the KNN distance.

Parameter	Fixations to	Fixations to Targets	P-Value
	Distractors		
Saccade	32.0([24.0 38.0])	32.0([24.0 38.0])	0.96
Duration(ms)			
Horizontal Saccade	4.7([2.3 8.1])	4.6([2.3 8.2])	0.86
Amplitude (deg)			
Vertical Saccade	2.4([1.1 4.7])	2.5([1.2 4.8])	0.81
Amplitude (deg)			

Following the matching procedure, the different parameters matched were shown to have similar distributions across both target and distractor condition (see Figure 4.14). The new matching procedure has shown that the parameters have no significant differences (see Table 4.2), which was a pre-requisite of the original matching procedure. From the results the grand average fRPs were produced for the matched properties.



Figure 4.15 fRPs for matched eye movement properties across midline channels: fixations onset at 0ms and the fRPs were baseline corrected [-200 -100]. The distractor condition the fixations were of >=400ms durations and <1000ms. Channels Fz, Cz, Pz and Oz are shown for the target (red) and distractor (blue) conditions. The horizontal and vertical saccade amplitude, as well as the saccade duration preceding the fixation of interest; were matched. The colour bar at the top of each channel plot, shows the p-values for the significant differences using cluster-based permutation tests in FieldTrip (Oostenveld et al. 2010).

The notable result in Figure 4.15 is that there are still early differences at the level of the VPP, and the expected P300 is also still existent. Compared to Figure 4.7 the shapes of the potentials remain very similar and the peak amplitudes are also very similar.

The results from the matching procedure have found that it is possible to match properties with free-viewing eye movements, in a robust way. However, with the exhaustive replacement method, found the unique matches for the two conditions were 827 (out of 1895). Hence, depending on the paradigm, the number of available statistics (especially in unrestricted freeviewing); could be a factor to consider when performing a matching procedure. The differences discovered, compared to the previous work presented in Chapter 3 and in Kamienkowski et al. 2012; seem to suggest a possible difference in the ecology of the eye movements involved in the paradigm of the current chapter. In the earlier studies instructions had been made in order to increase the length of fixations, and as a consequence created more predictable and "matchable" eye movements. However, it has been shown that matching properties can be applied in a robust approach, matching three parameters (vertical and horizontal saccade amplitudes as well as saccade duration); using the smallest distances in the three dimensional distributions based on a KNN algorithm. Furthermore, the results found that low level of control on eye movements creates a different ecology of eye movements across target and distractor conditions. Using matching properties, the early differences at the level of the VPP were not found to be eliminated. Therefore it can be assumed that the differences are not due to a superposition of eye movement artefacts, as the shape and amplitudes of the potentials do not show big signs of change, and visible differences in amplitudes between targets and distractors still exist.

4.8 Full Trial Analysis

Up to this point in the chapter, the main direction of the analysis has followed that of previous studies in order to compare the potentials produced; and to get a basic understanding of how the response in this experiment differed. The results until now have shown the fRPs in an exclusively local sense, the focus mainly on the similarity between the conditions of targets and distractors. However, with new paradigms, comes new perspective and ideas; perhaps out of these ideas, novel approaches can be made.

A full trial method was developed, stemming from a desire to see if there are any trends that can be seen. Trends that can possibly explain the local properties of the fRPs produced, as well as justifying a baseline; should it be chosen to apply one. By looking at the full range of the trial, there may be potentials produced that can be explained by the paradigm (in the visual stimulus presented), or the average behaviour of subjects in their approach to the task.

There were a few ways to construct the trials, an issue raised was the duration of each trial (unless the target is not found and the maximum trial time elapses), was variable. There were two trial structures that would overcome the problem, and be the most valuable to analyse. One, align the trials to the onset of the trial exploration. Two, align the trials to the onset of the target fixation.

In order to conduct the alignment, a matrix of the data was created. Only trials that contained a target were used and the focus remained on the midline electrode channels. To account for the "extra space" in the trials, all the trials aligned to the start of the exploration had 'Not a Number' (NaNs) added to the end, which brought them to the length of the longest trial which contained a target (all trials that did not finish with a target fixation were removed). Likewise, for the trials aligned to the target fixation, the same method of adding NaNs was applied but in this case to the beginning of the trial. Following this procedure, all trials were aligned for the averaging process. The method was run over all trials from all subjects to create one data matrix. For each trial, a 10 second period either side of the trial exploration onset and the target fixation onset was applied ([-10seconds "Exploration Onset" "Target Fixation Onset" +10seconds]). Furthermore, in order to keep any global effect from being lost, the trials were also not baseline corrected (as seen in section 4.6).

4.8.1 Exploration Onset Alignment

In order to avoid influences from previous trial processing or activity leading up to the current trial of interest, as well as any influences from target activity or further post-trial activity; trials were zero-padded between [-10 -5] seconds of the current trial, and also from the target onset onwards (i.e. to avoid including the response elicited from the target fixation). The rationale for zero-padding for this period, originates from the average time that it took between the end of one trials and the beginning of the next (see Figure 4.3D). There is a median duration of 5.5 seconds, and a mean of 6 seconds between the end of one trial and the exploration onset of the next trial. There is also very little variation as the SD is 1.7 seconds. The trial durations shown in Figure 4.2D show the mean duration of a trial to find the target was 5 (SD:3.9) seconds and median of 3.5 seconds. Thus there is a higher variance of trial durations than the variance of the duration of time from previous trial to start of the current. This therefore suggests that the potentials produced at ends of the previous trials are quite well aligned to the current trials. Therefore, this could create influences on the next upcoming trial. There are also very few trials that started within 5 seconds of the previous; therefore this would give a low likelihood of previous trial activity influences. Hence, all trials were zero-padded padded between [-10 -5] seconds of the current trial to avoid conclusions made on trends that could be the product of artefacts or superstition of potentials. The structure of the data for the target onset aligned with zero-padding: [Zeros Trial Duration Zeros (including 1 seconds post target fixation)].



Figure 4.16 Full trial amplitude across the midline electrodes aligned to the onset of the trial: Top Panel: The average ERP amplitude across all trials and subjects for electrodes Fz (orange), Cz (cyan), Pz (purple) and Oz (red). The vertical solid black line is representative of the onset of the exploration. The vertical dashed green line is the median onset of target fixation (2.5 seconds). The time window is [-5 5] seconds. **Middle Panel**: shows a closer look at the top panel, focussing on the pre-exploration between [-5 0] seconds. **Bottom Panel**: shows a closer view of the top panel, focussing on the onset of the exploration between [0 2.5] seconds.

In Figure 4.16 there are some potentials that onset shortly after t=0, at the onset of the exploration. This is an ERP not an fRP, as it is not aligning to fixations. In Fz, Cz and Pz there are three clear peaks and in Oz there is one peak followed by another more broad peak. Across

Fz, Cz and Pz. An increase in amplitude occurs between \sim [1 2.5] seconds and Oz from \sim [1.5 5] seconds. From \sim 2.5 seconds onwards in Fz, Cz and Pz there is a tendency towards zero; this was expected as the data was zero-padded, and trials in this region have heavily mixed durations.

Pre-onset of exploration, there is also some activity; in Oz at \sim - 4 seconds a peak occurs just before a peak in Fz. Then at \sim -1 seconds in all four electrode sites there is a peak, just before the onset of the exploration. In the Pre-Exploration of the paradigm (shown in Figure 4.1), the timeline begins with 3 seconds of target presentation + a random time (time taken for subject to fixate the dot) + 1 second of fixation on the random dot before the exploration begins. As can be seen in Figure 4.16, there is a peak at \sim -1 second, which can be presumed to be the processing of the fixation to the random dot. There is also a steady period between \sim [-4 -1] seconds, and this can be assumed to be the fixation to the target face for the 3 second period. The potentials produced in this part of the analysis for the pre-exploration appear to be consistent with the paradigm structure.



Figure 4.17 Activity at the onset of the trial: Top Panel: The averaged ERP, focussing on the initial exploration between [0 0.5] seconds. The four midline electrodes are shown again; Fz (orange), Cz (cyan), Pz (purple) and Oz (red). **Bottom Panel**: show the topographies of the activity shown in the top panel from [0 500] ms in a sequence of 50ms steps.

An interesting result (mentioned earlier) from Figure 4.16, were the peaks that occurred between $[0 \ 0.5]$. There are three clear peaks between $[0 \ 0.5]$ seconds after this the average amplitude dropped as the variances of the eye movements increased through the exploration. The potentials that elicit just after t=0, when the exploration begins, are assumed to be the processing (P100 potentials) of the first fixations made as the subjects try to gather information about the scene.

The bottom panel of Figure 4.17 further strengthens the hypothesis that the initial peaks in the top panel of Figure 4.17 are the processing of the first few fixations made in the exploration (this was confirmed using a single trial analysis later in chapter). Starting at ~250ms after the exploration onset there is a large activity in the occipital region (most likely the P100). Later in the chapter this will also be analysed in terms of the eye movement properties.

In figure 4.16, at ~2.5 seconds there are steady increases in amplitude across all the 4 midline channels. As the trials making up the average are not the same length; one possibility for the increase in amplitude could be that, for the grand average, the trial durations are mixed. Therefore, the superposition of different trends throughout the trial could be causing the increase at that onset at ~1 second; as this was around the median of the trial durations.



Figure 4.18 Trial duration quantile analysis, across the midline electrodes aligned to the onset of the trial exploration: A: Shows the grand average amplitude for all trials that contained trial durations between [0 1.23] seconds The onset of the exploration is shown with a dashed vertical black line and the median trial duration shown by the solid green vertical line (0.76 seconds). B: Shows the grand average amplitude for all trials that contained trial durations between [1.23 2.5] seconds The onset of the exploration is shown with a dashed vertical black line and the median trial duration is shown with a dashed vertical black line and the median trial duration is shown with a dashed vertical black line and the median trial duration shown by the solid green vertical line (1.78 seconds). C: Shows the grand average amplitude for all trials that contained trial durations between [2.5 5.5] seconds The onset of the exploration is shown with a dashed vertical black line and the median trial durations between [>5.5] seconds The onset of the exploration is shown with a dashed vertical black line and the median trial duration shown by the solid green vertical line (3.66 seconds). D: Shows the grand average amplitude for all trials that contained trial durations [>5.5] seconds The onset of the exploration is shown with a dashed vertical black line and the median trial duration shown by the solid green vertical line (3.65 seconds). For all figures, the four midline electrodes are shown again; Fz (orange), Cz (cyan), Pz (purple) and Oz (red).

By splitting trials into quartiles n=500 dependent on trial duration ($[0 \ 1.23]$ seconds, $[1.23 \ 2.5]$ seconds, $[2.5 \ 5.5]$ seconds and [>5.5] seconds it was investigated whether the hypothesis of a trend in amplitude with trial duration; should this be the case, there ought to be a shift in the onset of the amplitude increasing.

In this part of the analysis, the first observation was the zero-padding can be seen quite clearly in the figures when there isn't any trial data. This was used as a sanity check for the data. The quartile [0 1.23] seconds, shown in Figure 4.18A, for Fz and Cz the increase trend in amplitude onsets at ~0.5 seconds, while for Pz and Oz an increase onsets later ~0.75 seconds (the median trial duration). One point to note; is that the investigation in this case focussed on the trend prior to the median trial duration, as this guaranteed trials with no zero padding. For each case (Figure 4.18A-D) Fz and Cz showed a trend, which onsets at ~0.5 seconds, increasing amplitude as the trial progressed. In Figure 4.18B-D, Pz and Oz showed a steady increase that onset at ~1.25 seconds. The results suggest that trends do exist over the full trial, regardless of the trial duration.

The majority of the peaks shown in the full trial analysis aligned to the onset of the exploration were accounted for. To get a well-rounded understanding of what processing occurs during the average trial in this task, the full trial with the alignment to the target fixation was then investigated.

4.8.2 Target Onset Alignment

Following the procedure discussed earlier, with regards to aligning the trials to the target fixation onset. The next steps were to produce a comprehensive understanding of the full trial analysis. One of the main findings in the previous parts of this chapter was the discriminating property of the P300; which elicited to target fixations. Therefore, as the trials were all aligned to the target fixation, the fRP (not an ERP as trials were aligned to the onset of the fixation) for the target should be clearly seen; as the midline electrode channels were the main focus (Fz, Cz, Pz and Oz). To evade potential pitfalls, the data was also zero-padded; to provide a consistent analysis (avoiding false conclusions). The structure of the data for the target onset aligned with zero-padding would be: [Zeros Trial Duration (including 1 seconds post Target fixation) Zeros].



Figure 4.19 Full trial amplitude across the midline electrodes aligned to the start of the target fixation: Top Panel: The average ERP amplitude across all trials and subjects for electrodes Fz (orange), Cz (cyan), Pz (purple) and Oz (red). The vertical solid black line is representative of the onset of the target fixation. The vertical dashed green line is the median onset of trial exploration (2.5 seconds). The time window is [-9 1] seconds. **Middle Panel:** shows a closer look at the top panel, focussing on the exploration between -5 0] seconds. **Bottom Panel:** shows a closer view of the top panel, focussing on the onset of the target fixation between [-1 1] seconds.

With the current alignment and data structure (see Figure 4.19), there are two areas of activity noticeable. The most observable is the activity at t=0 (fixation to target onset, see the figure in bottom panel of Figure 4.19). This was expected as all the trials align to this fixation, and the

fRP that was produced; is the response to the target. The other area of notable activity occurs between \sim [-5 0] seconds (see the figure in middle panel of Figure 4.19), where there is a steady trend leading up to the target; this is a decreasing amplitude trend across the midline channels. This embodies what was previously seen in the grand average fRPs in Figure 4.13 and will also be further investigated in the global properties analysis later in the chapter.



Figure 4.20 Activity at the onset of the target: Top panel: The averaged fRP, focussing on the target fixation between [0.1 1] seconds. The four midline electrodes are shown again; Fz (orange), Cz (cyan), Pz (purple) and Oz (red). Bottom panel: show the topographies of the activity shown in the top panel from [0 400] ms the sequence of steps is highlighted below.

There are clear potentials seen here (see top panel of Figure 4.20); the P100 in the Oz electrode, the N100, the face processing VPP (manifestation of the N170) and the target discriminating P300 in the Fz, Cz and Pz electrodes. The topographies in the bottom panel of Figure 4.20 illustrate the target processing across the whole scalp. There is a P100 observable at 100ms. As well as the central-parietal VPP at 180ms, and the target discriminating P300 that onsets at 300ms. The potentials can be likened to those found in Kaunitz et al. 2014.

Looking at the figure in middle panel of Figure 4.19, there is a negative drift that onset \sim 5 seconds before the target is fixated. If this is a build-up of potential, or a dynamic of global properties; then the drift should be shifted depending on the trial duration. As before, for Figures 4.18A-D, the method of splitting the trials using quartiles based on trial duration was applied.



Figure 4.21 Trial duration quantile analysis, across the midline electrodes aligned to the onset of the target fixation: A: Shows the grand average amplitude for all trials that contained trial durations between [0 1.23] seconds. The onset of the target is shown with a solid black vertical line and the median trial duration shown by the dashed green vertical line (0.76 seconds). **B:** Shows the grand average amplitude for all trials that contained trial durations between [1.23 2.5] seconds. The onset of the target is shown with a solid black vertical line and the median trial duration shown by the dashed green vertical line (1.78 seconds). **C:** Shows the grand average amplitude for all trials that contained trial duration shown by the dashed green vertical line (1.78 seconds). **C:** Shows the grand average amplitude for all trials that contained trial duration shown by the dashed green vertical line and the median trial duration shown by the dashed green vertical line and the median trial duration shown by the dashed green vertical line and the median trial duration shown by the dashed green vertical line (3.66 seconds). **D:** Shows the grand average amplitude for all trials that contained trial duration shown by the dashed green vertical line (8.65 seconds). For all figures, the four midline electrodes are shown again; Fz (orange), Cz (cyan), Pz (purple) and Oz (red).

The trend is slightly difficult to see from Figure 4.21A-D. As the data is zero padded where the shift seems to occur, however it seems due to the shift of trial onset. A negative drift is present in each of the plots, more noticeable in Figure 4.21A, B and C. It is difficult to establish if this is a genuine property, nonetheless a more thorough global analysis later in the chapter illustrates this property more clearly.

The full trial analysis, as well as being quite a novel approach, has given more of a wellrounded view on how a trial progresses; the information gained through this method has been quite interesting. It has been seen that there could be properties of potentials lost through baseline correction. The results suggest the possibility that there is a potential for more global effects to be locked within the fRPs themselves, and should be further investigated (and will be later in the chapter).

Co-registration of EEG and ET has had the task to create dynamic tasks whilst being able to account for the eye movement artefacts. Whilst improvements have been made to artefact correction (Plöchl et al. 2012; Henderson et al. 2013; Bigdely-shamlo et al. 2013), one advantage of the full trial analysis, is the little effect eye movements will have; as the main purpose would be to investigate global effects and random eye movements would be averaged out. Whilst there has been an increase in fRP study utilising co-registration (Dimigen et al. 2011; Kretzschmar et al. 2013; Kornrumpf et al. 2016; Léger et al. 2014; Savage et al. 2013; Kovalenko & Busch 2016; Ehinger et al. 2015), the focus has broadly been to concentrate on changes in local potentials by isolating fRPs. However, as the present study has shown, for tasks in which global changes occur during the course of a trial, such as visual search, full trial analysis could be beneficial. The full trial analysis aligned to the appropriate point in the trial has already been shown to elicit the similar potentials to the isolated fRP in the aforementioned studies. The other advantage of the full trial analysis is that it requires no extra experiment, as the only aspect that would require some careful strategy; would be what epoch lengths and alignments of the trials to use. Hence, studies such as (Kamienkowski et al. 2012; Brouwer et al. 2013; Ušćumlić & Blankertz 2016; Ossandón et al. 2010; Nikolaev et al. 2011; Kaunitz et al. 2014; Devillez et al. 2015) could all see global effects without having to run a separate study. Currently, to the best of knowledge no such study utilising co-registration has investigated full trials in this method.

4.9 Local and Global Dynamics

In the previous section it was shown that there may be some global effects locked in fRPs that could have been lost in the baseline correction. One observation, given the result of the previous section and the grand average fRPs with no baseline correction in Figure 4.13; there appears to be a change in the level of amplitude from the distractors up to the target.

4.9.1 Isolated Fixation to Target fRPs

To find out whether this global effect is locked within the fRPs an analysis was applied; by categorising each fixation to distractor, by the number of fixations that the distractor of interest is before the target fixation; the distractors can be averaged as a function of this category to create an fRP that has a distinguishing global property.



Figure 4.22 Baseline corrected Vs. No baseline: There are two boxes, on the left there is a representation of one trial (depicted by the Tstart and Tend). On the left black box, there are two examples of one trial (Top left:Baseline corrected trial and Bottom Left: No baseline trial). Within these trials there are a number of fixations (blue dots) made before finding the target (F_N), and each fixation before finding the target is categorised by the number of fixations left to finding the target, i.e. F_{N-1} is one fixation to the target, F_{N-2} is two fixations from the target etc. On the right there are is another black box, which shows two examples of averaged fRPs. The top right are the averaged fRPs for the baseline corrected fixations to distractors. The bottom right shows the averaged fRPs for the categorised fixations to distractors.

The motivation behind Figure 4.22 was to find a way to link the full trial response global response to the local fRPs produced for fixations to distractors. A very clear result of applying baseline correction; any drift in the voltage of the signal would be eliminated; as discussed in section 4.6. Using the approach laid out in Figure 4.22, by isolating the individual fixation to distractors categorised by the number of fixations left to the target ($F_{N-1...}$ etc); the average fRPs for each of these categories were calculated. Global properties locked within the fRPs were then seen, as hypothesised in Figure 4.22.

The focus for this analysis was on the midline electrode site Fz, Cz and Pz in period [-500 200] ms. Due to the focal interest that the analysis investigated (only the amplitude level vs. Fixation number to target was of interest), fixations to distractors >200ms in duration were used for the averaging process. Therefore the number of trials making up the average should not be a factor for concern. No baseline correction was applied to the amplitude of the individual fRP trials; retaining global properties.



Figure 4.23 Averaged fRPs categorised as fixation number to target across midline electrode channels: Top Panel: Shows the fRPs in the Fz electrode. Top Panel: Shows the fRPs in the Fz electrode. Middle Panel: Shows the fRPs in the Cz electrode. Bottom Panel: Shows the fRPs in the Pz electrode. Each averaged fRPs is plotted from one fixation to target (N-1) to eleven fixations to target (N-10) all colour coded in the legend. Also plotted are the grand average fRP for the target mean (dashed red) and the distractor mean (dashed blue). They are made up of the following statistics N-1: 67, N-2: 584, N-3: 704 and N-4: 759 range between N-5 and N-10 699>n>338.

It is evident from all figures in the Figure 4.23, that there is a trend from the averaged fRPs of the isolated N-10 to N-1. The N-10 fRPs have larger amplitude on average; with larger total amplitude than the distractor mean. Then it seems as the fixation made gets closer to the target, through N-9 to N-1; the amplitude decreases towards the target mean. The result may initially appear like an obvious trend, however the fact that there was such a clear pattern across all the channels in the analysis; suggests that there are global properties locked within local fRPs.



Figure 4.24 Average amplitude of the baseline of fRPs vs. fixation to target across the Fz, Cz and Pz: The baseline period averaged was [-200 -100] ms for the fRPs. Each grey bar is the average of the baseline period of each averaged fRP categorised by its number of fixations to target. The dashed blue line is the average of the baseline period of the mean distractor fRP. The dashed red line is the average of the baseline period of the mean target fRP.

The trend is more prominent in Figure 4.24; it is clear that the amplitude for the fixations closer to the target is more similar to the target mean amplitude. Another point to note; the trend occurring is within the baseline period of the fRPs. Thus, the result suggests that there could be influences from baseline correction due to global changes in the signal. Therefore, there are global properties affecting fRPs, as well as methodological confounds that should be addressed when researching this kind of task.

4.9.2 Single Trials

In order to validate that the initial positive peaks are due to the first eye movements, and to continue the line of linking the global, full trial analysis with local dynamics. Single trial raster plots sorted by onset of the first two saccades were investigated. The region of interest is around the onset so a window of [-0.5 1] seconds.



Figure 4.25 Single Trials Aligned to the onset of the exploration sorted by 1st and 2nd saccade onset for Electrode Channel Cz: Top Panel: shows single trial raster plot, sorted by the 1st saccade onset after the exploration has begun the black line shows the time of the onset. **Middle Panel:** shows the single trial raster plot, each trial sorted by the onset of the 2nd saccade and the black line shows the onset. **Bottom Panel:** shows the Grand Average ERP highlighted in light blue for the Cz electrode, also with the distribution of 1st and 2nd saccade onsets, blue and black respectively. The raster plots are smoothed by a 20 trial average window.



Figure 4.26 Single Trials Aligned to the onset of the exploration sorted by 1st and 2nd saccade onset for Electrode Channel Oz: Top Panel: shows single trial raster plot, sorted by

the 1st saccade onset after the exploration has begun the black line shows the time of the onset. **Middle Panel:** shows the single trial raster plot, each trial sorted by the onset of the 2nd saccade and the black line shows the onset. **Bottom Panel:** shows the Grand Average ERP highlighted in red for the Oz electrode, also with the distribution of 1st and 2nd saccade onsets, blue and black respectively. The raster plots are smoothed by a 20 trial average window.

There are very strong potentials relating to saccadic eye movements in Figure 4.25 and 4.26; channel Cz and Oz shows a similar response (Fz and Pz also showed similar responses). However, the potentials elicit strongest in the Oz electrode in Figure 4.26. At ~0.1 seconds there is a small peak, which aligned very well to the onset of the exploration across all trials, and it can be assumed that this is the very first P100 ERP. A point to note is that the large peak at between ~[0.15 4] seconds (see ERP in the bottom panel of Figure 4.26) of the grand average ERP, is actually a superposition of multiple processes as well as eye movements. There is activity from the first saccade, the processing of the first saccade, as well as the activity from the saccades occur is also quite broad; shown in the histograms in the bottom panel of Figures 4.25 and 4.26. The result would not have been seen without the use of the single trial raster plots. Consequently, care must be taken, in relation to ERPs, as the grand average may not tell the entire story; thus cannot be taken at face value.

Another area raster plots would be interesting to use, was the trials that are aligned to the target fixation. As the previous fixation duration has shown to influence the dynamics of the following saccade (Findlay et al. 2001); the single trials were sorted by the previous fixation duration. The main focus of the analysis was on the central region of the scalp; as this is where it was expected for the response to elicit very strongly.



Figure 4.27 Single Trials Aligned to the onset of the Target Fixation sorted by the trial duration before Target for Electrode Channel Cz: Top Panel: shows the single trials sorted 104

by the trial duration before the target, the blue line shows the time of the onset of the fixation. **Bottom Panel:** shows the Grand Average ERP highlighted in orange for the Fz Electrode, also with the distribution of the last fixation durations in blue. The raster plots are smoothed by a 20 trial average window.

As a sanity check, the same analysis was performed on the occipital region of the scalp at the Oz electrode; as there was no P300 response expected in this region.



Figure 4.28 Single Trials Aligned to the onset of the Target Fixation sorted by the trial duration before Target for Electrode Channel Oz: Top Panel: shows single trials sorted by the trial duration before the target, the blue line shows the time of the onset of the fixation. **Bottom Panel:** shows the Grand Average ERP highlighted in orange for the Fz Electrode, also with the distribution of the last fixation durations in blue. The raster plots are smoothed by a 20 trial average window.

In the analysis there were also the observations of the grand average not really telling the whole story. In Figure 4.27 and 4.28, a small slightly broad peak can be seen just before t=0. However, the raster plots show that this was a product of the different saccades having different durations. The P300 fRPs in Fz, Cz and Pz showed a similar activity, although the amplitude was more prominent in the Cz and Pz electrode. There was not any influence that could be seen in the P300 potential that came as a trend of the duration of the fixation prior to the target fRP; shown to have quite a variance from the histogram in the bottom panel in Figure 4.27. In the same result, the VPP was also very well aligned across all trials in Cz (as well as Fz and Pz). The same can be said for the P100 in Oz (seen in Figure 4.28), the potentials were expected to be consistent across trials, and this was confirmed using raster plots.

From the single trial analysis, it has been shown that the grand average cannot be taken at face value, with respect to the broad peak just before the target fixation (mentioned earlier), as well as the superposition of activity from saccadic movement and processing. The results add to the

methodological confounds of grand averages. However, there are also some confirmations of previously assumed, consistent alignments of certain potentials such as the P100 and the VPP.

4.10 Expectancy and Surprise?

It is well known in fixed-gaze, that there is a target discriminating potential (the P300) when performing odd-ball tasks (Squires et al. 1977). There is also modulating property of the P300 that affects the amplitude with increase in the inter-stimulus interval (ISI) (Gonsalvez & Polich 2002). This has been related to the concept of expectancy; as the subject is anticipating a target to be presented. During this anticipation, a build-up of expectant potential is thought to occur; this can be related to the P3b subcomponent of the P300 (discussed in Chapter 1). Conversely, there is also the concept of surprise, which can be related to the P3a subcomponent of the P300. Therefore, a question raised was whether fRPs in free-viewing visual search tasks show properties that relate to classic concepts of expectancy and surprise?



Figure 4.29 Example of the progression of an oddball task: The blue circles are the distractors and the square is the target. The trial progresses from the left at T_{start} and finishes on the right at T_{end} .

The oddball task (as discussed in Chapter 1) is a fixed-gaze paradigm that flashes stimuli. The task normally contains two different stimuli. The distractor stimulus is flashed a number of times before a target stimulus is presented, as depicted in Figure 4.29. The target stimulus should elicit a response from the subject in the form of the P300 potential on the EEG.

From the current work, there was no control of the ISI; this was attributed to the free-viewing nature of the task. Furthermore, the differences in the shapes of fixed-gaze ERPs and dynamic fRPs (from Chapter 3) were quite obvious. Although, it was expected that the modulation

property of the P300 would still be contained within the signal produced in the fRPs, the ability to acquire that information may have been more challenging than its fixed-gaze counterpart.

Fixation rank is a property assigned to a fixation, and is defined by what number fixation it was within the trial. For example, the first fixation made in a trial would be fixation rank 1, the second fixation made within a trial would be considered fixation rank 2 etc. Looking back at the behaviour for this task, there was a median average of 8 fixations. Additionally, because there was no control over the ISI, a comparable option was to create the constraint for long and short trials based on the fixation rank of the target fixation. The foundations for the categorisation of long and short trials were the association that comes with them. The category of "short" trials, where any modulation occurs within, would be more associated with surprise; as within the small amount of fixations that were made the subject would not be "expecting". Whereas, within long trials the opposite could be said; the more fixations a subject made within a trial, the more a picture could be built to where the target is located. Therefore, subjects after fixating a large number of times would have a greater "expectancy". It would be anticipated that both categories would elicit an increased P300 potential. Although, there could be a superposition of the two; where each property would elicit an amplitude response and the sum total would be the P300 total amplitude. There could also be a factor in which one category of modulates, dominates the other

To remain statistically fair, the method of splitting the number of trials into quantiles was used so that the average fRPs were made up from a similar number of trials.



Figure 4.30 Midline Target P300 Amplitude as a function of Short and Long Trials: Top Panel: shows a bar chart; the amplitude is taken from the mean average between [250 400] ms subtracted by the mean average between [0 50]ms of the individual target fRPs the result of each subtraction was averaged across all trials and subjects. The grey bars represent the short trials (<4 fixations in a trial) and the black bars represent the long trials (>13 fixations in a trial). Short and long trials were made up from n=499 and n=502 respectively. **Bottom Panel:** shows a bar chart; the amplitude is taken from the mean average between [250 400]ms subtracted by the mean average between [0 50]ms of the individual target fRPs the result of each subtraction was averaged across all trials and subjects. The grey bars represent the short trials (<7 fixations in a trial) and the black bars represent the long trials (>7 fixations in a trial) and the black bars represent the long trials (<7 fixations in a trial) and the black bars represent the long trials (<7 fixations in a trial) and the black bars represent the long trials (>1 fixations in a trial). Short and long trials and subjects. The grey bars represent the short trials (<7 fixations in a trial) and the black bars represent the long trials (>7 fixations in a trial). Short and long trials were made up from n=983 and n=921 respectively. The two conditions, in top and bottom panels, were submitted to a Non-Parametric Rank-sum test and also a t-test, significant differences were shown by the asterisks above the bars and p-value shown in the key to the right of the figure.

The amplitude of P300, from the top panel in Figure 4.30, was much larger for shorter trials. This type of response would lead to the association, that there is a surprise effect that outweighs any effect of expectancy. There were very large significant differences between short and long trials when only comparing the outer quartiles of trial lengths. To confirm that this was not biasing the result, the same was applied to a fifty-fifty split of all the trials based on the trial length (see bottom panel of Figure 4.30). The significance, for the fifty-fifty split was extremely
strong. Therefore, the effect of surprise was not produced by a bias in the split of fixation numbers made within a trial, but actually a very strong effect that is still present when looking across the whole distribution of the number of fixation made within a trial.

For a fully comprehensive check for significance, the single trial amplitude distribution as a function of fixation rank was investigated.





 Table 4.3 Fixation Rank vs. Amplitude correlation significance: Left column: Electrode channel. Middle column: R values from Pearson's correlations test. Right column: resultant p-values from th Pearson's correlation test.

Electrode	Pearson R Value	P-Value
Fz	-0.11	1.2x10 ⁻⁶
Cz	-0.11	1.6x10 ⁻⁶
Pz	-0.11	1.7x10 ⁻⁶
Oz	-0.05	0.02

In this analysis shown in Figure 4.31, the significant effect found in Figure 4.30 was consolidated with a more robust method. In electrode Oz, the previous analysis found that the effect was not significant. However, from the current analysis a significant correlation was found (see Table 4.3). The result shows the usefulness of single trial methods; as in this instance a property that is lost in a grand average (Oz electrode significance) is retained within the single trial. The result confirms that the effect is very robust. Though there needs to be complete certainty that the effect is not a product of noise.

4.11 Single Trial Denoising

Finding effects within EEG signals can be problematic. In the past with EEG, a focus on control was a way to avoid arguments that any effects found were from artefacts within the signal or just noise in general. The control gave a baseline for comparison. However, in the context of the current work with the focus on a natural response, moving firmly away from control; there could be unexpected noise creating effects. In the previous section, the surprise effects were found to be dominating the effects of expectancy. To avoid any argument that the effect is a product of noise from the single trial, the data was denoised.

Using EP_den, a denoising software based on the wavelet transform (description in (Navajas et al. 2013)), the EEG signal was denoised by manually selecting wavelet coefficients to denoise each trial to form an average very similar to that of the original data.



Figure 4.32 Denoising Single Trials with selected coefficients for Cz electrode: At the top of the figure is the average fRP. Within that window is an epoch [-0.1 0.8] seconds and there are two traces; a grey (original average signal) and a red (denoised signal). Below the average fRP are the manually selected coefficients which were used to denoise the original signal.



Figure 4.33 Denoising Single Trials with selected coefficients for Oz electrode: At the top of the figure is the average fRP. Within that window is an epoch [-0.1 0.8] seconds and there are two traces; a grey (original average signal) and a red (denoised signal). Below the average fRP are the manually selected coefficients which were used to denoise the original signal.

In all the midline channels the denoised signals were very similar to the original, with exception to the last 0.2 seconds of the window. This appeared to be an issue more with length of the epoch, and perhaps the filtering involved in the denoising process. But as the regions of interest for this analysis are [0 0.05] seconds and [0.25 0.4] seconds then this was not influencing results. Examples can be seen of the denoised signal in Figure 4.32 and 4.33. Figure 4.33 was also used as a sanity check as the P100 was expected in Oz; without the P300 that was present in Cz.

In the untouched data, there was a decrease in P300 amplitude found for higher fixation ranks. Using the resultant denoised data, the robust analysis used in Table 4.3 for single trial amplitudes as a function of fixation rank, was be explored to see if there was any significant trends; like that found in the untouched data.



Figure 4.34 Denoised single trial P300 amplitudes vs. fixation rank at Cz electrode: Top Left Panel: shows the amplitude distribution histogram. Top Right Panel: shows a 2D histogram; each bin of the histogram contains single trial amplitudes of the P300 window ([250 400]ms), baseline corrected to the window [0 50]ms post target fixation. The amplitudes are plotted as a function of fixation rank (x-axis). The median amplitude for each fixation rank is plotted in red. There is a significant decreasing trend as the fixation rank increases. A Pearson's correlation was used to test for a trend, the result found (R=-0.11, p= 1.6×10^{-6}). Bottom Right Panel: shows the fixation rank distribution histogram.

Table 4.4 Fixation Rank vs. Amplitude correlation significance in Denoised data: *Left column:* Electrode channel. *Middle column:* R values from Pearson's correlations test. *Right column:* resultant p-values from th Pearson's correlation test.

Electrode	Pearson R Value	P-Value
		7
Fz	-0.11	9.8x10 ⁻⁷
Cz	-0.11	1.5x10 ⁻⁶
Pz	-0.10	6.8x10 ⁻⁶
Oz	-0.05	0.021

It is very clear from Figure 4.36 that the denoised data contains a very similar effect of surprise to that of the untouched data. This was clearer from Table 4.4, which showed huge similarities to Table 4.3; where there were significant decreasing effects in P300 amplitude as a function fixation rank across midline channels.

In a tried and test method for denoising data, the final results (Figure 4.34 and Table 4.4), showed that the previous finding of the effect of surprise modulating the P300 amplitude, was

not a factor of random noise. Thus, there are properties locked within fRPs in a naturalistic paradigm with free-viewing eye-movements that can compare to those found in static, fixed-gaze paradigms in EEG.

Summary

The results presented in this chapter form the large body of work accomplished through the 3 years of this study. The progression and the evolution of analysis from the previous study (in the previous chapter), is very novel and intriguing. This chapter started on a solid foundation, replicating robust fRPs and discovering there are very different conclusions that can be drawn if no baseline correction is applied to the individual trials. It has been shown that methods for matching eye movement properties are possible. However, the ecology of completely natural eye movements has been shown to differ from previous findings. There have also been some new methodological strategies analysed; such as the full trial analysis, which has given a new perspective and a greater view of the full trial progression of the EEG activity. In this analysis, two time lines were investigated; the alignment to the onset of exploration and the onset of the target fixation. Both resulted in some interesting findings. It has been determined that these types of studies can be more than just an analysis on the effects of individual fRPs. Both global and local dynamics can influence the activity elicited in a visual search. Eye movements were initially seen as a challenge for this study, though having free-viewing eye movements was crucial to trying to understand natural visual search. At the level of the single trial the effects of eye movement behaviour can be visibly seen and there are grand average potentials made from superposition of P100 responses of consecutive saccades. This was another cautious consideration discovered, that has to be made in these kinds of studies to avoid false conclusions. Finally, the results found also replicate those found fixed-gaze EEG studies in regards to modulations of the P300. Classical concepts of expectancy and surprise were seen. A decrease in amplitude of the P300 as a function of fixation rank was found, with a significant effect. The same result was also not a product of random noise. As an analysis involving a tried and tested method for de-noising was used, and the resultant effect was still significant. Overall, in this investigation new methods and effects have been revealed. Many methodological concerns have been put forward, as well as discovering properties locked within local and global dynamics of EEG activity. All of which are very useful for the advancement of the field of co-registration of technologies and visual search studies.

Chapter 5 The Progression of Co-Registration (Conclusion Chapter)

5.1 Join forces for greater strength

EEG and ET in the past existed as two separate fields in their own right. EEG focussed on the physiological responses of tasks, while ET concentrated on the behavioural aspects. The vast majority of visual processing studies involving EEG have had to restrict their investigations to fixed-gaze research. A challenge for EEG experiments is dealing with eye movements artefacts on the EEG signal traces, whether investigators removed noisy trial or corrected signals using ICA analysis; nonetheless considerations have to be made. ET however, has the luxury of free eye-movements. Though, there may be problems with calibration and setups, studies using ET have the potential to create much more imaginative, dynamic paradigms. Thus, EEG has the capability to view the physiological response, without an easy way of acquiring natural behaviour; while ET has some behavioural response, without the ability visualise the physiological response. Hence, the current thesis began with the objective of combining the two technologies. The main aim was to utilise the signals gathered from these two platforms for further understanding of natural visual processing.

During the process of combining the technologies there where firstly some methodological challenges that had to be overcome, the main challenge was finding the appropriate alignment of the signal. This was achieved by sending messages to the ET and immediately sending triggers to the EEG. This was done in order to be able to align the signal post hoc. As with all new challenges, this was not as smooth in practice as it appeared in theory. There was an expected delay, and while this was counteracted by artificial delay being added, there appeared a delay between the signals that was seemingly unexplained. This was subsequently investigated for common signal modulating factors; such as filtering, and the algorithm for the detection of saccades. Finally, using a lag vector to determine each individual subjects delay, and removing the artificial delay completely to correct the signal. This was also achieved on the back of clarifying the SP existed as a real part of the sRPs. This work provided a platform for the data analysis of the published work (Kaunitz et al. 2014), described in chapter 3. On further experiments on an updated paradigm there was also another signal problem; a miss alignment of the end of trial trigger. This was later found to be a coding error, where the final fixation to target did not pause the trial time counter; if the target was found within the last second of the trial. These findings clarified the validity of the results in the published work, and also show how valuable single trial raster plots can be as a security check on the data.

5.2 Finding fRPs in Visual search

Before co-registration, eye movements made it challenging to study EEG signatures from paradigms involving dynamic eye movements. Utilising the ability to know where and when a subject fixated brought a new method for studying EEG, in the form of fRPs. If a fixation could be isolated, because it is a steady form of gaze in which no eve movement is occurring, this signal during this time is clean. Therefore, a clear pathway illustrating the processing of a visual stimulus can be seen in these types of potentials. In EEG the P300 is known to elicit due to target discrimination. In the published work, described in Chapter 3, an investigation into whether this could be said with fRPs within a free-viewing visual search paradigm was made. Using a "Where's Waldo?" type paradigm; where subjects where tasked to find a target face amongst a crowded scene, an investigation was made to compare processing of fixations made to targets to that of fixations made to distractor faces. In order to compare late latency visual processing, subjects had to be trained, in order for distractor fixations to be elongated. Therefore, target and distractor fixations that were long enough to compare the period of the P300 potential (500ms fixations to distractors) were readily available. It was found that fixations made to targets elicited the P300 potential while fixations to distractors did not. The difference between targets and distractors used a false detection rate to account for the multiple comparisons problem (an issue that occurs when there are many sensor time pairs). The breakthrough finding of the P300 within a visual search task proved that classical potentials found within past EEG studies are also visible in the free-viewing counterparts. Armed with this discovery, further investigation was made into whether these potentials could be modulated by eye movements. A significant effect was also found, whereby the amplitude of saccades had a positive correlation with the amplitude of the P100 potential in the Oz electrode. It could be mentioned that this investigation involving co-registration could have been too complex for an initial study. A simpler design that could involve one face a random distance from an initial fixation point could have been implemented. This would be considered less natural but the control would allow for less concern for eye movement artefacts. However, the current study provided the platform for further investigation into the topic of free-viewing visual search.

5.3 Completely natural free-viewing visual search and robust fRPs

The main body of the current thesis was based on the topic of completely natural free-viewing visual search. A caveat of the initial work was the training involved to elongate fixations made to distractor faces. Naturally humans make a new fixation every ~210ms when immersed in a visual search. Hence, the training to elongate fixations in the published work could be perceived as "unnatural". Building on the solid platform that the previous work had started, an alteration of the "Where's Waldo?" paradigm was made; to remove the training and keep all fixations made completely up to the decision of the subject performing the task. This brought back the

naturalistic element to the task. The most pressing task initially was to discover if the same fRPs could be reproduced but under a natural context. This was accomplished by running the same analysis to compare the processing of target fixation to that of the distractors, using the 500ms fixations to distractors. A limitation of the complete natural paradigm is that the majority of fixation durations are ~ 200 ms, these are not long enough to compare the late processing of the P300. There were however enough fixations made that were >500ms to produce a noisy grand average distractor fRP. However, lowering the threshold for fixation duration to 400ms produced a grand average distractor fRP more similar in shape and variability to the initial published work (Kaunitz et al. 2014). For the comparison between targets and distractors different methods to find significant differences were assessed. The previous FDR method was compared to a tried and tested method to counter the MCP using Fieldtrip. Fieldtrip is a Matlab toolbox that uses cluster based permutation tests to test for significance. The two methods were found to be fairly similar in their assessment of significance. Hence, the natural, completely free-viewing visual search produced robust fRPs. However, even though robust fRPs were found, there were some early significant differences within fRPs that were not expected. A difference at the level of the N170/VPP was uncovered, this brought to light some concerns within fRPs. The concern was further investigated to eliminate any outside influences from eve movements, using a matching properties method. The method showed that even with freeviewing tasks, eye movement properties could be matched. Furthermore, different baselines were investigated and to some surprise, removing baseline correction found a pre-fixation difference between target and distractor fRPs. This brought another theory to the table, in which there could be activity throughout the trial causing this grand average pre-fixation difference.

5.4 New full trial methods

The Discovery of fRPs existing within the completely free-viewing tasks, albeit with different properties emerging led to the need for new angles of investigation. An interesting method of analysis was to look at the full trial aligned in two ways: the onset of exploration and the onset of the target fixation. Using these two alignments, the full trial behaviour could be seen in the EEG; each peak could be accounted for based on the trial structure. Firstly, in the start of exploration alignment, peaks are resultant from the presentation of the target face, followed by the fixation dot to start the exploration, and then first few saccades made in the trial. Single trial raster plots sorted by the onset of the first and seconds saccades were used to confirm the hypothesis. The result also showed a methodological confound in which the grand average was a large long peak. This could be miss-concluded as a single potential rather than the processing of concurrent saccades. In the target fixation alignment, the target fRPs were clearly visible. Single trial raster plots were also negative drifts in the averaged signals as they approached the target fixation. This was likened to the grand average fRPs without baseline correction,

which showed the target fRP to be more negative pre-fixation than the distractor fRP. This pressed for more understanding to how local and global dynamics affect the fRPs themselves.

5.5 New local and global dynamic discoveries

Continuing with the novel analysis, further investigation was made into the global dynamics of the fRPs during the course of a trial. A negative drift was found in the full trial analysis. This, combined with the grand average target fRP showing negative amplitude, steered the direction of analysis to focus on whether this negative global shift was seen in the fixations made within a trial. For this to be seen, each fixation as a function of how far it was from the target was isolated and averaged. For example, if the target was fixation N, then one fixation to target would be N-1; each N-1, N-2, N-3 etc. fixations were collected and averaged to see their average amplitude. This negative shift was seen locked within individual fRPs. The closer the fixations were to the target, the more negative the amplitude. After finding that there are global properties affecting fRPs, contemplation was made to whether classical concepts, such as expectancy and surprise found in EEG, were also locked within fRPs. In EEG it has been found that there is a correlation between the amplitude of the P300 and the length of the ISI (Gonsalvez & Polich 2002). This has been related to the P3b subcomponent of the P300 as well as the concept of expectancy. The other subcomponent of the P300 (the P3a), has been shown to elicit strongest for novel or unexpected stimuli. Therefore, has been related to the concept of surprise. With this in mind, the closest replication of ISI in visual search would be fixation rank (rank is given to the number the fixation is in the sequence of the trial i.e. the first fixation is rank 1 and the second rank 2 etc). By taking the average amplitude of the P300 window [250 400]ms and subtracting a baseline [0 50]ms for each fRP of the isolated rank, using a Pearson correlation test for amplitude vs fixation; a significant negative correlation was found in the midline electrodes. The results confirm that classical concepts of surprise are also locked within fRPs in natural completely free-viewing visual search. For further confirmation and to provide a well-rounded result, the data was also denoised; so that the trends found from local and global dynamics were not a by-product of random noise artefacts.

5.6 Work to be submitted for publishing

During the course of the current thesis, the research carried out could be very useful for many researchers in the field of co-registration. The vast majority of the work from the current thesis has been presented in some form to experts in the field, as well as industry and students interested in EEG and ET. Overcoming challenges in co-registration, discussed in Chapter 2, fundamentally contributed to the data acquisition and analysis discussed in Chapter 3 and 4. The investigation discussed in Chapter 3 was presented at an international conference ECEM in 2013, while also being part of published work (Kaunitz et al. 2014). The unrestricted free-

viewing fRPs, that reproduced similar potentials found in fixed-gaze and recent free-viewing studies; such as the P100, VPP and the P300 had been found shortly after the work from Chapter 3 was published. The difference between target and distractor fRPs at the level of the VPP, were also acknowledged and the findings were selected to be presented The Postgraduate Festival; hosted by The University of Leicester in June 2014. This was a county level event where academics and industry professionals, as well as students were able to view and ask questions about the work. It was also presented as a poster for Brain Awareness day in 2015, hosted by The University of Leicester Psychology department; as a way to inspire prospective students from local schools into neuroscience. During the event the public were free to view the work and ask questions. The investigation progressed to discover some methods of analysis that different fields may find advantageous, as well findings that will be of interest to EEG and ET fields. Discoveries such as how baseline correction, can lead to very different conclusions in terms of fRPs, as well as how local and global dynamics of the signal influence and modulate potentials; were important to the field. This was evident as the work was also selected to be one of the main presentations during a co-registration session at ECEM in 2015. During the presentation, the work was well accepted from experts in the field. Since being publicly presented there have been deeper analyses made. Firstly, a very novel approach looking at the full trial was accomplished; this is a method that could be utilised by other groups involved in EEG and co-registration. The methodological concerns found will be received well in many communities, in regards to baseline correction or single (full) trial raster plots sorted by saccades accompanying grand average ERPs/fRPs. Many will also be intrigued by the results that show local and global properties, as well as classical concepts of surprise locked within fRPs. Shortly after the submission of the current thesis a manuscript for submission to a suitable journal will be put together with the aforementioned content.

5.7 Future work and direction

The main body and findings of the current thesis built off the great foundation laid in Kauntiz et al. 2014. The investigation into co-registration of EEG and ET has allowed fRPs to be found in completely free-viewing tasks. In regards to the paradigm used in the current thesis, the research has explored many avenues over the course of the study. However, it is clear that co-registration must continue as it is a powerful tool for the investigation of the processing of visual information. Furthermore, the results have the potential for further application into a wide set of fields.

Visual search: It was shown that it is possible for unrestricted visual search to be researched using concurrent EEG and ET recording. This is a positive outlook for visual search research. It has been important to researchers to understand what drives a visual search, whether there are any features of a scene that guide the eye movement to the target. Attention driven behaviour

started off being modelled using aspects of salience (Itti & Koch 2001). But this has since progressed to show that the task demands can reverse effects of saliency (Einhäuser et al. 2008). Another area of interest to model; utilises saccadic eye movements. It is understood that when approaching a target, saccades elicit certain behaviour that could drive the search. Saccades have been found to be more accurate when made to an area that a target is expected (Shimozaki et al. 2005); therefore this has been an area utilised for modelling (Eckstein et al. 2006). However, it is clear that there are still problems involved with modelling in visual search; such as there being relatively little known about the neural mechanisms driving decisions for target prevalence (Eckstein 2011). Nevertheless, with the research from the current thesis the potential for modelling, not only behaviour, but also the physiological activity as a response; can only strengthen study into visual search modelling. Some results found during the course of the study such as the differences in targets and distractors at the level of VPP and P300 modulation as a function of fixation rank are clear areas in which the current work could be further investigated in terms of modelling.

Brain computer interfaces/human computer interfaces (BCI/HCI): A BCI is an arrangement in place to measure neural activity (Sajda et al. 2008), while HCI refers to any human interaction with technology, where the responses collected are processed by way of an algorithm or method in order to control an external computer or device. System such as these are readily used as assistive technologies for motor neuron diseases such as Amyotrophic Lateral Sclerosis (ALS), where patients have started to lose control of their limbs but still have eye movement functions. There has been research in EEG using speller paradigms (Sellers et al. 2006) and there are improvements in ET using the on screen eye keyboards (MacKay, David 2002) and research strive to vastly improve performance using different techniques; such as prefix highlighting (Diaz-Tula & Morimoto 2016) and gaze free eye swiping (Kurauchi et al. 2016). Assistive technologies are becoming more tuned to patient needs. There are already studies trying to utilise co-registration to assist motor neuron disease patients (Pasqualotto et al. 2015; Taher et al. 2015). For example, patients' suffering from ALS may have better motor control in the morning but may drift later in the day. The co-registration of EEG and ET has been beneficial for the understanding of natural visual processing. It is also beneficial in BCI/HCI, as they may reduce certain restrictions; particularly on eye movements. By using the two channels of information a feedback system could be implemented such that when ocular motor control decreases and the use of an eye tracker becomes difficult, then a BCI could take over as the main assistive technology. The P300, as discussed in Chapter 1, is an important potential in BCI as it is one of the easier to classify. Furthermore, the results found regarding modulations of the P300 in unrestricted visual search can only help improve and add to the knowledge of current systems; BCI would strengthen with further research into co-registration of EEG and ET.

Saccadic generators: There are still unanswered questions about the underlying reasons for different saccade and fixations made within natural viewing (Otero-Millan et al. 2008). If a time-window of EEG signal trace before the onset of a saccade was investigated, it could reveal the mechanisms involved in saccade generation. However, in practice this is much more difficult to implement. In a recent study (Nikolaev et al. 2011) investigated encoding failures in change detection. They found a relationship in pre-saccadic brain potentials to correct change detection. However they did not consider the possibility of influences of previous processing or superposition of saccade processing, which is of major importance as the current thesis has shown; as it has the potential to end in misleading results. Therefore, more control would be needed especially in free-viewing tasks to investigate anything relating to pre-saccadic activity, to look at backward and forward effects and how each influences the resultant potential.

Brain resetting: Another idea that could be explored would be to investigate whether there is a point at which the brain "resets" in visual search, when the target has not been located. There has been work to show local phase resetting after 100ms post stimulus (Wutz et al. 2014). It has also been found that there is a higher probability of phase resetting after difficult searches (Dugué et al. 2015). With the data collected in the current thesis, it would be feasible to investigate this idea. Although, there may have to be a move away from unrestricted eye movements and more controls implemented to avoid artefacts from eye movements influencing results.

Mistaken identity: A smaller investigation that would be interesting, building on the current paradigm would be to research how the brain responds to mistaken identity. In the current work there were a few fixations that were suspected to be mistaken identity (fixations to distractors >1000ms a criteria to end the trial). Although there were not enough fixations in the current work to be able to investigate, one hypothesis would be that a P300 would elicit, but if this potential would be the same produced as that of the actual target, remains to be seen.

Steady-State Visually Evoked potentials (SSVEP): Steady-State visually evoked potentials can be produced from a train of stimuli that are presented at a fixed "steady" rate. They are exogenous ERPs that have been shown to have very good signal-to-noise ratio (SNR) (Norcia et al. 2015). Through the course of the current thesis ERPs were the foundation to the discovery of fRPs. However, the main concerns have involved eye movement artefacts and noise reduction by averaging many trials. In the same context as ERP influenced the field of fRPs, SSVEPs could be the foundation for a new field that combines the freedom of design of fRP with the strong SNR of SSVEPs.

5.8 Conclusion

New understandings: The current Thesis produced fRPs that elicit similar properties to their fixed-gaze counter parts. Global and local dynamics were found to influence fRPs. Furthermore, properties similar to classical concepts in EEG were discovered locked within fRPs; properties such as surprise, which were thought to have been lost through ecological differences in the paradigms.

Practical implications: It has been established that co-registration of EEG and ET can be utilised for investigations into natural completely free-viewing tasks. New methodologies, such as full trial analysis and global dynamics, have been explored and could progress to be a standard in the visual processing EEG community. The current Thesis has highlighted the potential pitfalls that could be made in free-viewing visual search research in EEG and how to avoid those using different analyses.

Overall the current thesis provides a progression into natural completely free-viewing visual search processing, as well as providing a new angle for analysis methods used in EEG. The studies following the current Thesis in the field of co-registration will benefit from the findings made; allowing further progression into understanding visual processing that involves free eye movements.

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