# Investigating the effect of startle on cognitive performance using a n-back task and prepulse

Alex Bui

Supervisor: Dr. Anthony N. Carlsen

Committee: Dr. Erin K. Cressman & Dr. Dana Maslovat

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School of Human Kinetics

Faculty of Health Sciences

University of Ottawa

## Statement of Contribution of Collaborators

All the work contained in this document was carried out by the author in collaboration with Dr. Anthony Carlsen, and with input from Dr. Erin Cressman and Dr. Dana Maslovat.

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## Table of Contents

**Abstract**

The startle reflex is a whole-body response to a startling stimulus. The effects of a startle reflex elicited by a startling acoustic stimulus (SAS) on task performance have been reliably observed in motor tasks, with decrements in motor performance being attributed to the startle reflex. The presentation of a non-startling acoustic stimulus (prepulse), prior to a SAS has been shown to reliably decrease the amplitude of the startle reflex. This modulation, termed prepulse inhibition, has been used to diminish the startle reflex in motor performance tasks, resulting in a decrease in the decremental effects caused by startle. This phenomenon has been less examined in cognitive tasks. As such, the purpose of this experiment was to determine the effect of startle on cognitive performance, and examine whether a prepulse would reduce any decrements due to the startle reflex. In this experiment, participants performed twelve blocks of a five-minute continuous 2-back task. Throughout each block, a non-startling control stimulus (80dB), a SAS (120dB), and prepulse+SAS (80dB tone 100 ms prior to 120dB) was presented. Response accuracy (correct 2-back target acceptances and rejections) and response time was measured. The results revealed that cognitive performance was negatively affected by all stimulus types. Cognitive performance following a SAS was only mildly impacted, with participants able to maintain similar levels of accuracy to control at the cost of a slight increase in response time. Contrary to expectations, prepulse had no effect on reducing these detriments, but instead seemingly intensified them. These results suggest that any acoustic stimulus negatively impacts cognitive performance. Additionally, prepulse seems to cause more detriments perhaps due to the nature of it being two distinct acoustic stimuli, compared to one acoustic stimulus in control or SAS conditions. However, the addition of a prepulse reduced the frequency of a startle reflex, thus reducing the incidence of negative impacts on performance.

## Glossary of Terms

EMG: Electromyography

ISI: Interstimulus interval

nRPC: Nucleus reticular pontis caudalis

OOc: Orbicularis oculi

PPI: Prepulse inhibition

PP+SAS: Acoustic stimulus condition consisting of a prepulse and a SAS

RT: Reaction time

SAS: Startling acoustic stimulus or acoustic stimulus condition consisting of a startling acoustic stimulus

SCM: Sternocleidomastoid

SCM+: Data from SAS & PP+SAS conditions where a startle reflex was observed in SCM

SCM-: Data from SAS & PP+SAS conditions where no startle reflex was observed in SCM

## List of Figures

# Chapter 1: Literature Review

## 1. Introduction

In everyday life humans are required to perform a wide variety of movements, some of which require a high degree of precision and accuracy. These highly precise movements play an integral part in our lives from recreational activities like kicking a soccer ball to driving a car. However, unexpected events can play a role in how these movements are carried out. For example, the unexpected variables, like a pedestrian jumping out onto the road in the path of your car, may cause a startle reflex when performing these tasks. While the consequences of being startled are typically not severe, when occurring during specific scenarios (e.g., driving a car), they can cause catastrophic consequences.

The detrimental motor effects attributed to being startle have been examined using a startling acoustic stimulus (SAS) during the performance of various motor tasks (Vlasak, 1969; Thackray and Touchstone, 1970; May and Rice, 1971; Foss et al., 1989). Additionally, limited research has shown similar negative effects during a cognitive task (Vlasak, 1969; Woodhead, 1959). These experiments reported an immediate decrease in performance following a startle reflex as well as a prolonged negative effect for up to 30 seconds following a startle (Vlasak, 1969; Woodhead, 1959; Thackray and Touchstone, 1970).

The use of a weak prepulse stimulus can reduce the magnitude of the startle reflex, and has been shown to mitigate decreases in motor performance in discrete trials (Foss et al., 1989). However, it is unknown if similar improvements may be seen in cognitive performance decrements. The use of a cognitive task in combination with a SAS (and prepulse), can provide insight into the effect of startle on cognitive processes, and illustrate the effect of a prepulse on the startle reflex in a cognitive task paradigm. Additionally, the use of a continuous cognitive

task can provide more evidence for the prolonged effects of a startle as well as the potential reduction of these effects through prepulse.

First, we will discuss the information processing model of motor responses, and then will discuss how cognitive processes such as attention, decision making, and memory, contribute to motor responses. Next, we will examine some of the common cognitive tasks used to measure cognitive processes. Finally, we will examine the startle reflex, prior experimentation using startle within the context of task performance, and modulation of the startle reflex by prepulse.

## 2. Information Processing Model

One focus of motor behaviour research is to examine how sensory information from the environment, and the body are used to control movement, as well as how the central nervous system is organized to produce these movements (Schmidt & Lee, 2011). One of the most prominent models used to examine this interaction between sensory processing and motor control is the classical information processing model. This model is typically conceptualized as comprising three distinct stages that are completed between the onset of a stimulus and the start of a response, including stimulus identification, response selection, and response programming (Schmidt & Lee, 2011). The stimulus identification stage involves the detection and identification of a relevant stimulus from the environment. In the second stage, an appropriate response must be selected based off the stimulus identified in the previous stage. The third and final stage involves transforming the selected response in the previous stage to specific commands for the motor system and then initiating that response. Since information processing occurs within the central nervous system, it cannot be directly observed or measured. However, Donders (1969) was able to determine the duration of these mental processes using reaction time (RT) experiments. In his seminal experiments, Donders used simple RT, go/no-go, and choice RT

paradigms. In a simple RT task, there is only one possible response allowing it to be preprogrammed and therefore bypassing the response selection stage. In a choice RT task, there are at least two response options, requiring individuals to detect and identify the stimulus before selecting the appropriate response. In a go/no-go RT task, a response is required to one stimulus while no response is required to another stimulus (Donders, 1969), allowing for the preprogramming of the response. This task requires the individual to detect and identify the stimulus, and either execute the preprogrammed response or do nothing. The different requirements of each task allowed Donders to provide evidence for the existence of these stages, as well as their duration. This was done by examining the difference in RT between the tasks, now typically referred to as Donders' subtractive method.

### 2.1. Attention

Within the three-stage model of information processing, different cognitive processes play a role across the stages. Attention is a key cognitive process used within the information processing model; however, there are differing views on the effect of attention on the information processing stages. One view, known as the single channel theory, posits that processing constraints limit the ability to perform two tasks at once, as both tasks compete for a common processing resource (Broadbent, 1959; Welford, 1952). In relation to the information processing model, since the stages are organized in a serial order, this single-channel theory suggests that only one piece of information can be attended to and processed at one moment (Czyz, 2021). In contrast, another view of attention suggests that at a specific information processing stage, there is a bottleneck for information, requiring filtering. This theory, known as filter or bottleneck theory asserts that multiple pieces of information can be attended to and processed at any moment until reaching a bottleneck at a specific stage. However, where that bottleneck occurs

has been a matter of debate, with some suggesting that it occurs during either the detection phase (Broadbent, 1959) or identification phase (Deutsch & Deutsch, 1963) of the stimulus identification stage. Others suggest that this bottleneck exists at the response selection stage (Keele, 1973). Recent research by Maslovat and colleagues (2013) provided novel evidence that the bottleneck may occur in the response preparation stage. In their experiment, they used a psychological refractory period (PRP) paradigm to examine the overlapping period of response preparation and response initiation of two separate reaction time (RT) tasks by manipulating the time interval of the "go" stimuli. The results of their experiment showed that when the "go" stimuli for the two tasks were presented more than 500ms apart, there was no difference in the reaction time (RT) of the tasks compared to when they were done as individual tasks. However, when the stimuli were presented at shorter latencies (100-200 ms apart), reaction time of the second task was significantly slower. Maslovat and colleagues also used a SAS throughout their experiment to determine if the two responses could be prepared together. A SAS has been used to examine motor preparation in simple RT paradigms where advanced preparation can occur in numerous movements (see Carlsen et al., 2012). Maslovat and colleagues hypothesized that if preparation for both tasks in their dual-task paradigm had occurred, the SAS would trigger the second response at a latency similar to when performed as a single task paradigm. Alternatively, if both responses could not be prepared together, they hypothesized that the use of a SAS would result in a PRP (psychological refractory period) effect similar to the normal "go" signal. Their results showed that during SAS trials a PRP effect was still present during the short stimulus onset asynchrony. However, during the longer stimulus onset asynchrony, no PRP effect was observed in SAS trials. The PRP effect present in SAS trials suggests that only the first response was prepared in advance, and the preparation of the second response could only occur after

initiation of the first response as evidenced in the longer stimulus onset asynchrony condition. These results provide strong evidence that a bottleneck exists at the response preparation stage.

In summary, while attention is important for all the stages of information processing, it is important to note that the limitations of attention require individuals to (at some point) filter out irrelevant stimuli and attend to and process the relevant stimulus one at a time; and it appears this bottleneck likely occurs during the response preparation stage.

### 2.2. Working Memory

Another cognitive process that contributes to the information processing model is memory, and in particular, working memory. Memory can generally be thought of as the mental capacity to store and recall information. The integration of memory into the information processing model appeared in the 1960s as the field of psychology shifted towards an information-processing approach (Baddeley, 2010). This integration into the information processing model stemmed from the concept of working memory, which evolved from short-term memory (Baddeley, 2010). Early models of memory, like the one proposed by Atkinson and Shiffrin (1968), conceptualised working memory as a series of storage systems which information from the environment would pass through before being passed to a limited capacity short-term memory store and ultimately to long-term memory. Adaptation of these models led to a three-component model of working memory proposed by Baddeley and Hitch in 1974. This model proposed working memory as being comprised of three distinct systems interacting with one another to form working memory. These three components are the central executive system, the phonological loop, and visuospatial sketchpad. The central executive is thought to control the flow of information and direct attention, while the phonological loop and visuospatial sketchpad store and manipulate auditory and visual/spatial information respectively (Baddeley & Hitch, 1974). It is here in working

memory where information is processed for ongoing tasks or is assigned meaning and stored in long-term memory (Eggen, 2020). The transfer of information from working memory to long term memory is required as working memory capacity is limited, holding approximately seven plus or minus two unrelated items at a time (Miller, 1956). As working memory capacity approaches its limit, an increase in mental activity is required, which can be represented as cognitive load (Eggen, 2020). In summary, working memory in relation to the information processing model functions as a "workbench" upon which incoming information can be interacted with to generate an output during an ongoing task or be stored to long-term memory for later use.

### 2.3. Decision-making

One of the broader cognitive processes that is present in the information processing model is decision-making. As mentioned previously, the first stage of information processing typically involves the detection and identification of a relevant stimulus from the environment. Decision-making plays a role in this stage by selecting the relevant stimulus from the irrelevant stimuli. However, decision-making is even more important for the response selection stage, where an appropriate response must be selected based on information from the first stage. While early decision-making models (e.g., the expected utility theory proposed by Von Neumann & Morgenstern (1944)), emphasized maximizing expected value by probability of receiving a given outcome (and the value of the associated outcome), further additions to this general model (including subjective value and bias of people's perception of probability) have resulted in numerous models each with their own anomalies and exceptions. Over time a new approach to decision-making theory emerged based around information processing. These models argue that decision-making starts from basic cognitive processes, and that decision-making recruits from

cognitive processes such as memory and attention (for a review see Oppenheimer & Kelso, 2015). More specifically, these information processing models of decision-making posit that decisions arise from sampled evidence that is accumulated and integrated from cognitive processes such as memory and attention (Oppenheimer & Kelso, 2015). Further research into the temporal aspects of decision making has resulted in the consideration of "sequential sampling" or "bounded integrator" models of decision-making (Stone, 1960; Laming, 1968; Forstmann et al., 2016). The models suggest that incoming sensory stimuli are accumulated and temporally integrated up to a certain threshold, at which point a decision is made. Expanding on these models, Cisek and colleagues (2009) proposed a new model termed the urgency-gating model which suggest that rather than decisions being made when a threshold of sensory information is accumulated, decisions are made depending on the urgency to make a choice. While not completely neglecting sensory information, they argued that it is the combination of evidence for a given choice, combined with a motor signal related to the urgency to make a choice that drives decision-making. In summary, decision-making models suggest that decisions rely on integrating the outcomes of a given choice, which are derived from cognitive processes, along with (potentially) the urgency to make a choice.

3. Cognitive Tasks

Within the domains of clinical and experimental psychology, cognitive tasks serve as invaluable tools to assess different cognitive processes, such as attention, memory, executive function, and inhibitory control. While numerous tasks like the flanker, n-back, and Stroop task have been used to assess cognitive processes in healthy and patient populations (for reviews see Hamdam & Vieria 2022; Mullane et al., 2009; Nikolin et al., 2021), each of these tasks is aimed at assessing specific cognitive processes. For example, the flanker task, first implemented by

Eriksen & Eriksen (1974), is used to assess attention and inhibitory control. This task requires participants to respond to a visually relevant stimulus that is flanked by visually irrelevant stimuli. The findings from this seminal study demonstrated that attentional selectivity is unable to fully eliminate the effects of irrelevant stimuli, and that the effect of irrelevant stimuli on target reaction time is larger when the response is opposite or incompatible to the relevant stimuli.

Another commonly used task is the Stroop task which examines the ability to inhibit cognitive interference. This occurs as the processing of one stimulus feature impedes the simultaneous processing of another stimulus feature, otherwise termed the Stroop Effect (Scarpina & Tagini, 2017). This task, first proposed by Stroop (1935), required participants to read three different tables of words as fast as possible. In two of the tables, participants are required to read names of colours printed in black ink and name different colour patches. However, in the third table, participants were required to read names of colours printed in incongruent ink (e.g., the word RED printed in green ink). In this incongruent condition, participants were on average 2.3 seconds slower to complete reading through the table of words. These findings provided evidence of an interference effect between the colour that the word was printed in and the word itself implying that these two features competed for the same processing resources. This task is still commonly used in both experimental and clinical settings is to assess inhibitory processes such as the ability to inhibit cognitive interference (Scarpina & Tagini, 2017).

One of the most used cognitive assessments of working memory is the N-back task initially proposed by Kirchner (1958). The N-back task is a continuous task where subjects are presented with a sequence of stimuli and must decide whether the current stimulus matches the stimulus

presented N steps back in the sequence. Manipulation of this task involves increasing the value of N, thus increasing cognitive load by requiring participants to remember a larger, ever-changing sequence. Typically, it has been demonstrated that as the number of items "back" increases, the cognitive demand on working memory increases resulting lower accuracy and longer reaction times (Jaeggi et al., 2010, Scharinger et al., 2017). This can be attributed to the increased number of items needed to be concurrently stored in working memory. The N-back task can be considered a continuous task as it requires constant adaptation and updating of the items stored in working memory. This constant updating information as well as no definitive start and end point during the task distinguish it from discrete and even serial tasks. The N-back task can be classified a cognitive task due to the required input of working memory to constantly store and update a sequence of items. Many different versions exist with larger N's resulting in greater cognitive load, and even mental subtractions embedded within the N-back task (see Schwartz et al., 2025). However, what remains consistent between methodologies is the simplification of the motor response used to measures responses in the task, usually a button press (see Scharinger et al., 2017; Pergher et al., 2018; Schwartz et al., 2025).

4. The Startle Reflex

The startle reflex is a protective physiological response that occurs following the presentation of an intense, unexpected stimulus. It can be described as a diffuse response consisting of closing of the eyes, followed by the shoulders curling forward and up, and the shortening of the torso (Yeomans & Frankland, 1995); or otherwise characterized as a whole-body muscle flexion response (Landis, Hunt, & Strauss, 1939). The startle reflex can be elicited through a variety of stimulus modalities such as acoustic, tactile, vestibular, and electric (Blumenthal et al., 2005; Yeomans & Frankland, 1995). However, the modality that is most used is the startling acoustic

stimulus (SAS) due to its ease and reliability of implementation (Carlsen et al., 2011). The pathway by which a SAS elicits a startle reflex begins at the cochlear nucleus. Incoming sensory input is sent from the cochlear nucleus to the large neurons of the nucleus reticular pontis caudalis (nRPC). These neurons then act along the spinal cord, descending through the reticulospinal tract activating motorneurons via axons near the medial longitudinal fasciculus (Yeomans and Frankland, 1995). This pathway bypasses the auditory cortex where auditory stimuli would be processed, allowing for the rapid presentation of the startle reflex (14-151 ms) (Yeomans and Frankland, 1995). This activation produces the muscular contractions associated with the startle reflex which can then be measured using electromyography (EMG). The time of activation via the reticulospinal tract can be seen in measurements of EMG, where EMG latencies for different muscles increase proportionally to distance from the brainstem. This was observed by Brown and colleagues (1991) who recorded muscle activation following a startling stimulus in the following order: facial muscles, neck and paraspinal muscles, followed by upper limb, trunk, and lower limb activation in a proximal to distal pattern.

While the startle reflex produces contractions in muscles throughout the body, certain muscles like the orbicularis oculi (OOc) and sternocleidomastoid (SCM) are commonly used by researchers to ascertain whether a startle reflex has truly occurred (Brown et al., 1991). While it has been commonly used as an indicator of a startle reflex, OOc has been shown to be not as reliable of an indicator of a "true" startle reflex as previously thought (Brown 1991; Carlsen et al, 2007). Carlsen and colleagues (2007) demonstrated that the EMG pattern of the eyeblink was significantly different depending on whether a response was elicited in the SCM during the presentation of a SAS. Additionally, they showed that even when the rest of the startle reflex had habituated, presentation of a SAS still elicited a response in OOc. They proposed that activation

of the OOc, after the startle reflex had habituated, was due to the auditory eyeblink response rather than the auditory startle eyeblink. This is important as these two different responses are thought to involve different pathways (Brown et al., 1991). Following the eyeblink, the SCM is the next most frequently elicited and last to habituate muscular indicator of the startle reflex, and it serves as a more reliable indicator of whether a startle reflex has occurred (Brown et al., 1991). When examining the effect of startle on movement, it is important to ensure that a startle reflex has been observed. This is important as depending on whether a startle reflex has been observed (as indicated by the SCM muscles), it has been shown that reaction times are different and can be strongly differentiated (Carlsen et al., 2007; Carlsen and Maslovat 2019).

5. Previous Experimentation of Startle on Task Performance

Although relatively limited in scope, some previous research has assessed the effect of the startle reflex on cognitive task performance. Two examples of this include a continuous mental subtraction task used by Vlasak (1969) and the Mackworth multichannel test used by Woodhead (1959). In these studies, it was found the effect of the startle reflex on cognitive performance was deleterious, such that performance was impaired for up to 30 seconds following a startling stimulus (Vlasak, 1969; Woodhead, 1959). However, it is uncertain which specific processes were affected by the startle reflex as neither task accurately captured a specific cognitive process. The mental subtraction task used by Vlasak (1969) is similar to serial subtraction tasks. These tasks are useful based on the assumption that they measure attention and mental concentration. However, without standardized administration of the task, there are no recognized norms for these serial subtraction tasks, thus leading to some speculation of the usefulness of these tasks (Bristow, 2016). The Mackworth multichannel test, which was first proposed by Mackworth & Mackworth (1956), requires participants to match cards based on how many symbols on each

card were matching and was used to examine decision making. However, as previously mentioned decision making is a broad cognitive process, relying on other cognitive processes. While the findings from Vlasak (1969) and Woodhead (1959) do not suggest a specific cognitive process that is affected by a startle, the adverse effects on cognitive performance are clear. Performance decrements have also been shown in motor tasks (Vlasak, 1969; Thackray and Touchstone, 1970; May and Rice, 1971; Foss et al., 1989) and some limited research has shown that these motor decrements can be mitigated following the presentation of a prepulse prior to the startling stimulus (Foss et al., 1989; Bui et al., 2024). Specifically, the largest decrements in motor tasks have been observed in the initial 2-3 seconds following a startling stimulus (Vlasak, 1969; Thackray and Touchstone, 1970; May and Rice, 1971), with residual effects persistent up to 10 seconds following a startling stimulus (Thackray and Touchstone, 1970). Therefore, it is possible that cognitive performance decrements attributable to the startle reflex may also be mitigated by presenting a prepulse.

6. Prepulse Inhibition of Startle

One of the ways that the startle reflex can be modulated is through the phenomenon of prepulse inhibition. This is accomplished by delivering a weak, non-startling stimulus (called a 'prepulse') shortly prior (15 - 400 ms) to the startling stimulus (Graham, 1975). Presenting a prepulse prior to a startling stimulus has been shown to reduce the amplitude of the startle reflex (Graham, 1975). This modulation of the startle reflex has been termed prepulse inhibition (PPI) of the startle reflex (Valls-Solé et al., 2008). The presentation of a prepulse has been shown to not only reduce the amplitude of the startle reflex but also the perceived intensity of the startling stimulus (Heathcote et al., 2024). This reduction of the startle reflex has been attributed to sensory gating (Braff et al., 2001; Gómez-Nieto et al., 2020) which is an automatic process in

which the nervous system filters out (or "gates") irrelevant sensory information, allowing only relevant information to pass through to higher order conscious processing (Cromwell et al., 2008). The underlying mechanism that regulates the process of PPI is hypothesized occur at the brainstem (Fulcher et al., 2020). Here, the pedunculopontine tegmental nucleus receives sensory input, then sends inhibitory projections to the nRPC (i.e., the startle reflex generating apparatus). Inhibition of the nRPC limits the processing of any following stimuli such as a SAS (Fulcher et al., 2020). The dampening of the startle reflex is due to the nervous systems use of sensory gating to prioritize the processing of the prior prepulse stimulus and limit the interruption of ongoing processing (Graham, 1975).

While PPI can modulate the startle reflex, manipulation of prepulse properties such as duration, interstimulus interval (ISI), and intensity have been examined to determine what leads to the most effective inhibition. A study by Blumenthal and colleagues (1995) examined the effect of prepulse duration on PPI and demonstrated that the greatest magnitude of inhibition was obtained with a prepulse duration of 20 ms. Further research into the ISI between prepulse and startling stimulus revealed that the greatest startle reflex inhibition occurred with an ISI of 120ms (Graham, 1975; Braff et al., 1978). Finally, it has been shown that a greater probability of prepulse inhibition occurs with increasing prepulse intensity (Blumenthal, 1996) – up to a point. Specifically, Blumenthal found increased startle inhibition when using an auditory prepulse of 70 dB compared to a 60 dB prepulse, while Maslovat and colleagues (2012) showed that an 80 dB prepulse also produced a robust inhibitory effect. However, higher intensities (e.g. 90 dB or greater) have been shown to be less effective in producing prepulse inhibition (Swerdlow et al. 2007).

7. Research Question

While the effects of startle on reaction time have been widely investigated (see Carlsen and Maslovat 2019 for a review), there is little research examining whether the use of a prepulse can mitigate or eliminate cognitive and/or motor performance decrements caused by the startle reflex. One study that examined this was conducted by Foss et al., (1989) examining the disruption of a rifle aim by a startling acoustic stimulus along with the mitigating effects of prepulse. In a series of experiments, they found that the presentation of an 80 dB prepulse prior to the startling stimulus resulted in a two thirds reduction in error compared to just the startling stimulus alone (Foss et al., 1989). More recent evidence from Bui and colleagues (2024) showed a significant decrease in tracking error induced by a startle during a continuous motor tracking task when a prepulse was delivered prior to a SAS. Additionally, the data from Bui and colleagues (2024) also provided initial evidence that the prepulse also mitigated the duration of decrements attributable to the startle reflex. While these results demonstrate the effectiveness of a prepulse at mitigating the adverse effects of startle on performance, the tasks used in both experiments were motor tasks. There remains the question of whether similar effects can be observed on cognitive task performance when a prepulse is delivered prior to a SAS. While it has been previously demonstrated that a startle leads to decrements in cognitive processes and cognitive task performance, it is currently unknown whether prepulse inhibition can be used to mitigate the startle reflex's effect on these cognitive processes. The present experiment will look to address this question by examining continued performance of a cognitive task while delivering acoustic stimuli including both SAS alone and a SAS preceded by a prepulse stimulus.

## Chapter 2: Research Article

# An acoustic prepulse diminishes the probability of observing a startle reflex but seemingly exacerbates cognitive performance decrements when a startle reflex occurs.

Alex Bui and Anthony N. Carlsen

School of Human Kinetics, University of Ottawa, Ottawa, Canada

1. Introduction

In everyday life humans are required to perform a wide variety of movements, some of which require a high degree of precision and accuracy. These movements play an integral part in our lives from recreational activities like kicking a soccer ball to driving a car. However, unexpected events can play a role in how these movements are carried out. For example, a pedestrian unexpectedly jumping out onto the road in the path of your car may cause a startle reflex, which in turn could have an influence on performing driving tasks. While the consequences of being startled are typically not severe, when occurring during specific scenarios (e.g., driving a car), they can cause catastrophic consequences.

The startle reflex is a protective physiological response that occurs following the presentation of an intense, unexpected stimulus (Yeomans & Frankland, 1995). It can be described as a diffuse whole-body generalized flexion response consisting of closing of the eyes, followed by the shoulders curling forward and up, and the shortening of the torso (Yeomans & Frankland, 1995) (Landis, Hunt, & Strauss, 1939). Experimentally, the startle reflex can be elicited through various stimulus modalities (Blumenthal et al., 2005; Yeomans & Frankland, 1995); however, the most commonly used is the startling acoustic stimulus (SAS) (Carlsen et al., 2011). The pathway by which a SAS elicits a startle reflex begins at the cochlear nucleus. Incoming sensory input is sent from the cochlear nucleus to the large neurons of the nucleus reticularis pontis caudalis (nRPC). These neurons then conduct along the spinal cord, descending through the reticulospinal tract activating motoneurons via axons near the medial longitudinal fasciculus, bypassing the auditory cortex where auditory stimuli would be processed, thus allowing for the rapid generation of the startle reflex (14-151 ms) (Yeomans and Frankland, 1995). This activation produces the muscular contractions associated with the startle reflex

which can then be measured using electromyography (EMG) of key muscles, including the sternocleidomastoid (SCM) and orbicularis oculi (OOc).

One of the ways that the startle reflex can be modulated is through the phenomenon of prepulse inhibition. This is accomplished by delivering a weak non-startling stimulus (called a "prepulse") shortly prior (15 - 400 ms) to the startling stimulus, which has been shown to reduce the amplitude of the startle reflex (Graham, 1975). This modulation of the startle reflex has been termed prepulse inhibition (PPI) of startle (Valls-Solé et al., 2008). In addition to reducing the amplitude of the startle reflex the presentation of a prepulse has also been shown to decrease the perceived intensity of the startling stimulus (Heathcote et al., 2024). This reduction of the startle reflex has been attributed to sensory gating (Braff et al., 2001; Gómez-Nieto et al., 2020), an automatic process in which the nervous system filters out (or "gates") irrelevant sensory information, allowing only relevant information to pass through to higher order processing (Cromwell et al., 2008). The underlying mechanism that regulates the process of PPI of startle is hypothesized occur at the brainstem (Fulcher et al., 2020). Here, the pedunculopontine tegmental nucleus receives the sensory input related to the prepulse, then sends inhibitory projections to the nRPC (i.e., the startle reflex generating apparatus). Inhibition of the nRPC limits the processing of any following stimuli such as a SAS (Fulcher et al., 2020). By limiting the processing of the SAS this leads to a reliable decrease in the amplitude (attenuation) of the startle reflex. This reduction of the startle reflex by prepulse is thought to be due to the nervous system's use of sensory gating to prioritize and protect the processing of the initial prepulse stimulus and limit the interruption of ongoing processing (Graham, 1975). The introduction of prepulse as a form of modulation of the startle reflex presents an avenue to investigate the startle reflex and its effects

on numerous different processes. One body of research focuses on the relationship between startle and task performance.

The majority of research examining the effect of startle on human performance has mainly centered around motor tasks. Several studies have shown large performance decrements in manual tracking tasks in the initial 2-3 seconds following a startling stimulus (Vlasak, 1969; Thackray and Touchstone, 1970; May and Rice, 1971), with residual effects persisting up to 10 seconds following a startling stimulus (Thackray and Touchstone, 1970). A more limited amount of research has indicated that these motor decrements may be at least partially mitigated if a prepulse precedes the startling stimulus (Foss et al., 1989; Bui et al., 2024). Although it is understandable that motor performance may be impacted by the startle reflex, similar detrimental effects have also been seen in mental performance.

While the startle reflex is typically thought of as a subcortically-mediated response resulting in whole-body physical effects, there is evidence that the startle reflex can also have some cognitive effects at the cortical level. Previous literature has shown performance decrements in mental subtraction (Vlasak, 1969) and card matching tasks (Woodhead, 1959). However, few studies have investigated the potential of a prepulse to alter these cognitive effects. One recent study by Heathcote and colleagues (2024) examined the effect of prepulse on the startle reflex often induced by intense “call alerts” in emergency medical services. While they found a reduction in the amplitude of the startle reflex caused by the call alert with the addition of a prepulse, they also demonstrated that the addition of a prepulse prior to the call alert stimulus resulted in a reduction in perceived loudness of the call alert, as well as a reduction of dislike for the stimulus. These findings suggest that the startle reflex, and subsequent modulation

by prepulse have more than just a subcortical and physical response effect. They demonstrate the ability for prepulse to also modulate some of the cognitive effects of the startle stimulus.

The purpose of the present experiment was to investigate the effect of startle on a cognitively demanding mental performance task, as well as to determine whether the use of a prepulse would be effective at mitigating any changes in cognitive performance following a startling stimulus. It was hypothesized that the presentation of an intense stimulus capable of eliciting a startle reflex would result in cognitive performance decrements, as previously observed (Vlasak, 1969; Woodhead, 1959). Additionally, it was hypothesized that the addition of a prepulse would reduce the magnitude of the startle reflex, and reduce the impact of the SAS on cognitive performance.

## 2. Methods

### 2.1 Participants

A total of 21 participants were recruited to participate in this study, but one participant withdrew partway through data collection and the data were discarded. Thus, data from 20 participants (10F/10M; Mean age 24.7 years, SD = 7.4) were used for analysis. A power analysis completed a priori using G*Power (Faul, Erdfelder & Buchner, 2007) with an effect size d = 1.24 (Woodhead in 1959) indicated a sample size of 8 was required to observe a cognitive performance difference of this magnitude (alpha = .05, beta = .8) following a startle. Inclusion criteria for participants included normal or corrected-to-normal vision, and no history of sensory, motor, or cognitive disfunctions. Written informed consent was obtained from participants. All testing procedures were reviewed and approved by the University of Ottawa's Health Sciences and Science Research Ethics Board and adhere to the 2024 revision of the Declaration of Helsinki.

## 2.2 Apparatus & Task

Participants sat at a table facing a 24-inch LCD computer monitor (Asus VG248QE; 144 Hz refresh) placed approximately 50 cm in front of them, with a standard computer keyboard positioned on the table centered with the participant for recording keypress responses. Participants began with their left and right index fingers positioned over the Z and M keys, respectively. Participants performed a continuous 2-back task, requiring them to press the M key on correct 2-back targets, and Z key on non-targets (see below).

## 2.3 Experimental Procedure

Participants completed twelve 2.5-minute experimental blocks of the continuous 2-back task. At the beginning of each block a blank grey screen was shown on the computer monitor with a black fixation cross displayed centrally. All blocks began with the participant moving their index fingers onto the Z and M keys. Participants were then asked if they were ready to begin, and upon confirmation, the block was initiated by the researcher. The fixation cross then disappeared, and following a 3 s delay the first stimulus of the 2-back task was presented. During the task, the numerals 1-9 (excluding the number 7) were presented on screen for 1500ms with an interstimulus interval (ISI) of 500ms. The integers 1-9 were chosen for simplicity, with seven being excluded as it is the only two-syllable number, potentially increasing the demand of encoding 7 into working memory. The stimulus duration and ISI allowed a total of 75 stimuli to be presented throughout each 2.5 min block. Participants pressed the M key if the number on screen was the same as the number presented two stimuli earlier (2-back target) and pressed the Z key if the number on screen was different than the number shown two stimuli earlier (non-target). The frequency of targets (2-back repeats) was set at 33%, for a total of 25 targets per

block with the remaining 50 stimuli being non-targets. The numbers were presented in large black text (approximately 3 cm tall) inside of a 5.5 cm square white box presented in the middle of the grey screen. A customized program written in LabView (National Instruments Inc.) controlled the stimulus presentation and timing.

During each block three different acoustic stimuli were presented, with one occurring every 45-50s. The order of the stimulus presentations within each block was randomized and counterbalanced. The three acoustic stimulus conditions were: 1) An acoustic "control" stimulus (80dB, 1000 Hz tone, 25 ms); 2) A startling acoustic stimulus (SAS; 120 dB, broadband noise, 25 ms); and 3) A prepulse+SAS (PP+SAS) which consisted of the 25ms tone (80dB) presented 100 prior to the 25 ms SAS. The presentation of the primary acoustic stimulus was timed such that it always occurred 200ms following the appearance of a visual stimulus (i.e., number) on screen. In the case of the PP+SAS, the stimulus was timed such that the SAS occurred 200 ms following the visual stimulus onset. Acoustic stimuli were generated using a digital to analog converter (National Instruments PCIe-6321), which were then amplified and presented using a loudspeaker (M54-H, MG Electronics, Inc., Hauppauge, NY) placed 30 cm behind the participant's head. The intensities of the auditory stimuli were confirmed using a precision sound level meter (Cirrus Research Optimus, CR:162C; A-weighted, impulse setting). Prior to the first testing block, participants performed a 2-minute practice/familiarization trial that included two auditory control stimuli presented randomly across the 2 minutes. Participants were instructed that the acoustic stimuli were irrelevant to the task. The total duration of data collection was approximately 45 minutes per participant.

## 2.4 Recording Equipment

A Delsys Trigno (Delsys Inc.) wireless EMG system was used to collect startle reflex-related surface EMG data from the muscle bellies of the right and left sternocleidomastoid (SCM) and orbicularis oculi (OOc). Before applying the EMG sensors, the recording sites were cleaned to minimize electrical impedance using an abrasive gel (Nuprep) and alcohol wipes. Delsys Avanti Duo sensor electrodes were then placed parallel to the muscle fibres and attached to the skin using double-sided adhesive tape. Heart rate was collected using a Delsys Avanti EKG sensor. Adhesive EKG electrodes (3M 2560 Red Dot) were placed under the pectoral muscles and the sensor leads were attached to the electrodes using standard snap connectors. The EKG sensor was attached to the trunk below the electrodes using double sided adhesive tape. EMG and heart rate data were sampled at 2000 Hz by the Delsys Trigno base station. Acoustic stimulus data were sampled at 2000 Hz using a National Instruments analog to digital converter (PCIe-6321). Keypress responses were sampled at 100 Hz. All data were collected using a customized LabVIEW program (National Instruments Inc.) which synchronized the EMG, heart rate, acoustic stimulus, and keypress data. Data collection was initiated by the computer program at the beginning of each block and continued for 2.5-minutes.

## 2.5 Dependent Measures & Error Treatment

The dependent measures of interest can be separated into two categories, task performance measures, and startle reflex measures. The two task performance measures that were collected and analysed were response accuracy and response time (latency). Accuracy was measured as correct/incorrect responses to visual stimuli (correct rejections of non-2-back targets, and correct acceptances of 2-back targets). Within participants, mean accuracy was compared between the 15 responses around the onset of the acoustic stimulus: 5 responses preceding the stimulus and 10

responses subsequent to the stimulus. Mean accuracy was computed for each particular response number with respect to the acoustic stimulus onset across blocks for each acoustic stimulus type (e.g., accuracy for the first response following a SAS was calculated as the mean accuracy on the first response following a SAS across all 12 blocks). A response was deemed to be incorrect if a non-target response was made when a 2-back target was presented, and vice versa. Additionally, responses were deemed incorrect if no response was made within 2000 ms (a missed response).

Response time was calculated as the time between when the visual stimulus was presented and when a keypress response was detected. Similar to accuracy, response times were captured for analysis from the 5 responses preceding and 10 responses following an acoustic stimulus. When the participant failed to make a response within the 2000 ms window for a specific stimulus, a response time of 2000 ms was assigned.

EMG activity in the SCM and OOc muscles as well as heart rate were collected to characterize the startle reflex. EMG activity in the SCM was examined to detect whether a startle reflex has occurred, as well as its amplitude. Analysis of EMG in SCM (rather than OOc) was chosen as it serves as a better startle indicator and eliminates any false positive startle reflexs seen in OOc due to the auditory blink reflex. First, for comparison to previous studies, EMG amplitude in the left and right SCM muscles was computed irrespective of the observed presence of a startle reflex related burst by numerically integrating the raw rectified EMG in non-overlapping 10 ms bins for 150 ms following the onset of each of the acoustic stimuli. Second, a startle reflex in SCM was deemed to have occurred if a burst of EMG in SCM was detected between 20 ms and 120ms following the SAS. The presence of a burst of EMG in SCM as well as its onset latency was assessed by displaying the EMG pattern on a computer monitor and superimposing a marker onto the EMG pattern if rectified and filtered (using a zero-lag 25 Hz

low-pass elliptic filter) EMG activity increased to more than two standard deviations above baseline (i.e., the mean of 100ms of EMG activity preceding the acoustic stimulus) and remained elevated for at least 20 ms. The position of the marker was then manually verified and adjusted to the point at which activity first increased as necessary (see Carlsen et al., 2011 for a detailed review). EMG amplitude of an identified burst SCM was quantified as 1) the maximum value of the rectified and filtered EMG in a 100 ms window following onset, and 2) by numerically integrating the raw rectified EMG for a period of 100 ms following EMG onset. Heart rate data was examined using a moving average of 3 seconds of heart rate data (starting at the specific second), from 10 sec prior (i.e. 5 responses) to an acoustic stimulus to 20 seconds after a stimulus. Heart rate data was normalized for each participant as a percentage of the average of the first 3 heart rate measurements in each 30 s interval.

Data were discarded if a response was made prior to, or in less than 50 ms following a visual stimulus (anticipation, 5 trials), or if a data collection error occurred (1 trial), resulting in retention of 714/720 trials (99.2%) for analysis. Heart rate data were visually inspected, and trials were additionally discarded from heart rate analysis if the heart rate was not correctly captured (62 trials), resulting in retention of 652/720 (90.6%) of trials for heart rate analysis.

### 2.6 Statistical Analysis

Analysis of the proportion of trials on which a startle reflex was observed compared between startle and prepulse conditions was examined using a paired t-test. Analysis of EMG amplitude in SCM was initially analysed irrespective of whether a startle reflex was observed by examining the first 150 ms following an acoustic stimulus in 10 ms bins. A generalized linear mixed effect model was used to analyze integrated EMG as a function of time bin (15 levels), acoustic stimulus type (3 levels: Control, SAS, PP++SAS), and SCM side (2 levels: right and

left) as a control variable. Random intercepts were specified for participants, and the model used a Gamma distribution for the residuals with a log link function (e.g., Integrated EMG ~ timeBin * Stimulus + (1 | Participant), family = Gamma(link="log")). A Gamma model was chosen because integrated SCM amplitudes exhibited strong positive skew.

Startle reflex EMG amplitude was further examined by including only trials where a startle reflex was detected in either SCM. The amplitude quantified as the integrated area of the raw rectified EMG in the 100 ms following a detected onset in SCM ($Q_{100}$) in response to a SAS or PP+SAS. $Q_{100}$ was analysed using a generalized linear mixed effects model with acoustic stimulus type (SAS, PP+SAS) and side (right, left) as fixed factors with random intercepts specified for participants.

Normalized heart rate was also analyzed irrespective of whether a startle reflex was observed in SCM using a linear mixed effect model with time bin and acoustic stimulus type as fixed factors, and random intercepts specified for participants. To determine if any differences in normalized heart rate were present based on whether a startle reflex was observed (SCM+) or not (SCM-), data were first analysed from SAS or PP+SAS trials that were categorized as SCM- along with control trials. Following this, data from SAS and PP+SAS trials only, categorized as SCM+ or SCM- were analysed together. In both cases normalized heart rate was analysed using linear mixed effects models with time (15 levels) and acoustic stimulus type (3 or 2 levels) as fixed factors and random intercepts specified for participants.

Mean accuracy and response time were also analysed in a similar manner as heart rate. First, irrespective of whether a startle reflex was observed, then by separating the data by whether a startle reflex was observed, and then separate based on presence or absence of SCM activity (SCM+/SCM-). Accuracy and response time were analysed using linear mixed effects

models with response number (15 levels) and acoustic stimulus type (2 or 3 levels) as factors, with random intercepts specified for participants.

Data analysis of the dependent measures mentioned above was performed using R statistical software (R Core Team, 2020) and significance value set at $p < 0.05$ for all tests. Post-hoc pairwise comparisons were performed in R (R Core Team, 2020) using the emmeans function with the Benjamini-Hochberg method to control for the false discovery rate between multiple comparisons.

## 3. Results

### *3.1 Startle Reflex Measures*

#### *3.1.1 Proportion of SAS trials with SCM*

The proportion of trials where a startle reflex was observed in either the left or right SCM is presented in Figure 1. A paired t-test confirmed the proportion of trials with an observed startle reflex was significantly diminished (17.73%) when a prepulse stimulus preceded the SAS as compared to the SAS alone, $t(19) = 3.324$, $p = 0.004$.

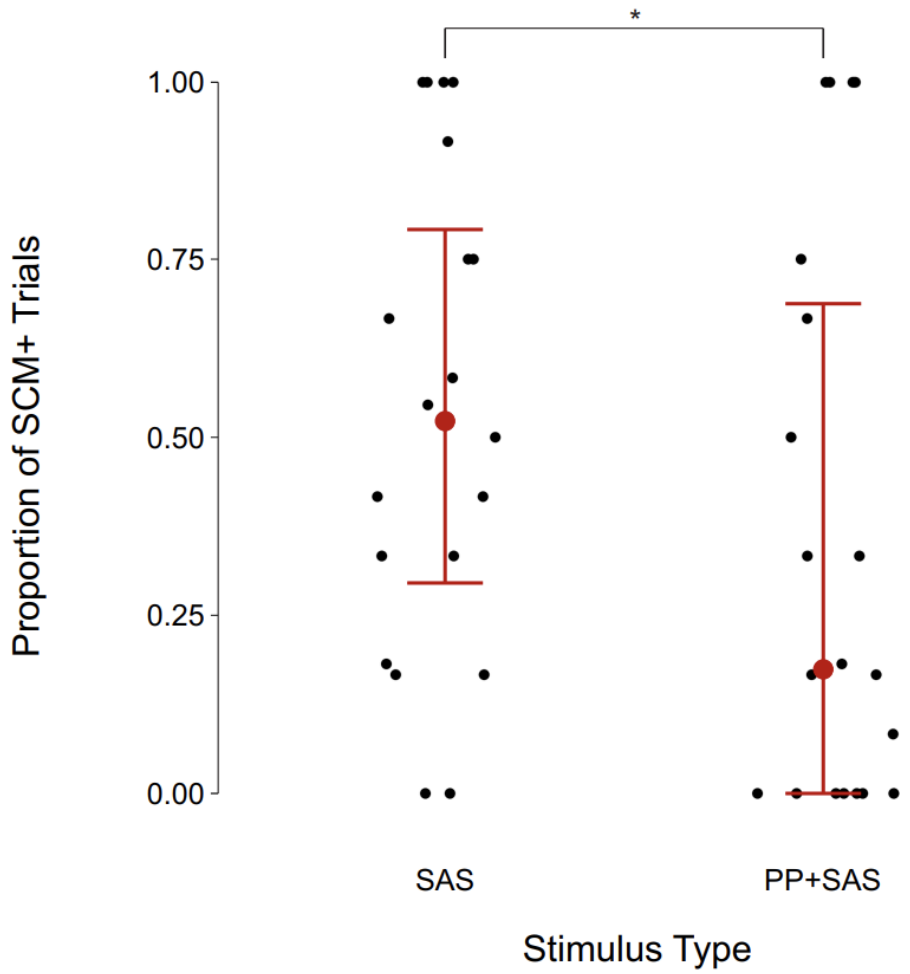


Figure 1. The proportion of startle reflexes observed in either right or left SCM following a SAS or PP+SAS. Large markers and error bars represent medians and upper / lower quartiles, while small markers indicate individual participant values.

*3.1.2 Amplitude of startle reflex in SCM*

The integrated EMG in SCM in the 150 ms following an acoustic stimulus was calculated in 10 ms bins for 150 ms irrespective of whether a startle reflex was observed (Figure 2). A significant interaction effect between time bin and acoustic stimulus type was observed $X^2(28) = 319.511$, $p < .001$. Pairwise comparisons revealed that compared to Control, iEMG was significantly larger at all times after 30 ms following a SAS (p-values < 0.006), and significantly larger following a PP+SAS at all times after 50 ms (p-values < 0.001). In addition, significant

differences in iEMG between the SAS and PP+SAS conditions were observed at times 90-120 ms whereby SAS showed larger iEMG than SAS+prepulse (p-values < 0.045).

On SAS or PP+SAS trials where a startle reflex was observed, integrated EMG in the 100 ms following EMG onset ($Q_{100}$) of both right and left SCM was calculated (Figure 3). On these trials $Q_{100}$ showed no significant differences based on acoustic stimulus type $X^2(1) = 0.127$, $p < .001$, $p = 0.722$ or side $X^2(1) = 0.007$, $p = 0.933$.

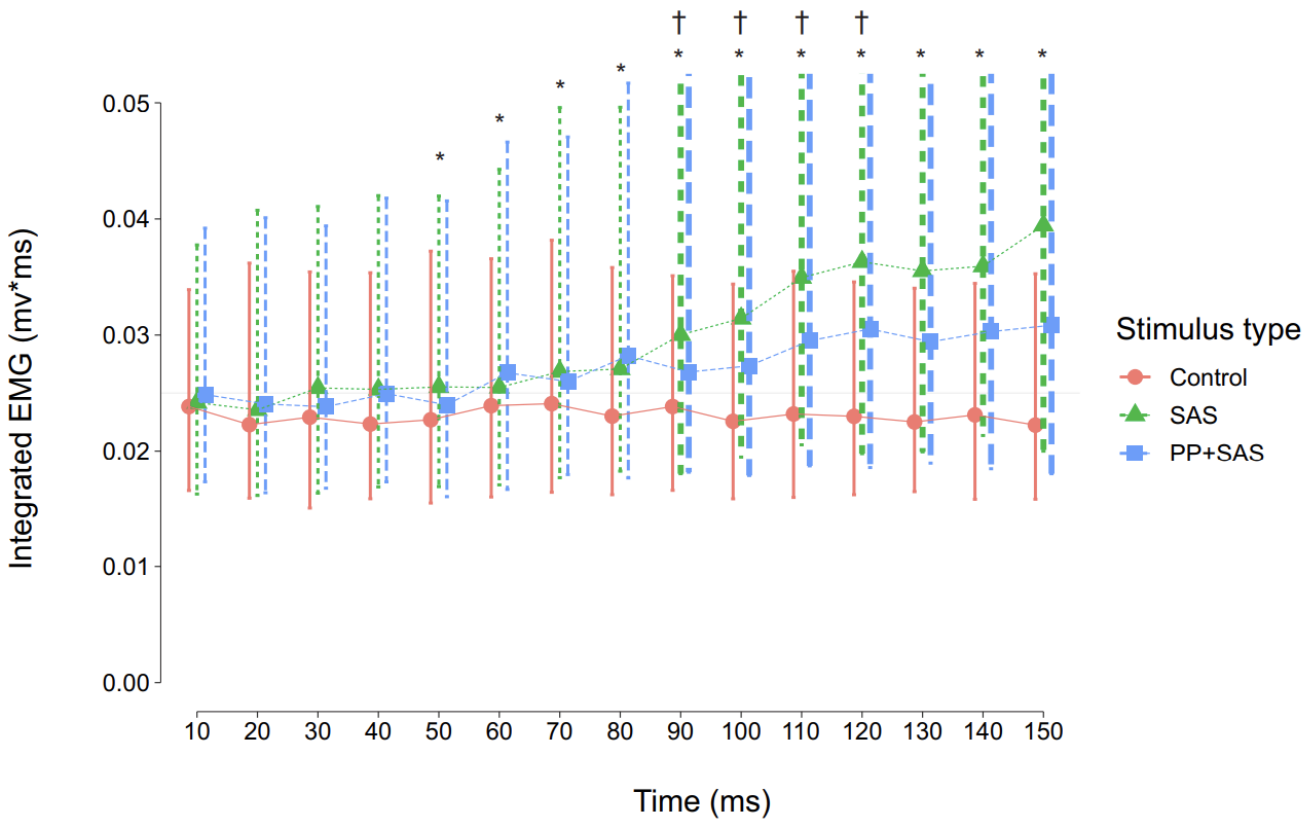


Figure 2. Integrated area of EMG (mV*ms) in SCM in the three acoustic stimulus conditions irrespective of whether a startle reflex was observed in the 150 ms following the stimulus. Note that data were integrated in 10-ms non-overlapping windows and that data are collapsed across the left and right side SCM. Note that (*) indicates significant differences between Control and SAS, and Control and PP+SAS conditions. Note that (†) indicates significant differences between SAS and PP+SAS conditions.

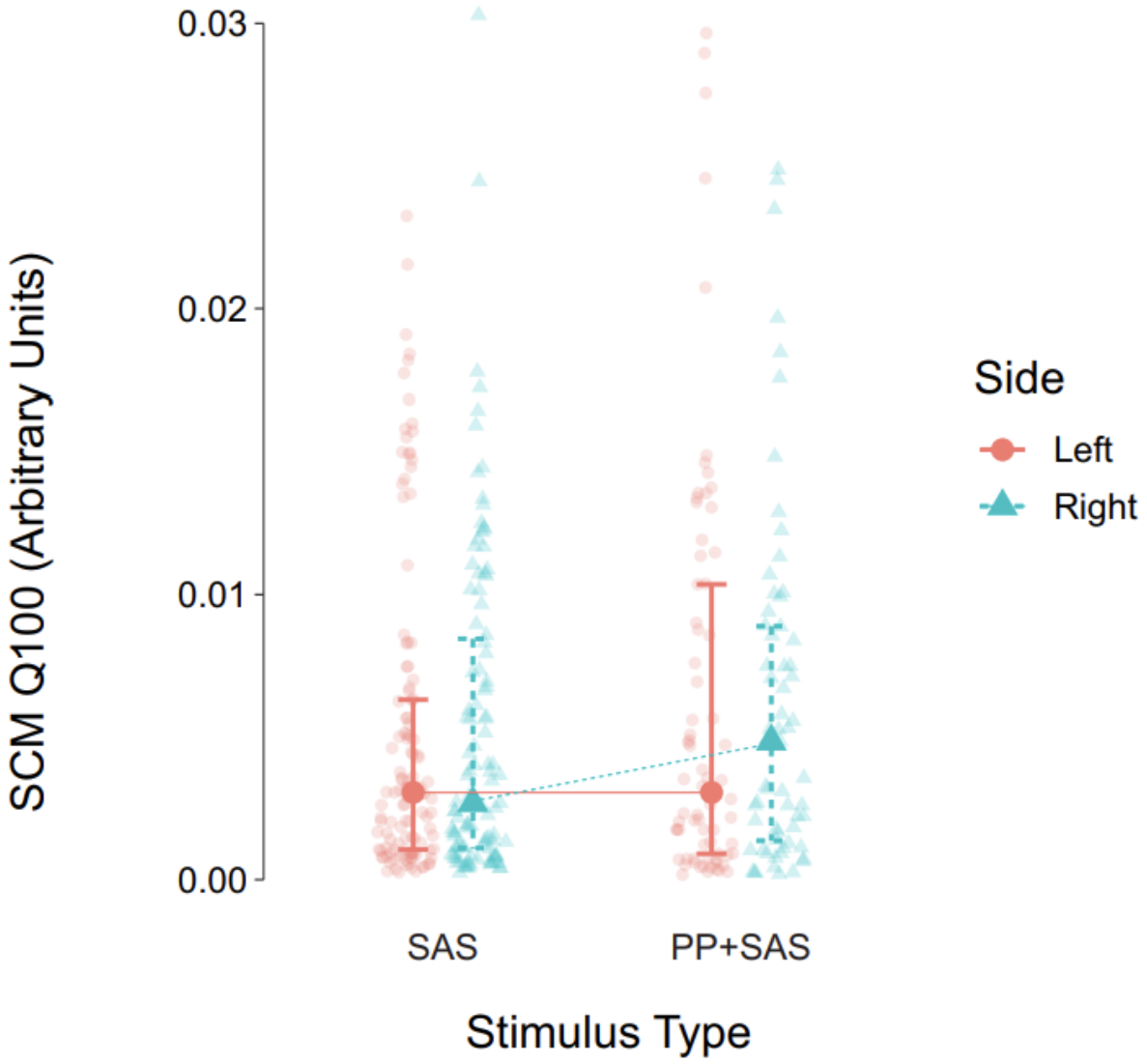


Figure 3. Integrated area of EMG activation in the 100 ms following an acoustic stimulus in the right and left SCM in SAS and PP+SAS trials where a startle reflex was observed. Markers and error bars represent medians and upper / lower quartiles.

*3.1.3 Heart Rate*

Normalized heart rate across the five responses prior and nine responses after an acoustic stimulus was examined irrespective of whether a startle reflex was observed (Figure 4). A significant interaction of acoustic stimulus type by response number effect was observed $F(26, 8978.5) = 2.363$, $p < 0.001$. Pairwise comparisons revealed that at responses 0 and 1 following

the acoustic stimulus, heart higher was higher following a SAS as compared to Control (3.48% and 2.84% respectively) and PP+SAS (2.34% and 2.19% respectively).

In control trials as well SAS and PP+SAS trials where no startle reflex was observed, normalized heart rate was analyzed across the five responses prior and nine responses following an acoustic stimulus (Figure 5). A significant effect of acoustic stimulus type was observed $F(2, 5760.3) = 8.540$, $p < 0.001$. Pairwise comparisons revealed a statistically significant difference in heart rate between Control and PP+SAS conditions. The mean difference in heart rate between Control and PP+SAS was 0.823% of normalized heart rate.

Normalized heart rate across the five responses prior to, and nine responses following an acoustic stimulus, was then analyzed for only SAS and PP+SAS trials, separated based on whether a startle reflex was observed in SCM (Figure 6). A significant interaction between response number and SCM presence was observed $F(13, 5887.2) = 2.905$, $p < 0.001$. Pairwise comparisons revealed that heart rate was larger at response numbers 0-5 following an acoustic stimulus when a startle reflex was observed (collapsed across acoustic stimulus type). There was also a significant interaction between acoustic stimulus type and SCM presence $F(1, 5904.1) = 5.142$, $p = 0.0234$. Pairwise comparisons revealed a difference within the SAS condition with higher heart rate (1.734%) on SCM+ trials compared to SCM- trials ($p < .001$). Similarly, within the prepulse condition, a higher heart rate was observed (0.887%) on SCM+ compared to SCM- trials, $p = 0.012$. Finally, a difference was observed across acoustic conditions when a startle reflex was observed. Specifically, heart rate was lower (1.351%) when a startle reflex was observed in the PP+SAS condition compared to SAS, $p < .001$.

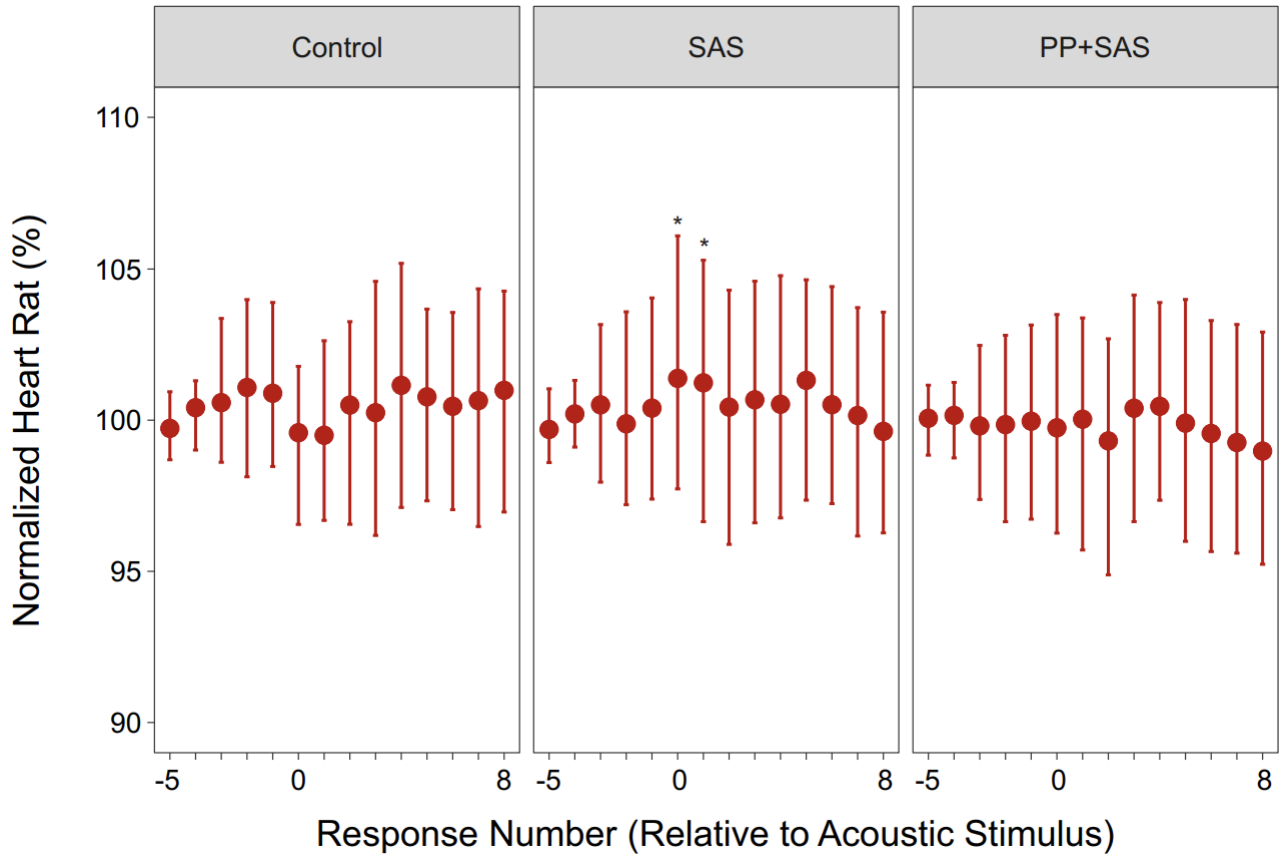


Figure 4. Heart rate (normalized to baseline for each participant), across the five responses prior to, and nine responses following an acoustic stimulus (response 0), regardless of whether a startle reflex was observed in SCM. Values represent 3 s moving average heart rate centred on each response time. Markers and error bars represent medians and upper / lower quartiles.

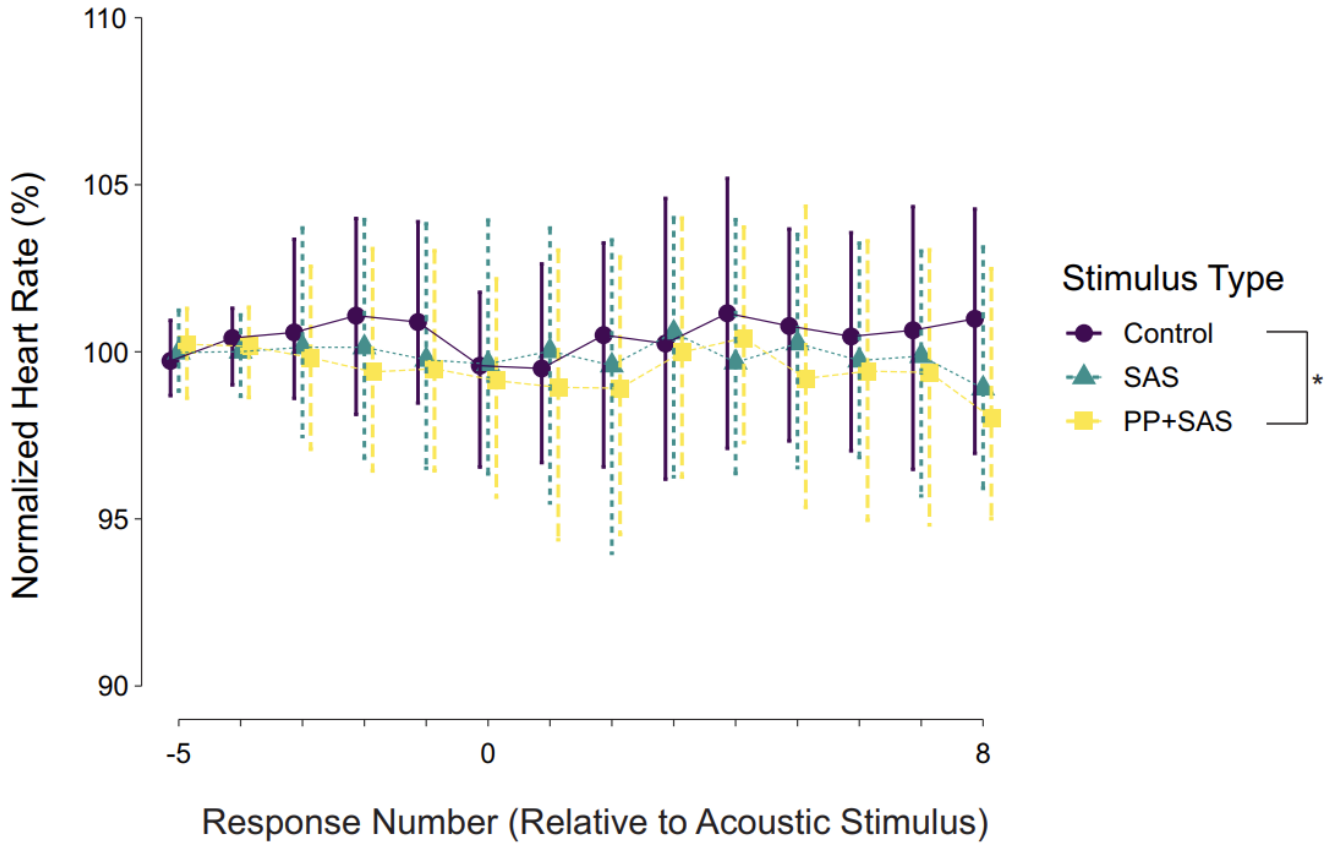


Figure 5. Normalized heart rate across the five responses prior to, and nine responses after an acoustic stimulus across all acoustic stimulus types when no startle reflex was observed. Values represent 3 s moving average heart rate centred on each response time. Markers and error bars represent medians and upper / lower quartiles.

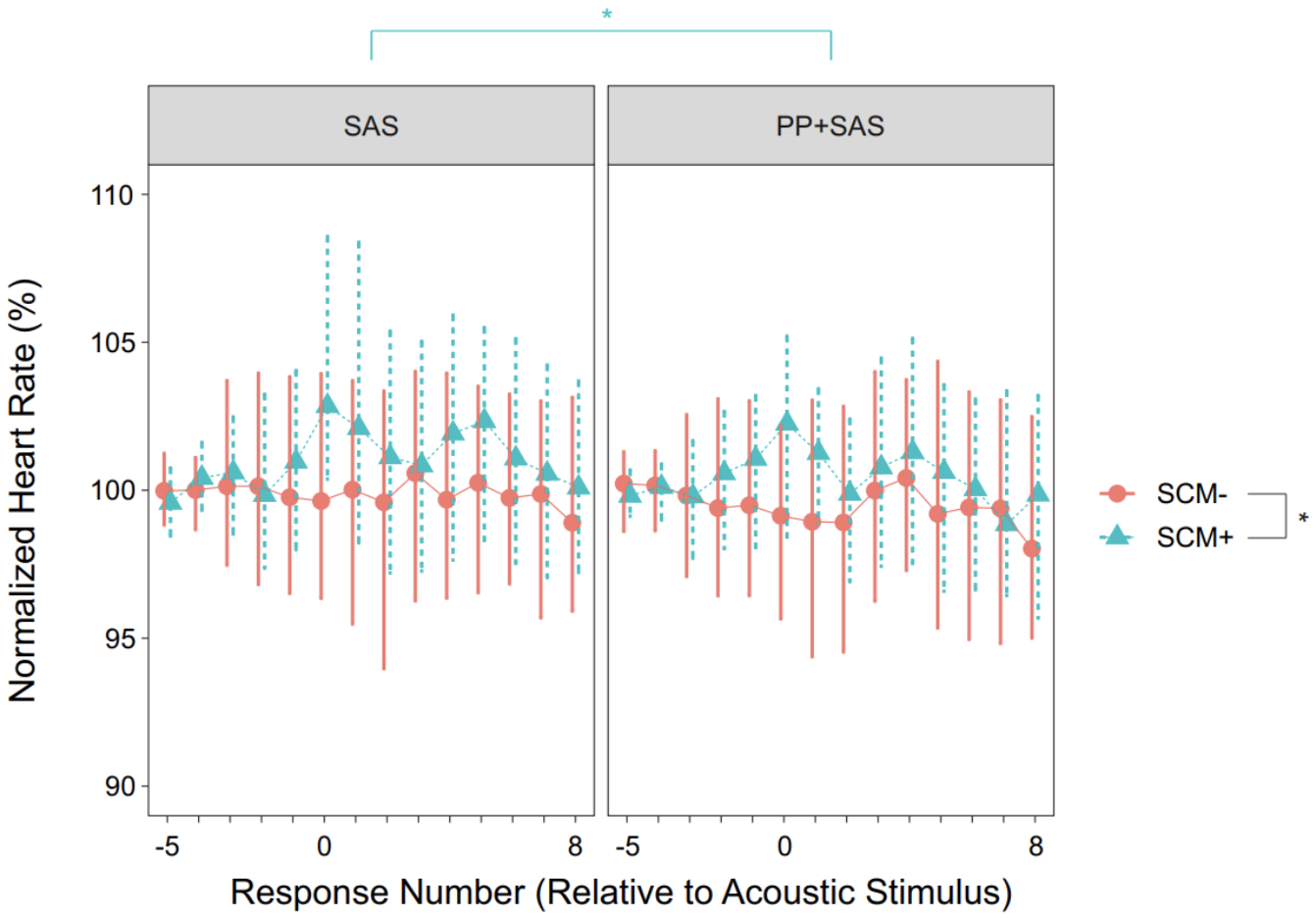


Figure 6. Normalized heart rate across the five responses prior to, and nine responses after an acoustic stimulus across SAS and PP+SAS stimulus types separated based on whether a startle reflex was observed in SCM (SCM+) or not (SCM-). Values represent 3 s moving average heart rate centred on each response time Markers and error bars represent medians and upper / lower quartiles.

### *3.2 Cognitive Performance Measures*

#### *3.2.1 Accuracy*

Mean accuracy was calculated as a mean proportion of correct responses at each response across the twelve blocks for the five responses prior to and ten responses subsequent to an acoustic stimulus irrespective of whether a startle reflex occurred, and are presented in Figure 7. A significant effect of response number was observed $F(14, 1181.3) = 3.720$, $p < .001$. Pairwise

comparisons revealed that accuracy of response number 1 was significantly lower than the five responses prior to acoustic stimulus (-5 to -1), and last seven responses (3-9).

Analysis of accuracy on Control trials and SAS and PP+SAS trials where no startle reflex was observed was further analyzed, and shown in Figure 8. A significant effect of response number was observed $F(14, 716.51) = 2.063$, $p = 0.012$. Pairwise comparisons revealed that mean accuracy on response number 1 following an acoustic stimulus was significantly lower than on responses -5 (7.73%), -4 (8.79%), -1 (9.17%), and 4 (7.76%), 5 (8.61%), 8 (8.16%), 9 (8.04%) (p-values < .05).

Mean accuracy of only SAS and PP+SAS trials, separated on whether a startle reflex was observed in SCM is shown in Figure 9. A significant interaction between SCM presence and acoustic stimulus type was observed $F(1, 872.25) = 11.252$, $p < 0.001$. Pairwise comparisons revealed that a significant difference within the PP+SAS condition where accuracy was higher (7.81%) when no startle reflex was observed ($p < .001$). Additionally, when a startle reflex was observed, accuracy was higher (5.04%) on SAS trials compared to PP+SAS ($p = .001$). A significant main effect of response number on accuracy was also observed $F(14, 866.51) = 4.123$, $p < .001$. Pairwise comparisons revealed accuracy on response numbers 0 & 1 following the acoustic stimulus was significantly lower as compared to response pre-acoustic stimulus (-5 to -1), and responses 4-9. A very near significant ($p = 0.0500546$) three-way interaction of response number, SCM presence, and acoustic stimulus type was observed.

A secondary analysis of mean accuracy irrespective of whether a startle reflex was observed or not was plotted across all three acoustic stimulus types separated based on 2-back target or non-target, or correct keyboard response (Figure 10). No significant difference was observed in keyboard response $X^2(1) = 0.0854$, $p = .7701$. A significant difference of acoustic stimulus type

was observed $X^2(2) = 19.2880$, $p < .0001$. Pairwise comparisons revealed that mean accuracy was higher (1.6320% and 1.7318% respectively) in the Control condition compared to both SAS, $t(97) = 3.686$, $p = .0011$, and PP+SAS $t(97) = 3.911$, $p = .0005$.

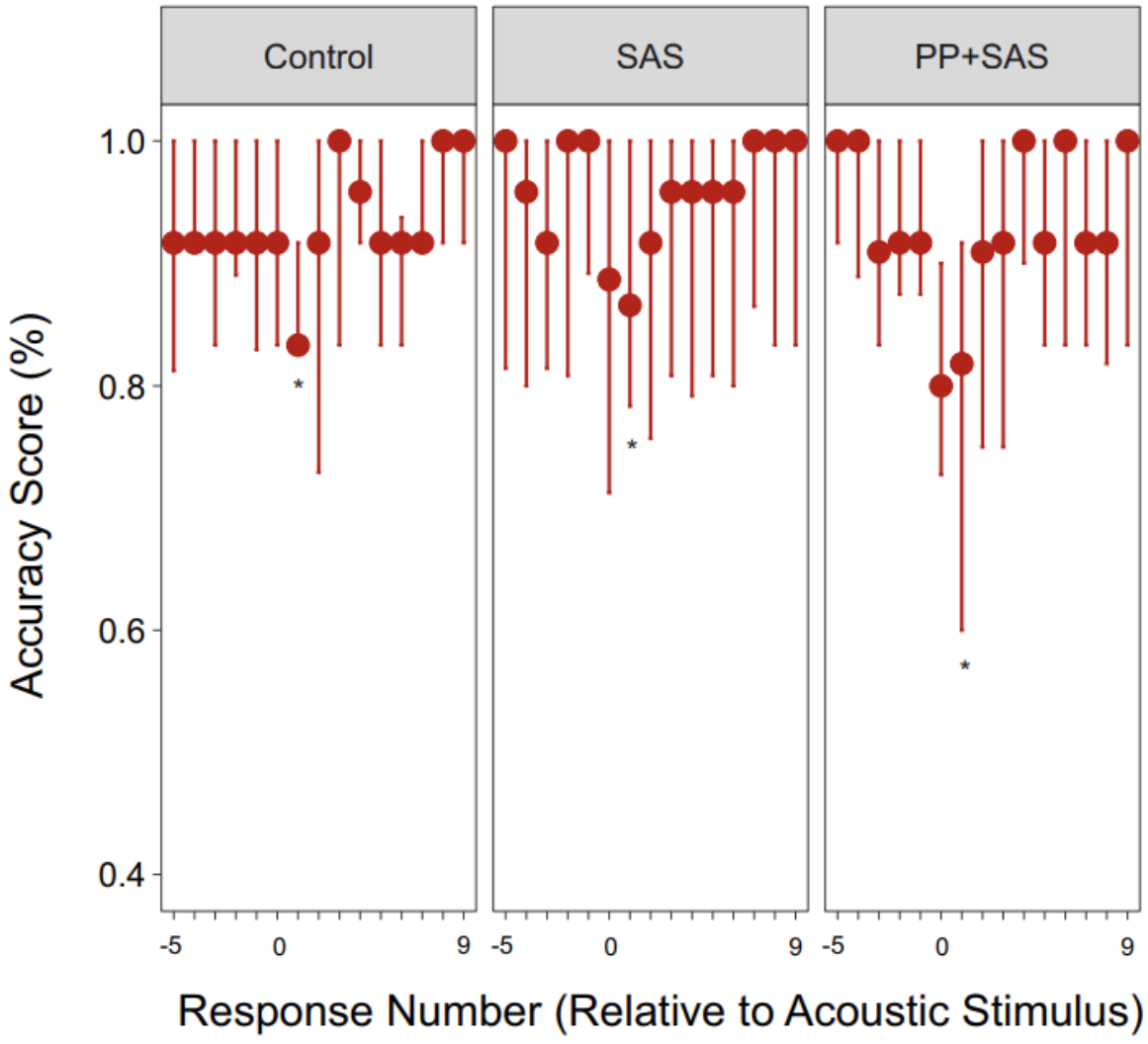


Figure 7. Median keypress response accuracy across the five responses prior to and ten responses after an acoustic stimulus irrespective of whether a startle reflex was observed. Markers and error bars represent medians and upper / lower quartiles.

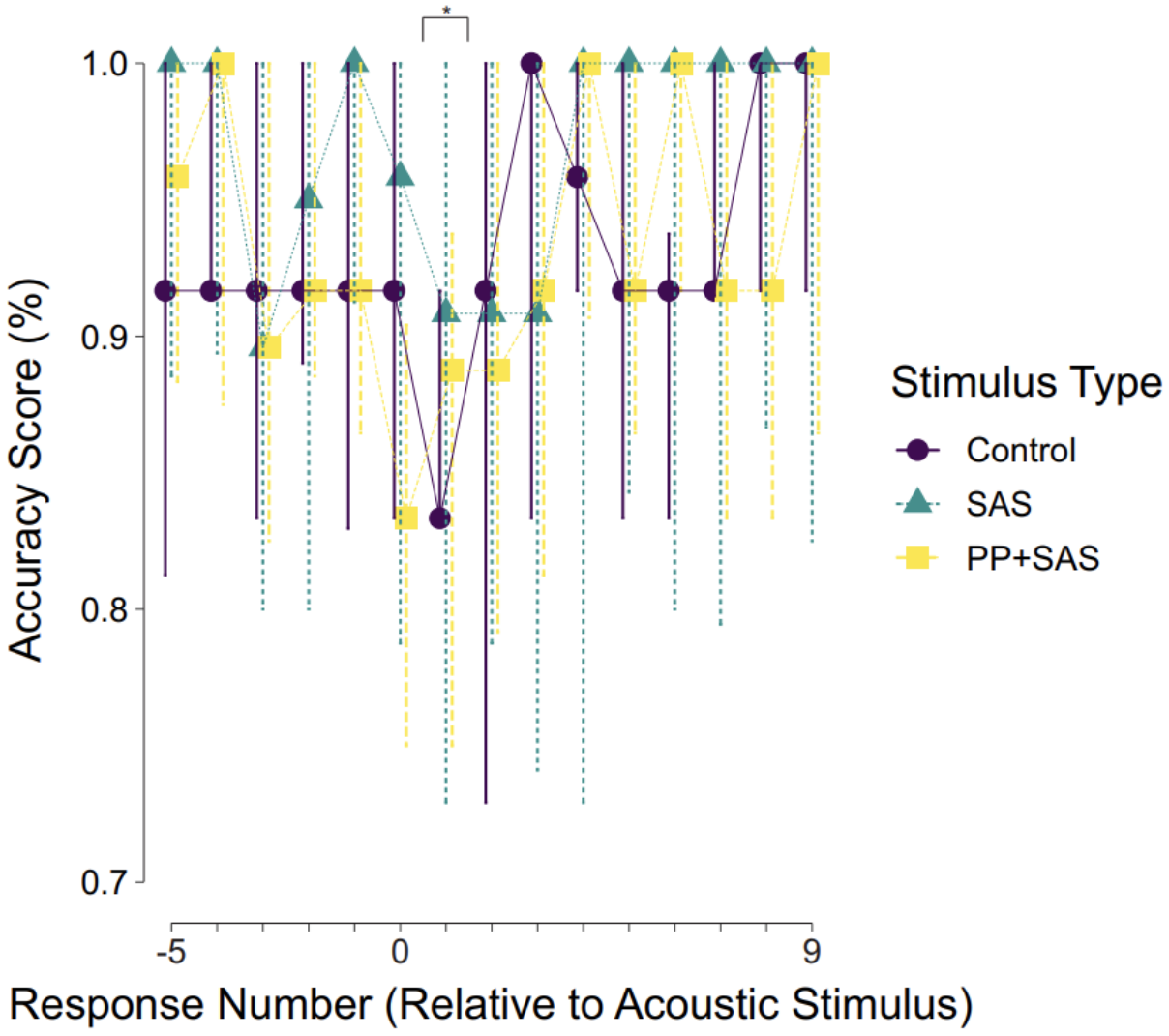


Figure 8. Median keypress response accuracy across the five responses prior to and ten responses after an acoustic stimulus across all acoustic stimulus types when no startle reflex was observed. Markers and error bars represent medians and upper / lower quartiles.

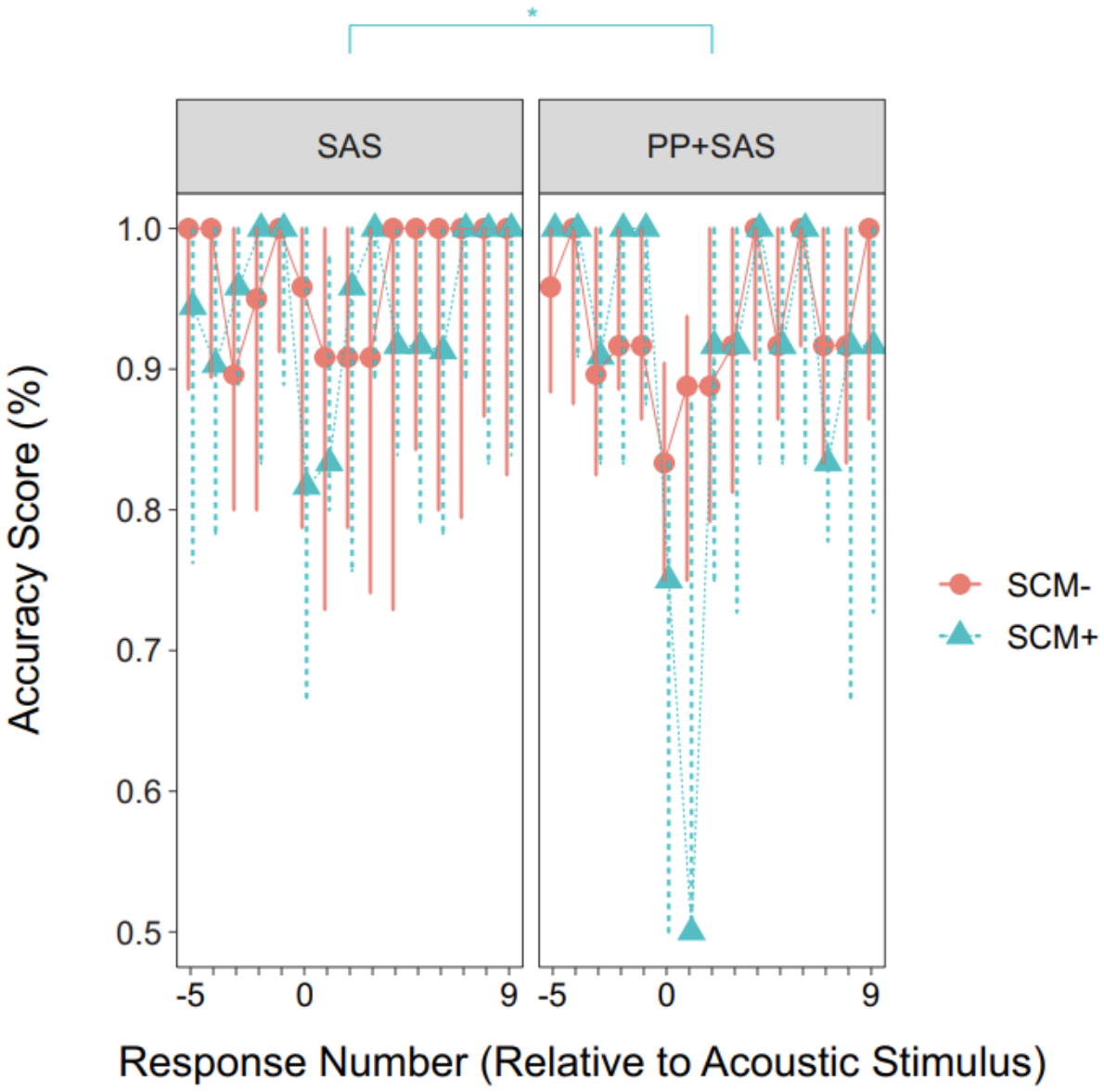


Figure 9. Median accuracy across the five responses prior and ten responses after an acoustic stimulus across SAS and PP+SAS stimulus types separated on whether a startle reflex was observed in SCM or not. Markers and error bars represent medians and upper / lower quartiles.

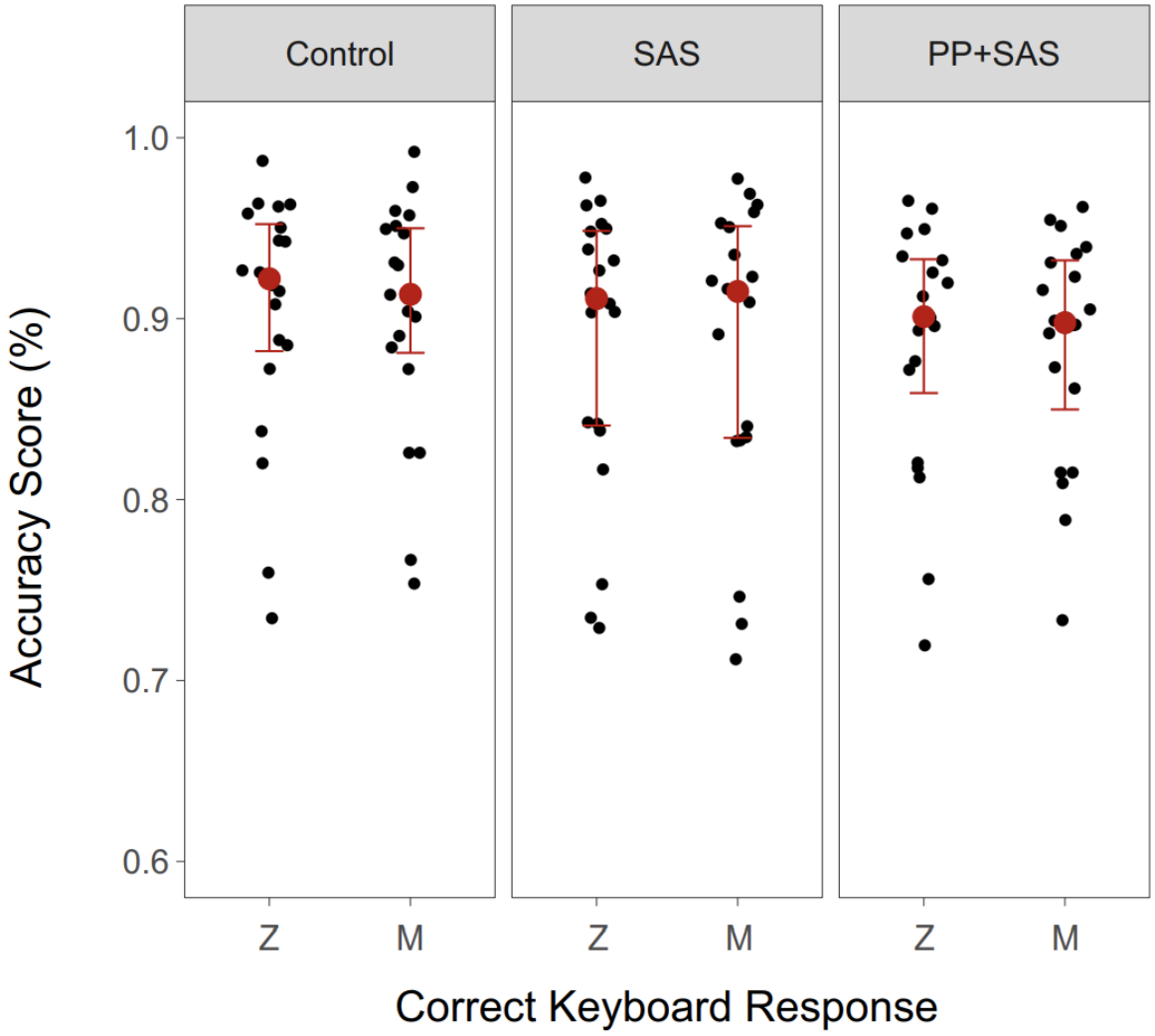


Figure 10. Mean accuracy irrespective of whether a startle reflex was observed or not across all three acoustic stimulus types separated based on whether the required keypress response was 2-back target (M-key) or non-target (Z-key). Large markers and error bars represent medians and upper / lower quartiles, while small markers indicate individual participant values.

*3.2.2 Response Time*

Mean response time was calculated for the five responses prior and ten responses subsequent to an acoustic stimulus, and analyzed irrespective of whether a startle reflex was observed (Figure 11). A significant response number effect was observed $F(14, 1181.0) = 4.341$, $p < .001$.

Pairwise comparisons revealed that mean response time of response number 1 was significantly higher than all other responses (all p-values < 005).

Mean response time for control trials, as well SAS and PP+SAS trials where no startle reflex was observed, was analyzed separately and is presented in Figure 12. A significant effect of response number was observed $F(14, 715.92) = 4.027$, $p = < .001$. Pairwise comparisons revealed that mean response time on response number 1 was significantly higher than all other responses (all p-values < .05), except response number 2.

Response time was also analyzed for only SAS and PP+SAS trials across responses, separated on whether a startle reflex was observed in SCM or not (Figure 13). A significant main effect of response number was observed $F(14, 866.05) = 3.987$, $p < .001$. Pairwise comparison revealed that mean response time for response number 1 was slower compared to all other responses (all $p < .05$). Another significant main effect of SCM+/- was observed $F(1, 878.62) = 74.996$, $p < .001$. Pairwise comparisons revealed mean response time was longer (99.3 ms) when a startle reflex was observed ($p < .001$).

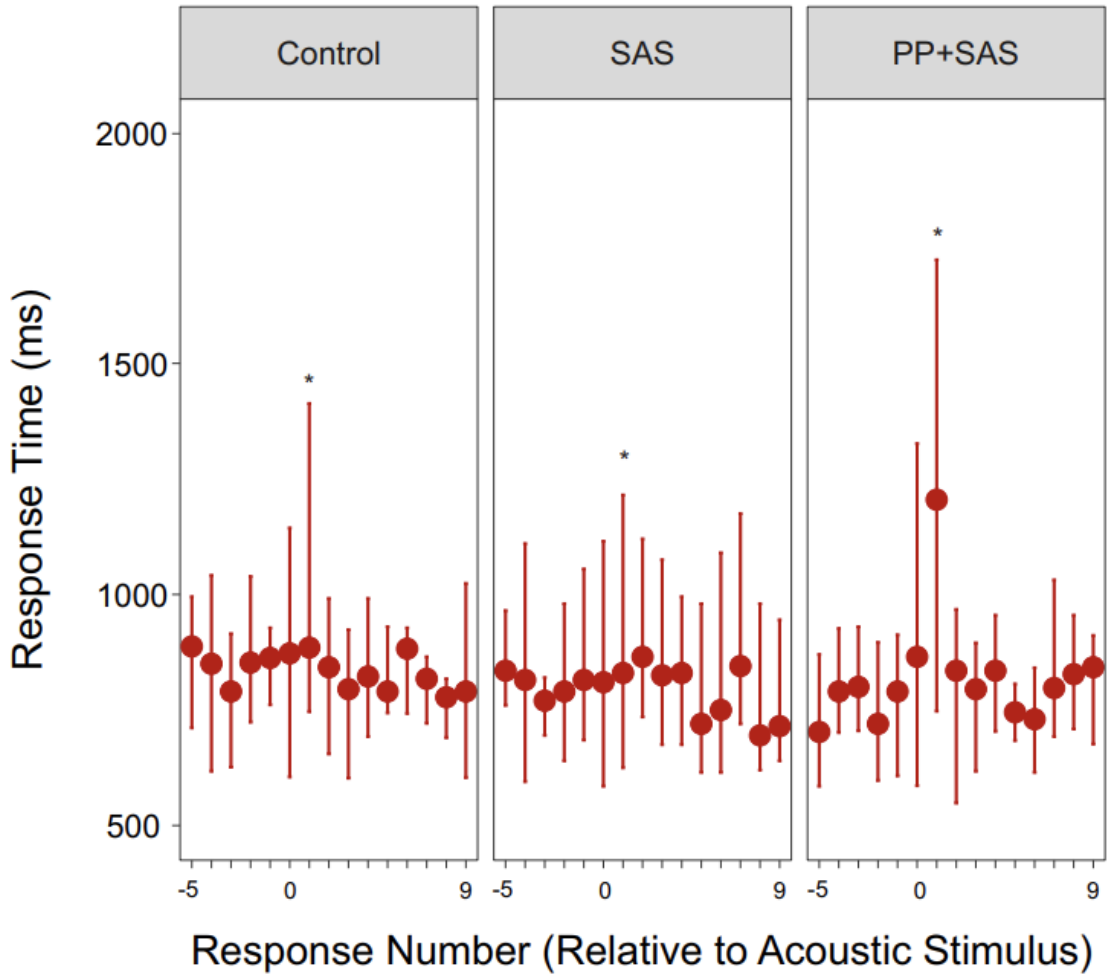


Figure 11. Mean response time across the five responses prior and ten responses after an acoustic stimulus irrespective of whether a startle reflex was observed. Markers and error bars represent medians and upper / lower quartiles.

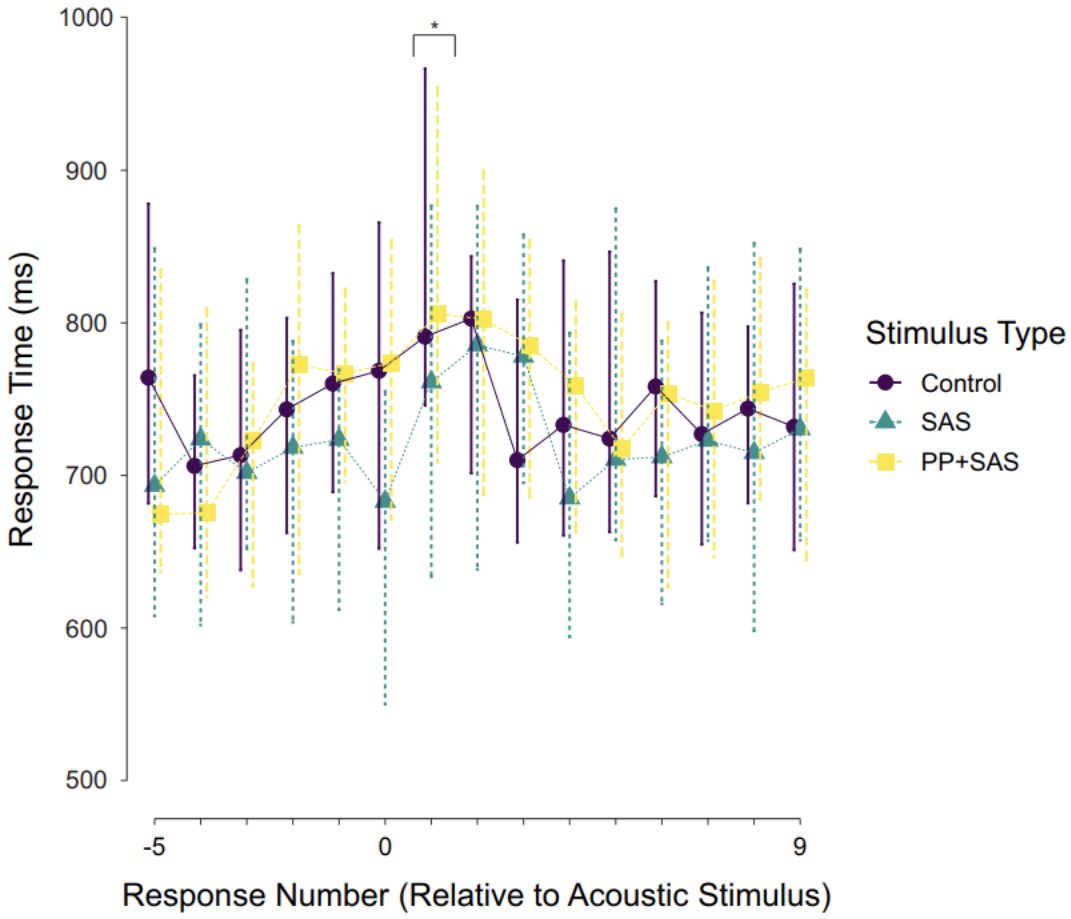


Figure 12. Mean response time across the five responses prior and ten responses after an acoustic stimulus across all acoustic stimulus types when no startle reflex was observed. Markers and error bars represent medians and upper / lower quartiles.

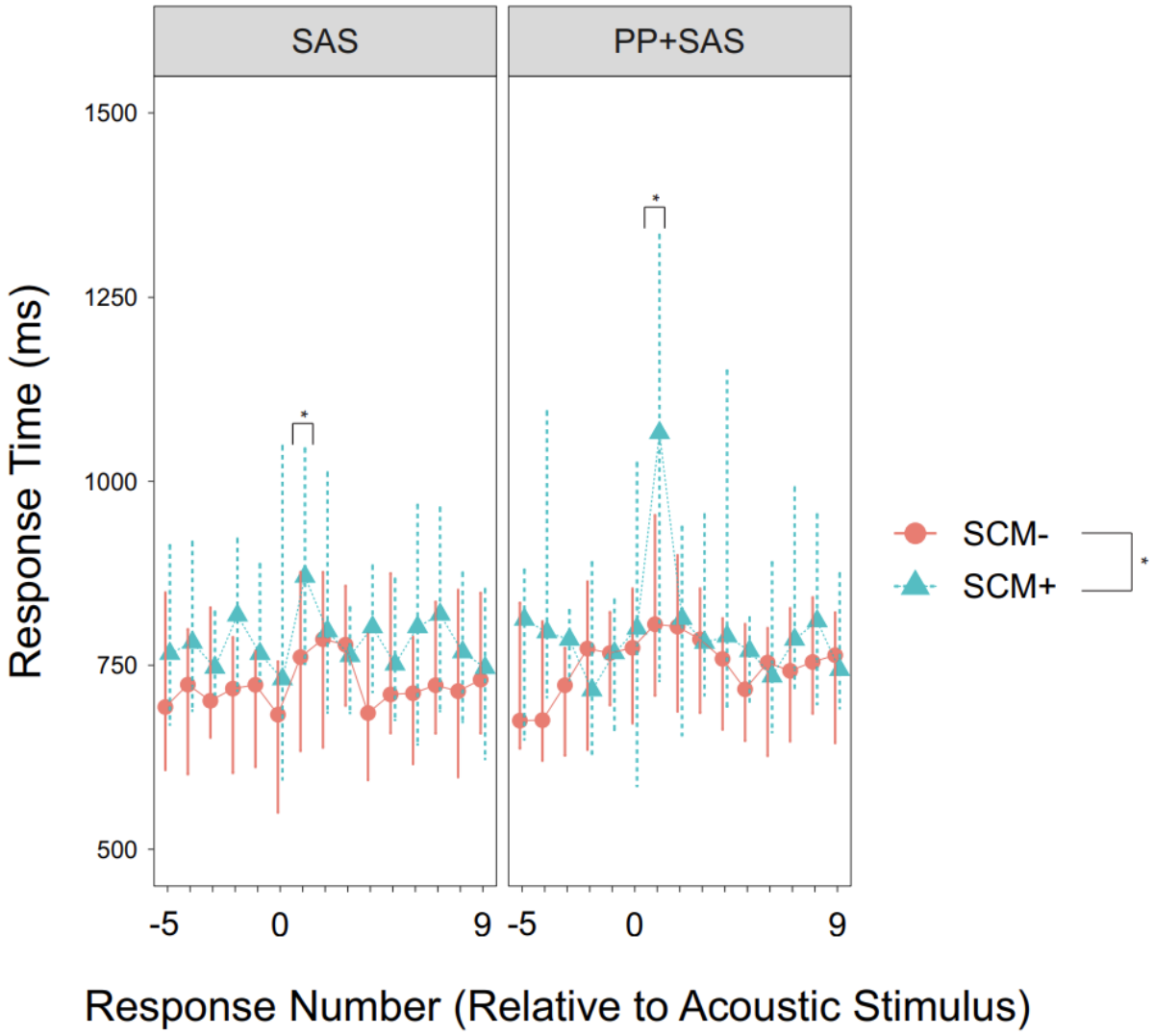


Figure 13. Mean response time across the five responses prior and ten responses after an acoustic stimulus across SAS and PP+SAS stimulus types separated on whether a startle reflex was observed in SCM or not. Markers and error bars represent medians and upper / lower quartiles.

## 4. Discussion

The purpose of the present experiment was to examine the effect of the startle reflex on cognitive performance, and to determine whether the use of a prepulse stimulus could diminish a presumed detrimental effect of startle on cognitive performance. A continuous 2-back (n-back) task was used to compare accuracy & response time prior to, and following either an 80dB

control tone, a 120dB SAS, or a prepulse stimulus presented 100ms prior to the SAS. As expected, the probability of observing a startle reflex in SCM was greatly reduced (-17.73%) when the SAS was preceded by a prepulse (Figure 1). Additionally, the amplitude of the startle reflex, measured using the integrated area of the first 150 ms of EMG in SCM, was observed to be diminished following a PP+SAS compared to a SAS alone (Figure 2). Response accuracy was shown to be negatively impacted in all acoustic stimulus conditions (Figure 7). Similarly with response time, an increase in response time in the response following any acoustic stimulus was observed (Figure 11). These results suggest that any acoustic stimulus, startling or not, may have a detrimental effect on cognitive performance. However, when looking at only trials where a startle reflex was seen (SCM+), no difference in accuracy was observed within the SAS condition (between SCM+ & SCM-), while accuracy within the PP+SAS condition was worse when a startle reflex was observed (Figure 9). Additionally, when comparing across conditions with SCM+ trials, accuracy was unexpectedly higher in the SAS condition compared to PP+SAS (Figure 9). When examining response time based on SCM presence in SAS and PP+SAS conditions, an increase in response time on the response following the SAS or PP+SAS (response 1) was observed (Figure 13). More interestingly, a main effect of SCM was observed, with longer response times in SAS and PP+SAS conditions when a startle reflex was observed (Figure 13). In summary, while prepulse did not mitigate the negative impact of startle on cognitive processes, it did decrease the incidence with which these negative impacts occur.

In the present study a distinct reduction in the probability of eliciting a startle reflex was observed when a prepulse was presented prior to the SAS, which is consistent with previous findings (Bui et al., 2024; Blumenthal, 1996). Additionally, startle reflex amplitude was significantly reduced with the addition of a prepulse prior to the SAS (Figure 2). To obtain this

result, the data were analyzed similar to previous studies using prepulse by examining integrated EMG activity in a short time window (150 ms) following the acoustic stimulus (Maslovat et al., 2012; Forgaard et al., 2018). Accordingly, this analysis produced the expected findings – a decrease in startle reflex amplitude. However, this method of observing the effect of prepulse on startle reflex amplitude does not account for whether a detectible startle reflex had actually occurred. Consequently, this may result in the false interpretation of the effect of prepulse. To observe the true effect of prepulse on startle reflex amplitude and heart rate, an analysis of the integrated area of EMG in both SCM, in the 100 ms window following the onset of a startle reflex was performed (Figure 3). This analysis only used data in which a startle reflex occurred, and showed that no difference in amplitude was observed between SAS and PP+SAS conditions. This is contrary to previous findings (Heathcote et al., 2024; Bui et al., 2024; Maslovat et al., 2012; Forgaard et al., 2018); however, it could potentially be attributed to the low proportion of prepulse trials that elicited a startle reflex. Another possibility is that in startle experiments looking at the startle reflex itself (in particular prepulse), trials have not been generally analyzed separately for SCM+/-.

Normalized heart rate, analyzed irrespective of whether a startle reflex was observed, revealed a significant increase following a SAS compared to Control and PP+SAS conditions in a short time window around the stimulus (Figure 4). Heart rate data was also separated and analysed based on whether a startle reflex was observed in SCM or not. This allowed for a comparison between the effect of any acoustic stimulus (e.g., control trials) to be fairly compared to SCM- data in SAS and PP+SAS conditions (Figure 5). This comparison revealed a statistically significant main effect, where a lower heart rate was present following a PP+SAS compared to the control stimulus across the 30 second window; however, the difference was small (0.823%)

and there was no interaction with time, suggesting the acoustic stimulus did not have a differential effect. Comparing heart rate in SAS and PP+SAS conditions separated by SCM presence (SCM+/-) revealed three interesting findings. First, within the startle and prepulse conditions, heart rate was elevated when a startle reflex was observed as opposed to when no startle reflex was observed (Figure 6). Second, heart rate in the six responses following a SAS and PP+SAS was elevated when a startle reflex was observed compared to no startle reflex. Finally, heart rate was elevated to a greater extent when a startle reflex was observed following a startle stimulus compared to the prepulse stimulus. Put together these findings suggest that heart rate is increased for six responses (approximately 14 seconds) following a startle or prepulse stimulus, when a startle reflex is observed. However, the increase in heart rate is not as high with a prepulse stimulus compared to control. These findings are consistent with previous findings (Bui et al., 2024) which examined the effect of startle and prepulse on a motor tracking task.

On initial examination, response accuracy irrespective of whether a startle reflex was observed, revealed an effect of response number (Figure 7). Specifically, accuracy of the response following an acoustic stimulus (80.7%, response number 1) was lower (≥5.4%) compared to the responses prior to acoustic stimulus (-5 to -1) and last seven responses following the stimulus (responses 3-9). A similar result was seen in response time (e.g. time between presentation of the visual target and the keypress), with a longer response times (≥148 ms) in the first response following an acoustic stimulus compared to all other responses (Figure 11). These initial findings suggest that an acoustic stimulus, even one that is non-startling has detrimental effects on cognitive performance. This finding can most likely be attributed to the irrelevant sound effect. Initially observed by Colle & Welsh (1976), this effect refers to the disruption of

processes involved in maintaining information in working memory. However, these results were independent of whether a startle reflex actually occurred.

Analysis of the SCM- data and control trial data revealed a decrease in accuracy on the first response after all acoustic stimuli, with no significant difference between conditions (Figure 8). This finding provides further evidence that an acoustic stimulus, even one that is non-startling has a negative effect on cognitive performance, providing additional evidence that the performance decrement is due to the irrelevant sound effect. Comparing accuracy across SAS and PP+SAS conditions after separating trial based on SCM+/- revealed novel, unexpected findings (Figure 9). While accuracy within the PP+SAS condition showed decreased accuracy on SCM+ trials (at trials 6 & 7), interestingly, no difference in accuracy existed within the SAS condition. Furthermore, a higher accuracy was observed in the SAS condition (specifically at trials 7 & 9) when a startle reflex occurred contrary to expectations. This finding is especially interesting as it was initially hypothesized that prepulse would mitigate any performance decrements; however, the present findings suggest the opposite. It seems prepulse may have actually exacerbated performance decrements when a startle reflex was observed. It is also unlikely that the decrease in performance in the PP+SAS condition can be attributed to a startle reflex alone, as no difference in performance was observed within the startle condition. One possible explanation for this may be a potential additive effect of the irrelevant sound effect due to the prepulse stimulus and an additive effect with the startle reflex. It is possible that the prepulse tone and SAS tone that make up the PP+SAS condition result in greater error compared to control and startle conditions due to the fact that the prepulse condition consists of two separate auditory stimuli, and this effect was worsened by the presence of a startle reflex. A

secondary analysis to determine if response accuracy was affected by whether the correct response was a 2-back target or non-target revealed no differences (Figure 10).

Similar findings to those seen in response accuracy were also seen with response time. Comparing SCM- data from startle and prepulse conditions to control revealed an increase in response time across all conditions in the response following the acoustic stimulus, with no differences between conditions otherwise (Figure 12). This finding provides further evidence that an acoustic stimulus, even if non-startling can have a negative effect cognitive performance. When examining response time across SCM+/- trials in the startle and prepulse conditions, a longer response time was seen in the response following both startle and prepulse stimuli, wherein for both startle and prepulse conditions, response time was on average 87.6 ms slower on SCM+ trials compared to SCM- (Figure 13). This finding could possibly explain the lack of the expected decrease in accuracy within the startle condition, whereby participants compensated by taking more time to respond, thereby maintaining task accuracy when a startle reflex occurred. However, this effect was not seen with the prepulse condition when a startle reflex was observed, where a decrease in accuracy was seen along with an increase in response time with a startle reflex suggesting no compensatory effect. This indicates a potential relationship the startle reflex, prepulse, and cognitive performance. While the startle reflex is thought to be primarily subcortical, there is evidence from neural imaging studies to suggests potential cortical effects. A study by Neuer and colleagues (2010) used functional magnetic resonance imaging (fMRI) to examine neural activation during the startle reflex as well as with prepulse inhibition and prepulse facilitation. In this study they identified primary and secondary somatosensory cortices, right thalamus, right insula, right temporal pole, cerebellum, and the middle part of the cingulum as essential parts of a common neural network for the processing of the startle reflex and its

modulation. They also identified the additional recruitment of prefrontal and parietal areas (left superior medial frontal cortex, anterior cingulate cortex, right caudate nucleus, and cerebellum) as important to prepulse inhibition. This is of particular importance as the prefrontal cortex plays a significant role in working memory (see Lara & Wallis, 2015 for a review). It is perhaps due to the additional recruitment of prefrontal/parietal areas required during prepulse inhibition, taking away from a limited cognitive resource, that results in poorer cognitive performance when prepulse inhibition occurs.

Another possible explanation for the observed results may be due to the habituation of the startle reflex throughout the experiment. A secondary analysis of the proportion of trials where a startle reflex was observed in either SCM was examined across twelve blocks, separated into quarters of three blocks each (Figure 18). While it is clear there was habituation of the startle reflex throughout the experiment, there is no change to the primary conclusions when analysing the data using only the first quarter.

The results from the present experiment provide possible insights into the processes affected by startle, through the timing of the acoustic stimulus, and the observed decrement in performance. The presentation of the acoustic stimulus occurred 200 ms into response 0, occurring prior to any keyboard response made during the task. However, decrements in performance were observed in response 1 (relative to acoustic stimulus), with performance in response 0 being relatively unaffected. These results suggest that the items within short term memory prior to the acoustic stimulus, the recall of those items and connection to the present visual stimulus was unaffected. However, the updating of the sequence, and formation of new memory in working memory may have been negatively affected, resulting in the delayed decrements in performance that were observed.

The findings from the present experiment suggest that a startling acoustic stimulus and subsequent startle reflex had minimal impact on cognitive performance compared to control, which is contrary to previous literature (Vlasak, 1969; Woodhead, 1959). In the present experiment, response accuracy was largely unaffected by a SAS and startle reflex, however, a small but significant increase in reaction time was observed when a startle reflex was observed. More recent work by Schwartz and colleagues (2025) found that cognitive performance was *improved* following a startle stimulus in a similar mental arithmetic n-back task. Additionally, in this study prepulse was observed to reduce the probability of observing a startle reflex, and therefore decrease the incidence of cognitive performance decrements. However, when a startle reflex was observed following a PP+SAS stimulus, cognitive performance seemed to be more negatively impacted. With the unexpected findings of the present study in relation to previous literature around startle and cognitive performance, it appears that the relationship between startle and cognitive performance may be more complex than previously thought.

## Chapter 3: General Discussion

The startle reflex has previously been shown to cause detrimental effects during continuous cognitive task performance (Vlasak, 1969; Woodhead, 1959). The purpose of this experiment was to replicate the detrimental effects caused by startle in a different cognitive task (2-back task), and determine if these detrimental effects could be mitigated by modulating the startle reflex with a prepulse. The present experiment found that the presence of an acoustic stimulus, whether it resulted in a startle reflex or not, can have a negative impact on cognitive performance. Additionally, the present study found no effect of a SAS on response accuracy different from control. However, when a SAS elicited a startle reflex, accuracy remained unchanged while a small decrement in response time was observed suggesting a compensatory effect. While the addition of a prepulse prior to the startling stimulus reduced the probability of observing a startle reflex, it had no effect on the magnitude of the response. In terms of its effect on performance, the present study found that the prepulse deteriorated performance more than that of just a SAS alone (when a startle reflex occurred), however, the probability of this occurring was reduced. This contradictory finding was unexpected, and may be attributed to an additive irrelevant sound effect as a result of the two distinct auditory tones in the PP+SAS. These findings are surprising in that only a small decrease in cognitive performance was observed following a startle contrary to previous literature (Vlasak, 1969; Woodhead, 1959), and the addition of a prepulse seemed to have a negative effect on performance, contrary to expectations. The findings of this study provide novel and unexpected insights into the relationship between startle, prepulse and cognitive performance.

The broader applicability of this research lies in its relevance to real-world scenarios in which consistently high cognitive performance is critical, and lapses can lead to catastrophic

outcomes. While the effects of startle on motor performance have been well-documented (Bui et al., 2024; Vlasak, 1969; Thackray & Touchstone, 1970; May & Rice, 1971; Foss et al., 1989), the influence of startle on cognitive task performance remains insufficiently understood. This experiment specifically examined a working memory task, but startle may have broader implications for other cognitive processes, such as attention, executive function, and decision-making. Understanding how startle impacts these processes is essential, especially in high-stakes environments, such as operating a vehicle, where a lapse in cognitive performance can lead to consequential outcomes.

One limitation of this experiment lies in the temporal resolution of the cognitive performance measures. Response accuracy and reaction time were recorded for each trial, which occurred at two-second intervals. As a result, acute effects of the startle reflex on cognitive performance, particularly those with a duration shorter than the inter-trial interval (i.e., <2 seconds), may not have been adequately captured. Furthermore, the specific impact of the startle reflex on cognitive performance remains ambiguous due to potential interference from the irrelevant sound effect. This is evidenced by the observed decline in cognitive performance following a non-startling control stimulus, indicating that the control stimulus itself, even though non-startling, likely affected task performance. Future research should further investigate the effects of startle and prepulse stimuli on cognitive performance using a broader range of cognitive tasks and methodological approaches to more precisely characterize their relationship. Specifically, employing an alternative modality for eliciting the startle reflex, such as a startling electrical stimulus, may help isolate or rule out the confounding influence of the irrelevant sound effect observed in auditory paradigms. Moreover, utilizing cognitive tasks that do not predominantly rely on working memory, in conjunction with a startling acoustic stimulus, may

provide a clearer assessment of startle-related effects on cognitive performance as the irrelevant sound effect affects processes of working memory.

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## Appendix A

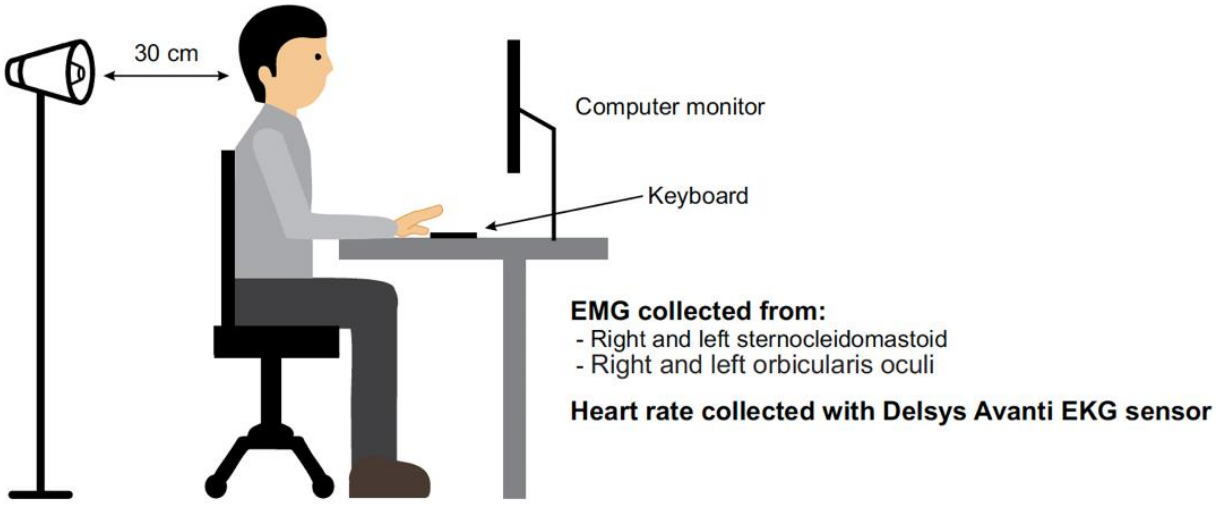


Figure 14. Experimental setup. Participants sat comfortably at a desk with both hands placed on the keyboard facing a computer monitor. A speaker was placed 30 cm behind the participant, centered with the midline of participant. Wireless EMGs (Delsys Trigno Wireless EMG system) were placed on both right and left SCM and OOc muscles. Heart rate was collected using a Delsys Avanti EKG sensor. Adhesive EKG electrodes were placed under the pectoral muscles. The EKG sensor was attached to the trunk below the electrodes.


Figure 15. Time course of an experimental block. Participants completed twelve blocks each lasting 2.5-minutes in duration. Throughout each block three different acoustic stimuli were presented every 45-50s. The order of the stimulus presentations within each block was randomized and counterbalanced. The three acoustic stimulus condition were: 1) an acoustic control stimulus (80dB); 2) a 120dB startling acoustic stimulus (SAS); and 3) a prepulse condition (PP+SAS) consisting of an 80dB control tone presented 120ms prior to a SAS. The presentation of the control stimulus was tied to the presentation of the visual stimulus. In the SAS and PP+SAS conditions, the presentation of the acoustic stimulus was synchronised such that the SAS occurred 200 ms following the presentation of the visual stimulus.

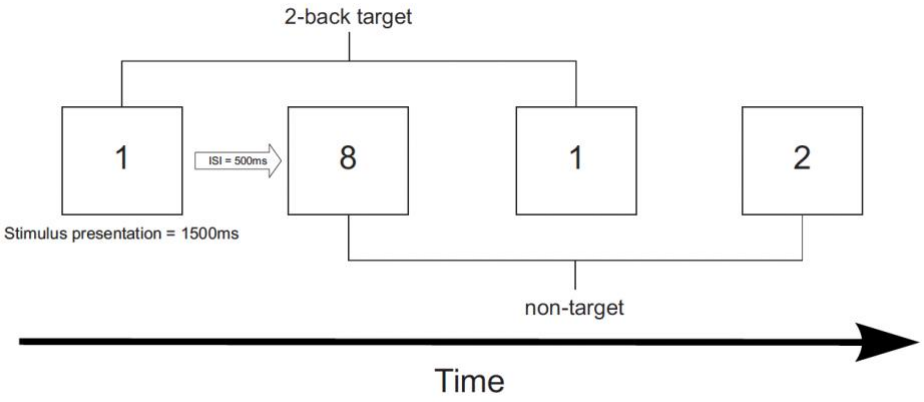


Figure 16. Time course for a series of four responses. A visual stimulus was presented for 1500ms (ex., '1'), followed by a 500ms blank screen ISI. The next visual stimulus (ex., '8') was then presented. On 25 trials per block (33%), a 2-back target was presented, the remaining 50 trials presented as non-targets.

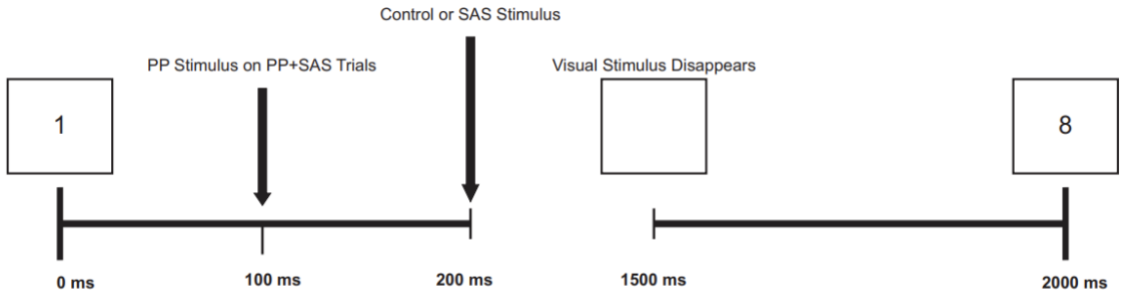


Figure 17. Timing of auditory stimulus. The primary auditory stimulus was presented 200ms following the visual stimulus. In PP+SAS conditions, the prepulse was presented 100ms following the visual stimulus, and the SAS was presented 200ms following the visual stimulus.

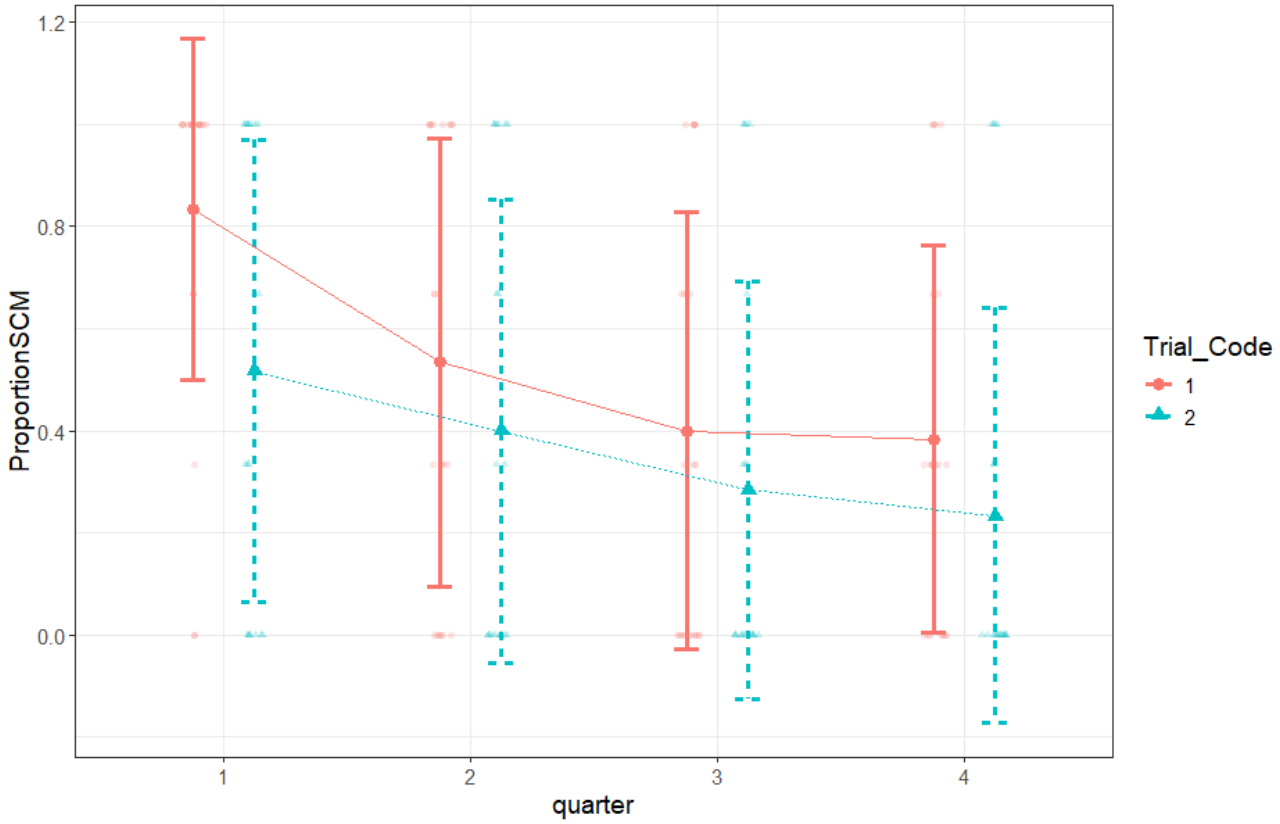


Figure 18. Habituation of the startle reflex throughout the experiment. The proportion of startle reflexes observed in either right or left SCM following a SAS or PP+SAS separated across four quarters of the twelve total blocks (3 blocks each). Large markers and error bars represent means and standard deviations, while small markers indicate individual participant values. Trial code 1 represents the SAS condition and trial code 2 represents the PP+SAS condition.