

The Effect of Snake and Spider Images on the Oculomotor System: An Eye-tracking Study

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A report submitted as a partial requirement for the degree of Bachelor
of Psychological Science with Honours
at the University of Tasmania, 2021

Statement of Sources

I declare that this report is my own original work and that contributions of others have been duly acknowledged.

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Date: 19/11/2021

Acknowledgments

Firstly, I want to thank both Jason Satel and Luke Johnson for their supervision. Their expertise and feedback were enormously helpful when designing the experiment. Jason's knowledge of eye-tracking experiments enabled me to design a dot probe task. Also, his broad knowledge of the equipment (computers and eye-tracker) software (MATLAB) made the experience seamless. Whenever we experienced occasional technical issues, Jason was always willing drop what he was doing and go out of his way to resolve the problem. I also need to thank Alfred Lim for taking the time to write the code for the dot probe task. He did a really good job of understanding what we were trying to do, as the dot probe task was quite complicated and hard to explain.

I also need to thank all who helped out in the lab and with data collection, especially Samuel Moore, Alyssa Marshall, Hannah Jones and Jason Satel. Thank you. Data collection would not have been possible without your help. Also, a special thanks to Samuel Moore, who taught me how navigate MATLAB and use the eye-tracker. He also helped resolve many of the technical issues that occurred. Thank you to Ezra de Vos for hanging out in lab, helping with little jobs, and keeping me company.

Lastly, I need to thank Mum. Without her, none of this would have been possible. I am so grateful that she allowed me to move in with her, enabling my move from Wynyard to Launceston. Her amazing roast lambs fuelled me throughout the entire degree.

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Word count: 9648

Abstract

Previous studies have demonstrated attentional bias to emotional images, including images of snakes and spiders. However, researchers are yet to compare attentional bias to snake and spider images in an eye-tracking study. Furthermore, there is evidence that suggests that primates have a predisposition to detect snakes, as they are evolutionarily relevant. The aim of this study was to assess attentional bias to spider and snake images via eye-tracking, and to see if such images are capable of inducing Pavlovian conditioning. Twenty-five participants underwent two dot probe tasks. It was hypothesised that emotional images would elicit faster oculomotor responses, that there would be no difference between spider and snake images, that there would be a conditioning effect, and that snakes would elicit a stronger conditioning effect given their ancestral relevance. Instead, results revealed that participants directed their gaze towards neutral images more quickly than emotional images. It is likely that the current study observed a later stage of attentional processing than previous work, which can be explained by the vigilant-avoidant hypothesis. It was also found that spider images elicited a greater emotional response than snake images. Lastly, Pavlovian conditioning to coloured shapes paired with the images was not observed.

The oculomotor system plays an essential role in the allocation of attention (Öhman et al., 2001). For example, in everyday life, we disregard much of the visual information we receive and perceive only what is deemed important (Diano et al., 2017; Matsunaka & Hiraki, 2019). This prioritisation may relate to a task we are consciously performing, or it may relate to our survival (Lipp & Derakshan, 2005; Mulckhuyse, 2018; Nikolaidou et al., 2019). It has been theorised that the oculomotor system has evolved to prioritise the detection of emotional stimuli, as this bias would support survival (Öhman et al., 2001). Emotional stimuli are stimuli that elicit an emotional response, such as changes in subjective feeling, behaviour, or action tendencies (Brosch et al., 2010). The theory that the oculomotor system evolved to prioritise emotional stimuli is supported in the literature, where studies have shown that humans process emotional stimuli more efficiently, resulting in faster oculomotor responses (Bannerman, Milders, De Gelder, et al., 2009; Mulckhuyse, 2018). Faster oculomotor responses to emotional stimuli is likely a mammalian defence system that has developed to aid the propagation of genes (Öhman & Mineka, 2001), as earlier detection allows more time to respond to threats in one's environment, increasing chances of survival. Inborn defence responses and Pavlovian conditioning are two of the possible selective mechanisms that contribute this defence system (Bertels et al., 2020; LoBue, 2010; Subra et al., 2018).

Attentional Bias and Cueing Tasks

In the literature, attentional bias is generally considered to be the facilitation of attention toward specific stimuli (Bannerman et al., 2010a; Hopkins et al., 2015; Nummenmaa et al., 2009). For example, if an individual has a bias towards particular stimuli, they will prioritise orienting to, and identifying, such stimuli (Bannerman et al., 2010a; Belopolsky, 2015; Nikolaidou et al., 2019). Attentional bias indicates that an individual deems the stimulus to be important, as there is neural circuitry supporting its detection

(Bertels et al., 2020; Diano et al., 2017). Attentional bias may vary from person-to-person in the case of addiction and personal interest (Nikolaidou et al., 2019), or it may be a hard-wired survival mechanism (Le et al., 2013). When it comes to emotional images, attentional bias is well documented (Mulckhuyse, 2018). This includes images of threatening animals, emotional faces and fear-full body postures (Bannerman et al., 2010b, 2010a; Carlson et al., 2009; Mulckhuyse, 2018). There have been mixed findings in regard to an emotional effect of such images, but Mulckhuyse's (2018) literature review attributed this to differences in cue duration.

Inconsistent results have primarily been seen in studies that have used manual button presses to assess reaction times, rather than using eye movements (Mulckhuyse, 2018). Because tasks that use manual button presses normally prohibit eye movements, they operate via covert attention. Overt attention, on the other hand, is assessed via eye movements (Mulckhuyse, 2018). This distinction is important because manual tasks require additional cognitive processing (Hommel & Schneider, 2002), which increases reaction time (Bannerman et al., 2010b). Bannerman et al. (2009) also found that the effect of emotional images is dependent on different cue durations. For manual responses, 500 millisecond (ms) cue durations elicited an emotional response; for saccadic responses, only 20 ms cue durations elicited attentional bias. This disparity suggests that activation of the oculomotor system has a significant impact on the processing of emotional visual stimuli. Furthermore, in real-world situations, the oculomotor system is rarely inhibited, so making inferences about visual processing based on manual button presses could result in inaccurate conclusions (Mulckhuyse, 2018).

Cueing tasks, including the dot probe task, have been used to measure attentional bias in response to emotional images (Bannerman, Milders, De Gelder, et al., 2009). By displaying two rivaling stimuli (one emotional, the other neutral) and measuring reaction

times, bottom-up processing can be observed (Mulckhuyse, 2018). When measuring saccadic reaction times, faster orienting to emotional cues is typically observed if the display time of the cues is brief (Bannerman et al., 2010b). Nummenmaa et al., 2009, whose study conducted multiple saccade cueing tasks, found that a cue duration of 150 ms elicited faster saccades to emotional stimuli. This bias in early attentional allocation is likely mediated by subcortical structures, such as amygdala, pulvinar, and superior colliculus (SC; Godijn & Theeuwes, 2002), and may occur independent of conscious perception (Diano et al., 2017; Hopkins et al., 2015). In fact, studies have provided evidence that cortically blind individuals are still capable of processing emotional visual stimuli, both conditioned and unconditioned (Hamm et al., 2003; Morris et al., 2001). Additionally, dot probe tasks that have used techniques such as backward masking, which impacts cortical processing of stimuli, have demonstrated attentional bias (Carlson et al., 2009; Lojowska et al., 2019). Together, this adds support to the theory that there are subcortical structures involved in visual processing (Diano et al., 2017). Attentional bias has also been demonstrated with previously neutral stimuli via Pavlovian conditioning (Hopkins et al., 2015). However, conditioning has commonly been induced by an electric shock or an aversive noise (Hopkins et al., 2015; Schmidt et al., 2017), rather than using only emotional visual stimuli.

Endogenous and Exogenous Attention

Attention can be categorised as being either endogenously driven or exogenously driven (Mulckhuyse, 2018). Endogenously driven saccades refer to voluntary attention in response to a task. For example, scanning an environment for one's car keys would constitute endogenous attention. This requires additional (top-down) processing and takes longer to execute than exogenously driven saccades (Garrett, 2016; Mulckhuyse, 2018). On the other hand, exogenously driven saccades are automatic and rely on bottom-up processing (Garrett,

2016; Mulckhuyse et al., 2013). An example of this would be when you see red and blue flashing lights in your rear vision mirror while driving. Given the importance of red and blue flashing lights in our society, this would likely be highly salient, involuntarily capturing your attention. Generally, reaction times less than around 200 ms are believed to represent exogenously driven saccades (Mulckhuyse, 2018). However, measuring exogenous attention in a lab-based setting is not straightforward, as cueing tasks require a degree of endogenous attention. In order to measure exogenous attention, however, a task-irrelevant distractor can be included (Mulckhuyse, 2018). A distractor, such as an emotional image, has a theoretical basis as to why it would capture attention (Belopolsky et al., 2011). This capturing of attention will be observed by slower saccades away from the distractor and faster saccades toward the distractor (Bannerman, Milders, de Gelder, et al., 2009; Belopolsky et al., 2011). Therefore, by measuring reaction times when a distracter image is paired with a neutral image, when images are task irrelevant, exogenous attention can be inferred (Mulckhuyse, 2018).

Inborn Defences System and Snake Detection Theory

When it comes to the selection of visual stimuli, humans appear to have a predisposition for the detection of fear-related animals, such as snakes and spiders (Lipp & Derakshan, 2005; Öhman & Mineka, 2001). The detection of such animals is often just as efficient as the detection of modern threats, such as guns and knives (Blanchette, 2006; Carlson et al., 2009), even though guns and knives arguably pose a greater threat in current times (Kawai & Koda, 2016). This is thought to be due to an inborn defence response, which operates via a bottom-up pathway (Bertels et al., 2020). Because fear-related animals have been a common danger to primates, it is believed that we have inherited a readiness to act upon their presence (Isbell, 2006). This detection mechanism seems particularly strong with

snakes, which were a common ancestral threat (Kawai, 2019). Lipp and Derakshan (2005), who looked at attentional bias in regard to snakes and spiders, found attentional bias to snakes to be unrelated to self-reported fear. That is, people who reported low fear of snakes still demonstrated attentional bias to images of snakes, whereas the detection of spiders relied more upon a subjective fear of spiders. This suggests the detection of snakes may be inborn, whereas fear of spiders may be learned (Luo et al., 2015).

In order for a species to survive, it must be able to adapt to its environment (Isbell, 2006). The visual system of primates is superior to that of most other mammals, which is likely a result of a need for depth perception to navigate and move through trees (Isbell, 2006). Also, in order to live among the trees, the visual system would likely need to evolve to identify dangerous inhabitants quickly (Kawai, 2019). This competition between predators and non-predators may have aided the development of the primate visual system and, as a result, contributed to human evolution (Devue & Grimshaw, 2017; Isbell, 2006; Kawai & Koda, 2016). As pointed out by Isbell (2006), this theory is supported by the fact that Madagascar monkeys have poorer vision compared to other primates. Although the vision of Madagascar monkeys is better than most mammals, they have no fovea, which prevents them from seeing the detail that other primates see (Isbell, 2006; Kawai, 2019). This is attributed to the fact that Madagascar monkeys were not in contact with venomous snakes, which put less pressure on the development of their visual system (Isbell, 2006; Kawai, 2019).

Snake detection theory (SDT) postulates that primates have evolved to efficiently detect snakes (Hayakawa et al., 2011; Kawai, 2019; Kawai & Koda, 2016). This theory is further supported by research with Japanese monkeys, which suggests that snakes are detected more efficiently due to their ancestral significance (Kawai & Koda, 2016). Kawai and Koda (2016) found that monkeys responded faster to images of snakes than other animals, including spiders. Even among young children who have had little to no exposure to snakes, snakes are

identified more quickly compared to non-threatening stimuli (Hayakawa et al., 2011).

Although evidence suggests young children are not afraid of snakes (Thrasher & LoBue, 2016), it does appear that they are predisposed to detect them (LoBue et al., 2010). This early attentional bias in children adds more support to the idea that the detection of snakes is inborn in humans, and that it is likely related to an evolutionary process (Isbell, 2006). Furthermore, ophidiophobia (a fear of snakes) is considered to be among the most common phobias, yet this fear often occurs independent of experience (Kawai, 2019). This somewhat contradicts behaviourist ideas around classical conditioning, where fear acquisition is considered to be a result of learned behaviour (Kawai, 2019). Although experience undoubtedly plays a role in fear learning, evidence suggests that there may also be an innate factor involved in the development of phobias (Kawai, 2019).

Like snakes, spiders are also commonly feared, with arachnophobia being among the most common phobias (Vetter, 2013). Spiders are also considered to be a fear-relevant animal, with studies showing that they elicit faster reaction times compared to non-threatening animals (Fabio & Caprì, 2019; Lipp & Derakshan, 2005). Interestingly, although there are some mixed results (Carlson et al., 2009; Gomes et al., 2017; Öhman et al., 2012), dot probe task experiments have shown that attentional bias to spiders can be as strong as it is with snakes (Lipp & Derakshan, 2005). However, the cause of this attentional bias has caused debate, with some claiming it to be an inborn mechanism (LoBue et al., 2010), while others suggest that it is learned (Carlson et al., 2009; Kawai & Koda, 2016). Many studies that have looked at attentional bias with spiders suggests that they are evolutionary relevant (Blanchette, 2006; Luo et al., 2015). It is possible that these mixed findings are a result of different sample populations. For example, if spiders are more common in a particular region, their relevancy may be increased, therefore their detection may be prioritised and rival that of inborn detection mechanisms. When Öhman and Mineka (2001) initially developed the fear

module theory, fear of spiders was considered to be evolutionarily primed. That is, humans form fear based on association, yet some fears are primed based on evolutionary relevance (Kawai, 2019; Öhman & Mineka, 2001). However, Öhman et al. (2012) later revised the fear module theory after the publication of *The Fruit, the Tree, and the Serpent* (Isabell, 2009), along with mounting evidence that primates do not possess a fear of spiders akin to that of snakes (Soares et al., 2009).

Pavlovian Conditioning and the Fear Module

Typically, fear is thought to develop via Pavlovian conditioning (Kawai, 2019). Pavlovian conditioning (often termed classical conditioning) is a learning procedure that occurs by association (Pishek-Simpson et al., 2009). For example, in the classic little Albert experiment (Watson & Rayner, 1920), Albert is conditioned to fear a previously neutral stimulus after repeated pairing with an aversive noise. This method of learning has been demonstrated in visual cueing tasks, where participants are quicker when orienting to previously neutral visual stimuli once they have been paired with an aversive noise or electric shock (Mulckhuyse et al., 2013; Pishek-Simpson et al., 2009; Stussi et al., 2020). This demonstrates that participants have consolidated two separate stimuli, with the previously neutral stimulus now indicative of an unconditioned stimulus, which triggers an emotional response (Pishek-Simpson et al., 2009).

Pavlovian conditioning is an important component of the fear module theory (Öhman et al., 2012; Öhman et al., 2001). The fear module is a mechanism that activates defensive, survival-oriented behaviours, such as fight or flight (Öhman & Mineka, 2001). Rather than suggesting that humans have a predisposition for the detection of specific fear-relevant animals, fear module theory postulates that humans are primed to form specific fears (Kawai, 2019; Öhman & Mineka, 2001). So, although there is an inborn component in the fear

module theory, it is not emphasised (Kawai, 2019). Instead, it has been suggested that humans have evolved a specialised fear module, which enables the efficient categorisation of specific stimuli, absent of cortical processes (Öhman et al., 2012). It may be the case that there are specific features that spiders possess that facilitate the formation of fear, however they may not be as primed as snakes (Soares et al., 2009). It has been shown that humans have the capacity to associate neutral stimuli, such as photos of mushrooms and flowers, with an electric shock (Öhman & Soares, 1993). Once this association has been established, the detection of such images rivals that of snake and spider images, suggesting that evolution has equipped humans with a nervous system that has the capacity to rapidly associate a neutral stimulus with an unconditioned stimulus (Mulckhuyse et al., 2013; Öhman & Soares, 1993).

When it comes to inducing conditioning via images, it is likely more difficult to do so than when using an electric shock or aversive noise. This is because aversive noise and electric shock often elicit an unavoidable physiological response (Diano et al., 2017), whereas visual stimuli in the form of images do not (Mulckhuyse, 2018). To our knowledge, inducing conditioning by using images as the primary unconditioned stimulus is yet to be done. Although physiological responses to images may not be as great as responses to aversive noises and electric shocks, brief presentation of emotional stimuli in cueing tasks show that physiological responses do exist (Bannerman et al., 2010b; Mulckhuyse, 2018; Nummenmaa et al., 2009). Additionally, early posterior negativity (EPN) amplitudes have been demonstrated in response to snake images. EPN amplitudes represent an event related potential component that indicates the processing of emotional stimuli within the visual system (He et al., 2014). If snake images are eliciting increased EPN amplitudes, it suggests an emotional response may be occurring, thus a conditioning effect may be possible (Kawai, 2019).

Because brief presentation of emotional images may speed saccades independent of conscious perception (Diano et al., 2017), briefly presented emotional images may facilitate the consolidation of neutral and unconditioned stimuli, as the context of the images is yet to be processed by cortical regions. Given the physiological responses elicited by images of snakes, which have been demonstrated to decrease reaction times (Carlson et al., 2009), as well as increased EPN amplitudes (He et al., 2014), it seems plausible to suggest such images can be capable of inducing Pavlovian conditioning. This ties in with a point Kawai (2019) suggested – that innate fear may be an important factor in the development of phobias. Also, if Pavlovian conditioning is to be induced by visual stimuli alone, it is likely that snakes will elicit the strongest response given their ancestral relevance (Kawai, 2019).

In the experiment conducted by Carlson et al. (2009), it was found that both snakes and guns elicited an emotional response. That is, both snake and gun images were attended to faster than neutral images. Snakes are considered to be evolutionary relevant, whereas guns are considered to be evolutionarily irrelevant (Carlson et al., 2009; Kawai & Koda, 2016). That means, for guns to elicit an emotional response, a learned association had to occur (LoBue, 2010). This demonstrates that conditioned stimuli can elicit a similar emotional response to that of supposedly unconditioned stimuli, perhaps via the same neural circuit (Koller et al., 2019). Furthermore, cues in the Carlson et al. (2009) experiment were all briefly displayed for 33 ms, demonstrating that conditioned stimuli (guns) can be efficiently processed. This is in contrast to what has been theorised in regard to evolutionary relevant stimuli (Isbell, 2006; Öhman et al., 2001). For conditioned stimuli to be attended to with such a short duration, it suggests that evolutionary relevancy may not dictate prioritisation within the oculomotor system. Rather, prioritisation may be dictated by perceived importance, regardless of whether it is inborn or conditioned (Carlson et al., 2009). One theory that has

been suggested is that both bottom-up and top-down information compete in what is referred to as a priority map (Mulckhuyse, 2018).

The priority map is responsible for space and object representation, which are both necessary for saccade programming in the SC (Mulckhuyse, 2018). The theory suggests that a saccade is made once a specific location within the priority map reaches threshold, receiving input from both bottom-up and top-down processes. For example, if one is to encounter a stimulus that represents a snake, a threshold will likely be reached via a bottom-up pathway, competing with stimuli that are relevant to the task being performed. Even if the stimulus is actually a fallen tree branch, incorrect object recognition may still reach threshold, as the snake-sensitive neurons of the pulvinar are potentially being activated (Belopolsky, 2015; Le et al., 2013). The priority map theory explains why saccades directed to emotional images are executed faster in dot probe tasks, and why saccades away from emotional images are slower (Bannerman et al., 2010a; Belopolsky et al., 2011), as there is a readiness to detect and respond to stimuli that are irrelevant to the task (Bannerman, Milders, De Gelder, et al., 2009). Additionally, if an individual has been conditioned to perceive a specific stimulus as threatening, this will also be prioritised by the oculomotor system (Mulckhuyse et al., 2013).

Neural Circuitry

A subcortical loop, consisting of the SC, the amygdala and the pulvinar, is thought to be responsible for the detection of threatening stimuli, which increases the speed of eye-movements toward such stimuli (Mulckhuyse, 2018). The amygdala is well known for its involvement in fear. It is typically thought to be responsible for fear memory and learning (Morris et al., 2001), as well as responding to threat (Diano et al., 2017; Kawai, 2019; Koller et al., 2019). If the medial pulvinar is damaged, its connection to the amygdala is then severed, resulting in an absence of a fear response (Kawai, 2019). This is thought to be

because the pulvinar, which is responsible for detecting threatening stimuli, is no longer sending information to the amygdala (Kawai, 2019). The amygdala is also associated with threat assessment, and it is believed to play an important role in identifying emotional visual stimuli (Amaral, 2003). It is well established that humans and monkeys can identify emotional faces among neutral ones (Landman et al., 2014; Peltola et al., 2013), as such stimuli are deemed important for survival and sociability (Stussi et al., 2018). Amaral (2003) showed that damage to the amygdala causes an inability to identify angry faces, suggesting that the amygdala is involved in threat assessment.

Although the amygdala is well-known for its involvement in fear (Amaral, 2003), it does not directly activate the oculomotor system (Mulckhuyse et al., 2013). The amygdala likely modulates oculomotor behaviour via the pulvinar and the SC (Koller et al., 2019; Tamietto et al., 2012). The SC is directly connected to the retina (Tamietto et al., 2012). It is involved in the allocation of visual attention and retinotopically represents one's environment (Godijn & Theeuwes, 2002). It is believed that the SC is responsible for detecting predators so they can then be avoided (Sewards & Sewards, 2002). When the SC of hamsters is stimulated, freezing and rushed movements are displayed (Northmore et al., 1988). If the SC is damaged, these responses do not occur, suggesting that the SC is also involved in threat response (Kawai, 2019). The SC also receives input from cortical regions, including V2 and V4 (Isbell, 2006; Kawai, 2019). V2 and V4 areas appear to be important for texture and depth perception, and they may also assist in the detection of contours and corners of snakes' scales (Hegd  & van Essen, 2007; Merigan, 2000; Peterhans & von der Heydt, 1993), which could contribute to the detection of specific animal features (Kawai, 2019).

From the SC, visual information is then sent to the pulvinar, which also appears to play a pivotal role in visual attention (Le et al., 2013). The pulvinar, which is much larger in primates compared to other animals (Kawai, 2019; Le et al., 2013), is believed to be involved

in the detection of salient and threatening visual stimuli (Koller et al., 2019). Studies suggest that the pulvinar acts as a filter for the visual system, discarding irrelevant visual information and processing only what is deemed important (Isbell, 2006; LaBerge & Buchsbaum, 1990). This involves recognition of shape, colour and movement (Corbetta et al., 1991; Kawai, 2019). Interestingly, cells in the pulvinar of humans and felines have been shown to respond to grid pattern movements (Dumbrava et al., 2001; Villeneuve et al., 2005). It may be the case, as SDT suggests, that such grid pattern movements are indicative of snake scales. SDT postulates that the development of the pulvinar was an important evolutionary edition in primates, which supported survival and further evolutionary advancement of the visual system (Kawai, 2019), namely binocular vision (Isbell, 2006; Le et al., 2013).

Van Le et al. (2013), who looked at activity in the medial and dorsolateral pulvinar in two monkeys, found evidence that suggests the pulvinar may be involved in inborn threat detection. They found increased activation of neurons in response to images of snakes versus monkey hands and angry monkey faces. Interestingly, these monkeys were born in captivity, never experiencing snakes in the wild. Furthermore, it has been well established that primates have a predisposition for face detection (Landman et al., 2014), as identifying such stimuli is important. The fact that the snake images were responded to faster than the monkey faces, without ever experiencing snakes before, suggests that snake detection is inborn, and that it has priority over faces (Le et al., 2013). These results support SDT, indicating that primates may have snake-sensitive neurons located in the pulvinar (Kawai, 2019; Le et al., 2013).

Aims and Hypotheses

Using a dot probe task, this study intends to investigate emotional visual stimuli (images of snakes and spiders) when simultaneously presented with neutral stimuli (Bannerman, Milders, de Gelder, et al., 2009; Belopolsky et al., 2011; Carlson et al., 2009;

Mulckhuyse, 2018). However, there are few studies that have measured saccadic reaction times (SRTs) to assess attentional bias to fearful stimuli (Mulckhuyse, 2018). All studies that have assessed reaction times in response to snake and spider images have used manual button presses rather than saccades (Kawai, 2019). This gap in the literature gives reason to investigate the impact snake and spider images may have on the oculomotor system. As stated before, the oculomotor system is rarely, if ever, disengaged in real-world scenarios, so measuring saccades will give us a greater understanding of the impact emotional stimuli have on attentional bias within the visual system (Mulckhuyse, 2018). Given the results of similar studies when looking at attentional bias to other types of emotional images, when the presentation of cues are brief (Mulckhuyse, 2018), it is expected that SRTs will be shorter when cued by emotional images in our study. That is, orienting to a target that succeeds an emotional image will be quicker than when orienting to a target that succeeds a neutral image.

When it comes to snakes versus spiders, things are not as clear. Although both snakes and spiders are considered threatening and are commonly feared, snakes are generally considered to be evolutionarily relevant, whereas spiders are not (Kawai, 2019). Spiders were once considered to be evolutionarily relevant, but this is no longer the case (Öhman et al., 2012; Öhman et al., 2001). However, previous studies that have relied on manual button presses to assess differences in attentional bias between spiders and snakes have found mixed results (Blanchette, 2006; Carlson et al., 2009; Gomes et al., 2017; Lipp & Derakshan, 2005). As stated before, attentional bias to spiders may be the result of Pavlovian conditioning (LoBue et al., 2010), which may be contributing to these mixed findings. The use of different images may also be a factor, as some images may emphasise specific features that elicit an emotional response (Carlson et al., 2009), whereas others may not. Previous studies have shown that conditioned stimuli can elicit faster oculomotor responses (Armstrong et al., 2019;

Mulckhuyse et al., 2013), so it is plausible to suggest that spider images may elicit shorter SRTs compared to neutral images, even if fear of spiders is due to Pavlovian conditioning. Therefore, we hypothesise that there will not be a significant difference in SRTs between snakes and spiders.

This study also intends to investigate whether emotional visual stimuli presented in the dot probe task can induce Pavlovian conditioning, and if so, assess the magnitude of such conditioning. Given humans' reliance on the visual system for the detection and encoding of emotional stimuli (Ehlers et al., 2002; Nummenmaa et al., 2009; Schmidt et al., 2017), we hypothesise that there will be a conditioning effect seen when viewing coloured shapes (conditioned stimuli), absent of the unconditioned stimuli (emotional images). In a similar experiment, Carlson et al. (2009) demonstrated attentional bias in response conditioned stimuli. In their dot probe task, guns were briefly presented for 33 ms, which elicited faster button presses. However, guns are a very modern threat, so this emotional response is likely the result of Pavlovian conditioning. Therefore, if conditioning to a coloured shape is to occur, it will likely be observed when presented briefly. Given the evolutionary relevance of snakes (Kawai & Koda, 2016), it is expected that the conditioning of snake images to be stronger than that of spider images (Bramwell et al., 2014; Stussi et al., 2020). Again, this ties in with the theory that there is an inborn component involved in conditioning (Kawai, 2019).

Method

Participants

For this study, we recruited 25 participants (15 male, 10 female) between the ages of 19 and 45 (Mean age = 25, SD = 6.12). Participants were psychology undergraduates from the University of Tasmania or members of the general public, participating for either course

credit via SONA or a financial reward of \$15/hour. All participants were required to be over 18 years of age and have normal or corrected vision.

Materials

EyeLink 1000 Plus eye tracking system was used to monitor eye position and record saccades. Two sets of Windows computers and monitors were used: one set to operate the eye tracking system, the other dedicated to the dot probe task.

Design and procedure

The setting for the research project was a physical site at the University of Tasmania's Newnham campus. Participants were permitted to sit at a computer monitor and complete task-related saccades in a dot probe task. An eye-tracking device recorded participants' saccadic reaction times. This study had two phases. Phase 1 (see figure 1) assessed attentional bias and attempted to induce Pavlovian conditioning by having images appear within a coloured shape. For this study, validated images were used, which were sourced from the Geneva Affective Picture Database (Dan-Glauser & Scherer, 2011). Snake, spider and neutral images were used.

There were three different coloured shapes, which were coupled with either spider, snake or neutral images. Coloured shapes were either a blue circle, a green square or a purple hexagon. Coloured shapes/image type associations were counterbalanced in order to eliminate a potential bias to a specific coloured shape, leading to six variations of the task. In phase 2 (see Figure 2), another dot probe task was completed but without the images, consisting of only the coloured shapes. This allowed us to assess whether pairing emotional visual stimuli (snakes and spiders) with neutral visual stimuli (coloured shapes) during the

dot probe task facilitates Pavlovian conditioning, as well as assessing the magnitude of the conditioning.

For the task in the first phase, participants were required to fixate on a marker in the centre of the screen for 1000 ms. Then, coloured shapes appeared left and right of the fixation for a total duration of 200 ms. Once the coloured shapes had been on the screen for 50 ms, images then appeared within the coloured shapes for 150 ms: snake vs. neutral or spider vs. neutral. Then, after a 200 ms interstimulus interval (ISI), a target cross appeared left or right of fixation until a saccade was made. Targets appeared for a total of 3000 ms or until a saccade was made. In phase 2, a fixation point was on screen for 1000 ms. Then coloured shapes appeared left and right of fixation for 150 ms: snake-paired vs neutral-paired or spider-paired vs neutral-paired. ISI and target durations in phase 1 and phase 2 were identical. Both phases also included a random intertrial interval ranging from 750 ms to 1250 ms.

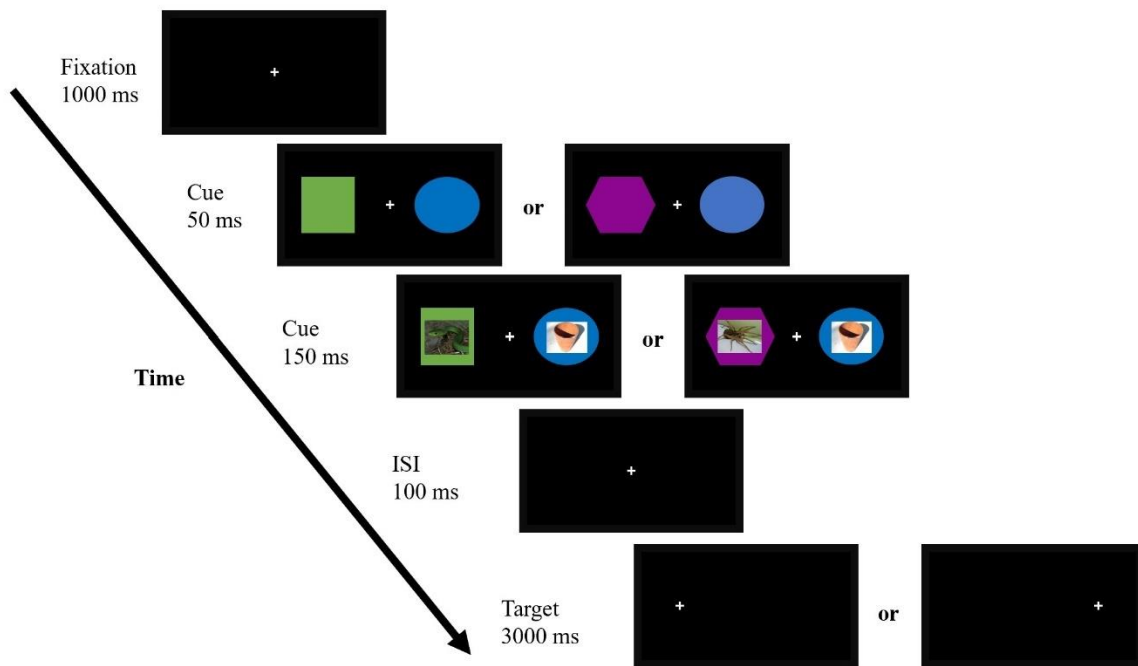


Figure 1. Schematic representation and time sequence of phase 1. A fixation point is present for 1000 ms, followed by the presentation of two coloured shapes for 50 ms. An emotional image (snake/spider) and neutral image will then appear within the coloured shape for 150 ms. After a 100 ms blank screen, a target cross (+) will appear left or right of fixation for 3000 ms or until saccade.

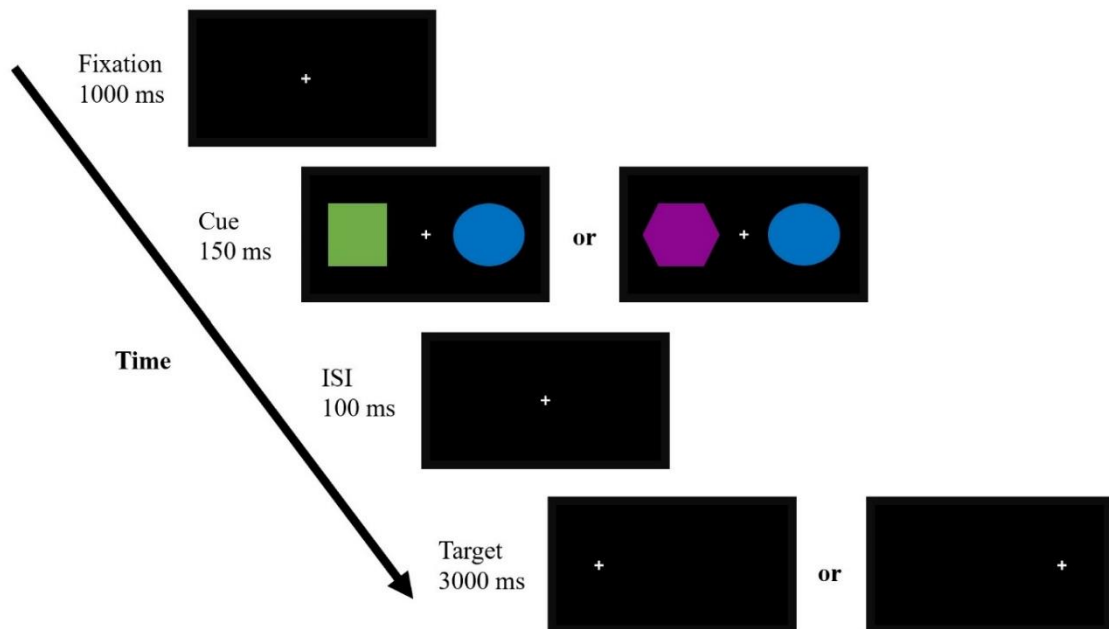


Figure 2. Schematic representation and time sequence of phase 2. A fixation point is present for 1000 ms, followed by the presentation of coloured shapes (snake-paired/spider-paired) and neutral-paired coloured shapes for 150 ms. After a 100 ms blank screen, a cross will appear left or right of fixation for 3000 ms or until saccade.

At the halfway point in the task, after the completion of phase 1, participants were given a break to rest their eyes. Participants were also encouraged to pause at any point throughout the task if they felt the need to rest their eyes. There were a total of 240 trials in each phase and 480 trials in total. It was estimated that experimental duration for each participant would last between 35-40 minutes when including recycling of incorrect (i.e., trials when an incorrect eye movement was made at any stage were randomly recycled) trials and a planned 5-minute break at the completion of phase 1. An additional 10-20 minutes was allocated for setting up equipment and informing participants. There were eight variations of trial type when factoring in left and right target presentations.

Analysis

This study used several types of statistical analyses. Descriptive statistics were analysed first (e.g., means, standard deviations, etc.). This was then followed up with a 2x2 within-subjects analysis of variance (ANOVA), with cue category (emotional/neutral) and phase (phase 1/phase 2) as the IVs and SRT as the DV. This was intended to get a broad view of the results, analysing all SRTs in both phases.

Then, a 2x2 within-subjects ANOVA was conducted to assess phase 1, with emotional congruency (congruent/incongruent) and emotional image (snake/spider) as the IVs. Emotional congruency indicates whether the target succeeds an emotional image. For example, an emotional congruent trial is when a target succeeds a snake or spider image. An emotional incongruent trial is when a target succeeds a neutral image. Emotional image indicates the emotional image used in the trial.

Then, a 2x2 within-subjects ANOVA was conducted to assess if snake and spider images had a conditioning effect, with emotional congruency (congruent/incongruent) and emotional coloured shape (snake-paired/spider-paired) as the IVs. In phase 2, emotional

congruent trials refer to coloured shapes that were paired with an emotional image in Phase 1, whereas emotional incongruent trials refer to coloured shapes that were paired with neutral images. Emotional coloured shape indicates whether the trial included snake-paired or spider-paired coloured shapes. Snake-paired refers to coloured shapes that were paired with snake images, whereas spider-paired refers to coloured shapes that were paired with spider images. In order to assess the extinguishing of Pavlovian conditioning, a follow up one-way within-subjects ANOVA with quartile (quartile 1, quartile 2, quartile 3 and quartile 4) as the IV was planned if a conditioning effect was observed in phase 2. Also, a one-way within subjects ANOVA with three levels (coloured shape: green square, blue circle and purple hexagon) was conducted to assess whether coloured shape had an effect on SRTs.

Ethical Considerations

Given this study contained negative emotional images, there was a potential risk that this could cause distress among some individuals. However, as shown by similar studies (Bannerman et al., 2010b; Carlson et al., 2009; Nummenmaa et al., 2009), emotional images in this context appear to be completely safe. Nevertheless, in the information sheet and consent form, participants were notified that some images will contain negative emotional content. Participants were instructed to notify the researcher if they experienced distress. Participants may also experience fatigue. For this reason, the experiment included a break at the conclusion of phase 1, in addition to participant-mediated breaks if they felt the need. Participants were also informed that they can discontinue the experiment without explanation.

Results

First, a histogram of all participants' SRTs were plotted to determine the cut-off for outliers. This is because extraordinarily fast response times are likely to be anticipations rather than reactions. Long reaction times, on the other hand, do not constitute a saccade – a

rapid, uninterrupted, eye movement made from one point in an individual's visual field to another (Wong, 2014). After assessing the histogram at numerous high and low cut-offs, it was decided that all SRTs below 120 ms and above 600 ms should be removed. There were 108 trials under 120 ms (0.91% of total trials) and 415 trials over 600 ms (3.49% of total trials). This resulted in removal of 523 trials, which equated to 4.39% of total trials of all participants. Similar portions of data have been removed from other studies that have measured reaction time, usually having relatively similar cut-offs (Carlson et al., 2009).

In order to eliminate coloured shapes as a potential confounding variable, a one-way within-subjects ANOVA with three levels (green square, blue circle and purple hexagon) was conducted. This analysis used SRT data from phase 2. Mauchly's test revealed that the test of sphericity was violated, $\chi^2 = 0.68$, $p = .011$. An ANOVA with Greenhouse-Geisser corrections revealed no significant main effect for coloured shapes ($F[24,1.51] = 0.73$, $p = 0.453$, $\eta^2_p = .03$), indicating that coloured shapes did not have an influence on SRTs.

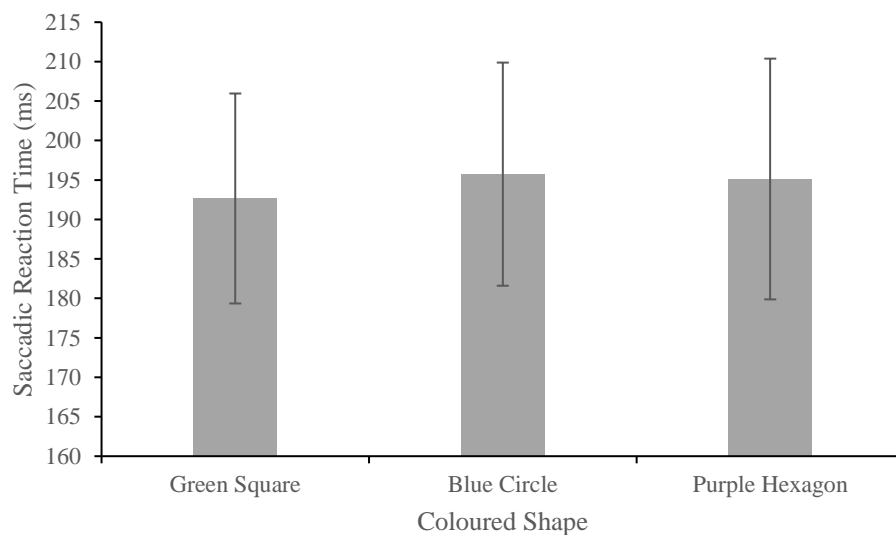


Figure 3. Mean saccadic reaction times for coloured shapes (error bars represent 95% CIs).

Emotional Versus Neutral

A 2 (cue category: emotion/neutral) x 2 (phase: phase 1/phase 2) within subjects ANOVA was conducted. This was intended to assess the hypothesis that SRTs would be shorter when orienting to a target that succeeds an emotional image versus a neutral image and to get a broad look of the results across both phases. Results revealed a significant main effect for cue category ($F[1,24] = 9.49, p = .005, \eta^2_p = .28.$), with shorter SRTs in the neutral category ($M = 201$ ms, $SD = 34.60$ ms) compared to emotional ($M = 204.82$ ms, $SD = 33.71$ ms). There was also a significant main effect for phase ($F[1,24] = 47.88, p = <.001, \eta^2_p = .67.$), with shorter SRTs in phase 2 ($M = 194.11$ ms, $SD = 33.80$ ms) compared to phase 1 ($M = 211.71$ ms, $SD = 35.40$ ms). The interaction between cue category and phase was not significant, $F(1,24) = 1.08, p = .310, \eta^2_p = .04.$

To analyse the effect of emotional versus neutral in each phase, post-hoc comparisons with Bonferroni corrections were conducted. Results revealed a significant difference for cue category in Phase 1, with shorter SRTs to targets that succeeded neutral images ($M = 209.07$ ms, $SD = 35.88$ ms) compared to emotional images ($M = 214.35$ ms, $SD = 35.26$ ms), $t(24) = 3.74, p = .006, d = .75.$ However, cue category was not significant in phase 2, $t(24) = 1.04, p = 1.00, d = .21,$ suggesting conditioning did not occur.

Table 1

Means and standard deviations for saccadic reaction times for emotional and neutral trials for phase 1 and phase 2.

	Emotional	Neutral
Phase 1	214.35(35.26)	209.07(35.88)
Phase 2	195.29(33.63)	192.94(34.89)

Note. Standard deviations are presented in parentheses.

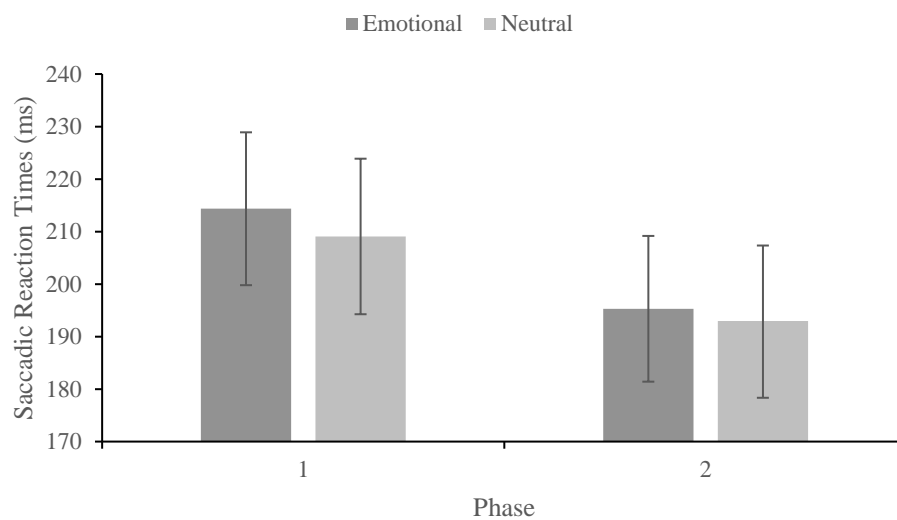


Figure 4. Mean saccadic reaction times for emotional and neutral trials in phase 1 and phase 2 (error bars represent 95% CIs).

Phase 1

To analyse phase 1 specifically, a 2 (emotional congruency: congruent/incongruent) x 2 (emotional image: snake/spider) within subjects ANOVA was conducted. Results revealed a significant main effect for emotional congruency, with faster SRTs for congruent trials ($M =$

209.07 ms, $SD = 35.88$ ms) compared to incongruent trials ($M = 214.35$ ms, $SD = 35.26$ ms), $F(24,1) = 14.02$, $p = .001$, $\eta^2_p = .37$. Main effect for emotional image type was marginal, ($F[24,1] = 0.03$, $p = .0865$, $\eta^2_p = 0.00$). There was not a significant interaction, $F(1,24) = 2.39$, $p = .0135$, $\eta^2_p = .09$.

Post-hoc comparisons with Bonferroni corrections were then conducted in order to investigate these effects further and to assess the hypothesis that there would not be a significant difference between snake congruent trials ($M = 212.96$ ms, $SD = 35.77$ ms) and spider congruent trials ($M = 215.74$ ms, $SD = 35.47$ ms). This was justified given that there was a significant main effect for emotional congruency and a marginal effect for emotional image type. The hypothesis that there would not be a significant difference between snake congruent and spider congruent was supported as the results were not significant, $t(24) = -1.37$, $p = 1.00$. Furthermore, there was not a significant difference between snake images ($M = 212.96$ ms, $SD = 35.77$ ms) and neutral images ($M = 210.11$, $SD = 36.42$), $t(24) = 1.88$, $p = .032$, $d = .38$. However, it was found that saccades were significantly faster if the target succeeded a neutral image ($M = 208.2$ ms, $SD = 36.92$ ms) when simultaneously presented with spider images ($M = 215.74$ ms, $SD = 35.47$ ms), $t(24) = 3$, $p = .003$, $d = .6$.

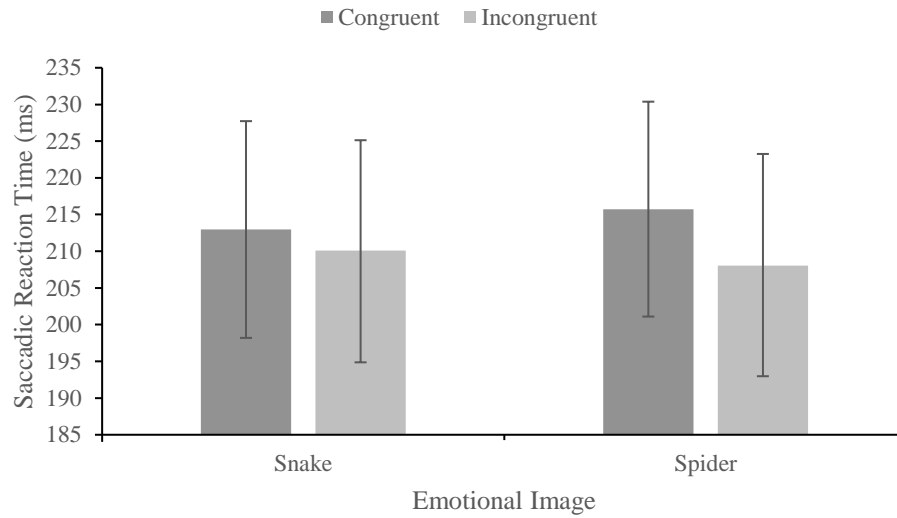


Figure 5. Mean saccadic reaction times for snake congruent, snake incongruent, spider congruent and spider incongruent trials (error bars represent 95% CIs).

Phase 2

To assess whether there was a conditioning effect in phase 2, a 2 (emotional congruency: congruent/incongruent) x 2 (emotional coloured shape: snake-paired/spider-paired) within-subjects ANOVA was conducted. Results revealed no significant main effects for emotional-paired congruency ($F[24,1] = 1.09, p = .307, \eta^2_p = .04.$) and emotional coloured shape, $F(24,1) = 3.28, p = .082, \eta^2_p = .12$. There was not a significant interaction, $F(24,1) = 0.83, p = 0.372, \eta^2_p = .03$.

Table 2

Mean saccadic reaction times for emotional congruent and incongruent trials in phase 1 and phase 2.

	Snake/Snake-Paired		Spider/Spider-Paired	
	Congruent	Incongruent	Congruent	Incongruent
Phase 1	212.96(35.77)	210.11(35.77)	15.74(35.47)	208.02(36.92)
Phase 2	195.94(35.19)	195.64(38.57)	194.64(33.51)	190.24(32.54)

Note. Standard deviations are presented in parentheses.

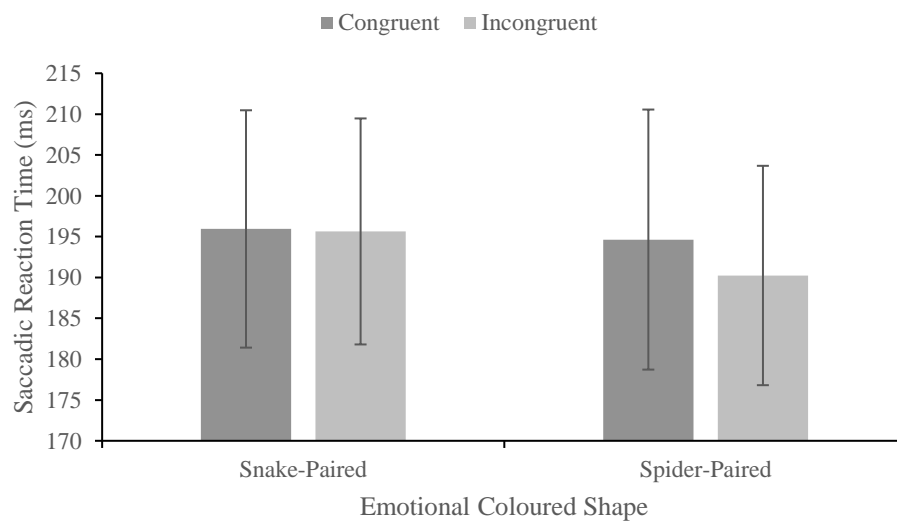


Figure 6. Mean saccadic reaction times for snake-paired congruent, snake-paired incongruent, spider-paired congruent and spider paired incongruent (error bars represent 95% CIs).

Discussion

This study aimed to investigate the effect of emotional stimuli on the oculomotor system in a dot probe task, and if such an effect can induce Pavlovian conditioning. The hypothesis that there would be an attentional bias toward emotional stimuli was not supported. This hypothesis was based on results from numerous manual reaction time studies (Blanchette, 2006; Carlson et al., 2009), as well as some studies that used SRTs (Bannerman et al., 2010b; Bannerman & Hibbard, 2012; Nummenmaa et al., 2009). However, the results from the current study suggest that the emotional images did elicit an emotional response but in the opposite direction. That is, participants were significantly faster when orienting their gaze to targets that succeeded neutral images compared to targets that succeeded emotional images. Further investigation revealed that this effect was only significant when spiders were presented. Mean SRTs were lower for neutral images compared to snake images, however the effect did not reach statistical significance. Analysis of phase 2 revealed that Pavlovian conditioning did not occur. Interestingly, it was also found that there was a significant main effect for phase, with SRTs in phase 2 being quicker compared to phase 1.

Dot Probe Task Design

It is not entirely clear why the current study observed the opposite effect of what was hypothesised. This goes against what has been demonstrated in studies with similar dot probe designs (Nummenmaa et al., 2009). It may be the case that longer cue durations and interstimulus intervals (ISI) impacted our results. The cue duration in our study was based on the study of Nummenmaa et al. (2009), who found that SRTs were faster to emotional images when images were cued at 150 ms. This is quite long relative to other studies that have assessed attentional bias (Bannerman et al., 2010a; Bannerman et al., 2009). Also, unlike Nummenmaa et al. (2009) and Bannerman et al. (2009) we included an ISI. It may be the

case that we did not see faster SRTs directed toward emotional images because the ISI was too long (200 ms; Carlson et al., 2009). There is likely a small window of time in which attentional bias to emotional stimuli occurs (Carlson et al., 2009). Nevertheless, although our study did not observe an attentional bias to emotional stimuli, it does appear an emotional effect was captured. Perhaps the design of our dot probe task facilitated for the observation of a different stage in attentional processing, rather than the intended vigilance stage in attentional processing (Armstrong et al., 2014; Pflugshaupt et al., 2005; Rinck & Becker, 2007).

Vigilant-Avoidant Hypothesis

The trend of orienting more quickly away from emotional stimuli may be explained by the vigilant-avoidant hypothesis (Kishimoto et al., 2021). The vigilant-avoidant hypothesis postulates that the oculomotor system prioritises the detection of emotional stimuli, which is then followed by attentional avoidance (Kishimoto et al., 2021). Such avoidance tendencies have been observed in numerous studies in response to emotional images, including spider images (Armstrong et al., 2014; Pflugshaupt et al., 2005; Rinck & Becker, 2006, 2007).

Rinck and Becker (2007) demonstrated that spider fearful participants were quicker to push forward a joystick in response to spider images, which simulated the act of pushing them away. When monitoring eye-movements, studies have shown that people who fear spiders quickly avoid them after detecting them (Rinck & Becker, 2006). Baum et al. (2013) provided evidence for the vigilant-avoidant hypothesis in a dot probe task, where they presented emotional images (faces expressing anger, pain and joy) at 100 ms and 500 ms. They found that participants' reaction times to anger and pain images were faster than the joy images when presented for 100 ms. However, when images were presented for 500 ms, reaction times to the anger and pain images were significantly slower than joy images.

Similar results have also been observed in a dot probe task when measuring saccades in response to emotional faces at 100 ms and 500 ms cue durations (Kishimoto et al., 2021), where attentional bias is observed at 100 ms cue durations, and avoidance is observed at 500 ms cue durations. Both Kishimoto et al. (2021) and Baum et al. (2013) concluded that this was evidence for the vigilant-avoidant hypothesis – that there are two rivaling stages of attentional processing (Weierich et al., 2008).

Although the images in our dot probe task were displayed for just 150 ms, the 200 ms ISI may have allowed for enough processing time to enable an avoidance response to spider images. As mentioned before, 150 ms cue duration is relatively long compared to other studies that have assessed attentional bias to emotional images (Bannerman et al., 2010b; Bannerman & Hibbard, 2012). For example, some studies have used cue durations as short as 20 ms and 40 ms (Bannerman et al., 2010b). Nevertheless, Nummenmaa et al. (2009) observed attentional bias for cue durations of 150 ms, so it was hypothesised that this was an appropriate cue duration in the current study. Furthermore, cue duration of 150 ms is much shorter than cue durations used in dot probe tasks to assess attentional avoidance, with 500 ms being the shortest (Kishimoto et al., 2021; Weierich et al., 2008). Since the current study had an ISI of 200 ms, cue-target onset asynchrony (CTOA) was 350 ms. This is still relatively short in comparison to studies that have observed avoidance with a CTOA of 500 ms (Rinck & Becker, 2007; Weierich et al., 2008). However, results from the current study suggest CTOAs of 350 and 500 may be within same stage of attentional processing (Kishimoto et al., 2021; Weierich et al., 2008).

Additionally, results found that SRTs were significantly faster in phase 2 when the images were not present, with a large effect size ($\eta^2_p = .67$). This effect suggests that the presence of emotional images may have been slowing saccades in the phase 1, which may add more evidence for the occurrence of an avoidance response. However, we cannot confirm

whether this was specifically due to the presence of emotional images or images in general. It may also be the case that the 50 ms difference in cue duration caused this effect.

Interestingly, the results of the current suggest that short cue durations (<500) may be capable of eliciting avoidance behaviour within the oculomotor system. Vigilance is thought to occur without conscious perception and is the result of bottom-up processing (Weierich et al., 2008; Mulckhuyse, 2018), with studies demonstrating attentional bias at short cue durations (20ms – 150 ms; Bannerman et al., 2010b; Nummenmaa et al., 2009). Avoidance, on the other hand, has not been observed for cue durations under 500 ms (Weierich et al., 2008), which is likely enough time to consciously perceive the stimuli (Meador et al., 2002). However, results in the current study potentially demonstrate that avoidance occurs independent of conscious perception, as cue durations of 150 ms may be too fast for many to consciously perceive the content of the images (Meador et al., 2002; Weierich et al., 2008).

Snakes Versus Spiders

The second hypothesis was that there would not be a significant difference between snake and spider images. This was based on mixed results from studies that used manual button presses, and the fact that fear of spiders is likely the result of Pavlovian conditioning (Blanchette, 2006; Carlson et al., 2009; Kawai, 2019; Kawai & Koda, 2016), making their effect difficult to predict. Although this hypothesis was supported, it was made based on the presumption that faster congruent saccades would be indicative of an emotional response. However, the results from this study suggest that an emotional response was characterised by slower congruent saccades and faster incongruent saccades. Therefore, it may not make sense to compare snake congruent and spider congruent responses. Instead, it may be better to measure emotional responses based on the difference between congruent and incongruent images. Based on this, the results from the current study suggest that spiders elicited a greater

emotional response than snakes. This is not surprising given the variability of other studies, some of which have demonstrated an attentional bias to spiders that has rivalled snakes (Fabio & Caprì, 2019), which is indicative of an emotional effect (Mulckhuyse, 2018; Mulckhuyse et al., 2013).

As mentioned previously, arachnophobia is among the most common phobias (Vetter, 2013). Numerous studies have confirmed that spiders have a significant influence on attention and the oculomotor system (Gerdes et al., 2009; Rinck et al., 2010; Rinck & Becker, 2006). This includes dot probe task studies that have demonstrated an emotional response to spider images. However, the cause of this fear is not so clear. Some studies claim that spiders are evolutionarily relevant and that there is an inborn component relating to their fear (Gerdes et al., 2009; Luo et al., 2015), whereas others claim that their fear is due to Pavlovian conditioning (Kawai, 2019; Kawai & Koda, 2016). Kawai and Koda's (2016) study of Japanese monkeys provides compelling evidence that the primate visual system has a bias to snakes but not spiders, which indicates that fear of snakes may be the result of conditioning. Nevertheless, it is clear that spiders are capable of eliciting an emotional response (Blanchette, 2006; Gerdes et al., 2009; Mulckhuyse et al., 2013; Pflugshaupt et al., 2005; Rinck et al., 2010), which is further supported by the current study. Although the current study cannot make inferences on whether fear of spiders is inborn or learned, our results do suggest that spiders may elicit a greater emotional effect than snakes. This indicates that Pavlovian conditioning can have a considerable impact on the oculomotor system and allocation of attention (Mulckhuyse et al., 2013).

It is possible that geographical location played a part in our results, as snakes in Tasmania are timid (Department of Primary Industries, Parks, Water and Environment, n.d.), and death due to snake bite is a rare occurrence (Fisher & Howarth, 2020). Spiders, on the other hand, are commonly encountered in homes, so perhaps relevance of spiders increased

their emotional effect among participants (Lindner et al., 2019). Image database may also contribute to mixed results found between snakes and spiders, with some images emphasising specific features that may be important for detection (Kawai, 2019), which may diminish the realism of images. For example, the spider images presented in the dot probe task better represent the size of a spider, whereas snake images do not accurately represent the size of the typical snake. Previous studies have suggested that snake scales are an important feature by which they are identified (Isbell & Etting, 2017; van Strien & Isbell, 2017). Perhaps that is why an emotional response was not observed, as image size and resolution did not emphasise contours and corners of snake scales, which may be crucial for their detection (Kawai, 2019). Additionally, it has been suggested that movement may aid the detection of snake scales (Kawai, 2019; Peterhans & von der Heydt, 1993). So, it may be the case that our study did not facilitate for snake detection.

Another thing to consider is that, as found by Thrasher and LoBue (2016), young children have demonstrated attentional bias to snakes absent of fear. So, it is possible that attentional bias to snakes in a dot probe task may occur independent of an emotional response (Mulckhuyse, 2018; Thrasher & LoBue, 2016). Instead, attentional bias to snakes may be mostly due to neurons in the Pulvinar that aid their detection (Le et al., 2013; Soares et al., 2017). This may explain why we did not observe a significant effect for snakes, because our study unintentionally observed avoidance rather than attentional bias. In order to observe attentional avoidance, an emotional response may need to occur (Weierich et al., 2008). Additionally, it has also been found that emotional stimuli can elicit sustained engagement (Belopolsky et al., 2011; Weierich et al., 2008). That is, individuals detect emotional stimuli quicker, sustain engagement, then seek to avoid (Armstrong et al., 2014; Kishimoto et al., 2021; Pflugshaupt et al., 2005; Weierich et al., 2008). So, it may even be the case that an emotional effect did occur but, due to the timing of the dot probe task, there was interference

between two rivalling attentional systems – sustained attention and avoidance (Belopolsky et al., 2011; Koster et al., 2004; Weierich et al., 2008). This is plausible given that the current study is likely assessing attentional avoidance in its early stages. Although there was not a significant difference between snake and neutral images, mean SRTs were lower for neutral images versus snake images, which may indicate delayed avoidance due a stronger vigilant response.

Pavlovian Conditioning

We hypothesised that there would be a conditioning effect seen in phase 2 of the dot probe task. That is, participants would orient to coloured shapes quicker if they were previously paired with an emotional image. This was based on humans’ reliance on the visual system for the detection and encoding of emotional stimuli (Ehlers et al., 2002; Nummenmaa et al., 2009; Schmidt et al., 2017), as well as evidence of physiological responses to emotional images (Bannerman et al., 2010b; Bannerman & Hibbard, 2012; Nummenmaa et al., 2009). Given that both snakes and spiders are commonly feared (Blanchette, 2006; Kawai, 2019; Rinck et al., 2005; Subra et al., 2018), it was thought that snake and spider images may be capable of inducing Pavlovian conditioning. However, this hypothesis was not supported, with no significant difference found for coloured shape category. Admittedly, it is likely that images alone do not elicit an emotional effect strong enough to induce Pavlovian conditioning, as images do not produce a physiological response as strong as an electric shock or aversive noise, which is typically what is used to induce Pavlovian conditioning (Armstrong et al., 2019; Hopkins et al., 2015; Stussi et al., 2020).

We also hypothesised that snake images would induce a stronger conditioning effect than spiders. This was based on the ancestral relevance of snakes (Isbell, 2006), and the theory that there may be an inborn component that contributes to the consolidation of fear

(Kawai, 2019). This is plausible given that snakes are often feared independent of previous encounters with them (Kawai, 2019; Lipp & Derakshan, 2005). However, given that the current study did not observe an emotional effect for snakes, we cannot tell if there was a conditioning effect. As mentioned before, it may be that case sustained engagement due to a vigilant response prevented us from observing an emotional response, as we were likely observing attentional avoidance in its early stages (Weierich et al., 2008).

Summary of Limitations

The current study has several limitations. First, we are unable to make inferences on the impact snake and spider images have on attentional bias because we likely observed a later stage in attentional processing (Kishimoto et al., 2021). Second, because an emotional effect was not observed for snakes, we are unable to determine if Pavlovian conditioning for snakes occurred or not. This is because a strong vigilant response and sustained engagement may have delayed the onset of avoidance (Koster et al., 2004; Weierich et al., 2008). As a result, we may have observed a period in which vigilance and avoidance for snakes crossed paths. Also, as mentioned above, the snake images used in this study may have been incapable of producing an emotional effect due to their low resolution and low realism.

Future Research

Again, the aim of this study was to observe an attentional bias in response to spider and snake images, and to see if these images could induce Pavlovian conditioning. This study did not facilitate for attentional bias to either of the emotional images, perhaps due to the duration of the ISI. Future studies that intend to investigate attentional bias to snakes and spiders in eye-tracking studies may want to consider designing a dot probe task without an ISI. Future studies may also want to consider increasing the realism of snake trials (Kawai & Qiu, 2020), perhaps by increasing the resolution of images. Also, given the potential importance of

movement in snake detection (Kawai, 2019), future studies may want to consider developing experiments that use moving images, commonly referred to as GIFs (graphics interchange format). This may also be a method in which Pavlovian conditioning to visual stimuli alone can be induced. Additionally, virtual reality may even provide an avenue in which Pavlovian conditioning via visual stimuli is induced, as this would likely produce a more realistic experience (Kawai & Qiu, 2020), perhaps eliciting a stronger physiological response. Even if visual stimuli alone are incapable of inducing Pavlovian conditioning, investigating snake and spider images when paired with an aversive noise or electric shock will allow inferences to be made in regard to their influence on Pavlovian conditioning. This would test the theory that there is an inborn component that is involved with the fear of snakes (Kawai, 2019).

The results from this study suggest that spider images displayed for just 150 ms can elicit an avoidant emotional response. Future research should investigate different timing parameters for dot probe tasks in order to investigate attentional avoidance, gaining greater understanding of the stages in attentional processing. More research in this area could focus on the impact of ISI durations, which may reveal to what degree time after cue presentation impacts attentional avoidance. Results from this study also prompt future research into attentional avoidance at short cue durations. Because conscious perception of the images varied depending on the individual (Lojowska et al., 2019; Meador et al., 2002), short cue durations with long ISI durations should be investigated. Masking the emotional images could also be an option, which is a common technique that aims to stop cues from being perceived (Carlson et al., 2009). This would test whether avoidance can be observed independent of conscious perception. For example, eye-tracking studies have demonstrated that images displayed for 20 ms can elicit an emotional response (Bannerman et al., 2009). Perhaps a dot probe task with a similar cue duration with a CTOA of 350 ms can observe attentional avoidance. More research in this area is required to understand this stage of

attentional processing (Weierich et al., 2008). Furthermore, the visual system plays an important role in anxiety disorders (Ehlers et al., 2002; Kleim et al., 2021; Lissek et al., 2005; Yiend, 2010). Better understanding the role of the oculomotor system and its relationship with emotional images and Pavlovian conditioning will assist with strategies that aim to address anxiety disorders in the future.

Conclusion

The current study intended to assess attentional bias in response to snake and spider images in a dot probe task, and to investigate whether such images are capable of inducing Pavlovian conditioning. Although attentional bias was not observed, an avoidance effect was, which was significant for spider trials. This indicates that spiders can cause an emotional response (Brosch et al., 2010), and it may demonstrate that they are capable of eliciting an emotional response greater than that of snakes. This also suggests that attentional avoidance can be observed at short cue durations of 150 ms. This finding is of considerable importance given previous studies have not observed attentional avoidance with cue durations lower than 500 ms (Weierich et al., 2008). This suggests that CTOA may be more important than cue duration when it comes to observing attentional avoidance. However, future research is needed to confirm this effect. Furthermore, the current study suggests that emotional images may not be capable of inducing Pavlovian conditioning. However, it may be the case that Pavlovian conditioning via images is possible, but limitations may have prevented us from observing such an effect.

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Appendices
Appendix A
Ethics Approval Letter



Ethics Approval Letter

28/06/2021

To: Dr Satel

Project ID: 24601

Project Title: The Effect of Emotional Images on the Oculomotor System and Pavlovian Conditioning

The above named project has been approved by the University of Tasmania Human Research Ethics Committee on 28 June 2021.

Approval has been granted for the following documentation:

Submission Document Name	Submission Document File Name	Submission Document Type	Submission Document Date	Submission Document Version
ethics_protocol_24601	ethics_protocol_24601.pdf	PROTOCOL	11/05/2021	2
ethics_ad_24601	ethics_ad_24601.pdf	ADVERTISING MATERIAL	11/05/2021	2
ethics_consent_24601_v3	ethics_consent_24601_v3.docx	PARTICIPANT INFORMATION AND CONSENT FORM	18/06/2021	3
ethics_info_24601_v3	ethics_info_24601_v3.docx	PARTICIPANT INFORMATION AND CONSENT FORM	18/06/2021	3

ethics_response_24601_R4 ethics_response_24601_R4.docx OTHER PROJECT-	25/06/202 4 1
RELATED DOCUMENTATION	

The University of Tasmania Human Research Ethics Committee has provided approval for the project to be conducted at the following sites:

- UTAS - Newnham

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the University of Tasmania Human Research Ethics Committee. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

In accordance with the [National Statement on Ethical Conduct in Human Research](#), it is the responsibility of institutions and researchers to be aware of both general and specific legal requirements, wherever relevant. If researchers are uncertain they should seek legal advice to confirm that their proposed research is in compliant with the relevant laws. University of Tasmania researchers may seek legal advice from Legal Services at the University.

The University of Tasmania Human Research Ethics Committee (HREC) operates under and is required to comply with the National Statement on the Ethical Conduct in Human Research.

Therefore, the Chief Investigator's responsibility is to ensure that:

- (1) All investigators are aware of the terms of approval, and that the research is conducted in compliance with the HREC approved protocol or project description.
- (2) Modifications to the protocol do not proceed until **approval** is obtained in writing from the HREC. This includes, but is not limited to, amendments that:
 - (i) are proposed or undertaken in order to eliminate immediate risks to participants; (ii) may increase the risks to participants;
 - (iii) significantly affect the conduct of the research; or
 - (iv) involve changes to investigator involvement with the project.

Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.

- (3) Reports are provided to the HREC on the progress of the research and any safety reports or monitoring requirements as indicated in NHMRC guidance.

Guidance for the appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located under the ERM "Help Tab" in "Templates". All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested.

- (4) The HREC is informed as soon as possible of any new safety information, from other published or unpublished research, that may have an impact on the continued ethical acceptability of the research or that may indicate the need for modification of the project.
- (5) All research participants must be provided with the current Participant Information Sheet and Consent Form, unless otherwise approved by the Committee.
- (6) This study has approval for four years contingent upon annual review. A Progress Report is to be provided on the anniversary date of your approval. Your first report is due on the anniversary of your approval, and you will be sent a courtesy reminder closer to this due date. Ethical approval for this project will lapse if a Progress Report is not submitted in the time frame provided.
- (7) A Final Report and a copy of the published material, either in full or abstract, must be provided at the end of the project.
- (8) The HREC is advised of any complaints received or ethical issues that arise during the course of the project.
- (9) The HREC is advised promptly of the emergence of circumstances where a court, law enforcement agency or regulator seeks to compel the release of findings or results. Researchers must develop a strategy for addressing this and seek advice from the HREC.

Kind regards,

Ethics Executive Officer



Appendix B

Information Sheet



Faculty of Health, University of Tasmania

Information Sheet (H0024601)

The Effect of Emotional Images on the Oculomotor System and Pavlovian Conditioning

Honours Student: Samuel Wilson;

Supervisors: Luke Johnson & Jason Satel;

Research Assistants: Samuel Moore, Alyssa Marshall, & Hannah Jones

1. Invitation

You are invited to participate in a research study looking at how emotional images influence the visual system. This study is being conducted by Samuel Wilson as a component of his honours degree.

2. What is the purpose of this study?

The aim of the proposed study is to investigate eye movement response times when looking at emotional images. For example, do people look at negative emotional images quicker than neutral or positive images? If so, we also want to know if coloured shapes can be condition with these images. Will people look at a coloured shape quicker if it was previously paired with negative emotional images?

3. Why have I been invited to participate?

You are eligible to participate in this study if you are over the age of 18 and have no existing uncorrected visual disabilities or psychiatric/neurological disorders. Corrected vision through the use of glasses or contact lenses still makes you eligible to participate. All participation is voluntary and there are no consequences either personally or academically if you do not wish to participate.

4. What will I be asked to do?

You will be asked to complete a computerised task, called the dot probe task, which requires you to make a series of eye movements to dots that appear either left or right. These dots will appear after two images/shapes briefly flash upon the screen.

The experimental session will take about 45 minutes to complete, and it will take place in room O008 on the Newnham campus.

5. Are there any possible benefits from participation in this study?

There are no direct benefits to participants or the community. However, this study will add to a body of knowledge that may help in understanding the detection and encoding of visual stimuli, as well as the underlying mechanisms. In exchange for participation, you will receive \$15 per hour or SONA course credit (1 point/hour).

6. Are there any possible risks from participation in this study?

It is possible to experience fatigue after sitting and viewing the screen. If you feel that you need a break, you may inform the researcher of your discomfort and a break can be scheduled where possible. The negative images presented in the experiment are unlikely to cause distress. Please notify the researcher if you do feel distressed or concerned during or after the experiment.

7. What if I change my mind during or after the study?

You are free to withdraw at any stage during the experiment. You have no obligation to complete the experiment and no explanation is needed if you choose to withdraw.

8. What will happen to the information when this study is over?

All data collected during this experiment will be confidential and will be destroyed after 5 years after publication.

9. How will the results of the study be published?

At the end of the study, results will be published in an honours thesis and potentially in academic journals. You can access such articles through the UTAS academic websites.

10. What if I have questions about this study?

This study has been approved by the Tasmania Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, you can contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 2975 or email ss.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote H0024601. The individual researcher can be contacted via email at jason.satel@utas.edu.au.

Appendix B

Consent Form

Faculty of Health, University of Tasmania

Consent Form (H0024601)



The Effect of Emotional Images on the Oculomotor System and Pavlovian Conditioning

Honours Student: Samuel Wilson;

Supervisors: Luke Johnson & Jason Satel;

Research Assistants: Samuel Moore, Alyssa Marshall, & Hannah Jones

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. I understand that this project is being conducted as part of honours research.
5. I understand that the study involves emotional images.
6. I understand that I am to notify a supervisor if I experience distress during or after the experiment.
7. I understand that participation involves no foreseeable risks.
8. I understand that all research data will be securely stored on the University of Tasmania premises for five years from the publication of the study results and will then be destroyed.
9. Any questions that I have asked have been answered to my satisfaction.
10. I understand that the researcher(s) will maintain confidentiality and that any information I supply to the researcher(s) will be used only for the purposes of the research.
11. I understand that the results of the study will be published so that I cannot be identified as a participant.
12. I understand that my participation is voluntary and that I may withdraw at any time without any effect.
13. I understand that I will not be able to withdraw my data after completing the experiment as it has been collected anonymously.

This study has been approved by the Tasmania Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, you can contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226

2975 or email ss.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote H0016857. The chief investigator can be contacted via email at jason.satel@utas.edu.au.

Participant's name: _____

Participant's signature: _____

Date: _____

Faculty of Health, University of Tasmania

Consent Form (H0024601)



Statement by Investigator

☐ I have explained the project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

Investigator's name: _____

Investigator's signature: _____

Date: _____