Abstract

The autonomic nervous system controls numerous effectors within the human body, working to adapt to changing environments. It is composed of three divisions: the sympathetic, parasympathetic, and enteric nervous systems. Sympathetic and parasympathetic activity have been related to emotion, cognition and behaviour. Sympathetic and parasympathetic influence on behavioural performance has been examined extensively; however, the autonomic modulation of performance in tasks involving distinct voluntary and automatic behaviours has not been directly investigated. Interleaved pro- and anti-saccade tasks (IPASTs) are regularly used to evaluate voluntary and automatic behaviour, respectively. The pro-saccade task requires an automatic visuomotor response whereas the anti-saccade task requires the suppression of an automatic response and the generation of a voluntary response in the opposite direction. We sought to investigate parasympathetic and sympathetic modulation of voluntary and automatic saccade behaviour using an IPAST. Thirty healthy human controls completed five blocks of trials (each block: 40 pro / 40 anti = 8 minutes) over 45 minutes. We explored the relationship between experiment duration, autonomic activity and performance in the context of this task as arousal varied. Eye movements, pupil size, electrodermal activity and electrocardiogram were measured throughout. The consecutive measurement of these peripheral autonomic indices further allowed for the investigation of the role of autonomic drive in their coordination. A clear effect of time-on-task on arousal was present in all three measures with arousal increasing across the first three blocks. This modulation of arousal however,
was unrelated to task performance, which was unaffected by block number. Lastly, a task effect on performance was found, replicating previous studies. A task effect on arousal was also demonstrated. The changes in sympathetic and parasympathetic activity underlying this effect however are unclear. These findings suggest that the development of cognitive fatigue is not a concern in the use of the IPAST subtending 45 minutes. Additionally, further investigation of autonomic modulation of performance in the IPAST may contribute to the interpretation and analysis of related fMRI data and its application towards investigations of autonomic dysfunction in various neurological disorders. Moreover, the effects of task and block on arousal demonstrated here warrant further investigation.
Co-Authorship

This research project in this report was conceived by Dr. Chin An Wang and Dr. Douglas Munoz. Dr. Brian Coe and Donald Brien developed marking software for classifying and defining eye movement parameters. Donald Brien further contributed to experiment setup. Donald Brien and Talia Baird coded the biometrics data analysis software. Talia Baird completed all data collection, performed data and statistical analyses and wrote this thesis in its entirety.
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List of Abbreviations

**ACh**  Acetylcholine

**ANOVA**  Analysis of Variance

**ANS**  Autonomic Nervous System

**BOLD**  Blood Oxygen Level Dependent

**CG**  Cardiac Ganglion

**DLPFC**  Dorsolateral Prefrontal Cortex

**DVN**  Dorsal Vagal Nucleus

**ECG**  Electrocardiogram

**EDA**  Electrodermal Activity

**FEF**  Frontal Eye Fields

**fMRI**  Functional Magnetic Resonance Imaging

**HBF**  Heart Beat Frequency

**HR**  Heart Rate

**HRV**  Heart Rate Variability

**IPAST**  Interleaved Pro- and Anti-Saccade Task

**RMSSD**  Root Mean Square of Successive Differences

**NA**  Nucleus Ambiguus

**NE**  Norepinephrine

**NTS**  Nucleus of the Solitary Tract
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PNS</td>
<td>Parasympathetic Nervous System</td>
</tr>
<tr>
<td>SA</td>
<td>Sinoatrial</td>
</tr>
<tr>
<td>SEF</td>
<td>Supplementary Eye Field</td>
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<td>SG</td>
<td>Stellate Ganglion</td>
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<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
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<td>SRT</td>
<td>Saccade Reaction Time</td>
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Chapter 1

General Introduction

Levels of arousal constantly change throughout the day in response to various stimuli. For example, if a person jumped out from around a corner your heart rate (HR) and pupil size would quickly increase as your body prepares itself to fight or for flight. In addition to initiating basic physiological responses, increased autonomic arousal influences behaviour. Arousal, driven by an increase in the ratio of sympathetic: parasympathetic activity has been associated with performance on cognitive and motor tasks. The influence of autonomic arousal however, has not been investigated in the context of voluntary and automatic behaviour. The anti-saccade task enables the evaluation of voluntary oculomotor behaviour while the pro-saccade task facilitates the investigation of automatic oculomotor responses. Sympathetic and parasympathetic activation can be indexed by electrodermal activity (EDA) and HR or heart rate variability (HRV) respectively. Pupil size is controlled by both the parasympathetic (PNS) and sympathetic nervous systems (SNS) and reflects arousal however, the relative contribution of these systems to changes to pupil size in the context of anti- and pro-saccade task is unknown. In order to evaluate parasympathetic and sympathetic modulation of voluntary and automatic behaviours, eye movements, pupil size, EDA, and cardiac electrical activity via electrocardiogram (ECG) were concurrently measured as healthy human controls completed an interleaved anti- and pro- saccade task across five consecutive blocks. The effects of time-on-task on arousal and performance and their relationship were additionally evaluated. Lastly, the consecutive recording of ECG,
EDA, and pupil size allowed for the evaluation of the relationship between autonomic indices (pupil size, HRV and EDA).

1.1 The Autonomic Nervous System

The autonomic nervous system (ANS) is essential in maintaining homeostasis and exerts considerable control within the human body. It is the component of the peripheral nervous system that controls visceral activities, cardiac muscle contraction and glandular functions of the body, acting below the level of consciousness (reviewed in Gordan, Gwathmey & Xie, 2015). It is controlled by centers located in the hypothalamus, brain stem and spinal cord (reviewed in Gordan et al., 2015).

The ANS is closely linked to many emotions, behaviours and the immune system (Fisher, Young, & Fadel, 2015; Gibbins, 2013; Jänig, 2006; Kenney & Ganta, 2014; Llewellyn-Smith & Verberne, 2011; Sladek, Michelini, Stachenfeld, Stern, & Urban, 2015). The list of unconscious processes under ANS regulation includes body temperature, HR, and blood flow amongst others (Jänig, 2006; Llewellyn-Smith & Verberne, 2011; Loewy & Michael Spyer, 1990). As such, it is crucial for adaptation to environmental stressors and other challenges. In turn, its failure severely compromises these abilities (Garland & Robertson, 2009; Mathias & Freeman 2003).

The ANS consists of three divisions: the SNS, the PNS and the enteric nervous system. The enteric nervous system works with the PNS and SNS to control digestion. While the PNS is most active under restful conditions, promoting conservation and restoration of energy, the SNS prepares the body for energy expenditure, stressful or emergency situations by increasing metabolic output. In preparation for motor action,
increased SNS activity results in pupil and bronchiole dilation, sweat secretion, elevated HR, blood pressure, and pupil size in addition to the redirection of blood from the gastrointestinal tract towards the heart, lungs, brain and skeletal muscles. In contrast, increased PNS activity results in changes such as decreased HR, bronchiole diameter and pupil size.

To control their many effector organs, the SNS and PNS can function antagonistically, synergistically or independently (Jänig, 2006; Llewellyn-Smith & Verberne, 2011; Loewy & Spyer, 1990). The SNS and PNS function synergistically to control pupil size (via the smooth muscles of the iris). In the heart, these two divisions function as physiological antagonists, with parasympathetic tone dominating. Whereas, as in the case of sweat glands, innervated solely by the SNS, some organs are controlled independently by one system. The activity of sweat glands can be measured via EDA recording. As such, pupil size, HR or HRV, and EDA are regularly used to index the activity of the SNS and/or PNS. HR and HRV are accepted indices of PNS activity (Akselrod et al., 1981; Hirsch & Mackintosh, 2003) while EDA is an accepted index of SNS activity (Boucsein, 2012).

1.1.1 ANS control of the pupil

Pupil size is determined by sympathetic and parasympathetic balance (Fotiou, Fountoulakis, Goulas, Alexopoulos, & Palikaras, 2000) (Fig. 1). The dilatory and constrictor pupillae muscles of the iris control pupil size, respectively producing pupillary dilation and constriction. While the constrictor pupillae muscles are primarily innervated by the PNS, the dilatory pupillae muscles are innervated by the SNS.
Parasympathetic innervation of the constrictor pupillae comes from preganglionic neurons in the Edinger-Westphal nucleus in the midbrain, which is the autonomic subdivision of the third cranial nerve nucleus (reviewed in McDougal & Gamlin, 2015). These preganglion neurons of the Edinger-Westphal nucleus project to pupilloconstrictor postganglionic neurons in the ciliary ganglion. In turn the ciliary ganglion controls constrictor pupillae muscles directly through a short projection (Lowenstein & Loewenfeld, 1950).

Sympathetic control of the dilatory pupillae begins in the spinal cord. Preganglionic sympathetic neurons located in the ciliospinal center of Budge, the C8-T2 segments of the spinal cord, project to sympathetic chain ganglia and travel to the superior cervical ganglia through the sympathetic trunk to the superior cervical ganglion (reviewed in McDougal & Gamlin 2015). Here, post-ganglionic sympathetic neurons project to the dilatory pupillae via long and short ciliary nerves (Ruskell, 2003).
Figure 1. Autonomic control of pupil size. See text for details.
1.1.2 ANS control of the heart rate and heart rate variability

While cardiac automaticity is intrinsic to various pacemaker tissues, the autonomic nervous system exerts a large degree of influence over heart rate and rhythm (Jalife, 1994). The parasympathetic and sympathetic nervous systems work antagonistically to control HR and HRV (Fig. 2). Autonomic control of cardiac activity begins in the medulla. Here, the nucleus of the solitary tract activates the nucleus ambiguus (NA) (Agarwal & Calaresu, 1992) and parasympathetic dorsal vagal nucleus (DVN) and inhibits the sympathetic rostral ventrolateral medulla (Klabunde, 2011). Preganglionic neurons in the DVN and NA form the vagus nerve (Benarroch, 2012) and project to cardiac ganglia where post-ganglionic neurons project to the heart, releasing acetylcholine (ACh) (Klabunde 2011; Pardini, Lund, & Schmid, 1990). The rostral ventrolateral medulla projects to preganglionic neurons in the spinal cord which in turn project to post-ganglionic paravertebral stellate ganglion neurons which innervate the heart (Pardini et al., 1990). Sympathetic pre-ganglionic neurons in the spinal cord also innervate the adrenal medulla, a modified sympathetic prevetebral ganglion, stimulating the release of epinephrine and norepinephrine into the blood stream, which then travels to the heart (Feher, 2012).

Membrane processes of the sinoatrial (SA) node, whose activity is modulated by the PNS and SNS (Levy and Warner, 1994; Randall, 1994), control the normal rhythm of the heart. Postganglionic parasympathetic terminals at the SA node release ACh which binds to type two muscarinic (M2) receptors, slowing the rate of SA node depolarization and discharge through the activation of transmembrane potassium channels (Sakmann, Noma & Trautwein, 1983). This action further produces an
increase in HRV (Zaza & Lombardi, 2001). Contrastingly, norepinephrine (NE), released by sympathetic terminals at the SA node, bind to β1 adrenergic receptors, inducing an acceleration of the SA node rhythm and therefore HR. This increase in HR results in decreases in HRV (Kazmi et al., 2016).

Further cardiac autonomic effects occur outside of the SA node. Conduction and contractility are additionally affected. The conduction of SA node electrical signal is affected by the PNS and SNS. Atrioventricular node conduction velocity is increased through the activation of β1 adrenergic receptors and decreased through the activation of M2 cholinergic receptors (reviewed in Gordan et al., 2015). Contractility is affected through the activation of M2 and β1 adrenergic receptors found on atrial and ventricular cardiomyocytes. Activation of β1 receptors on these cardiomyocytes leads to increases in intracellular calcium. Moreover, while the vagus nerve only directly innervates cardiomyocytes at the SA and AV nodes, it is currently believed that parasympathetic effects are exerted through M2 receptors located primarily in the atria with sparse representation in the ventricles, reducing contractility (reviewed in Gordan et al., 2015).

The latencies of sympathetic and parasympathetic effects on cardiac activity are distinct. Studies evaluating the functional effects of phasic modulation of sympathetic and vagal activities have concluded that the cardiac response to sympathetic activity is characterized by a time delay and slower response while the cardiac response to vagal activity is rapid (de Boer, Karemaker & Strackee, 1985; Somsen, Molenaar, Van der Molen, & Jennings, 1991). Spear Kronhaus, Moore and Kline (1979) investigated heart period responses to brief bursts of sympathetic and vagal activity and reported a latency of maximum vagal effect at 0.5 s and a latency of maximum sympathetic effect of 4 s.
These findings align with those of Berger, Saul and Cohen (1989) and Penaz (1962) using frequency-domain signals.

While both of these systems regulate cardiac activity, vagal tone prevails under resting conditions (Craft & Schwartz, 1995; Levy, 1971) and is the main determinant of heart period (Chess, Tam, & Calaresu, 1975). When both cardiac sympathetic and vagal inputs are pharmacologically blocked, intrinsic HR is higher than the normal resting HR (Craft & Schwartz, 1995; Jose & Collison, 1970). The interaction of sympathetic and vagal activity may explain this. Due to the high concentration of acetylcholinesterase at the sinus node, the effects of vagal impulses are short lived. Parasympathetic influences exceed sympathetic effects none-the-less and this is probably accomplished through two independent mechanisms. In response to sympathetic activity, NE release is reduced cholinergically. Additionally, cardiac adrenergic responses are attenuated cholinergically. Taniguchi, Fujiwara, Lee and Hiduka (1979) found that when ACh and NE are presented simultaneously in the SA node region, cholinergic influence on nodal activity, mediated primarily by muscarinic receptors, influenced adrenergic signaling pathways, delaying and reducing the positive chronotropic effects of NE.
Figure 2. Autonomic control of heart rate. See text for details.
1.1.3 ANS control of the electrodermal activity

EDA is controlled independently by the SNS (Fig. 3). It is a sensitive and convenient measure of assessing changes in SNS activity reflected in sweat gland activity. Sympathetic control of sweat gland activity begins in the preoptic sweat nucleus of the hypothalamus. A projection from this hypothalamic nucleus travels through the nucleus raphe pallidus extending further, in the intermediolateral spinal column where it activates pre-ganglionic neurons (Smith & Johnson, 2016). These fibres then exit the spinal cord in the ventral root, synapsing with a preganglionic sympathetic neuron in the paravertebral sympathetic chain ganglia. This postganglionic sympathetic neuron in turn projects to a sweat gland (Sato, Kang, Saga, & Sato, 1989). These sympathetic terminals surround sweat glands (Sato et al., 1989) and their activity modulates sweat secretion which can be measured as EDA. Sugenoya, Iwase, Mano, & Ogawa (1990) reported a linear relationship between mean sudomotor burst voltage amplitude and the rate of rise of the corresponding sweat expulsion. They further found that sweat expulsion was consistently preceded by bursts of sympathetic activity in cutaneous nerves with latencies of 2.4 to 3s. Since, post-stimulus onset skin conductance latencies of 1 to 3 s have been reported (Zhang et al., 2012). As sweat is a weak electrolyte and good conductor, its excretion increases the conductance of an applied current through the introduction of low-resistance parallel pathways.
Figure 3. Autonomic control of sweat gland activity. See text for details.
1.2 Sympathetic and Parasympathetic Activity and Performance

Sympathetic and parasympathetic activation have both been associated with cognitive and motor task performance. In 1965, Eason, Beardshall and Jaffee recorded skin conductance as participants completed a vigilance task. They found a positive correlation between relative task performance and relative skin conductance magnitude. Later, in 1973, Cowles found that mean skin conductance level was significantly correlated with reaction time in participants completing a simple signal response task, while HR was not. In 2001, however, Tremayne and Barry found that pre-shot skin conductance levels were lower and HR deceleration was longer and more systematic for the best shots among elite pistol shooters compared to poor shots.

ANS activity has been linked to cognitive function. Polyvagal theory (Porges, 2007) and models of neurovisceral integration (Thayer & Lane, 2000; Thayer Hansen, Saus-Rose, & Johnsen, 2009) have described frameworks in which autonomic activity is critical for higher-order behaviour and cognition. Furthermore, the central autonomic network is believed to play a key role in the link between autonomic activity and cognitive function. The central autonomic network supports goal-directed behaviour and adaptability (Benarroch, 1993, 2012). This network includes prefrontal and limbic structures: the anterior cingulate, insular, orbitofrontal, and ventromedial prefrontal cortices, the central nucleus of the amygdala, the paraventricular and related nuclei of the hypothalamus, the periaqueductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract, the nucleus ambiguous, the ventrolateral medulla, the ventromedial medulla, and the medullary tegmental field. Functional units within this network have been identified which serve executive, social, affective, attentional and
motivated behaviour in humans and animals (Damasio, 1998; Devinsky, Morrell, & Vogt, 1995; Masterman & Cummings, 1997; Spyer, 1989). Through prefrontal modulation of bottom-up sensory inputs, the central autonomic network is associated with the generation of context appropriate responses and regulates psychophysiological resources related to goal-directed behaviour (Thayer & Friedman, 1997; Friedman & Thayer, 1998).

Vagally mediated cardiac functions (indexed by HR and HRV) have been associated with cognitive, affective, and physiological regulation (Thayer & Brosschot, 2005) which have in turn been linked with right hemisphere prefrontal activity (Ahern et al., 2001; Aron, Robbins, & Poldrack, 2004; Kalisch et al., 2005). Prefrontal cortical activity and vagally mediated HRV have also been associated using pharmacological and neuroimaging approaches (Ahern et al., 2001; Lane, McRae, Reiman, Ahern, & Thayer, 2007; Lane, Reiman, Ahern, & Thayer, 2001; Lane, Weidenbacher, Fort, Thayer, & Allen, 2008; Nugent, Bain, Thayer, & Drevets, 2007; Nugent, Bain, Thayer, Sollers, & Drevets, 2008). Pharmacological blockade of the prefrontal cortex produces a downstream disinhibition of medullary cardioacceleratory circuits and an increase in HR (reviewed in Thayer & Lane, 2009). Additionally, a meta-analysis of fMRI studies found higher resting-state vagally mediated HRV was found to be associated with greater activity in executive brain regions (Thayer et al. 2012). Behavioural connections are also apparent. Hansen et al. (2003) found that high HRV (reflecting greater parasympathetic influence) is associated with better performance specifically on tasks involving executive function. Moreover, Park, Vasey, Van Bavel & Thayer (2013) reported a load-dependent association of cardiac vagal tone and selective attention control. Under low cognitive
load, HRV was not associated with task performance while a clear positive association between HRV and performance was seen when participants completed more complex tasks.

Sympathetic activity, indexed by EDA, has also been linked to cognitive activity. In 2000, Critchley, Elliott, Mathias, & Dolan reported a positive correlation between skin conductance and medial prefrontal cortical activation in a decision-making task. In a separate study, the skin conductance of patients with prefrontal damage were recorded as they played a card game stimulating real-life decision making (Bechara, Tranel, Damasio, & Damasio, 1996). While controls began to generate skin conductance responses prior to their card selection, the patients with prefrontal damage did not. This absence of anticipatory skin conductance responses in patients with prefrontal damage further correlated to their insensitivity to future outcomes. Additionally, neural activity in the ventromedial prefrontal cortex was later found to be inversely correlated with skin conductance level as subjects completed biofeedback arousal and relaxation tasks (Nagai et al., 2004). Lastly, in 2003, Asahina, Suzuki, Mori, Kanesaka & Hattori measured sympathetic sweat responses in patients with multiple system atrophy, a syndrome featuring autonomic dysfunction, during a mental arithmetic task. They found that mean amplitudes of the sympathetic sweat response were significantly reduced in these patients as compared to healthy controls.

While both sympathetic and parasympathetic activity are linked to cognitive function, sympathetic and parasympathetic responses to cognitively challenging tasks are distinct. In response to cognitive challenge, PNS activity has been found to decline (Melis & van Boxtel, 2001, 2007). Moreover, a positive relationship between task
difficulty and degree of high frequency HRV (parasympathetic measure) withdrawal has been repeatedly demonstrated (Backs & Seljos, 1994; Byrd, Reuther, McNamara, DeLucca & Berg., 2014; Lenneman & Backs, 2009). In 2010, Mathewson et al. observed faster response times on the stroop test associated with greater HF-HRV withdrawal. While PNS activity appears to decline with cognitive challenge, cognitive challenge appears to elicit increases in SNS activation (Allen & Crowell, 1989; Backs & Seljos, 1994; Berntson et al., 1994; Berntson, Cacioppo, & Fieldstone, 1996).

Interestingly, Giuliano, Gatzke-Kopp, Roos & Skowron (2017) found that individual differences in performance on a challenging working memory task were associated with interactions between SNS and PNS function. The positive relationship between parasympathetic function and behaviour was dependent on resting sympathetic activation.

Many studies have considered the relationship between SNS or PNS measures and performance on cognitive tasks. Most studies exploring associations between cognitive performance and autonomic physiology have focused on either SNS (e.g. Hajcak, McDonald, & Simons 2003, 2004) or PNS measures (e.g. Thayer et al., 2009; Park, Vasey, Van Bavel & Thayer, 2014; Williams, Thayer & Koenig, 2016). Moreover, the consideration of multiple autonomic measures would enable the evaluation of their interaction. Additionally, although the question of SNS and PNS influence on performance has been approached from many avenues, the modulation of both SNS and PNS of performance in tasks requiring distinct voluntary and automatic and behaviours has not been directly investigated.
1.3 Voluntary and Automatic Behaviour and the Oculomotor System

Voluntary behaviours are goal-directed conscious actions determined using perceptual information in a flexible manner. By contrast, automatic behaviours result from fast, inflexible, unconscious, automatic responses. The “visual grasp reflex” first introduced by Hess, Burgi & Butcher (1946), provides a clear example of an automatic behaviour within the oculomotor system. This response involves rapid saccadic eye movement that are made to newly appearing objects in the peripheral visual field. Humans and monkeys however, can suppress this automatic response under instruction to use the target as a landmark for a saccade to its mirror location (Hallett, 1978).

The visual grasp reflex and its suppression are demonstrated in the performance of pro- and anti-saccade tasks respectively. These tasks are commonly used to investigate voluntary and automatic behaviours. Prior to stimulus appearance, participants are instructed to generate either an anti-saccade (look in opposite direction of the stimulus) or a pro-saccade (look at the peripheral stimulus). The pro-saccade task requires an automatic visuomotor response, whereas the anti-saccade task requires suppression of this automatic response in addition to the generation of a voluntary response in the opposite direction of the stimulus. The active inhibition of automatic responses required to correctly perform the anti-saccade task enables the evaluation of executive function.

While similar basic neural circuitry is involved in both voluntary and automatic saccades, voluntary saccades require the support of higher level processes (McDowell, Dyckman, Austin, & Clementz, 2008) while pro-saccades operate largely independently of executive control (Pierrot-Deseilligny, Rivaud, Gaymard, Muri & Vermersch, 1995).
When subjects perform blocks of anti-saccade trials, compared with blocks of pro-saccade trials, cerebral blood flow increased in a large network of cortical areas including the frontal lobe, dorsolateral prefrontal cortex (DLPFC), supplementary eye fields (SEF), superior and inferior parietal cortex and primary visual cortex (Doricchi et al., 1997; O’Driscoll et al., 1995; Paus, Petrides, Evans, & Meyer, 1993; Sweeney et al., 1996). Correspondingly, patients with frontal lobe damage often fail to suppress the initial automatic visuomotor response (Fukushima, Fukushima, Miyasaka, & Yamashita, 1994; Guitton, Buchtel, & Douglas, 1985; Pierrot-Deseilligny, Rivaud, Gaymard, & Agid, 1991; Walker, Husain, Hodgson, Harrison, & Kennard, 1998). Moreover, greater activation of the frontal eye field (FEF) has also been reported by fMRI studies during preparation for anti-saccades compared to pro-saccades in humans (Connolly et al. 2002; DeSouza, Menon, & Everling, 2003; Manoach et al., 2007). The significance of this finding is furthered by the existing link between greater preparatory activity within the FEF and decreased saccade reaction times (SRTs) (Alahyane, Brien, Coe, Stroman, & Munoz, 2014; Connolly, Goodale, Goltz, & Munoz, 2005).

Neural correlates for different preparatory sets between anti and pro saccades have also been identified. When monkeys receive saccade response instructions, differences in the baseline discharge rate are present in single neuron recordings in the FEF (Everling & Munoz, 2000) and SEF (Schlag-Rey, Amador, Sanchez, & Schlag, 1997) have been reported. Desouza et al. (2003) further demonstrated greater activity in the right FEF and DLPFC during the instruction period for anti-saccade trials as compared to pro-saccade trials. No differences however were found in response to the
peripheral stimulus or saccade in these areas. This suggests that differences between anti- and pro-saccades primarily originate from the modulation of preparatory activity.

1.4 Relationships Between Electrodermal Activity, Heart Rate, Heart Rate Variability, and Pupil Size

HR, HRV, EDA and pupil size have all been extensively used in their own right to index aspects of autonomic activity. Their associations however have not received as much attention. Pupil size and HR changes have been consistently correlated while correlations between galvanic skin conductance and pupil size, HR or HRV are less consistent. Separately, HR and HRV are negatively correlated (Kazmi et al., 2016).

Numerous studies have evaluated concurrent changes in pupil size and HR, HRV and or EDA, far fewer however, have specifically evaluated their relationship. Bradley, Miccoli, Escrig, and Lang (2008) found that during affective picture viewing pupil size was related to HR but not skin conductance. Further, in 2016 Kuzinas, Noiret, Bianchi, and Laurent. found no significant correlations between pupil size and skin conductance during a free viewing task of IAPS stimuli. Wass, de Barbaro and Clackson (2015) evaluated pupil size, HR and GSR through as infants freely viewed various videos. They reported covariations of pupil size and EDA and pupil size and HR. No significant relationship however, was found between EDA and HR. Finke, Deuter, Hengesch and Schachinger (2017) concurrently measured HR, skin conductance and pupil size responses to the free viewing of erotic stimuli. Using PCA analysis, they identified early and late component of the pupil response which were further correlated to HR and skin conductance changes respectively. This suggests that earlier
parasympathetic inhibition may induce pupil dilation while later SNS activation leads to further dilation.

Like most others, these studies, have a notable limitation. Saccadic eye movements influence not only the accuracy of pupil size measurement in any video-based eye tracking systems, but also the PNS and SNS activity via the pathway through the midbrain superior colliculus (Wang & Munoz, 2015). This factor was not adequately controlled by the previous investigators and may have confounded their findings. In the current study, we investigate the relationships between pupil size, SNS, and PNS activation over a fixation period to control for this influence.

1.5 Effect of Time-On-Task on Arousal and Performance

Time-on-task has been associated with changes in arousal and performance. Here, time-on-task is defined as the total amount of time spent completing trials of a task or tasks. The effect of time-on-task on performance is more popularly known as cognitive or mental fatigue. Cognitive fatigue is defined as a failure to complete mental tasks requiring internal cues and self-motivation in the absence of motor weakness or cognitive failure (Chaudhuri & Behan, 2000). It has been linked to autonomic activity and its effects have implications when considering the design of experiments involving cognitive tasks. Changes in arousal were studied independently of performance over an eight hour period in which various cognitive tasks were completed (Mizuno et al., 2011). High frequency HRV power, a measure of parasympathetic activity and the ratio of low frequency: high frequency HRV power, a measure of sympathetic activity were significantly changed from baseline at the end of the eight hour task period.
Furthermore, compared to the relaxation group, participants who completed the cognitive task had significantly higher low frequency:high frequency HRV power ratios and significantly lower high frequency HRV power. Further, the effect of time-on-task on task performance was evaluated by Mockel, Beste and Wascher (2015). They found that performance on a Simon task decreased with time-on-task.

Arousal and performance have also been associated in the context of time-on-task. Few studies however have evaluated the association of cognitive fatigue and autonomic activity. In 2009, Tanaka, Mizuno, Tajima, Sasabe and Watanabe found that during a 30 minute 2-back task session, high frequency component power decreased while low frequency component power increased. Neither reaction time nor accuracy, the performance metrics, however significantly changed. Later in 2015, Gergelyfi, Jacob, Olivier and Zénon evaluated autonomic activity throughout a 120 minute period in which participants completed sudoku tasks, inducing mental fatigue. Further, a significant effect of block was found on working memory performance which was evaluated at intervals throughout the 120 minute experiment. Task performance gradually decreased over time. Further, HRV increased over the course of the experiment while pupil size and skin conductance remained constant. In this study, in order to investigate the influence of time-on-task on arousal and performance in the context of the interleaved anti- pro-saccade task, a 400 trial, 45 minute task, consisting of five consecutive blocks, was designed.
1.1 Aims and Hypotheses

The primary aim of this thesis is to investigate the modulation of voluntary and automatic task performance by the parasympathetic and sympathetic branches of the autonomic nervous system. The coordination of EDA, pupil size and HRV, whose values reflect the activation of autonomic effectors, in the context of the interleaved anti-pro-saccade task is also of interest. Lastly, the third aim of this thesis is to investigate the influence of time-on-task on arousal and performance in the context of the interleaved anti-pro-saccade task.

In order to investigate the modulation of voluntary and automatic task performance by the parasympathetic and sympathetic branches of the autonomic nervous system, the relationships of the following values over the fixation periods of anti and pro-saccade trials will be evaluated: percent direction error (PDE), SRT, pupil size, skin conductance, root mean square successive difference (RMSSD; a measure of HRV) and HR. It is expected that sympathetic activity will be greater and parasympathetic activity smaller on anti-saccade as compared to pro-saccade trials. Considering this, it is expected that larger EDA, HR, and pupil size and smaller HRV will be associated with anti-saccade trials.

With increasing block number, sympathetic activity is expected to increase while parasympathetic activity decreases across blocks one to four while the reverse is expected in block five as its identity as the final block is declared. Decreased EDA and pupil size and HRV increases are expected across blocks one to four while the reverse is expected in block five. Considering this, performance is expected to decrease across
blocks 1-4 and increase in block five. As for the association of EDA, HR, HRV and pupil size, it is expected that HRV will be associated with pupil size.
Chapter 2
Correlating Sympathetic and Parasympathetic Control of Saccade Behaviour

2.1 Introduction

Levels of autonomic arousal are in constant flux, changing in response to various stimuli. The autonomic nervous system plays an important role in the maintenance of homeostasis, adapting to changing settings and situations. It acts below the level of consciousness to control visceral activities, cardiac muscle contraction and glandular functions of the body (reviewed in Gordan et al., 2015). Sympathetic and parasympathetic activation can be indexed by electrodermal activity (EDA) and heart rate (HR) or heart rate variability (HRV), respectively (Akselrod et al., 1981; Boucsein, 2012; Hirsch & Mackintosh, 2003). Pupil size is controlled by both the parasympathetic (PNS) and sympathetic nervous systems (SNS) and reflects autonomic arousal. While autonomic control of these indices is well understood, the relationships of EDA, pupil size and HR or HRV are not firmly understood. While HR and pupil size changes have been consistently correlated (e.g. Bradley et al., 2008; Wass et al., 2015), correlations between EDA and pupil size, HR or HRV are less consistent (e.g. Finke et al., 2017; Korn, Staib, Tzovara, Castegnetti, & Bach, 2017; Wass et al., 2015).

The ANS has been associated with many behaviours, emotions, and the immune system (Fisher et al., 2015; Gibbins, 2013; Jänig, 2006; Kenney & Ganta, 2014; Llewellyn-Smith & Verberne, 2011; Sladek et al., 2015). Moreover, performance on cognitive and motor tasks has been associated with autonomic arousal. In 2001, pre-shot HR deceleration (parasympathetic index) was found to be longer and more
systemic and skin conductance levels (sympathetic index) were found to be lower for best shots among elite pistol shooters compared to poor shots (Tremayne and Barry, 2001). Further, high HRV (reflecting greater parasympathetic influence) has been found to be associated with better performance specifically on tasks involving executive function (Hansen et al., 2003). Additionally, Benikos et al. (2013) found that skin conductance level increased with block number while percent error decreased on a NoGo task for low and medium task difficulty. The influence of autonomic arousal however, has not been investigated in the context of voluntary and automatic behaviour. Voluntary and automatic oculomotor behaviour can be investigated using the anti and pro-saccade tasks, respectively (Munoz and Everling 2004; Coe and Munoz 2017). Another factor which may influence performance on cognitive tasks is time-on-task. This effect, known as cognitive fatigue, is defined as a failure to complete mental tasks requiring internal cues and self-motivation in the absence of motor weakness or cognitive failure (Chaudhuri & Behan, 2000). Cognitive fatigue has been linked to autonomic activity; however few studies have concurrently evaluated these elements. In 2009, Tanaka et al. used HRV to measure autonomic activity as subjects completed a 30 minute 2-back task session. They found that high frequency component power decreased (parasympathetic index) while low frequency component power (sympathetic index) increased. Neither of the performance metrics, reaction time nor accuracy, significantly changed however. Conversely, Gergelyfi et al., (2015) found an effect of time-on-task on performance on a working memory task, yet while HRV increased throughout the experiment, pupil size and skin conductance remained unchanged. They evaluated autonomic activity throughout a 120 minute period in which participants
completed sudoku tasks, inducing mental fatigue. At the beginning of each of the 14 blocks, participants completed working memory tasks from which the performance scores were taken.

Here, we investigate the modulation of voluntary task performance by the parasympathetic and sympathetic branches of the autonomic nervous system. In order to evaluate parasympathetic and sympathetic modulation of voluntary and automatic behaviours, eye movements, pupil size, EDA, and cardiac electrical activity via electrocardiogram (ECG) were concurrently measured as participants completed an interleaved anti- and pro-saccade task. The concurrent measurement of these indices further allows for the investigation of their relationships. Additionally, in order to evaluate changes in arousal and performance with time-on-task, 400 trials were divided into five blocks of 80 trials.

2.2 Materials and Methods

2.2.1 Participants

This study was conducted with an experimental protocol approved by Health Sciences Research Ethics Board, which adheres to the principles of the Canadian Tri-council Policy Statement on Ethical Conduct for Research Involving Humans and the principles of the Declaration of Helsinki (1964). Thirty participants (eighteen female) ranging between twenty and twenty-eight years of age ($M= 21.93$, $SD =2.067$) were recruited for this study. All participants were naïve regarding the purpose of the experiment, had good or corrected vision and did not report any history of any neurological or psychiatric conditions. They gave written consent prior to investigation and received compensation.
for their participation. One male participant was excluded due to an average PDE on the anti-saccade task of greater than 50% due to concerns that he did not understand the task.

2.2.2 Recording and apparatus

Eye movements, pupil size, ECG, and EDA were measured using three independent pieces of apparatus. A video-based eye tracker (Eyelink-1000 binocular-arm, SR Research, Osgoode, ON, Canada), was used to measure eye position and pupil size with binocular recording at a sampling rate of 500 Hz. A wireless heart rate monitor (S182 Wireless Exercise Heart Rate Monitor, Qubit Systems Inc., Kingston, ON, Canada), worn as a chest band was used to measure cardiac activity at a sampling rate of 200 Hz. Ultrasound gel (Revitive Ultrasound Gel, Actegy Ltd., Quebec, Canada) was placed between the sensor and skin to facilitate recording. A galvanic skin conductance sensor (Q-S222 Galvanic Skin Response Sensor, Qubit Systems Inc., Kingston, ON, Canada) was used to sample electrodermal activity at a sampling rate of 200 Hz. Stimuli were presented on a 17-inch LCD monitor at a screen resolution of 1280 x 1024 pixels (60 Hz refresh rate), subtending a viewing angle of 32° x 26°, as the distance from the eyes to the monitor was set at 58 cm.

2.2.3 Task

Participants performed an interleaved pro- and anti-saccade task with an extended initial fixation period (3000 ms compared to the typical 1000 ms) (Fig. 4). Skin conductance responses are typically observed between 1-3 seconds after stimulus
presentation which necessitated this adjustment (Zhang et al., 2012). Participants completed five consecutive ~8 minute blocks consisting of 40 anti and 40 pro-saccade trials randomly interleaved. Each trial involved an initial 3000 ms fixation period in which subjects fixated a central fixation spot, followed by a 200 ms gap of no stimuli, and then a 1000 ms epoch in which a visual stimulus was presented 10° left or right of centre. The intertrial interval was approximately 2000 ms in duration. Task instruction (pro vs anti) was conveyed by the colour of the fixation spot (green = pro-saccade; red = anti-saccade). Participants were instructed to look towards the peripheral stimulus in the pro-saccade and to look away to the opposite position in the anti-saccade condition. Between blocks, participants were instructed to close their eyes and relax for a short variable period (~1 minute) as biometrics files were saved and a new recording session was opened.

2.2.4 Data analysis

Pupil, eye movement, ECG, and EDA data were obtained for analysis. A signal indicating the beginning of a new trial was included in biometric (EDA and ECG) data files and was used to align eye tracking and biometric data. Data was not collected due to technical issues over one block for three participants. Of the remaining data, six blocks were excluded due to failure to meet biometric data criteria. Blocks in which biometrics files did not contain 80 trial were excluded analysis along with blocks in which irregular spacing of these signals was detected. Trials containing eye loss and/or no response or an incorrect response to the peripheral stimulus were excluded from analysis. Overall, 38% of trials were excluded from analysis. The proportion of trials
excluded from analysis per participant ranged from 20.5% to 62.4%. Outliers were identified using the MATLAB boxplot function. They were removed and replaced with mean values prior to analysis.

Trial EDA values were divided by the standard deviation of the block and baseline corrected according to the average EDA value calculated from 50 ms prior to fixation point appearance (start of trial). Pupil size was similarly baseline corrected with reference to pupil area at the time of fixation point appearance. RMSSD was calculated across blocks and trial fixation periods while HR was calculated across each block.

The effects of block number and task on these block values were evaluated via repeated-measures ANOVAs with pairwise, Bonferroni-corrected post hoc t-tests. PDE and SRT were additionally calculated to reflect task performance. PDE was calculated as the proportion of viable trials in which corrected or uncorrected direction errors were made. SRT is defined as the saccade latency following the presentation of the target stimulus.

The pupil constriction effect was calculated as pupil area change from fixation point appearance to maximum constriction. The latency from fixation point appearance to the point of maximum constriction was additionally calculated. Pupil dilation effect was calculated as pupil area change from maximum constriction to the end of the fixation period. To evaluate the influence of block and task on these three values, two-way repeated-measures ANOVAs were performed with pairwise, Bonferroni-corrected post hoc t-tests.

Two epochs were considered for EDA, pupil size and heart beat frequency. Pupil responses in the tasks showed considerable divergence at approximately 600 ms post-
fixation point onset, the epoch of 100-600 ms post-fixation point onset was selected to represent task-independent effects (epoch one) because it was from the start of the trial. Epoch one means were calculated from unnormalized data. As task-dependent divergence was most apparent through the 2500 to 3000 ms epoch, this epoch (epoch two) was selected to reflect task effects. Twenty-nine participants were included in the statistical analysis. Repeated measures, two-way ANOVAs with pairwise, Bonferroni-corrected post hoc t-tests were performed to assess the effects of block number and task on EDA and pupil size in each epoch, in RMSSD over the fixation period and PE and SRT. To evaluate the coordination of autonomic indices and their association with performance, bivariate Kendall’s tau-b correlations were conducted between RMSSD, epoch two pupil size and EDA, PE, and SRT throughout anti-saccade and pro-saccade trials.
Figure 4. Task design. (A) Schematic of experimental paradigm presented to participants on the visual screen. Each trial began with the appearance of a central fixation point (0.5° diameter) on a black background. After 3000 ms, a blank black screen was presented for 200 ms. A target was then presented 10° right or left of centre for 1000 ms. Trials were separated by a variable intertrial interval of approximately 2000 ms. (B) Time course of trial events. Two epochs were selected for pupil analysis: epoch one, 100-600 ms post fixation point appearance and epoch two, 2500-3000 ms post fixation point appearance.
2.3 Results

2.3.1 Pupil size

Pupil dynamics across the trial can be seen in Fig. 5. Following fixation point appearance, the pupil constricted to reach a minimum size after 500-800 ms post fixation point appearance. This initial constriction was largely attributed to the pupillary light reflex. The same pattern was observed for each block of trials but the magnitude of the response changed across blocks. Average maximum constriction was reached at 571 ms (SD=489) post fixation point appearance for pro-saccade trials, and 714 ms (SD=566) post fixation point appearance for anti-saccade trials. In addition to task variation, maximum constriction latency varied according to block (Fig. 6). A two-way repeated-measures ANOVA was performed with block and task as independent variables to investigate differences in the latency of maximum trial constriction. A significant effect of block was revealed, $F(4,25)=42.9, p=.000$. Pairwise comparisons between the blocks showed that block one maximum constriction latency ($M=1039, SD=728$) was significantly greater than those of blocks two ($M=688, SD=447$) ($p=0.006$), three ($M=607, SD=472$)($p=.000$), four ($M=512, SD=387$)($p=.000$) and five ($M=519, SD=364$) ($p=.000$).

Pupil area change from the time of fixation point appearance to maximum constriction also varied with task and block number (Fig. 7A). A two-way repeated-measures ANOVA was performed with block and task as independent variables to investigate differences in constriction size. Significant effects of block, $F(4,25)=5.36, p=.003$ and task $F(1,28)=20.3, p=.000$ were found. Pairwise comparisons between the blocks showed that block four constriction size ($M=-100, SD=70.4$) was significantly
greater than that of block two \((M=-138, SD=94.5)\) \((p=.023)\). Pairwise comparisons between tasks showed that constriction size was greater in pro-saccade trials \((M=-109, SD=80.9)\) as compared to anti-saccade trials \((M=-138, SD=96.6)\) \((p=.000)\).

Following maximum pupil constriction, pupil size gradually increased at similar rates in blocks one to four until the end of the fixation period (Fig. 7B). Block five pupil size however, increased more rapidly than those of earlier blocks. A repeated-measures two-way ANOVA, with block number and task as independent variables was performed to evaluate their influence on the difference in pupil size from the point of maximum pupil constriction to the end of the fixation period. No significant effects were found. In anti- and pro-saccade trials, pupil size increases following maximum constriction at approximately the same rate (Fig. 5C).

To evaluate increases in arousal related to block number independently of task, a two-way repeated-measures ANOVA was performed with block number and task and independent variables for epoch one pupil size. A significant effect of block was found, \(F(4,25)=4.60, p=.006\). Pairwise comparisons between the blocks showed that block two pupil size \((M=1607, SD=594)\) was significantly greater than that of block one \((M=1772, SD=481)\) \((p=.006)\).

Block and trial effects on epoch two pupil size can be seen in Fig. 8A. Pupil size values increased over blocks one to three and plateaued for the duration of the experiment. A two-way repeated-measures ANOVA was performed considering epoch two pupil size to evaluate task and block effects on pupil size. Significant effects of block \(F(4,25)=7.49, p=.000\) and task \(F(1,28)=10.73, p=.003\) was revealed. Pairwise comparisons between the blocks showed that, block one pupil size \((M=-51.840, SD=32.4)\)
SD=56.6) was significantly smaller than those of blocks three (M=-5.43, SD=53.9) (p=.004), four (M=-8.53, SD=40.7) (p=.000), and five (M=-5.73, SD=54.6) (p=.000). Additionally, block two pupil size (M=-35.10) was significantly smaller than those of blocks three (p=.007), four (p=.035), and five (p=.006). Pairwise comparisons between tasks showed that pupil size was significantly greater in pro-saccade trials (M=-10.51, SD=56.5) as compared to anti-saccade trials (M=-32.0, SD=54.6) (p=.003). These findings suggest that an increased in arousal related to time-on-task over exists, however this effect is isolated to the first three blocks of the experiment. They further suggest that the pro-saccade task is associated with greater levels of arousal.

2.3.2 Electrodermal activity

EDA dynamics across the trial can be seen in Fig. 9. On average, EDA decreased across the trial in both anti and pro-saccade trials (Fig. 9C). Pro- and anti-saccade trial EDA values decreased together until ~2000 ms post fixation point appearance, at which point they diverged. Following this divergence point EDA continued to decrease in pro-saccade trials while it plateaued in anti-saccade trials. Block effects on EDA differed in anti- and pro-saccade trials. In pro-saccade trials (Fig.9A), from fixation point appearance to ~1500 ms post fixation point appearance, EDA decreased together across all five blocks until ~1500 ms post fixation point appearance at which point EDA block averages diverged. Blocks one and two continued to decrease, blocks three and five plateau while block four increases. In anti-saccade trials (Fig. 9B) EDA block averages remain consistent until a point of divergence ~1000 ms post fixation point
appearance. Beyond this point, EDA decreased in block one and two while the signal remained stationary in blocks three to five.

To evaluate increases in arousal related to block number independently of task, a two-way repeated-measures ANOVA was performed with block number and task as independent variables for epoch one EDA. No significant effects were found.

Block and trial effects on epoch two EDA can be seen in figure 8B. EDA values did not change from blocks one to two however, they increased in block three and plateaued for the duration of the experiment. A two-way repeated-measures ANOVA was performed considering epoch two EDA to evaluate task and block effects on EDA. A significant effect of block was revealed, $F(4,25)=6.70, p=.001$. Pairwise comparisons between the blocks showed that block five EDA ($M=-0.02, SD=0.019$) was significantly greater than that of block one ($M=0.012, SD=0.014$) ($p=.008$). There was also significant interaction of block and task, $F(4,25)=7.03, p=.001$. These findings further support effect of time-on-task on sympathetic activity, limited to the first three blocks of the experiment.

2.3.3 Heart rate and heart rate variability

HR was calculated over each block (Fig. 10A). HR did not appear to change across the experiment, and there was no clear task effect. Further, a repeated-measures two-way ANOVA with block number and task as independent variables was performed to evaluate their influence on HR. No significant effects were found.

RMSSD was calculated over two periods, the 3000 ms fixation period (Fig. 8C) and the entire block (Fig. 10). RMSSD values calculated from trial and block periods
followed the same trend. RMSSD values decreased over the first three blocks plateauing for the remainder of the experiment. When considering trial RMSSD, little task differentiation was seen. A repeated-measures ANOVA with block number as the independent variable was performed for block RMSSD values to evaluate the effect of block number. A significant effect of block was found $F(4, 25)=2.77, p=.049$. Pairwise comparisons between the blocks showed that block one RMSSD ($M=12.8, SD=10.4$) was significantly greater than that of block five ($M=6.58, SD=5.34$) ($p=.019$). For trial RMSSD, repeated measures two-way ANOVA revealed significant effects of block, $F(4, 25)=46.9, p=.000$ and task $F(1,28)=120, p=.000$. Pairwise comparisons between the blocks showed that block five RMSSD ($M=0.804, SD=0.804$) was significantly greater than those of block one ($M=0.230, SD=0.183$) ($p=.000$), block two ($M=0.195$) ($p=2 \times 10^{-12}$), block three ($M=0.193, SD=.151$) ($p=.000$) and block four ($M=0.196, SD=.136$) ($p=.000$). Pairwise comparisons between tasks showed that RMSSD was significantly greater in anti-saccade ($M=0.431, SD=0.526$) as compared to pro-saccade trials ($M=0.216, SD=0.170$) ($p=.000$). Furthermore a significant interaction of block and task was found $F(4, 25)= 33.0, p=.000$.

These findings suggest that a decrease in parasympathetic activity related to time-on-task may exist, limited to the first three blocks of the experiment. They further suggest that the pro-saccade task may be associated with lower levels of parasympathetic activity.
2.3.4 Performance

Task and block effects on saccade performance measures can be seen in Fig. 11. Both SRT (Fig. 11A) and PDE (Fig. 11B) are distinctly higher in anti-saccade trials however, there is no clear block effect. Repeated measures two-way ANOVAs with task and block number as independent variables were performed to evaluate their influence on PDE and SRT. For PDE, repeated measures two-way ANOVA revealed a significant effects of task, $F(1,28)=81.4, p=.000$. Pairwise comparisons between tasks showed that PDE was significantly greater in anti-saccade trials ($M=27.0, SD=3.98$) as compared to pro-saccade trials ($M=5.94, SD=16.2$) ($p=.000$). There was also a significant interaction of task and block $F(4,25)= 4117, p=.012$. For SRT, a repeated measures two-way ANOVA revealed a significant effect of task, $F(.28)=248, p=.000$. Pairwise comparisons between tasks showed that SRT was significantly greater in anti-saccade ($M=259, SD=44.5$) as compared to pro-saccade trials ($M=177, SD=35.0$) ($p=.000$). There was also a significant interaction of task and block, $F(4,25)= 5.24, p=.003$. These findings suggest that the anti-saccade task is associated with poorer saccade performance. No effect of time-on-task however was found.

2.3.5 ANS correlations

Bivariate Kendall’s tau-b correlations were run to determine the relationship between RMSSD, epoch two pupil size and epoch two EDA values during pro-saccade and anti-saccade trials. Pupil size and EDA were significantly positively correlated across pro-saccade trials ($\tau_b = .265, p = .045$) while all other correlations across both pro- and anti-saccade trials failed to reach significance.
2.3.6 ANS and performance correlations

Bivariate Kendall’s tau-b correlations were run to determine the relationship between autonomic indices (HRV, EDA and pupil size) and performance on anti and pro-saccade tasks. No significant correlations were found between autonomic indices and SRT or PDE on anti-saccade or pro-saccade trials.
Figure 5. Pupil trial dynamics. (A) Average pupil size across pro-saccade trials in each block. (B) Average pupil size across anti-saccade trials in each block. (C) Average pupil size across anti and pro-saccade trials. Pupil area values were baseline corrected with reference to pupil area at the time of fixation point appearance.
Figure 6. Maximum constriction latency. Red circles represent values associated with anti-saccade trials. Blue circles represent pro-saccade trial values. Open circles represent subject average values. Filled circles represent overall average values.
Figure 7. Constriction (A) and dilation (B) size. Constriction size was calculated as pupil area change from fixation to maximum constriction. Dilation size was calculated as pupil area change from maximum constriction to end of fixation. Red circles represent values associated with anti-saccade trials. Blue circles represent pro-saccade trial values. Open circles represent subject average values. Filled circles represent overall average values.
Figure 8. Task effect on autonomic measures. Average (A) baseline corrected pupil area from 2500-3000 ms post-fixation point appearance (B) baseline corrected electrodermal activity from 2500-3000 ms post-fixation point appearance and (C) RMSSD values. Red items represent anti-saccade trial values. Blue items represent pro-saccade trial values. Unfilled circles represent participant averages. Solid circles represent overall average values.
Figure 9. Electrodermal activity trial dynamics. (A) Average EDA across pro-saccade trials in each block. (B) Average electrodermal activity across anti-saccade trials in each block. (C) Average electrodermal activity across anti- and pro-saccade trials. Electrodermal activity values were divided by the standard deviation of the block and baseline corrected with reference to the average electrodermal activity value calculated from 50 ms prior to fixation point appearance.
Figure 10. Block effect on (A) heart rate and (B) heart rate variability. Unfilled circles represent participant averages. Solid circles represent median values.
Figure 11. Saccade performance. (A) Saccade reaction time. (B) Percent direction error. Red items represent anti-saccade trial values. Blue items represent pro-saccade trial values. Open circles represent participant averages. Filled circles represent overall median values.
2.4 Discussion

In the present study the sympathetic and parasympathetic modulation of voluntary and automatic task performance was investigated. Changes in sympathetic and parasympathetic activity and performance with time-on-task and their relationship were also evaluated. Lastly, the association of autonomic measures was investigated to test the assumption of their coordination by autonomic drive. Eye movements, pupil size, EDA and ECG were concurrently measured as participants completed a five block, 45 minute long, interleaved anti- and pro-saccade task. Task was found to influence levels of arousal with pupil size and HRV reflecting this modulation. Furthermore, across the first three experimental blocks, parasympathetic activity decreased in conjunction with increases in sympathetic activity. Performance however, was unaffected by block number and unrelated to autonomic activity. Nonetheless it was affected by task. Lastly, EDA and pupil responses were found to be coordinated in pro-saccade trials.

2.4.1 Block effect on arousal

A trend of increasing arousal was seen across the first three blocks of the experiment. Significant effects of block were found on HRV (calculated as RMSSD), pupil size and EDA. EDA increased over the first three blocks, reflecting an increase in sympathetic activity. HRV decreased over the first three blocks, reflecting a decrease in parasympathetic activity. Further, pupil size increased over the same period, reflecting an increased in the ratio of sympathetic:parasympathetic activity, determining arousal.
From this we can conclude that time-on-task influenced arousal, resulting in increases over the first three blocks.

Time-on-task has previously been linked to increases in arousal. In 2013, Benikos found that skin conductance level increased over a thirty minute Go/Nogo task training session. Tanaka et al. (2009) has further demonstrated increases in low frequency HRV component power (sympathetic index) and decreases in high frequency HRV component power (parasympathetic index) across a thirty minute 2-back task session.

It has been suggested that skin conductance elevations reflect the effortful mobilisation of mental resources, directed towards a task (Dawson et al., 1990). The increase in arousal over the first few blocks could be related to increases in participant effort to maintain an alert state. Moreover, in a feedback form, several participants reported struggling to maintain alertness throughout the experiment in the general feedback section. Further, the amount of participant effort to maintain alertness may have reached a ceiling during block three.

A plateauing of autonomic response changes has previously been reported by Mehler, Remier, Coughlin and Dusek (2009). They investigated changes in HR and skin conductance over time in three difficulty conditions, involving the completion of 0-back, 1-back or 2-back tests and reported significant effects of condition on HR and skin conductance. HR increased throughout each of the sensory load states with significant increases between 0-back and 1-back and 1-back and 2-back conditions however, additional HR changes between the 1-back and 2-back condition were modest. When considering skin conductance increases between the 0-back and 1-back condition
reached significance however, increases between the 1-back and 2-back condition failed to reach significance. When considering the response curve, a clear increase in arousal is seen when comparing the lower (0-back) and medium difficulty (1-back) conditions. Little or no change is seen in HR and skin conductance however, when comparing the medium and high (2-back) difficulty conditions. They suggest that during the 1-back condition, participants reached a threshold relative to the amount of additional effort that they were willing or able to invest.

This concept can be applied in the context of this experiment. Over the first three blocks participants may have increased their level of effort to maintain a state of alertness and task focus. By the end of block three however, participants may have provided the maximum amount of additional effort that they are willing or able to invest, resulting in the subsequent plateau of autonomic measures over the remaining two blocks. This possibility could be investigated in future studies through the evaluation of participant fatigue at regular intervals throughout the experiment.

An additional possibility is that the initial three block trend in arousal could be related to stress associated with the task, compounded by the lack of awareness of the remaining duration of the experiment. This effect may have reached its ceiling during block three. This possibility is strengthened by participant feedback at the end of the experiment. A question was added to a participant feedback form for the last 12 participants asking them to rank how stressful they found the experiment from 1-5. A majority of participants rated the experiment three or above indicating that they found the experiment stressful. This possibility could be better investigated in future studies by collecting participant stress level feedback throughout the experiment.
2.4.2 Task effect on performance

A clear task effect was reflected in both performance measures, SRT and PDE. Anti-saccade trials were associated with poorer performance reflected in longer reaction times and higher error rates as compared to pro-saccade trials. These findings align with many previously published results (e.g. Munoz & Everling, 2004; Pratt & Trottier, 2005). Correct performance of the pro-saccade task requires the generation of an automatic saccade. To correctly perform the anti-saccade task however, an automatic saccade must be suppressed and a voluntary saccade in the opposite direction must be generated.

Most direction errors on the anti-saccade task are result of failure to suppress the visual grasp reflex. Munoz and Everling (2004) found that most anti-saccade direction errors occur in the express saccade period. Further, after a short intersaccadic interval, most direction errors are corrected. Moreover, the suppression of the visual grasp reflex, which generates unwanted automatic responses in anti-saccade trials, is associated with fixation-related activity, in turn linked to top-down inhibition (Everling, Dorris, Klein, & Munoz, 1999). Top down inhibition is further required before stimulus appearance during the fixation period for correct anti-saccade task performance (Everling, Spantekow, Krappmann, & Flohr, 1998). Insufficient fixation-related activity before stimulus appearance therefore contributes to direction errors in anti-saccade trials.

Human functional magnetic resonance imaging (fMRI) studies have shown that greater FEF activation is associated with preparation for anti-saccades as compared to pro-saccade preparation (Connolly et al., 2002; DeSouza et al., 2003). Furthermore,
SRTs are negatively correlated with this preparatory activity (Alahyne et al., 2014; Connolly, Goodale, Goltz, & Munoz, 2005) which facilitates the generation of anti-saccades. These findings have also been demonstrated in monkey single neuron recordings. Similarly, higher pre-stimulus fixation-related activity was found before anti-saccades, compared to pro-saccades in the superior colliculus and FEF (Everling & Munoz, 2000; Munoz & Everling, 1998). Further, levels of pre-stimulus activity in these studies have also been found to negatively correlate with SRTs (Everling et al., 1999, Everling & Munoz, 2000).

2.4.3 Time-on-task, arousal and performance

While time-on-task was associated with increases in arousal, no effect on performance was found in this experiment. Block number, an indicator of time-on-task, influenced pupil size over both pre-trial effect (100-600 ms post fixation point appearance; epoch one) and trial effect (2500-3000 ms post fixation point appearance; epoch two) epochs, EDA over the trial effect epoch and RMSSD calculated over the entire fixation period. No effect of block on either of the performance measures, PDE and SRT, however, was found, indicating that participants did not experience mental fatigue. Further, no significant correlations were found between autonomic indices and performance measures. This lack of correlation may indicate that arousal and performance were not linked throughout this experiment. Alternatively, the absence of this link may be related to the lack of sensitivity of the measures.

These findings strengthen those of Tanaka et al. (2009) who reported time-on-task autonomic effects unaccompanied by performance effects. In 2009, they found that
high frequency component HRV power (parasympathetic index) decreased while low frequency component HRV power (sympathetic index) increased across a thirty minute 2-back task session, which involved working memory. Neither accuracy nor reaction time however significantly changed. Furthermore, effects of time-on-task on arousal have been reported independently of performance investigation. Mizuno et al., 2011 demonstrated significant changes in high frequency HRV frequency component power and the ratio of low frequency: high frequency component power (sympathetic measure) after 8 hours of cognitive tasks.

These findings however run contrary to those of Gergelyfi et al. (2015). They found that performance on a working memory task decreased with mental fatigue induced by sudoku task completion over 120 minutes. They further reported accompanying changes in arousal indexed by HRV. A notable distinction between the present study and that of Gergelyfi however is experiment duration. The present study involved an experiment duration of 45 minutes while that of Gergelyi took 120 minutes. Furthermore, the 45 minute experiment duration may not have been sufficient to induce cognitive fatigue. Future studies involving various experiment durations including a 120 minute condition could be performed to investigate the presence of a time threshold for the appearance of cognitive fatigue.

2.4.4 Coordination of peripheral autonomic indices

Neural control of the autonomic nervous system originates centrally in the hypothalamus which acts as an integrator for autonomic function, receiving inputs from upstream cortical areas (Cechetto & Chen, 1990, Ulrich-Lai & Herman, 2009).
Considering their common central control, it could be expected that the activity of peripheral autonomic indices such as HRV, EDA and pupil size would be coordinated (Fig. 12A). Previous studies however, have inconsistently reported relationships among these peripheral indices. In the present study, the relationships of these peripheral autonomic indices were evaluated to explore whether a common underlying neural process drove these responses. A single correlation reached significance, that of EDA and pupil size in pro-saccade trials. Previously, correlations of this nature have been interpreted as an indication of the role of sympathetic drive in observed differences. In 2008, Bradley et al. recorded HR, skin conductance and pupil size during affective picture viewing. They reported effects of image valence on HR, skin conductance and pupil responses, indicating that certain conditions were associated with higher levels of arousal. They further found that pupillary changes co-varied with skin conductance and no covariation of pupil size and HR. Considering these findings, they concluded that the sympathetic nervous system modulates changes in arousal the context of affective picture viewing. Considering the findings of this study, it could similarly be concluded that the sympathetic nervous system modulates changes in arousal in the context of the interleaved anti- and pro-saccade task (Fig. 12B).

An alternate interpretation of these data involves the presence of an additional influence or influences on the HRV signal. EDA and pupil size may be coordinated by the sympathetic component of autonomic drive however condition-related changes in arousal may not be driven specifically by changes only in sympathetic activity. This option is supported by observed task effects on autonomic indices. A task effect was found with respect to HRV and pupil size however, no effect was found with respect to
EDA. Pro-saccade trials were associated with larger pupil size and less HRV, both indicating a general increase in arousal. The existence of this effect in HRV and pupil size measures, to the exclusion of EDA, suggest that these changes were mediated by decreases in parasympathetic activity in pro-saccade trials. EDA is the only index used in this experiment which independently reflects the activity of the sympathetic nervous system. The lack of trial effects in the presence of block effects further suggests that the changes in sympathetic activity may not have been responsible for observed task effects on pupil size. Furthermore, RMSSD, here used to reflect HRV, has previously been correlated with the high frequency component of HRV and is an accepted index of vagal activity (Kleiger, Stein & Bigger, 2005). The existence of task effects on this measure further indicates that changes in parasympathetic activity may be responsible for related changes in pupil size and general task effects (Fig. 12C). Nevertheless, it is unclear whether task-related effects on arousal are mediated by changes in sympathetic and or parasympathetic activity. This question should be explored in future studies. Moreover, additional analysis of the current data may further elucidate this question. One element which may provide further clarity is the consideration of the ratio of low frequency: high frequency power HRV or its time domain surrogate, the standard deviation of normal to normal R-R intervals:RMSSD ratio. This measure of sympathovagal balance may indicate whether task related changes in HRV are related to increased sympathetic influence.

An additional interesting element of the correlation of pupil size and EDA is its isolation to pro-saccade trials. This isolation may be associated with observed task effects on arousal. A higher level of arousal was associated with pro-saccade trials. This
finding aligns with the task bias on autonomic correlations. Moreover, arousal dependent relationships between autonomic indices have been previously reported. In 2015, Wass et al. found an effect of arousal on the association of autonomic measures. EDA was only significantly related to HR and pupil size when arousal levels were high.

2.5 Conclusion

An influence of task on arousal has been established within a typical oculomotor experiment. The present results however, do not provide a clear indication of predominant sympathetic or parasympathetic modulation of these effects. The existence of a correlation between EDA and pupil size in the absence of significant relationships with HRV found only in pro-saccade trials may suggest that changes in sympathetic activity drive observed task differences in arousal. The lack of task effects on EDA in the presence of effects on HRV and pupil size however, support the possibility that changes in parasympathetic activity were responsible for observed effects on arousal. In this case, a parallel non-autonomic influence on HR may explain the absence of correlations of HRV with pupil size.

Arousal was also affected by time-on-task, reaching a ceiling midway through the experiment. This modulation of arousal however, was not found to be related to performance which was unaffected by block number. A task effect on performance however, was found replicating the findings of previous studies. Considering the lack of performance effect of time-on-task and its absent relationship with arousal, the development of cognitive fatigue with the use of longer experimental periods, up to 45 minutes should not be of concern.
Figure 12. Sympathetic and parasympathetic control of autonomic indices. (A) Autonomic control of peripheral autonomic indices. (B) Task-related changes driven by increased sympathetic drive. (C) Task-related changes driven by decreased parasympathetic drive. Red arrows represent sympathetic influence. Blue arrows represent parasympathetic influence.
Chapter 3

General Discussion

ANS activity is constantly changing in response to the environment allowing it to maintain homeostasis throughout the human body. Links between sympathetic and/or parasympathetic activity and performance on cognitive and motor tasks have been previously demonstrated. Sympathetic and parasympathetic modulation of voluntary and automatic behaviours, however, have not been investigated. The interleaved anti- and pro-saccade task facilitates the study of voluntary and automatic behaviours, respectively. Additionally, time-on-task has previously been associated with the development of cognitive fatigue, reflected in performance deficits, as well as changes in arousal. Further, autonomic activity and performance have previously been linked. Moreover, the coordination of peripheral autonomic indices, reflecting changes in arousal have been inconsistently reported.

The aims of this study were to use concurrent measurement of EDA, pupil size and HRV throughout a five-block, 45 minute interleaved anti- pro-saccade task to investigate: (1) sympathetic and parasympathetic modulation of voluntary and automatic task performance, (2) the effect of time-on-task on sympathetic and parasympathetic activity and performance and (3) investigate the association of autonomic measures.

The results indicated that an influence of task on arousal exists within a typical oculomotor experiment. They do not provide a clear indication of predominant sympathetic and parasympathetic modulation of these effects however. The overall absence of significant relationships of HRV with other autonomic indices together with the selective presence of a correlation between EDA and pupil size in pro-saccade trials
may indicated that changes in observed task differences in arousal were driven by changes in sympathetic activity. The absence of task effects on EDA together with the existence of these effects on pupil size and HRV, however, support the role of modulation of parasympathetic activity in driving these changes. The effect of time-on-task on arousal over the first three blocks were unrelated to performance which did not change throughout the experiment.

3.1 Summary of thesis aims and related findings

**Aim 1:** Investigate the modulation of voluntary and automatic task performance by the sympathetic and parasympathetic branches of the autonomic nervous system.

**Finding 1:** A task effect on arousal was demonstrated. The role of sympathetic and parasympathetic nervous systems in driving this effect is unclear.

**Aim 2:** Evaluate the association of pupil size, sympathetic and parasympathetic measures in anti and pro-saccade trials.

**Finding 2:** The only significant association found occurred between electrodermal activity and pupil size and was isolated to pro-saccade trials.

**Aim 3:** Investigate the influence of time-on-task on arousal and performance.

**Finding 3:** Significant effect of block number on arousal were found. No effect on performance however was demonstrated.
3.2 Implications for fMRI analysis

Effects of block and task on autonomic activity hold implications for the analysis and interpretation of blood oxygenation level-dependent (BOLD) signals in future functional magnetic resonance imaging studies utilizing this experimental paradigm. Physiological factors mediate the transfer function between neural and BOLD fluctuations (Logothetis, 2008) and play important roles in interpretation of BOLD data. These factors represent potential confounds. The impacts of physiological noise sources on the BOLD signal are being actively researched (e.g. Bianciardi et al., 2009). Physiological parameters reflecting ANS activity represent one of the main external factors known to covary with BOLD measurements. The correlation of functions such as perspiration, HR and blood pressure with the BOLD signal have been established in the literature (Lund, Madsen, Sidaros, Luo & Nichols, 2006). Further, the impact of these factors may increase in higher field strengths (Triantafyllou et al. 2005). Considering this, unless these factors are appropriately controlled for, higher strength measurements may provide a marginal advantage (Iacovella & Hasson, 2011).

3.3 Autonomic dysfunction in clinical populations

An additional obvious extension of this work is the study of patterns of autonomic activity in the context of the interleaved anti-pro-saccade task in clinical populations. Following a more thorough investigation of autonomic control of performance in the interleaved anti-pro-saccade task, the understanding of relationships in healthy control populations can be compared to those of different clinical populations. The inclusion of
autonomic activity recording in experiments involving interleaved anti- and pro-saccade tasks may help elucidate the source of behavioural differences from control groups. This comparison may inform the understanding of underlying disease processes along with the development of biomarkers. Autonomic dysfunction is being identified in an increasing number of clinical populations. A few examples of disorders in which autonomic dysfunction has been studies include parkinson’s disease (Siddiqui, Rast, Lynn, Auchus & Pfeiffer, 2012), multiple sclerosis (Haench and Jorg, 2006), anorexia (Kreipe, Goldstein, DeKing, Tipto & Kempski 1994), depression (Bajko et al., 2012) and type one diabetes (Rosengar-Barlund et al., 2009).

3.4 Considerations related to the extended fixation period

While the extended fixation period of the interleaved anti- pro-saccade task used in this experiment was essential for the capture of EDA clean responses that take up to three seconds to fully develop, it reduced the applicability of these finding to those of previous interleaved anti- and pro-saccade results. The extended fixation period may have altered preparatory effects initially reported in Wang, Brien and Munoz (2015). Saccades with faster reaction times were found to accompany larger pupil dilation prior to stimulus appearance. Here, we found no correlation between pupil size and SRT. Another consideration which may have affected this relationship however is the data analysis approach. Wang et al. (2015) compared pupil size and SRT on a trial-by-trial basis. All correlations performed in this thesis however, were performed using participant average values which may have affected the findings and reduced the
strength of these results. Evaluation of this and future data using trial-by-trial analysis may strengthen these findings.

3.5 Compounded trial effects and response latencies of autonomic effectors

The extended 3 s fixation period may not have captured the complete autonomic task effects. The latencies of responses seen within each of the peripheral autonomic measures are distinct. Pupil response latencies to fixation point appearance in the context of the interleaved anti and pro-saccade task have been previously reported. Initial constriction responses have been seen within 400 ms of fixation point appearance. Further, changes in HRV induced by sympathetic and parasympathetic activity occur at distinct latencies. Changes in vagal firing rate occur after a negligible delay inducing changes in beat to beat activity. Changes in cardiac sympathetic outflow however induce changes after a significant time delay of 1.7 s, as estimated in dogs by Berger et al. (1989) with a rapidly decreasing gain. EDA has been associated with response latencies similar to those related to cardiac sympathetic outflow, however recovery of baseline values appear to be longer. In 2012, Zhang et al. evaluated changes in skin conductance in the context of Go and Nogo task trials. They found that skin conductance responses occurred 1-3 s post stimulus onset however, skin conductance was still elevated from baseline 10 s post stimulus onset. Considering that each trial runs for 4.2 s followed by an intertrial interval of approximately 2 s, it is likely that changes in skin conductance from at least one previous trial will be compounded, shaping the observed trial changes. While previous trial effects are minimized by the existence of an approximately equal ratio of anti and pro previous trials, these
compound influences could be parsed out through analysis of one-back, two-back and three-back previous trial effects. It has further been suggested that repetition effects can be dealt with in the framework of a linear convolution model (Bach, Flandin, Frison & Dolan, 2010). Nevertheless, it may be beneficial to evaluate these effects in future studies.

3.6 Influence of task irrelevant stimuli

One feature of peripheral autonomic indices which makes them especially useful in research is their sensitivity to environmental changes. This same sensitivity makes recordings susceptible to confounding effects from small changes in the experimental environment. These include street noises such as loud honking, the physical presence of an individual and the delivery of instructions. During the setup process in this experiment skin conductance recordings were presented on a screen. Jumps in skin conductance were noted following the entry of the experimenter, interaction from beyond an opaque barrier and sudden street noise. In this experiment, during the EDA calibration process the experimenter waited for a stable baseline value to be established. This process was protracted by repeated entries of the experimenter into the separate space in which the participant was seated. As a result, throughout piloting, the setup was altered to facilitate the calibration of this recording from the other side of the barrier. These effects should be considered in future studies. Efforts should be taken to reduce interference by outside noises and human interaction when possible.
3.7 Uncontrolled participant factors

Multiple factors are understood to influence autonomic function. Caffeine and nicotine are two examples relevant to this experiment. Caffeine consumption has been shown affect ANS responsivity (Zahn & Rappaport, 1987). Further, nicotine has been shown to broadly affect the autonomic nervous system. In habitual smokers, smoking has been shown to completely reset vagally mediated arterial baroreceptor-cardiac reflex responses and reduce baseline vagal cardiac nerve activity (Niedermaier et al., 1993). Additionally, sympathomimetic, sympatholytic, parasympathomimetic and parasympathetolytic drugs by definition affect autonomic activity. The use of these substances was not controlled for and may have influenced the results. It has been recommended that caffeine and nicotine be withheld for 24-48 hours and anticholinergics (parasympatholytic medications) be withheld 48 hours before testing (Jaradeh & Prieto, 2003). These recommendations should be considered in future studies. The requirement of caffeine abstinence however, may make participant recruitment more difficult.

3.7 Sampling effects

The sample use in this study may have biased the results. Further, the limited diversity of this sample limits the generalizability of these findings. This study only included staff and students from Queen’s University, a majority of which were in their early 20s. The recruitment a more diverse group of participants may strengthen future studies. If this increased diversity extends to age however, reports of changes in sympathetic
modulation of HRV with age should be considered (Yeragani, Srinivasan, Vampati, Pohl & Balon, 1993). The influence of this factor is further supported by significant declines in sympathetic and parasympathetic responses associated with increasing age group (Parashar, Amir, Pakhare & Chaudhary, 2016).
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