The space around us: Utilizing the Hand-Blink Reflex to model Defensive Peripersonal Space, and exploring interactions with State Anxiety.

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Abstract

Personal Space is a complex field, including boundaries used in social interactions, navigation and defence. The Defensive Peripersonal Space (DPPS) is a close proximity personal space used for defence, thought to be located within reaching distance. The DPPS is measured in individuals by the Hand-Blink Reflex (HBR), a blink reflex triggered by electrical stimulation of the median nerve at the wrist. By triggering the HBR while varying the hands distance from the face, an individual's DPPS can be approximated. The field of DPPS and HBR research is however new and the HBR method is highly complex. This study replicated a HBR testing method, finding support for the presence of the DPPS, while finding the method extremely delicate, and in need of refinement and clarification. It is anticipated that this study will add help refine the HBR testing method, and be a useful addition to the field.

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List of Abbreviations

AUC – Area under the Curve

BR – Blink reflex

DPS – Defensive Personal Space

DPPS – Defensive Peripersonal Space

EEG – Electroencephalography

EMG – Electromyography

EOG – Electrooculography

HBR – Hand-Blink Response/Reflex

ISI – Inter-Stimuli Interval

MUAP – Motor Unit Action Potential

MUAPT – Motor Unit Action Potential Train

MSC – Magnetic Search Coil

OO/OOM – Orbicularis Oculi/Orbicularis Oculi Muscle

PS – Personal Space

PZ – Polysensory Zone

SBR – Somatosensory Evoked Blink Response/Reflex

STAI – State-Trait Anxiety Inventory

VIP – Ventra Intraparietal

Chapter 1 Introduction

In the seminal paper "Defensive peripersonal space: The blink reflex…" by Sambo, Liang, Iannetti, and Cruccu (2012), a novel blink reflex (BR) was identified and theorized to be a useful measurement for a type of defensive personal space. The blink reflex called the Hand-Blink Reflex (HBR) was elicited by electrically stimulating the median nerve at the wrist and it was found that the HBR magnitude changed based on the hands relative distance to the face. The authors identified that this enhanced response could be explained by a close proximity personal space called the Defensive peripersonal space (DPPS). These findings allowed for further exploration of this reflex response and the investigation into the specific components of the brain mediating this change in response that construct the DPPS for the purpose of self-defence. Further refinement by Sambo and Iannetti (2013) clarified the HBR testing method by measuring the HBR at four hand positions and exploring the relationship between DPPS size and trait anxiety. Seeing the importance of this newly discovered space and method of testing, this study aims to replicate the procedure used in Sambo and Iannetti (2013) with two key aims. Firstly to act as a methodological replication, adding to the field of research and to further test the validity of the HBR testing method, and secondly to explore state anxiety in relation to the DPPS, complementing the original work that investigated trait anxiety.

The field of HBR and DPPS research is growing with numerous recent papers, (R. Bufacchi, Sambo, Stefano, Cruccu, & Iannetti, 2017; Rory John Bufacchi, 2017; S. B. Wallwork, Bufacchi, Moseley, & Iannetti, 2017; S. B. Wallwork, Talbot, Camfferman, Moseley, & Iannetti, 2016). Replicating the underlying method would support all past and future research and exploring state anxiety would act as a useful and interesting comparison to existing trait anxiety research.

Chapter 2 Literature

2.1 Personal Space

Personal space has long been known to both researchers and the layperson, but often underappreciated for its complexity. The field is broad, and encompasses many different concepts with different names depending on context. Descriptors such as social distance, personal distance, territory and simply space are common, with meanings and terminology varying within disciplines.

The first traces of personal space can be found in Sociology in the mid-1800s, with reference to the concept of "Social distance" (Laing, 1850). Here social and physical distance were distinguished, with Liang calling steam power a force to "annihilate the conventional distinctions, differences and social distance between man and man…" (1850, p. 1). These early definitions held emphasis on social rather than physical interaction, but over time the concept broadened to encompass more fields.

Work into personal space as it is understood now, specifically a social and defensive region surrounding a person, began in the 1930s (Ryan, 1938). The field grew slowly and was recognised as lacking, with Sommer (1959) putting it simply by stating "Surprisingly little is known about the way people use space" (1959, p. 247) and expanding later by noting the lack of studies regarding human interaction and space usage outside the lab. In their study Sommer (1959) sought to remedy this by conducting an exploratory study observing how patients from a mental hospital interacted with each other, based on their seat position at their cafeteria table. They discovered gender differences in seating, finding females sitting closer to females than males and schizophrenic patients appearing to present an impaired ability to respect others personal spaces.

During this time the field began to expand with the formalization of Proxemics by anthropologist Edward Hall (1963). Proxemics aimed to decode human interaction, and methods for this were constructed in a series of papers and books (Hall, 1963, 1966). These explored aspects such as body position, movement, breath and loudness of voice to construct a notation scheme for the "transaction" of human language. The papers aimed to construct a notation method that described the movements, positioning and attributes of people during interaction that could be used to formally and objectively describe human to human interactions.

2.1.1 Defensive Personal Space

Defensive personal space or defensive space is a form of personal space used for self-preservation and defence of an animal or human. Defensive personal spaces are constructed in the brain (Holt et al., 2014), and are used to delineate important zones that require defence, such as the face, used in protecting key organs such as the eyes. Intrusions into these Defensive personal spaces can trigger reflexes such as startle responses, defensive attacks or large movements away from the encroachment in addition to mental responses like anxiety, anger or sadness (Meisenhelder, 1982).

Defensive space is often monitored intuitively and many animals, including humans, have evolved systems to assist in self-defence to ensure a greater chance of survival. Examples of these systems are nociception which is responsible for pain and responses to pain, and proprioception which is the ability to intuitively determine the position and movement of an organisms own limbs, a system key to movement and spatial awareness. Nociception in particular has been known to exist in many animals and outwardly present itself in similar or identical ways to humans (Besson, Guilbaud, & Ollat, 1994). In humans these systems are typically intricate, with individuals intuitively keeping track of the movement, size, shape and sound of objects, and when keeping track of other humans, language and facial movements

are monitored. In humans the brain integrates useful information from many sources like proprioception, with visual, auditory and olfactory stimuli to assist self-defence and navigation. This information is used to intuitively determine a safe boundary space around the individual to give the best chance of self-defence from perceived threats, and allow the brain to pre-emptively prepare against these potential threats.

The specific brain structures or substrates that perform these tasks are however not well understood, with theories tending to focus on general regions and combinations of regions of the brain instead of specific structures. This field is however evolving, and in recent years many more functions related to specific brain regions are being identified and studied in depth. One case study of a patient (SM) with bilateral amygdala damage showed a smaller comfortable approach distance than healthy controls, providing support that the amygdala may help moderate personal space boundaries (Kennedy, Gläscher, Tyszka, & Adolphs, 2009). Mirroring this result, rhesus monkeys with bilateral amygdala lesions were shown to be more impulsive and spent more time close to novel objects (Mason, Capitanio, Machado, Mendoza, & Amaral, 2006). These findings support the idea that the amygdala acts as a protection system or "brake", helping moderate personal space and safety, and when this region is damaged, our internal constructions of personal space are weakened. Conversely, amygdala damage has been theorised to be related to Autism with autistic children maintaining a larger personal distance than typically developed children (Baron-Cohen et al., 2000). These findings together indicate that the amygdala is clearly involved in personal space but the specific interactions are likely complex and not well understood yet.

Other areas such as the Ventral Intraparietal (VIP) area and Polysensory Zone (PZ) in monkeys have been found to be related to Extrapersonal Space (Bremmer, Bremmer, Kaminiarz, Hoffmann, & Schlack, 2013) and defensive responses including startles (Cooke & Graziano, 2003; Graziano & Cooke, 2006). Other more complex interactions have been

observed such as connections between the Dorsal Intraparietal Sulcus and Ventral Premotor Cortex being correlated with personal space size (Holt et al., 2014).

Together these findings make it clear that defensive personal space and defensive responses are highly complex and the brain structures that govern them are heavily interconnected and nuanced, requiring more research to uncover the precise interactions.

2.2 Reflexes

One important system used in defending personal spaces are reflexes, which are fast involuntary reactions to stimuli, typically aversive stimuli. Neural pathways called Reflex Arcs trigger these responses, and allow for reactions to be triggered without involvement of the higher conscious areas of the brain. By bypassing conscious thought, it allows for responses at a speed not possible through conscious decision making, allowing an organism to react faster, to better protect itself and be safer.

These reflexes can serve a multitude of different purposes and can range in complexity. A well-known reflex, the knee jerk reflex or Patellar reflex was observed by Sir Michael Foster (1897) and is typically triggered by tapping the tendon below the Patellar, causing the leg to involuntarily jerk upwards. More complex reflexes like the Diving Reflex (Hurwitz & Furedy, 1986) exist in humans and other vertebrates, which triggers cardiovascular changes and blood flow redistribution when rapidly submerged in water. The purpose of this particular reflex helps minimize oxygen usage and temperature loss when diving into and being submerged in water.

2.2.1 Blink Reflexes

One important reflex is the Blink Reflex, a subtype of blinking behaviour used to protect and maintain the eyes due to their extreme importance in survival. Blinking broadly occurs as one of three types, Spontaneous, Voluntary and Reflex. Spontaneous blinks occur regularly at a

rate of approximately 17 blinks per minutes at rest (Bentivoglio et al., 1997), with variations based on an individual's current task in addition to individual differences, like age. These occur for the purpose of clearing the eyes of foreign bodies and small particles to help ensure vision is unobstructed and allow the eyes to function properly. In comparison voluntary blinks occur when the eye muscles are consciously contracted, which can occur for a variety of reasons based on the particular needs of a situation. Reflex blinks in contrast are a reflex and therefore occur automatically in response to a variety of different stimuli, including visual, auditory, or as a reaction to touch or to pain.

Attributes of the three types of blinks can vary, with voluntary blinks typically lasting longer with greater muscle activation, with spontaneous showing the least muscle activation (Kaneko & Sakamoto, 1999). Additional studies have shown that electrically induced blink reflexes were shorter than both voluntary and spontaneous blinks, and attributed age as a factor contributing to lower onset duration (VanderWerf, Brassinga, Reits, Aramideh, & Visser, 2003).

The blink reflex is controlled primarily by the Orbicularis Oculi muscle (OOM) that surrounds the eye and the reflex is comprised of two components called the R1 and R2. The R1 component is an early impulse that passes to the same side eye as the stimuli (ipsilateral side), with the later R2 component passing to both eyes (bilaterally) and is responsible for closing the eyes (Berardelli, Cruccu, Kimura, Ongerboer de Visser, & Valls-Sole, 1999). Measuring this specific blink reflex and other related eye movements can be performed in a variety of ways, each with their own strengths and weaknesses. Electromyography (EMG) can measure electrical activity in the muscles around the eye, typically the OOM, while Electrooculography (EOG) can measure electrical activity across the eyeball itself. More direct methods include using a Magnetic Search Coil (MSC), which involves placing a contact lens-type device containing a magnetic coil on the eye in order to measure electrical

activity as the eye and eyelid move. Other methods include manual coding and categorisation of blinks potentially assisted by high speed video footage, and using infrared light bounced off the back of the eye to determine blink occurrences (Cruz, Garcia, Pinto, & Cechetti, 2011). The method used in a given study will vary based on the requirements of the study, with each method having better or worse qualities and no method perfect in all cases. Studies that require concealment or non-invasive methods may use manual detection or EMG, while studies requiring more specific recording of the eyeball movement and qualities or gaze tracking could use the MSC method. This study utilizes EMG as used in the original study (Sambo & Iannetti, 2013), which benefits from high data clarity and being less invasive than the MSC method.

2.2.2 Electromyography

Electromyography (EMG) has been used since as early at 1952 (Kugelberg). Electromyography measures Motor Unit Action Potentials (MUAP) occurring inside a muscle during muscle contraction (Fridlund & Cacioppo, 1986). Potentials can occur in rapid success called a Motor Unit Action Potential Train (MUAPT). This electrical activity is measured using an electrode attached to the skin surface over a muscle (SEMG), or via a needle electrode inserted into the muscle itself. Surface electrodes are potentially inferior to needle electrodes, as they can lack specificity regarding which muscle group is being activated and measured (Perry, Easterday, & Antonelli, 1981). Despite this, SEMG and Needle EMG are very different procedures, useful for different purposes and can even be complimentary procedures (Sella, 2007). Another important factor in selecting between EMG methods is considering the invasiveness of each procedure and what is allowable for a given study. While SEMG can be less specific, it is significantly less invasive and simpler to implement than needle EMG, and the invasiveness of needle EMG can prohibit certain types of experiments or measurements.

EMG electrodes are typically placed in pairs, measuring the electrical difference that occurs between them. The resulting electromyogram is a stream of MUAPs superimposed over each other, ranging in frequency from 6Hz to 500Hz (Konrad, 2006). The raw data requires significant care and processing in order to be interpretable. Filtering of specific signal frequencies in addition to reducing data noise is required, followed by amplification, rectification and various smoothing procedures depending on experiment context (Konrad, 2006).

2.2.3 Hand-Blink Reflex

The Hand-Blink Reflex (HBR) is a specific type of Blink Reflex discovered recently, that is triggered by an electrical stimuli delivered to the median nerve at the wrist. This electrical pulse is calibrated for an individual to be non-tissue damaging and applied via an electrode on the wrist in order to excite the median nerve. The median nerve is one of three major nerves including the ulnar and radial nerves that send and receive impulses for the hand.

The HBR was first identified where it was theorized to constitute a novel and fundamentally different form of blink reflex (Hideto Miwa, Imamura, Kogahara, Ohori, & Mizuno, 1995). In this work, authors called the reflex a Somatosensory Evoked Blink Response (SBR) and identified that the SBR had longer durations and was more variable than typical startle responses, providing support that the SBR was a separate phenomenon worthy of study. They identified that this reflex could be triggered via stimulating the peripheral nerves, including through the anterior chest or neck in patients with Miller Fisher Syndrome. The reflex habituated (reduced after repeated exposure) rapidly and was not consistent across those tested. In further testing it was identified that stimulation of the lower peripheral limbs also triggered the SBR, however the response was smaller than via upper limb stimulation (H. Miwa, Yamaji, Abe, & Mizuno, 1996). In addition, they identified that the SBR produced significantly different EMG responses as compared to startle blinks in both clinical patients

and healthy controls. The procedure was also considered by the authors as a “delicate phenomenon”, specifically that habituation occurred easily with the SBR disappearing shortly after repeated stimuli. Similarly to the previous study, they identified the SBR was limited to only a small portion of patients and controls alike, with the reasons for the response inconsistencies remaining unknown.

Similar median-nerve triggered blink reflexes were studied by Valls-Sole, Valldeoriola, Tolosa, and Marti (1997) who investigated the differences between healthy controls and patients with Progressive Supranuclear Palsy, Ideopathetic Parkinson’s disease, Multi-system atrophy and Corticobasal Ganglionic Degeneration. Their method was more clearly specified, involved only median nerve based electrical stimulations and featured a unique method where the working intensity was searched for by progressively scaling up the intensity until a response was identified or consent was withdrawn. Using this new process, 40% of participants presented measurable facial responses from the orbicularis oculi and mentalis in response to the electrical stimuli.

Further refinement of Somatosensory based reflex testing was performed by Alvarez-Blanco, Leon, and Valls-Sole (2009) who investigated differences in response based on stimulation of upper or lower limbs. They identified that stimuli to upper limbs resulted in shorter response latency as compared to lower limbs, theorized to be caused in part due to shorter nerve conductance distance as the upper limbs are closer to the brain. This theory was tested explicitly by Sambo, Liang, et al. (2012) where support was found for an alternative theory, that the different blink responses between upper and lower peripheral limb stimulation was instead due to the limbs distance to the face and not due to nerve conductance distance. To do this, median nerve stimulated blink responses were triggered with the hand in two different positions, far and near the face, and authors found significant differences in responses between the different positions despite the nerve conductance distance remaining the same.

To support this alternative theory, stimulation of the suborbital nerve to elicit a blink response was performed and responses were measured finding no differences in responses based on hand position. In addition, EEG responses specifically the N20 wave associated with the arrival of the response to the brain, found no differences across different hand positions, which excluded mediation via higher brain structures. These findings together indicate that hand position mediates blink responses for median nerve stimulation, and that this mediation was potentially triggered by lower brainstem level circuits, not higher order cortical structures or within the eyes themselves. Additionally the testing procedure used a similar scaling up procedure to Valls-Sole et al. (1997) and by using higher intensities, lower habituation and a greater responder rate of 60% of participants was observed.

2.3 Defensive Peripersonal Space

During the study by Sambo, Liang, et al. (2012) it was theorized that one explanation for the differing responses based on hand position could be due to the presence of a type of defensive personal space. They called this new space the "Defensive Peripersonal Space" and theorized that blink responses were changing as the hand passed from outside to inside this space. They theorized that this defensive personal space was located within peripersonal or reaching space, acting as a close proximity defensive mechanism and that based on their findings this mediating relationship was potentially controlled by some form of brain stem level circuitry.

To explore the DPPS further, the HBR testing method adapted by Valls-Sole et al. (1997) was refined by Sambo, Iannetti, Forster, and Williams (2012) and explored how expectations of stimuli and presence of a vision blocking screen could modify HBR responses. They identified crucially that the HBR was mediated by expectations of stimuli, with HBR magnitude greater when the participants expected a stimuli to their hand located within their DPPS as opposed to outside their DPPS. In addition, they identified that HBR mediation was

removed when a screen was placed in front of the face, based on the theory that the screen modified and reduced the size of the DPPS as compared to simply blocking vision.

2.4 The HBR and DPPS research landscape

In recent years new and valuable research has been conducted regarding the DPPS, investigating its specific properties and its relation to other psychophysiological variables. A key investigation into the properties of the DPPS was conducted by testing four different hand positions with respect to the face, allowing for significantly greater data granularity and the ability to investigate whether the boundaries of the DPPS were gradual or abrupt (Sambo & Iannetti, 2013). This new method allowed for greater approximation of the shape of the DPPS, with the authors identifying that the DPPS had sharp boundaries, with the mean boundary location existing between 20 and 40cm from the face. Due to the greater granularity in measurement, the DPPS shapes could be approximated through statistical modelling, classified between four possible models of “Small”, “Large (Ramp)”, “Large (Step)” or “Extra Large”. These models described different possible boundary locations, with “Small” representing a boundary located between 4 and 20cm from the face, “Large (Step)” representing a boundary 20-40cm, “Extra-large” representing 40-60cm and “Large (Ramp)” as an alternative model. 40% of responding participants were classified as “Small”, 53% as “Large (Ramp)” and one participant as “Large (Step)”.

More specific attributes and interactions regarding the DPPS have also been investigated, such as gravitational effects (R. J. Bufacchi & Iannetti, 2016) by measuring the HBR when the hand is in various vertical positions in sitting and supine positions. It was identified that gravitational cues, specifically when the hand was vertically above the head as opposed to below, altered HBR magnitude. This indicates the DPPS adjusts to gravitational cues, helping defend against threats from above that may fall downwards to the face and body as we would expect objects to do in our natural environment.

This concept of threat expectation was expanded on in further work that explored the DPPS and HBR magnitude changes in relation to a moving arm (S. B. Wallwork et al., 2016). In addition to finding further support that HBR magnitudes were greater when the hand was inside as opposed to outside the DPPS, there were significantly different response magnitudes when the hand was moving away as opposed to towards the face. Response magnitudes were similarly large across distances when the hand was moving towards the face however they were diminished when the hand was moving away. It was theorized that HBR magnitudes were modified in real time based on the predicted position of the hand.

Further clarification of the shape of the DPPS has been performed by R. J. Bufacchi, Liang, Griffin, and Iannetti (2016), specifically that the shape of the DPPS could be modelled in a head-centred ellipsoid shape extending outwards, finding that defensive responses such as the HBR that occur within this space tended to increase non-linearly. This was supported by collecting past HBR experiments in addition to new experiments, to test HBR magnitudes when the hand is in a variety of positions surrounding the head, ranging from vertical, horizontal and frontal positions, as well as behind the head and positions where the head was rotated. This allowed for a comprehensive analysis of HBR measurements surrounding the head and a higher resolution estimate of the shape of the DPPS than was previously possible.

More specific neurological interactions involving the DPPS have also been investigated, such as trigeminal neuralgia (TN), a unilateral pain condition involving the facial trigeminal nerves (R. Bufacchi et al., 2017). It was theorized that because TN affected only one side of the face, this could result in an asymmetrical representation of the DPPS. This was based on the fact that TN could cause pain that was both very strong and triggered by mundane stimulation like gentle touching, making it unpredictable and startle-like. Using a similar four hand position method used by Sambo and Iannetti (2013), TN patients showed a larger DPPS surrounding the affected side of the face compared to their healthy side, while controls

showed an expected symmetrical DPPS. This finding further supports the theory that DPPS shape is affected by expectation, due to the developed expectation of greater pain on the affected side causing an expansion of the DPPS.

The specific brain circuitry involved in the HBR was also investigated in greater depth by measuring HBR magnitude across different hand positions in patients with early and late-onset blindness (S. B. Wallwork et al., 2017). It was identified that HBR mediation did not occur in a patient with Early-Onset blindness, while the mediation did occur in patients with Late-Onset blindness. This finding indicated that while vision is not a requirement to HBR mediation, the timing of the formation of the visual system was critical to how HBR was mediated. It was theorized that because the visual system does not fully develop until 3-7 years of age, if vision was lost after complete formation that the HBR mediation would remain stable despite this loss.

Developments in this field are wide and ongoing, featuring dramatic shifts in theory and understanding regarding the DPPS and its underlying mechanics. One such occurrence by Rory J. Bufacchi and Iannetti (2018), aims to reconceptualise peripersonal space and consequently defensive peripersonal space research. Both authors are key researchers in the field of DPPS and HBR research and have identified critical issues of definitions and terminology, in addition to growing evidence that these spaces may not have hard boundaries but could instead be reconceptualised as layered graded fields. This particular theory is of key interest as it contrasts the original paper featuring G.D. Iannetti (2013) a principle author, making this a dramatic shift in their own findings. This reconceptualization and desire to clarify terminology stems from the fields high complexity, and how interconnected the concept of personal space is with many brain structures, defensive behaviours, navigation and social interaction.

One area covered infrequently within DPPS research are interactions with psychophysiological variables. The original study by Sambo and Iannetti (2013) investigated trait anxiety and claustrophobia, finding support for an interaction between DPPS side and trait anxiety levels, however little else has been studied. This was theorized to be attributed to anxious individuals incorrectly perceiving threats closer than they actually were located.

2.5 Anxiety

The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013) describes Anxiety in its relation to fear. It describes fear as the specific response to a threat as compared to anxiety, which is the anticipation of a threat. Both anxiety and fear are useful tools, with anxiety and fear helping prepare reflex and defensive responses to ensure safety and survival (Marks, 1994). Anxiety however is not useful in all cases, and prolonged or strongly heightened levels of anxiety can cause distress and can be both physically and mentally debilitating. These are distinguished as clinical or diagnosable forms of anxiety. These can be diagnosed through the DSM-5 (American Psychiatric Association, 2013) or the International Classification of Diseases and Related Health Problems (ICD) (World Health Organization, 1992).

Diagnosable types of anxiety include Generalised Anxiety Disorder (GAD) indicated by excessive worrying that is frequent and lasting for a long period of time, usually 6 months or longer (Antony & Stein, 2008). Other types include Panic Disorders, characterised by periods of brief but intense anxiety, and Specific Phobias characterised by intense feelings of fear such as the commonly known condition arachnophobia, the fear of spiders (American Psychiatric Association, 2013).

To quantify levels of anxiety, three main inventories and scales are used, the Beck Anxiety Inventory (BAI), State-Trait Anxiety Inventory (STAI) or the Hospital Anxiety and

Depression Scale (HADS-A) (Julian, 2011). The STAI and the State-Trait Anxiety Inventory Short Form (Marteau & Bekker, 1992) in particular break anxiety down into two components, State Anxiety and Trait Anxiety. State Anxiety is a measure of Anxiety for the immediate situation whereas Trait Anxiety is a persisting measure of anxiety based on individual differences (Spielberger, 1966).

2.5.1 Anxiety and Personal Space

Anxiety, both clinical and non-clinical has long been known to be influenced by personal space. At a basic level, anxiety is triggered by anticipation of threat present in the world, and the location of this potential threat related to a person's personal space boundaries will affect how a person feels and their response.

Specific investigation into this relationship was explored by Taffou and Viaud-Delmon (2014) where fear inducing looming stimuli were found to cause an expansion of Peripersonal Space. This is believed to be achieved by individuals selectively underestimating the collision time of threatening stimuli, believing them to be closer or faster than they actually are. This would serve an important function by aiding survival and self-preservation, as it allows for an earlier and faster response, and therefore greater likelihood of safety.

A similar finding was found in the original paper by Sambo and Iannetti (2013), where Trait Anxiety was explored in relation to DPPS size. Authors identified that scores of Trait Anxiety had a positive relationship with DPPS size, with larger scores indicating larger DPPS size. It was theorized that anxious individuals having a larger DPPS size would allow for faster response to threatening stimuli, and allow for greater protection.

Chapter 3 Current research

The current research experiment is comprised of two major components:

1. To perform a partial replication of "Better Safe Than Sorry? The Safety Margin Surrounding the Body Is Increased by Anxiety" by Sambo and Iannetti (2013)
2. To manipulate participant State Anxiety levels and to investigate the effect on the HBR magnitude and the DPPS

3.1 Purpose of this research

The primary rationale for this research is to act as a replication of the HBR testing method used in many existing and modern studies in order to test its validity as it is currently described. The field of DPPS research is presently very new, and therefore understandably localised to a small set of research labs and groups of researchers. This results in a clustering of a small number of researchers across a substantial portion of papers present in the field. In particular, present and past members of the Iannetti research lab (Iannetti Lab, 2009), notably Dr Giandomencio Iannetti, Dr Rory Bufacchi and Dr Chiara Sambo have contributed substantially to many papers in the field. This, like any new field of research engaging in novel testing methods can result in scepticism both of the new methods and of the theories they relate to.

The current research therefore aims to provide an isolated replication, with no ties to any existing researcher or lab. Additionally, as the research is independent from existing work, it reduces both perceived and actual bias towards any particular research result. This research has the benefit of standing separate to existing work, while acting as a useful comparison. This decision does have flaws, due to my relative inexperience in the field and lack of direct support from the principle authors, it is possible I could perform methodological errors or

misunderstand qualities of the research. In order to counteract these difficulties I have chosen to replicate an existing experiment as closely as possible, with some alterations and expansions. Additionally I have elected to provide full raw data and an explanation of all statistical methods used throughout this experiment to limit problems of multiple comparisons or "p-hacking". I believe based on these factors my work would prove useful to the field even in the event of errors or flaws.

The specific four hand distance method by Sambo and Iannetti (2013) was chosen based on a balance of practicality and granularity of possible results. The method utilizes 4 hand positions, while earlier methods utilize only two distances (Sambo, Iannetti, et al., 2012), and later methods use 3D positional tracking of the hand (S. B. Wallwork et al., 2016) or used a variety of methods with different hand positions across several experiments (R. J. Bufacchi et al., 2016). These later methods are not practically feasible for a Masters level project and earlier methods would not provide a satisfactory depth of analysis. Based on these factors the 4-position method by Sambo and Iannetti (2013) was considered an adequate compromise of data granularity and practicality for a project of this level.

Finding support for the HBR and DPPS testing method would prove valuable to the overall field, helping underpin and support all consequent research, whereas failing to find support would act as an interesting contrast to existing research in the field.

The second component of the research, the State Anxiety manipulation, was chosen as a complement to the Trait Anxiety research from the original study by Sambo and Iannetti (2013). Presently investigation into DPPS in relation to psychological measures is very limited and no known research has explored the relationship between state anxiety and DPPS size yet. In order to investigate state anxiety the current experiment was required to be modified as compared to the original study by Sambo and Iannetti (2013). In the original

study trait anxiety was measured with no specific manipulations, as it is broadly a measure of long term person-specific anxiety. Because state anxiety is situation specific a manipulation must be included in order to induce higher levels of state anxiety as compared to a control group. Inducing anxiety and other emotions has been achieved through the use of an Autobiographical Writing Task (Baker & Guttfreund, 1993), with the task being reasonably short and unintrusive. This Writing Task has participants write for approximately 10 minutes about the saddest moment in their lives. The theoretical basis behind this task is that by having participants relive a particular memory, specifically an extremely sad memory, the memories can induce emotions felt during that event. Therefore I adapted this task, whereby participants recount an extremely anxiety inducing memory and pairing this with a short-form of the STAI used to specifically measure state anxiety. This method was chosen due to the experiments long length, a task that was quick to complete was desirable as it would allow for all testing to be completed in a single session. Additionally both the task and the manipulation check of the STAI-Short form are easy to understand and easy to score.

3.2 Specific Objectives of Research

The primary objectives of this research are composed of two confirmatory hypotheses, aimed at testing the existing HBR method and evaluating evidence for a non-linear relationship that would indicate support of the DPPS. The second component includes the Exploratory Hypotheses concerned with replication of the specific DPPS models, and the exploratory element investigating the relationship between state anxiety and DPPS. All confirmatory and exploratory hypotheses stated here were pre-registered through Open Science Framework (OSF) (Colville, Philipp, & Barnes, 2018).

3.2.1 Confirmatory hypotheses

Hypothesis 1 - The EMG magnitude recorded from the orbicularis oculi muscle is positively correlated with nearness of hand position to the face when a calibrated electrical stimulation is applied to the median nerve of the positioned wrist.

Hypothesis 2 - The relationship in Hypothesis 1 is non-linear.

3.2.2 Exploratory hypotheses

Exploratory hypothesis 1 - For specific participants where Hypothesis 1 and Hypothesis 2 apply, the participant specific relationship in Hypothesis 1 can be described by one of 4 non-linear piecewise functions. The models are described in arbitrary units across the four hand distances “ultra-far”, “far”, “near” and “ultra-near”, also called Position 1, 2, 3 and 4.

- Small. In arbitrary units, (-1, -1, -1, 3). Constituting an abrupt change between the near and ultra-near hand position.
- Large (ramp). (-1.5, -1.5, 0.5, 2.5). Constituting an abrupt change between far and near, followed by a linear relationship.
- Large (step). (-1, -1, 1, 1). Constituting an abrupt change between far and near.
- Extra-large. (-3, 1, 1, 1). Constituting an abrupt change between ultra-far and far.

Exploratory hypothesis 2 – Does the manipulation of State Anxiety affect the way participants respond? Specifically does it affect factors such as HBR magnitude, the type or strength of model that best describes the relationship in Hypothesis 1 or increase or decrease DPPS boundary distances?

Chapter 4 Method

4.1 Research Design

The design is a 4x2 mixed experimental design consisting of one within subject independent variable, participants hand distance from face (4cm, 20cm, 40cm and 60cm), and one between subjects variable, completion of the Anxiety writing task or the neutral writing task. The design is based on the design from the original paper by Sambo and Iannetti (2013).

4.2 Participants

4.2.1 Sample size

The targeted sample size at pre-registration was 50 participants. This was not decided based on a power analysis, but was chosen based on the maximum number participants that could be compensated with the available budget for the study. Participants were compensated with $20NZD, which was deemed appropriate for the 1-2 hours required of a participant's time, to offset participant's potential unwillingness to participate due to the presence of electrical stimulation and as reasonable compensation due to such electrical stimulations. A financial grant from Massey University consisted of $1000NZD and 50 participants at $20 with no other costs would constitute the entirety of this $1000.

Additionally, because the study is a replication the sample size of the original research must be considered with respect to the target sample size. The original study by Sambo and Iannetti (2013) used 25 participants, therefore a minimum of 25 participants was necessary with 50 participants as an ideal maximum. Ultimately 32 participants were recruited.

4.2.2 Recruitment

Participants were recruited from the general student population of Massey University Manawatū campus. Participants were required to be 18-40 years of age, healthy and available

for 1-2 hours. Advertising posters were placed on noticeboards in lecture and department buildings, common areas and study areas. The posters were present from June 6th to August 31st. The posters stated the time and age requirements, requested that participants be healthy, the intention to study reflexes, that electrical stimulation was involved and the compensation provided. Experiment scheduling was processed through the website Setmore, however participants could contact me via email at any stage. Through email participants could request more information on what was involved, however specific information about the purpose of the study or what was being measured was not provided until the conclusion of the experiment.

4.2.3 Participant criteria

Participants were required to pass a health screening form, see Appendix 2 – Health Form. The health screening form was adapted from the Physical Activity Readiness Questionnaire (PAR-Q), requirements from the Massey University Code of Ethical Conduct and research specific conditions. The health screening form was used to highlight any potential health concerns for the purpose of participant safety. Questions included presence of any cardiovascular conditions, pain or injury located in upper limbs, torso neck and head, medication usage, and objections to physiological measurement. If participants indicated any condition that I did not fully understand, I consulted with my supervisors to determine if the participant could safely participate in the experiment. Participants were also encouraged to write down any major injuries, conditions or presence of pain, even if the conditions only held a small possibility of being relevant to the study. These conditions could then be discussed with me in order to determine if they could pose a safety concern.

All participants were open to discussing health issues, and to the best of my knowledge clearly understood the purpose of the health screening form. In general participants were

healthy, with the most common health concern being generalized back pain and miscellaneous sprains and aches.

Several participants presented health conditions which were either considered non-serious or were screened as low risk via my supervisors. Due to an underlying medical issue, one participant was excluded from the study based on my decision, supported by supervisor consultation.

No participants disclosed any medications that could have interfered with their ability to sense discomfort or pain, or disclosed any cultural or religious sensitivities regarding physiological measurement.

4.2.4 Stopping conditions

Recruitment was slated to cease on August 31st or after a maximum of 50 participants. Recruitment ended on August 31st with 32 participants tested.

4.3 Ethics

4.3.1 Informed voluntary consent

Participants were provided with an information sheet, a health screening form and a consent form, in that order. The information sheet contained a broad overview of the study, and explained the specific discomfort that may be involved as well as ethics committee notifications, contact and legal information. Participants were encouraged to ask questions for any aspect that they did not understand to ensure that participants were knowledgeable about the experiment that they were participating in, and knew their rights as participants before they agreed to participate.

To the best of my knowledge no participant was lacking for information, or brought to my attention that there was information lacking. Several participants requested experiment

information before the experiment which I could not answer, but were satisfied with the information being provided at the conclusion of the study.

Several participants were curious about the precise purpose of the experiment at its conclusion. I answered any and all questions that participants asked, only requesting that they not discuss the study openly if they knew others that were interested in participating.

4.3.2 Minimisation of physical harm

The study contained two elements of potential physical harm to participants.

The first element was in the use of Electromyography. Prior to beginning the experiment I received training for EMG preparation from Dr Michael Philipp. When attaching EMG electrodes to a participant, electrodes must have a clear connection to the skin. Because of individual differences such as skin cleanliness and texture, the skin must be sufficiently prepared to improve the accuracy of the equipment. This process includes a cleaning of the electrode sites with cleaning products, followed by alcohol wipes, followed by abrasion and repeating this process several times until a sufficient connection is made. The combination of alcohol wipes and skin abrasion can cause discomfort or mild pain in some participants, especially due to the EMG location under the eyes being a particularly sensitive location. Minimisation of discomfort was achieved by clear communication with the participant during preparation, and if the participant was particularly uncomfortable, I ceased skin preparation then tested and used the equipment as is. Presence of allergies to any cleaning products was also screened at this time.

No participant refused skin preparation and a majority of participants showed no difficultly with skin preparation, and for the participants with sensitive skin this was prepared as best as participants allowed.

The second potential for harm was in the use of Electrical Stimulation. Prior to beginning the experiment I received training on the use of the DS7A electrical stimulator from Dr Matthew Barnes and sought out and received Comprehensive First Aid Training through the New Zealand Red Cross. Discomfort from electrical stimulation is highly subjective and varies based on many factors such as skin thickness, fat and muscle deposits, pain tolerance, specific electrical intensity and the stimulation location. Electrical stimulation when used improperly may also lead to burns or serious health issues (Jones & Johnson, 2009). To mitigate as many concerns as possible, electrical stimulation intensity began at below a perceptible level of 0.5mA in all participants, and was progressively increased with consent until a working intensity was found. No participants were able to detect the 0.5mA level. Participants could withdraw consent at any point and it was emphasised to participants that they should communicate clearly and frequently about their level of comfort.

Because of individual differences in wrist structure and the difficulty of electrical stimulation application, positioning of the stimulator over the median nerve proved to be difficult. To assist this, I asked participants to indicate if the stimulations felt “sharp”. Sharpness was defined as the feeling the stimulations to be more pinpoint and focused at the stimulator site, as opposed to feeling the stimulations spread more generally throughout the hand. I identified that feelings of sharpness indicated the electrode was positioned incorrectly, and was transmitting the stimulation primarily into the skin and muscle of the wrist, not into the median nerve, leading to this stronger discomfort. If participants stated that the stimulations felt sharp, I adjusted the position of the electrode up to three times and requested the participant compare the stimulations to the prior position. This served to reduce feelings of sharpness which could be uncomfortable, and to ensure the stimulator was positioned as close to the median nerve as feasible.

Because working intensities varied from as little as 6mA to as much as 80mA, the scaling procedure varied across participants. In general intensities would begin below the perceptible level and increase in small increments with the increments increasing over time. A generalised intensity increment schedule can be seen in Table 1 - Intensity increment schedule.

Table 1 - Intensity increment schedule

Intensity	Increasing increment
1.0 – 8.0mA	0.5 – 1.0mA
8.0 – 18.0mA	1 - 2mA
18.0 – 30.0mA	2 – 4mA
30.0 – 80.0mA	4 – 6mA

When participants indicated the intensity was at or close to their tolerance, they were asked if they could tolerate more and were allowed to request the intensity be scaled back if it exceeded their tolerance. Because of individual differences in tolerances as well as differences in personal definitions of discomfort and pain, the terms “discomfort” and “pain” were de-emphasised throughout the experiment. Instead “tolerance” was emphasised, which was found to better encapsulate the objectives of the study while balancing ethical concerns of harm.

Participant 6 presented an adverse reaction to the stimulations during calibration not due to any malfunction or experimenter error. The participant was compensated fully and debriefed and did not continue in the experiment, but is included in the study for demographic statistics.

4.3.3 Minimisation of mental harm

Potential for mental harm was present through the anxiety writing task. Triggering feelings of anxiety can be unpleasant depending on the strength of these feelings. Conversation with participants during the writing task was minimized where possible, limited to brief instructions to avoid influencing participant's feelings or mood in unintended ways.

At the conclusion of the experiment all participants regardless of anxiety or control task were debriefed and provided with any clarifying information regarding the task. No participants had observable levels of distress or discomfort at the conclusion of the study, and no participants directly expressed discomfort due to the writing task.

4.3.4 Confidentiality

Data obtained in the study is comprised of two types, Electromyography data, and writing task data. EMG data is not directly identifiable to any given participant and therefore there were no concerns regarding its storage. The writing task data consisted of writing from the participant about either their trip to the University or about a time they felt strong feelings of anxiety. They were encouraged to use initials instead of full names, and the form itself only recorded the date and their participant number. The second component was their answers for the STAI Short Form, which also only indicated the participant number.

The only method of identifying individual responses would be to correlate either consent forms or the payment acknowledgment form with individual responses. Forms were kept separate and at the conclusion of the study, the payment form, consent forms and health forms were provided to Massey University School of Psychology for storage and security.

Additionally participants were entitled to a copy of their results and individual responses, which was requested by four participants.

4.3.5 Deception

Participants were not directly deceived about any element of the study, however concealment was used. The precise purpose of the study was not disclosed to participants, however they were made aware of this fact, and told truthfully that knowing the precise purpose could interfere with results. Specifically, participants were told that the study aimed to "measure their reflexes".

All participants were happy with this explanation, and in the event participants wanted more information they were provided with a full explanation of the study and its purpose at the conclusion of the study.

This concealment was necessary because of the possibility of participants becoming self-conscious of their eye movements if they were aware of the studies aim to measure their eye activity. If participants became self-conscious of their eye movements, this could supress natural or reflex blinking, or result in abnormal blinking patterns.

4.4 Measures

4.4.1 Writing task

Participants completed either an anxiety writing task, or a control writing task, see Appendix 4 – Anxiety writing task and Appendix 5 – Control writing Task. The writing task was based on work by Baker and Guttfreund (1993) and aimed to induce feelings of anxiety, or act as a control. The anxiety writing task asked participants to write about an incident that caused strong feelings of anxiety, and the control task asked participants to write about their trip to the University. Participants were given 5 – 10 minutes or until they had nothing else to write about.

4.4.2 State Trait Anxiety Inventory (STAI) Short Form

Following the writing task, participants completed the STAI short form by Marteau and Bekker (1992) as a manipulation check for the writing task. The STAI short form contains 6 questions from the state scale of the full STAI form. Three of these questions are positively coded, “I am tense”, “I feel upset” and “I am worried”. Three questions are negatively coded, “I feel calm”, “I am relaxed” and “I feel content”. Possible responses are 1 = “Not at all”, 2 = “Somewhat”, 3 = “Moderately” and 4 = “Very Much”. The questionnaire is scored by adding up the positively coded scores, and adding the inverse of the negatively coded scores (e.g. a score of 1 becomes 4, and 3 becomes 2). This gives a score range of 4 indicating the lowest state anxiety, and 24 indicating the highest state anxiety.

4.5 Equipment

A Powerlab 4/25T unit with Chart software (AD Instruments, Australia) and associated PC were used to record electromyography data. The unit and EMG electrodes, conductive gel and cleaning equipment were supplied through Massey University School of Psychology and School of Sport, Exercise and Nutrition.

A Digitimer Constant Current Stimulator (DS7A) unit was used and supplied through the Massey University School of Sport, Exercise and Nutrition, paired with a bipolar surface electrode.

4.5.1 Equipment settings

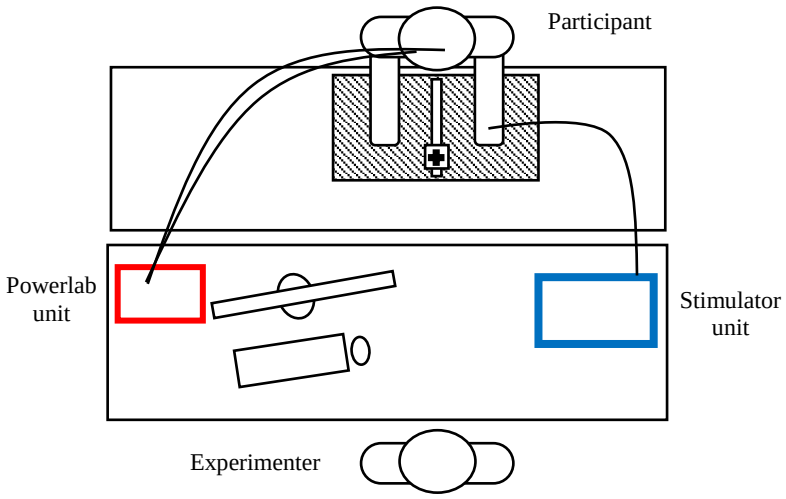
Electromyography settings a 10000 Hz sampling rate, a hardware 10Hz high pass filter, followed by a digital 50 Hz high pass and 500 Hz low pass filter setting. Additional software based full-wave rectification and smoothing settings were used for visual inspection only.

4.5.2 Setting

The experiment was conducted at the Practical Teaching Lab at the Manawatū campus of Massey University.

Participants were seated in a comfortable armless adjustable chair, sitting with their chest close to a large table. Seat height was adjusted individually to satisfy arm position requirements. Participants placed their elbows and forearms on a piece 2-3in thick foam. A piece of tape was placed along the centre of the foam to align participants and along the tape a black fixation cross measuring 1.5x1.5cm was marked at 45° vertically from the estimated eye position. The experimenters table was positioned in front of the participants table, and held the recording PC, Powerlab unit and Stimulator unit. From this position, I could view live data on the PC and adjust stimulation intensity via the Stimulator unit with full view of the participant.

Figure 1 - Drawing of experimental layout



4.6 Procedure

1. Present participant the Information Sheet, see Appendix 1 – Information Sheet, Health screening form, see Appendix 2 – Health Form and Consent form, see Appendix 3 – Consent Form. Reiterate procedure to participant and answer questions.
2. Prepare EMG and electrostimulation sites and attach electrodes, test for impedance and repeat if necessary.
3. Begin "**Scaling up procedure"** for first hand for that participant.
    a) Settings are a 200 microsecond square wave single pulse, beginning at ~500µA (0.5mA) to the wrist site.
    b) Increase stimulation intensity progressively based on participant feedback, allowing for 10 – 20 between stimulations. If stimulations feel sharp, reposition the electrode. Once participants indicate they can feel the stimulations, give participants headphones playing white noise, and ensure they can still hear instructions.
    c) Participants should rest their arms on a soft surface with palms face up, staring at the fixation cross 30cm away at a 45 degree downward angle.
    d) Continue increasing stimulation intensity until either a blink response is observed, the participant withdraws consent to further increases or 80mA is reached. If a blink is observed, repeat the stimulation 2 more times to ensure consistency, increasing and repeating if responses are inconsistent.
    e) If a consistent response is identified, participants are classified as Responders. Participants who withdraw consent or reach 80mA are classified as Non-Responders. Both complete the full experiment but all Non-Responder data is considered exploratory. The working intensity used for stimulated wrist is the maximum intensity the participant could tolerate.

4. Begin **"HBR procedure"** for the wrist used in the scaling up procedure.
    a) Position arm marker board by participant and instruct them on the four arm positions and the procedure, adjusting chair height where needed such that the hand never touches the face. Play white noise.
    b) Using the participants predetermined hand position order (pseudorandom such that no more than 2 of the same distances are repeated consecutively), announce the first location using Position 1 to Position 4. Wait 30 seconds before triggering a stimulus to wrist at the previously recorded working intensity. Repeat 32 times using the participants hand position order allowing 30seconds between each stimulus.
5. Following the first Block, disconnect EMG cables from equipment but keep electrodes attached to participant and allow for a 5 minute break, then reconnect equipment.
6. Perform "**Scaling up procedure"** for second hand. Working intensity may be different.
7. Begin **"Writing task"**
    a) Participant completes their assigned task, either the anxiety writing task or the control writing task, see Appendix 4 – Anxiety writing task and Appendix 5 – Control writing Task
    b) Participant complete the State Trait Anxiety Inventory short form, see Appendix 6 – STAI Short Form.
8. Perform **"HBR procedure"** for second hand.
9. Experiment conclusion. Disconnect all equipment, debrief and compensate participant.

4.7 Procedure alterations

Due to the large time and resource requirements to test participants, it was not possible to identify all procedure errors prior to beginning testing, and consequently there are differences between the pre-registered procedure and final study.

During testing the custom specific data reduction and analysis script was adapted over time as edge cases were identified and requirements of the study changed. It was not possible to construct this script prior to collecting data due to lack of experience analysing data of this type.

Procedural changes include introduction of a break between Blocks 1 and 2 for participants after Participant 1, based on feedback. Additional changes include rephrasing and clarification of requirements for participants after Participant 8 due to confusion and misunderstanding of requirements. The layout of the experiment was changed at this time to limit participant view of live data, and to provide a better view of the participant throughout the experiment.

Chapter 5 Data Reduction

Data for the experiment is primarily EMG data. Data reduction for EMG data is non-trivial, requiring multiple stages of data transformation for data to be useable or interpretable with many transformations not having objective parameters, with various approaches and perspectives on EMG data reduction possible.

Raw EMG data is comprised of stochastic positive and negative values sampled at 10000 Hz (10,000 samples per second). The structure of data reduction is first reduction of noise in the raw data through hardware and software filtering, followed by full-wave rectification, application of a smoothing function followed by trimming to 200ms sections per trial. At this stage data reduction is complete, and can be followed by onset/offset calculations, blink validity determination and Area under the Curve (AUC) calculations, followed finally by higher order statistical analysis.

5.1 Noise reduction

The first step when processing physiological data is noise reduction. Noise with regard to physiological measurement is any signal or component of a signal that is considered undesirable (Cacioppo, Tassinary, & Fridlund, 1990). Noise can originate from many different sources, such as physical limitations of equipment, imprecise or limited recording specificity and limitations due to human physical factors such as high skin conductance or inconsistent responses.

5.1.1 Reduction of noise from Equipment

The most pervasive type of noise is 50 – 60 Hz electrical noise from AC power present in electrical equipment such as the Powerlab recording unit, the Digitimer Stimulator unit and the PC recording unit. To minimize this a specified notch filter is used. AC noise is also

minimized by using electrically shielded high-grade equipment with up to date electrical certification, which was present for this experiment. In addition the Digitimer unit, Powerlab unit and PC were placed as far away from each other as practical, with the stimulator cables and EMG recording cables kept separated and not touching at all times. EMG cables were separated from all power and data cables where possible. These measures ensure limited electrical interference between the different devices and cables.

To minimise noise from the electrodes, each pair of recording electrodes was tested before and during the experiment using a Model 1089 Mk III Checktrode unit to ensure they were working correctly. During the experiment, electrodes were exchanged if they were shown to have inconsistent or unusual readings.

5.1.2 Noise due to skin

Build-up of dead skin cells, dirt and hair on the skin can interfere with measuring electrical activity via EMG by acting as a barrier and disrupting measurement. In order to achieve the clearest measurement possible, this debris should be removed and the skin prepared to ensure the clearest connection possible. Therefore as recommended by Konrad (2006) and broad EMG best practises, a skin preparation procedure was performed with each participant. The skin sites were firstly cleaned with soap, followed by iterations of alcohol wipes and abrasion. The soap and alcohol wipes ensured the skin was clean and sterile, and the abrasion process allowed for an improved recording surface by roughening the skin and increasing available surface area for recording. Following the skin preparation process, electrodes were attached and tested for impedance using a Model 1089 Mk III Checktrode unit, with an ideal target of 5-10 kΩ (Konrad, 2006, p. 21). Skin preparation was repeated up to four times based on participant feedback, and for participants who expressed discomfort the skin was prepared as much as possible.

5.2 Raw Data

During the experiment, the Chart software by AD Instruments recorded and presented live EMG data for both the left and right eye. It also presented the marker channel that indicated when the stimulator had been triggered, and presented rectified and smoothed left and right eye readings for visual reference. The raw electromyography data is comprised of positive and negative values measured in either Microvolts (mv) or Millivolts (μV).

5.2.1 Sampling rate

The minimum required sampling rate is determined by the Nyquist frequency for EMG based muscle activity recording. The Nyquist theorem states that the sampling should be double the highest frequency you wish to sample (the low-pass filter frequency). Based on a maximum useful frequency and low-pass filter of 500Hz, this results in a minimum sampling rate of 1000Hz.

The original study by Sambo and Iannetti (2013) used a higher sampling rate of 8192Hz. Sampling rates above the Nyquist frequency are beneficial due to the increased resolution of the data, however higher sampling rates increase overall data size and therefore data processing and analysis time, which can become prohibitive depending on the resources available. The Chart software I used allowed for sampling at 1000Hz or 10000Hz, and it was initially deemed impractical to sample at 10000 Hz, therefore 1000Hz was used. After testing Participant 1 I sufficiently improved the data analysis code to allow for 10000 Hz sampling and consequently all following participants were tested at a 10k Hz sampling rate. To simplify data reduction and analysis I decided to up-sample Participant 1s data to 10k Hz. This was achieved by duplicating each millisecond of data and stretching it over each set of ten 0.1ms intervals present in 10k Hz data. This method is imperfect, and unusual edge cases

can occur during analysis, however this was considered an effective compromise due to the potentials errors associated with analysing data at different sampling rates.

5.2.2 Filtering

Electrical activity data is composed of many different overlapping signals with different frequencies. Depending on the goals of EMG, only specific frequencies are considered useful and therefore specific frequencies are included or removed, or bands of frequencies included or removed.

In line with the original paper by Sambo and Iannetti (2013), a similar filtering process was used using a 50 Hz high pass filter, and a 500 Hz low-pass filter, which restricts frequencies to a band of 50 – 500 Hz. A notch filter was also used to counteract noise from AC power sources.

5.2.3 Data export and import

For each participant, raw data of Blocks 1 and 2 from the Chart software was exported as a tab-delimited text file. Each row of the file constituted a measurement containing the time, marker value (used to identify stimulator activation), left eye raw value, right eye raw value and comments (used for identifying the distance for each trial). Data was then imported into a custom data analysis code.

5.3 Initial EMG processing

Blocks 1 and 2 of raw data were first combined into a single dataset. The filenames were formatted to include useful information, to avoid repeated manual entry e.g. “Participant 8 - NR - L - T - Block 1 - 10k”. This format indicated the participant number, responder status of “RS” or “NR” (Responder or Non-Responder), initial tested hand of “L” or “R”, testing condition of either “T” or “C” (test or control), block number and sampling rate.

Marker values used for identifying stimulator activation were analysed for values in excess of 1 volt. The marker channel typically reads ~0.00V and peaks at ~5.00V when a stimuli is triggered. This marker channel in line with EMG data allows for identification of useful data windows. 400ms of data was taken, 100ms prior to stimulation, 200ms of target data post stimulation and 100ms of further data, with an expected 64 sets of data. Excess data was taken as smoothing functions destroy half their smoothing width of data at the beginning and end of datasets, and to allow for various useful pre-stimulus and post-data thresholds to be calculated.

5.4 Rectified Data

Raw data is not generally useable, and must be reduced in stages in order to be interpretable. The first stage is rectification, which transforms the data by taking absolute values, removing any positivity or negativity as these features are not usually useful to analysis.

Left and right 400ms data windows were then full wave rectified separately. Full Wave Rectification is the process centring the data to a baseline of zero by subtracting the mean from each value, followed by taking the absolute value. Centring the data to zero helps to correct for offsets due to equipment issues, and rectification allows for overall magnitude of data to be more interpretable.

5.5 Smoothed Data

After rectification, data is still not readily interpretable as it is heavily stochastic in nature. Because of this a smoothing function is used. Typical smoothing functions used are rolling or moving mean or median filters that use a specified window width. This function takes a specified window width of values and calculates the mean or median, then steps one value along repeating this process for the full dataset.

Left and right rectified data windows were then smoothed using a rolling mean function with a width of 10ms (101 0.1ms time intervals). These parameters were based on those used by Sarah B Wallwork (2016), and were determined to be an effective smoothing method and potentially similar to what was used in the original paper by Sambo and Iannetti (2013). Following this the left and right data windows are averaged to an overall reading across both eyes. Smoothed data for left and right eyes were kept stored for further analysis and can be paired with tested hand data to classify them as ipsilateral eye (same side as tested hand) or contralateral eye (opposite to tested side) data. Trials are then marked for hand position, specific tested hand (left or right), and trial and block number.

Chapter 6 Results

6.1 General Experiment statistics

For clarity, as Participant 6 did not complete any trials, they are included in all Demographic and Impedance statistics but treated as a Non-Responder with no valid trials in all other analyses.

6.1.1 Demographic statistics

The mean participant age was 26.5 years old, with a standard deviation of 6.8 years with one participant not stating their age which was compared to expected ages (Massey University, 2018a). Gender distribution was predominantly female as expected (Massey University, 2018b) with 59% female, 19 out of 32 participants.

Ethnicity of participants was not recorded. Handedness of the participants was not recorded due to experimenter error. However previous studies do not consider handedness a variable of significance, and a majority of data analyses use pooled left and right hand results which would remove any artefacts related to handedness if they exist.

6.1.2 Group allocation

Starting hand allocation was 47% (15/32) for left hand and 53% (17/32) for right hand, with an expected value of 50%. Condition allocation was 53% (17/32) for the writing task condition, and 47% (15/32) for the control condition with an expected value of 50%. The allocations are not precisely 50% due to recruitment ending prior to the 50 desired participants, and starting hand and condition allocation was coded prior to recruitment based on expected participant number. Hand and condition allocation only diverged by one participant from the 50% expected split and therefore is not considered a problem.

6.1.3 Impedance statistics

Table 2 – Mean impedance statistics

Group	Left eye	Right Eye	Reference	Range
Overall	14.9 kΩ	7.2 kΩ	6.9 kΩ	1.2 – 36 kΩ
Responder	14.7 kΩ	7.6 kΩ	7.4 kΩ	1.2 – 32 kΩ
Non-Responder	15.1 kΩ	6.9 kΩ	6.4 kΩ	1.5 – 36 kΩ

Impedances of the three sets of electrodes were recorded for each participant to identify any potential problems with skin preparation.

A noticeable pattern was observed for the left eye with impedances being higher than the right eye impedances, with the reference impedance being the lowest. The lower reference impedance can be attributed to the forehead site being less sensitive, therefore able to be cleaned more without discomfort and less likely to have makeup present as compared to underneath the eyes, and therefore lower average impedance. The higher left eye impedance can be attributed to a flaw in my specific skin preparation method. For each participant, I arranged cleaning equipment on the table on the right hand side of where the participant was sitting and cleaned the skin sites with the participant either facing forward or slightly towards me. This meant in almost all cases I was standing on the participant's right side, reaching over the participants face to clean the left eye site and likely meant the left eye site was not cleaned as well as the closer more easily accessible right eye site. Unfortunately I was not aware of this systematic issue until the conclusion of the experiment. Left and Right impedances differences are statistically significant with $p = .0013$. Despite the significant differences in impedance values I do not believe it affected the data quality in a noticeable way. Best case EMG impedances are recommended to be 1 – 10 kΩ (Konrad, 2006, p. 21) with a maximum of 30 kΩ, and therefore a mean of ~15 kΩ falls within this range.

Additionally, the responses in this study are large scale blink responses and therefore are less affected by higher impedance values compared to muscle groups with finer or smaller activity. Furthermore the signal for each eye in every participant was inspected and additional cleaning was performed whenever possible, ensuring the lowest impedance for each eye where possible.

6.1.4 Intensity Statistics

Table 3 – Mean intensity statistics

	Block		Hand		Overall
	1	2	Left	Right	
Thumb-twitch					
All	16.1 mA	16.4 mA	15.5 mA	17.1 mA	16.3 mA
Responder	18.4 mA	17.6 mA	17.1 mA	18.9 mA	18.0 mA
Non-Responder	14.0 mA	15.1 mA	13.9 mA	15.2 mA	14.6 mA
Working intensity					
All	33.3 mA	31.0 mA	31.6 mA	32.7 mA	32.2 mA
Responder	36.0 mA	34.5 mA	35.6 mA	34.9 mA	35.3 mA
Non-Responder	30.4 mA	27.0 mA	27.1 mA	30.4 mA	28.8 mA

Electrical stimulator intensities were recorded for each participant, specifically the intensity needed to elicit a supramaximal thumb-twitch (1-2cm thumb-twitch), and the working intensity used throughout the main experiment for a given participant. An electrical stimulation sufficient to elicit a thumb twitch was expected to occur before a working intensity was found, but this was not required or observed in all cases.

Responders overall had higher working intensities than Non-Responders as expected, and somewhat higher thumb twitch intensities. Of interest was that while Responders had higher working intensities, there was no significant difference between Responder working intensities and Non-Responder working intensities, $p = .357$. This can be explained by the extremely wide spread of intensities, and the ceiling effect of the 80mA maximum allowed intensity. For both Responders and Non-Responders, the range of intensities was almost identical, 6.5 – 80.0 and 2.2 – 80.0 respectively, and due to the ceiling of 80.0 this range does not accurately reflect actual maximum tolerances. This range was understandable and necessary for safety reasons, however because of its implementation I cannot know for certain whether intensity is significantly different between the groups if I cannot measure the full range of actual working intensities.

The mean working intensity of 35.3mA and the range of 6.5 – 80mA is generally in line with past research. The lower overall working intensity of 35.3mA can potentially be explained by a decreased intensity tolerance of participants based on the experiment context. By working alone and by visibly being a junior researcher as compared to the senior researchers conducting past studies, I potentially lacked the professional context that could put participants more at ease with higher electrical intensities. This is only my estimation, and based on the varied range of working intensities observed, the lower working intensity could simply be explained by chance and individual differences.

Table 4 - Working intensities (mA) of current and past studies

Study	Mean	Range
Current	35.3	6.5 - 80
Sambo, Liang, et al. (2012)	43.5	20 – 80
Sambo, Iannetti, et al. (2012)	42.5	20 – 80
Sambo and Iannetti (2013)	42.3	13x – 53x perceptible
R. J. Bufacchi and Iannetti (2016)	37.5	-
R. J. Bufacchi et al. (2016)	53.8, 48.2, 39.0, 55.8	-
Sarah B Wallwork (2016)	16.7	3.5 – 70
R. Bufacchi et al. (2017)	43.7	14 - 70

There were no significant difference between left and right hand working intensities or Block 1 and Block 2 working intensities across all participants. There were no significant differences between Left and Right or Block 1 and Block 2 working intensities across Responder and Non-Responders.

6.2 Preliminary Analysis

6.2.1 Onset/Offset threshold determination

Prior to any confirmatory analyses, the data must first be analysed for various useful qualities, the primary of these is identifying the onset and offset (beginning and end) of a blink of a particular trial. Previous work has found that an automated or objective method of identifying blink onset and offset has flaws (Sarah B Wallwork, 2016) and suggested visual trial analysis as a form of expert analysis. However based on my relative lack of experience visually analysing EMG waveforms, I thought it best to use an objective method as I was not confident my visual analysis would be reliable. One method of achieving this is by using a

threshold to classify the beginning and end of what is defined as a blink, and is defined as a point significantly greater than baseline muscle activity and therefore the beginning or end of genuine eye movement. First a baseline of EMG activity is taken by taking the mean value between 100ms before stimulus and 20ms before stimulus and averaged across all trials. This was considered to be the baseline value of rest EMG activity, and -20ms to 0ms was excluded due to the presence of electrical interference from the stimulator unit. The threshold is then calculated by taking 2 standard deviations above this baseline. This threshold is adapted from Sarah B Wallwork (2016) which used a 2sd above baseline of -100ms to -20ms of an individual trials data. My method takes a mean baseline of pre-stimulus data but averaged across all trials. This version has advantages, as calculating a threshold based on the pre-stimulus baseline per trial can create an unusable threshold if pre-stimulus data is unusual or significantly above expected baseline. One potential problem is that it results in a single threshold value per participant, and not individual thresholds per trial. Based on experimentation I do not believe this constitutes a meaningful difference, but there may be cases where individual trial thresholds are beneficial.

Datasets were then trimmed to 200ms beginning at stimuli trigger. Total participant level data at this stage is comprised of 64 trials of 200ms (2000 data points) of raw left and right, rectified left and right, smoothed left and right, and smoothed averaged.

By identifying peak amplitudes and searching forward and backwards until a threshold point is reached, envelopes of assumed blink responses can be identified.

For this blink envelope, Onset duration, Peak duration, Offset duration, total Blink Duration and Peak value (μV) are identified. Interstimuli-intervals (ISI) were also calculated, with an expected value of ~30seconds. Variations did occur in trial to trial ISI, because of delays in

between trials due to communicating with the participants, equipment difficulties or discomfort.

6.2.2 Onset/offset statistics

Table 5 – Averages of Valid trials.

	Responder		Non-Responder	
	Mean	SD	Mean	SD
Onset(ms)	56.9	10.1	69.1	22.1
Peak(ms)	77.5	11.6	84.4	22.8
Offset(ms)	102.8	20.2	98.5	24.2
Duration(ms)	45.9	21.4	29.4	15.6
Peak(µV)	28.9	17.4	8.1	5.3

For Responders Onset and Peak durations were the most consistent, with Offset and consequently Duration being less consistent with a higher standard deviation.

For Non-Responders, values were inconsistent for Onset, Peak, Offset and Duration. Non-Responder Peak (µV) values were consistent and noticeably lower than Responder Peak (µV) values, and were statistically significantly lower with $p < .001$.

Table 6 - Means of valid Responder trials by position

	Position 1		**Position 2**		**Position 3**		**Position 4**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Onset (ms)	56.5	8.1	56.2	8.0	57.0	9.5	57.4	11.5
Peak (ms)	75.5	8.2	75.6	10.0	77.0	10.7	80.8	12.6
Offset (ms)	99.3	16.3	98.5	16.1	101.0	17.8	110.2	22.2
Duration (ms)	42.9	18.8	42.3	18.1	44.0	20.0	52.9	23.2
Peak(µV)	25.6	13.6	26.4	14.9	27.9	15.6	34.6	20.6

Splitting the averages for valid Responder trials shows various interesting qualities. In particular, variability of all values tends to increase progressing from Position 1 to Position 4. This is explained by larger responses seen in the increases of both duration and peak (μV) values as they progress from Position 1 to Position 4, leading to greater potential range of values.

Position 4 increased duration appears caused by a later Offset duration, which appears to stretch the entire blink envelope, and consequently Peak (ms) is also slightly later than seen for other positions.

6.2.3 Trial classification explanation

A further quality of EMG blink analysis is determining whether the blink was a genuine response to the stimuli (a valid response) or whether the blink was due to other facts such as generalized blinking, or even if the activity was simply noise (an invalid response).

Similar to onset and offset detection, automated methods are imperfect (Sarah B Wallwork, 2016) and again visual expert analysis is suggested to subjectively decide whether a blink is legitimate or not. As with onset/offset determination, I believed my own visual analysis was unlikely to be reliable, therefore using a small sample of pilot data and over the course of the experiment, I developed a set of simplistic exclusion criteria to assist in data analysis. The criteria were specifically made to be broad, to avoid any false classification of actual valid trials, and intended just to capture a majority portion of invalid trials. Many of these criteria are based entirely on observations of data and their patterns during the experiment itself and therefore could be subject to bias. This was however determined to be a better alternative than visual analysis where I believe bias would have been significantly more prevalent and problematic.

The blink envelope for each trial is analysed for validity, with blinks meeting any of the following criteria being considered invalid:

1. Onset duration earlier than 20ms.
    - Due to nerve conduction velocities it is improbable that reactions as fast as or faster than 20ms were caused by the stimulation at the wrist.
2. Onset duration later than 120ms
    - Base on the observation that blinks occurring after ~120ms are unlikely to be caused by the stimulus, and were typically pain or discomfort related flinch responses.
3. Duration less than 10ms
    - Blinks shorter than 10ms were typically this short due to “peaking” above the threshold, were extremely small in size and occasionally were only identified from data noise.
4. Duration greater than 120ms
    - Typically blinks classified as longer than 120ms were due to a failure of the threshold detection. Typically EMG activity did not return to baseline after a visually observable blink, therefore this excludes such envelopes.
5. No peak greater than the previously calculated threshold

6.2.4 Trial classification statistics

Table 7 – Trial Classifications

	Responder	Non-Responder
Valid	844 (82.4%)	277 (29.9%)
Invalid	180 (17.6%)	650 (70.1%)
No Reaction	68	513
Too Early	14	52
Too Late	16	26
Too Short	34	66
Too Long	54	3

Note. Invalid classifications are partially non-exclusive. Invalid Responses are either "No Reaction" or a combination of 4 semi-exclusive "Too…" classifications.

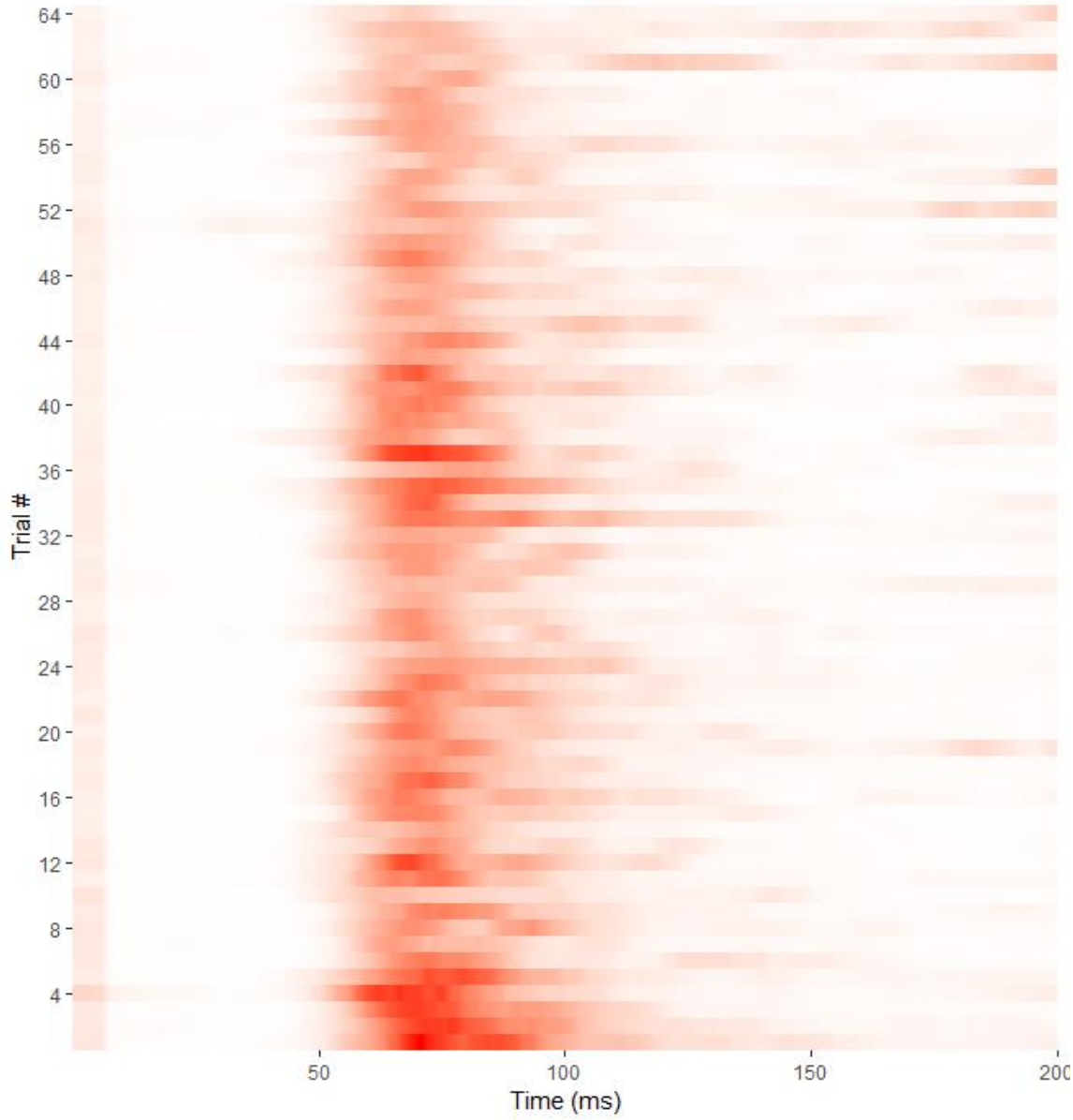
Responders presented 844/1024 (82.4%) valid trials and 180/1024 (17.6%) invalid trials. Non-Responders presented 277/927 (29.9%) valid trials and 650/927 (70.1%) invalid trials. Total trial counts were different despite the same number of Responder and Non-Responders due to one missing Trial from Participant 4, all 64 trials missing from Participant 6 and 32 missing trials from Participant 27.

Validity classifications were noticeably different between Responders and Non-Responders. Because Non-Responder responses were typically smaller in size in terms of peak magnitude, this led to responses being classified as shorter, and less likely to be classified as "Too Long" and more likely as "Too Short". Additionally, because Non-Responders responses were both smaller and less likely to occur, a vast majority of invalid classifications were of the type "No reaction". The "Too Early" classification was also more prevalent for Non-Responders, with it typically triggering in trials with no genuine blink response and the analysis code incorrectly identifying the stimulator interference located at 0ms as a blink response. The

"Too Long" classification being more prevalent for Responders could also be explained by trials where large and long lasting generalised eye activity occurred and the analysis code failing to identify the onset and offset of the blink correctly.

6.2.5 Habituation

Figure 2 - Habituation plot of Valid Responder trials

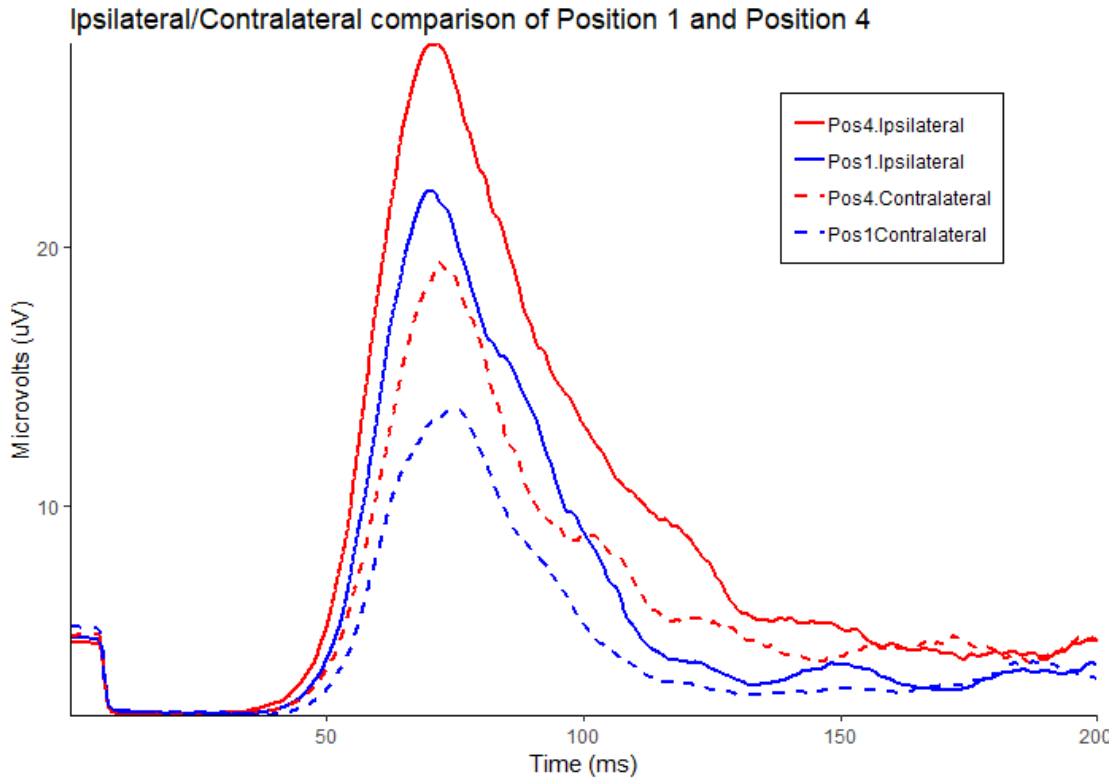


Above is a stacked gradient plot with each row representing the average response of that trial across all valid Responder trials, with the gradient representing response magnitude.

Responses are noticeably higher during the first series of trials in each block (Trials 1-6 in Block 1, and trials 32-36 in Block 2). This is an expected feature, as there was a small time gap between intensity calibration and the first Trial, and a significant gap between Blocks 1 and 2. I suspect this gap allowed for some small sense of heightened expectation related worry regarding the stimulations, which then stabilised as participants experienced them throughout the experiment.

6.2.6 Ipsilateral and Contralateral analysis

Figure 3 - Ipsilateral/Contralateral comparison of Position 1 and Position 4



Ipsilateral and contralateral Position 1 versus Position 4 results are plotted to compare directly with the same analysis performed by Sambo, Liang, et al. (2012). As expected, ipsilateral responses were on average larger than their contralateral counterparts and the overall pattern is almost identical as found by Sambo, Liang, et al. (2012). This is likely due to the hand being closer to the ipsilateral side, and therefore theoretically of greater threat, warranting a larger and faster response. One key observation is how similar the Position 1

Ipsilateral responses and Position 4 Contralateral responses were too each other, with these two pairings being more similar to each other than the two Ipsilateral/Contralateral pairings are within themselves.

6.3 Confirmatory Analysis

6.3.1 Area under the Curve calculations

In order to test the confirmatory hypotheses, the magnitude of valid blink responses must be quantified. Objective magnitudes of blink activity (μV measurements) can vary dramatically person to person, therefore a measure needed to be identified that allowed for both within and between participant comparisons.

The original study by Sambo and Iannetti (2013) utilized an Area under the Curve (AUC) calculation of each blink waveform as a classification of HBR magnitude. It was not immediately clear whether the method used in the original study measured area under the curve for the full 200ms duration, or only between the onset and offset durations. Based on information identified after pre-registration and after beginning the experiment (Sarah B Wallwork, 2016), it appears the likely method used was to measure area under the curve between onset and offset. For the purpose of pre-registration, the method I elected to use was to measure area under the curve of the full 200ms. This was based on the assumption that the automatic onset and offset detection was liable to be inaccurate and could pose problems when wishing to compare invalid blink envelopes and valid blink envelopes.

Because of these factors, I have opted to explore area under the curve using both methods and perform all consequent analyses using both area under the curve results in order to compare them. The limited 200ms method was chosen to be used, as it was pre-registered and the onset-offset method was additionally chosen to be used at it appears likely it was used during the original study. For the purpose of this paper and for clarity, I refer to the full 200ms AUC

method as the “Full” AUC method, and the Onset to Offset AUC method as the “Constrained” AUC method.

Once AUCs were calculated for each trial, these were transformed into z-scores using valid trials across the participant. These could then be averaged for each distance per participant, resulting in four averaged z-score AUC values for each participant, one z-score AUC value for each distance. Z-scores allow for measurements to be directly comparable between participants, as objective measurement comparisons can be misleading. Graphing these values provides a view of how an individual participant’s response changes over the four distances, with each value an arbitrary unit with an overall mean of zero across the four values.

6.3.2 Hypothesis 1 results

Hypothesis 1 - The EMG magnitude recorded from the orbicularis oculi muscle is positively correlated with nearness of hand position to the face when a calibrated electrical stimulation is applied to the positioned wrist.

To test Hypothesis 1 and satisfy the pre-registered analyses a simple linear regression was performed on mean Area under the Curve (AUC) using the “Full” method, for each distance across all Responder participants using valid trials. This indicated an R^2 value of .35 which is considered weakly positive, with $F = 33.41_{(1,62)}$ and $p < .001$ which is considered significant.

The “Constrained” AUC method was not pre-registered but results were extremely similar with an R^2 value of .38 which is considered weakly positive, with $F = 37.0_{(1,62)}$ and $p < .001$ which is considered significant.

In order to ensure the validity criteria I established were not biased towards any particular result, AUC responses regardless of validity were also tested. Using all “Full” AUC values indicated R^2 value of .39, with $F = 39.22_{(1,62)}$ and $p < .001$ which is considered significant.

Using all "Constrained" AUC values indicated an R^2 value of .37, with $F = 37.42_{(1,62)}$ and $p < .001$ which is considered significant. Due to the extreme similarity and significance across all measurements, this indicates the validity criteria are not biased towards a particular result and that this is a comprehensive effect.

6.3.3 Hypothesis 2 explanation

A one-way repeated measures ANOVA is performed for HBR magnitude. Paired t-tests are performed for the combinations of hand positions, primarily adjacent hand positions. Differences between magnitude significance across t-tests of adjacent distance pairings would indicate support that the relationship is non-linear, as significance of difference is not consistent.

Additionally, a Point-by-Point ANOVA and Point-by-Point t-tests are performed of the previous analyses. A Point-by-Point ANOVA or t-test is performed by taking comparisons of each time point of the data, which returns either an f or t value waveform, coupled with a p-value waveform. A consecutivity threshold is then applied, where every value within 10ms (100 time points) of consecutive time must be significant in order for that region of time to be considered significant, to account for multiple comparisons. This results in regions of time greater than 10ms that can be considered significantly different either for a particular variable for ANOVAs, or between particular groups in the case of t tests. For this study, regions of time that are identified would indicate time regions where a majority of blink activity is occurring and whether this region is significantly different between comparison groups. If these significant time regions were similar to the time regions identified with the threshold technique, this would indicate support that the different techniques were comparing similar constructs, specifically that they were measuring HBR magnitude.

6.3.4 Hypothesis 2 results

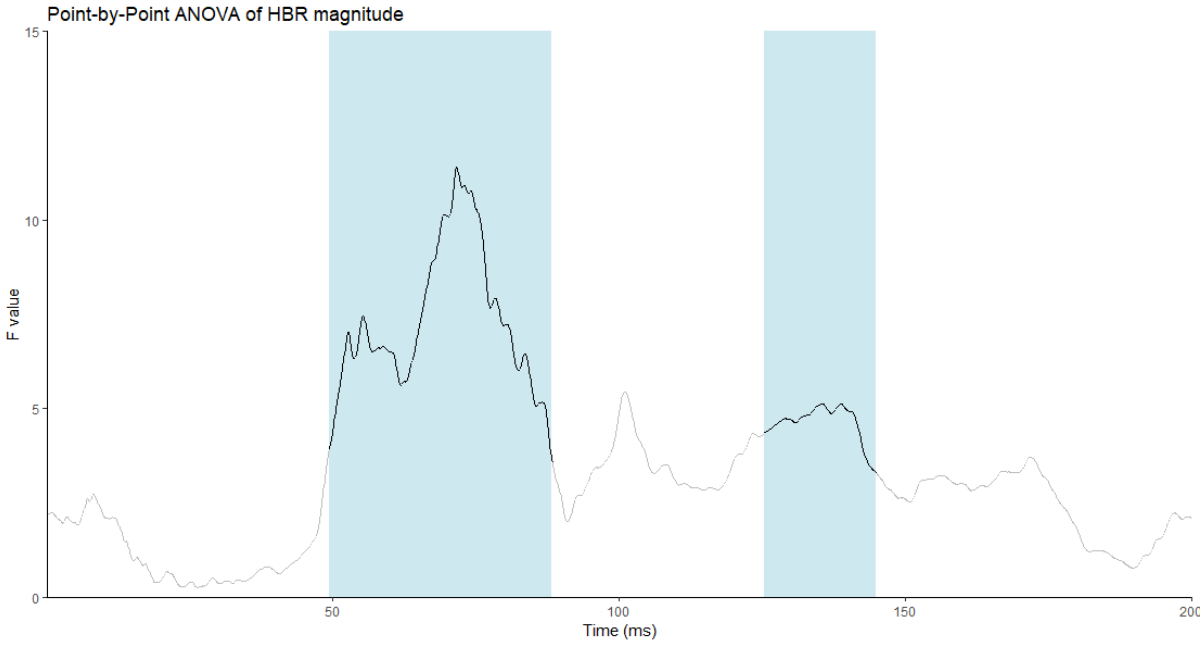
Hypothesis 2 - The relationship in Hypothesis 1 is non-linear.

Hypothesis 2 was tested through a variety of analyses, with results from all analyses taken together to determine support for the Hypothesis. The pre-registered analyses include a one-way repeated measures ANOVA of HBR magnitude, paired t-tests of various hand positions, a Point-by-Point ANOVA of HBR at each time point and Point-by-Point t-tests of various hand positions.

A one-way repeated measures ANOVA was performed using HBR magnitude using the "Full" AUC method. This indicated a significant effect of hand position on the HBR, $F = 15.96_{(3,45)}$ with a $p < .001$.

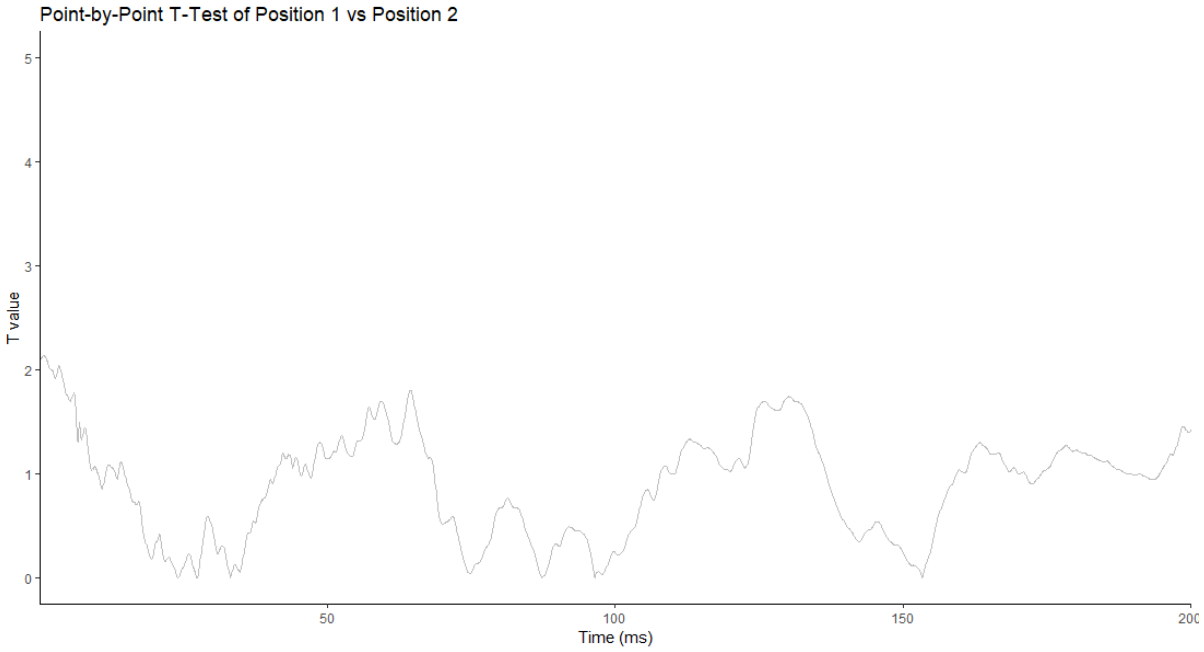
T-tests were performed of various different relevant pairings of distances, particularly adjacent distances, and various comparisons including Positions 4, as this was expected to present the most different response. Position 1 compared to Position 2, was not significantly different with $t = 0.262_{(15)}$ and $p = .797$. Position 2 compared to Position 3 was not significantly different with $t = -1.53_{(15)}$ and $p = .146$. Position 3 compared to Position 4 was significantly different, $t = -3.98_{(15)}$ with $p = .0012$ (significant as $p < .05$). An additional comparison of Position 1 compared to Position 4 was statistically significant with $t = -4.99_{(15)}$ and $p < .001$.

Figure 4 - Point-by-Point ANOVA of HBR magnitude



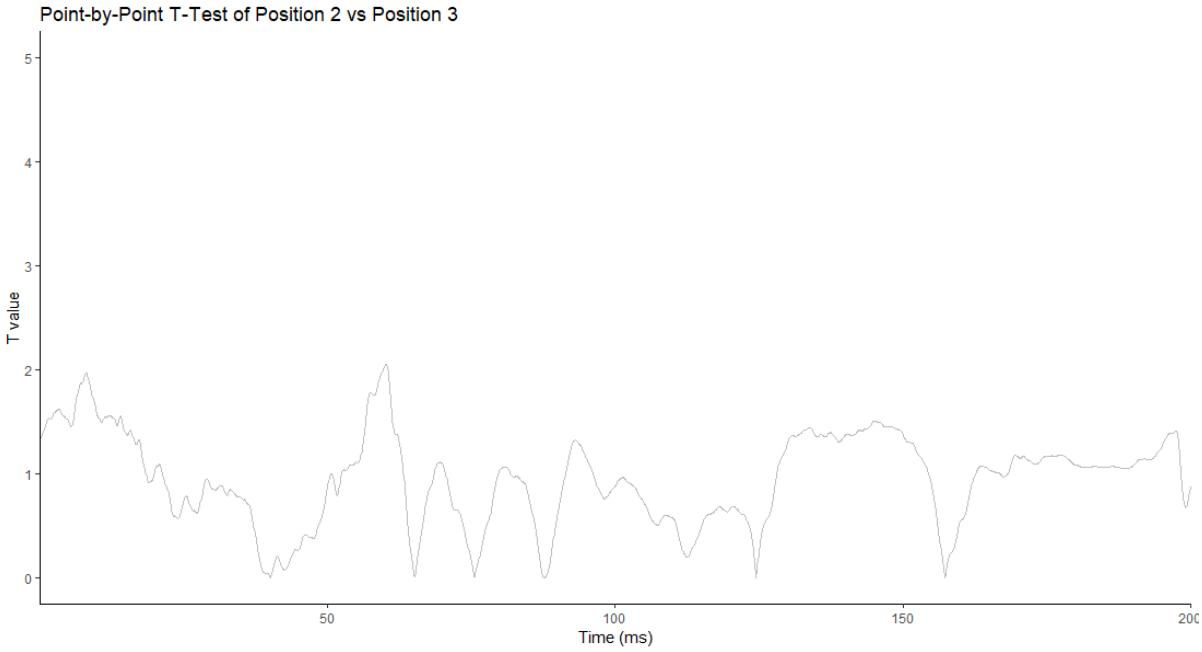
Performing a Point-by-Point repeated measures ANOVA of HBR at each time point indicated 2 time regions where HBR was significantly different across the four hand positions. The first region 49.3 – 88.2ms, and the second region 125.3 – 144.8ms. This first region is comparable to the mean onset and offset durations established using the threshold method of 56.9 – 102.8ms, indicating this region is significantly different regardless of statistical method used.

Figure 5 - Point-by-Point t-test of Position 1 vs Position 2



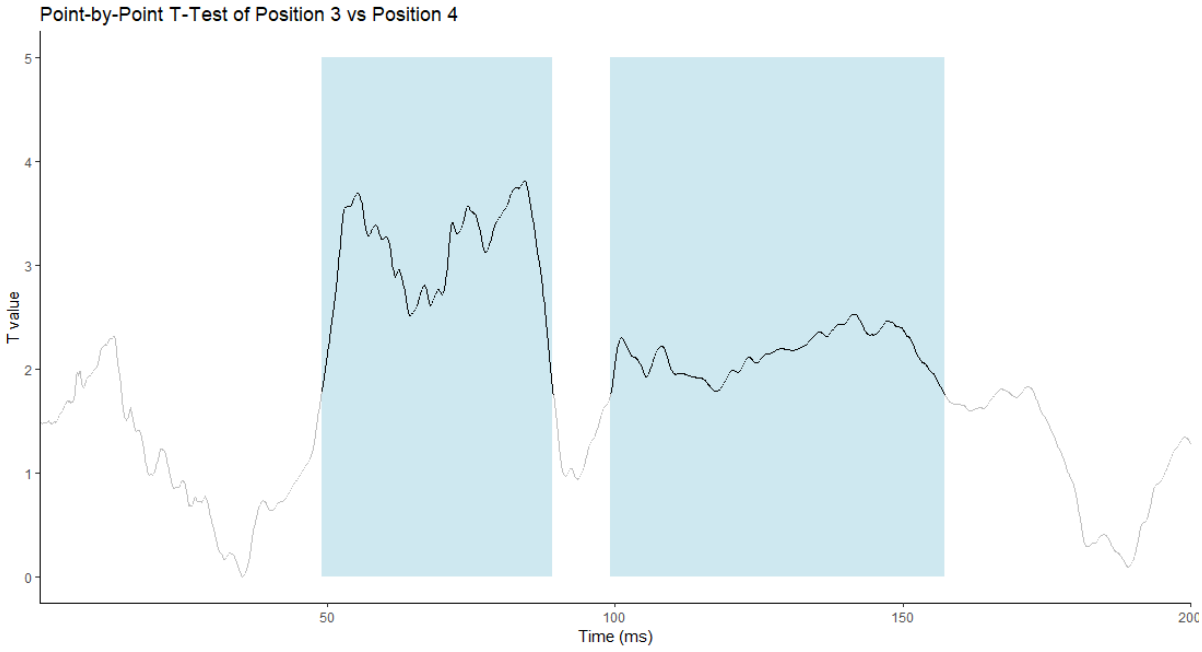
The Point-by-Point t-test of Position 1 versus Position 2 contained no regions of time that were statistically significant.

Figure 6 - Point-by-Point t-test of Position 2 vs Position 3



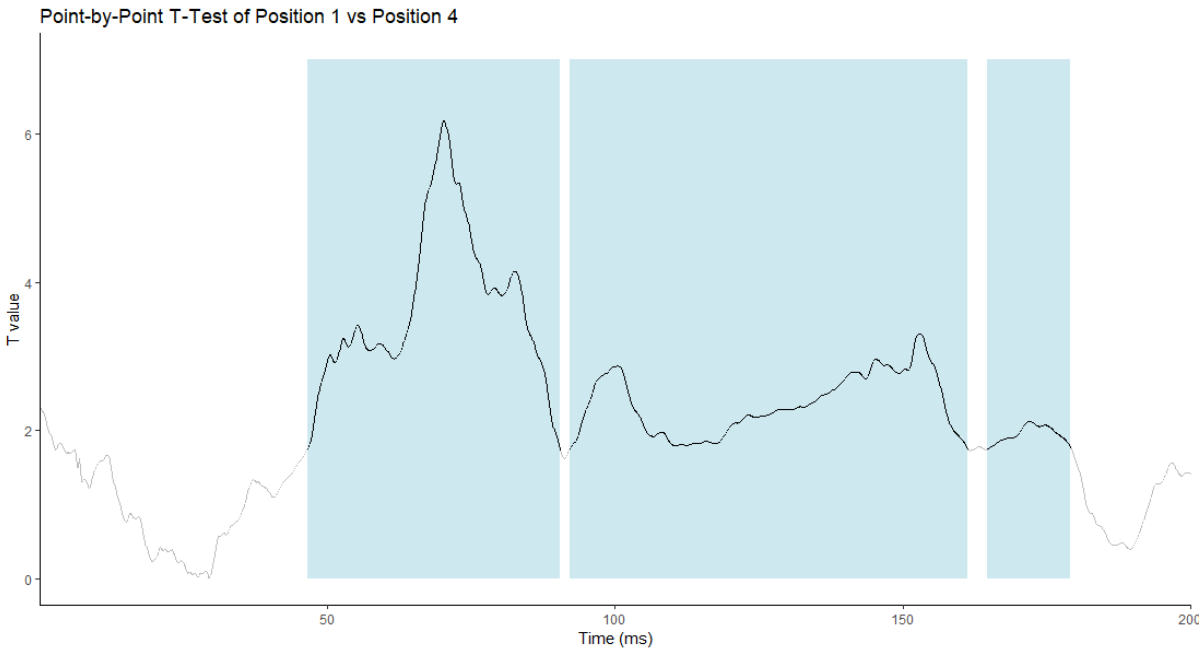
The Point-by-Point t-test of Position 2 versus Position 3 contained no regions of time that were statistically significant.

Figure 7 - Point-by-Point t-test of Position 3 vs Position 4



The Point-by-Point t-test of Position 3 versus Position 4 yielded two regions of significant difference, 49.0 – 89.2ms and 99.2 – 157.2ms. The first region corresponds very closely to the first region identified in the Point-by-Point ANOVA of 49.3 – 88.2ms.

Figure 8 - Point-by-Point t-test of Position 1 vs Position 4



The Point-by-Point t-test of Position 1 versus Position 4 yielded three regions of significant difference, 46.6 – 90.4ms, 92.2 – 161.2ms and 164.7 – 179.1ms. Similar to the Position 3 versus position 4 t-test and ANOVA, the first identified region is similar in terms of onset and offset, but with a longer duration, and again corresponds closely to the mean onset/offset of 56.9 – 102.8 established using the threshold method.

Table 8 – Point-by-Point significant regions of time from current and original study

Analysis	Current study	Original study
ANOVA: HBR Magnitude	49 – 88 , 125 – 145	37 – 128
T-Test: Position 1 vs Position 2	None	None
T-Test: Position 2 vs Position 3	None	55 – 65, 73 - 97
T-Test: Position 3 vs Position 4	49 – 89 , 99 – 157	48 – 95, 99 – 110
T-Test: Position 1 vs Position 4	47 – 90, 92 – 161, 165 – 179	42 – 125

Comparing Point-by-Point results from the current and original study (Sambo & Iannetti, 2013), the regions identified were reasonably consistent within each study, but differed in interesting ways between the studies. The original study in particular identified a narrower and somewhat earlier band of significant time points across all tests, identifying the 37-128ms region to have some significance whereas the current study identified 47-179ms region as containing significance. This broader band of significance can be explained by the primary flaw of Point-by-Point analyses requiring extremely consistent trials in order to be compared reliably. It is reasonable to assume responses in the current study were somewhat less consistent than the original study due to the experiment context, and therefore occurred over a more variable timeframe. This could cause a greater range to be compared, and for the t-tests to identify a larger range as significantly different.

6.4 Exploratory Analysis

6.4.1 Contrast Analyses explanation

Once an AUC model is determined for a participant, a contrast analysis can be performed to compare this model to a set of pre-defined models in order to categorize it. A contrast analysis uses contrasts, a combination of variables that can be used to test predefined models

that describe interactions and combinations of variables. These contrasts are a set of values that must sum to zero.

The 5 models are taken as described by Sambo, Liang, et al. (2012) and are used to represent the various positions of where the edge of the DPPS could occur. Model 1 – Linear is used to as a counterexample to the existence of the DPPS as it contains no abrupt shifts in reaction and therefore responses are fully explainable by distance alone. Model 2 – Small uses an abrupt shift between positions 3 and 4, representing the DPPS transition located approximately in this region. Model 3 – Large (Ramp) is used as an alternative model. Model 4 – Large (Step) uses an abrupt shift between positions 2 and 3, representing the DPPS boundary in this region. Model 5 – Extra Large uses an abrupt shift between positions 1 and 2, representing the DPPS boundary in this region.

These 5 models therefore cover potential DPPS locations in all 3 possible locations (between 1 and 2, 2 and 3, 3 and 4), an alternative DPPS model and a counterexample to the DPPS.

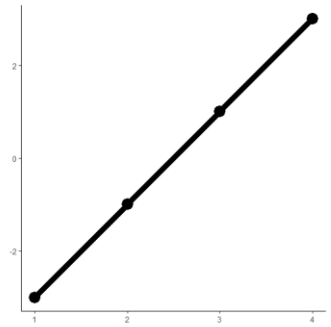


Table 9 - Model 1 - Linear

Model 1 - Linear			
Position 1	Position 2	Position 3	Position 4
-2.5	-0.5	0.5	2.5

Table 10 - Model 2 - Small

Model 2 - Small			
Position 1	Position 2	Position 3	Position 4
-1.0	-1.0	-1.0	3.0

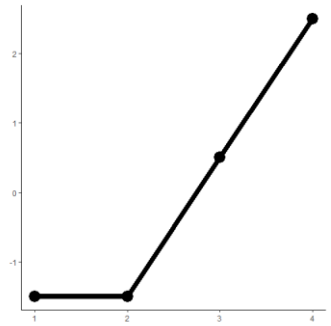


Table 11 - Model 3 - Large (Ramp)

Model 3 – Large (Ramp)			
Position 1	Position 2	Position 3	Position 4
-1.5	-1.5	0.5	2.5

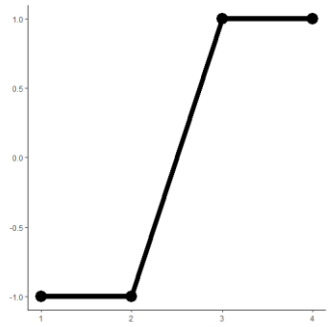


Table 12 - Model 4 - Large (Step)

Model 4 – Large (Step)			
Position 1	Position 2	Position 3	Position 4
-1.0	-1.0	1.0	1.0

Table 13 - Model 5 - Extra Large

Model 5 – Extra Large			
Position 1	Position 2	Position 3	Position 4
-3.0	1.0	1.0	1.0

To perform the contrast analysis, an ANOVA is performed and a Contrast Analysis is performed on the results.

6.4.2 ANOVA selection

In order to perform the Contrast Analysis an ANOVA must be performed, and therefore the type of the ANOVA must be specified. Broadly two types of ANOVAs exist, between subjects and within subjects, also called repeated measures. In the original study by Sambo and Iannetti (2013) a repeated measures ANOVA was used.

One difficulty in selecting the type of ANOVA for this study is in identifying the comparison space. In typical studies, participants are taken from a population and are presented with conditions and compared, whereas in this study individual trials at each hand position are compared, with participants acting as "populations" from which the data originates.

Based on this a repeated measures ANOVA is indeed the most appropriate, as each trial can be considered an independent event sampled from the population and subjected to every level of the independent variable distance. However there is a significant difficulty associated with a repeated measures as it is extremely sensitive to excluded or missing data. Because trials are subjected to my established validity criteria, they can be excluded if they meet these criteria which results in missing data. When performing a repeated measures ANOVA with excluded or missing data, the number of instances per condition must be made equal in order to be compared. This means that if a particular condition by chance has many more excluded trials, valid data from other conditions must also be excluded until the conditions are equal in length. If for example a participant's data contains 16 valid trials at a particular distance and 10 valid trials in another, 6 valid trials must be excluded from the first distance. Practically this has the potential to exclude an extremely large portion of valid data from a participant's analysis, and could result in conclusions that are not representative of the data analysed.

Based on this conclusion, if single trials are excluded without imputation a repeated measures ANOVA is not an appropriate statistical test, and instead a within subjects ANOVA should be used, as this handles missing data without the exclusion of valid data.

To complicate this decision, Sarah B Wallwork (2016) has stated that for their analysis "Single trials were excluded if a voluntary or spontaneous blink occurred concurrently with the stimulus onset, or if there was excessive background noise."(2016, p. 65) and that "Close visual inspection of the waveforms was undertaken before all analyses were conducted."(2016, p. 70). Further clarification however is not readily available and while it is assumed this procedure is present in many of the related studies, inspection of all other recent HBR research shows no indication of whether trials were excluded, or how excluded data may have been processed. Therefore based on the lack of existing best practises, and choosing between using the most appropriate statistical method compared to using the most appropriate dataset, I conclude it is better to instead test only valid trials using the inferior between-subjects ANOVA. For transparency, a repeated measures ANOVA is included for comparison in Appendix 8 – Repeated-Measures Contrast Analyses.

6.4.3 Responder Analyses

Table 14 - Responder trial classifications

n	Valid (%)	Valid	Invalid	Too Early	Too Late	Too Short	Too Long	No Reaction
1	79.7%	51	13	2	0	3	2	7
3	98.4%	63	1	1	0	0	0	0
10	81.2%	52	12	1	0	0	10	2
11	78.1%	50	14	0	2	5	0	8
12	73.4%	47	17	0	5	1	8	3
13	93.8%	60	4	1	0	0	1	2
14	70.3%	45	19	0	0	9	1	9
15	95.3%	61	3	1	0	0	2	0
18	84.4%	54	10	2	0	0	9	0
19	73.4%	47	17	0	0	0	13	4
22	90.6%	58	6	2	1	0	2	1
24	82.8%	53	11	1	0	0	6	4
26	53.1%	34	30	3	3	7	0	18
30	73.4%	47	17	0	2	6	0	10
31	90.6%	58	6	0	3	3	0	0
32	100%	64	0	0	0	0	0	0

6.4.3.1 Full wave Contrast Analysis

Table 15 – Responder Contrast Analysis – Full AUC method

n	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity
1	0.9629	0.8723	0.9323	0.7864	**0.7817**	79.7%
3	0.0127	**0.0031**	0.0092	0.0768	0.1524	98.4%
10	0.7313	0.6145	0.9829	0.6912	**0.3733**	81.2%
11	0.0108	**0.0007**	0.0026	0.0458	0.3980	78.1%
12	0.6394	**0.1068**	0.1864	0.4342	0.1672	73.4%
13	0.0195	**0.0015**	0.0064	0.0753	0.4476	93.8%
14	**0.0558**	0.0727	0.0598	0.1053	0.1870	70.3%
15	0.2616	**0.0710**	0.1043	0.2593	0.7952	95.3%
18	0.9262	0.5857	0.5402	0.5899	**0.3429**	84.4%
19	0.3462	0.8715	0.4147	**0.2280**	0.3891	73.4%
22	0.0088	**0.0009**	0.0042	0.0723	0.1710	90.6%
24	**0.0134**	0.0288	0.0191	0.0398	0.0510	82.8%
26	0.0200	**0.0015**	0.0042	0.0326	0.3268	53.1%
30	0.8680	**0.4026**	0.8611	0.6740	0.9282	73.4%
31	0.1541	0.2806	0.2885	0.3990	**0.1061**	90.6%
32	<0.0001	**<0.0000**	<0.0000	0.0020	0.1018	100%

Footnote a: Bolded values indicate smallest p value and model of best fit

For the Full AUC method Contrast Analysis, 7 Responder participants had models with p < .05. These were participants 3, 11, 13, 22, 24, 26, and 32.

6.4.3.2 Constrained Contrast Analysis

Table 16 – Responder Contrast Analysis – Constrained AUC method

n	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity
1	0.9023	0.7575	0.9418	**0.7071**	0.8610	79.7%
3	0.0040	**0.0005**	0.0025	0.0411	0.1034	98.4%
10	0.7614	0.6002	0.9675	0.7018	**0.4088**	81.2%
11	0.0266	**0.0007**	0.0055	0.1058	0.6766	78.1%
12	0.2034	**0.0218**	0.0562	0.2456	0.6296	73.4%
13	0.0133	**0.0009**	0.0036	0.0504	0.4472	93.8%
14	0.1387	0.1597	**0.1367**	0.1976	0.3401	70.3%
15	0.2497	**0.0666**	0.1013	0.2602	0.8317	95.3%
18	0.6457	0.4287	**0.3503**	0.3926	0.5155	84.4%
19	0.3444	0.8356	0.4070	**0.2342**	0.3986	73.4%
22	0.0107	**0.0008**	0.0050	0.0962	0.1927	90.6%
24	**0.0238**	0.0536	0.0344	0.0586	0.0670	82.8%
26	0.0532	**0.0044**	0.0137	0.0813	0.4916	53.1%
30	0.6619	**0.2722**	0.6634	0.8218	0.7750	73.4%
31	0.0965	0.1338	0.1774	0.3351	**0.0915**	90.6%
32	0.0002	**<0.0000**	<0.0000	0.0089	0.1720	100%

Footnote b: Bolded values indicate smallest p value and model of best fit

For the Constrained AUC method Contrast Analysis, 8 participants had models $p < .05$. These were participants 3, 11, 12, 13, 22, 24, 26 and 32.

6.4.3.3 Participant 1

Participant 1 was the first Participant tested and therefore procedures were less precise and less consistent as other participants. One primary difference with Participant 1 was that their data was recorded at 1k Hz as opposed to 10k Hz and for other participants the introduction of a break between Blocks 1 and 2 was not present for Participant 1. Participant 1 presented with 51 Valid Responses and 13 Invalid, 7 of which had no blink response. This participant was fitted with an “Extra Large” model for the Full method and “Large (Step)” model for the Constrained method with all p-values non-significant. Due to the high and inconsistent p-values neither of these models are considered a reliable classification for this participant.

6.4.3.4 Participant 3

Participant 3 is considered the model Responder participant. Responses were visibly consistent and waves had almost no noise surrounding the primary blink response making detection of the onset and offset of blinks extremely accurate. Participant 3 presented 63 valid responses and 1 invalid responses. This participant was classified as fitting the "Small" model for both Full and Constrained Contrast analyses. In addition due to only having a single invalid trial excluded, within-subject ANOVA based Contrast Analyses also could be utilized which indicated identical model fittings, all of which were similarly statistically significant. Based on these factors the model fitting of a "Small" model is a very reliable classification for this participant.

6.4.3.5 Participant 10

Participant 10 presented consistent blink responses, however it was evident the threshold detection was not effective with this participant. Many of the blink responses did not completely return to baseline, and due to the very large magnitude of the blink responses, this led to incorrect offset detection and in one incidence incorrect onset detection. Because of this, 10 out of 12 invalid trials were classified as "Too Long" and one trial as both "Too Long" and "Too Early". Additionally the responses from the Participants right eye were observably larger than the left eye across both blocks. The participant was classified as fitting the "Extra Large" model for both Full and Constrained Contrast Analyses but these were not significant. Due to the high p values and previous issues the "Extra Large" model is not considered a reliable classification for this participant.

6.4.3.6 Participant 11

Participant 11 presented with small but moderately consistent responses. The participant presented with 14 invalid trials with 5 trials classified as "Too Short", 2 as "Too Late" and 8

with no reaction. Due to the small response magnitude it is visibly clear that the threshold detection had difficulty with some responses, but overall a large portion of trials were valid. For both Full and Constrained Contrast Analyses, this participant was fitted with the "Small" model with significant p values in both cases, making the model a reliable classification for this participant.

6.4.3.7 Participant 12

Participant 12 presented extremely unusual responses. Trials 1 – 22 presented clear and consistent responses, however trials 23 – 32 were substantially smaller in magnitudes. Following this in Block 2, trials 33 – 38 were a mixture of smaller and normal responses, however trials 39 – 64 presented very small responses in conjunction with very large sections of eye activity beginning ~150ms after stimulation. Checking outside the standard 200ms of data, 19 trials between 39 – 64 presented large scale eye activity centred at ~200ms after stimulation, and several earlier trials also present additional activity between 200 – 300ms which would normally be trimmed from all analysis. My immediate assumption is that the later eye activity was a type of painful flinch in response to the stimuli, however the intensity used in this block was only 19.2mA, somewhat below the mean working intensity. The participant was classified for both Full and Constrained Contrast Analyses as fitting a "Small" model, with the Constrained method being statistically significant. Due to the significant problems surrounding a large portion of trials, I would not consider this a reliable classification for this participant.

6.4.3.8 Participant 13

Participant 13 presented consistent responses with Block 1 indicating somewhat larger habituation than Block 2. The threshold detection showed errors for some trials due to long response decay similar to Participant 10, however a majority of responses were classified

appropriately, with 60 valid responses and 4 invalid. Right eye activity was also noticeably larger than left eye activity. Both Full and Constrained contrast analyses fitted a “Small” model, and these were shown to be significant, and therefore this is considered a reliable classification for this participant.

6.4.3.9 Participant 14

Participant 14 presented with consistent but generally small responses. In each Block, the first 5 trials were noticeably larger than the remaining 27. The participant presented with 45 valid and 19 invalid responses, of which 9 of which were “Too Short”, 9 had no reaction and 1 was “Too long”. Visibly the responses were consistent with the mean responses for each distance having similar onset, peak and offset durations, however a noticeable difference between the initial larger responses and the more typical smaller responses makes comparison difficult. The Full Contrast Analysis indicated a non-significant “Linear” model, and the Constrained method indicated a non-significant “Large (Ramp)” model. Based on these factors I would not consider either model a reliable fit for this participant.

6.4.3.10 Participant 15

Participant 15 presented consistent and clear responses. 61 trials were valid, and 3 were invalid. Similar to some participants, the onset and offset threshold presented inaccuracies due to the blink response decaying slowly, and the offset of the blink not being correctly identified. In addition absolute response magnitude was very large, leading to greater variability in potential response magnitude. Position 1 also indicated a higher than expected HBR, greater than Position 2 and 3, with the reason for this being unknown. Despite this problem, responses between eyes and between both blocks were extremely consistent. For both Full and Constrained methods, the “Small” model was fitted with non-significant p-values of .071 and .066. Based on the extremely stable and consistent responses, I would

expect the non-significance to be attributed to the higher than expected Position 1 results causing a less accurate fit across all models. Based on this fact I believe the “Small” model to best fit the participant despite the non-significant p-values. I cannot however claim the model to be a reliable fit or to be confident of the fit due to the insignificant p-values. To confirm the best model for this participant I would recommend either more in-depth analysis of Participant 15s results, or further data to reliably classify the participant.

6.4.3.11 Participant 18

Participant 18 presented moderately consistent blink responses presenting 54 valid trials, and 10 invalid, but containing significant amounts of unwanted eye activity noise after many blink responses. Many responses while having a visually clear blink response, had eye activity above baseline preceding the blink up to the full 200ms, resulting in many blinks with an “Offset” duration of 200ms and consequently being classified as “Too Long”. These issues makes the Full Contrast Analysis problematic as it includes all additional noise, and also problematic for the Constrained method as Onset/Offset durations are highly inaccurate and therefore invalid. In contrast, averaging and graphing the 200ms waveforms across the four distances and comparing them visually shows clear onset and offset durations, as well as clear patterns in peak duration and blink shape. Based on this I believe the participant could have a significant model fitted to them based on their results, however the current AUC methods are not capable of capturing this, due to the significant amount of noise present. The Full and Constrained methods were both non-significant, indicating “Extra-Large” and “Large (Ramp)” models. Because of these problems, a reliable model cannot be determined with the current method.

6.4.3.12 Participant 19

Participant 19 presented unusual responses. Trials 1 – 4 presented extremely large responses, which appeared to habituate rapidly with many much smaller results in Block 1. In Block 2, similar very large responses were present from trials 33 – 48 where the responses appeared to habituate. Responses indicated a very consistent Onset and Peak duration, but an inconsistent Offset Duration. The participant also presented a noticeably higher response in their right eye than their left eye across all trials, a magnitude difference similar to the expected and observed between Ipsilateral and Contralateral. Analysing left vs right eye activity per trial indicates that despite Block 1 stimulating the left hand, the contralateral right eye activated more strongly than the ipsilateral left eye in many cases. In Block 2, ipsilateral right eye activity was greater than contralateral left eye activity as expected. One possible explanation is equipment failure, or significantly different electrode placement on one of the eyes. A further explanation could be significant physical differences between the participant's eyes, however I believe the more likely explanation to be incorrect electrode placement or equipment error. Both Full and Constrained methods indicated non-significant fitting for the "Large (Step)" model. Based on the problems identified with this participant, I cannot determine what model is a reliable fit for this participant.

6.4.3.13 Participant 22

Participant 22 presented consistent responses, with 58 valid and 6 invalid responses. Similar to previous participants, the onset/offset threshold produced some errors regarding offset durations due to response not returning to baseline quickly. Responses had very low noise, were consistent between Blocks 1 and 2 and across both eyes, and showed minimal habituation. Position 1 responses were later in onset duration than Position 2 and 3, and Position 4 responses were both earlier, and lasting longer than all other distances in addition to being larger in peak HBR. Both Full and Constrained contrast analyses indicated

significant fitting of the “Small” model. Based on these factors, this is considered a reliable model for this participant.

6.4.3.14 Participant 24

Participant 24 presented somewhat consistent responses, with 53 valid and 11 invalid. Responses were unusual in that many responses presented with a repeated multiple peak pattern. Viewing the averaged responses across distances it is possible to see a similar 4 peak structure for Positions 1 and 2, and a concurrent 3 peak structure that lines up for Positions 3 and 4. However based on high standard deviation in onset, peak, offset and duration statistics it is clear that responses were inconsistent in their specific timing. Habituation also occurred rapidly across the experiment, with many extreme responses occurring in trials 1 – 16, and all other trials including early trials of Block 2, showing significantly reduced responses. Both Full and Constrained contrast analyses indicate a significant fit for the “Linear” model. Despite the significant model fit I would not consider the “Linear” model a reliable fit for the participant due to the dramatic differences between blocks, as well as due to the presence of the unusual response pattern making determination of blink qualities very difficult. Deeper analysis of the data would likely not be useful as the data itself is incredibly inconsistent and varied. In order to verify if the model is appropriate and improve the ability to classify the participant, a greater sample of data for this participant would be needed in order to isolate the specific unique qualities of this participants responses.

6.4.3.15 Participant 26

Participant 26 presented small and inconsistent responses. 34 trials were valid, 30 invalid with 3 “Too Early”, 3 “Too Late, 7 “Too Short” and 18 with no reaction. Additionally several responses identified as valid were visually not valid, as they appear to contain multiple overlapping responses occurring within the 200ms timeframe. Both Full and Constrained

contrast analyses indicated fitting a “Small” model, with significant p-values. Visually it is likely the “Small” model was fitted due to Position 4 having a significantly high enough HBR to counteract the noise from invalid responses, and not due to actually fitting the model well. Therefore despite the significant model fitting, the model is not a reliable fit of the participant due to the extremely high number of invalid responses.

6.4.3.16 Participant 30

Participant 30 presented initially with consistent responses during Block 1, but with significantly reduced responses during Block 2, and a visual comparison of Block 1 vs Block 2 mean responses supports this. This is further supported by 47 trials being valid and 17 invalid, with 15 invalid trials in Block 2. A further unusual feature was the substantially different response of Position 3 as compared to all other positions. The mean Position 3 response was earlier, shorter and smaller than all other responses. Both Full and Constrained contrast analysis indicate non-significant fitting of the “Small” model. The lack of fitting is most certainly due to the significantly reduced response at Position 3 with no models able to account this. Despite these problems, responses were extremely consistent with low standard deviation for Onset, Peak and Offset durations, and mean responses for each position showing strong overlap between the responses indicating consistency of response. Since there are no theoretical explanations for a reduced Position 3 response and due to the significantly lower than average working intensity used for this participant (9mA Block 1 and 11mA Bock 2) I believe the lower response at Position 3 to be either due to an error, or as a result of irregular Position 3 data. Based on these factors I believe participant 30 can be fitted with a significant model, however more data would be required and more careful testing regarding the working intensity.

6.4.3.17 Participant 31

Participant 31 presented consistent but unusual blink responses. 58 trials were classified as valid, with 6 as invalid, none of which were classified as no reaction. This is problematic as visually several trials have no clear blink response, and are primarily noise of sufficient magnitude above threshold detection which results in a "blink" being detected. The visually distinct blink responses also have an unusual 3 peak pattern similar to Participant 24 with an initial peak clearly similar across all four distances, and the further 2 peaks similar across distances 2 – 4. Because of this unusual pattern of responses and smaller more typical responses, overall AUCs of any kind appear to be unreliable. The Full AUC calculation would be inaccurate because of the large amount of noise activity in many trials, and the Constrained AUC method is inaccurate as the onset and offset for the 3 peak pattern response is unlikely to be identified correctly. Both Full and Constrained methods fitted the "Extra-Large" model and both were non-significant. Based on the aforementioned difficulties it cannot be determined what model best fits this participant. To identify a reliable model, more data would likely be required and an improved threshold detection method would be necessary to more clearly identify and classify each blink response.

6.4.3.18 Participant 32

Participant 32 presented extremely consistent responses, being the only participant with all 64 trials classified as valid. Despite this it is visually difficult to determine the presence of blink responses in some trials at the conclusion of Block 2, as they cannot be distinguished from background noise. Responses for all four distances had a consistent 2 peak shape, with Position 4 responses being earlier, longer and significantly larger. Both Full and Constrained methods were noticeably significant and fitted a "Small" model in both cases, which is considered a reliable fit for this participant. One noteworthy concern is Participant 32s age being 44, over the specified age range of 40. The primary reason for the age limit of 40 was

firstly due to that limit being used in the original study by Sambo and Iannetti (2013), which is based on the potential for increased muscle, nerve and pain related conditions over this age. Participant 32 however indicated no serious health concerns that would lead me to believe their age could impact their results in a substantive way, and therefore I believe their data can reasonably be included.

6.4.3.19 Summary of Responders

Table 17 - Responder model fitting

n	Full model significant?	Constrained model significant?	Subjectively and contextually valid?	Final model (if valid)
1	NO	NO	NO	
3	YES	YES	YES	Small
10	NO	NO	NO	
11	YES	YES	YES	Small
12	NO	YES	NO (REJECTED)	
13	YES	YES	YES	Small
14	NO	NO	NO	
15	NO	NO	YES (ACCEPTED)	Small
18	NO	NO	NO	
19	NO	NO	NO	
22	YES	YES	YES	Small
24	YES	YES	NO (REJECTED)	
26	YES	YES	NO (REJECTED)	
30	NO	NO	NO	
31	NO	NO	NO	
32	YES	YES	YES	Small

Based on the results from all contrast analyses and analysis of individual participant data, 6 Responders were considered to have valid models and in all cases they were fitted with the "Small" model. For Participants 12, 24 and 26, one or both contrast analyses indicated significant models, but these were subjectively not considered valid models. For participant 15, neither Contrast Analyses was considered significant, but the model was considered

subjectively valid. For Participants 3, 11, 13, 22 and 32, both Contrast Analyses indicated valid models which were considered subjectively valid.

6.4.4 Non-Responder Analyses

Table 18 - Non-Responder trial classifications

n	Valid (%)	Valid	Invalid	Too Early	Too Late	Too Short	Too Long	No Reaction
2	14.1%	9	55	3	4	9	1	40
4	6.2%	4	59	3	0	4	0	52
5	4.7%	3	61	21	0	3	0	37
6	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
7	34.4%	22	42	4	0	0	0	38
8	20.3%	13	51	1	0	9	0	41
9	6.2%	4	60	3	0	0	0	57
16	96.9%	62	2	0	1	1	0	1
17	73.4%	47	17	1	0	2	0	14
20	68.8%	44	20	0	1	5	1	14
21	21.9%	14	50	4	3	8	0	35
23	4.7%	3	61	2	5	2	0	53
25	26.6%	17	47	0	1	9	0	38
27	3.1%	2	30	1	0	2	0	27
28	12.5%	8	56	5	1	4	0	48
29	39.1%	25	39	4	10	7	1	19

6.4.4.1 Full Contrast Analysis

Table 19 – Non-Responder Contrast Analysis – Full AUC method

n	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity
2	*NA*	*NA*	*NA*	*NA*	*NA*	14.1%
4	*NA*	*NA*	*NA*	*NA*	*NA*	6.2%
5	*NA*	*NA*	*NA*	*NA*	*NA*	4.7%
6	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
7	0.2647	**0.1418**	0.2047	0.3635	0.7489	34.4%
8	0.9640	**0.2374**	0.8408	0.4664	0.5819	20.3%
9	*NA*	*NA*	*NA*	*NA*	*NA*	6.2%
16	0.0016	**0.0003**	0.0010	0.0209	0.0551	96.9%
17	0.0824	0.4321	0.2324	0.2320	**0.0357**	73.4%
20	0.1691	0.3738	0.1772	**0.1575**	0.3202	68.8%
21	0.7025	0.7932	0.9985	0.7981	**0.3238**	21.9%
23	*NA*	*NA*	*NA*	*NA*	*NA*	4.7%
25	0.3014	0.2447	**0.2432**	0.3513	0.5898	26.6%
27	*NA*	*NA*	*NA*	*NA*	*NA*	3.1%
28	0.7912	**0.3665**	0.4389	0.6759	0.3738	12.5%
29	0.1296	0.4013	0.2961	0.2981	**0.0606**	39.1%

Footnote c: Bolded values indicate smallest p value and model of best fit

For the Full AUC method Contrast Analysis, 2 Non-Responders had models with $p < .05$, Participant 16 and 17. For 7 participants a contrast analysis could not be performed as that participant lacked valid trial data for one or more distances, resulting in modelling of those positions to be impossible.

6.4.4.2 Constrained Contrast Analysis

Table 20 - Non-Responder Contrast Analysis – Constrained AUC method

n	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity
2	*NA*	*NA*	*NA*	*NA*	*NA*	14.1%
4	*NA*	*NA*	*NA*	*NA*	*NA*	6.2%
5	*NA*	*NA*	*NA*	*NA*	*NA*	4.7%
6	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
7	*NA*	*NA*	*NA*	*NA*	*NA*	34.4%
8	*NA*	*NA*	*NA*	*NA*	*NA*	20.3%
9	*NA*	*NA*	*NA*	*NA*	*NA*	6.2%
16	0.0008	**0.0001**	0.0003	0.0118	0.0698	96.9%
17	0.2018	0.4980	0.4723	0.5772	**0.0669**	73.4%
20	0.1185	0.3180	0.1340	**0.1183**	0.2327	68.8%
21	0.6763	0.7563	0.9135	0.9213	**0.3805**	21.9%
23	*NA*	*NA*	*NA*	*NA*	*NA*	4.7%
25	*NA*	*NA*	*NA*	*NA*	*NA*	26.6%
27	*NA*	*NA*	*NA*	*NA*	*NA*	3.1%
28	*NA*	*NA*	*NA*	*NA*	*NA*	12.5%
29	0.1132	0.3886	0.2359	0.2101	**0.0691**	39.1%

Footnote d: Bolded values indicate smallest p value and model of best fit

For the Constrained AUC method contrast analysis, only Participant 16 had a model with $p < .05$. For 11 participants a contrast analysis could not be performed as that participant lacked valid trial data for one or more distances, resulting in modelling of those positions to be impossible.

6.4.4.3 Participant 2

Nothing interesting of note. 9 valid trials, 55 invalid trials, 40 of which were no reaction.

6.4.4.4 Participant 4

Nothing interesting of note. 4 valid trials, 59 invalid trials of which 52 were no reaction. Participant 4s data contained only 63 trials, appearing as though the stimulator did not trigger for the 64th trial. This is not a concern due overall lack of response.

6.4.4.5 Participant 5

Nothing interesting of note. 3 valid trials, 61 invalid of which 58 were “Too Early” or no reaction. The “Too Early” classification was due to many responses detecting part of the stimulator interference as a blink response.

6.4.4.6 Participant 6

Participant had no data due to exclusion.

6.4.4.7 Participant 7

Participant presented a small selection of visually and detectable valid responses. 22 trials were classified as valid and 42 invalid, of which 38 were no reaction which agreed with visual inspection. Additionally based on visual inspection, it appears the HBR occurred albeit inconsistently. One possibility for triggering responses more readily would be to increase the working intensity, however this could prove to be intolerable or highly painful to the participant and therefore could not be easily ethically explored, resulting in no clear way to test these assumptions.

6.4.4.8 Participant 8

Participant presented 13 valid responses and 51 invalid of which 41 were no reaction. Similar to participant 7, it appears visually that the HBR occurred infrequently, but triggering a consistent response would be difficult or impractical.

6.4.4.9 Participant 9

Nothing interesting of note. Participant presented 4 valid trials with 60 invalid, of which 57 were no reaction. Visual inspection suggests almost all trials classified as valid were not genuine blink responses and were only classified due to artefacts of noise or random eye

activity. Participant 9s raw data showed 65 trials instead of 64 and the invalid trial (Trial 36) was removed.

6.4.4.10 Participant 16

In contrast to previous Non-Responders, participant 16 presented consistent small blink responses that appeared similar in pattern to Responder participants. 62 trials were classified as valid with 2 invalid, 1 of which had no reaction. The onset/offset threshold detection showed some errors but despite this mean onset, peak and offset durations were somewhat consistent. Responses also showed very low habituation, little differences between blocks, however noticeable differences between eyes with stronger responses in the right eye. Both Full and Constrained contrast analyses indicated fitting for the "Small" model with $p < .05$. Based on the noticeably high number of valid trials and visual inspection suggesting a reliable response, I would consider the "Small" model a reliable fit for this participant despite the classification as a Non-Responder. I believe this participant was likely misclassified as a Non-Responder in error.

6.4.4.11 Participant 17

Participant 17 presented somewhat consistent blink responses, with 47 valid trials and 17 invalid, of which 14 were no reaction. Visual inspection suggests that the HBR was triggered reliably in Block 1 however this appeared to habituate dramatically across the blocks. Responses when present were noticeably larger in trials 1 – 18, and significantly reduced in the remaining trials. Of interest and in contrast to previous habituation, the final set of trials (53-64) visually presented moderately consistent responses. Full and Constrained contrast analyses indicated fitting for an "Extra-Large" model, with the Full method being significant, and the Constrained method non-significant with $p = .067$. I believe this participant falls somewhere between a Responder and Non-Responder, and that current methods and results

cannot reliably fit the participant to a model. With further testing and more data I believe the participant could be fitted to a significant model and likely classified as a Responder with further testing.

6.4.4.12 Participant 20

Participant responded somewhat consistently across trials 1 – 12, inconsistently across trials 13 – 47 and failed to respond in trials 48 – 64. 44 trials were classified as valid, 20 as invalid with 14 as no reaction. Due to the rapid and dramatic habituation, no real conclusions can be drawn for this participant. It is clear that the HBR was triggered early in the experiment, but it is not clear why the participant habituated to the stimulations so dramatically. One possibility is due to the very low working intensity of 9mA and 8mA being too low to produce a consistent response in this participant. More testing and data would be required to draw further conclusions.

6.4.4.13 Participant 21

Participant responded somewhat consistently from trials 1 – 10, and subsequent responses were either inconsistent or non-existent. 14 trials were classified as valid, 50 as invalid with 35 as no reaction. Several trials contain large amounts of activity that visually do not appear to be valid blink responses. Because of this habituation cannot be determined easily.

6.4.4.14 Participant 23

Nothing interesting of note. 3 valid trials, 61 invalid of which 53 were no reaction. Visually there was no presence of genuine blink responses.

6.4.4.15 Participant 25

Participant presented extremely inconsistent but visually apparent responses between 50-100ms range where blink responses are expected. More consistent responses could potentially be obtained by higher intensities, however this would be difficult or impractical to achieve.

6.4.4.16 Participant 27

Participant only completed 1 Block and had no consistent blink responses. 2 trials were valid, 30 were invalid of which 27 were no reaction. Visual inspection suggests no genuine blink responses.

6.4.4.17 Participant 28

Participant presented 8 valid trials and 58 were invalid of which 48 were no reaction. Visual inspection indicates three instances of blink-like activity between 65-85ms that show expected ipsilateral-contralateral differences, but it cannot be determined if these are a genuine HBR-like response, due to their infrequency.

6.4.4.18 Participant 29

Participant presented eye activity with a significantly higher degree of noise than typical. Visual inspection cannot draw any real conclusions due to the extreme level of noise, however there are three possible trials that exhibit blink-like activity. Onset/Offset threshold detection was extremely inaccurate for this participant, classifying 25 as valid, and 39 as invalid of which 19 had no reaction.

6.4.4.19 Summary of Non-Responders

Table 21 - Non-Responder Summary

n	HBR present?	Non-Responder status valid?
2	NO	YES
4	NO	YES
5	NO	YES
6	N/A	N/A
7	LOW	SOMEWHAT
8	LOW	SOMEWHAT
9	NO	YES
16	YES	NO
17	HIGH	SOMEWHAT
20	LOW	SOMEWHAT
21	LOW	SOMEWHAT
23	NO	YES
25	LOW	SOMEWHAT
27	NO	YES
28	NO	YES
29	NO	YES

Of the 15 Non-Responders (excluding Participant 6), 8 participants were clear Non-Responders with either an extremely low or non-existent response rate. 6 participants presented an algorithmically and visually detectable HBR that indicates the potential of re-classification to Responder if more testing were possible. Participant 16 presented extremely consistent responses, such that I am confident the participant was misclassified as a Non-Responder and their results are therefore treated as similar to Responder data.

6.4.5 Summary of all participants

Based on the previous summaries, participants can be classified subjectively into four groups. The first group are participants with strong and clear blink responses able to be classified with a significant model, and where I consider the model to be both contextually appropriate and reliable. This group consists of 6 Responders and 1 Non-Responder. The second group

are participants with strong or moderate blink responses but no appropriate model. These participants are some of the most valuable, in that they help identify problems and flaws with the procedure and the data analysis process. This group contains 10 Responders and 2 Non-Responders. The third group contains participants with extremely small or inconsistent blink responses, but which are visually and algorithmically detectable. These participants I believe are distinct from the second group in that their small responses are not due to flaws or errors, but instead due to the specific participant's physiology, or due to a working intensity that was not strong enough to elicit a consistent response. This group contains 4 Non-Responder participants. The forth group contains participants who exhibited either no blink response whatsoever, or blink responses too inconsistent to be considered reliably caused by the stimulations in question. This group contains 9 Non-Responders.

6.4.5.1 Group 1

Table 22 – Group 1 - Clear HBR and significant valid model fitted

n	Status	Valid trials	Model
3	Responder	98.4%	Small
11	Responder	78.1%	Small
13	Responder	93.8%	Small
15	Responder	95.3%	Small
16	Non-Responder	96.9%	Small
22	Responder	90.6%	Small
32	Responder	100%	Small

Participants in this group are the participants previously described in the Responder analyses. These participants presented strong, and consistent blink responses and generally presented very low background noise. The trial validity of these participants was extremely high in a majority of cases, over 58 out of 64 trials valid with the exception of Participant 11.

6.4.5.2 Group 2

Table 23 – Group 2 - Moderate-Strong HBR with no significant valid model

n	Status	Valid trials	Notes
1	Responder	79.7%	• New testing methods (lack of rest/break) and lower 1k sample rate
10	Responder	81.2%	• Threshold detection unable to account for long response decay
12	Responder	73.4%	• Threshold detection unable to account for long response decay and inconsistent responses.
14	Responder	70.3%	• Inconsistent response magnitude
17	Non-Responder	73.4%	• Somewhat Inconsistent responses
18	Responder	84.4%	• Extremely high noise
19	Responder	73.4%	• Differences between eyes and strong habituation
20	Non-Responder	68.8%	• Strong habituation inconsistent responses
24	Responder	82.8%	• Unusual multiple peak blink pattern
26	Responder	53.1%	• Low trial validity
30	Responder	73.4%	• Elevated Position 3 response
31	Responder	90.6%	• Unusual multiple peak blink pattern

Participants in this group showed a mixture of strong to moderate blink patterns, but either inconsistency of responses, flaws in the data analysis method or procedural flaws resulted in an inability to reliably classify these participants with specific models. The most common difficulty was due to inconsistency of responses, typically due to inconsistency of the presence of a response, the size of the response or due to unusual patterns of blinks. A further difficulty was in the threshold detection method failing to properly classify many responses. Habituation was present for many participants, but was not considered a significant problem except in two participants, where habituation and other factors contributed to lower data quality. Trial validity was overall moderately high excluding Participant 26 where it was significantly lower, resulting in lower data quality. Participant 30 also presented unusual results with an elevated Position 3 response.

6.4.5.3 Group 3

Table 24 – Group 3 - Small HBR response

n	Status	Trials valid
7	Non-Responder	34.4%
8	Non-Responder	20.3%
21	Non-Responder	21.9%
25	Non-Responder	26.6%

Participants in this group showed very small but detectable blink responses. Analysing mean responses across the four distances indicate extremely small but visually detectable activity around the expected 50-100ms region. However due to the significantly lower trial validity rate, the lowered response is likely due to either working intensities being lower than necessary to elicit a sufficient response, or participants physical qualities preventing a sufficient response.

6.4.5.4 Group 4

Table 25 – Group 4 - No HBR present

n	Status	Trials valid
2	Non-Responder	14.1%
4	Non-Responder	6.2%
5	Non-Responder	4.7%
6	*NA*	*NA*
9	Non-Responder	6.2%
23	Non-Responder	4.7%
27	Non-Responder	3.1%
28	Non-Responder	12.5%
29	Non-Responder	39.1%

Participants in this group showed either no discernible blink responses or their eye activity was not distinguishable from background noise. In a majority of cases the trial validity rate was extremely low, and trials were typically only considered valid due to the detection

method attributing significance to background noise by chance. For Participant 29, more trials were considered valid than expected due to extremely large and erratic eye activity that was likely due to a pain or discomfort related eye-twitch as opposed to a genuine HBR.

6.4.6 Writing task and STAI Analyses

Between Blocks 1 and 2, participants completed either the anxiety writing task, or a control writing task. The writing task was based on work by Baker and Guttfreund (1993) and aimed to induce feelings of anxiety, or act as a control.

Data analysis of the writing component is therefore comprised firstly of a comparison of STAI sort form scores between the anxiety and control groups as a manipulation check, to see if the task worked as intended. Following this, comparisons between Blocks 1 and 2 can be performed, including comparisons of overall average HBR and average HBR for each block and hand position.

6.4.6.1 STAI scores

Table 26 - STAI scores

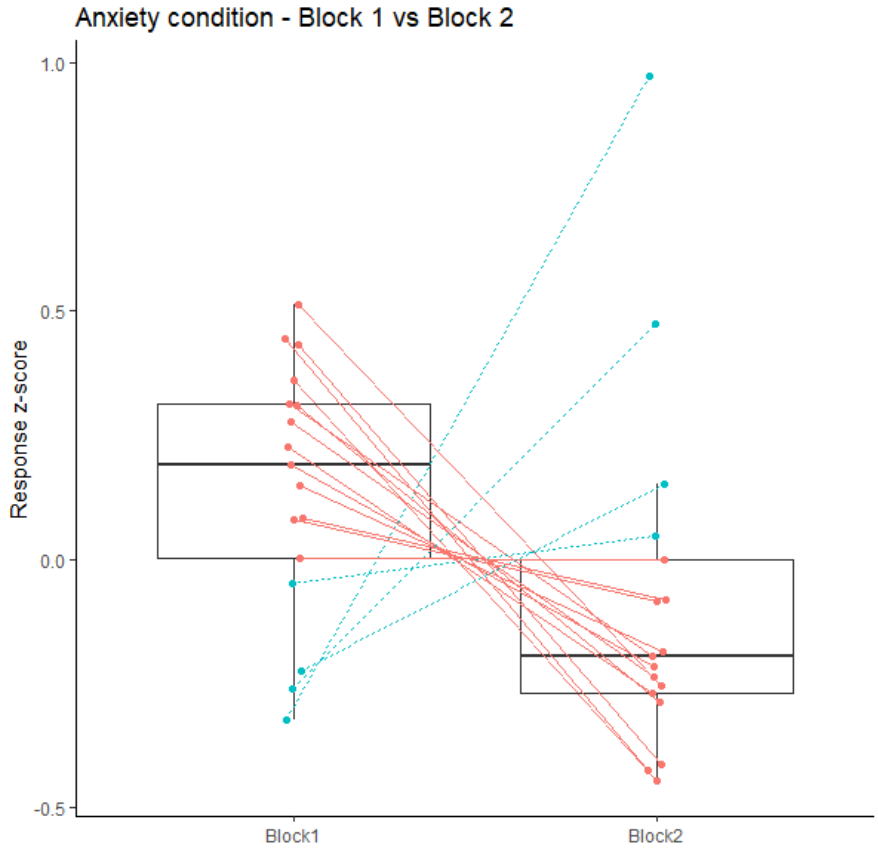
Group	Mean	Standard Deviation	Range
Overall	11.7	3.54	6 - 21
Anxiety	11.7	4.03	6 - 21
Control	11.6	2.93	7 - 18

The anxiety and control group means were nearly identical, differing by only 0.09, and were not significantly different with $p = .944$. The Anxiety group showed slightly greater spread and range of results as compared to the Control group. Based on the extreme similarity between the groups, it is clear the writing task did not function as desired.

6.4.6.2 Pre-Post writing task response comparison

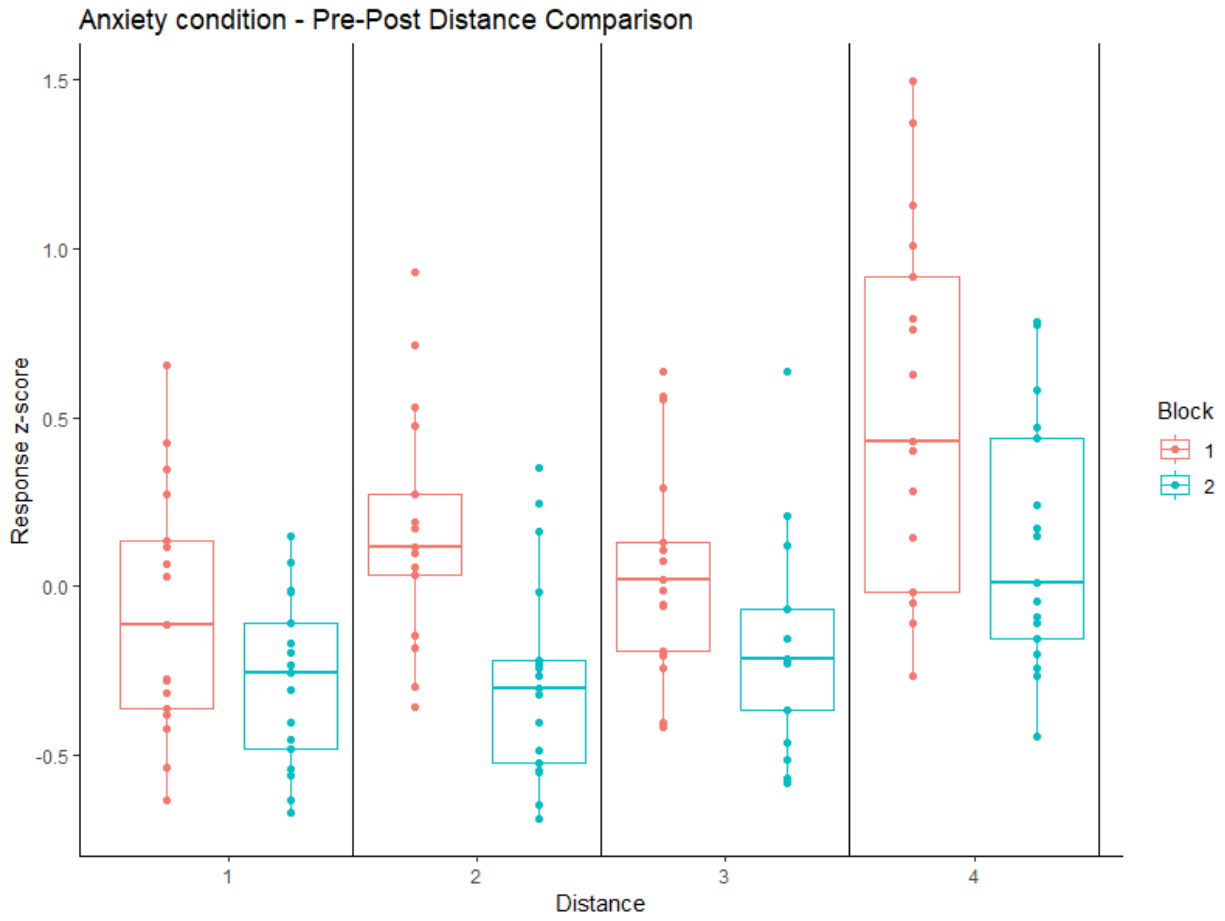
6.4.6.2.1 Anxiety condition

Figure 9 – Anxiety condition – Block 1 vs Block 2



Comparing Anxiety group Pre-Post writing task responses indicates a trend of a reduced response from Block 1 to Block 2 with variability of responses remaining somewhat constant, with Block 2 appearing to have several large outliers. Performing an exploratory paired t-test of Block 1 and Block 2 responses indicates $t = 1.63_{(16)}$ with a $p = .123$ which is considered not significant.

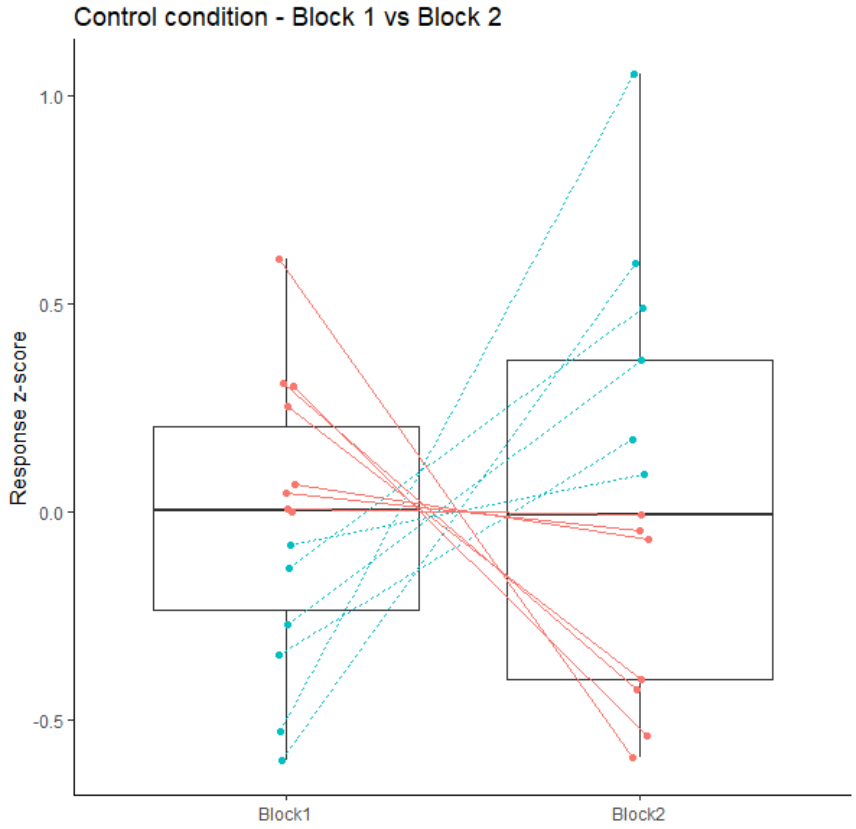
Figure 10 – Anxiety condition – Pre-Post Distance Comparison



Breaking down Block 1 and Block 2 differences in mean response over each distance indicates a reduced response across all 4 hand positions, with a dramatically reduced response for Position 2 and Position 4.

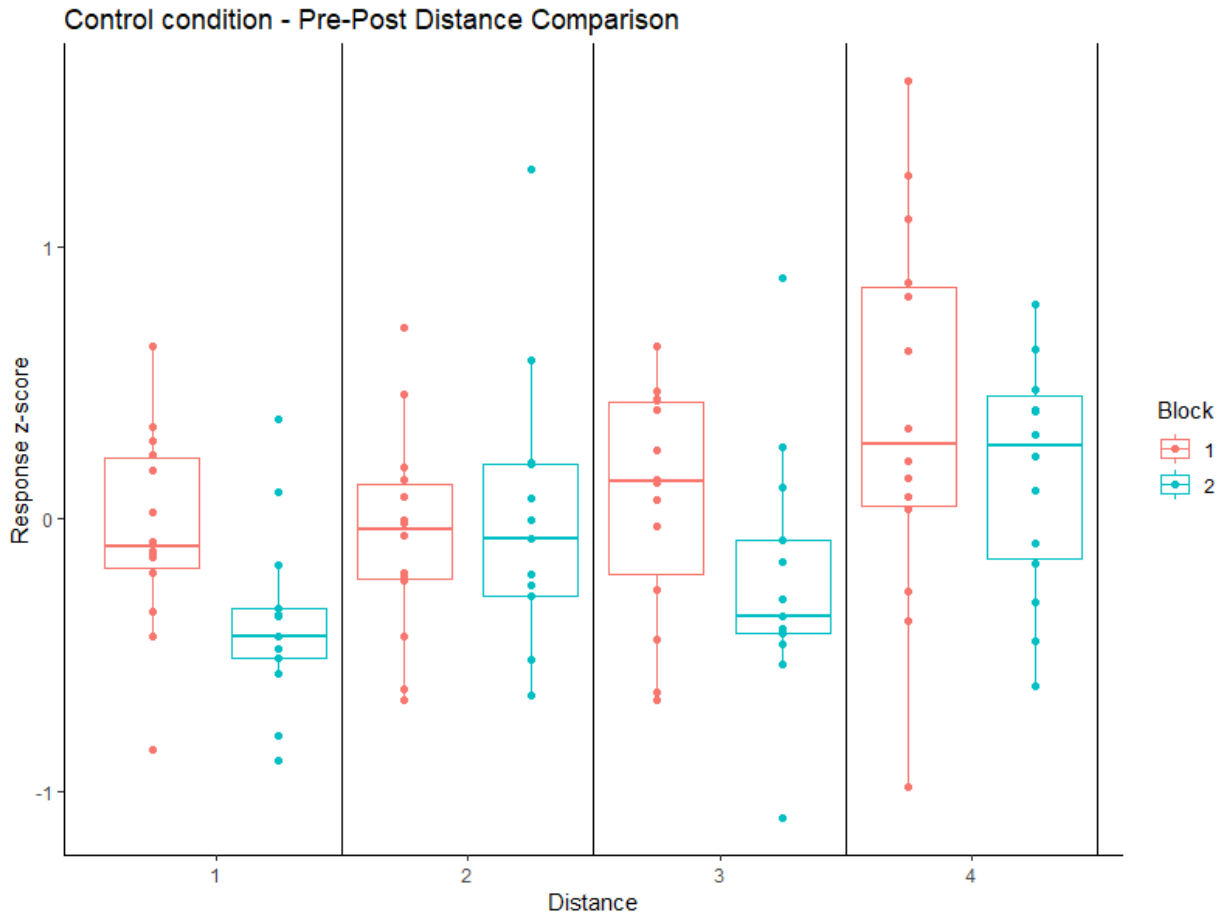
6.4.6.2.2 Control condition

Figure 11 – Control condition – Block 1 vs Block 2



Comparison of the Control group pre-post writing task responses indicate a balance of increases and decreases for a given participant, with an overall pattern of increased variability of responses in Block 2.

Figure 12 – Control condition – Pre-Post Distance Comparison



Breaking down the control group responses by Block and distance indicates that for Positions 2 and 4 responses were similar between blocks, and for Position 1 and 3 that responses were reduced. For the control group it appears responses have more variability due to the greater presence of outliers, which makes more in-depth interpretation difficult.

6.4.6.2.3 Pre-Post writing task summary

There do not appear to be any consistent or significant pre-post differences between either groups. Across all participants it appears that responses were somewhat reduced and it appears unlikely this was related to participation in either group, as decreased responses were observed seemingly randomly across all participants.

Chapter 7 Discussion

For this study a replication of the HBR procedure by Sambo and Iannetti (2013) was performed to measure the HBR, a blink reflex triggered via median nerve electrical stimulation at four different hand positions. Participants additionally completed a State Anxiety inducing or control writing task to explore its effects on HBR magnitude and potential changes in DPPS. Thirty-two participants were tested, 16 of which were classified as Responders and therefore had data testable via pre-registered analyses. Several key findings were identified, firstly that the HBR is positively correlated with hand distance indicating basic support for the presence of HBR mediation, secondly that this relationship appeared to be non-linear indicating support for the presence of the DPPS mediating HBR across distance. Additionally it was found that the state anxiety writing task failed to induce feelings of anxiety, and no comprehensive differences between the anxiety writing task and control task participants were identified. Furthermore it was identified that individual differences in blink patterns, impedances and electrical tolerances likely contributed to the HBR being difficult to measure, and alterations and refinements to the testing procedure could improve data quality significantly in future studies.

7.1 HBR is positively correlated with hand distance

The first confirmatory hypothesis, stating a positive correlation between hand distance and HBR was supported by the finding of a moderately positive and significant relationship between the nearness of the hand to HBR magnitude. This hypothesis was established to ensure the replication was functioning at its most basic level. By identifying a positive correlation between hand position and HBR, this supports the wide spectrum of DPPS related research that has shared this finding (Rory John Bufacchi, 2017; R. J. Bufacchi & Iannetti, 2016; R. J. Bufacchi et al., 2016; Sambo & Iannetti, 2013; Sambo, Iannetti, et al., 2012;

Sambo, Liang, et al., 2012; S. B. Wallwork et al., 2016). Showing support for this relationship additionally supports the conclusion of an overall increased blink response to an approaching and therefore more threatening stimuli. This is an intuitive conclusion, indicating that as the threat nears the face the magnitude of the response to the stimuli increases, allowing for faster and more effective protection of the body by threat avoidance.

The findings lend support to the existing understanding of threat response and reflexes, suggesting that the testing method is effective at measuring this specific type of threat response.

7.2 HBR relationship is non-linear

The second confirmatory hypothesis, stating a non-linear positive correlation between hand distance and HBR magnitude was supported by various analyses. Firstly it was indicated that hand position was a significant factor for HBR magnitude, and that standard t-tests were shown to be significant for combinations involving position 4 (pairings of 1 and 4, 2 and 4, and 3 and 4). This indicates that significant differences are primarily related to Position 4, and therefore changes in response are not consistent across all hand positions and non-linear.

Point by Point analyses were also performed, with these indicating a primary region of significant differences between hand positions from 49.3 – 88.2ms, and Point by Point t tests showing significance difference between Position 3 and Position 4 in a similar region of 49.0 – 89.2ms. These analyses together indicate a similar conclusion to the standard ANOVA and t-tests specifically that the differences between Position 3 and Position 4 are greater than other successive pairings, and therefore the relationship over all distances is non-linear. One primary flaw with the Point by Point method as illustrated by Sarah B Wallwork (2016) is that if multiple blinks compared do not have similar onset and offsets, the comparison can provide misleading results as it is not accurately comparing similar parts of each blink to one

another. Despite this problem, the region of time found to be significant corresponds closely to the mean onset and offset values identified with the 2sd threshold method of 56.9 – 102.8ms. Together, these distinct analyses show a similar ~50ms region of time where blinks are occurring and that these blinks are significantly different between Positions 3 and 4, and therefore the relationship as the hand is closer to the face is non-linear across the four hand positions.

7.3 The study as a replication

A key aspect of this study was to act as a replication of the HBR testing method by Sambo and Iannetti (2013). One important difference between this study and the original is the experiment and experimenter context. In particular, it should be emphasized the greater experience and expertise present for the original researchers as compared to myself. While this difference should not be overstated or used against the findings of this study, it is not a factor that can be ignored. In particular, because this study likely had less resources and personnel in addition to my relative inexperience performing the HBR procedure, I believe it reasonable to assume that results would be somewhat less consistent as compared to the original study. The current study has identified similar conclusions as the original study, however it has diverged in a few noteworthy ways.

One notable difference to the original study by Sambo and Iannetti (2013) was that they identified significant differences between Position 2 and 3. The original studies standard t-tests showed clear significance at $p < .0001$ whereas this study identified non-significance at $p = .146$. Further exploratory analysis using t-tests using Constrained AUC values show identical results, specifically significance only with regard to Position 4 and its pairings. Further compounding this finding, the current studies point by Point t-tests also indicated no significant difference between Positions 2 and 3 in contrast to the original study by Sambo and Iannetti (2013). I believe this difference is due to a lack of consistency in responses for

Position 3 in the present study. My arm position apparatus for this study was rudimentary and anecdotally I observed the most consistent positions to be Position 1 due to the arm lying flat, and Position 4 due to it needing to be checked for each participant based on its potential to be performed incorrectly.

In contrast, using Table 8 – Point-by-Point significant regions of time from current and original, there appear to be similarities in the regions of time identified between this and the original study. In particular the onset times appeared the most similar and consistent between the studies, indicating support for the HBR method being executed effectively. In contrast, this study identified later time periods to be significantly different than the original, which can be attributed to lower consistency of blink offset durations. This is likely due to limitations of the onset/offset detecting method used for this study, and could be improved with future refinements. Additionally, because Point-by-Point analyses require compared time regions to overlap, lowered consistency of offset durations results in a wider region of time being considered significant and less accurate results.

One further difference between the current and the original study by Sambo and Iannetti (2013) is in the model fitting for participants. In particular the current study found that a maximum of 8 participants out of 16 Responders had significant models prior to subjective analysis (6 Responder and 1 Non-Responder after subjective analysis), whereas all 15 Responders in the original study had significant models. Furthermore p-values for the current study were noticeably less consistent with greater range and variability. I believe this can be attributed to the lower consistency of both triggering and detecting of blink responses in participants. It is clear that for participants with higher rates of valid trials, p values in general are lower due to the more consistent responses. This is caused by the fact that consistently low Position 1 and high Position 4 responses will result in all models fitting somewhat effectively, as all models feature low Position 1 and high Position 4.

Despite the limitations present, I believe the replication was effective, and provides strong support for the presence of the HBR and its mediating relationship, and moderate support for the presence of the DPPS.

7.4 The anxiety writing task was ineffective

The purpose of the anxiety writing task was to induce feelings of anxiety in the participant and to investigate the effects of increased levels of state anxiety as compared to the control condition. The anxiety writing task failed to achieve this goal as the STAI scores between anxiety and control conditions were not significantly different with $p = .944$, indicating high similarity between the two conditions. This suggests the task failed to induce state anxiety in for the anxiety group as compared to the control group, and therefore did not produce different groups capable of being compared.

There are two explanations for the failure to increase state anxiety levels. Firstly it is reasonable to assume that the task may not have been taxing enough or anxiety inducing enough for participants. During the original construction of the STAI-Short form (State), non-clinical mean responses ranged from 1.85 to 2.43 (prorated to scores ranging 1 – 4). Furthermore in a comparison of non-clinical and anxiety diagnosed participants, mean nonclinical anxiety responses of 1.85 and 2.75 for the clinical sample were observed (Ortuño-Sierra, Garcia-Velasco, Inchausti, Debbane, & Fonseca-Pedrero, 2016). The current study showed a mean response of 2.07 for the anxiety group and 2.05 for the control. Based on these lower than expected scores it seems reasonable that the anxiety writing task was not taxing enough on participants, and did not induce strong feelings of anxiety as intended.

An alternative explanation is that the context of the experiment could have interfered with the writing task by itself inducing higher state anxiety in all participants. It is reasonable to assume that the context of the experiment, specifically receiving electrical stimulations in an

unusual situation was itself an anxiety inducing experience. The somewhat elevated scores of 2.07 and 2.05 as compared to the non-clinical past scores of 1.85 could support this theory. However solving this problem would prove difficult, as familiarising participants with the equipment or the procedure too greatly could interfere with their responses in unexpected and unusual ways.

7.5 No significant differences between anxiety and control groups

Due to the writing task not performing as intended, comparisons between groups are limited. Comparing pre and post writing task HBR responses identified no significant differences either between anxiety and control conditions, or differences within the groups for a given hand position. The only visible observed difference between groups was a slight decrease in HBR magnitude across both groups, likely due to participants somewhat habituating to the stimuli across the experiment, however this decrease was non-significant and could therefore be an artefact.

7.6 Individual differences

The HBR procedure as replicated in this study presented a wide range of individual differences. Broad demographic statistics were as expected, consisting of a typical university student population. Impedance and intensity statistics were highly varied, with no significant interactions between demographic statistics, impedances and intensities being identified. Despite previous findings of gender differences in tolerance of transcutaneous electrical nerve stimulation (TENS) (Rocha et al., 2011) I observed no significant differences in this study. The likely explanation is a lack of specificity on these particular measurements during the study, and the stimulations in this study being single pulse, as opposed to the repeated-pulse type used with TENS. Additionally there appeared to be little difference between Responder and Non-Responder participants with regard to working intensities, with

differences being non-significant likely due to the wide spread across participants. Working intensities were somewhat lower than past research, however due to the wide range of intensities it is difficult to determine how similar or dissimilar the intensities are.

In contrast, the desired HBR in response was identified extremely reliably across Responders and identified somewhat reliably for Non-Responders. The process of classifying participants as Responders or Non-Responders appears to be an effective and accurate process, with only 1 Non-Responder being considered misclassified, and results showing clear differences of HBR magnitude between the two groups.

The threshold detection and trial classifications I developed, despite being largely arbitrary and intentionally broad, showed dramatic trial classification differences between Responders and Non-Responders, demonstrating their effectiveness and showing great promise. I believe that if refined, a highly effective EMG blink classification analysis method could be created, potentially removing the need for much of the subjective judgement currently used in EMG blink analysis.

Further analysis of the HBR using point by point analyses was also very encouraging, indicating similar results to the threshold method. This suggests the method is effective, and its potential as an alternative should be considered for future research.

7.7 Limitations and Future considerations

While the study on its own and as a replication has shown great promise, it features various limitations which must be acknowledged to put the findings in context.

One key limitation that was identified early in the study, was the selection bias with regard to the participants tested. Due to the study openly featuring electrical stimuli and participation being entirely voluntary, it is reasonable to assume individuals with low pain tolerances or a fear or dislike of electrical stimuli would be less likely to participate. It is possible that the

results therefore do not reflect the general population and that individuals with higher pain tolerances could react in fundamentally different ways to individuals with lower tolerances. One solution to this could be to use a questionnaire about perceived pain tolerances and fear of electrical stimuli, which could be compared to a general population to ensure a representative sample is obtained.

With regard to impedances and data recording, it was noticed during data analysis that impedance values for the left eye were systematically higher than right eye impedances. This is due to a flaw in the cleaning and skin preparation procedure, which was caused by the positioning of myself and the participant causing less effective left eye cleaning and preparation, resulting in higher impedances. For future studies care should be made to ensure all electrode sites be prepared with equal care to avoid systematic differences.

Consistency of electrical stimulations was also identified as a key difficulty during the study. Identifying the best stimulator position and limiting overall participant discomfort was extremely challenging, as these elements were extremely sensitive to small shifts in stimulator position. I observed that small adjustments to the stimulator position at the wrist could elicit very different perceived discomfort in participants, and for some participants three or four adjustments were necessary to minimize feelings of sharpness or discomfort. In some participants, discomfort could not be minimized as much as desired and for these participants discomfort was minimized as much as possible. This limitation I believe to be an issue of experience on my part. With more personal experience and time to pilot electrical stimulation procedures, I believe many of these issues could be solved and in future studies these difficulties are likely to be minimal.

A further key limitation in the study is in the failure of the anxiety writing task and determining the specific cause. Because of the context of the experiment, it is difficult to

untangle whether scores on the STAI-Short form are directly due to the writing task or due to elevated feelings of anxiety from the experiment itself or a mixture of both. It is likely not possible to completely isolate or remove feelings of anxiety for an experiment of this type, as the procedure will always be considered unusual to participants and will somewhat influence levels of state anxiety. One solution for future testing would be to use a new task or sufficiently alter the existing writing task such that it induces significantly higher feelings of anxiety in participants. This could ensure significant differences between the anxiety and control conditions even accounting for anxiety induced by the experiment itself.

Several difficulties would be present in making this change. The new or altered task would still be required to be located before the second block of testing, and experiment length is an important factor to consider due to problems associated with alertness and tiredness in participants. In addition significantly increasing the intended levels of anxiety has ethical considerations and participant wellbeing should always be considered.

If the new or altered task was sufficiently large in scope, participants could be tested across multiple sessions in order to limit negatives associated with experiment length. This could be beneficial, allowing for larger blocks for each session and consequently more data, leading to clearer analysis of individual blink patterns. It would however require significantly more time for participants and researchers, and therefore additional resources.

Furthermore, based on the length of the experimental sessions it would also be necessary to determine how long the effects of the anxiety task would last. A decay time for any induced emotion is always expected, and inducing a sufficiently large enough anxiety response with limited decay could prove difficult for longer experiments. However with a dedicated experiment I believe the associated difficulties could be overcome, and the relationship

between state anxiety and HBR tested in the future with greater resources and a more focused experiment.

7.8 Conclusion

This study sought to replicate the procedures by Sambo and Iannetti (2013) and despite the many difficulties identified succeeded at this task. It identified the presence of the HBR in participants and the procedure provided support for its positive correlation to hand distance, as well as its non-linearity which suggest presence of the DPPS. Specific exploratory modelling was as expected different to the original study, however extremely recent developments in the field of DPPS research suggest that these models may not be very useful (Rory J. Bufacchi & Iannetti, 2018). This does not detract from the findings of this study, as replicating the original study and identifying the presence of the HBR is an important act of support to the field. Additionally, by identifying limitations and difficulties with the HBR testing method, this study will help guide future research in order to construct clearer and more specific testing methods for higher data quality and improved results.

It is unfortunate that the state anxiety component of the study did not function as intended, however it is reasonable to assume that it may have exceeded the possible scope of the project. However I believe investigating the relationship between HBR and state anxiety is both achievable and worthwhile, and hope that this study will help guide such work.

Research into the field of the DPPS is growing dramatically, and based on new work it is clear the field is wide and complex. The field shows great promise and it is clear that its findings are key to our understanding of threats and personal space, and to a deeper understanding of how we safely navigate this complex world with such ease.

Chapter 8 Appendices

8.1 Appendix 1 – Information Sheet

Participant Information Sheet

Title of experiment: Reflex response experiment

Kia ora. My name is Mark Colville and I am performing a research experiment for my Master's Thesis. I am interested in studying how we process threats and reflexes, and hope that the findings can help make us feel safer in our everyday lives.

How can you help?

Before you decide whether or not to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take the time to read the following information carefully and decide if you want to take part in this experiment. Please feel free to ask questions if there is anything that is not clear or if you would like more information.

What is involved?

In this study you will experience two blocks of non-painful electrical stimulations delivered to your wrist with your arm in various positions. The intensity will be calibrated to your particular tolerances. Sensors will be attached under your eyes to record biological processes. You will also complete a writing task about an experience in your life. The experiment will take approximately 1 to 2 hours to complete.

What discomfort is there?

The skin cleaning process under your eyes can cause discomfort for those with sensitive skin. This is necessary for the sensors to record properly.

Electrical stimulations to the wrist may be uncomfortable or mildly painful in some individuals. During the experiment the intensity will be calibrated for each wrist and will not change during each block.

It is important that the stimulations be the highest intensity you are able to tolerate.

Please tell the experimenter if the stimulations are sharp or stronger than you can tolerate.

Do I have to take part?

Participation in this study is entirely voluntary. You are under no obligation to take part in this study. If you decide to participate you will be asked to sign a consent form that

confirms that you understand this information sheet and what is involved in the experiment. You have the right to withdraw from the study at any time and without giving a reason. At the conclusion of the study you will receive a $20 voucher as koha in thanks for your participation.

What happens to my data and information I provide?

All data and information you provide will be confidential and will be anonymized. Only the researcher (Mark Colville), the research supervisor (Dr. Michael Philipp) and the appropriate Massey Staff will have access to consent forms and specific participant information. These consent forms and personally identifiable data will be kept for 5 years and then destroyed.

Who do I contact for questions or concerns?

Researcher contact – Mark Colville – [redacted]

Supervisor contact – Dr. Michael Philipp - M.Philipp@massey.ac.nz

Co-Supervisor contact – Dr. Matthew Barnes - M.Barnes@massey.ac.nz

We encourage you to contact either myself, Dr. Michael Philipp or Dr. Matthew Barnes at the above addresses if you have any questions or concerns.

Committee Approval Statement

- *This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 18/20. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 356 9099 x 85094, email humanethicsoutha@massey.ac.nz .*

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

8.2 Appendix 2 – Health Form

Health screening questionnaire

Title of experiment: Reflex response experiment

Please read the following questions carefully. If you have any difficulty, please advise the researcher who is conducting the study. If you answered yes to any of the questions below more information may be requested to accurately assess your suitability to participate in this study.

The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), Massey University Human Ethics Committee requirements and conditions specific to the study. The information provided by you on this form will be treated with the strictest confidentiality.

Age: _____ **Gender:** _____

- ***Have you had or do you have a known heart or cardiovascular condition?***

  Yes ***No***

- ***Have you had or do you have any nerve/muscle injuries located in your hands, arms, neck, or head?***

  Yes ***No***

- ***Have you had or do you have an injury or medical condition that you think may affect your ability to sense pain or discomfort?***

  Yes ***No***

- ***Are you taking prescribed medication that may affect your cognition, or ability to feel pain or discomfort?***

  Yes ***No***

- ***Have you had or do you have persistent or regular lower back pain?***

  Yes ***No***

- ***Have you suffered from any painful injury or condition that lasted more than one week in the last 6 months?***

  Yes ***No***

- ***Have you been hospitalized in the last 6 months?***

  Yes ***No***

- ***Do you have any cultural or religious sensitivity regarding measurements of the human body?***

  Yes ***No***

- ***Do you have any other health concerns that may be relevant?***

  Yes ***No***

Participant Initials Experimenter Initials

8.3 Appendix 3 – Consent Form

Consent form

Title of experiment: Stimulation induced reflex response experiment

Thank you for your interest in this study.

The data you provide will be used only for research purposes. As a participant in this research you will never be personally identified by any of the outputs that arise from this project (e.g., reports, research articles) and your data will never be identifiable to anyone outside the research team.

For each of the following statements, please tick either *agree* or *disagree*:

Agree	*Disagree*	
[ ]	[ ]	I confirm that I have read and understand the information sheet for the above experiment.
[ ]	[ ]	I have had the details of the study explained to me.
[ ]	[ ]	I have had opportunities to ask questions and my questions have been answered to my satisfaction. I understand that I may ask further questions at any time.
[ ]	[ ]	I understand that my participation is voluntary and that I am free to withdraw at any time, without providing any reason.
[ ]	[ ]	I understand I may be requested to write about events that are uncomfortable or potentially distressing and that I have the right to refuse the writing task at any time.
[ ]	[ ]	I agree to have my de-identified data included in a public, online repository that will be available to other researchers.
[ ]	[ ]	I agree to take part in the above named experiment.

Participant

Full name: _____

Date: _____

Signature: _____

Researcher

I have explained to the best of my ability the experiment to the above participant.

Full name: _____

Date: _____

Signature: _____

8.4 Appendix 4 – Anxiety writing task

Participant number: _____ **Date: _____**

Please read the instructions carefully.

In this writing task you will answer some questions and write a short essay about a situation where you felt **strong feelings of anxiety**. You should focus on the feelings of anxiety and the reasons why you think the situation made you feel anxious. You may include as many details as you think are necessary, and you may replace names with initials for privacy if you desire. You are encouraged to write for up to 10 minutes and the experimenter will let you know when the time is complete.

Please let the experimenter know you have finished the instructions before beginning.

What year did the event take place?

What were the initials of the people involved?

Describe the event.

8.5 Appendix 5 – Control writing Task

Participant number: _____ **Date: _____**

Please read the instructions carefully.

In this writing task you will answer some questions and write a short essay about **your trip to Massey University today**. You may include as many details as you think are necessary, and you may replace names with initials for privacy if you desire. You are encouraged to write for up to 10 minutes and the experimenter will let you know when the time is complete.

Please let the experimenter know you have finished the instructions before beginning.

Describe the event.

8.6 Appendix 6 – STAI Short Form

Participant number: _____ **Date: _____**

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you feel **right now, at this moment**.

There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe you present feelings best.

	Not at all	*Somewhat*	*Moderately*	*Very much*
1. I feel calm	1	2	3	4
2. I am tense	1	2	3	4
3. I feel upset	1	2	3	4
4. I am relaxed	1	2	3	4
5. I feel content	1	2	3	4
6. I am worried	1	2	3	4

Please make sure that you have answered **all** the questions.

8.7 Appendix 7 – Full procedure script

Setup

1. Prepare electrodes. Attach collars and insert gel.
2. Lay out EMG preparation equipment, alcohol wipes, abrasion, cleaning etc.
3. Software running, pre-sets loaded.

Introduction Script

10. Have participant be seated, introduce self.
    a) *"Thank you for your interest, my name is Mark Colville. To begin I will have you read this information sheet, and let me know if you have any questions"*
11. Present participant the Information Sheet, see Appendix 1 – Information Sheet and instruct them to read it thoroughly and that when they are done they can ask questions. Allow for 2-3 minutes and for questions if required.
12. Provide participant with Health screening form, see Appendix 2 – Health Form and instruct them to complete it. Use form to check for any health concerns, and check for any potential allergies or sensitivities.
    a) *"This is a health form to screen for any injuries, illnesses or conditions that I need to know about. Please include as much details as you can and if you think it may be relevant, please include it."*
13. Provide Consent form, see Appendix 3 – Consent Form, and reaffirm to participant to ask any questions they want.
    a) *"This is a consent form for you to complete that indicates you understand the study and are participating voluntarily. Please make sure to ask me about anything you are unsure of."*

14. Reiterate precise procedure (approximate script used for all participants)
    a) *"To begin with I will be connecting these sensors underneath your eyes here (indicate) and here (indicate), and one in the centre of your forehead here (indicate). To do that I need to clean the skin by using soap, alcohol wipes and some light scrubbing. I'll do several iterations then attach the sensors, then check their connection. Do you have any skin allergies or sensitivities?*

       After I have the sensors attached I will attach the stimulator to your wrist. We are starting with you LEFT/RIGHT wrist today. Once the stimulator is attached, I will start the stimulations below what you can feel and I need you to tell me when you can start to feel them. Once you are able to feel them, you will put on this set of headphones that will be playing noise, and then I will be triggering stimulation ***without telling you and increasing them over time****. It is important during this part that you tell me when the stimulations get to the highest level you can tolerate or if they are above what you can tolerate. Also, if the stimulations feel sharp please tell me, and I will attempt to adjust the stimulator to be more comfortable.*

    b) *Once we get to an intensity we agree on we will begin Block 1. For this, all you need to do is move your arm between several positions, hold it there, then there will be a wait followed by a stimulation, and this will repeat for about 15 minutes.*

       Following this you will have a small break, followed by a writing task, followed by Block 2 testing your other wrist, then you are done. Do you have any questions?"

Preparation procedures

1. Prepare participant for EMG and electrostimulation testing.

2. Clean orbicularis oculi and wrist electrode sites of dirt and skin by-products with soap or skin wash. The orbicularis oculi electrode sites are located 1 - 2cm underneath each eye, and the wrist site is over the median nerve. Clean orbicularis oculi electrode sites with alcohol wipes or equivalent. Skin at the orbicularis oculi electrode sites must then be exfoliated. Allow for 2-3 repetitions of alcohol and abrasion for the orbicularis oculi site based on participant feedback.
    - *"Please let me know if the cleaning is uncomfortable or if you would like me to stop"*
3. Connect EMG electrodes to participant. Test electrodes for impedance with a target of below 10kΩ. If impedance is limited due to discomfort, continue but make note of greater than desired impedance. If impedance is high, but participants consent to more cleaning, remove the electrodes and repeat cleaning procedures and retest impedance.
4. Attach stimulator electrode to wrist used in Block 1.

Responder/Non-Responder screening procedure

1. Software should be running with live data viewable.
2. Electrical stimuli settings are a 200 microsecond square wave consisting of a single pulse, beginning at an initial intensity of ~500µA (0.5mA), a sub-perceptible level.
3. While informing participant of stimulations, increase intensity in 0.5-1.0mA increments based on participant feedback allowing ~10-20 seconds inter-stimuli duration. Instruct participants should indicate if the stimulations are "sharp" or when they become intolerable/greater than their tolerance.
    - *"Now I will be triggering stimulations to your wrist and telling you as I do so. Please let me know when you are able to feel them"*

4. Continue increasing stimuli intensity to the wrist until participant indicates they can feel the stimulations. At this intensity, have participants wear headphones playing white noise and indicate that stimulations will be triggered without informing them. Ensure participants can hear the experimenter but not hear any mouse/keyboard noises to indicate stimulations. Have participants relax their arms with palms facing up, and to look at fixation cross 30cm away at a 45 degree angle downwards.
    - *"From this point I will be triggering stimulations **without telling you** and I will be **increasing them over time.** It is important you tell me when the stimulations are the highest you can tolerate, or if the stimulations are more than you can tolerate. Let me know if the stimulations feel sharp, and I can adjust the stimulator so the stimulations do not feel sharp"*
5. Continue increasing intensity based on participant feedback. For participants with low tolerances increment by 1-2mA and for higher tolerances increment up to 5mA.
6. During increases participants will typically respond with a supra-maximal thumb-twitch in their stimulated hand. For participants with tolerances higher than this intensity, this indicates a good stimulator electrode placement.
7. Increase intensity until a blink response is observed. The blink response must occur approximately 20-150ms after the stimuli was triggered, with a minimum duration of 10ms. In addition to the blink response itself, it can visually be accompanied by a body startle response including full body movement and vocalisations. Reflex blink responses on average present higher magnitudes than spontaneous blinks.

8. Once a response is identified, the stimuli should be repeated two more times at the same intensity. If a consistent response is observed across all 3 instances, record the intensity as the working intensity, and the participant is classified as a "Responder" and the intensity is marked as that participants working intensity.
9. If a consistent response is not observed, repeat the process of increasing stimuli intensity and testing with 3 instances, either to the highest tolerable intensity, until 3 consistent responses are identified, or the intensity reaches 80mA. Participants who reach 80mA intensity or withdraw consent prior a consistent blink response are marked as "Non-Responders".
10. Participants are not informed of their status as "Responder" or "Non-Responder". Both continue to the main experiment however "Non-Responder" data will be excluded from all confirmatory analysis. The working intensity for Non-Responders is at 80mA, or the intensity at which they withdrew consent to increasing intensity.

Main experiment procedures

a) Check all electrodes for firm connection.
b) Position arm marker board by participant on tested side. Instruct participant on the four arm positions. Instruct participant that these positions are called Position 1, Position 2, Position 3 and Position 4, respectively Ultra-Far, Far, Near, Ultra-Near. The arm positions have the hand located at 60cm, 40cm, 20cm and 4cm from the participant's eyes, respectively. Approximate arm angles are 120 for Position 4 (Ultra-Far) and 75 degrees for Position 1 (Ultra-Near), with Position 2 and 3 at intermediary angles. Adjust chair height if necessary to ensure when the hand is in Position 4 that the fingertips are not visible in peripheral vision and ensure that the hand or fingers do not touch their face.

c) Have the participant perform several practise arm movements between the four distances to ensure understanding, calling them out as Position 1 to Position 4. The participant's non-tested hand should remain on the table relaxed with palm up.
    - *"Looking at this board, you need to tilt your arm up towards you keeping your elbow in the same spot, moving your arm in line with these positions. For position four, you need to place your hand in front of your LEFT/RIGHT eye, a few centimetres from your face, making sure your hand is not touching your face. Are you able to see your fingertips?"*

d) Instruct participant that they will have Positions announced to them during the experiment, and for them to position their arm accordingly and to hold it in that position. Instruct that they may check the board to correctly position their arm, and once positioned they should focus on the fixation cross located at a 45 degree angle downwards approximately 30cm from their face.

e) Instruct that the experiment will begin and play white noise via headphones ensuring participant is able to hear instructions but unable to hear any mouse/keyboard sounds. Ensure software is recording.
    - *"For the main experiment, I will call out a position to you, one two three or four, and you need to place your arm into that position then focus on the cross. You can check the board to ensure your arm is in the right place if you need to. After each position there is a wait, then a stimulation at the agreed intensity, then I will call out another position and this repeats for about 15 minutes. Do you have any questions?"*

f) Using the individual participants predetermined hand position order (pseudorandom such that no more than 2 of the same distances are repeated

consecutively), announce the first location using Position 1 to Position 4. Once the participant has placed their hand in the correct position, wait 30 second before triggering a 200 microsecond electrical stimulus at the previously recorded working intensity. This procedure of positioning, wait, stimulation is performed 32 times using the participants predetermined hand position order for the initial hand (left or right). If complications occur such as talking, or breaks are needed, allow for a full uninterrupted 30 seconds before stimulation.

g) Once complete, disconnect stimulator electrode. Disconnect EMG electrodes from system (leaving the electrodes attached to the participant) and provide cords to participant. Allow participant have a break for 5 minutes, and encourage them to walk around and stretch.
    - *"Now you have a few minutes break, I encourage you to stand up and stretch and wake yourself up as the experiment can be tiring"*

h) Reconnect participant and connect stimulator electrode to the next hand, repeating the Responder/Non Responder process for the new hand. (Participants classification should not change, however the working intensity can change from the previous hand)
    - *"Now we will calibrate the intensities for your LEFT/RIGHT wrist, using the same procedure as we did before" (Repeat previous instructions if necessary)*

i) After calibration but before beginning Block 2, instruct that the participant will perform a writing task. Keep all electrodes attached to participant.
    - *"Before we start Bock 2, you'll complete a writing task. Please read the instructions carefully, and feel free to ask any questions"*

- Participant completes the Writing Task component. This form comprises either the Anxiety or Control writing task Appendix 4 – Anxiety writing task and Appendix 5 – Control writing Task.

j) . The Anxiety task asks the participant to write about an incident that caused strong feelings of anxiety, and the Control task asks the participant to write about their trip to the University. The specific writing task is randomized across all participants prior to beginning.

k) Afterwards participants complete a manipulation check, the State Trait Anxiety Inventory short form, see Appendix 6 – STAI Short Form. This task contains 6 statements and asks participants to rate their agreement on a 4-point Likert scale. This concludes the writing task component.

l) After the writing task, repeat main experiment (32 stimuli) with the alternative hand, using the predetermined positions. Minimize conversation and interaction with the participant where possible.

m) Experiment conclusion. Disconnect all electrodes. Debrief participant where necessary. Thank participant for their time and provide compensation.

8.8 Appendix 8 – Repeated-Measures Contrast Analyses

Table 27 - Within Subjects Contrast Analysis - Full AUC method

n	Status	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity (%)
1	RS	0.1734	0.1154	0.1054	0.1839	0.6856	79.7
2	NR	0.7101	0.5957	0.9397	0.7380	0.2722	14.1
3	RS	0.0002	<0.0001	0.0002	0.0152	0.0106	98.4
4	NR	0.8917	0.8336	0.6927	0.6359	0.6852	6.2
5	NR	0.4163	0.0265	0.0673	0.2674	0.1479	4.7
6	NR	*NA*	*NA*	*NA*	*NA*	*NA*	0
7	NR	0.1487	0.1089	0.1291	0.2573	0.4088	34.4
8	NR	0.9624	0.8863	0.9975	0.8974	0.8983	20.3
9	NR	*NA*	*NA*	*NA*	*NA*	*NA*	6.2
10	RS	0.4287	0.9020	0.3772	0.1770	0.7246	81.2
11	RS	0.0005	<0.0001	0.0001	0.0085	0.1616	78.1
12	RS	0.0860	0.0210	0.0151	0.0397	0.7616	73.4
13	RS	0.0487	0.0071	0.0451	0.3339	0.2058	93.8
14	RS	0.2470	0.2041	0.1631	0.2230	0.7558	70.3
15	RS	0.2583	0.0774	0.1265	0.3147	0.9797	95.3
16	NR	0.0004	0.0001	0.0003	0.0093	0.0203	96.9
17	NR	0.0068	0.0052	0.0099	0.0604	0.0355	73.4
18	RS	0.9494	0.3622	0.7887	0.7278	0.7270	84.4
19	RS	0.0952	0.0712	0.1375	0.3693	0.1392	73.4
20	NR	0.0679	0.1347	0.1048	0.1607	0.1037	68.8
21	NR	0.0411	0.0650	0.0522	0.1020	0.1140	21.9
22	RS	0.0500	0.0135	0.0841	0.4912	0.0747	90.6
23	NR	0.8119	0.7598	0.4182	0.2821	0.3490	4.7
24	RS	0.0022	0.0002	0.0009	0.0221	0.1244	82.8
25	NR	0.2097	0.1176	0.1590	0.3260	0.5893	26.6
26	RS	<0.0001	<0.0001	<0.0001	0.0004	0.0085	53.1
27	NR	*NA*	*NA*	*NA*	*NA*	*NA*	3.1
28	NR	0.1621	0.2475	0.1180	0.1105	0.5393	12.5
29	NR	0.4830	0.8647	0.7565	0.5090	0.2255	39.1
30	RS	0.5664	0.0408	0.3768	0.7324	0.8286	73.4
31	RS	0.0392	0.1162	0.0818	0.1247	0.0436	90.6
32	RS	<0.0001	<0.0001	<0.0001	0.0010	0.0769	100

Table 28 - Within Subjects Contrast Analysis - Constrained AUC method

n	Status	Linear	Small	Large (Ramp)	Large (Step)	Extra-Large	Validity (%)
1	RS	0.4856	0.2716	0.4548	0.7783	0.7117	79.7
2	NR	*NA*	*NA*	*NA*	*NA*	*NA*	14.1
3	RS	0.0001	<0.0001	0.0001	0.0097	0.0064	98.4
4	NR	*NA*	*NA*	*NA*	*NA*	*NA*	6.2
5	NR	0.2988	0.3817	0.3264	0.3688	0.3929	4.7
6	NR	*NA*	*NA*	*NA*	*NA*	*NA*	0
7	NR	0.2877	0.1724	0.2448	0.4126	0.6915	34.4
8	NR	0.7834	0.2599	0.5560	0.9480	0.6087	20.3
9	NR	*NA*	*NA*	*NA*	*NA*	*NA*	6.2
10	RS	0.7315	0.9684	0.6691	0.4585	0.9465	81.2
11	RS	0.0910	0.0144	0.0445	0.2305	0.6353	78.1
12	RS	0.0211	0.0197	0.0065	0.0112	0.4879	73.4
13	RS	0.1413	0.0556	0.1650	0.5273	0.2501	93.8
14	RS	0.2031	0.5576	0.3021	0.2292	0.1863	70.3
15	RS	0.2376	0.0732	0.1218	0.3102	0.9437	95.3
16	NR	0.0007	0.0001	0.0003	0.0134	0.0520	96.9
17	NR	0.0467	0.0683	0.0834	0.1896	0.0588	73.4
18	RS	0.9148	0.3392	0.8106	0.6644	0.4634	84.4
19	RS	0.0937	0.0964	0.1540	0.3554	0.1057	73.4
20	NR	0.0543	0.1087	0.0755	0.1150	0.1095	68.8
21	NR	0.0454	0.0213	0.0297	0.0893	0.3258	21.9
22	RS	0.0496	0.0066	0.0681	0.5415	0.1083	90.6
23	NR	*NA*	*NA*	*NA*	*NA*	*NA*	4.7
24	RS	0.0102	0.0013	0.0048	0.0579	0.2206	82.8
25	NR	0.4085	0.2818	0.3207	0.4723	0.7424	26.6
26	RS	0.0060	0.0022	0.0037	0.0210	0.1082	53.1
27	NR	*NA*	*NA*	*NA*	*NA*	*NA*	3.1
28	NR	0.1114	0.0777	0.0611	0.1254	0.8746	12.5
29	NR	0.4478	0.2528	0.5086	0.9868	0.4741	39.1
30	RS	0.7501	0.1522	0.7302	0.4872	0.8714	73.4
31	RS	0.0267	0.0269	0.0425	0.1453	0.0607	90.6
32	RS	<0.0001	<0.0001	<0.0001	0.0034	0.1205	100

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