Using Fixation-Related Potentials to investigate cognitive processes

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Contents

Thesis summary

The aim of this research program is to explore and develop a relatively new technique for measuring the neurological processing of naturalistic visual images. Fixation-related potentials (FRP) combine eye-movement recordings with electroencephalographic recordings (EEG) so that brain responses can be measured to points of interest within a whole ecologically valid stimulus (e.g., to eyes within a whole face, or to a word within an entire paragraph). The first paper reports an experiment that used FRPs to investigate the neurological processing of faces when subjects' eye fixations were directed to eyes and mouths within a whole face. This experiment revealed that neural activity to faces is modulated by point of gaze. The second paper presents the results of an experiment that used FRPs to examine the neurological processing of faces when subjects' eye fixations were free to roam freely within a whole face. This experiment revealed that early occipito-temporal activity associated with face processing (N170) is elicited upon presentation of a face, and is not present in subsequent fixations, supporting suggestions that this brain potential reflects a face-detection process. In the third paper, the focus of the research program shifts from faces to words. Specifically, it reports an experiment that tracked the development of neural responses to novel words as they were repeatedly presented within a paragraph. This experiment revealed that unfamiliar words elicit different neural activity compared to familiar words when read for the first time, but that upon subsequent encounters this difference is no longer apparent. The fourth paper provides a methodological review of the combination of the FRP technique, using the insights gained from the first three experiments in this research program to build upon the seminal literature utilising this new neurological technique.

Statement

I certify that the work in this thesis entitled "Using Fixation-Related Potentials to investigate cognitive processes" has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself has been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis. The research presented in this thesis was approved by the Macquarie University Ethics Review Committee, reference numbers: **5201000123D** and **5201000124D** on 22nd of April, 2010.

Signed:

Peter de Lissa

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General Introduction

1.1 Introduction

Our knowledge about the functioning of the human brain has progressed markedly in the last century due to the development of non-invasive brain measurement devices such as electroencephalography (EEG), event-related potentials (ERPs), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI). In a typical EEG, ERP, MEG, or fMRI experiment, participants are presented a repeated stimulus or stimuli, and their average brain response to each stimulus is calculated. This necessitates exact knowledge about when the brain is processing each stimulus. This can be difficult to ascertain if a stimulus of interest is presented within a naturalistic complex scene (e.g., a pair of eyes is presented within a face, or a written word is presented within a paragraph of text). Thus, many EEG, ERP, MEG, and fMRI experiments present stimuli in isolation (e.g., just present a pair or eyes, or a single word).

A limitation of this approach is that brain responses measured may not accurately reflect the neural processing of the stimulus in the "real world". What is needed is a technique that allows the measurement of brain responses to stimuli presented in complex naturalistic settings. This is the goal of a relatively new technique called fixation-related potentials (FRPs), which co-registers eye-movement and EEG recordings to create ERPs that are time-locked to eye-fixations to stimuli presented within a naturalistic scene (e.g., to the eye region within a face, or to a written word within a paragraph).

To date, FRPs have been used in a limited number of published studies, which have investigated the old-new word effect (Hutzler et al., 2007); the effect of parafoveal preview in reading, (Baccino & Manunta, 2005; Dimigen, Kliegl, & Sommer, 2012; Simola, Holmqvist, & Lindgren, 2009); the effect of semantic violations in natural reading (Dimigen, Sommer, Hohlfeld, Jacobs, & Kliegl, 2011; Kretzschmar, Bornkessel-Schlesewsky, & Schlesewsky, 2009); object recognition (Rama & Baccino, 2010); change detection (Graupner, Pannasch, & Velichkovsky, 2011; Nikolaev, Nakatani, Plomp, Jurica, & van

Leeuwen, 2011); proof reading (Takeda, Sugai, & Yagi, 2001); and presaccadic activity relating to information processing during visual fixations (Graupner, Pannasch, & Velichkovsky, 2011; Ossandon, Helo, Monefusco-Siegmund, & Maldonado, 2010; Pannasch & Velichkovksy, 2009; Rajkai et al., 2008). Because FRPs have been used in relatively few experiments, researchers are still addressing a number of methodological challenges relating to merging of eye-movement and EEG data. The aim of this thesis is to address a number of these challenges by using the FRP paradigm to investigate the neural processes involved in two domains of visual processing: face processing (Studies 1 and 2) and word reading (Study 3). These domains were chosen because both are encountered regularly in everyday life, and because both are areas of intense research in visual cognition. The studies serve to demonstrate the application of the FRP paradigm, and illustrate the methodological problems and solutions that relate to the FRP paradigm (discussed in detail in Study 4).

1.2. How are FRPs created?

As mentioned above FRPs involve the co-registration of an eye-movement recording with an EEG recording to calculate ERPs that are time-locked to eye-fixations on a stimulus presented within a naturalistic scene. Thus, in order to understand FRPs, it is important to understand eye-movement recordings, EEG recordings, and ERPs.

1.2.1. Eye-movement recordings.

Eye-movement recordings are made by an eye-tracker that records the time (in milliseconds) and location (i.e., co-ordinates of a stimulus screen) of fixations (i.e., periods of time when the eyes are effectively static) and saccades (i.e., changes in eye-position that exceed 30 degrees/s velocity and 8000 degrees/s^2 acceleration; Stampe, 1993) as participants freely view an image on a computer screen. The timing and locations of fixations and saccades can be "time-stamped" into an EEG recording (see below) to indicate when the eyes fixate (and hence when the brain starts to process) a part of a complex scene.

1.2.2. EEG recordings.

An EEG is a continuous recording of electrical activity detected by electrodes placed on a participant's head. The EEG for each electrode comprises two types of activity: signal (i.e., electrical activity that relates to experimental stimuli) and noise (i.e., all other electrical activity). The brain is a very noisy place and so the signal-to-noise ratio is poor for a single stimulus. However, since electrical activity related to noise is randomly distributed, while electrical activity to the signal is "time-locked" to a stimulus, averaging together EEG responses to a number of stimuli starts to smooth (i.e., cancel out) the noise, leaving an average response that better represents the electrical potential related to the stimulus event. This is called an event-related potential (ERP).

1.2.3. ERPs.

In a typical ERP experiment, an ERP is time-locked to the presentation of an experimental stimulus or stimuli. This is achieved by sending a "time-stamp" (or trigger or pulse code) to the EEG recording indicating the point in time that each stimulus is presented. However, in a typical FRP experiment, instead of representing the point in time at which a stimulus is presented, the time-stamps represent the point in time at which the eyes fixate on a stimulus within a complex scene, which is at the point in time at which the brain starts to process that stimulus (e.g., the eye region within a face, or the word within a paragraph).

It is noteworthy that the process of time-stamping EEG recordings from eye-movements via an eye-tracker represents a progression from a previous technique of time-stamping an EEG via electrical activity recorded at the eye-muscles through electrooculography (EOG). In the latter technique, a sudden change in voltage associated with ocular activity denotes the timing of fixation onset or offset, which is stamped into the EEG recording (Gaarder, Krauskopf, Graf, Kropfl, & Armington, 1964). This technique has been successfully applied to investigate the early lambda components elicited in response to fixation onset and pre-fixation saccadic activity (Kurtzberg & Vaughan, 1977; Yagi, 1979), and higher-order cognitive processes such as the P300 peak elicited by oddball tasks

(Marton, Szirtes, & Breuer, 1984), and word recognition processes (Marton, Szirtes, & Breuer, 1985). However, this technique is limited in scope since the nature of the eye-movements has to be inferred by the experimental design, such as the placing the stimuli of interest in specific places of a computer monitor in order to elicit a large saccade at a specific time (Marton & Szirtes, 1988).

1.3. Variable viewing position paradigm (VVPP) – ERP technique

As well as producing FRPs, the combination of eye-movement and EEG recordings allows the use of the VVPP to manipulate the timing of the presentation and spatial positioning of stimuli (O'Regan & Jacobs, 1992; O'Regan, Lévy-Schoen, Pynte, & Brugaillere, 1984). In the VVPP, a fixation on a specific portion of a presentation screen for a certain period of time (e.g., 150 ms on a centrally-located fixation cross) can be used to trigger the presentation of a stimulus that is positioned so that the eye falls directly on an area of interest within a complex scene (e.g., the mouth in a face). Hence, an ERP can be measured to a specific area of interest. This VVPP-ERP paradigm, which is used in Study 1, has been used to investigate brain potentials associated with the processing of words in the parafovea, (Baccino & Manunta, 2005); and the optimal viewing position effect on ERP measures of lexicality (Hutzler, Braun & Jacobs, 2008). The VVPP technique has also been used to reduce the influence of parafoveal information to reduce the likelihood of eye-movement contamination within critical time-periods (Hutzler et al., 2007). The ability to control the initial fixation point relative to a stimulus also allows for naturalistic eye-movement behaviour in response to specific stimuli, by presenting the stimulus outside of the centrally fixated region. This may avoid interfering with the natural patterns of fixations made to certain stimuli, such as faces (Arizpe, Kravitz, Yovel, & Baker, 2012). It is noteworthy that the VVPP-ERP technique represents an intermediate step between the traditional ERP and FRP paradigms, where the timing of the presentation of stimuli can be controlled by natural fixations, while retaining the ability to manipulate the specific regions

of a stimulus that are centrally fixated. This contrasts with a free-viewing FRP paradigm where participants control how visual stimuli enters their visual system through the timing and the landing positions of natural fixations. Thus, the VVPP can be used to address research questions that would otherwise fall outside the methodological constraints of either technique.

1.4. Studies in this thesis

1.4.1. Study 1: The neural processing of face parts in ecologically valid stimuli.

Previous research suggests that the size of the face-sensitive N170 ERP peak is larger to eyes presented in isolation than faces without eyes, and whole intact faces (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Itier, Alain, Sedore & McIntosh, 2007). These findings have lead to the suggestion that configurally intact faces recruit only face-specific neurons, while disruption of face configuration leads to the recruitment of eye-specific neurons as well as face-specific neurons (Itier & Batty, 2009; Itier et al., 2007; Itier & Taylor, 2004). A limitation of this interesting research is that most studies have presented faces and eyes centrally, such that participants' fixations fall in-between the eyes (Eimer, 1998; Itier et al., 2007; McPartland, Cheung, Perszyk & Mayes, 2010) or over the nose region (Rossion et al., 1999). This does not match the natural t-pattern scan-path for faces, where most fixations fall on the eyes and mouth (Althoff & Cohen, 1999; Blais, Jack, Scheepers, Fiset & Caldara, 2008).

The aim of Study 1 was to use the VVPP-ERP technique to determine if there is a larger N170 to eyes when (1) intact whole faces are used as stimuli (both upright and inverted), and (2) fixations are directed to naturalistic scan-path destinations. The results showed a significantly larger N170 peak to fixations to eyes than mouths in whole upright faces, suggesting that eye-sensitive neurons may be recruited even in the absence of configural disruption. Further, inversion of the faces lead to a larger N170 increase when mouths were centrally fixated compared to eyes. Overall, these VVPP-ERP findings suggest

that whole faces do recruit eye-sensitive neurons, but only when a viewer is actually fixating on the eye region itself, which is typically the case under naturalistic viewing conditions (Blais, Jack, Scheepers, Fiset & Caldara, 2008; Groner, Walder & Groner, 1984; Walker-Smith, Gale & Findlay, 1977; Williams & Henderson, 2007).

1.4.2. Study 2: Face-sensitivity of the N170 is limited to initial presentation: fixation-related potentials during naturalistic scanning of faces.

Behavioural studies have shown that faces capture people's attention more than any other object category (Crouzet, Kirchner, & Thorpe, 2010; Palermo & Rhodes, 2007). The elevated salience of faces as a category has also been observed in ERP research, where a larger N170 response to faces has been interpreted as the reflection of a face detection process (Bentin et al., 1996; George, Jemel, Fiori, Chaby, & Renault, 2005; Rousselet, Husk, Bennett, & Sekuler, 2005). If this interpretation is correct, the N170 should only demonstrate "face-sensitivity" effects to the initial presentation of a face (i.e., the N170 is larger to faces than non-face stimuli, and is larger to inverted than upright faces), and not to subsequent fixations on the same face. This was tested in Study 2, where we compared FRPs to upright and inverted faces and wrist-watches (a non-face stimulus) during initial presentation, and during subsequent fixations. The results support the suggestion that the N170 reflects a face-detection process, since the N170 FRP was only larger to faces compared to watches, and to inverted than upright faces, for the initial presentation.

1.4.3. Study 3: Orthographic learning in the brain: New insights from fixation related potentials.

Behavioural studies suggest that typically developing readers can establish a representation of a new written word in a single exposure, and that the strength of this representation can increase in strength for up to four exposures (Bowey & Muller, 2005; Nation, Angell & Castles, 2007). Further, this "orthographic learning" occurs during silent

reading as well as reading aloud (Bowey & Muller, 2005; de Jong & Share, 2007; de Jong, Bitter, & van Setten, 2009).

Less is certain about orthographic learning in the brain. Most ERP studies of orthographic learning have compared brain responses to unfamiliar and familiar words that are presented one at a time (e.g., Bentin, Mouchetant-Rostaing, Giard, Echallier, & Pernier, 1999; Hauk et al., 2006; Maurer, Brandeis, & McCandliss, 2005; Proverbio, Vecchi, & Zani, 2004; Sereno, Rayner, & Posner, 1998; Sereno, Brewer & O'Donnell, 2003; Simon, Petit, Bernard, & Rabai, 2007; Taroyan & Nicolson, 2009). These studies have produced inconsistent findings, which may stem from task-related differences between studies (Maurer et al., 2005). One way to address this problem is to measure orthographic learning under natural reading conditions.

To this end, in Study 3, we used the FRP paradigm to record FRPs to unfamiliar and familiar names in paragraphs of text. Each name was repeated four times within the paragraph. The results showed that the first encounter of an unfamiliar word in a paragraph engages neural processes (1) in the frontal-central region at around 150 ms, which respond to the novelty of an unfamiliar word as a visual stimulus, and (2) in the parietal region at around 300 ms, which are related to the allocation of attention to novel or unexpected stimuli. By the second encounter, a new word is no long processed as an unfamiliar class of visual stimulus (i.e. the P150 effect has disappeared) but still attracts extra attention as a novel stimulus (i.e., the P300 effect is still present). By the third encounter, a new word no longer attracts extra attention as a novel stimulus. Additionally, a significant attenuation of early P1 peaks with each encounter of both familiar names and unfamiliar names may be indicative of increasingly strong assumptions made about the upcoming words. These findings suggest that orthographic learning in the brain reflects – at least in part - shifts in processes relating to visual recognition, which in turn guide attention and processing resources.

1.4.4. Study 4: Fixation-related potentials: Some methodological insights.

An advantage of the FRP technique is that it allows the measurement of brain responses to stimuli that are presented in complex naturalistic settings. This is achieved via the co-registration of eye-movement and EEG recordings. However, the integration of eye-movements with EEG raises a number of methodological issues that that do not affect typical EEG or ERP experiments. Study 4 provides a review of the methodological factors that should be considered when conducting FRP experiments. These factors relate to the offline versus online co-registration of eye-movement and EEG recording, ocular artefact avoidance and removal, stimulus type and presentation, offline FRP processing, and recording instruments. In addition to these considerations, factors influencing the interpretation of the resulting FRP waveforms are discussed, such as the nature of the eye-movements associated with experimental conditions and overlapping potentials in the waveforms.

1.4.5. Author contribution

The chapters outlining the experimental studies and methodological review included in this thesis are primarily the works of the author of this thesis. The collaborators on each chapter made contributions to the design, construction and implementation of the paradigms discussed herein. They also contributed via revisions of the resulting manuscripts. T0he contributions of the co-authors have been credited accordingly.

1.5. Summary

The general aim of this thesis is to progress the development and application of the FRP paradigm. To this end, we use FRPs to investigate the neural processes involved in the domains of face processing and word reading. In Study 1, we use the VVPP-ERP technique to measure ERPs to eyes or mouths in upright and inverted whole intact faces to determine if eye-sensitive neurons are involved in processing more naturalistic whole intact faces. In Study 2, we use FRPs to determine if the N170 reflects a face-detection process that occurs

only at initial presentation of faces and not in subsequent fixations. In Study 3, we explored orthographic learning in the brain by measuring FRPs to repeatedly encountered familiar and unfamiliar words in a natural contextual narrative. And in Study 4, we provide an overview of the FRP technique, a discussion about relevant factors to consider when designing and implementing the paradigm, and a means of addressing confounding variables such as ocular artefacts.

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Paper 1

The neural processing of face parts in ecologically valid stimuli

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The neural processing of face parts in ecologically valid stimuli

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Abstract

The current study used event-related potentials (ERP) in combination with a variable viewing position paradigm (VVPP) to direct fixations to specific face parts (eyes or mouths) in upright or inverted whole faces. The N170 elicited by the VVPP was greater to faces than to non-face objects (wristwatches); was larger and earlier when gaze was directed toward the eyes than the mouth in whole faces; and was larger and later to inverted than upright faces. Face inversion had a greater effect on N170 amplitude for the mouth and on N170 latency for the eyes, suggesting that point of fixation within a face modulates brain potentials due to a complex interaction between featural and configural processing.

Keywords:

Face; Eyes; N170

Inversion

Fixation

Perception

The neural processing of face parts in ecologically valid stimuli

2.1. Introduction

Data from a large body of behavioural studies suggests that face perception involves the interplay between two levels of processing: Featural processing of the individual parts of a face, principally the eyes, the mouth, and the nose; and configural processing, which refers to the integration of information across the whole face (see McKone & Yovel, 2009, for a review; Sergent, 1984). The neural processing of faces has been measured with the N170 event-related potential (ERP). This ERP occurs bilaterally over occipito-temporal regions between 130 and 200 ms, and is often larger in the right hemisphere. The N170 is typically larger to faces than non-face stimuli (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Eimer 1998; Rossion et al., 2000); and is typically delayed and enhanced for inverted faces compared to upright faces (Itier, Latinus & Taylor, 2006; Itier & Taylor, 2004; Linkenkaer-Hansen et al. 1998; Rossion et al., 2000). The delayed and enhanced N170 to inverted faces has been interpreted as reflecting the disruption of configural processing (e.g., Itier & Batty, 2009; Robbins & McKone, 2007; Rossion & Caharel, 2011; Van Belle de Graef, Verfaillie, Rossion & Lefèvre, 2010).

Behavioural studies have found evidence for a hierarchy of facial-feature processing. The most important facial features appear to be the eyes (Shepherd, 1981, for review). This is supported by ERP studies reporting that eyes presented in isolation trigger a larger N170 than faces without eyes (Itier, Alain, Sedore & McIntosh, 2007). It has also been reported that eyes in isolation evoke a larger N170 than whole faces (Bentin, Allison, Puce, Perez, & McCarthy, 1996). According to Itier and colleagues, these findings suggest an interaction between face sensitive neurons and eye sensitive neurons (Itier & Batty, 2009; Itier et al., 2007). Specifically, Itier et al. have hypothesized that eyes presented in configurally-disrupted face stimuli (i.e., isolate eyes or eyes in inverted faces) trigger a larger N170 than upright faces (that comprise eyes) because the latter recruits face-sensitive neurons alone,

while the former elicits responses from eye-sensitive neurons in addition to face-sensitive neurons. This would explain why faces with no eyes evoke similar N170 amplitudes to faces with eyes, since face configuration for eyeless faces is sufficient to recruit face-sensitive neurons alone (Itier et al., 2007). Further, eyeless faces do not exhibit the face inversion effect (delayed and enhanced N170), lending support to the hypothesis that the enhanced N170 to inversion is a result of the additional recruitment of eye-sensitive neurons.

While the similar N170 to upright faces with and without eyes appears to be a reliable effect when investigated with similar paradigms (Eimer, Kiss & Nicholas, 2010; Nemrodov & Itier, 2011), the conclusion that eye-selective neurons do not respond to upright faces is questionable for two reasons. First, while whole faces form a common category of visual stimulus, and are frequently encountered in social interactions, it is questionable whether the stimuli used by previous ERP studies to measure the N170 to eyes (eyes presented in isolation) form an ecologically valid stimulus. These experimental stimuli may not engage the same types of neural processing as real faces. Second, the point of visual fixation used in previous ERP studies may have restricted the degree of eye-specific processing in upright faces. Most studies asked subjects to focus their attention on a fixation point that fell either between the eyes in a face (Eimer, 1998; Itier et al., 2007; McPartland, Cheung, Perszyk & Mayes, 2010) or over the nose region (Rossion et al., 1999). Eye-tracking studies using more natural free-viewing paradigms have shown that people: (1) almost always fixate on the eyes when they first see a face; (2) fixate many more times on the eyes than any other part of a face; (3) spend most of their time shifting their gaze from the left eye to the right eye and back again; and (4) seldom fixate on a point between the two eyes (e.g., Althoff & Cohen, 1999; Blais, Jack, Scheepers, Fiset & Caldara, 2008; Groner, Walder & Groner (1984); Heisz & Shore, 2008; Walker-Smith, Gale & Findlay, 1977; Williams & Henderson, 2007). Thus, forcing participants to fixate between the eyes or on

the nose whilst processing a face may artificially inhibit the eye-specific processing within an upright face.

Given the apparent attraction of the eyes to humans, and given the methodological limitations of studies that suggest that eye-specific neurons are not engaged in processing upright faces, the aim of this study was to determine if eye-specific neurons are involved in processing whole upright faces using a paradigm that allows the use of (1) more ecologically valid stimuli, and (2) natural eye fixations. This paradigm integrated ERPs with the variable viewing position paradigm (VVPP), which is frequently used in the domain of visual word recognition (O'Regan, Lévy-Schoen, Pynte, & Brugaillere, 1984; O'Regan & Jacobs, 1992). In each trial of our VVPP-ERP paradigm, participants were asked to fixate on a central fixation cross. A 150-ms fixation triggered the presentation of a whole face. The face was positioned so that the participants' gaze fell directly on an eye or on the mouth. As well as measuring the N170 to eyes and mouths in upright faces, we measured the N170 to eyes and mouths in inverted faces. If Itier et al.'s (2007) hypothesis is correct then eye-selective neurons should not be active during the processing of upright faces, and so the N170 to eyes and mouths should be similar in upright faces. Further, fixations to eyes in inverted faces should trigger a larger N170 than eyes in upright faces. Alternatively, if the point of fixation to a face does mediate the strength of the N170, then the N170 to eyes should be larger than the N170 to mouths in both upright and inverted faces.

2.2. Methods

The Human Ethics Committee at Macquarie University approved the methods and procedure used in this study.

2.2.1. Participants

Eighteen participants (13 females, 16 right-handed), aged between 19 and 30 years (mean age = 24.2 years), took part in the study. While the strength of laterality of N170 responses has previously been found to be modulated by participant sex, the face-sensitive

nature of the N170 has not found to be similarly modulated (Proverbio, Riva, Martin & Zani, 2010). Accordingly, it is unlikely that the imbalance of females to males in the current study would confound the interpretation of patterns of face-sensitivity. All participants had normal or corrected-to-normal vision, and gave their informed consent before participating in the study. Sixteen participants were Caucasian. Two participants were Asian but had resided in Australia for at least three years, and thus had extensive exposure to the race of the face stimuli (i.e., Caucasian faces), decreasing the likelihood that processing of the face stimuli would be modulated by inexperience with Caucasian faces (Rhodes et al., 2009). Participants volunteered or were reimbursed $30 for their time.

2.2.2. Stimuli.

Face stimuli consisted of 200 grey-scale images of Caucasian individuals (100 female, 100 male). The faces were presented twice each: Once upright and once inverted. Faces were emotionally neutral, and cropped within a standard sized oval frame where only the internal face parts were visible (see Figures 1 & 3a). The face images were obtained from seven databases: NimStim (Tottenham, et al., 2002), the Karolinska Directed Emotional Faces (KDEF; Lundqvist, et al., 1998), Gur et al. (2002), Computational Vision Archive (courtesy of Caltech), the MIT-CBCL (Weyrauch, et al., 2004), the Ekman and Friesen face set (Ekman & Friesen, 1976), and a set from Kieran Lee and David Perrett of St Andrews University.

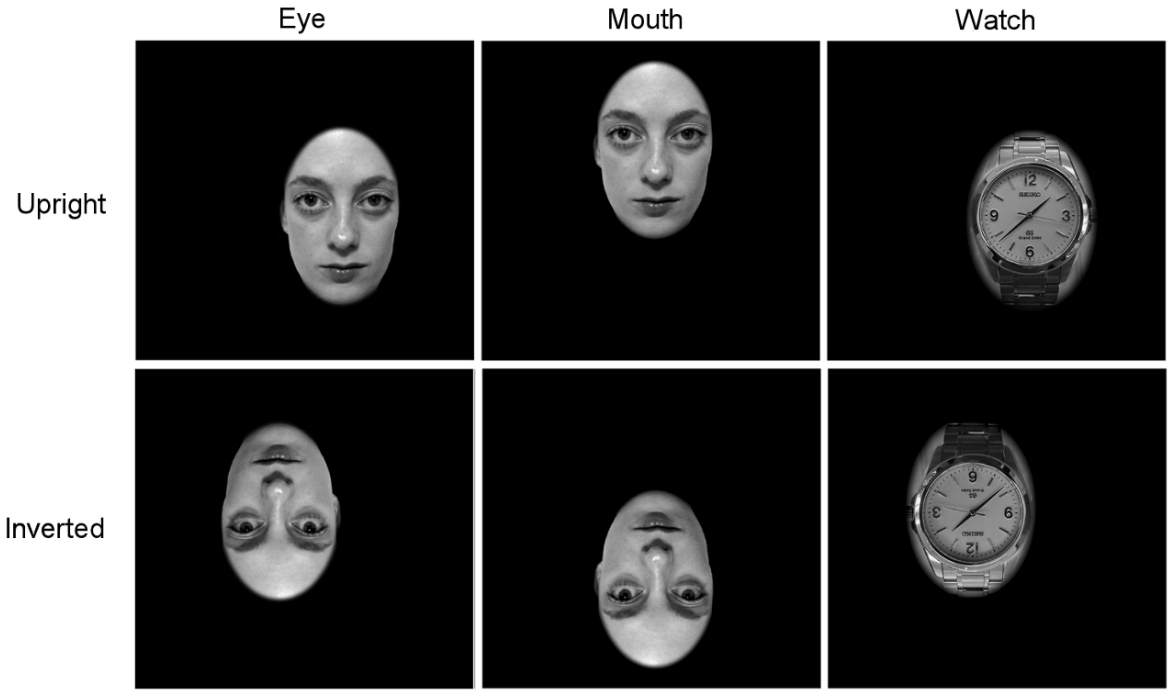

Fig. 1. *Examples of presented stimuli (stimuli were presented relative to a central fixation cross where a participant's gaze was directed to be over the eye, mouth or corresponding regions of a wristwatch to upright and inverted faces and watches)*

As well as measuring the N170 to upright and inverted faces, we measured the N170 to upright and inverted non-face stimuli to ensure that our N170 was analogous to the face-sensitive N170 indexed by previous face processing studies. The non-face stimuli were grey-scale images of 50 different wristwatches, sourced from the University of Kansas Information and Telecommunication Technology Center database. The images were presented once in an upright condition and once in an inverted condition. Watches were chosen because they were (1) familiar objects, (2) similar in shape to the faces (i.e., round), and (3) have been used as non-face stimuli in previous ERP studies (e.g., Bentin, DeGutis, D'Esposito & Roberston, 2007). Like the face stimuli, each wristwatch was cropped to fit within a standard size oval frame.

The face and watch stimuli were presented on a 19" CRT computer monitor with a refresh rate of 100 Hz at a distance of 50cm from the participant. As such, each image was 17.4^0 x 12.7^0 degrees of visual angle.

2.2.3. On-line electroencephalogram (EEG) and eye-tracking

The EEG was recorded using 30 Ag-AgCl sintered electrodes embedded in an elastic cap (EasyCap) positioned according to the 10-20 system. The left and right earlobes were used as online and offline references, respectively. The ground electrode was located between the Fz and FPz electrodes. Electrode impedances were kept below 5kΩ. Ocular movement was recorded with bipolar electrodes placed at the outer canthi, and above and below the left eye. The online EEG was sampled with a Synamps II amplifier at a sampling rate of 1000 Hz, with an online band-pass filter of 1 to 100 Hz, and a notch filter at 50 Hz Participants' eye-movements were tracked and recorded with a monocular (right eye) Eyelink 1000 eye-tracker sampling at 1000 Hz.

2.2.4. Procedure

Participants were fitted with the EEG cap and positioned in the eye-tracking headrest. Stimulus presentation was controlled by Experiment Builder software (version 1.6.1) utilising a gaze-contingent central fixation cross to initiate the presentation of the images. Participants were instructed to judge whether the presented stimulus was a female face, male face, or watch using buttons on a keypad. Each trial started with a centrally-presented white fixation cross on a black background. Once the participant had fixated on the cross for 150 ms, a port code was sent to the EEG recording device, and an upright or inverted face or watch was presented for 200 ms. Each stimulus was positioned so that the participant's gaze fell on the right eye, the left eye, or the mouth of a human face, or the corresponding regions of a watch face. This was followed by a blank black screen which lasted until a button response was recorded. Finally, a "blink now" screen was displayed for 1500 ms before the next trial started. Accuracy and response latencies of button responses were recorded. The

onset of each stimulus sent a serial port code to the online EEG recording. The order of stimuli presentation was randomised.

There were 500 trials in total: 200 for upright faces (100 with gaze directed to either right and left eye, 100 with gaze directed toward the mouth), 200 for inverted faces (with fixations directed to the eyes and mouth in the same manner), 50 trials with upright watches (25 with gaze directed to locations corresponding the eye on faces, and 25 corresponding to the mouth on faces) and 50 trials with inverted watches (with fixations directed in the same manner as in upright watches). A smaller number of trials was used in the watch conditions because (1) 50 trials is an adequate number of trials to create a reliable waveform in adults, and (2) we wanted to minimise the length of the already long testing session (1 hour) as much as possible for the participants' sakes.

2.2.5. Offline ERP processing

The EEG data was analysed offline with Neuroscan 4.3 software. Re-referencing of scalp EEGs was achieved through averaged mastoid electrodes, following the removal of VEOG artefacts from EEG sites using a standard ocular reduction algorithm. The port codes (see above) were used to form epochs and condition averages time-locked to the onset of the fixation-driven stimulus presentation. These -100 to 600 ms EEG epochs were baseline corrected using the period 100ms prior to stimulus onset, and were filtered through a band-pass of 0.1 – 30 Hz with 12 dB/octave roll-off. Trials containing EEG artefacts exceeding +/-80 μV were excluded from analysis. Stimulus presentation was triggered through eye-fixations, which are associated with electrical artefacts resulting from ocular movement. Contamination from this electrical artefact was avoided by ensuring that the eyes remained static on the fixation cross for a minimum of 150 ms before the stimulus was presented. This reduced the likelihood of introducing ocular artefacts that would have needed correction.

Visual analysis of the condition average waveforms revealed the N170 peak latencies and amplitudes to be maximal at the occipito-temporal left P7 and right P8 electrodes (see

Figure 2), which is similar to the findings of previous studies (see Rossion & Jacques, 2007 for review). A grand average formed from all upright stimuli showed a distinct N170 peak occurring at around 160 ms, and accordingly a peak detection algorithm was applied over a time window of 130-190 ms (30 ms either side of this N170 peak in the grand average waveform) in order to extract the latency and amplitude measures for the N170 peaks. Visual analysis of the peak detection process confirmed that each participant's N170 peak was maximal during this 60 ms time-window.

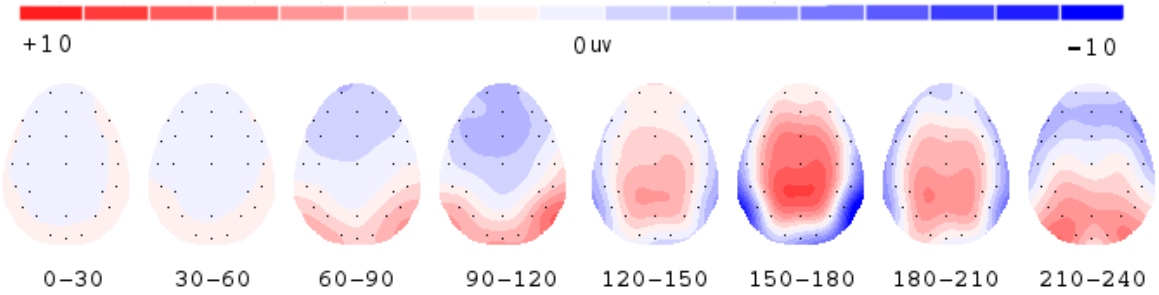

Fig. 2. *Topographical illustration of grand-averaged neural activity in response to the presentation of the stimuli (faces and watches), where maximal negative activity corresponding to the N170 was observed in occipito-temporal P7 and P8 electrode sites at approximately 160ms.*

2.2.6. Data analysis

Levene's test of normality found the distributions of the N170 peak amplitude and latency data to be normally distributed. Thus, repeated measures ANOVAs were used to test the main effects of hemisphere (P7 (left) or P8 (right)), stimulus type (eye, mouth, or watch) and stimulus orientation (upright or inverted), and the interactions between stimulus type and orientation, for N170 amplitudes and latencies separately. A Bonferroni correction for multiple comparisons was applied to analyses involving a comparison of fixations on watches with fixations made to eyes and mouths in faces. Accuracy and correct response

times on the behavioural identification task were also analysed separately using repeated measures ANOVAs and post-hoc t-tests. Greenhouse-Geisser corrected values were used when sphericity was violated. The threshold for statistical significance was taken at p <= .05.

2.3. Results

2.3.1. N170 amplitude

The hemisphere (P7 versus P8) by stimulus (eyes, mouths, watches) by orientation (upright, inverted) repeated measures ANOVA revealed a significant main effect of hemisphere, with N170 peaks significantly larger at P8 than P7 [$F(1, 17) = 10.671$, $p = .005$, $\eta^2 = .386$]. There was significant effect of stimuli [$F(1.1, 18.6) = 64.645$, $p < .0005$, $\eta^2 = .808$], with eyes eliciting a larger mean amplitude (-9.9μV) than mouths (-8.6μV), and both eyes and mouths eliciting larger amplitudes than watches (-2.8μV). There was also a significant main effect of orientation ([$F(1, 17) = 32.893$, $p < .0005$, $\eta^2 = .722$], with the N170 significantly larger to inverted than upright stimuli. There was an interaction between stimuli and hemisphere [$F(1.1, 18.7) = 4.880$, $p = .037$, $\eta^2 = .223$] because there was a greater difference between the size of the N170 between stimuli in the right hemisphere (-8.47μV) than the left hemisphere (-5.74μV). To better understand this interaction, we conducted separate stimulus (eyes, mouths, watches) by orientation (upright, inverted) ANOVAs for P7 and P8. The statistics for the significant effects are outlined in Table 1. A significant main effect of stimulus was found at both sites, with eyes producing the largest N170 followed by mouths and then watches at both P8 (see Figure 3b) and P7. A significant main effect of orientation was also present at P7 and P8, with inverted conditions producing a larger N170 than upright conditions. There was a stimulus by orientation interaction at both sites, and further analysis with t-tests revealed that at both P7 and P8: (1) the N170 elicited by upright eyes and mouths was larger than the N170 elicited by upright watches; (2) eyes elicited a significantly larger N170 than mouths; (3) inverted eyes and mouths elicited a significantly larger N170 than upright eyes and mouths; (4) this inversion effect was larger

for mouths than eyes; and (5) this inversion effect did not exist for watches (see Figures 4 & 5a).

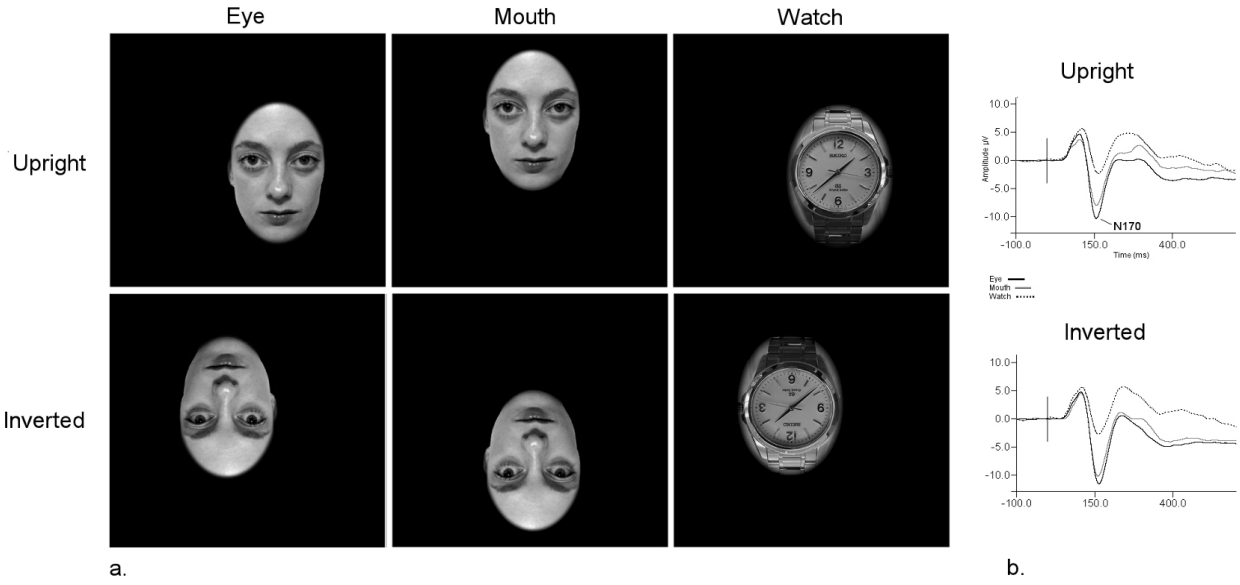

Fig. 3. (a) *Example stimuli and (b) corresponding ERP waveforms of the N170 peaks for each condition, recorded at the P8 electrode.*

Table 1. Statistics for N170 amplitude and latency effects of point of fixation and orientation

N170 amplitude								
	Electrode: P7				Electrode: P8			
ANOVA	*F*	*p*	η^2	t-tests	*F*	*p*	η^2	t-tests
Stimuli	47.48	< .001	.736	Eye > Watch† Mouth > Watch† Eye > Mouth**	41.97	< .001	.712	Eye > Watch† Mouth > Watch† Eye > Mouth†
Orientation: Inv > Up	66.84	< .001	.797	Eye*** Mouth†	16	.001	.485	Eye* Mouth†
Interaction: Effect of inversion	8.55	.003	.517	Mouth > Eye*	12.98	< .001	.619	Mouth > Eye*
N170 latency								
Stimuli	ns	ns		ns	ns	ns	ns	Eye < Watch*** Mouth < Watch* Eye < Mouth*
Orientation: Inv > Up	31.94	< .001	.653	Eye† Mouth***	29.66	< .001	.636	Eye† Mouth***
Interaction: Effect of inversion	5.95	.013	.416	No sig difference	11.17	.001	.583	Eye > Mouth**

Note. *p < .05, **p < .01, ***p < .005, †p < .0001

2.3.2. N170 Latency

A repeated measures ANOVA was used to test the effects of hemisphere (P7 versus P8) stimuli (eyes, mouths, watches) and orientation (upright, inverted) on the latency of the N170. While there was no significant main effect of stimuli or hemisphere on N170 latency, there was a significant main effect of orientation because inversion delayed the N170 [$F(1, 17) = 79.474$, $p < .0005$, $\eta^2 = .824$]. There was also a significant interaction between stimuli and orientation [$F(1.4, 23.2) = 15.935$, $p < .0005$, $\eta^2 = .484$]. To better understand this interaction one-way ANOVAs (and appropriate post-hoc t-tests) were conducted for P7 and P8 separately for the upright and inverted stimuli. In the upright condition there was a significant main effect of stimulus at P8 [$F(2, 16) = 9.297$, $p = .002$, $\eta^2 = .537$] because eyes elicited a significantly earlier N170 than mouths, and watches elicited a significantly later N170 than mouths (and hence eyes). There was no effect of stimulus at P7. No significant differences were found between the N170 latency to eyes, mouths, and watches in the inverted condition at either P7 or P8 (see Figure 5b).

The significant main effect of orientation was investigated with one-way ANOVAs and post-hoc t-tests within the eye, mouth, and watch conditions, and for P7 and P8 separately. At both electrode sites inversion caused a significant N170 delay for both eyes and mouths. The inversion effect on N170 latency was significantly larger for eyes than mouths at the right P8 electrode. There was no inversion effect at P7 or P8 for watch stimuli, thus, inversion had a significant effect only on face stimuli.

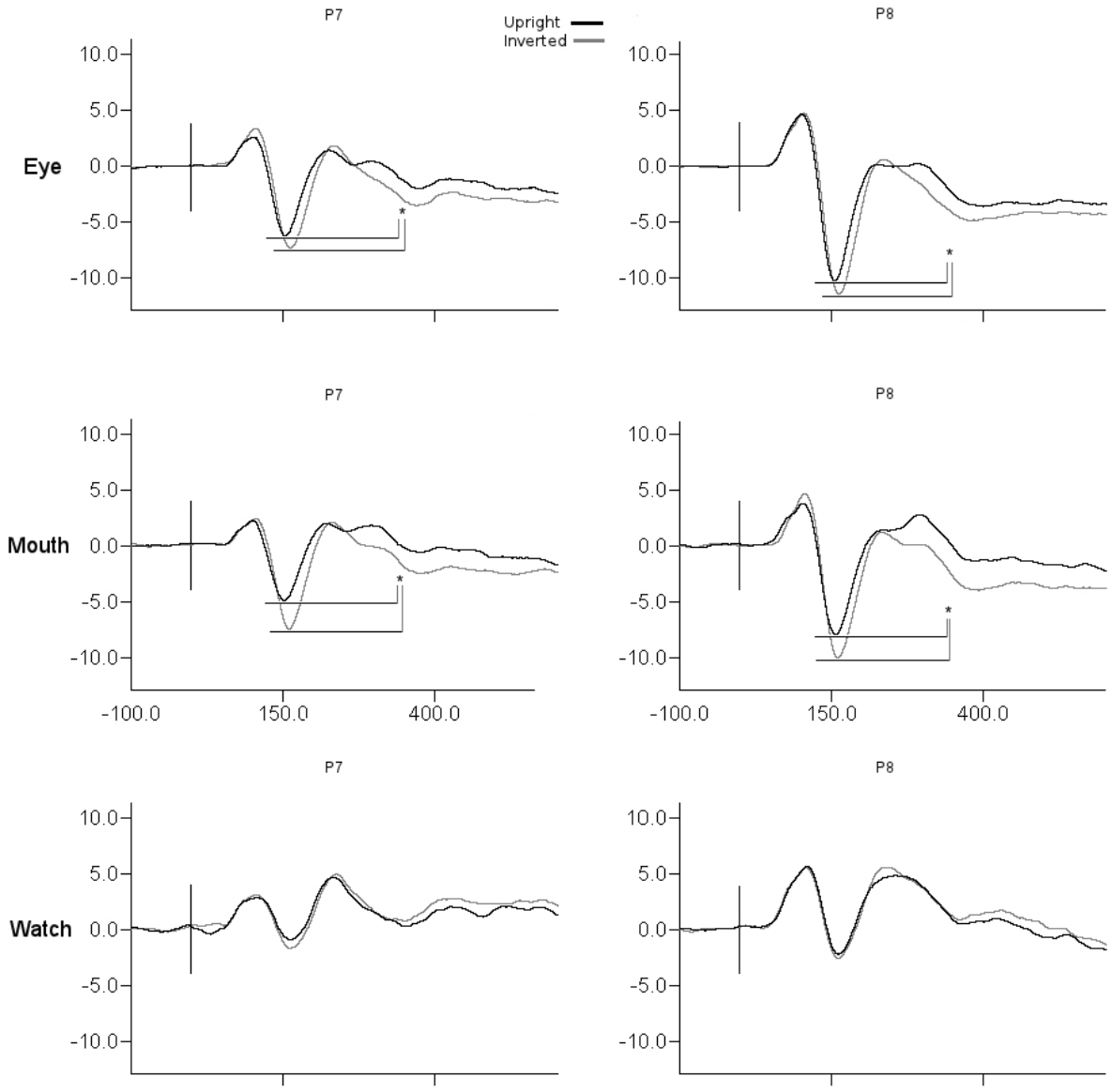

Fig. 4. *N170 waveforms of effects of inversion for fixations to eyes and mouths within whole faces, and to watches (*p < .05).*

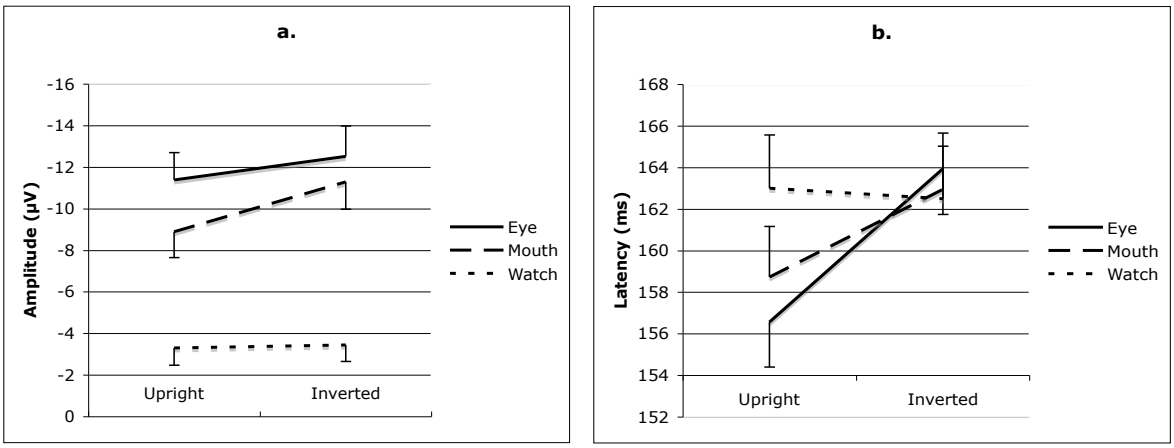

Fig. 5. *N170 amplitudes (a) and latencies (b) for eyes, mouths and watches in upright and inverted orientations. Values taken at P8 electrode.*

2.3.3. Behavioural Responses

Participants' judgements of whether the image was of a male, female or watch were analysed with repeated measures ANOVAs. For both accuracy and reaction time, there were significant main effects of stimuli and orientation, and an interaction between the two (see Table 2 for statistics). Follow up t-tests found no inversion effect for watch judgements. However, inversion of faces lead to significantly decreased accuracy and increased reaction times for gender judgements. Further, post-hoc ANOVAs revealed an effect of fixation, where fixations to the eye lead to more accurate and faster judgments than fixations to the mouth. Further exploration of the interaction between orientation and point of fixation revealed that fixating on the eye of an upright face lead to faster and more accurate judgements. In contrast, there was no difference between the accuracy and latency of judgements to eyes and mouths in inverted faces.

Table 2. Statistics for gender judgement effects of point of fixation and orientation

Accuracy				
ANOVA	*F*	*p*	η^2	t-test comparisons (Face parts)
Stimuli	5.03	.039	.228	Upright: Eye > Mouth† Inverted: Eye = Mouth
Orientation	191.89	< .001	.919	Upright Eye > Inverted Eye† Upright Mouth > Inverted Mouth†
Interaction: Effect of inversion	24.73	< .001	.593	Eye > Mouth†
Reaction time				
Stimuli	ns	ns	ns	Upright: Eye < Mouth * Inverted: Eye = Mouth
Orientation	63.64	< .001	.799	Upright Eye < Inverted Eye† Upright Mouth < Inverted Mouth **
Interaction: Effect of inversion	4.85	.043	.233	Eye > Mouth†

Note. *p < .05, **p < .01, ***p < .005, †p < .0001

2.4. Discussion

The aim of the current study was to use the VVPP-ERP paradigm to present more ecologically valid face stimuli to determine if eye-specific neurons are involved in processing whole upright faces. The VVPP-ERP paradigm in the present study replicated well-established effects of face processing. Specifically, there was a distinct face-sensitive N170 - irrespective of orientation or fixation location – which indicates that we indexed the same type of face-sensitive N170 that has been measured in previous face processing studies (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Eimer 1998; Rossion et al., 2000). Furthermore, the ERP-VVPP paradigm replicated the typical face inversion effect, with a larger and delayed N170 in response to face inversion (Itier, Latinus & Taylor, 2006; Itier & Taylor, 2004; Linkenkaer-Hansen et al. 1998; Rossion et al., 2000).

A critical and unique finding in the present study was that the N170 was larger and earlier to eyes than mouths in upright faces. One interpretation of this result is that eye-specific neurons are recruited in the processing of whole upright faces, contrary to Itier et al.'s (2007) hypothesis. An alternative interpretation is that eye-sensitive neurons are only recruited when a person fixates on the eyes directly. This would explain why previous studies, which focused subjects' attention on the eye region in general and not the eyes specifically, found no evidence of eye-sensitive neuron activation in response to whole upright faces.

A second critical finding in the present study was that the effect of face inversion was larger for fixations to mouths than for fixations to eyes. One explanation for this relates to differences in the activation of eye-sensitive neurons in upright and inverted faces. Specifically, upright faces may only recruit eye-sensitive neurons when a fixation is made directly to an eye, while inverted whole faces may recruit eye-sensitive neurons regardless of whether the eye or mouth is fixated (i.e., due to a disruption in configural processing; Itier et al., 2007). Consequently, the effect of face inversion would be greater when viewing the

mouth, since eye-sensitive neurons would already be recruited by fixations to the eye in upright faces.

There are three methodological factors that might explain why the outcomes of the present study differed from previous studies. First, studies by Itier et al. (2007), Eimer, Kiss and Nicholas (2010) and Nemrodov and Itier (2011) compared the N170 to different stimuli: isolated eyes, faces with eyes, and eyeless faces. In the current study, the same stimulus was used in all conditions (i.e., intact whole faces). Further, the stimuli were more ecologically valid than isolated eyes and eyeless faces. If we are to understand the importance of the eyes to face processing, it is necessary to recreate the context in which eyes are normally perceived as closely as possible - that is, within a whole face. As outlined above, the stimuli used in the previous studies may have been problematic because they were unnatural and so may have triggered cognitive processes that may have disrupted or confounded natural face processing.

Second, in most previous studies of the face-specific N170 component, the (initial) fixation location was set between the eyes or above the nose rather than on one of the eyes. A previous study investigating the effect of point of gaze showed that fixations to the mid-region of a face lead to a smaller N170 amplitude compared to the upper and lower regions, which both elicited comparable activation (McPartland, Cheung, Perszyk & Mayes, 2010). However, again in that paradigm attention was directed only to the eye region in general rather than an eye specifically. This may have disrupted or confounded natural face processing since eye-tracking experiments indicate that people prefer to shift their focus from one eye to another, seldom fixating between the eyes.

Third, a consequence of the VVPP-ERP paradigm was that the stimuli had to be presented in different locations relative to the fixation cross, which was always at the centre of the display screen. This means that there were differences in the low-level visual characteristics of the visual input between the conditions (for example, when a participant's

fixation was on the mouth of an upright face, then the visual stimuli extended more in the upper visual field than in the lower visual field). Could this explain why the current study produced different findings to previous studies? This seems unlikely. If different locations of the visual stimuli had a confounding effect on the N170 due to low-level visual differences, then this same effect should have been present in the wristwatch conditions which were presented in the same way positions as the face stimuli (i.e., fixations on spatial regions corresponding to the eyes and mouths of human faces). There were no differences in the ERPs in response to the watch-faces between these conditions. Thus, the existing evidence suggests that the unique outcomes of the current study are not simply due to low-level visual differences related to the location of the stimuli on the screen between conditions.

2.5. Conclusions

The outcomes of the present study support the use of the VVPP-ERP paradigm to investigate face processing. Using this paradigm, a number of well-established effects of face processing on the N170 were replicated, with two theoretically relevant exceptions: For the processing of upright faces, the VVPP-ERP paradigm revealed that the N170 to eyes was different to mouths in upright faces, which may reflect the recruitment of eye-sensitive neurons by upright faces. Further, an inversion effect was found when viewing either eyes or mouths, and this effect was greater when viewing mouths than eyes. This suggests that the inversion effect may not be solely driven by the additional recruitment of eye-sensitive neurons. Together, the outcomes of this VVPP-ERP study of face processing suggest that eye-specific neurons are involved in processing both upright and inverted faces. As the point of fixation was found to mediate the timing and strength of the face-sensitive N170 peak, further studies investigating the neural processing of faces might benefit from controlling the initial point of fixation.

Acknowledgements

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Paper 2

Face-sensitivity of the N170 is limited to initial presentation: fixation-related potentials during naturalistic scanning of faces

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Face-sensitivity of the N170 is limited to initial presentation: fixation-related potentials during naturalistic scanning of faces

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Abstract

The current study investigated the N170 peak elicited by face stimuli, to determine whether this face-sensitivity is observed in subsequent fixations made to static face images as well as at initial presentation. This was achieved with the use a fixation-related potential paradigm, where EEG recordings were time-locked to fixations made to faces and objects (wrist-watches). The results indicate that the face-sensitive nature of the N170 is only apparent at the initial presentation of a face, and not in follow-up fixations made to faces. This adds support to the suggestion that the N170 reflects face-detection processes, which is undertaken once.

Keywords:

Face

FRP

N170

Inversion

Fixation

Perception

Face-sensitivity of the N170 is limited to initial presentation: fixation-related potentials during naturalistic scanning of face

3.1. Introduction

We are biased to attend to faces more than other object categories (see review by Palermo & Rhodes, 2007). For instance, when nested within a complex scene, faces tend to attract our attention more than other visual stimuli (Yarbus, 1967). When asked to saccade either left or right towards specific categories of objects, people tend to look more quickly at images of faces than images of other objects (Crouzet et al., 2010). When asked to saccade in the *opposite* direction to specific categories (anti-saccades) people tend to make more errors when required to look away from faces (Morand et al., 2010). When faces are inverted, the anti-saccade error-rate is smaller (Gilchrist & Proske, 2006), a finding which is consistent with decades of research showing that inverted faces, despite containing the same visual information as upright faces, are processed differently. Upright faces are processed holistically, with information across the whole face integrated at a perceptual level, whereas face inversion disrupts holistic processing (e.g., Busigny, Joubert, Felician, Ceccaldi, & Rossion, 2010; McKone & Yovel, 2009). Upright faces are also processed more holistically than non-face objects, so the effects of inversion are disproportionate for faces (e.g., Yin, 1969; McKone & Yovel, 2009).

Studies with patients with brain lesions and functional magnetic resonance imaging (fMRI) work indicates that discrete regions of the brain, particularly in the occipital and temporal lobes (e.g., fusiform face area, FFA, Kanwisher et al., 1997), respond preferentially to faces rather than other objects (see review by Kanwisher & Barton, 2011). Event-related potential (ERP) studies also support the suggestion that faces are treated differently than other classes of objects. The presentation of faces has been found to elicit a strong negative electrical peak recorded through scalp electrodes over occipitotemporal areas, which is generally larger than to other objects approximately 170 ms after stimulus onset (Bentin et

al., 1996; Rossion & Jacques, 2007; although cf Thierry, 2007). This "N170" peak has been found to be sensitive to the disruption of holistic processing, with inverted faces eliciting a later N170 peak than upright faces (Itier & Taylor, 2004; Itier, Latinus & Taylor, 2006; Linkenkaer-Hansen et al. 1998; Rossion et al., 2000). In some studies, the increase in N170 latency has also been accompanied by an increase in N170 amplitude (Itier & Taylor, 2004; Itier, Latinus & Taylor, 2006; Rossion et al., 2000). It is generally considered that the N170 ERP peak reflects the recruitment of face-sensitive areas of the brain, and indexes processes related to face-detection and early stages of face processing (George et al., 2005).

A limitation of the N170 as a face-sensitive measure is the way in which it is measured. Due to intrinsic stimulus presentation constraints imposed by ERP paradigms, participants are typically asked to view a series of brief images of faces on a computer screen (e.g., successive faces presented foveally for approximately 250 ms each; Jeffreys, 1989; Bentin et al., 1996). The N170 ERP is typically measured from the onset of each face image (i.e., is "time-locked" to the stimulus onset). This type of presentation differs from people's natural viewing of faces, which often involves the shift of focus from non-face stimuli to a face (Yarbus, 1967), followed by self-guided fixations around that face (usually in a 'T' pattern between the eyes and mouth; Althoff & Cohen, 1999). This self-directed scanning of faces has been found to facilitate the encoding and recognition of identity (Sekiguchi, 2011; Wilson, Palermo & Brock, 2012). In line with this, atypical scan-paths have been found to be associated with a reduction in activity of the FFA (Morris et al., 2007) and impaired ability to recognise emotion in faces (Kliemann et al., 2010).

Given that our understanding of the N170 principally stems from ERP studies that have had to employ less naturalistic face viewing procedures, it is not yet clear what role the N170 plays in naturalistic face processing. The data from ERP studies has been taken to suggest that the N170 reflects a face detection process (Bentin et al., 1996; George et al., 2005) that occurs even when a face is presented outside the central visual field (Rousselet et

al., 2005). If the N170 peak does reflect a face detection process, we might expect that the N170 in naturalistic viewing settings is triggered when a face is first detected somewhere in a visual scene, but would then no longer be present in subsequent explorations (i.e., fixations) of that face. The primary aim of this study was to test this prediction. To this end, we employed a relatively new electrophysiological paradigm - fixation-related potentials (FRPs) - in addition to ERPs. FRPs enable the measurement of brain potentials to eye-fixations in different regions of interest within a complex visual image (e.g., to a word within a sentence; to a tree within a country scene). The FRP paradigm has been used to investigate neural processes involved in word reading (Baccino & Manunta, 2005; Hutzler et al., 2007; Dimigen et al., 2011) and object identification (Rama & Baccino, 2010). However, to our knowledge, FRPs have not yet been used to investigate face processing.

Given that we aimed to measure the N170 with a new electrophysiological technique, it was important that we established that the N170 that we measured in this study was analogous to the face-sensitive N170 measured in previous ERP studies. This was the secondary aim of this study. We tested this in two ways. First, we measured the N170 to non-face stimuli (i.e., watches) as well as faces. In line with previous ERP studies, we predicted that the N170 would be larger to faces than non-faces (Bentin et al., 1996; Rossion & Jacques, 2007). Second, we measured the N170 to inverted face and non-face stimuli in addition to upright face and non-face stimuli. As mentioned above, previous ERP studies have found that inverting faces modulates the N170 amplitude and latency to a greater degree than inverting non-faces, which suggests that the N170 may relate to the holistic or configural processing of faces (Itier & Taylor, 2004; Itier, Latinus & Taylor, 2006; Rossion et al., 2000). In line with these ERP studies, we predicted that we would find a greater effect of inversion on the N170 for faces than non-faces.

To reiterate, this novel FRP study of face processing had two aims. The first was to test whether the N170 reflects a face detection process in more naturalistic face-viewing

situations. We predicted that this would be the case, with a clear N170 triggered by a face first presented in the periphery of a visual scene, but not in subsequent self-guided fixations around that face. The second aim was to confirm that the N170 measured in this experiment was analogous to the N170 measured in previous ERP experiments. We again predicted that this would be the case, with a larger N170 elicited by faces than non-face stimuli, and with a greater effect of inversion on the N170 of faces than non-faces.

3.2. Methods

The Human Ethics Committee at Macquarie University approved the methods and procedure used in this study.

3.2.1. Participants

Sixteen participants (11 females, 14 right-handed), aged between 19 and 30 years (mean age = 23.5 years, SD = 2.9), took part in the study. Whereas sex differences have been found for the strength of the laterality of N170 responses, the face-sensitive nature of the N170 has not found to be modulated by sex (Proverbio, Riva, Martin & Zani, 2010). Accordingly, it is not likely that the imbalance of females to males in the current study is problematic in light of the aim to investigate face-sensitivity, rather than N170 laterality. All participants had normal or corrected-to-normal vision, and gave their informed consent before participating in the study. Participants volunteered or were reimbursed $30 for their time.

3.2.2. Stimuli

Face stimuli consisted of 100 grey-scale images of Caucasian individuals (50 female, 50 male). Faces were emotionally neutral, and cropped within a standard sized oval frame where only the internal face parts were visible (see Figure 1). The face images were obtained from seven databases: NimStim (Tottenham, et al., 2002), the Karolinska Directed Emotional Faces (KDEF; Lundqvist, et al., 1998), Gur et al. (2002), Computational Vision Archive (courtesy of Caltech), the MIT-CBCL (Weyrauch, et al., 2004), the Ekman and

Friesen Pictures of Facial Affect (Ekman & Friesen, 1976), and a set from St Andrews University (courtesy of David Perrett).

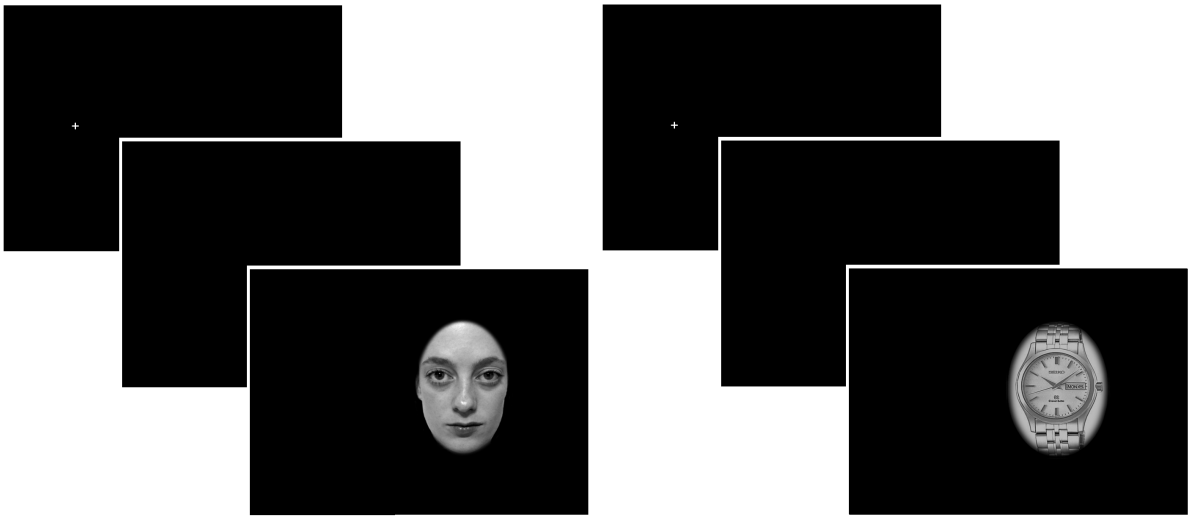

Fig. 1. *Examples of the type of presented stimuli (upright condition). An initial fixation cross was presented to the left of centre, followed by the presentation of upright and inverted images of faces and watches in the participants' right periphery. A fixation on the initial cross triggered the presentation of the stimuli, whereupon participants were free to saccade to the images and make self-guided fixations.*

As well as measuring the N170 to faces, we measured the N170 to non-face stimuli to test if the N170 was larger to faces than non-faces. As mentioned above, this would help confirm that the N170 study measured in this study was analogous to the face-sensitive N170 indexed by previous ERP face processing studies (Rossion & Jacques, 2007 for review). The non-face stimuli were grey-scale images of 50 different wristwatches, sourced from the University of Kansas Information and Telecommunication Technology Center database. Watches were chosen because they were (1) familiar objects, (2) similar in shape to the faces (i.e., oval), and (3) have been used as non-face stimuli in previous ERP studies (e.g., Bentin, DeGutis, D'Esposito & Roberston, 2007). Like the face stimuli, each wristwatch was cropped to fit within a standard size oval frame.

The face and watch stimuli were presented in upright and inverted orientations to test whether the amplitude and latency of the N170 was modulated to a greater degree by inversion of faces than non-faces. If true, this would further confirm that the N170 study

measured in this study was analogous to the face-sensitive N170 indexed by previous ERP face processing studies (Itier & Taylor, 2004; Itier, Latinus & Taylor, 2006; Rossion et al., 2000).

There were 300 trials in total: 100 for upright faces, 100 for inverted faces, 50 trials with upright watches, and 50 trials with inverted watches. A smaller number of trials were used in the watch conditions because (1) 50 trials is an adequate number of trials to create a reliable waveform in adults, and (2) we wanted to minimise the length of the already long testing session.

The stimuli were presented in a single block, with short breaks after every 100 trials. The order of stimuli presentation was randomised. The face and watch stimuli were presented on a 19" CRT computer monitor with a refresh rate of 100 Hz at a distance of 50cm from the participant. As such, each image was 17.4 x 12.7 degrees of visual angle.

3.2.3. Procedure

Participants were fitted with the EEG cap and positioned in an eye-tracking headrest. Stimulus presentation was controlled by Experiment Builder software (version 1.6.1) utilising a gaze-contingent white fixation cross in the central-left part of a computer monitor to initiate the presentation of stimuli. Once the participant had fixated on the cross for 150 ms, an upright or inverted face or watch was presented 9.5 degrees of visual angle to the right of the fixation cross and then the participants freely viewed the images for 5000 ms. We measured participants' neural activity in relation to the initial peripheral image presentation (ERP) and to the first and second fixations of each image (FRP). These "presentations" of the stimuli to participants' visual systems are hereafter termed "first observation", "second observation" and "third observation". We presented the stimulus in the periphery of the visual field, rather than in the centre of the visual field, for two reasons. First, because this more closely represents a naturalistic face-viewing situation, whereby a viewer shifts their focus from non-face stimuli (in this case, the fixation cross) to a face

(Yarbus, 1967), and then executes self-guided fixations around that face. Second, we did not want to interfere with participants' natural scanpaths to faces, which can be modulated by directing the initial fixation to a face (Arizpe et al., 2012). Each trial was followed by a "blink now" screen that was displayed for 1500 ms before the next trial started. The testing session lasted for approximately 1 hour for each participant.

3.2.4. On-line electroencephalogram (EEG) and eye-tracking

Participants' eye-movements were tracked and recorded with a monocular (right eye) Eyelink 1000 eye-tracker sampling at 1000 Hz. This sent trial-specific serial port codes, which indicated the onset of the stimulus (first observation) and the two subsequent fixations on that stimulus (second and third observation), to an online EEG recording. The EEG was recorded by a Synamps II amplifier at a sampling rate of 1000 Hz, an online band-pass filter of 0.01 to 100 Hz, and a notch filter at 50 Hz. The EEG recording comprised electrical activity detected by 30 Ag-AgCl sintered electrodes embedded in an elastic cap (EasyCap) that was positioned on participants' heads according to the 10-20 system. The left and right earlobes were used as online references. The ground electrode was located between the Fz and FPz electrodes. Electrode impedances were kept below 5kΩ. Ocular movement was recorded with bipolar electrodes placed at the outer canthi, and above and below the left eye.

3.2.5. Offline FRP processing

The EEG data was analysed offline with Neuroscan 4.3 software in eight steps. First, eye-blink VEOG artefacts from each EEG channel were removed using a standard ocular reduction algorithm (Semlitsch et al., 1986). Second, each EEG channel was referenced to linked mastoids, excluding ocular sites. Third, the EEG data from each site was cut into segments (i.e., epochs), each starting 150 ms before an eye-tracker port code and ending 350ms after the same port code. Fourth, we baseline corrected each epoch. For the stimulus presentation waveform, the average of the 100ms interval preceding stimulus presentation was used as the baseline value. The fixation-related potentials formed by the first and second

fixations on the images captured the saccadic movement immediately preceding fixation onset, (time zero) which made baseline correction relative to the pre-stimulus activity inappropriate. Thus, for the two fixation waveforms, each epoch was baseline corrected to the average activity immediately following fixation onset (0-20 ms), in line with previous FRP studies (Hutzler et al., 2007; Dimigen et al., 2011). Fifth, each epoch was filtered with a band-pass of 0.1 – 30 Hz with 12 dB/octave roll-off. Sixth, trials containing EEG artefacts exceeding +/-80 μV were excluded from analysis. In the seventh step, for each participant, we created separate waveforms for the first three "stimulus observations" (i.e., stimulus presentation, first stimulus fixation, second stimulus fixation) for the upright and inverted faces and watches at P7 and P8 (see below for justification for these sites).

In the final step, we removed eye-movement artefacts from the FRPs to the first and second fixations on the stimuli (i.e., second and third stimulus exposures). This artefact was introduced by saccadic activity that followed the initial presentation of the stimuli and preceded fixations to the stimuli. To ascertain whether saccadic activity was modulated by stimulus category or orientation, an omnibus ANOVA incorporating these factors was conducted. This revealed that the reaction times of participants' saccades to the watch and face images (181ms and 182ms, respectively) were not modulated by stimulus category [$F(1, 15) = 2.03$, $p = .177$, $\eta^2 = .13$], or stimulus orientation [$F(1, 15) = .113$, $p = .742$, $\eta^2 = .01$], suggesting that the latencies of the ocular noise related to this saccadic activity was comparable across conditions. Saccades leading into the second fixation within faces were observed to be larger than within watches, though this did not generate a different pattern of ocular artefact. Further, the duration of fixations on the images were not modulated by either stimulus category [$F(1, 15) = 1.54$, $p = .236$, $\eta^2 = .106$], or stimulus orientation [$F(1, 15) = 4.19$, $p = .061$, $\eta^2 = .244$],. However, follow-up fixations to the images were significantly longer than the initial fixations, with an average duration of 210ms for initial fixations, and 267ms for second fixations to the images [$F(1, 13) = 25.59$, $p < .001$, $\eta^2 = .66$].

The ocular activity associated with saccadic activity was corrected through the application of independent component analysis that incorporated the EOG channels, as carried out by previous FRP studies (Hutzler et al., 2007; Vigario, 1997). Figure 2a provides a topographic illustration of the average saccadic noise removed from waveforms time-locked to stimulus presentation, corresponding to activity relating to initial eye-movements to the images. Figure 2b shows a topographical map of the ocular noise that was removed from the waveforms time-locked to eye-fixations on the images, where saccadic movement noise preceded fixation onset and followed fixation offset.

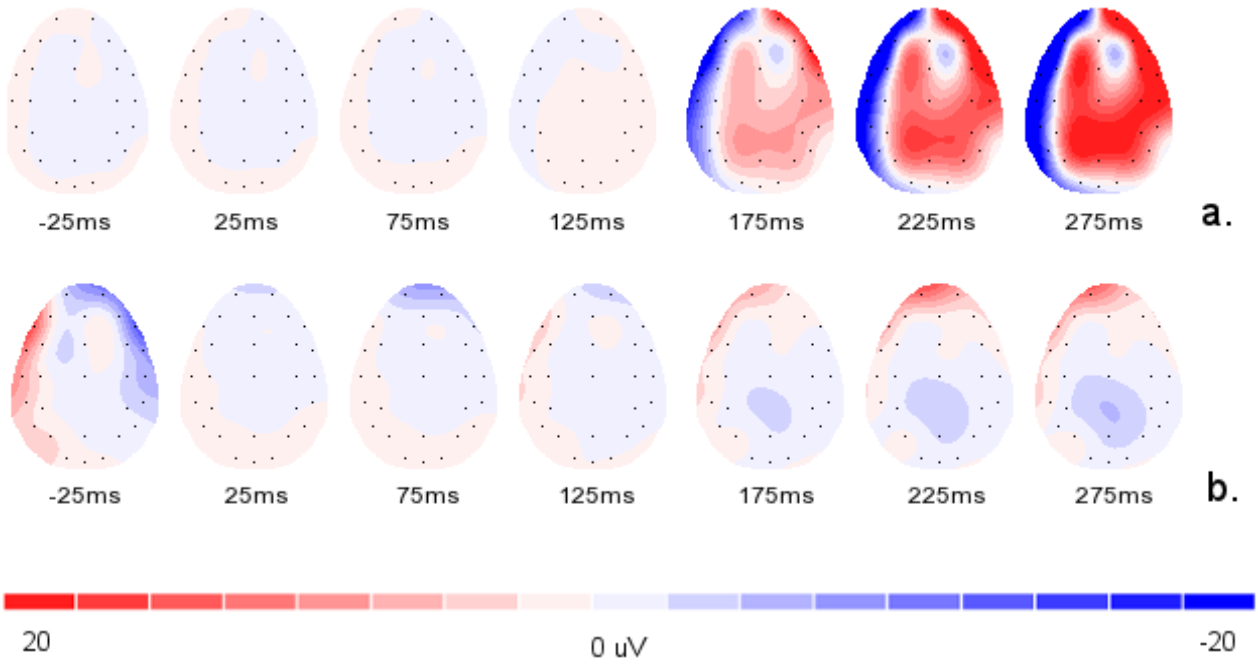

Fig. 2. *Average saccadic movement noise removed from initial presentation (a.) and fixation-related potential (b.) waveforms. Lateral-frontal ocular activity was isolated with independent component analyses, corresponding to eye-movements made to the stimuli.*

3.2.6 Data analyses

Examination of the average waveforms for the group revealed that the mean N170 peak occurred at around 160 ms over occipito-temporal areas, corresponding to the left P7 and right P8 electrode sites (Figure 3), similar to the findings of many previous ERP studies (see Rossion & Jacques, 2007 for review). Thus, maximal peak amplitudes and latencies

were extracted over a time window of 110-210 ms for the N170 peak at P7 and P8. The latency values for the N170 peaks elicited by the watch stimuli possessed a high degree of variance, which was most likely due to the indistinct nature of the peaks for watches (see Figure 4). Thus, in the interest of statistical reliability (Luck, 2005), we restricted our analyses to amplitude data.

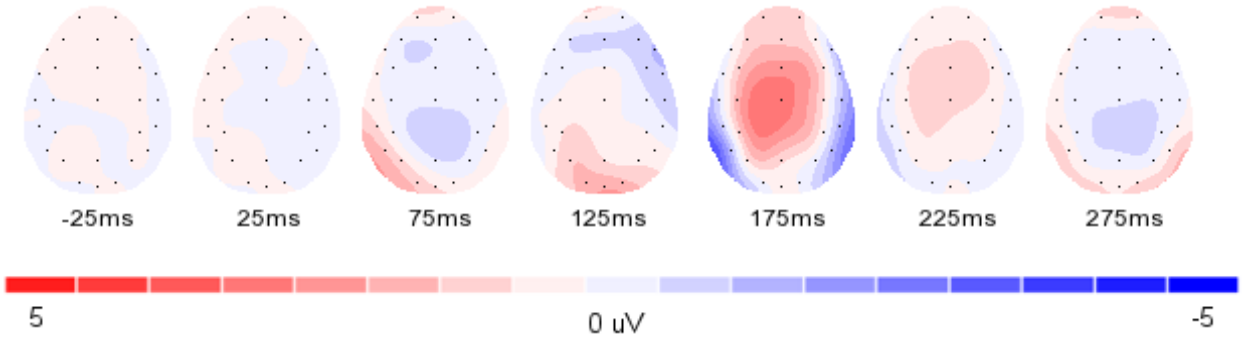

Fig. 3. *Topographic illustration of neural activation in response to initial stimulus presentation (watches and faces averaged together). An initial positive peak at approximately 100ms was observed, followed by strong negative peaks corresponding to the N170 over occipito-temporal areas.*

A $\log_{10}$ transform was applied to the N170 amplitude values to correct for a non-normal distribution of data. The N170 amplitude data was using an omnibus ANOVA comparing the effects of stimulus type (faces, watches), stimulus observation (stimulus presentation, first stimulus fixation, second stimulus fixation), stimulus orientation (upright or inverted), and electrode site (P7, P8). Any statistically significant main effects were further examined by pair-wise t-tests between levels. Any significant interactions were explored by ANOVAs or t-tests, depending on the complexity of the interaction. Greenhouse-Geisser corrected values were used when sphericity was violated. The threshold for statistical significance was taken at $p <= .05$.

It is noteworthy that visual inspection of the group average waveforms suggested a difference in the P1 response (i.e. the first positive peak in the waveforms) to faces and watches. Thus, P1 peak amplitudes were extracted from a 50-120 ms time window, and the

log10 data analysed in an omnibus ANOVA. The outcomes revealed a significant main effect of stimulus type [$F(1, 15) = 24.11,\ p < .001, \eta^2 = .62$], with a larger P1 elicited by watches than by faces; and an interaction between stimulus observation and electrode [$F(2,14) = 9.00,\ p = .003, \eta^2 = .56$]. The latter was further analysed with ANOVAs conducted at each level of stimulus observation. These showed that at the first observation (i.e., stimulus presentation), the P1 was larger at the left P7 electrode site than the right P8 site. At the second observation (i.e., the first fixation to the stimulus) no amplitude difference was found between the P7 and P8 electrodes, and that at the third observation (i.e., second fixation to the stimulus) a larger P1 amplitude was found at the P8 than P7 electrode. The fact that the P1 was larger to watches than faces, and did not show an inversion effect, supports previous research in suggesting that the P1 amplitude most likely reflects low-level visual properties and do not reflect face sensitivity (Ganis, Smith & Shendan, 2012). Thus, only the N170 results are considered from this point onwards.

3.3. Results

The omnibus ANOVA on the N170 amplitude data revealed significant main effects for all four factors. Specifically, there was a significant main effect of stimulus type [$F(1, 15) = 23.25,\ p < .001, \eta^2 = .61$] because the N170 was larger to faces than watches. There was a significant main effect of stimulus observation [$F(2, 30) = 22.17,\ p < .001, \eta^2 = .59$] because the N170 to the first observation (stimulus presentation) was significantly larger than to the second observation ($p = .002$) and third ($p < .001$) stimulus observations (first and second fixations on the images, respectively), which did not differ from each other (Figure 4). There was a significant main effect of orientation [$F(1, 15) = 15.41,\ p = .001, \eta^2 = .51$] because inverted stimuli elicited a larger N170 than upright stimuli. And there was a significant main effect of electrode site [$F(1, 15) = 6.77,\ p = .02, \eta^2 = .31$] because the N170 was overall larger at the left P7 electrode than the right P8 electrode.

The analysis revealed significant two-way interactions between stimulus type and stimulus observation [$F(2, 30) = 20.98$, $p < .001$, $\eta^2 = .58$], stimulus type and orientation [$F(1, 15) = 5.14$, $p = .039$, $\eta^2 = .25$], stimulus observation and orientation [$F(2, 30) = 16.20$, $p < .001$, $\eta^2 = .52$], and stimulus observation and electrode site [$F(1.2, 17.5) = 8.03$, $p = .009$, $\eta^2 = .35$].

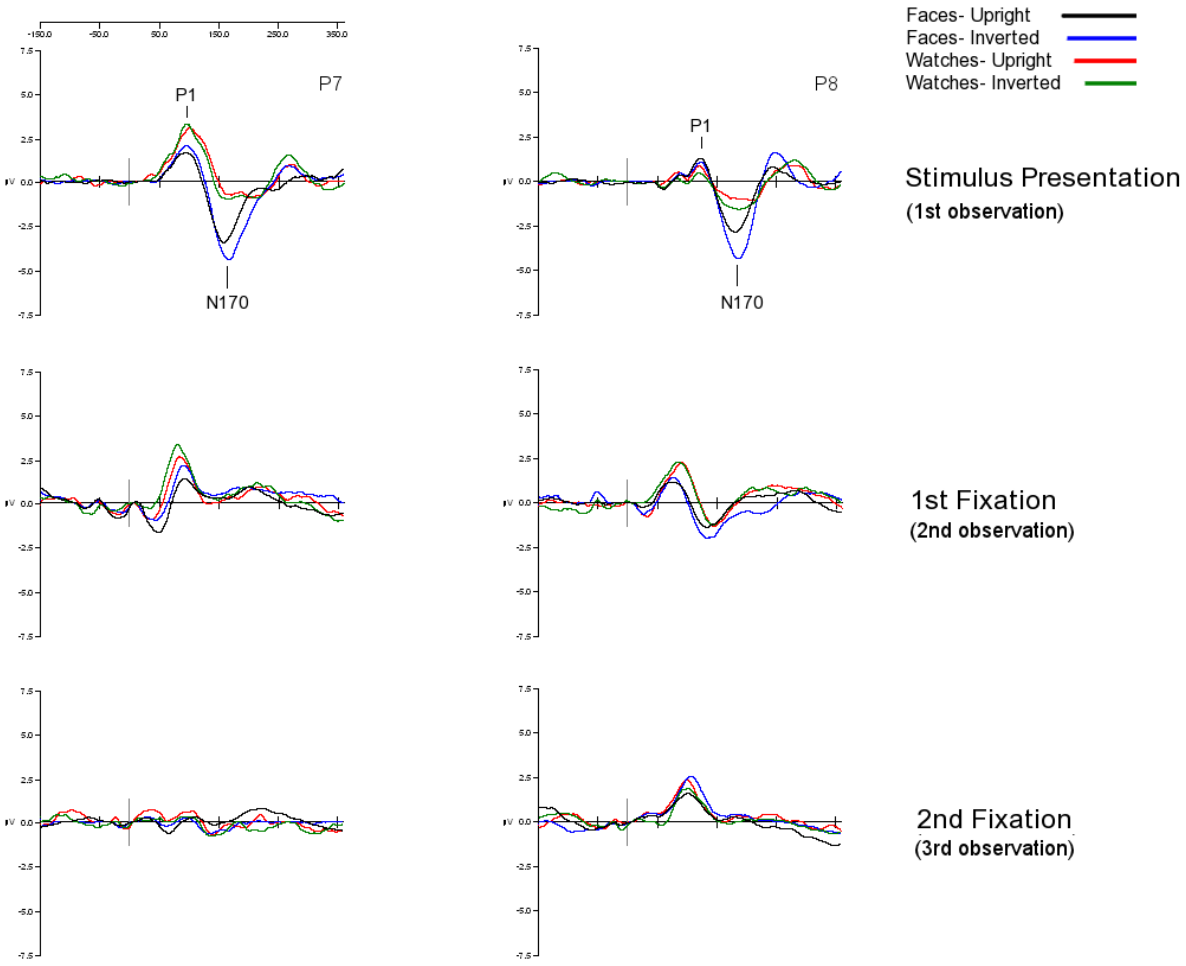

Fig. 4. *N170 amplitudes in response to: stimulus presentation, 1st fixation on the images and 2nd fixation on the images. Measured at left P7 and right P8 electrodes.*

There were also three-way interactions between stimulus type, stimulus observation, and electrode [$F(2, 30) = 4.66$, $p = .017$, $\eta^2 = .24$], and between stimulus type, stimulus observation, and orientation [$F(2, 14) = 7.93$, $p = .005$, $\eta^2 = .53$]. Further analyses of these interactions were conducted through ANOVAs performed at each level of stimulus observation separately. For N170 to the first observation (stimulus presentation), there was a

significant main effect for stimulus type [$F(1, 15) = 34.61$, $p < .001$, $\eta^2 = .69$] because faces elicited larger N170 peaks than watches (Figure 5). There was also a significant main effect of stimulus orientation because inverted stimuli elicited a larger N170 than upright stimuli [$F(1, 15) = 37.14$, $p < .001$, $\eta^2 = .71$]; and a significant main effect of electrode site because the N170 for the first stimulus fixation was larger at the right P8 electrode than the left P7 electrode [$F(1, 15) = 7.92$, $p = .013$, $\eta^2 = .35$].

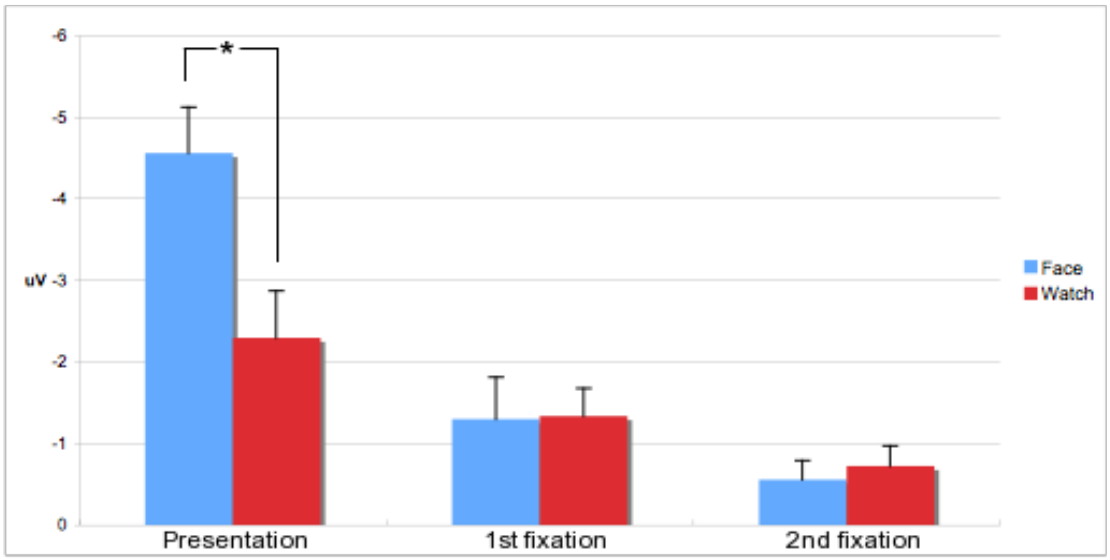

Fig. 5. *N170 amplitudes elicited by upright faces and watches by initial image presentation, and by subsequent 1st and 2nd fixations to the images*

Finally, there was a stimulus orientation by stimulus type interaction [$F(1, 15) = 13.19$, $p = .002$, $\eta^2 = .47$] because there was a significant inversion effect for faces at P7 and P8 but not watches for the N170 to the initial stimulus presentation (Figure 6).

In contrast, for the second and third stimulus observations (i.e., first and second self-guided fixations on the stimuli), there was no effect of stimulus or orientation because the N170 was not larger to faces than to watches, and there was no face inversion effect. However, for the first fixation on the stimuli, the N170 was larger in the right P8 electrode than the left [$F(1, 15) = 7.61$, $p = .015$, $\eta^2 = .34$]. This pattern reversed for the second fixation, where a larger peak was observed in the P7 than P8 electrode [$F(1, 15) = 6.50$, $p = .022$, $\eta^2 = .30$].

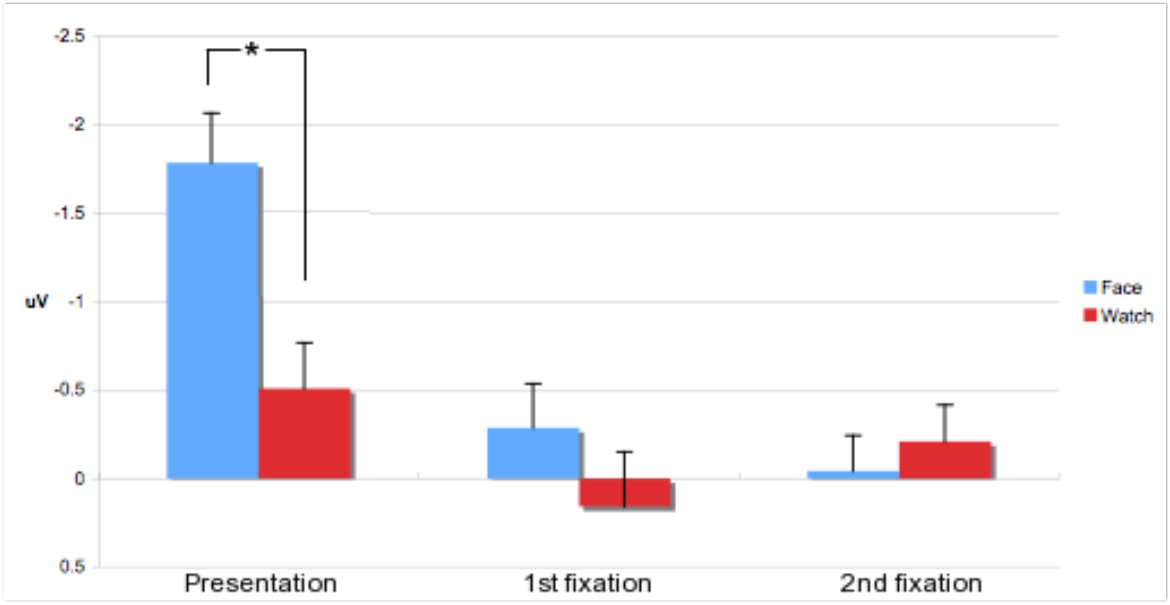

Fig. 6. *Stimulus inversion effect (inverted stimuli N170 amplitudes minus upright stimuli amplitudes) at initial image presentation, and by subsequent 1st and 2nd fixations to the images*

3.4. Discussion

From previous ERP studies, we made three predictions about the outcomes of this study. In line with ERP research suggesting that the N170 reflects a face detection process, our first predicted that under more naturalistic viewing conditions an N170 would be elicited by an initial observation of a face (i.e., at stimulus presentation) but not to subsequent observations of that face (i.e., first and second self-guided stimulus fixations on the face). This prediction was confirmed by the results. The onset of a face in the periphery of a viewer's visual field evoked a distinct N170 in the occipito-temporal regions. When the viewer then fixated on the face directly, the N170 amplitude was significantly reduced - almost to the point of absence in the left occipito-temporal region. When the viewer fixated on the face a second time, no distinct N170 peak was apparent at all. These results suggest that the N170 in more natural face-viewing situations is only observed at the initial presentation of faces, supporting the view that this peak reflects the activation of face-detection and encoding processes (Bentin et al., 1996; George et al., 2005).

Our second prediction was that the N170 would be larger to faces than non-faces. This prediction was also supported by the data. In line with previous ERP studies, we found that the N170 to the peripheral presentation faces was larger than to non-face stimuli – in this case watches (Rousselet et al., 2005). This suggests that the N170 measured in this study illustrated a degree of face-sensitivity similar to that found in previous ERP paradigms investigating face processing (Bentin et al., 1996, Rossion et al., 2000). Further, the fact that the N170 was strongly attenuated or absent in first and second fixations to both faces and watches suggests that the characteristic face-sensitivity of the N170 to the initial presentation of a face no longer exists in subsequent explorations of that face.

Our third and final prediction was that inverting faces would affect the N170 to a greater degree than inverting non-faces. This too was supported by the results. Inverted faces elicited larger N170 peaks than upright faces. In contrast, there was no significant effect of inversion for the watch stimuli. This further indicates that the N170 elicited by the face stimuli in the current experiment reflects similar face-sensitive processes to that observed in previous ERP experiments (Itier & Taylor, 2004; Itier, Latinus & Taylor, 2006; Rossion et al., 2000).

It was interesting to discover that the effects of face inversion were only observed at the stage of the initial stimulus presentation (i.e., in the stimulus presentation waveform) and not in the waveforms for the fixations on the stimuli. This suggests that the disruption to holistic processing during the initial perception of the faces does not impair the face processing in follow-up fixations. These results are consistent with proposals for two levels of holistic processing: one involved in the rapid detection of face stimuli as faces (as measured here), and a later stage of holistic processing where more detail is extracted to identify faces (which might be indexed by later ERPs) (Busigny, et al., 2010; Busigny & Rossion, 2011).

The attenuation of the N170 peak after initial exposure observed in the current study appears similar to that seen in neural adaptation studies, in which neural populations responsible for processing specific categories of stimuli, including faces, respond to a lesser degree when preceded by similar or the same images (Amihai et al., 2011; Campanella et al., 2002). This attenuation has been found to be greater when the stimulus onset asynchrony (SOA) is reduced (Fu et al., 2012). It is important to note that in adaptation studies the similarity and SOA between subsequent images is under the control of the experimenter, whereas the current study employed a naturalistic design, where participants' were effectively allowed to control the SOA, leading to an average of 220ms asynchrony between image observations. One interpretation of the results of the current study could be that the greatly attenuated N170 amplitude in the second and third observations of a face is a result of short self-induced SOAs, leading to greater adaptation to the stimuli within trials, to which the N170 is particularly sensitive. The question of whether such an adaptation could be viewed as an artefact of the present methodology or an intrinsic feature of self-guided fixations invites future investigation.

3.5. Conclusions

In this study – apparently the first to use FRPs to investigate face processing – we discovered that under more naturalistic viewing situations, the N170 to faces is elicited by the initial presentation of a face, but not in subsequent fixations of a face. This simple, yet clear, finding provides direct support for the idea that the N170 reflects a face detection process, and that this process is present in more naturalistic face viewing situations than those typically allowed by ERP paradigms.

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Paper 3

Orthographic learning in the brain: New insights from fixation related potentials

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Orthographic learning in the brain: New insights from fixation related potentials

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Abstract

This paper presents the results of an investigation into the neural activity elicited by unfamiliar words that are repeatedly read in a naturalistic context. We used fixation-related potentials (FRPs) to measure brain responses to familiar and unfamiliar words that were presented four times in paragraphs of text. The FRPs indicated that when read for the first time, familiar words produced a stronger positive peak than unfamiliar words over the frontal-right scalp region at approximately 150 ms (P150), whereas unfamiliar words elicited a larger positive peak than familiar words over the right parietal region at approximately 300 ms (P300). The early P150 effect was only observed for the first encounter of the words in the text. In contrast, the P300 effect was observed at the first and second encounters. These patterns suggest an interplay between perceptual and attentional demands relating to the initial stages of integrating new orthographic forms into the lexicon.

Keywords

Fixation-related potentials; event-related potentials; eye-movements; orthographic learning

Orthographic learning in the brain: New insights from fixation related potentials

4.1. Introduction

When a fluent reader first encounters an unfamiliar word in a book - such as "Dumbledore" or "Expelliarmus" - they are confronted by an unfamiliar string of letters that has no matching representation in their orthographic lexicon of written words. Consequently, this unfamiliar string cannot be read using the same processes as familiar words, such as "Harry" or "wizard", that have existing representations in the orthographic lexicon. According to the Self-Teaching Hypothesis (Share, 1995, 1999), to read an unfamiliar word, a reader has to use a "phonological recoding" strategy that transposes each grapheme (letter or letter cluster) into its corresponding phoneme (speech sound) and then merges those phonemes into a single phonological (spoken) representation. The process of phonologically recoding a word sets up a representation of that word in the orthographic lexicon. Each time the reader encounters the same word, its orthographic representation strengthens and the need for phonological recoding diminishes.

At the level of behaviour, research suggests that typically developing readers can establish an orthographic representation of a new word in a single exposure, and that the strength of this representation can increase in strength for up to four exposures (Bowey & Muller, 2005; Nation, Angell, & Castles, 2007). Further, orthographic learning occurs during silent reading as well as reading aloud (Bowey & Muller, 2005; De Jong & Share, 2007; De Jong et al., 2009).

There is less certainty regarding orthographic learning at the level of the brain. This has typically been studied using event-related potentials (ERP) to compare brain responses to orthographic stimuli that vary in familiarity. Studies have reported an occipito-temporal negative peak at 150-200 ms (N170) that is larger to (1) low

frequency words than high frequency words (Hauk & Pulvermueller, 2004; Hauk et al., 2006; Sereno, Rayner, & Posner, 1998; Sereno, Brewer, & O'Donnell, 2003); (2) familiar words than pseudowords and symbols (Maurer et al., 2005; though the reverse pattern has also been observed, Hauk et al., 2006); and (3) orthographic stimuli (letter strings) than other objects (Bentin, Mouchetant-Rostaing, Giard, Echallier, & Pernier, 1999). These findings are further complicated by studies reporting no difference between the N170 to pseudowords and real words (Bentin et al., 1999; Simon, Petit, Bernard, & Rebai,, 2007).

At around the same time as the N170, there is a positive ERP peak in the frontal-central region – the P150 – that has also been found to be larger to high-frequency words than low-frequency words (Proverbio et al., 2004), and larger to parafoveal words than nonwords (Baccino & Manunta, 2005), but not to differ between low-frequency words and pseudowords (Proverbio, Vecchi, & Zani, 2004).

Around 150 ms later (i.e., 300 ms), there is another positive ERP peak at parietal and occipito-temporal regions that has been found to be larger to pseudowords than words (Taroyan & Nicolson, 2009, denoted as the P4 occurring between 300 and 500 ms). There is also a negative ERP peak (the N300) in the same region that is larger to pseudowords than familiar words (Hauk et al., 2006). Finally, at around 450 ms, there is a negative peak at the frontal-central region – the N450 – that is larger to pseudowords than known words when a subject's task involves semantic processing, but not when their task is limited to phonological, lexical, phonetic or orthographic processing (Bentin et al., 1999).

In sum, studies that have compared ERPs in response to orthographic stimuli that vary in familiarity suggest that orthographic learning in the brain may relate to processes that occur at around 100 to 200 ms (the occipital-temporal N170 and

frontal-central P150) and around 300 to 450 ms (the parietal P300, occipital-temporal P300, and frontal-central N450). However, the validity of the suggestion of orthographic sensitivity in these brain potentials is limited by inconsistent outcomes between studies. Maurer, Brandeis, and McCandliss, (2005) have suggested that these mixed outcomes might be explained by differences in task demands (such as the likelihood of automatic phonological decomposition taking place), which is also suggested by Bentin et al.'s (1999) finding that the N450 is affected by orthographic familiarity under some conditions (i.e., during semantic processing) but not others (i.e., phonological, lexical, phonetic or orthographic processing). Why have ERP studies of orthographic familiarity used different reading tasks when reading in the "real world" is a relatively uniform procedure (i.e., reading sentences or passages of text)? This may stem in part from an inherent methodological limitation of the ERP technique. When measuring ERPs to a stimulus, it is important to know exactly when the brain starts processing that stimulus. This is difficult to know for words that are presented within a sentence or a paragraph. Thus, most ERP studies of orthographic familiarity have solved this problem by measuring brain responses to words that are presented one at a time.

An alternative solution to this problem is to co-register eye-movement data with ERP responses to pinpoint when the eyes fixate on a particular word within a paragraph, and measure the brain's response from that point in time. This relatively new technique –called fixation related potentials (FRPs) - has been used to investigate various facets of reading such as parafoveal preview (Baccino & Manunta, 2005; Dimigen, Kliegl, & Sommer, 2012; Simola et al., 2009), the old-new effect (Hutzler et al., 2007) and the effect of semantic violations in natural reading (Dimigen, Sommer, Hohlfeld, Jacobs, & Kliegl, 2011; Kretzschmar, Bornkessel-Schlesewsky, &

Schlesewsky, 2009). The aim of the current study was to use the FRP paradigm to measure brain responses to repeated unfamiliar and familiar names within naturalistic paragraphs of text to determine: (1) when (in time) and where (on the scalp) FRPs differ between unfamiliar and familiar words (Aim 1); and (2) if differences between FRPs to unfamiliar and familiar words disappear as unfamiliar words became more familiar (Aim 2).

4.2. Methods

The Human Ethics Committee at Macquarie University approved the methods and procedures used in this study.

4.2.1 Participants

Sixteen native English speakers took part in the study, however one participant's data was excluded from analysis due to heavy contamination from alpha-wave activity. Fifteen participants were included in data analysis (9 females, 13 right-handed), aged between 22 and 40 years (M =25.6 years, SD = 4.5). Previous findings have suggested that males and females do not differ significantly in language processing laterality (Frost et al., 1999). It is therefore unlikely that the over-representation of females in the current study would yield condition-specific patterns of activation.

All participants had normal or corrected-to-normal vision and gave informed consent before participating in the study. Participants were reimbursed $30 for their time.

4.2.2 Stimuli

The stimuli in this study consisted of high-frequency familiar proper nouns (e.g., Pamela) and unfamiliar proper nouns (e.g., Padela) embedded in meaningful paragraphs of text (see Figure 1 for an example, appendix for full list). Proper nouns were used instead of common nouns because unfamiliar proper nouns are a common

type of unfamiliar word encountered by mature readers in text, and because proper nouns minimise semantic differences between unfamiliar and familiar word stimuli. For example, there is a smaller semantic difference between two unknown characters called “Pamela” and “Padela” than between “jam” (a sweet, fruit-based conserve) and “jad” (which has no meaning at all).

The familiar names (N = 144; 50% female) were 3 to 7 characters long, and were selected from databases of high-frequency names compiled from the 1990 US Census. The 144 unfamiliar names were constructed by substituting one letter in each familiar name (for example “Pamela” was used to create “Padela”, and “Frank” became “Frask”). This ensured familiar and unfamiliar names were matched for length and number of syllables. The 144 familiar names and 144 unfamiliar names were each divided into two subsets in such a way that no two related stimuli were presented in the same subset. For example, a participant who was presented with Pamela would be presented with Frask, while another participant would be presented with Frank and Padela. Thus, each participant was exposed to 72 familiar names and 72 unfamiliar names that were unrelated to each other.

The 72 familiar and 72 unfamiliar names were embedded in 72 paragraphs, which comprised 80-120 words that formed coherent narratives. Each narrative involved two characters (one with a familiar name and one with an unfamiliar name), with each character’s name occurring four times in each paragraph. The order in which the characters were introduced in the paragraphs was counter-balanced within participants, where half of the paragraphs presented characters with familiar names first, while the other half involved the presentation of characters with unfamiliar names first. In addition, the introduction of familiar and unfamiliar character names was counterbalanced between participants by the use of two-subsets of paragraphs

were created, where the order of familiar and unfamiliar names in the text was swapped so that each name (familiar or unfamiliar) was the first-named character in one subset, and the second-name character in the other subset. Accordingly, each participant read the 72 paragraphs once. This addressed the potential confound of order-related semantic effects on the processing of each name, where there is more information relating to the subjects as the sentence progresses. For example, when a name is the first character in a paragraph ("When Padela stormed out of the room Frank was left confused") it may be processed differently to when it is the second character in the paragraph ("When Frask stormed out of the room, Pamela was left confused"). Due to an uneven number of participants in the two paragraph conditions (7 + 8 for a total of 15), counterbalancing between participants was imperfect. However, this was likely mitigated by the within participant counterbalancing.

4.2.3 Stimulus presentation

The paragraphs were presented on a 19" CRT computer monitor with a refresh rate of 100 Hz at a distance of 50 cm. The white text was presented against a black background, with letters of 1.5 degrees of visual angle in height. The familiar and unfamiliar words were matched for length, luminosity, contrast, colour, and font.

Stimulus presentation was controlled by Experiment Builder software (version 1.6.1). This used a gaze-contingent white fixation cross in the upper-left part of the computer monitor to initiate the presentation of a paragraph in the middle of the screen. A gaze-contingent fixation cross at the bottom-right of the screen allowed participants to end the trial after they had finished reading a paragraph. Each trial was followed by a "blink now" screen that was displayed for 1500 ms before the next gaze-contingent pre-trial fixation cross was presented in the top-left part of the screen.

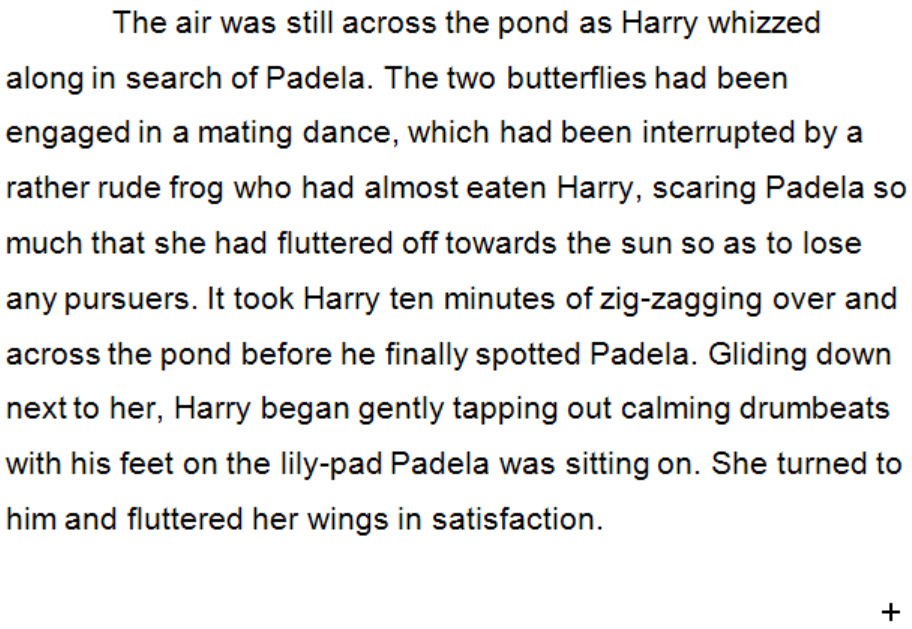

Fig. 1. *Example familiar and unfamiliar word stimuli embedded in paragraph as character names, each repeated four times.*

4.2.4 Task procedure

Participants were told that they would silently read a series of narratives at their own pace, and that the narratives included characters with names that may or may not be familiar to them. The aim of the latter instruction was to foster the impression that the unfamiliar names were rare existing names rather than fabricated names.

Participants were also told that they would be asked questions about the characters at regular intervals during the testing session. This facilitated participants' attention to the content in the paragraphs, and supplied a behavioural index of the familiarity of the orthographic stimuli. The tests, which were administered 7.5 minutes after the names were encountered (on average), and which comprised half of the unfamiliar names, presented participants with four choices: a previously exposed

unfamiliar name (e.g., Padela), two orthographic distractors (e.g., Paleda), and a phonological distractor (e.g., Pidela). The subject selected the appropriate name using a keypad. Participants accurately recognised unfamiliar names 74.1% of the time, and binomial tests for each participant showed that all participants performed above chance (25%) with all *p*-values below 0.003. The pattern of errors aligned with that of chance.

4.2.5 On-line eye-tracking and electroencephalogram (EEG) recording.

Whilst participants read the experimental paragraphs, their eye-movements were tracked and recorded with a monocular (right eye) Eyelink 1000 eye-tracker, sampling at 1000 Hz. This sent trial-specific serial port codes, which indicated the onset of the trials to an online EEG recording and the eye-tracking computer, and were used as an index for offline eye-movement analysis (see Step 8 in the following section).

At the same time, each participant's EEG was recorded using 30 Ag-AgCl sintered electrodes positioned in an elastic cap (EasyCap) in line with the 10-20 system (Jasper, 1958; Fp1, Fp2, F7, F8, FT7, FT8, T7, T8, TP7, TP8, P7, P8, F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4, CP3, CPz, CP4, P3, Pz, P4, O1, Oz, O2). We recorded this activity with a Synamps II amplifier, using a sampling rate of 1000 Hz, an online band-pass filter of 1 to 100 Hz, and a notch filter at 50 Hz. The EEG activity recorded at the left and right mastoids was used as online and offline references, respectively. The ground electrode was located between the Fz and FPz electrodes. Electrode impedances were kept below $5k\Omega$. Ocular movement was recorded with bipolar electrodes placed at the outer canthi, and above and below the left eye. The experimental session was approximately 1 hour for each participant.

4.2.6 Offline FRP processing

The EEG data was analysed offline with Neuroscan 4.3 software in eight steps:

1. Eye-blink VEOG artefacts from each EEG channel were removed using a standard ocular reduction algorithm (Semlitsch, Anderer, Schuster, & Presslich, 1986).

2. Each EEG channel was referenced to mathematically linked mastoid electrodes, excluding ocular sites.

3. Participants' trial-by-trial eye-movement data was used to insert triggers (i.e., port codes) into their EEG indicating the times that the target words were first fixated upon at the 1st, 2nd, 3rd, and 4th appearance of each familiar name and unfamiliar name within the paragraphs. Using these triggers, each participant's EEG was cut into segments (i.e., epochs) that started 150 ms before each fixation-related port code and ended 400 ms after the same port code.

4. We baseline-corrected each epoch. This process was complicated by the fact that FRPs to a stimulus are confounded by ocular noise related to the saccade that immediately precedes stimulus fixation (i.e., time zero). Thus, baseline-correction could not be based on the average pre-stimulus activity, which is typically used for ERPs. Instead, in line with previous FRP studies, each epoch was baseline corrected to the average activity immediately following fixation onset (0-20 ms; Dimigen et al., 2011; Hutzler et al., 2007).

5. Each epoch was filtered with a band-pass of 0.1 – 30 Hz with 12 dB/octave roll-off.

6. Trials containing EEG artefacts exceeding +/-80 μV were excluded from analysis.

7. FRP epochs were averaged according to stimulus type (familiar name and unfamiliar name) and by the number of times that they had been encountered in the paragraph (1st, 2nd, 3rd, 4th).

8. We removed eye-movement artefacts from the FRPs caused by ocular noise. This artefact was introduced by saccadic activity that (1) preceded fixations to a word of interest and, (2) followed fixations to the same word of interest (fixation-offset). This saccadic activity was corrected through the application of independent component analysis (ICA) that incorporated the EOG channels, as carried out by previous FRP studies (Hutzler et al., 2007; Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997; Vigario, 1997). This procedure was conducted on individual participant's FRPs for 1st, 2nd, 3rd, and 4th exposures to familiar names and unfamiliar names, to measure and account for any condition-specific ocular artefacts. An example of oculomotor activity removed through ICA can be found in Figure 2.

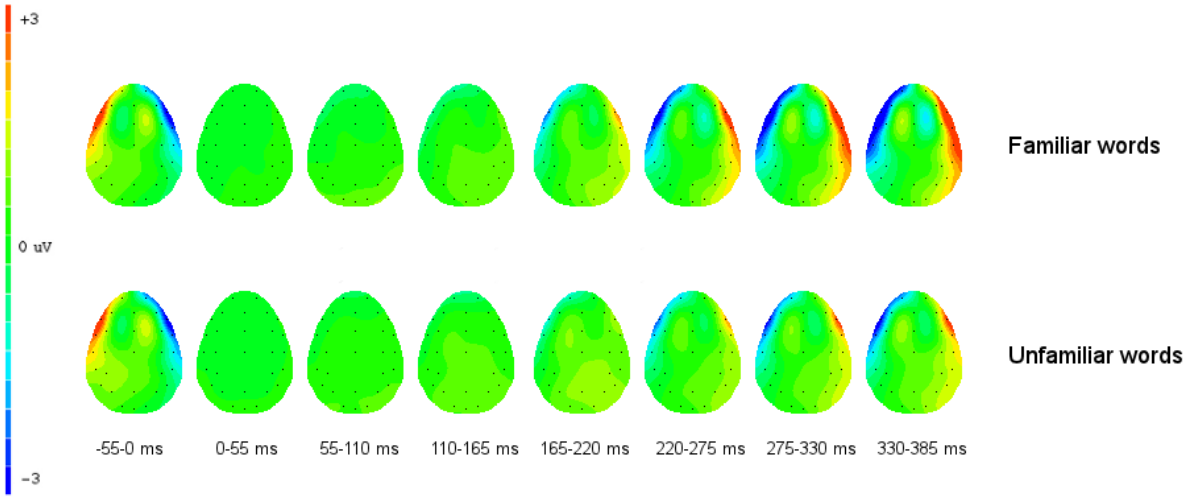

Fig. 2. *Example of oculomotor activity removed through independent component analysis for familiar and unfamiliar words at first encounter.*

4.2.7 Eye-movement factors

Since FRPs are derived from the eye-movements made to stimuli, some dimensions of eye-movements can interfere with the interpretation of the FRP waveforms. It is therefore necessary to either (1) correct for these factors, or (2) analyse these factors to try to determine the extent of their potential influence on FRPs.

One eye-movement factor to consider is eye-fixation duration variability. The average duration of eye fixation (defined as a static eye-position for at least 50 ms) to a stimulus was 203 ms. Any neural activity in an FRP waveform after this time may include neural activity related to fixation to the next stimulus due to the short stimulus onset asynchrony that characterises sequential fixations and thus producing overlapping brain potentials (Baccino, 2011). If fixation durations to a stimulus are highly variable, the neural activity relating to fixation to the next stimulus will be jittered, and hence poorly defined in the FRP waveform to the current stimulus. However, if fixation durations are uniform, the neural activity related to fixation to the next stimulus will be more temporally uniform, and hence may be well-defined in the FRP waveform. This is also true for variability in the duration of fixations made to words preceding the target stimuli (word n-1). In this study, we measured variation of eye-fixation duration by computing the standard deviation of the duration of fixations made to the familiar and unfamiliar names at each level of stimulus encounter, as well as for pre-target fixations (word n-1). We analysed these measures to determine if the effect of stimulus type (familiar names and unfamiliar names) and encounter (1^{st}, 2^{nd}, 3^{rd}, and 4^{th}) was the same or different as the FRP measures (see Eye-fixation duration variability analysis in section 4.3.3.1 of Results below). Similar patterns in the fixation-duration variability and FRP effects might suggest a link between the two, and a possible confound. However, condition-specific patterns of eye-movements that

would explain the observed brain potentials in the FRP measures were not found, suggesting that eye-fixation duration variability did not account for the pattern of FRP results in this study.

Another eye-movement factor to consider is amplitude of saccades. The size of the preceding saccade leading to the fixation on a stimulus has been found to modulate the size of the early occipital ERPs (e.g., the P1), with larger saccades leading to larger occipital activity (Dimigen et al., 2011). This is thought to reflect lambda activity related to the offset of saccades and a "priming" of the visual cortex (Yagi, 1979; Kazai & Yagi, 2003). If incoming saccade lengths are modulated by word-type (e.g., if saccades are larger to unfamiliar than familiar names; or if saccades are larger to 1st encounter than 4th encounter) then this may generate a larger P1 occipital peak in certain conditions. Thus, we measured the length of the incoming saccades leading into fixations on the stimuli, reported in degrees of visual angle. Saccades were defined as eye-movements exceeding 30^0/sec velocity and 8000^0/sec^2 acceleration (Stampe, 1993). We analysed these measures to determine if the effect of stimulus type (familiar names and unfamiliar names) and encounter (1st, 2nd, 3rd, and 4th) was the same or different as the FRP measures (see Saccade amplitude analysis in section 4.3.3.2 of Results below). A difference would suggest that saccade amplitude did not account for the pattern of FRP results in this study.

A third eye-movement factor to consider is eye-fixation duration. Based on previous research, we expected to find longer fixations to unfamiliar than familiar words (in line with word frequency and word/pseudoword effects, Hutzler & Wimmer, 2004; Rayner & Raney, 1996); and a decrease in fixation duration with each encounter of an unfamiliar word (Hautala, Hyönä, Aro, & Lyytinen, 2011). Since this pattern of results was also expected for relevant FRPs, we corrected for eye-fixation

duration using an ICA procedure developed by previous FRP/ERP studies (Hutzler et al., 2007; Makeig, et al. see Step 8 in the Offline FRP processing section). Although we controlled for this eye-movement factor in FRPs, we analysed the effect of stimulus type (familiar names and unfamiliar names) and encounter (1st, 2nd, 3rd, and 4th) on eye-fixation duration measures for researchers who are interested in eye-movement data (see eye-fixation duration analysis in section 4.3.3.3 of Results below).

4.3. Results

4.3.1 Aim 1: When (in time) and where (on the scalp) do FRPs differ between unfamiliar and familiar names?

We used sample-by-sample two-tailed t-tests (Guthrie & Buchwald, 1991) on voltage values at the 30 scalp electrodes (outlined in section 4.2.5) to pinpoint when and where FRPs differed between familiar and unfamiliar words (stimulus type) at 1st encounter. Figure 3 shows a topographical representation of the temporal and spatial differences between familiar and unfamiliar names at first encounter. T-tests revealed the positive peak to familiar names was significantly larger than to unfamiliar names at the fronto-central FC4 and FCZ sites ([$t(14) > -2.15$, $p < 0.05$] for 6 consecutive time points starting at 158 ms ("P150"). There was also a significantly larger positive peak to unfamiliar names than familiar names (for which the peak was missing entirely) at 295 ms ("P300") at parietal P3, Pz and P4 electrode sites ([$t(14) > -2.15$, $p < 0.05$] for 12 consecutive points). We found no evidence of a stimulus type effect on the parieto-temporal N170 or N450. Thus, when first encountered within a naturalistic context, unfamiliar names elicited a different pattern of neural activity to familiar names in the central-frontal P150 FRP and the parietal P300 FRP.

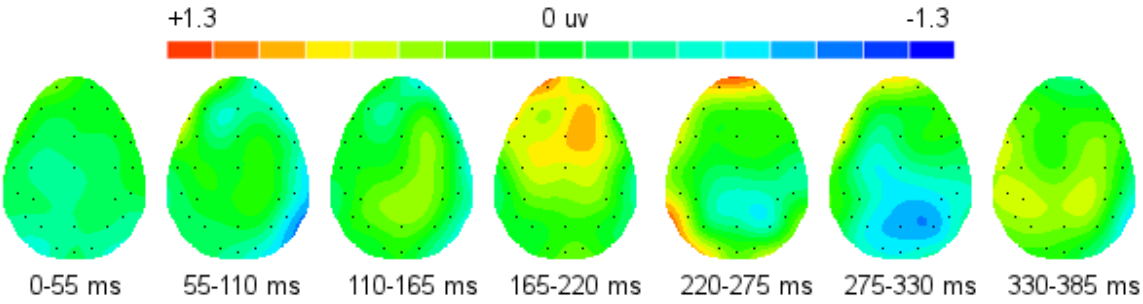

Fig. 3. *Topographical plot of unfamiliar word waveforms subtracted from familiar word waveforms, indicating temporal and spatial points of difference between the conditions (words and pseudowords) at the first encounter with the stimuli in the paragraphs.*

4.3.2 Aim 2: Do differences between FRPs at first exposure disappear as unfamiliar names become more familiar?

For the 1st, 2nd, 3rd, and 4th encounters of unfamiliar names and familiar names, we took amplitude measures of the (1) P150 peak 140-200 ms post-fixation onset at FCz and FC4 electrode sites; and (2) P300 peaks 250-350 ms post-fixation onset at P3, Pz and P4 sites, based on the sample-by-sample two-tailed t-tests performed on the waveforms as outlined in section 4.3.1. We also took amplitude measures of a very clear peak at occipital sites (O1 and O2) at 100 ms ("P1"), which changed markedly between encounters but did not differ between unfamiliar names and familiar names at 1st encounter (and hence was not relevant to Aim 1). We applied a $\log_{10}$ transform to the P150, P300, and P1 amplitude values at each relevant site to correct for non-normal distribution of data. These amplitude values were analysed separately using omnibus ANOVAs comparing the effects of stimulus type (familiar name, unfamiliar names), stimulus encounter (1st, 2nd, 3rd, 4th encounter in the paragraphs), and electrode site (FCz and FC4 for P150; P3, Pz, and P4 for P300; 01 and 02 for P1). Statistically significant main effects were further examined by pair-wise *t*-tests between levels and, for a significant main effect of encounter, within-

subjects contrasts (i.e., to determine the trajectory of the stimulus encounter effect). Significant interactions were explored by ANOVAs or *t*-tests, depending on the complexity of the interaction. Greenhouse-Geisser corrected values were used when sphericity was violated. The threshold for statistical significance was taken at $p <= .05$.

4.3.2.1 The frontal-central P150. The stimulus type (familiar name, unfamiliar name) by stimulus encounter (1^{st}, 2^{nd}, 3^{rd}, 4^{th}) by electrode site (FCz, FC4) ANOVA revealed no significant main effect of stimulus type ($p = .56$) or stimulus encounter ($p < .89$), and no significant interaction. There was a significant main effect of electrode site because P150 was significantly larger at FCz than FC4 [$F(1, 14) = 29.11$, $p < .01$].

The presence of a significant main effect of stimulus type on the frontal-central P150 at 1^{st} encounter (Aim 1), paired with the absence of such an effect in the omnibus ANOVA (Aim 2), suggested that the stimulus effect at 1^{st} encounter must have rapidly disappeared in subsequent encounters. We examined this suggestion using paired *t*-tests to compare the P150 to familiar and unfamiliar names at FCz and FC4 for each stimulus encounter (1^{st}, 2^{nd}, 3^{rd}, 4^{th}). The outcomes showed a significantly larger P150 peak to familiar names than unfamiliar names [$t(14) = -2.15$, $p = 0.04$] at FC4 (see Figure 4 & 6) at 1^{st}, (see Figure 4) but not subsequent, encounters [$t(14) = .47$, $p = 0.64$; 1.54, $p = 0.14$; 1.21, $p = 0.24$, 2^{nd}, 3^{rd} & 4^{th} respectively]. Thus, effect of stimulus type on the P150 at 1^{st} encounter disappeared by the 2^{nd} encounter. Although a visual inspection of the waveforms at the 4^{th} encounter with the stimuli suggested a reversal of the P150 pattern observed in the 1^{st} encounter, where unfamiliar names appeared to elicit a larger positive peak than familiar words, this pattern did not approach significance.

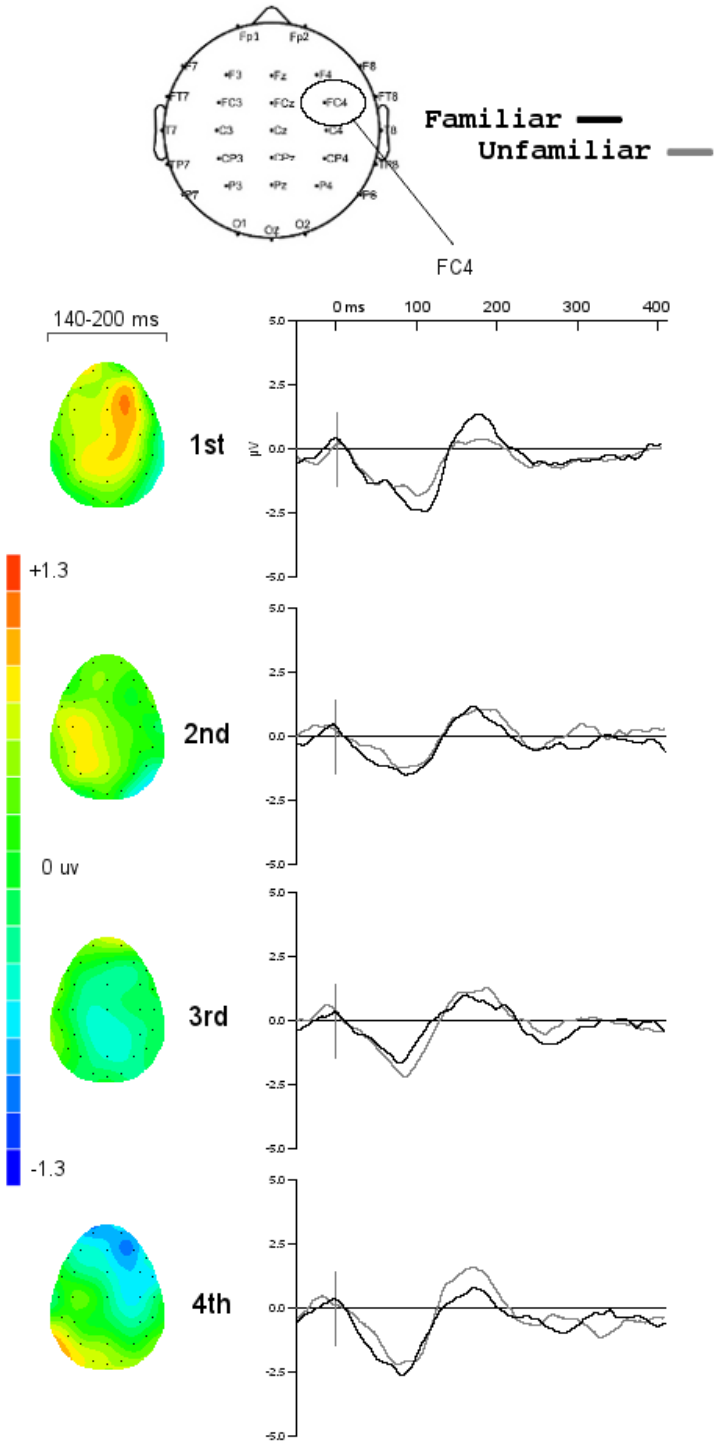

Fig. 4. *Waveforms for early P150 peaks in frontal areas at each encounter with the familiar and unfamiliar words in the paragraphs. Point-by-point t-tests suggested a significant difference at right FC4 electrode at the first encounter, which did not survive correction for multiple comparisons.*

4.3.2.2 The parietal P300. The stimulus type (familiar name, unfamiliar names) by stimulus encounter (1^{st}, 2^{nd}, 3^{rd}, 4^{th}) by site (P3, PZ, P4) omnibus ANOVA

revealed a significant main effect of electrode [$F(2, 28) = 21.19$, $p < .01$] because the P300 at the left P4 electrode site was significantly larger than the P300 at Pz [$p < .01$] and P3 [$p < .01$].

There was also an interaction between electrode and stimulus type [$F(2, 28) = 3.55$, $p = .04$] because the P300 was larger at the right P4 parietal electrode to unfamiliar names than familiar names [$F(1, 14) = 4.90$, $p = .04$], suggesting a lateralization of the effect to the right hemisphere. A follow-up ANOVA focusing on the right P4 electrode site further revealed an interaction between stimulus type and encounter [$F(3, 42) = 2.90$, $p = .046$]. This occurred because there was a significant main effect of encounter for unfamiliar names [$F(3, 42) = 3.25$, $p = .03$] but not for familiar names [$F(3, 42) = .67$, $p = .57$]. Similarly, the within-subjects contrasts showed a linear diminution for P300 amplitude to unfamiliar names [$F(1, 14) = 8.3$, $p = .01$] but not familiar names [$F(1, 14) = .02$, $p = .88$]. Paired t-tests showed that the P300 was larger to unfamiliar names than familiar names at the 1st and 2nd encounters [$t(14) = 2.87$, $p = .01$ & $t(14) = 2.23$, $p = .04$, respectively], but not the 3rd and 4th encounters [$t(14) = -1.35$, $p = .2$ & $t(14) = .15$, $p = .88$, respectively] (see Figure 5 & 6).

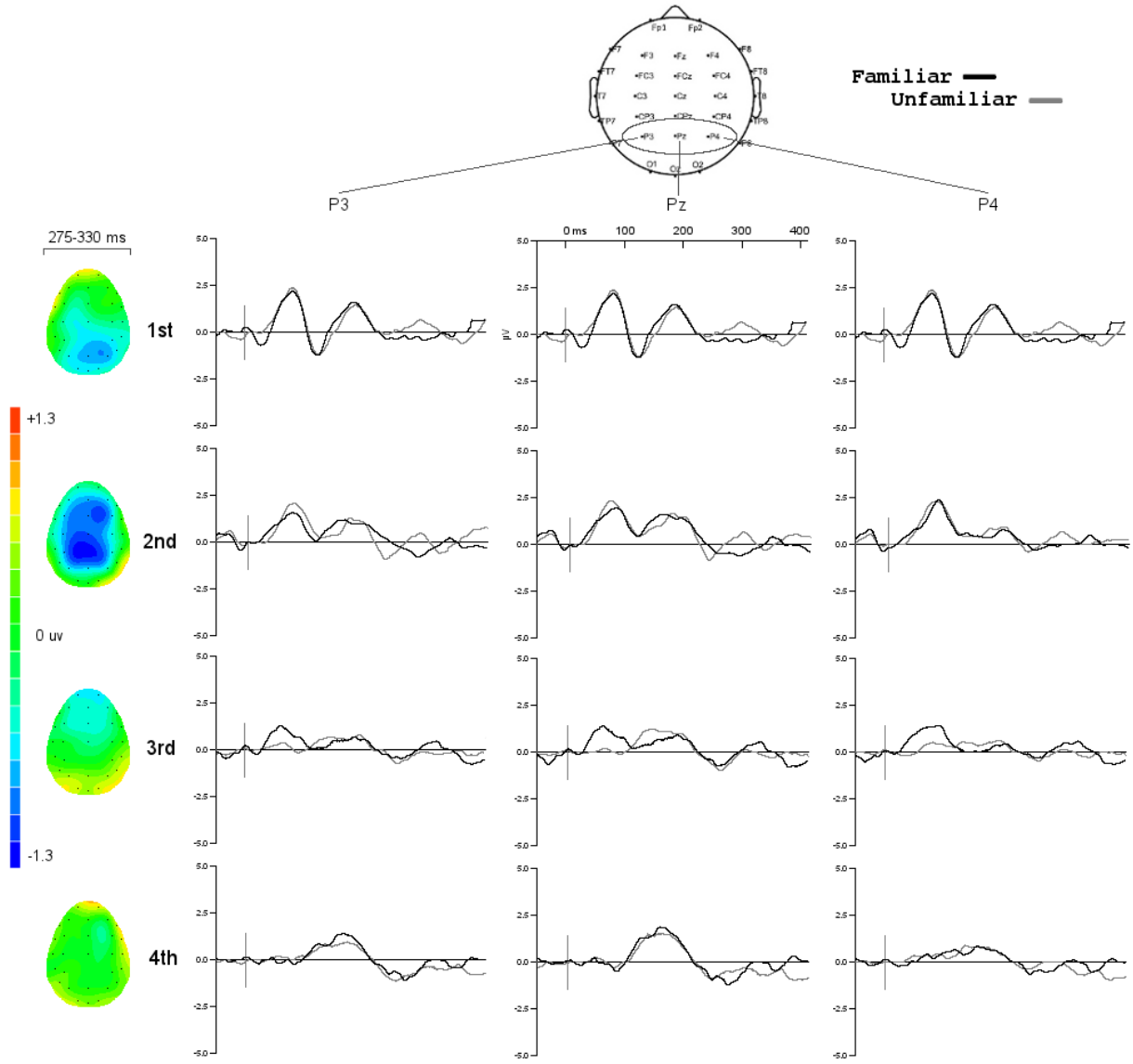

Fig. 5. *Activity present at parietal Pz & P4 electrodes at approximately 300 ms revealing a significant difference between familiar and unfamiliar names at the 1st and 2nd encounters (P300), which was no longer differentiated in the 3rd and 4th encounters.*

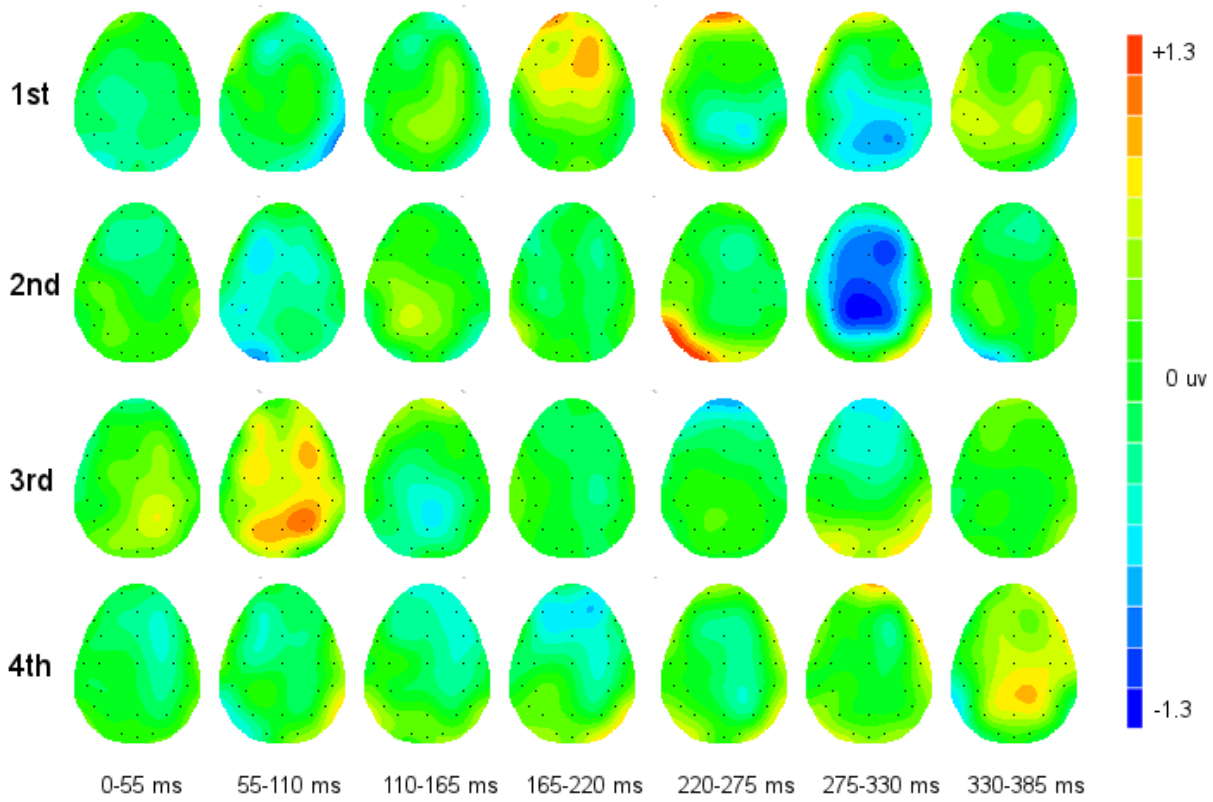

Fig. 6. *Topographical representation of the difference of activity (familiar words subtracted from unfamiliar words) across the scalp elicited at each encounter with the stimuli.*

4.3.2.3 The occipital P1. The stimulus type (familiar name, unfamiliar names) by stimulus encounter (1^{st}, 2^{nd}, 3^{rd}, 4^{th}) by site (O1 and O2) omnibus ANOVA revealed a significant main effect of stimulus encounter [$F(3, 42) = 14.53$, $p < .01$] but not stimulus type [$F(1, 14) = .84$, $p = .84$] or site [$F(1, 14) = .08$, $p = .78$]. A follow-up within-subject contrast for the encounter effect revealed a strong linear trend [$F(1, 14) = 57.81$, $p < .01$, indicating that there was a steady decrease in the size of the P1 between each encounter (see Figure 7).

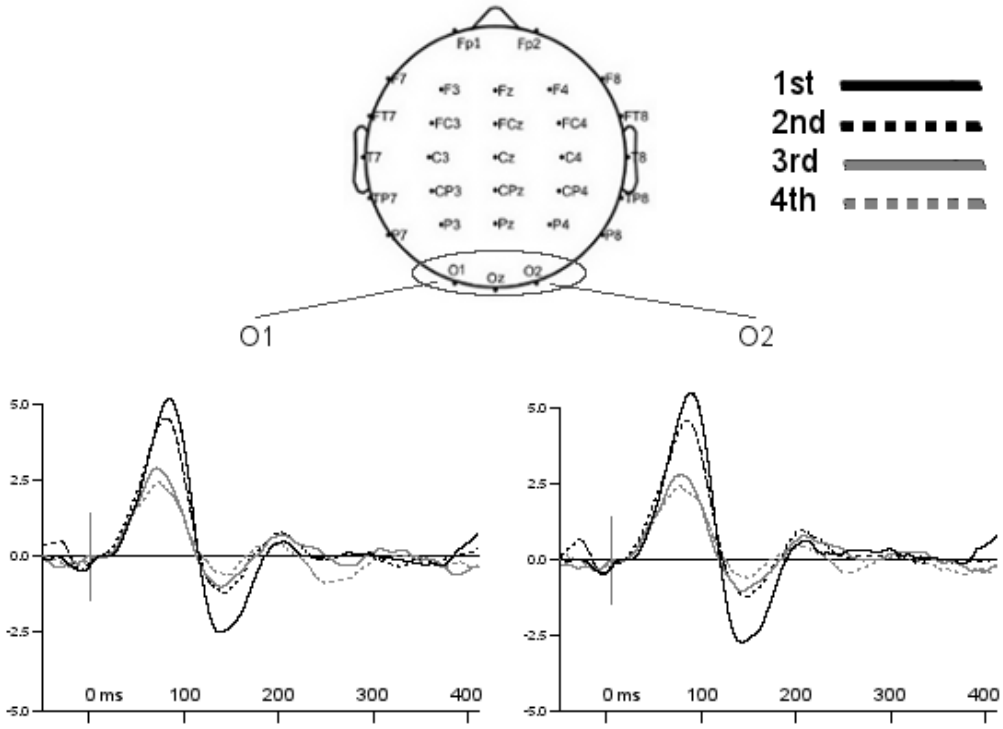

Fig. 7. *The early occipital positive peaks attenuated in strength with each encounter with the stimuli (the peaks were not modulated by stimulus type). Familiar and unfamiliar word waveforms averaged together.*

4.3.3 Eye-movement factors

As outlined in the Methods, there are two eye-movement factors in this study that could potentially confound the FRPs: fixation duration variability and amplitude of saccades. For these factors, we used (1) t-tests to examine the effect of stimulus type at 1st encounter (to compare the eye-movement outcomes with the central P150 FRP results at 1st encounter), and (2) ANOVAs to examine the effect of stimulus type (familiar name, unfamiliar names) and stimulus encounter (1st, 2nd, 3rd, 4th). In addition, we provide the results of the same analysis for eye-fixation duration (i.e., corrected for in the FRPs) for researchers who are interested in eye-movement measures.

4.3.3.1 Eye-fixation duration variability. In contrast to the central P150 FRP results at 1st encounter, a t-test revealed no significant effect of stimulus type (familiar

names, unfamiliar names) at 1st encounter on fixation duration variability. Further, in contrast to the parietal P300 and occipital P1 FRP results, the stimulus type (familiar names, unfamiliar names) by stimulus encounter (1st, 2nd, 3rd, 4th) ANOVA revealed no significant main effects on fixation duration variability. Thus, it seems unlikely that the P150, P300, or P1 FRP effects in this study could be explained by the undue influence of eye-fixation duration variability on FRPs. The variability of the duration of fixations made to words immediately preceding fixations on the target stimuli (word n-1) were not significantly different when the target stimuli were familiar or unfamiliar names ($p = .23$), and were not modulated by stimulus encounter ($p = .60$). This suggests that that it is unlikely that the observed FRP effects were the result of a differential overlap of brain potentials from pre-target fixations.

4.3.3.2 Saccade amplitude. A stimulus type (familiar names, unfamiliar names) by stimulus encounter (1st, 2nd, 3rd, 4th) ANOVA revealed no significant main effect of stimulus type [$F(1, 14) = .04$, $p = .84$) on saccade amplitude. However, there was a significant main effect of stimulus encounter [$F(3, 42) = 10.29$, $p < .01$] because saccades increased with each stimulus encounter. An interaction between stimulus type and stimulus encounter was also observed [$F(3, 42) = 6.16$, $p = .02$, which was further investigated via separate ANOVAs for unfamiliar names and familiar names. These revealed a significant effect of stimulus encounter for both unfamiliar and familiar names [$F(3, 42) = 4.29$, $p = .01$ & $F(3, 42) = 11.47$, $p < .01$, respectively]. Further post-hoc t-tests revealed larger saccades for unfamiliar than familiar names at the 1st encounter [$t(14) = 2.95$, $p = .01$], smaller saccades to unfamiliar than familiar names at 2nd encounter [$t(14) = -3.27$, $p = .01$], and no difference at 3rd or 4th encounter [$t(14) = -1.48$, $p = .17$ & $t(14) = .78$, $p = .45$,

respectively]. This unexpected reversal in effect direction between 1st and 2nd encounter was not observed for any of the FRPs.

4.3.3.3 Eye-fixation duration. As mentioned above, we analysed the eye-fixation duration data for the interest of eye-movement researchers. The stimulus-type-by-encounter ANOVA revealed a significant main effect of stimulus type [$F(1, 14) = 31.47$, $p < .01$] because unfamiliar names were fixated upon longer than familiar names ($p < .01$). There was also a significant main effect of encounter [$F(3, 42) = 9.33$, $p < .01$] because fixation durations decreased with each encounter within the paragraphs in a linear [$F(1, 14) = 19.35$, $p < .01$] and quadratic [$F(1, 14) = 7.38$, $p = .02$] trend.

There was an interaction between stimulus type and encounter [$F(3, 42) = 3.77$, $p < .02$] because there was a significant effect of encounter for both familiar and unfamiliar names [$F(3, 42) = 4.34$, $p = .01$; and $F(3, 42) = 9.53$, $p < .01$, respectively]. Interestingly, the effect of encounter produced a quadratic trend of attenuation of fixation duration for familiar names [$F(1, 14) = 11.75$, $p = .01$], and a linear trend of attenuation for unfamiliar names [$F(1, 15) = 36.12$, $p < .01$]. This interaction between stimulus-type and encounter can be seen in Figure 8. Paired t-tests revealed significantly longer durations for unfamiliar than familiar names at the first [$t(14) = 4.87$, $p = .01$], second [$t(14) = 2.74$, $p = .02$] and third [$t(14) = 2.56$, $p = .03$) encounter, with no difference at the fourth encounter. [$t(14) = .01$, $p = .99$].

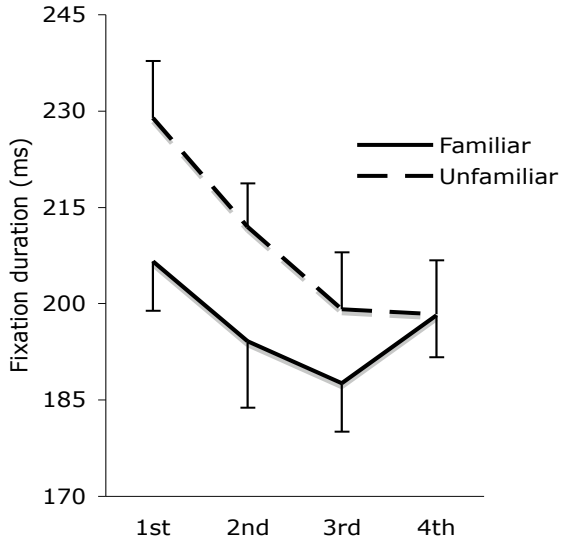

Fig. 8. *Average fixation durations made to familiar and unfamiliar words at the 1st, 2nd, 3rd and 4th encounters within the paragraphs.*

The eye-fixation duration outcomes supported our predictions from previous research that fixations would be longer to unfamiliar than familiar words, and that fixation duration would decrease with each encounter of an unfamiliar word. Since this pattern of results was also expected for relevant FRPs (in this case, the P300), we corrected for eye-fixation duration using an ICA procedure developed by previous FRP/ERP studies (Hutzler et al., 2007; Makeig et al., 1997; Vigario, 1997). If the P300 was simply a reflection of eye-movement activity, it would have been (1) found near the frontal regions associated with oculomotor dipoles, or the occipital lobes associated with the initial processing of a visual stimulus; or (2) 20 ms later for unfamiliar words than familiar words, since this was the difference in fixation duration for the two types of words. Neither was the case. In addition, the duration of fixations made to the words immediately preceding fixations made to the target stimuli (n – 1) was analysed, to determine whether any condition-specific patterns of neural activity were due to a differential overlap of brain potentials from pre-stimulus processing. No significant difference was observed in the duration of fixations made

to words immediately preceding familiar and unfamiliar words (p = .42), nor was this measure modulated by stimulus encounter (p = .13). Thus, the FRP outcomes, which were corrected for eye-fixation duration, do not appear to be just a function of eye-fixation duration in either target or pre-target fixations.

4.4. Discussion

The aims of the current study were to determine (1) when (in time) and where (on the scalp) FRPs differ between unfamiliar and familiar names; and (2) if differences between FRPs to unfamiliar and familiar names disappear as unfamiliar names become more familiar. To this end, we tested 15 adults for their FRPs to unfamiliar and familiar names that were each presented four times in realistic paragraphs of text. Below, we use the FRP outcomes to address each aim in turn. We then discuss an unexpected insight offered by the FRP data, the importance of considering eye-movement data when interpreting FRPs, and potential limitations of the current study.

4.4.1. Aim 1: When (in time) and where (on the scalp) do FRPs differ between unfamiliar and familiar names?

The FRP data in the present study suggest that when words are read in a naturalistic setting (i.e. paragraphs of text), the first encounter of an unfamiliar word elicits different neural activity to a familiar word at around 150 ms in the frontal-central region. This frontal-central P150 has been found to be sensitive to the familiarity of visual object class, suggesting that it may reflect activity of the posterior fusiform gyrus that is sensitive to familiar categories of objects (Schendan, Ganis, & Kutas, 1998). Thus, the larger P150 peak to unfamiliar words than familiar words may reflect sensitivity to word familiarity during self-paced reading.

In addition, the current study found a significantly larger positive FRP to the first encounter of unfamiliar names in the parietal region at around 300 ms. This parietal 300 FRP was absent to the familiar words (see Figure 6). The P300 FRP in this study appears to be similar to the P3b ERP, which is elicited by the presentation of a task-related stimulus that is unexpected or uncommon (Polich, 2007). The P3b is regarded as an indicator of novelty and significance, and an index of attentional resources influenced by the prediction of the occurrence of a stimulus (Donchin, 1981; Polich, 2007). Thus, the P3b is thought to reflect neural activity relating to event-categorisation where external stimuli are compared with internal representations (See Kok, 2001 for review). Such activity is therefore related to attentional factors, and the influence of working memory resources that may subserve this event categorisation process. The results of the current study fall in line with a previous study that found that the P3b was modulated by the degree to which word forms were expected according to context (Osterhout, McKinnon, Bersick, & Corey; Kutas & Hillyard, 1980). Interpreted within the P3b framework, the results of this study suggest that unfamiliar words in paragraphs are processed as unexpected stimuli that conflict with an event categorisation process related to the recognition of word stimuli. For fluent readers, the probability of encountering unfamiliar names in a paragraph of text in English is low relative to the probability of encountering familiar names. Thus, when reading a string of words in a sentence, the likelihood of recognising the majority (if not all) of the words is high. This implicit assumption about word familiarity is violated when a reader encounters an unfamiliar word in a paragraph, leading to neural activity relating to novelty and the allocation of extra attention (i.e., the P3b). However, given that there was no specific task in the current paradigm, it is a matter for future research to test this interpretation by manipulating

task demands to determine whether such an implicit assumption about word familiarity is a plausible explanation for the observed P3b patterns.

Paired with the P150 results, this interpretation suggests that FRPs differ between unfamiliar and familiar words in the frontal-central area of the brain at around 150 ms, and in the parietal region of the brain at around 300 ms. This in turn suggests that differences between the brain's response to novel and known words are due – at least in part – to different amounts of processing relating to the familiarity of objects (the frontal-central P150) and novelty and allocation of extra attention (the parietal P300).

4.4.2. Aim 2: Do differences between FRPs to unfamiliar and familiar names disappear as unfamiliar names became more familiar?

The differences between FRPs to unfamiliar and familiar words disappeared when as the unfamiliar words were repeatedly encountered in the paragraphs of text. Specifically, the difference between the frontal-central P150 peak to unfamiliar and familiar names that was apparent at the 1st encounter was not longer present in the 2nd encounter. This result suggests that by the 2nd encounter, an "unfamiliar word" is familiar enough to be processed as a familiar visual stimulus in fluent reading adults.

The difference between the parietal P300 FRP to unfamiliar and familiar words also decreased with repeated encounters of the unfamiliar names. However, in contrast to the P150 peak, which showed a difference at 1st but not 2nd encounter, the differences between the P300 FRP to familiar and unfamiliar words was observed in the first two encounters of the names within the paragraphs, but was no longer apparent in the third and fourth encounters.

If the P300 FRP observed in the present study is indeed a measure of stimulus novelty or allocation of attention, it is pertinent to consider what tasks are carried out

during fluent reading so as to determine which qualities of a novel word attract increased attention, and why this would occur. Naturalistic reading takes place in a left to right manner in English, generally involving fixations on words and eye-movements between words (i.e., saccades; Rayner, 1998 for review). Fluent reading involves a cascade of processes relating to orthographic, phonological and semantic processes, as well as the integration of the timing of these processes with oculomotor control (Sereno, Rayner & Posner, 1998). The latency of the P300 peak (*M* = 290 ms) in the present study suggests that activity relating to this peak occurs after the eyes have already moved on to the next word (*M* = 221 ms). Considering that (1) subsequent words were matched between conditions via counterbalancing (i.e., the positions of the unfamiliar and familiar names were switched between stimulus subsets as outlined in Stimuli section 4.2.2), and (2) there was no evidence that eye-fixation duration variability explained the FRP outcomes (see section 4.3.3.1 above), it seems unlikely that the P300 reflects activity related to the next word. Instead, the disappearance of the P300 FRP by the third encounter of a new word may reflect the formation of an orthographic representation in the first two encounters, which reduces the novelty of the word, and hence a diminution in the allocation of attention (i.e., the parietal P300). Alternatively, the 1st and 2nd encounters of the new word may have formed an episodic representation that was stored or recognised as a logograph (Frith, 1985) where grapheme–phoneme relations are of lesser salience to the reader (Aghababian, Nazir, Lancon, & Tardy, 2001).

Unfortunately, the current study was not designed to adjudicate between these two explanations. However, the outcomes of this study certainly suggest that in fluent adult readers, the brain recognises a new word as a familiar visual stimulus by the second encounter of that word (as reflected by the fronto-central P150), and that by

the third encounter, a new word no longer attracts the type of attention that is usually dedicated to a novel stimulus (as reflected by the P300), possibly because its orthographic or episodic representation is complete.

4.4.3. The importance of considering eye-movement when interpreting FRPs

As outlined in the Methods, because FRPs are triggered by eye-fixations on stimuli, eye-movement factors might interfere with the interpretation of the FRP waveforms. In the current study, we corrected for eye-fixation duration using an ICA procedure developed by previous FRP/ERP studies (Hutzler et al., 2007; Makeig et al., 1997; Vigario, 1997; see Step 8 in the Offline FRP processing section). This appeared to work for reasons outlined in section 4.3.3.3 of the results. Thus, the FRP outcomes, which were corrected for eye-fixation duration, do not appear to be just a function of eye-fixation duration.

In the current study, we also analyse two other eye-movement factors – eye-fixation duration variability and saccade amplitude – to determine if they could account for the FRP data. As outlined in the results, in contrast to the FRP measures, stimulus type and stimulus encounter had no reliable or interactive effects on fixation duration variability. More interestingly, the analysis of the saccade amplitude data revealed larger saccades for unfamiliar than familiar names at the 1st encounter, smaller saccades to unfamiliar than familiar names at 2nd encounter, and no difference at 3rd or 4th encounter. This reversal in effect direction between 1st and 2nd encounter was not observed for any of the FRPs. Further, the results of previous studies predict that an increase in saccade amplitude at each encounter should lead to an increase in FRP size at each encounter, particularly for an early FRP peak like the occipital P1 (Dimigen et al., 2011). However, the P1 FRP in this study decreased in size with encounter. Thus, it seems very unlikely that the FRP effects observed in this study

were influenced by saccade amplitude, eye-fixation duration variability, or eye-fixation duration.

4.4.4. Unexpected findings

An unexpected finding in the current study was the attenuation of the early occipital P1 peak to both unfamiliar and familiar names with each stimulus encounter. There were no differences in the low-level visual properties of the stimuli that could readily explain this effect (see Methods). Similarly, it is unlikely that this effect is due to saccade amplitude, since, as discussed above, if anything this would have lead to an increase in P1 amplitudes (Dimigen et al., 2011), rather than a decrease. One possible explanation for this pattern of attenuating activity might relate to the specific procedure and stimuli utilised in the current study. Specifically, a decrease in P1 to repeated encounters of unfamiliar and familiar names might be explained by the parafoveal preview benefit afforded by fixations on the words immediately preceding the target stimuli. Previous studies have found that the P1 is not modulated by typical manipulations of parafoveal preview (Dimigen, Kliegl & Sommer, 2012). However, the parafoveal stimuli in the current study were atypical in that they capitalised proper nouns, and thus the left-most letter in each target stimulus provided insight into the word function (character name) as well as the word identity. In addition, the parafoveal stimuli were repeated throughout the paragraphs. This parafoveal preview may not have been of much utility at the first encounter since the character names had not yet been introduced (hence there was a relatively large P1 to both types of names). However, at the second encounter, parafoveal preview might provide an insight into which name is about to be fixated upon, based on the previous encounter with the character name. This may reduce responses from irrelevant neurons, and hence trigger a slightly smaller P1. Increased familiarity with the character names gleaned from

each encounter may allow increasingly strong assumptions to be made about the upcoming words from parafoveal preview, and hence further reduce the size of the P1 FRP. Therefore, the decrease in the P1 response to both unfamiliar and familiar words with repeated encounters may reflect a decrease in response from irrelevant neurons due to improved prediction about the identity of a word from parafoveal preview.

4.4.5. Limitations

To our knowledge, this is the first study to use FRPs to examine neural processing associated with orthographic learning using highly naturalist setting (i.e., reading new words in paragraphs). To maximise the ecological validity of the stimuli, familiar and unfamiliar proper nouns (i.e., character names) were used as stimuli for three reasons. First, there is less semantic difference between familiar and unfamiliar proper nouns than between names of object categories, which minimised the impact of semantic-related processes on the FRP responses. Second, names form a category of novel words that are routinely encountered in silent reading, and are thus an ecologically valid type of word to introduce to fluent adult readers. Third, names are repeatedly referenced throughout narratives, which make them a good category of word type to repeat in a narrative of limited length (80-120 words). However, it is possible that proper names – though ecologically valid for fluent adult readers - represent a different class of word stimulus than common nouns. Apart from a potential difference in the type and degree of semantic information associated with proper nouns, their capitalisation may alter the way in which they are read and recognized in unconstrained natural reading. Additionally, the neural processing of common nouns, which are more likely to be unfamiliar to beginning readers than fluent adults, may prove different to proper nouns due to differences in reading

experience. We are keen to address this empirical question in future studies with young readers who are less familiar with proper nouns.

It is also noteworthy that like some previous studies (Bentin et al., 1999; and Simon et al., 2007), but unlike others (Maurer et al., 2005; Sereno & Rayner, 2003), we did not find an effect of orthographic familiarity on negative FRP peaks. Work by Bentin et al. (1999) suggests that unfamiliar and familiar words have different effects on the N450 depending upon task demands. Further, Maurer et al. has hypothesised that orthographic familiarity may have different effects on the N170 in shallow languages that depend more on phonological decoding (e.g., German) than deep languages (e.g., English), where phonological decoding may be less automatic. Thus, unfamiliar and familiar words may only have differential effects on the negative FRPs under certain task- and language-related conditions, which are not present when fluent adult readers are reading naturalistic paragraphs of text in English.

Individual variation in word recognition skills may modulate the reliability or manifestation of the patterns of the brain potentials observed in the current study. Future studies might benefit from measuring participants' existing word recognition skills to investigate whether this factor modulates the patterns of brain activity as novel orthographic stimuli are encountered and become familiar.

4.5. Conclusion

The goal of the current study was to address a dearth of knowledge about orthographic learning in the brain under naturalistic conditions. The FRPs measured in the current study suggest that for a fluent adult reader, the first encounter of a new word engages neural processes (1) in the frontal-central region at around 150 ms that respond to the novelty of an unfamiliar word as a visual stimulus, and (2) in the

parietal region at around 300 ms that are related to the allocation of attention to novel or unexpected stimuli. By the second encounter, a new word is no longer processed as an unfamiliar class of visual stimulus (i.e. the P150 effect has disappeared) but still attracts extra attention as a novel stimulus (i.e., the P300 effect is still present). By the third encounter, a new word no longer attracts extra attention as a novel stimulus. Additionally, a significant attenuation of early P1 peaks with each encounter of both familiar names and unfamiliar names may be indicative of increasingly strong assumptions made about the upcoming words. These findings suggest that orthographic learning in the brain reflects – at least in part - shifts in processes relating to expectations and assumptions, which in turn guide attention and processing resources.

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Appendix

ADAR
ADY
ALAD
ALET
ALNERT
AMLER
ANNIK
ANTHUR
ARANDA
ATRIL
BALTER
BANNY
BARLA
BEP
BILTY
BIM
BINA
BIRL
BISA
BLEN
BRACY
BREDDA
BRUNE
BUNE
CADMEN
CAROP
CARTIE
CHRIN
CONDY
CRANG
CURTIG
DAKE
DALID
DARRY
DASON
DAWT
DAYNE
DEAB
DEBBIN
DELISE
DENNIT
DEREB
DETRA
DOYCE
EDDIN
ELLET
ELMA
EMIN
ENNA
ERID
ERITA
FRASK
FREG
GART
GENALD
GOBIN
GOIL
GORBON
GORIS
GRAKE
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HABEL
HAGOLD
HANDRA
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LETTY
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MANICE
MANTIN
MAPTHA
MARIK
MARIP
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MARNUS
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MONIDA
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NOOMA
NORPAN
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OSTAR
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LISA
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CINDY
CRAIG
CURTIS
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LARRY
JASON
DAWN
WAYNE
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DEBBIE
DENISE
DENNIS
DEREK
DEBRA
JOYCE
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ELLEN
EMMA
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ERIC
ERICA
FRANK
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JEAN
BETH
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JOE
JULIE
JUSTIN
KAREN
KATIE
KELLY
KEVIN
LAURA
LAURIE
LEE
BETTY
LYNN
LUCY
JANICE
MARTIN
MARTHA
MARIE
MARIA
MARY
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Paper 4

Fixation-related potentials: Some methodological insights

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Fixation-related potentials: Some methodological insights

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Abstract

Fixation-related potentials (FRPs) are a relatively new non-invasive technique for measuring neural processing in the human brain. FRPs are created by co-registering an eye-movement recording and an electroencephalograph (EEG) recording so that brain responses can be measured to the onset of eye-fixations to stimuli when presented within a complex scene (e.g., eyes within a face, words within a paragraph). Given that FRPs are newer than other neuroscientific techniques, researchers are still grappling with methodological challenges that arise from integrating eye-movement and EEG data. The aim of this paper is to outline what we have learned (thus far) about the methodological factors that need to be considered when conducting FRP experiments. It is our hope that this information will make it easier for researchers to adopt the FRP paradigm into neuroscientific laboratories around the world.

Keywords

Fixation-related potentials; EEG; eye-movements; eye-tracking;

Fixation-Related Potentials: A methodological guide

5.1. Introduction

Over the last two or three decades, there has been a boom in human brain research. This has been triggered, in part, by significant advances in the engineering of machines that can measure the brain in a non-invasive way (i.e., from outside the head). At this point in time, the most widely used techniques in brain research are functional and structural magnetic resonance imaging (fMRI and MRI), event-related potentials (ERPs), and magnetoencephalography (MEG). While fMRI and MRI have excellent spatial resolution (i.e., are accurate at pinpointing the location of active brain cells), ERPs and MEG have excellent temporal resolution (i.e., are accurate at tracking brain-cell activity across time). This makes ERPs and MEG particularly useful for scientists who are interested in how the brain processes information across time.

ERPs and MEG represent the average pattern of electrical (ERPs) or magnetic (MEG) activity in response to a stimulus of interest (e.g., human eyes, a written word). When measuring ERPs or MEG to a stimulus, it is important to know exactly when the brain starts processing that stimulus. This can be difficult to ascertain if a stimulus is presented within a naturalistic complex scene (e.g., a pair of eyes is presented within a face, or a written word is presented within a paragraph of text). A common approach to this problem is present stimuli in isolation. A limitation of this approach is the resultant ERP and MEG responses may not accurately reflect the neural processing of those stimuli in “the real world”.

What is needed is a technique that allows us to measure ERP and MEG responses to stimuli that are presented within naturalist settings. This is the goal of fixation-related potentials (FRPs), which co-registers an eye-movement recording with an electroencephalograph (EEG) recording in order to create ERPs that are time-locked to the onset of eye-fixations to particular stimuli of interest presented within a naturalistic scene

(e.g., an eye within a face, or a written word within a paragraph; see next section for more detail about how this is done).

To date, the FRP paradigm has been used in a handful of published studies, and have been used to investigate the old-new word effect (Hutzler et al., 2007); the effect of parafoveal preview in reading, (Baccino & Manunta, 2005; Dimigen, Kliegl, & Sommer, 2012; Simola, Holmqvist, & Lindgren, 2009); the effect of semantic violations in natural reading (Dimigen, Sommer, Hohlfeld, Jacobs, & Kliegl, 2011; Kretzschmar, Bornkessel-Schlesewsky, & Schlesewsky, 2009); visual search (Kamienkowski, Ison, Quiroga, & Sigman, 2012); object recognition (Rama & Baccino, 2010); change detection (Nikolaev, Nakatani, Plomp, Jurica, & van Leeuwen, 2011); proof reading (Takeda, Sugai, & Yagi, 2001); and presaccadic activity relating to information processing during visual fixations (Graupner, Pannasch, & Velichkovsky, 2011; Ossandon, Helo, Monefusco-Siegmund, & Maldonado, 2010; Pannasch & Velichkovksy, 2009; Rajkai et al., 2008). Given that FRPs are newer than ERPs, MEG, or fMRI, FRP researchers are still grappling with certain methodological challenges created by integrating eye-movement and ERP data. For example, as well as capturing brain activity related to the processing of a particular stimulus within a complex scene (e.g., eyes within a face), FRP waveforms can capture electrical activity related to the programming and execution of eye-movements. Further, because FRPs depend upon the co-registration of eye-movement and EEG recordings, eye-movement and EEG machines must work in concert in terms of timing. Thus, the design of FRP studies, and the analysis of FRP data, differs from typical ERP studies in a number ways. The goal of this paper is to outline what we have learned (thus far) about the methodological factors that need to be considered when conducting FRP experiments. It is our hope that this information will make it easier for researchers to adopt the FRP paradigm into their ERP laboratories. This in turn will produce many new insights into the neural processing of stimuli in naturalistic contexts.

5.2. Eye-movement and EEG co-registration: offline versus online

The integration of EEG and eye-movement recordings to form FRPs essentially provides an EEG recording with markers that pinpoint the times that a participant fixates on certain locations (e.g., the eyes, a written word) within an image (e.g., a whole face, a paragraph of text). To illustrate the co-registration procedure, consider the presentation of the sentence: “The pen is the tongue of the mind” while a participant’s eye movements and EEG are being recorded. The following sequence characterises the online and offline co-registration of the two data streams:

1. A participant views a computer monitor (fixating on a particular region, if there is a need to control attention). The computer screen updates to present the sentence, and simultaneously a transistor-transistor logic (TTL) pulse is sent to the EEG recording device event-file and a message is recorded in the eye-movement recording, identifying the onset time of the sentence and thus the beginning of the trial.
2. The participant reads the sentence according to the instructions given (at their own pace, for example).
3. The participant is freed from the shackles of the scalp electrodes and sent home with either a small sum of money or the giant satisfaction of contributing to scientific advance.
4. The eye-movement data is analysed, yielding the times at which the participant fixated upon each unique word in the sentence relative to the onset time of the sentence. For example, “pen” may have been fixated upon 1000 ms after the sentence onset, while “tongue” may have been fixated upon 2000 ms after the sentence onset.
5. The event-file of the EEG recording in this example would contain one port code, which would correspond to the time of sentence onset, and the time in milliseconds that the code was received (the time since the EEG file started recording). To create an epoch time-locked to the fixation on the word “pen”, a new port code can be

created in the event-file by adding the time that it took to fixate on that word from the sentence onset (1000 ms) to the existing sentence onset code in the file. This can also be done for when the word "tongue" was fixated upon in the same manner, by adding 2000 ms to the initial port code time (see Figure 1).

6. The new event-file containing the two new port codes and associated times can be used to create epochs time-locked to the fixations on the words of interest, which are averaged to create ERP brain potentials elicited by those words.

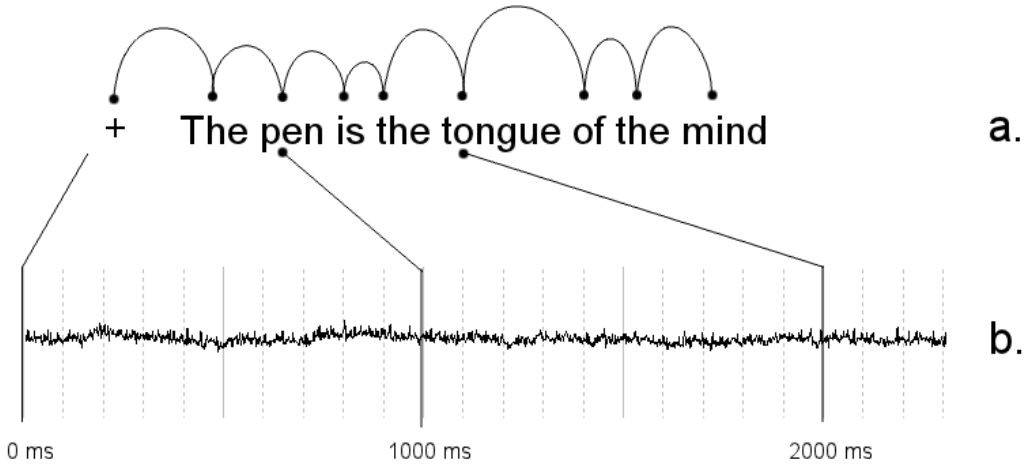

Fig. 1. *Co-registration of recorded eye-movement (a.) and EEG data (b.). The onset of the stimulus presentation is used as a point of reference to create new port codes associated with fixations on individual words ("pen" and "tongue").*

An alternative approach to the above offline co-registration of eye-movements and EEG recordings involves online communication between the eye-tracker and the EEG event-file, whereby TTL port codes are sent when the eye-tracker detects a fixation on a predefined area of interest. In the previous example, the areas of the screen corresponding to the words "pen" and "tongue" can be pre-programmed into the experiment file, so that when the eye-tracker detects a fixation in these areas a port code is sent to the EEG event-file. This has the benefit of eliminating the need to create artificial port codes offline, and thus simplifies the procedure to a degree.

However, there are potential weaknesses to this online procedure, depending on the dynamics of the eye-tracker. In order for a fixation event to be detected by the eye-tracker, the eye must be effectively static for a specified period of time (movement below a threshold of 30^0/sec velocity and 8000^0/sec^2 acceleration; Stampe, 1993), which is typically 35 or 50 ms. Thus, a true fixation will trigger the sending of a port code 35 or 50 ms after the fixation began, since this time is required in order to satisfy the definition of a fixation. This leads to all of the codes arriving 35 or 50 ms later than the actual time of fixation, and thus the derived fixation-related EEG epochs will be similarly offset. This is not in itself problematic, since the times of the individual codes in the event file can be artificially offset by 35 or 50 ms and hence corrected for any systematic lag. However this method relies on the perfect operation of the eye-fixation detection and TTL port code generation of the eye-tracking machine, and thus any variability between the fixation detection process and the TTL port code generation can lead to variability in the accuracy of the timing of the port-codes received by the event-file. This variability can be measured and therefore accounted for in offline-processing, depending on the capacity of the eye-tracking device and software, and so this procedure may be implemented while also preserving the capacity to implement the offline co-registration technique. Thus, the choice of offline or online co-registration of eye-movement and EEG recordings for an FRP experimental should take into account the timing accuracy of TTL port codes generated by the eye-tracking machine. This choice has implications for programming of the experimental procedure in the relevant presentation software. The physical connections between the EEG, eye-tracker and stimulus presentation devices will depend on the specifics of the hardware chosen, however an example of a typical set-up of the hardware involved is illustrated in Figure 2.

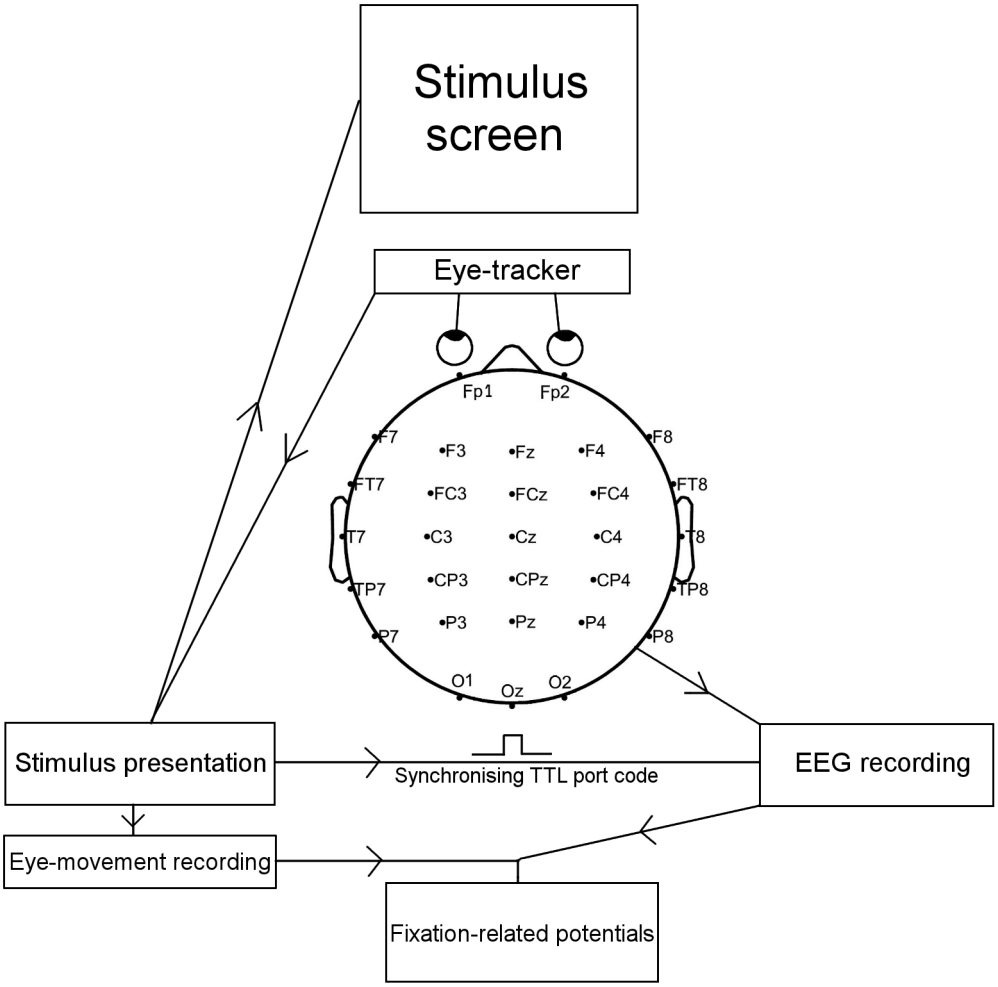

Fig. 2. *Example of a typical hardware configuration for co-registering eye-movements and EEG online and offline.*

5.3. Ocular artefacts

FRPs are derived by time-locking to the beginning of a fixation, which is also the end of a saccade. This means that FRPs are necessarily time-locked to the saccadic activity that precedes fixation-onset. Thus, the time-period immediately preceding time-zero (fixation onset) for an FRP may contain electrical artefact. The strength of this artefact is greatest at electrode sites spatially close to the eyes and the muscles associated with their movement, and decreases with distance from this source. These ocular artefacts can be decomposed into three general component sources: (1) contraction of peri-ocular muscles responsible for or associated with the movement of the eyeballs, which elicits a negative presaccadic spike potential ipsilateral to the direction of movement (Thickbroom & Mastaglia, 1985; 1986;

Moster & Goldberg, 1990); (2) movement of the eyes themselves, which due to a disparity of electrical charge within the structure of the eye, forms a corneoretinal dipole (Berg & Scherg, 1991); and (3) movement of the eyelids across the corneoretinal dipole in vertical eye-movements and blinks (the manifestations of which are dissociable, Lins et al., 1993; Picton et al., 2000).

The influence of ocular artefacts on FRP waveforms should be considered carefully when planning the experiment. If the nature of the eye-movements is not directly related to the research question, ocular artefacts can be dealt with in at least three ways in FRP experiments. First, the effect of ocular artefacts can be minimised by restricting the analysis of the FRP waveforms to time periods when the eyes are stationary, and thus do not propagate electrical noise through the scalp (Baccino & Manunta, 2005; Simola, Holmqvist, & Lindgen, 2009). This includes early time-windows of FRPs that are time-locked to the onset of the eye-fixation to a stimulus of interest (e.g., the first 200 ms in which the eyes fixate on a individual word within a paragraph), and later time-windows of FRPs waveforms that are time-locked to the last word of a sentence, where there is no following word to attract an eye-movement (Hutzler et al., 2007).

A second approach to minimising ocular artefact effects on FRPs involves the isolation and removal of the artefacts through either a combination of principal component analysis (PCA) and dipole models of cortical and ocular activity, or independent component analysis (ICA). Both approaches determine the influence of ocular artefacts on the activity recorded at EEG channels, and remove this pattern of influence from the EEG recordings whilst retaining the cortical activity. The combination of dipole source modelling and PCA has been used successfully to remove ocular artefact in an FRP paradigm (Dimigen, Kliegl, & Sommer, 2011; Dimigen et al., 2012) through the application of Surrogate Multiple Source Eye Correction (MSEC, Berg & Scherg). This involves recording the EEGs and EOGs of participants when they make eye-movements in specified directions. PCA is then

used to deconstruct the topographical sources and influences of the ocular activity. This is applied in the presence of dipole models of cortical activity to model the typical artefact while retaining underlying cortical activity. This allows for the removal of ocular artefacts in experimental data that are unique to each participant.

ICA similarly involves the deconstruction of the artefacts related to eye-movements found in EEG and ERP recordings into statistically independent components, allowing a blind separation of components satisfying higher-order independence (Vigario, 1997; Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997). ICA decomposes the EEG or ERP data into weighted components, indicating temporal and spatial patterns across channels. These can be used to isolate the contribution of ocular activity to EEG channels as it propagates across the scalp, which can be retained as components reflecting this activity, or removed from all other component combinations to leave the EEG, ERP or FRP data cleansed of the ocular artifacts (de Lissa, McArthur & Brock, 2012; de Lissa et al., 2012a; Hutzler et al., 2007). This process is applied to individual participant's EEG data, since the nature of ocular artefacts are specific to the physiology of the individual. In the case of ICA applied to ERP data, the process can be applied to condition-specific data to account for patterns of ocular activity that are correlated with the varying conditions (Makeig et al., 1997). For the sake of methodological transparency, the results of a systematic isolation and removal of ocular artefacts can be measured and represented topographically to provide insight into the source and strength of these patterns of influence (see Figure 3).

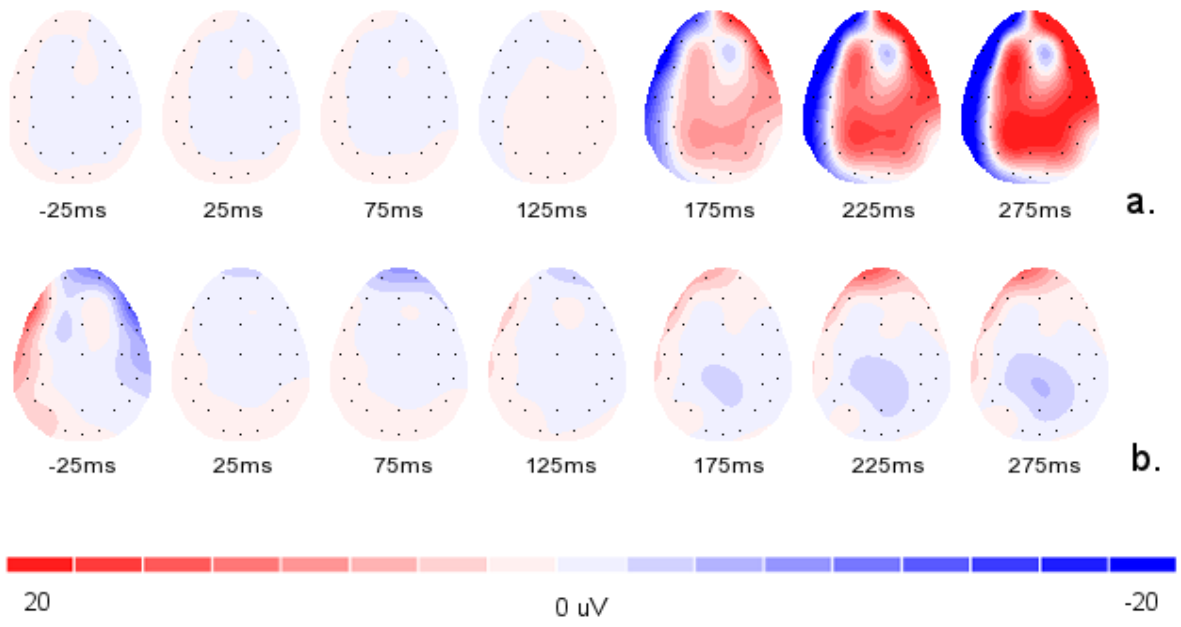

Fig. 3. *Example of ocular artifacts removed through ICA for (a.) saccadic movement beginning at approximately 160 ms in response to image presentation in the periphery, and (b.) saccadic activity associated with the onset and offset of a fixation made within a face (de Lissa et al., 2012a).*

In contrast to minimising or correcting for ocular artefacts in FRPs, a third way to deal with ocular artefacts in FRP experiments is to analyse the pattern of ocular artefact data across conditions to see if this matches the pattern of FRP data across conditions. If this is not the case, then ocular artefacts are unlikely to explain the FRP outcomes. There are at least two types of ocular data that can be considered in this way. One is eye-fixation duration variability. Any neural activity in an FRP waveform that falls after the average amount of time (e.g., 200 ms) spent fixating on that particular stimulus (e.g., a word within a sentence) may include neural activity related to fixation to the next stimulus (e.g., the next word), due to an overlap of brain potentials from what is effectively a short stimulus onset asynchrony (Baccino, 2011, Dimigen et al., 2011). If fixation durations to a stimulus are highly variable, the neural activity relating to fixation on the next stimulus will be jittered and hence poorly defined in the FRP waveform to the current stimulus. However, if fixation durations are uniform, the neural activity related to the fixation on the next stimulus will be more temporally uniform, and hence may be well-defined in the FRP waveform to the current

stimulus. Thus, it is appropriate to analyse possible differences in the variability of the duration of fixations made to target stimuli, where there may be condition-specific differences in overlapping brain potentials from fixations on post-target stimuli (n + 1), as well as fixations immediately preceding targets (n - 1), as this might also introduce a differential of overlapping brain potentials that would confound the interpretation of the FRPs. It is possible to measure eye-fixation duration variability by computing the standard deviation of the duration of fixations to each level of each condition. These standard deviations can then be analysed as a behavioural measure, in the same way as the FRP data to determine if the pattern of outcomes is the same as the FRPs. As mentioned above, if there is not a systematic difference in fixation duration variability related to the experimental conditions, then this dimension is less likely to be attributed as the cause of any condition-effects in the FRP waveforms.

Another ocular artefact to consider is amplitude of saccades. The size of the preceding saccade leading to the fixation on a stimulus has been found to modulate the size of the early occipital ERPs (e.g., the P1), with larger saccades leading to larger occipital activity (Dimigen et al., 2011). This is thought to reflect lambda activity related to the offset of saccades and a "priming" of the visual cortex (Yagi, 1979; Kazai & Yagi, 2003). If different levels within a condition modulate incoming saccade lengths (e.g., to eyes versus mouths within a face), then this may generate larger FRPs in certain conditions. With saccades defined as eye-movements exceeding 30^0/sec velocity and 8000^0/sec^2 acceleration (Stampe, 1993), saccade size can be measured as the length of the incoming saccades leading into fixations on the stimuli, reported in degrees of visual angle. Yet again, these measures can then be analysed in the same way as the FRP data to determine if the pattern of saccade size could account for observed effects in the FRP waveforms, such as enhanced P1 peaks.

5.4. Stimuli

Different sized stimuli may elicit different sized eye-movements as they are viewed, which may lead to differences in neural responses related more to saccade-size than to the categorical differences between the stimuli. Additionally, the complexity or attentional salience of the stimuli may produce different sized eye-movements, even when the stimuli sizes are comparable. For example, faces have been found to elicit larger eye-movements when they are freely scanned compared to wrist-watches (non-face control category; de Lissa et al., 2012a). Thus, an observed increase in early occipital P1 peak in response to fixations on faces may relate to the salience of faces as a category in terms of occipital activation, or it may merely be due to the effects that larger saccades have on this early visual peak (Dimigen et al., 2011).

There are at least three approaches to address the potential confound stimulus-related differences in saccade size: (1) select stimuli that are likely to elicit comparable eye-movements, (2) remove trials that contain different saccade sizes across conditions, and (3) counterbalance stimuli to reduce any bias between conditions. The first two approaches have their weaknesses. Selecting stimuli to elicit comparable eye-movements necessitates an appreciation of what participants' eyes will do, which may indeed be part of the purpose of the experiment in the first place. Further, pruning the eye-movement data to match saccade size may lead to an imbalance of trials between experimental conditions. Thus, at this stage, the best way to address the influence of stimulus-related difference in saccade size on the FRP waveforms may involve a combination of all three approaches outlined above.

Another stimulus-related feature that is important to consider in FRP experiments, like ERP experiments, is the low-level visual properties of the stimuli. ERP studies have established that stimulus features such as the brightness and contrast of visual stimuli influence ERP peaks, such as the early occipital P1 (80-100 ms) peak and the following face-sensitive occipito-temporal N170 (100-200 ms) peak (Rossion & Caharel, 2011). Thus, a systematic modulation of the size and contrast of the stimuli may yield patterns of neural

activity that reflect these low-level factors (e.g., long words versus short words), as well higher-level processing factors (effects of word frequency, word neighbourhood size). Thus, as is true for ERP studies, it is important for FRP studies to match or control for differences in stimulus features between stimuli.

5.5. Stimulus presentation

Another factor to consider in an FRP paradigm is the manner in which the stimuli are presented. Co-registration of eye-movements with EEG allows at least three types of stimulus presentation. First, it allows brain responses to be measured to specific stimuli (e.g., a written word) in a complex scene (e.g., a paragraph of text), which is a traditional FRP paradigm. Second, it allows for the online manipulation of when and where a stimulus is presented, and hence when and where a participant's fixation falls on the stimulus. This technique is called the variable viewing position paradigm (VVPP; O'Regan & Jacobs, 1992; O'Regan, Lévy-Schoen, Pynte, & Brugaillere, 1984). In the VVPP, a fixation triggers an update of the computer monitor so that a stimulus (e.g., a word) is presented in a specific location (e.g., within a sentence) relative to the eye-position of the participant (e.g., a fixation cross). This can be used to direct visual attention to specific parts of stimulus, such as certain letters in words, to investigate the optimal viewing position effect in reading (Hutzler, Braun, & Jacobs, 2008), or to modulate visual attention related to parafoveal preview effects in reading (Baccino & Manunta, 2005). The VVPP paradigm also allows attention to be directed away from the stimuli of interest at the beginning of a trial, so as to allow the stimuli to be viewed through natural fixations (de Lissa et al., 2012a). As such, the VVPP paradigm represents an intermediate step between traditional ERP procedures and free-roaming FRP procedures. Specifically, the EEG is time-locked to stimulus presentation, as in ERP. However, the timing of stimulus presentation is triggered by eye-fixations made

to specific regions of a presentation screen. Thus, the VVPP paradigm involves both a temporal time-locking to stimulus presentation times as well as to natural eye-fixations.

A strength of the VVPP paradigm is that it can be used to minimise confounding effects of eye-movement-related activity in FRPs. To explain via example, in face-processing studies, the presence or absence of the eyes in a face significantly modulates the face-sensitive N170 ERP peak (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Itier, Alain, Sedore & McIntosh, 2007), leading to suggestions that eye-specific neurons play a prominent role in this peak (Itier & Batty, 2009; Itier et al., 2007; Itier & Taylor, 2004). To test this hypothesis in a naturalistic setting (i.e., not employing eyeless faces, which are unnatural enough to be slightly disturbing), we compared FRPs to the eyes within faces to FRPs to mouths within faces (de Lissa, 2012b). We found that because the fixations to the various face parts were under the control of the participant and not the experimenter, subjects made significantly more fixations to the eyes than to the mouths. This would not pose a problem if we knew that order of fixation to the face parts does not modulate the N170 response. Such an assumption was found to be erroneous, where the results of a free-viewing FRP experiment indicated that the N170 is significantly reduced after initial presentation of faces (de Lissa et al., 2012a). Fortunately, the VVPP paradigm can be used to present faces in such a way that the same number of fixations are made to the eyes and the mouth of a face. This circumvents the confounding effect of eye-movement-related activity in FRPs, while still allowing for full faces to be presented, rather than eyeless faces or disembodied face parts.

A third type of stimulus presentation allowed by co-registration of eye-movements and EEG relates to parafoveal preview. Since the FRP paradigm allows for natural fixations to be made, parafoveal information can also be used to modulate when certain stimuli appear to a participant during a trial. Examples of this kind of stimulus presentation include the gaze-contingent moving window and boundary paradigms (Reder, 1973; McKonkie &

Rayner, 1975). This contrasts with the procedure employed by Baccino and Manunta (2005), where parafoveal preview was investigated by directing a fixation to a target word that was flanked by words of differing categories in the right parafoveal region. The manipulation of what parts of a stimulus are available to be viewed by participants can also be used to influence eye-movement behaviour, such as reducing the likelihood of regressions being made to previously fixated stimuli (Hutzler et al., 2007) by removing previously read words from view. While these paradigms do not address the confounding effect of eye-movements on FRP data per se, they are potentially useful stimulus presentation paradigms to consider when designing FRP experiments.

5.6. Offline FRP processing

Most of the steps involved in converting co-registered eye-movement and EEG recordings into FRPs are the same as converting EEG recordings into ERPs, such as correcting eye-blinks through ocular artefact reduction algorithms involving the VEOG channel and frequency filtering. However, the choice of offline re-referencing can potentially influence FRPs if pre-fixation saccade-related ocular artefacts propagate across the scalp in an asymmetrical way. These ocular artefacts (see Ocular Artefact section above) can suffuse the activity recorded in scalp electrodes, which forms a distinct topographical pattern whereby the source may be discerned to be the eye-region. If a common average of all scalp electrodes is used as an offline reference, any asymmetry in the spread of ocular artefact across the channels can inject noise into the EEG channels, which accordingly has the potential to distort all the EEG channels. This can be addressed through the selection of an offline reference spatially distant from the source of the ocular artefact, such as the mastoid region, or the artefacts caused by the ocular movement can be isolated and removed through noise-reduction ICA or PCA processes (see Ocular Artefacts section) before the re-referencing process is applied.

A similar consideration relates to the time-window used for base-line correction. A common time period for baseline correction in ERPs is the time-window immediately preceding stimulus presentation. However, when epochs are time-locked to the onset of a fixation, the preceding time period includes ocular artefacts relating to saccadic movement, which provides an inappropriate reference point for the rest of the waveform. A similar distortion may arise if the entire epoch time-window is used a baseline, if saccades preceding or following fixation to a stimulus systematically differs between conditions. In lieu of a perfect solution, the period immediately following fixation onset (0-20 ms) provides a relatively uncontaminated reference point from which to perform baseline correction (Hutzler et al., 2007; Dimigen et al., 2011).

5.7. Recording instruments

The co-registration of eye-movement and EEG recordings entails a combination of data from two recording devices. The resulting FRP data is therefore as accurate as the least accurate recording device involved. The sampling rate of modern EEG devices is typically high, yielding resolutions up to half a millisecond (2000 Hz). However, this high temporal sensitivity can be limited in the FRP paradigm by the sampling rate of the eye-movement recording device. Specifically, the co-registration of the eye-movement data and the EEG data is most straightforward if the sampling rates of the eye-tracker and the EEG devices are the same. If an eye-tracker is sampling once every four milliseconds (250 Hz) then the EEG can only be indexed once every four milliseconds as well. Thus, when measuring FRPs, it is important to use an eye-tracker with a high sampling rate in units.

Many eye-trackers are capable of recording the position of both eyes at the same time, which may or may not lead to a reduction in temporal sensitivity (halved by alternating sampling of each eye and subsequent averaging of the two). The question of whether to track both eyes and use the position of each eye separately to index the EEG data (to investigate fixation disparity between the eyes when reading, for example), or to choose the dominant

eye for monocular recording, is a matter of experimental aim and compromise, depending on whether divergence during fixation may confound the results. Thus, when measuring FRPs, the selection of monocular versus binocular recording will depend on the experimental design, the desired temporal resolution, and the desired acuity of the spatial resolution of the eye-tracker.

In addition to the recording of eye-movements with an eye-tracker, it is advisable to record electrical activity associated with vertical and horizontal eye-movements through VEOG and HEOG bipolar electrodes. These EEG channels can be used to remove ocular artefacts caused by eye-blinks through ocular-artefact reduction algorithms, and saccadic activity through ICA and PCA (see Ocular Artefacts section). When EEG epochs are time-locked to the onset of a fixation, the horizontal EOG channel exhibits a distinct cessation of electrical activity. Previous experiments have utilised this sudden change in voltage level as a means to time-lock EEG epochs without the use of an eye-tracker, by inferring that this indicates the end of a saccadic event and the beginning of a fixation (Gaarder, Krauskopf, Graf, Kropfl, & Armington, 1964; Kurtzberg & Vaughan, 1977; Marton, Szirtes, & Breuer, 1984; Yagi, 1979). Accordingly, the HEOG channel provides a means through which to determine the accuracy of the eye-movement-EEG time-locking process, by comparing the voltage-change in this channel at time-zero as defined by the eye-tracker. In addition, the HEOG channel also provides a point of comparison when determining the efficacy of the correction of ocular artefacts by correlating the activity in this and scalp channels with the eye-position recorded through the eye-tracker (Dimigen et al., 2011).

It is noteworthy that the communication between an eye-tracker and an EEG recording device involves the sending and receiving of port codes in the form of transistor-transistor logic pulses (TTL). This is a common interface with EEG recording devices, and is increasingly utilised in eye-tracking devices for synchronisation purposes. In the FRP paradigm, the TTL allows trial-specific information to pass between the presentation device,

the eye-tracker, and the EEG device, in various combinations of configurations if required. It is therefore necessary to utilise hardware that allows for TTL (or equivalent) pulses to be sent and received through ports that do not involve a significant degree or variability of timelag.

It is also noteworthy that an EEG recording device and an eye-tracking device will operate from different computers, with different processor clocks operating in each. Small differences in CPU clock speeds between computers are almost indiscernible at any one moment in time. However, if both recordings commence at the same time, any difference between the two CPU clocks will become increasingly more pronounced as this clock-drift occurs (Marouani & Dagenais, 2008). This drift may be corrected by determining the effects of the systematic differences between the computer clocks on the recording, and manually offsetting one of the files to align more accurately to the other. However, a more efficient approach is to synchronise the two recordings in each trial by providing trial-specific indexes to both the eye-movement and EEG recording simultaneously. This involves sending TTL port codes from the presentation device to the EEG device at specific time-points of a trial (e.g., at the beginning of each trial), whilst also writing into the eye-movement file the time at which these port codes were sent. The EEG device stores these port codes in an "event-file", which details the numbers corresponding to the port-codes and the time in milliseconds that they were recorded. The timing of these port codes corresponds to the start of the trial in the eye-movement file, whereby the timing of fixations and saccades within the trials can be referenced. This allows the recorded eye-movements relative to the start of a trial to inform the event-file of when fixations occurred, and thus allow the time-locking of the EEG to those events.

5.8. Summary

The co-registration of eye-movements and EEG recording dramatically expands the realms of cognition and visual processing open to investigation. The methodological issues

involved in combining these two streams of data to form FRPs are one important aspect of the FRP technique. The other equally important aspect is the imagination of the experimenter utilising the FRP paradigm; along with the expanded contexts of visual processing open to investigation comes an expanded sphere of theoretical considerations. The increased number of variables that the FRP paradigm encompasses, such as ocular artefacts, requires a balance between the factors under investigation and the factors to be controlled. It is the hope of the authors that this guide will aid in the consideration of these factors, and hence expand the successful implementation of the FRP paradigm around the world.

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General Discussion

6.1 Discussion

The general aim of this thesis is to progress the development and application of the FRP paradigm. To this end, we used FRPs to investigate the neural processes involved in the domains of face processing and word reading. To recap, in Study 1, we used the VVPP-ERP technique to determine if eye-sensitive neurons are involved in processing whole faces by measuring ERPs to eyes and mouths in upright and inverted intact faces. In Study 2, we used FRPs to determine if the N170 brain response reflects a face-detection process, which only occurs at the initial presentation of a face, and not in subsequent fixations on that face. In Study 3, we explored orthographic learning in the brain by measuring FRPs to repeatedly encountered familiar and unfamiliar words in paragraphs of text. And in Study 4, we considered methodological challenges associated with the FRP technique, and offered solutions to those challenges.

In section 6.2 below, I provide a more detailed summary of the aims, methods, and results of studies 1 to 3 (sections 6.2.1, 6.2.2, and 6.2.3), as well as the methodological issues outlined in Study 4 (section 6.2.4). Since Study 4 was written as a stand-alone manuscript for submission for publication, we minimised reference to studies 1 to 3, since the latter are under review. Thus, in section 6.3 below, I explicitly link the four studies that comprise this thesis by providing examples of how some of the methodological issues outlined in Study 4 were addressed in studies 1, 2, and 3. I conclude with potential avenues for future FRP research (6.4) and a final brief summary (section 6.5).

6.2 Summary of studies

6.2.1 Study 1: The neural processing of face parts in ecologically valid stimuli.

Previous research has found that the face-sensitive N170 ERP is larger to eyes presented in isolation than (1) faces without eyes, and (2) whole intact faces (e.g., Bentin, Allison, Puce, Perez, & McCarthy, 1996). This has lead to the suggestion that whole intact faces recruit only face-specific neurons, while disruption of face configuration leads to the

recruitment of eye-specific and face-specific neurons (e.g., Itier & Batty, 2009). A potential problem with this hypothesis is that it is based on ERP studies that presented faces and eyes in such a way that participants' fixations fall in-between the eyes or on the nose (e.g., Eimer, 1998; Rossion et al., 1999). This is not where humans typically fixate when looking at faces (i.e. the eyes and mouth; e.g., Althoff & Cohen, 1999).

The aim of Study 1 was to use the VVPP-ERP technique to determine if whole intact faces recruit face-specific neurons, while configurally disrupted faces recruit face- and eye-specific neurons. To this end, we measured the N170 brain response to eyes and mouths in upright and inverted faces. The results showed that fixations to eyes in whole upright faces elicited stronger N170 peaks compared to fixations to mouths. This suggested that configurally intact faces recruit eye-sensitive neurons as well as face neurons. Further, inverting faces had a larger effect on the N170 for mouths compared to eyes, which would not be expected if configurally disrupted faces lead to the additional recruitment of eye-sensitive neurons. Overall, the findings of Study 1 suggest that there is a complex interplay between configural and featural processing of faces, whereby face-sensitive and feature-sensitive neurons are modulated by face configural disruption to different degrees in naturalistic contexts.

6.2.2 Study 2: Face-sensitivity of the N170 is limited to initial presentation: fixation-related potentials during naturalistic scanning of faces.

Previous ERP studies have suggested that the N170 brain response to faces reflects a face detection process (e.g., George et al., 2005; Rousselet et al., 2005). If this is true, the N170 should demonstrate "face-sensitivity" effects to the initial presentation of a face (i.e., the N170 is larger to faces than non-face stimuli, and is larger to inverted than upright faces) but not to subsequent fixations on a face. This was tested in Study 2, where we compared FRPs to the initial presentation of, and subsequent fixations to, upright and inverted faces and watches. The results support the suggestion that the N170 reflects a face-detection

process, since faces only elicited a larger N170 FRP than watches in response to the initial presentation, and since inverted faces elicited a larger N170 than upright faces in response to the initial presentation of a face, but not subsequent fixations.

6.2.3 Study 3: Orthographic learning in the brain: New insights from fixation related potentials.

In Study 3, the FRP paradigm was used to investigate another important domain of visual cognition: word reading. Behavioural studies suggest that readers can establish an representation of a new written word in a single exposure, and that the strength of this representation can increase in strength for up to four exposures (Bowey & Muller, 2005; Nation, Angell & Castles, 2007). Less is known about orthographic learning in the brain. Previous ERP studies have discovered differences in brain potentials elicited by familiar and unfamiliar words, such as modulation of the N170 ERP response (see Maurer et al., 2005 for review), the P150 ERP response (Proverbio et al., 2004), and the P300 and N300 ERP responses (Taroyan & Nicolson, 2009; Hauk et al., 2006 respectively). However, these ERPs were typically elicited by single words presented in isolation, which is an unnatural context for adult readers.

In order to investigate brain activity associated with orthographic learning under more natural conditions, we used the FRP paradigm to record brain potentials elicited by fixations made to familiar and unfamiliar words (character names) embedded and repeated within a paragraph of text. At first encounter, familiar words elicited a larger P150 FRP over the frontal-right region than unfamiliar words, and unfamiliar words triggered a larger P300 over the right-parietal region than familiar words. At the second encounter, familiar and unfamiliar words elicited a similar P150. However, the P300 peak was still significantly larger for unfamiliar than familiar words. This difference was no longer present at the third and fourth encounters. An additional, yet unexpected, finding was an early P1 peak that reduced in size across all four encounters for both familiar and unfamiliar words. Considered

together, these outcomes suggest that orthographic learning in the brain reflects – at least in part – a shift in processes relating to expectations and assumptions of word stimuli, which in turn guide attention and processing resources.

6.2.4 Study 4: Fixation-related potentials: Some methodological insight.

One significant advantage of the FRP technique is that it allows the measurement of brain responses to stimuli that are presented in complex naturalistic settings. This is achieved via the co-registration of eye-movement and EEG recordings. However, the additional domains open to investigation through FRPs also brings a number of methodological considerations, which influence the implementation of the paradigm, and the interpretation of the resulting waveforms. Study 4 provided a review of the methodological factors that should be considered when conducting FRP experiments. These factors relate to online versus offline techniques used to co-register eye-movement and EEG recordings, ocular artefact avoidance and removal, stimulus type and presentation, online eye-tracker/EEG device synchronisation, offline FRP processing, and the relationship between different recording instruments. In addition to these considerations, factors influencing the interpretation of the resulting FRP waveforms were discussed, such as the nature of the eye-movements associated with experimental conditions.

6.3 How some methodological issues in Study 4 were addressed in studies 1 to 3

6.3.1 Ocular artifacts.

As outlined in section 5.3 of Study 4, since FRPs are systematically time-locked to the saccadic ocular movement that precede fixation-onset, the time-period immediately preceding fixation may contain ocular artefacts. Such artefacts can be minimised in FRP experiments in at least three ways: (1) restricting the FRP analysis to time periods when the eyes are stationary; (2) isolating and removing ocular artefact via PCA and ICA; and (3) comparing the patterns of ocular artefact data across conditions to see if this matches the pattern of FRP data across conditions.

Studies 2 and 3 adopted the second method. In Study 2, saccades were made from a central fixation cross to the image of a face or watch in the visual periphery. Then, further saccades were made before and after subsequent fixations on the face and watch images. In Study 3, saccades were made before and after familiar and unfamiliar words were fixated upon within paragraphs of text. The influence of these ocular artefacts on FRPs was successfully addressed through the isolation and removal of the artefacts through ICA (Hutzler et al., 2007; Vigario, 1997, Makeig et al., 1997; de Lissa et al., 2012a) This involved determining the sources of the contamination as originating from the ocular region and subsequently removing the distorting patterns of activity from the scalp channels.

Figure 1 illustrates the pattern of ocular artefact generated in Study 2 for (a) the initial presentation of the face and watch stimuli and (b) the subsequent fixations on these stimuli. In these waveforms, ocular artefacts can be observed in the time immediately preceding fixation onset (at time zero), and later in the waveforms at the beginning of the offset of the fixation (at approximately 175 ms). Figure 1 also illustrates that the pattern of ocular artefact in the waveforms relating to (a) and (b) exhibit a different quality in terms of the strength and the time-course of the activity.

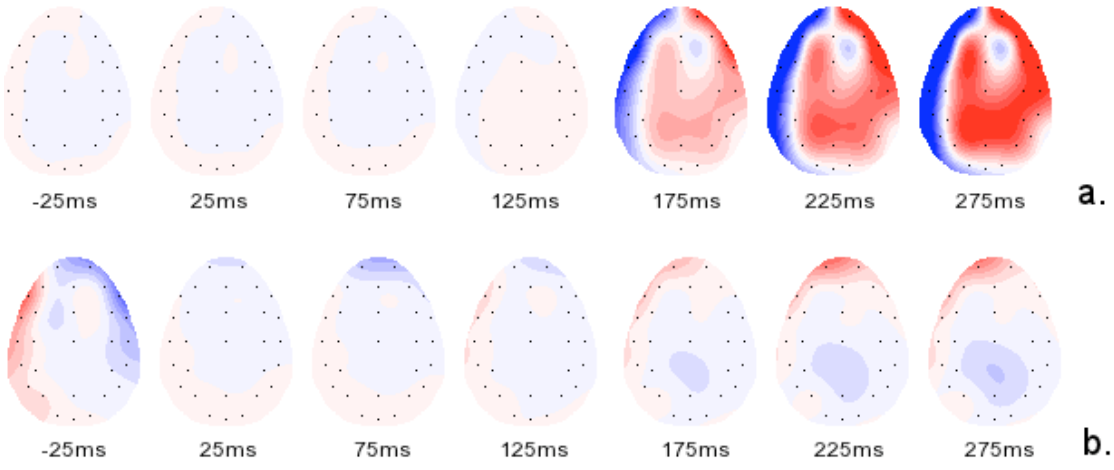

Fig. 1. *Average saccadic artefact removed from (a) initial presentation and (b) fixation-related potential waveforms in Study 2. Lateral-frontal artefact was isolated with independent component analyses, corresponding to eye-movements made to the stimuli (de Lissa et al., 2012a).*

Graphical presentations of the patterns of ocular artefact that have been isolated and removed are useful for at least two reasons. First, such graphs reveal the interaction between ocular activity and the FRP waveforms in terms of time-course and topography. This can be used to determine if the correction processes was successful or warranted. Second, graphical representations can be used to determine if ocular artefacts differ between stimuli within an experiment. For example, in Study 3, the waveforms representing ocular artefacts isolated by the ICA process (see Figure 2) revealed that the duration of initial fixations was 20 ms longer for unfamiliar words than familiar words. These differences in ocular artefact between stimuli elucidate the importance of addressing eye-movement artefacts on the FRP waveforms through their isolation and removal with either ICA (Hutzler et al., 2007; Vigario, 1997) or mixtures of ICA and PCA and dipole modelling (Dimigen et al., 2011, 2012).

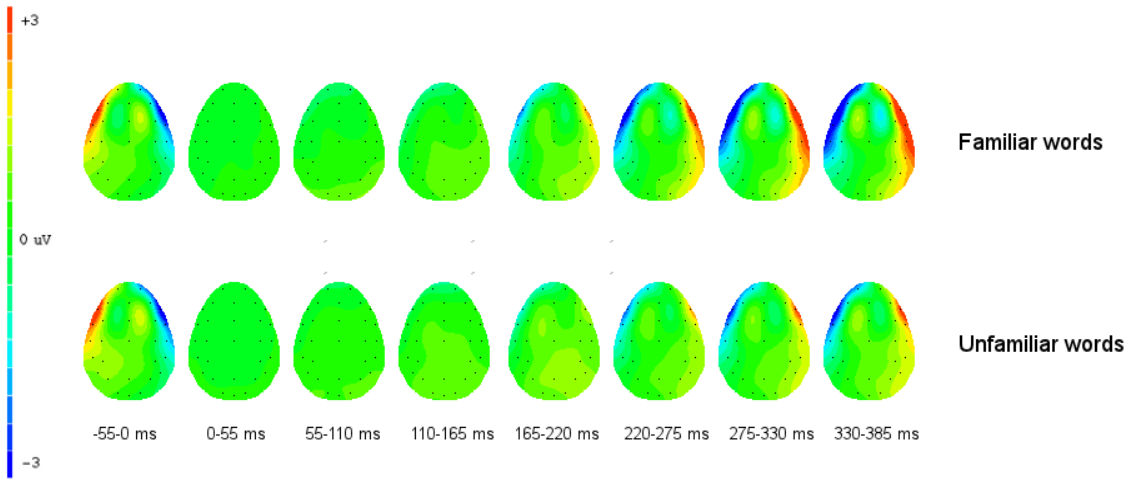

Fig. 2. *Example of oculomotor activity removed through independent component analysis in experiment 3, exhibiting differences in removed activity between familiar and unfamiliar words at first encounter (de Lissa et al., 2012b).*

In Study 3, we also adopted the third method to address ocular artefacts in FRP data. That is, we compared the pattern of ocular artefact data across conditions to see if it matched the pattern of FRP data across conditions. If this is not the case then ocular artefacts are

unlikely to explain the FRP outcomes. Study 3 analysed two types of ocular data in this way: eye-fixation duration variability and saccade amplitude. The artefact results were markedly different to the FRP outcomes, which suggested that the ocular artefacts were unlikely to explain the FRP outcomes.

6.3.2 Stimuli.

As outlined in section 5.4 of Study 4, stimuli that differ in size, complexity, or salience may elicit different sized eye-movements. This may lead to differences in neural responses related to saccade-size rather than the processing of the stimuli per se. There are at least three ways to address this potential confound: (1) select stimuli that are likely to elicit comparable eye-movements, (2) remove trials that contain different saccade sizes across conditions, and (3) counterbalance stimuli to reduce any bias between conditions.

Study 3 adopted the last approach. In this study, we measured FRPs to familiar and unfamiliar words presented in naturalistic paragraphs of text. If there had been a systematic difference in the saccade size that preceded fixations on the target stimuli, such as longer words preceding the familiar words and shorter words preceding the unfamiliar words, there may be a modulation of the early P1 peaks over occipital regions that relates to the incoming saccade sizes rather than the familiar and unfamiliar words themselves. This potential problem was addressed by counterbalancing the size of the target stimuli and the order of when the familiar and unfamiliar words are encountered in the paragraphs, which balanced the words that preceded these targets.

6.3.3 Stimulus presentation.

The co-registration of eye-movements with EEG allows at least three types of stimulus presentation for FRPs: (1) a stimulus is presented within a complex scene (typical FRP paradigm), (2) a stimulus is presented at a particular time and location once a participant has fixated on particular location for a certain period of time (VVPP-ERP paradigm), and (3) a stimulus is presented according to particular parafoveal information

(parafoveal paradigm). Study 1, which measured brain responses to eyes and mouths in upright and inverted faces, adopted the second (VVPP-ERP) method. This was done for two reasons. First, if participants were free to decide where their initial fixations fell on the stimuli, they may have made more fixations to the eyes than mouths. This would have resulted in noisier (and hence incomparable) FRPs to mouths than eyes. Second, at the time of Study 1, it had not yet been established whether the order of fixations to faces modulated the N170 response (this was the basis of the second study). Thus, the VVPP-FRP paradigm was chosen to ensure that participants' initial fixations fell on the eye or the mouth of a face the same number of times, and hence again produced comparably reliable FRP responses. This illustrates the necessity of using the appropriate stimulus presentation technique to eliminate the influence of eye-behaviours that may lead to methodological weaknesses.

Study 2 adopted the first (FRP) presentation paradigm to determine if the N170 reflected a face-detection process, in which case the N170 would present to the initial presentation of a face, but not to subsequent fixations on the face. This was done because while study 2 suggested that the eyes within a whole face elicit a larger N170 than mouths, it had not yet been established whether the order in which faces are viewed through natural fixations might modulate the N170. The neural response elicited when someone looks at an eye in someone's face might be larger than when they look at the mouth, eye-tracking studies have suggested that the majority of times the first fixations to a face land near or on the eye-region (Althoff & Cohen, 1999; Blais, Jack, Scheepers, Fiset & Caldara, 2008). If there was a pattern of difference in FRPs to fixations made to eyes and mouths it would not be clear whether this difference was due to the importance of the face part, or the order that the face parts were fixated upon. If the N170 did reflect a face-detection process as has been suggested (George et al., 2005) then the prediction would be that initial perception of a face might elicit the strongest N170 response. Thus, a stimulus presentation sequence was chosen whereby faces were presented peripherally, allowing a natural orienting response towards

the images for subsequent fixation. This allowed the initial N170 response to face presentation to be recorded, as well as the N170 responses to subsequent fixations. The results confirmed the predicted importance of the order in which faces are perceived, where the initial presentation alone elicits a face-sensitive N170 response, which is not apparent in subsequent fixations.

6.4 Future directions for FRP research

Within specific methodological boundaries, the FRP paradigm is well suited to investigate dynamic visual processing as it occurs through natural fixations. This expands the contexts in which cognition can be observed, and allows for a mapping between visual cognition and the eye-behaviours that cause and are caused by it. The ability to synchronise eye-tracking devices with neural recording devices is essentially in its infancy, and there is great potential for its successful application in a multitude of disciplines and contexts. For example, the results of studies 1 and 2 and previous ERP research suggests that the N170 response reflects a face detection process that is triggered by the initial presentation of a face but is no longer present in a subsequent fixation on a face. It would be interesting if future FRP studies measured the N170 FRP when the image of a face updated to a different stimulus (i.e., a watch, or an inverted face) in the middle of an eye-movement. This may provide insight into whether the initial perception of faces can modulate later neural processing when the images have been surreptitiously changed, such as with a boundary paradigm (Reder, 1973).

Another direction for future FRP research that may prove fruitful is the integration of FRPs with transcranial magnetic stimulation (TMS). Interesting face-sensitive effects have been observed through the application of TMS to areas of the occipito-temporal lobe at critical time periods immediately after stimulus onset (Pitcher et al., 2007). Further investigations of these effects may benefit from the co-registration of eye-movements, TMS

and EEG, whereby TMS pulses can be controlled and administered through an eye-tracker in response to fixations on specific stimuli while brain potentials are recorded.

Yet again, the FRP paradigm may prove well-suited to performing regression analysis on the relationship between eye-movements and elicited brain potentials during reading of extended text, whereby the volume of trials required by the averaging process in the FRP paradigm can be enabled through post-hoc analysis on complex eye-movement data rather than a strict control of the stimuli. This would allow for the collection of FRPs on a larger range of stimuli. For example, rather than manipulating the stimuli that is to be read in an FRP paradigm ahead of time, a range of texts may be selected by the participants, which can later be analysed to yield information about the words in the texts (such as word frequency, word length, cloze predictability, etc.) so that FRPs may be time-locked in conditions relating to these dimensions.

6.5 Final summary

The overall aim of this thesis was to progress the development and application of the FRP paradigm. To this end, we used FRPs to investigate the neural processes involved in the domains of face processing and word reading. In the process of conducting these studies, we gained insights into the methodological challenges of doing FRP experiments, and how those challenges may be met. It is hoped that the outcomes of the studies illustrate the considerable usefulness of the FRP paradigm. It is also hoped that the methodological insights provided by the research in this thesis, and by other FRP researchers, provide researchers interested in the FRP technique with the ability and willingness to address the range of experimental considerations that characterise the FRP methodology.

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