CORTICAL AND SUBCORTICAL CONTRIBUTIONS TO STRESS-INDUCED MUSCLE ACTIVITY IN THE UPPER TRAPEZIUS AND RELATIONSHIPS TO CHRONIC NECK PAIN

by

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Thesis directed by Associate Professor Katrina S. Maluf

ABSTRACT

Psychosocial stress is known to induce increased muscle activity in the upper trapezius. This stress-induced activity is commonly experienced and thought to contribute to chronic neck pain. Mechanisms behind this phenomenon, however, are currently unknown. The primary aim of this thesis was to investigate neurophysiologic contributions to stress-induced upper trapezius muscle activity and how some of these relate to chronic neck pain. The first two studies investigated cortical (Aim 1) and subcortical (Aim 2) mechanisms contributing to stress-induced upper trapezius muscle activity in a laboratory setting. A standardized protocol manipulating psychosocial stress was implemented during neurophysiologic testing. In all participants, responsiveness of the corticospinal tract increased during the stress protocol. Only healthy individuals had a corresponding increase in intracortical inhibition, whereas individuals with neck pain did not. Aim 2 found that the majority of healthy individuals receive inhibitory input to the upper trapezius from the reticulospinal tract, though individuals with high trait anxiety receive some excitatory input. The overall input from the reticulospinal tract decreased during exposure to the psychosocial stress protocol.

Aims 3 and 4 investigated relationships between autonomic nervous system (ANS) activity, upper trapezius muscle activity, and chronic neck pain development in ecologically valid settings in office workers via portable data monitoring. ANS activity
was assessed through investigation of heart rate variability (HRV). While no relationship was found between muscle and ANS activity, upper trapezius muscle activity was significantly greater on days where participants reported higher compared to lower levels of perceived anxiety. Finally, Aim 4 sought to investigate the contribution of ANS activity, independent of muscle activity, to the development of chronic neck pain, and found that HRV measures did not predict the development of chronic neck pain.

Overall the work on this dissertation shows that multiple mechanisms may contribute to the phenomenon of stress-induced upper trapezius muscle activity, and this activity exists in ecologically valid settings. The results of this thesis will help direct future healthcare for chronic neck pain, both in terms of what physiologic mechanisms to target, as well as when to implement these interventions, either for prevention or treatment of chronic neck pain.

The form and content of this abstract are approved. I recommend its publication.

Approved: Katrina S. Maluf
DEDICATION

This dissertation is dedicated to my wife, Micaela Marker. I would not be here, and this thesis would not exist, without your support.
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CHAPTER I

INTRODUCTION

Psychosocial stress, defined as a disruption in homeostasis caused by actual or perceived adverse emotional threats, has long been suspected of altering the way in which a human moves. Often thought of as a component of the “fight-or-flight” response, these stress-induced changes in movement (i.e. a tense muscle, a trembling hand, or a shaking knee) are commonly experienced, though the physiologic mechanisms underlying them are not always known. Specifically, psychosocial stress has been shown to increase muscle activity in the upper trapezius, a cervical muscle commonly associated with chronic neck pain. This stress-induced muscle activity has been hypothesized to contribute to chronic neck pain, either by causing it to develop, or perpetuating present pain. Despite this association, and the high prevalence of chronic neck pain, the mechanisms underlying stress-induced muscle activity in the upper trapezius are unknown. Quantification of this phenomenon in a real-world setting is also lacking.

The overall purpose of this project is to investigate different mechanisms that may contribute to stress-induced muscle activity in the upper trapezius, in laboratory and real-world settings, and also look at the relationship of some of these mechanisms to chronic neck pain. Aims 1 and 2 will look at cortical and subcortical contributions to stress-induced muscle activity in a laboratory setting. Aim 3 will investigate relationships between upper trapezius muscle activity, autonomic nervous system (ANS) activity, and perceived anxiety in a real-world setting. Finally, Aim 4 will investigate the contribution of ANS activity to the development of chronic neck pain.
Aim 1

To investigate changes in corticospinal excitability and intracortical inhibition in the primary motor cortex in individuals with and without chronic neck pain during exposure to low and high levels of psychosocial stress.

H1: All participants will show an increase in corticospinal excitability during exposure to high levels of psychosocial stress.

H2: Healthy individuals will have a compensatory increase in intracortical inhibition in the primary motor cortex during exposure to high levels of psychosocial stress, which will not be present in individuals with chronic neck pain.

Aim 2

To investigate changes in reticulospinal input to the upper trapezius during exposure to low and high levels of psychosocial stress in healthy individuals.

H1: All participants will show an increase in reticulospinal input to the upper trapezius during exposure to high levels of psychosocial stress compared to low.

Aim 3

To investigate the relationship between ANS and upper trapezius motor activity during workplace recordings and assess how they change in response to changes in perceived anxiety.

H1: Participants showing indications of decreased parasympathetic and increased sympathetic tone during the workday will demonstrate higher levels of upper trapezius muscle activity.

H2: Participants will demonstrate increased upper trapezius muscle activity and indications of increased sympathetic tone and decreased parasympathetic tone on days
Aim 4

To investigate changes in ANS activity during acute exposure to low and high levels of psychosocial stress and assess whether this change, or ambulatory levels of ANS activity during the workday, are predictive of developing chronic neck pain.

H1: Participants demonstrating indications of greater decreases in parasympathetic tone and increases in sympathetic tone in response to an acute stressor will be more likely to develop chronic neck pain.

H2: Participants demonstrating indications of lower levels of ambulatory parasympathetic and higher sympathetic tone during the workday will be more likely to develop chronic neck pain.

Summary

These studies will provide valuable information on the mechanisms of stress-induced upper trapezius, allowing the development of targeted interventions in patients with any condition where this phenomenon is deemed a problem, not just chronic neck pain. It will provide information on the validity of stress-induced muscle activity in a real-world setting, where it is commonly perceived but rarely quantified. Finally, it will provide information on contribution of ANS activity to stress-induced muscle activity and chronic neck pain development, informing the timing of future interventions; whether they should be implemented as preventative measures, or as interventions for those already experiencing pain.
CHAPTER II
BACKGROUND

The Human Motor System

The human motor system is capable of incredibly complex tasks and is of utmost importance in the human experience. It is movement that allows humans to interact with, and in many ways experience, the world and environment. This is accomplished through the coordinated control of skeletal muscle. Through the summation and interaction of multiple inputs to the muscles the human motor system becomes increasingly complex (Latash 2012). Indeed, despite the importance of the human motor system, our understanding is still not complete and the system continues to be the topic of many investigations. The objective of this section is to give a basic introduction of the human motor system and its primary components. Figure 2.1 shows several major components of the human motor system, and demonstrates the interconnectedness and complexity of the system, even when reduced to primary components.

Signaling in the Motor System

The primary type of signaling cell in the motor system is a neuron. Neurons consist of three main parts: the soma (cell body), dendrites, and the axon. Neurons receive signals at the dendrites, which are transferred to the soma, and new signals are sent along the axon. Signals are generated by electrochemical mechanisms. Electrical signals passed down the axon, called action potentials, are generated through the movement of ions across the axon membrane. At rest, the inside of the neuron has a negative voltage relative to the outside of the cell, typically -70 mV, though this voltage
differs between different types of cells (Kandel, Schwartz et al. 2012). This voltage is maintained through the accumulation of anions within the cell, and a larger concentration of Na\(^+\) ions outside of the cell. Also important to the generation of action potentials is a large concentration of K\(^+\) inside the cell.

The resting membrane potential of the neuron can be depolarized (increased) or hyperpolarized (decreased). Once a specific part of the soma, the axon hillock, is depolarized to a certain threshold, an action potential is initiated and propagated by the movement of ions across the cell membrane, transported through specific ion channels. The initial depolarization triggers the opening of voltage-gated Na\(^+\) ion channels, which allows rapid influx of Na\(^+\) ions into the cell along an electrochemical gradient, resulting in an increase in membrane potential to approximately +30 mV (Kandel, Schwartz et al.)

**Figure 2.1:** Representation of the primary components of the human motor system. Adapted from Figure 7.35 in Enoka (2004).
Slower acting K⁺ ion channels then open, allowing the flow of K⁺ out of the cell along an electrochemical gradient, decreasing membrane potential back towards the initial negative value. After an action potential, the initial resting membrane potential is restored and maintained by Na⁺/K⁺ pumps, which utilize energy to move Na⁺ out of the cell and K⁺ into the cell, maintaining the necessary electrochemical gradients for future action potentials. The action potential travels as a wave of depolarization down the axon to the axon terminal. It is important to note that an action potential is an all-or-nothing phenomenon, with a set amplitude of depolarization. Depolarization beyond the initial threshold at the axon hillock does not result in an increased action potential amplitude, but rather an increase in the rate at which action potentials are generated (Granit, Kernell et al. 1963), which is crucial in the control of skeletal muscles.

Once an action potential reaches the axon terminal, it triggers the release of chemicals called neurotransmitters into the synapse, the space between the axon terminal and the neuron to which the current neuron is signaling, the post-synaptic neuron. Axons most often, but not always, synapse onto the dendrites of the post-synaptic neuron. Each neurotransmitter will bind to a specific receptor on the post-synaptic neuron, typically resulting in the opening of ion channels resulting in either a depolarizing current which travels to the soma and makes the neuron more likely to generate an action potential (excitatory post-synaptic potential (EPSP)) or a hyperpolarizing current, making the neuron less likely to generate an action potential (inhibitory post-synaptic potential (IPSP)) (Eccles 1982). The neuromuscular junction is where a neuron synapses onto a muscle fiber instead of another neuron. Acetylcholine is released by the pre-synaptic
neuron, resulting in the generation of an action potential that travels down the muscle fiber, causing it to contract (Enoka 2008).

The Motor Unit

The fundamental unit of the human motor system is the motor unit. The motor unit is composed of the alpha motor neuron and the muscle fibers it synapses on. The force produced by a muscle at any given moment is controlled by two basic properties of motor units: the number of motor units generating action potentials, or recruited motor units, and the rate at which motor units are generating action potentials, or rate coding (Adrian and Bronk 1929).

Inputs from the central nervous system, or synaptic drive, are mainly integrated at the dendrites and soma of the alpha motor neuron, which are located in the ventral horn of the spinal cord. Inputs to the alpha motor neuron can be excitatory or inhibitory (from presynaptic inputs resulting in post-synaptic EPSPs or IPSPs, respectively), influencing the alpha motor neuron to generate more or fewer action potentials. Action potentials generated by the alpha motor neuron travel down the axon, which projects peripherally to synapse on the muscle fibers of the motor unit. The number of motor units composing all the muscle fibers in a given muscle varies greatly, from approximately 10 to over 1000 (Jenny and Inukai 1983). All the motor units innervating a specific muscle are referred to as a motor pool and the characteristics of motor units within each pool can vary widely (Kernell 2006). These different characteristics influence the recruitment and rate coding within the motor pool to inputs from the rest of the motor system (Heckman and Enoka 2012). One property of alpha motor neurons that contributes greatly to recruitment is the size. Smaller motor neurons are consistently recruited at lower levels of synaptic drive.
with larger motor neurons recruited with increasing synaptic drive, in a fixed order (Denny-Brown and Pennybacker 1938). This orderly recruitment is referred to as the size principle (Henneman 1957). Rate coding is controlled by the level of synaptic input. Once a motor unit is recruited, its discharge rate increases somewhat linearly with increasing synaptic drive (Kernell 1965).

The force produced by a motor unit is highly dependent on the muscle fibers innervated by the alpha motor neuron. The number of muscle fibers innervated by a motor neuron is the largest contributor to the force that can be produced, and ranges from 10 to over a thousand (Enoka and Fuglevand 2001) muscle fibers that all respond to a given alpha motor neuron. An action potential from the motor neuron will cause a single force-producing twitch in the innervated muscle fibers, but as the discharge rate of the motor neuron increases, the forces produced by muscle fiber twitches will summate, increasing the total force produced by the motor unit (Heckman and Enoka 2012).

Several methods exist to classify muscle fibers and motor units, such as force twitch characteristics, histochemical makeup, or molecular properties (Kernell 2006), however, none of these classifications are completely accurate in human motor units, where characteristics exist on a continuum (Heckman and Enoka 2012). All classifications tend to indicate that the first recruited motor units innervate fatigue-resistant muscle fibers that generate lower forces, but that these muscle fibers summate their force twitches at a lower discharge rate, reaching their peak force at lower levels of synaptic drive (Kernell 2006, Heckman and Enoka 2012). The muscle fibers associated with these motor units are also present in a higher percentage in muscles that are used more commonly throughout the day (Monster, Chan et al. 1978).
Referred to as the “final common pathway” by Charles Sherrington (Burke 2007), the motor unit is the integrator of all inputs within the motor system. The following sections briefly describe different sources of inputs within the system that are integrated by the motor unit. Some inputs synapse directly on the motor unit, such as those from muscle spindles or a portion of inputs from the primary motor cortex, though most inputs pass through interneurons within the brain or spinal cord prior to reaching the motor unit. Given the complexity of input integration within interneurons or at the motor neuron, a given input can produce varied responses in muscle activity (Hultborn 2001).

**Peripheral Inputs**

Sensory receptors in skin, joints, and muscles all convey information to the spinal cord, where they may synapse directly or indirectly on spinal motor neurons, initiating a change in motor unit behavior (Brooke and Zehr 2006). One of the most well studied peripheral inputs is the muscle spindle. The human body contains over 27,500 muscle spindles, with a high density of spindles in cervical muscles (Prochazka 1996). Muscle spindles provide information on muscle stretch, both static and dynamic (Matthews 1981). Neurons relaying information from muscle spindles (Ia afferents) are unique in that they synapse directly on alpha motor neurons as well as interneurons and ascending tracts within the spinal cord (Eccles and Lundberg 1958). This monosynaptic connection is excitatory, initiating or increasing the rate of action potential generation at the alpha motor neuron, resulting in an automatic response in the muscle, or a reflex (Enoka 2008). The responsiveness of a muscle spindle to a stretch can be modulated through the activation of gamma motor neurons, which exclusively innervate intrafusal muscle fibers within muscle spindles (Matthews 1981). Therefore, a quick stretch to a muscle will
result in an increase in muscle activity, though this stretch response can be modulated by a variety of other inputs. Ia afferents also synapse on interneurons which send inhibitory signals to motor neurons innervating antagonistic muscles (those producing an opposing motion to the initiating muscle, or the agonist).

Golgi tendon organs are other sensory receptors in the muscle providing information on muscle force, or load. Afferents from tendon organs synapse on interneurons and cause the opposite effect of afferents from muscle spindles, namely inhibition in the agonist muscle and some excitation in the antagonist muscle (Eccles, Eccles et al. 1957). Other sensory receptors providing peripheral input to the motor unit are in the joint and skin (Ruffini endings, Pacinian corpuscles, Golgi endings, Merkel disks, Meissner corpuscles) and synapse on interneurons in the spinal cord, relaying peripheral information to the nervous system and causing complex changes in behavior of motor units. Peripheral nociceptive inputs, contributing to pain perception centrally, also have complex effects on motor control and motor unit behavior, leading to several different theories of underlying mechanisms (Melzack and Wall 1965, Roland 1986, Lund, Donga et al. 1991, Hodges and Tucker 2011). Evidence does exist that acute experimental pain induces central and reflex mediated inhibition of motor unit discharge rate (Farina, Arendt-Nielsen et al. 2004).

Subcortical Inputs

In addition to peripheral inputs, human motor control is greatly influenced by inputs from subcortical areas, largely involved with tasks not consciously initiated. These inputs come from areas such as the brainstem and cerebellum. The cerebellum, not discussed in detail here, is crucial for fine motor control. This area of the brain integrates
peripheral and sensory information, along with information from other motor areas of the brain, to refine precision and coordination of voluntary movement (Fine, Ionita et al. 2002). Also not discussed in detail here, but of great importance to human motor control, is the presence of central pattern generators located in the spinal cord itself. These neuronal circuits are able to take tonic, or sustained, input from the periphery and central motor areas and convert it to a reciprocating or more complex output (Duysens and Van de Crommert 1998). These pattern generators are extensively involved in reciprocating motor tasks, such as gait (Rossignol and Frigon 2011), but may also be involved in more complex tasks (Zehr 2005).

The brainstem contains many nuclei which provide extensive inputs to alpha motor neurons. Some of these nuclei provide what is known as neuromodulatory input, with the primary neuromodulatory neurotransmitters being epinephrine (adrenaline), norepinephrine (noradrenaline), and serotonin. Noradrenergic input is received from nuclei such as the locus coeruleus, subcoeruleus, and the medial and lateral parabrachial nuclei, and serotonergic input is received from the raphe nucleus (Rekling, Funk et al. 2000). Instead of initiating EPSPs or IPSPs in alpha motor neurons, these neuromodulatory inputs alter the intrinsic excitability of the spinal motor neuron membrane, modulating the response to EPSPs and IPSPs from other inputs. Neuromodulatory inputs primarily increase the excitability of spinal motor neuron dendritic membranes through the activation of persistent inward currents (PICs) (Heckman, Lee et al. 2003). Activation of PICs has two main effects on motor unit activity: the amplification of synaptic input, and the initiation of bistable behavior (Heckman 2003). Amplification of synaptic input results in an increased firing rate of the
motor unit to a given synaptic input, and in some motor units a lower recruitment threshold. Bistable behavior refers to the presence of self-sustained firing, or continued action potential generation after the removal of synaptic input (Heckman and Enoka 2012). Neuromodulatory input to the motor unit increases with increased motor output, primarily from the serotenergic system (Jacobs, Martin-Cora et al. 2002), and with increased arousal, primarily from the noradrenergic system (Aston-Jones, Rajkowski et al. 2000). These descending inputs terminate widely throughout the spinal cord (Lemon 2008).

Descending inputs including tectospinal, medial and lateral vestibulospinal, and reticulospinal tracts form the ventromedial brainstem pathway, and have varied influences on motor unit behavior, though are mainly thought to be involved in postural control of the head, neck, and trunk, and proximal limb movements (Lemon 2008). Both the tectospinal and vestibulospinal pathways are involved in maintaining and adjusting head posture in response to stimuli. Tectospinal input adjusts head and neck posture reflexively in response to visual stimulation and assists with the coordination of gaze. The medial and lateral vestibulospinal tracts also assist with coordinating head posture with gaze as well as adjusting posture in relation to gravity (Kandel, Schwartz et al. 2012). Reticulospinal tracts innervate postural muscles and are able to both excite and inhibit these muscles, depending on task (Davidson and Buford 2004, Davidson and Buford 2006). The reticulospinal tract originates from nuclei within the reticular formation. The reticulospinal tract may also play a major role in many complex motor tasks, particularly in non-primate mammals (Lemon 2008), but also in primates, as monkeys have been shown to be able to run, balance, and climb after bilateral lesions of
the corticospinal tract (Rothwell 2006, Lemon, Landau et al. 2012). Respiratory nuclei within the reticular formation may also innervate motor units of respiratory muscles through the reticulospinal tract (Ford and Kirkwood 2006). Finally, the mesencephalic locomotor and diencephalic locomotor regions within the reticular formation project through the reticulospinal tract to central pattern generators associated with locomotion (Shik, Severin et al. 1966).

The basal ganglia are subcortical structures necessary for human movement. Most of the influence on motor control comes via inputs primarily to the motor cortex, but also brainstem structures, that subsequently provide input to alpha motor neurons, instead of directly projecting to the spinal cord (Calabresi, Picconi et al. 2014). The basal ganglia are able to inhibit and disinhibit the motor cortex, playing a major role in the initiation of voluntary movement. Dysfunction in the basal ganglia is the well-known cause behind Parkinson’s disease, which produces a myriad of motor symptoms (Nelson and Kreitzer 2014, Obeso, Rodriguez-Oroz et al. 2014).

**Cortical Inputs**

Cortical inputs in the human motor system are critical in the development, planning, and execution of voluntary movement. The majority of cortical inputs to the spinal cord and motor units arise from the primary motor cortex and descend through the corticospinal tracts, but can also arise from premotor, supplementary motor, and cingulate motor areas (Dum and Strick 2005). The majority of these inputs innervate the contralateral spinal cord and motor units after decussating at the medullary pyramids, forming the lateral corticospinal tract. The remainder of these inputs descend ipsilaterally forming the anterior corticospinal tract. Corticospinal inputs have
significant control over motor unit activity, particularly in humans, where corticospinal neurons synapse directly onto spinal motor neurons as well as spinal interneurons (Lemon 2008). Direct synapses of cortical neurons on spinal motoneurons do not exist in many mammals, such as cats, rats, or mice (Illert, Lundberg et al. 1976, Alstermark, Ogawa et al. 2004, Lemon, Kirkwood et al. 2004). These connections are present to a variable degree in non-human primates (Bortoff and Strick 1993), but are most developed in humans (Rothwell, Thompson et al. 1991, Palmer and Ashby 1992), indicating an increased reliance of cortical control on movement in humans not present in other species (Lemon and Griffiths 2005). The corticospinal tract also serves to control nociceptive, reflex, somatosensory, and autonomic function to some degree (Lemon 2008).

Organization of the primary motor cortex follows the location of the body part controlled by the innervated alpha motor neurons. More distal body parts, such as the wrist and hand, are represented on the more lateral aspects of the primary motor cortex, while more proximal body parts, such as the shoulder, are represented on medial aspects. This somatotopic representation is known as the motor homunculus (Snyder and Whitaker 2013). Within each representation in the primary motor cortex, different populations of pyramidal neurons (neurons with axons projecting down the corticospinal tract) are involved in different muscle actions, or joint motions (Evarts 1966, Georgopoulos, Kalaska et al. 1982). The primary motor cortex itself receives input from many other cortical and subcortical structures (see Figure 2.1), including the basal ganglia, discussed in the previous section, and supplementary motor area, which is involved in complex movement planning and execution (Roland, Larsen et al. 1980). The prefrontal cortex also has projections to the primary motor cortex (Takada, Nambu et al.
which may be primarily inhibitory, involved in muscle relaxation (Rollnik, Schubert et al. 2000, Spraker, Corcos et al. 2009) and response inhibition during choice tasks (Gangitano, Mottaghy et al. 2008). The primary motor cortex also contains interneurons which have both excitatory and inhibitory effects on the pyramidal neurons innervating spinal motor neurons (Ilic, Meintzschel et al. 2002).

**Methods of Measurement**

**Electromyography**

Electromyography (EMG) is one of the most common ways of measuring muscle activity and motor control and is used in a variety of professions (Enoka 2008). EMG recordings are often made utilizing two electrode surfaces which can detect the difference in electrical potential between them. As an action potential travels down a muscle fiber, initiating a force producing muscle twitch, the electrical difference between the two electrodes increases, as the action potential reaches one before the other if electrodes are properly positioned in alignment with the muscle fibers of interest (Freriks, Hermens et al. 1999). The recording of a single muscle fiber action potential is shown in Figure 2.2A.

The type of EMG signal recorded is dependent on the type of electrodes used (Enoka 2008). Two fine, insulated wires can be placed immediately adjacent to muscle fibers within the muscle itself, where the uninsulated tips of the wires act as the two electrodes for EMG recording and detect electrical differences in a very small section of the muscle. This *intramuscular EMG*, shown in Figure 2.2B, results in signals containing the action potentials of a single, or several muscle fibers, representing the activity of the motor units these muscle fibers are a part of. Analysis of intramuscular EMG recordings allows investigations of motor unit recruitment and discharge rate. When electrodes are larger
conducting surfaces placed on the skin over a muscle of interest, the recording area is greatly increased and the resulting EMG signal contains the summation of many muscle fiber action potentials (Sanders, Stalberg et al. 1996, Fuglsang-Frederiksen 2000). This *interference EMG* or *surface EMG* signal, shown in Figure 2.2C, provides a more global measurement of muscle activity.

![Figure 2.2](image)

**Figure 2.2:** Representative recordings of a single motor unit action potential by fine wire electrodes (A), intramuscular electromyography (EMG) (B), and interference EMG recorded by surface electrodes.

Given the larger recording area of surface EMG and the prevalence and characteristics of other electrical signals in the human body, these recordings often contain information not related to the activity of the muscle of interest (Farina, Merletti et al. 2014). For example, the electrical activity generating the contraction of cardiac muscle can travel through subcutaneous tissue and be recorded by surface electrodes, particularly those over trunk muscles (Butler, Newell et al. 2009). One method of detecting and
removing these contaminations is through examination of the frequency content of the surface EMG signal. This can be accomplished by performing a Fast Fourier Transform (FFT) on the signal, which identifies the amplitude of a range of frequencies contributing to the surface EMG signal. Frequencies contributing the most to actual EMG signal usually fall between 20 - 200 Hz (Winter 1990) and those frequencies outside this range, often contaminating factors, can be removed by filtering the signal, or removing most of the contribution to the signal from frequencies above or below given values (Smith 1997). Also, since muscle fiber action potentials are biphasic, containing both positive and negative components, the summation of these action potentials in the interference EMG signal does not always have a linear relationship with the actual amplitude of muscle activation or muscle force (Farina, Merletti et al. 2004, Keenan, Farina et al. 2005). Many different processing techniques are employed to account for this fact, including rectification, root-mean-square processing, and reference contraction normalization. Different summary measures are utilized depending on the purpose of the analysis, including mean amplitude, mean and median frequency of the signal, or Amplitude Probability Distribution Function (APDF) calculations (Merletti and Parker 2004).

Startle Response

It is possible to use EMG recordings to assess the responsiveness of an input to a muscle by eliciting a reflex via providing a stimulus to the given input and recording the evoked muscle activity. For example, the stretch reflex described in a previous section assesses the current responsiveness of muscle spindles by providing a brief, known stimulus (muscle stretch) to the muscle spindles and recording the resulting muscle
activity with EMG recordings. Similarly, the startle reflex, or startle response, can be used to assess the responsiveness of the reticulospinal input to a given muscle. The stimulus required to elicit a startle response is a large amplitude, unexpected, sudden onset sensory stimulus, which can take the form of tactile, visual, or most often auditory sensation (Blumenthal, Cuthbert et al. 2005). This sensory stimulation (from the cochlear nucleus in the case of auditory startle) is transmitted to the nucleus reticularis pontis caudalis (nRPC) in the pontomedullary reticular formation in the brainstem. The giant neurons in this nucleus are the primary initiating neurons of the startle reflex (Yeomans and Frankland 1995). The “startle pattern” is generally characterized as an automated, protective response consisting of forward thrusting of the head and a descending flexor wave extending through the trunk and knee (Landis and Hunt 1939, Nonnekes, Carpenter et al. 2015) transmitted by the medial reticulospinal tract (Valls-Sole 2012). The observed movements associated with this response are a blink, cervical extension, superior and anterior motion of the shoulders, and general shortening of total body length. The onset latency of these responses are highly variable, but generally between 14 – 151 ms, indicating only several synaptic delays (Yeomans and Frankland 1995). Finally, while the blink response during a startle reaction is one of the most commonly experienced and studied responses, evidence exists that this is most likely a separate reflex pathway involving the inferior colliculus and midbrain reticular formation (Valls-Sole 2012).

The startle response has also shown significant interaction with motor processes. Initial study of the startle response has been limited, primarily due to the significant amount of habituation that occurs to a given stimulus quickly after initial presentation (Aniss, Sachdev et al. 1998). This is likely due to modulation of the responsiveness of the
giant neurons in the nRPC by other cortical and subcortical structures (Rothwell 2006, Carlsen, Maslovat et al. 2011). However, the readiness to execute a voluntary movement has been found to facilitate the startle response and reduce habituation (Valls-Sole, Kumru et al. 2008, Carlsen, Maslovat et al. 2011). This phenomenon was initially studied in simple reaction tasks where reaction times are significantly decreased beyond what is voluntarily possible (Carlsen, Maslovat et al. 2012), but has recently been performed in more complex tasks such as head movements (Siegmund, Inglis et al. 2001, Oude Nijhuis, Janssen et al. 2007).

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) provides a non-invasive assessment of corticospinal and cortical responsiveness in humans (Petersen, Pyndt et al. 2003). TMS produces a strong, short-lasting magnetic field, which passes non-invasively through the scalp and skull, inducing a small electric current as a stimulus to the underlying brain structures, most often the motor cortex (Barker, Jalinous et al. 1985). This electrical current can stimulate both cortical interneurons and corticospinal neurons to produce action potentials, resulting in a muscle twitch in the innervated muscle (Terao and Ugawa 2002, Petersen, Pyndt et al. 2003). The type of TMS coil and orientation most commonly used induces action potentials in cortical interneurons, which then synapse on corticospinal neurons producing a series of descending volleys, or indirect waves (I-waves) (Di Lazzaro, Oliviero et al. 2003). When three or more I-waves summate at the spinal motor neuron, a motor evoked potential (MEP) is produced in the muscle which is recorded by EMG. The amplitude of this MEP reflects the overall responsiveness of the corticospinal tract. Using certain conditioning TMS stimuli prior to the test stimulus
allows the activation and assessment of specific types of cortical interneurons. For example, providing a subthreshold conditioning stimulus 2.5 ms prior to the test stimulus results in a decreased MEP amplitude (Chen 2004). This reduction in amplitude is the result of the conditioning stimulus activating GABAergic inhibitory interneurons (Boroojerdi, Battaglia et al. 2001) and is known as short-interval intracortical inhibition (SICI) (Chen 2004). Measuring SICI allows the assessment of and changes in intracortical inhibition.

**The Stress Response**

The term “stress” in reference to physiologic processes was first introduced by Hans Selye in the 20th century (Selye 1974). No one accepted medical definition of stress exists. Some prior definitions state that stress “is an evolutionarily conserved ability of an organism to deal with situations requiring vigilance, arousal, and/or action” (Neese and Young 2000) and “is defined as a state in which homeostasis is actually threatened or perceived to be so” (Chrousos 2009). The study of homeostasis was used in the early 20th century by Cannon (Cannon 1915) who studied the fight-or-flight response, more recently proposed as the freeze, flight, fight, or fright response (Bracha, Ralston et al. 2004). McEwen (McEwen 1998) utilized the term “allostasis”, which refers to an organism’s ability to “maintain stability through change.” This stability through change view of stress is one of the more commonly accepted views of stress and the stress response in modern literature (Conrad 2011).

Similar to the motor system, stress responses are mediated by a complex interaction of systems, some of which are shown in Figure 2.3. While the stress response involves changes in many bodily systems to maintain stability, this review will focus
primarily on two systems. The Hypothalamic Pituitary Adrenal (HPA) axis was the primary stress system studied by Selye (1974) and for many years considered the primary stress system (Conrad 2011). The effects of this system are primarily mediated by the release of glucocorticoids. The Sympathetic-Adrenal-Medulla (SAM) system is more involved in the initial fight-or-flight response studied by Cannon (1915). This system is a first response to a stressor and involves the rapid release of catecholamines through both the sympathetic nervous system and the adrenal medulla. Both systems have beneficial effects when activated in response to an acute stressor, but chronic activation of these systems may lead to many health detriments (McEwen 1998, Chrousos 2009). The response of systems more central to the HPA axis and SAM system will also be discussed.

Figure 2.3: Schematic of certain components involved in the stress response. Central Mediators of Stress
Before discussing the systems which act to prepare the body for stress, a brief discussion of the central structures assessing a stimulus as stressful and initiating physical responses must be made. Certain physical stressors can initiate reflexive, direct stress responses, such as alterations in circulating glucose and insulin, as well as visceral changes (Herman and Cullinan 1997). Increasingly more common in modern society are psychogenic stressors. These stressors often initiate a stress response in anticipation of a future stressor. If this future stressor never occurs, does not require the energy mobilization created by the stress response, or is a constant threat, this preparatory response can become detrimental (Passatore and Roatta 2006, Hannibal and Bishop 2014). This often occurs when fear, a response to an immediate stressor, becomes sustained. Anxiety is sustained fear in response to an unpredictable threat (Davis, Walker et al. 2010). Many central structures are involved in determining fear and anxiety, and different structures respond to different stressful stimuli, but most commonly involved are structures of the limbic system, especially the amygdala, which receives input from higher processing areas in the cortex (Davis, Walker et al. 2010). Different nuclei within the amygdala are activated with fear and anxiety stimuli. The medial central nuclei of the amygdala has projections to the hypothalamus and brainstem, initiating somatic and autonomic responses associated with fear, including enhanced startle reactions (Lang and Davis 2006, Davis, Walker et al. 2010). The lateral central nuclei of the amygdala project to the bed nucleus of the stria terminalis, which mediates most responses to anxiety via projections to initiators of the HPA axis and SAM systems (Davis, Walker et al. 2010).
Hypothalamic Pituitary Adrenal Axis

The HPA axis response to stress is initiated at the paraventricular nucleus (PVN) in the hypothalamus, which releases corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) into the portal blood system, where it travels to the anterior pituitary. The PVN can be activated directly, in response to physiologic stressors, or indirectly, in response to psychogenic stressors. The anterior pituitary synthesizes and secretes adrenocorticotropic hormone (ACTH). ACTH is released systemically where it eventually binds in the adrenal cortex and initiates the release of glucocorticoids, specifically cortisol in humans. Cortisol has many systemic effects, most involved in mobilizing energy in the body to prepare for a response to a given stressor: gluconeogenesis is stimulated and glucose transport to cardiac and central nervous system tissues is facilitated, neural regions associated with sensory processing, attention, and memory are primed, and the immune system and inflammatory processes are suppressed (Sapolsky, Romero et al. 2000). HPA axis activity is often self-limiting, as a negative feedback loop exists with cortisol inhibiting further secretion of CRH.

Sympathetic Adrenal-Medullary System

The SAM system includes the sympathetic nervous system (SNS), which is composed of preganglionic neurons primarily in the thoracolumbar spinal cord and postganglionic neurons located in ganglia adjacent to the spinal cord. These neurons project to a variety of organs and body structures, releasing primarily norepinephrine in response to a stressor. One of these organs is the adrenal medulla, which releases norepinephrine and epinephrine systemically. While the specific response can differ depending on the type of stressor (Pacak and Palkovits 2001), the SAM system initiates a
rapid response to a stressor. SAM activation typically results in increased heart rate, respiration, blood pressure, and skeletal muscle vasodilation. Body systems not immediately necessary to deal with a stressor have a reduced blood flow due to increased vasoconstriction, such as the digestive and reproductive systems. Glycogen to glucose conversion is also enhanced to provide the body with more energy. Finally, oxygen and glucose availability to brain tissue and skeletal muscle is increased in preparation for action (McCarty 2000).

Two related systems must be discussed in conjunction with the SAM system. The SAM system is activated centrally by the locus coeruleus, already discussed as a subcortical input in the human motor system. This structure not only provides norepinephrine input to the spinal cord, but also the brain, and it is highly involved in emotion and arousal (Holstege 1992, Berridge 2008). The locus coeruleus also projects to the PVN in the hypothalamus and is involved in the activation of the HPA axis as well. The other system to discuss is the autonomic nervous system (ANS). The SNS is one of two systems in the ANS, the other being the parasympathetic nervous system (PNS). While the SNS is involved in preparing the body for action, the PNS has the opposite effect and is often referred to as the “rest and digest” system, facilitating vasodilation and increased blood flow to the reproductive and digestive systems. Unlike the SNS, with more centralized ganglia around the spinal cord, the PNS has ganglia distributed throughout the body. One main effector within the PNS is cranial nerve X, or the vagus nerve, which among other organs innervates the heart, exerting the PNS effects on cardiac activity (Kandel, Schwartz et al. 2012).
Effects of Chronic Stress

As stated previously, while the effects of both the HPA axis and SAM system have beneficial effects when dealing with a stressor, chronic activation of these systems can lead to detrimental health conditions such as anxiety, depression, metabolic disorders, and neurovascular degenerative disease (Chrousos 2009). Chronic stress biases the HPA axis towards the hypersecretion of cortisol but different types of stressors may have specific effects. Cortisol levels and HPA axis activity return to baseline levels after several days with some forms of chronic stress (Kant, Bunnell et al. 1983). This reduction may reflect a decreased responsiveness of the system, as cortisol receptors are expressed less at target organs after the initial rise in cortisol. Other forms of chronic stress, such as chronic inflammation, lead to sustained increases in cortisol. Central changes that may contribute to this maintained cortisol level include a shift towards increased AVP and decreased CRH production at the PVN (Aguilera, Subburaju et al. 2008). Prolonged activation of the SAM system can also lead to health detriments. For example, increased heart rate and blood pressure are adaptive responses to acute stressor by preparing the body for mobilization, long term increases can lead to a greater risk for cardiovascular disease (Manuck, Marsland et al. 1995). Overall autonomic activity is also disrupted by chronic stress, with anxiety disorders being associated with decreased heart rate variability (HRV) (Chalmers, Quintana et al. 2014). This reduction often accompanies reduced parasympathetic and increased sympathetic nervous system activity (Berntson, Cacioppppo et al. 1994). Finally, long-term activation of both the HPA axis and SAM system via chronic stress can contribute to chronic pain symptoms, through cortisol
dysfunction (Hannibal and Bishop 2014) and sympathetically mediated pain (Janig and Baron 2006).

In addition to prolonged activation and deactivation, chronic stress will also affect how a system responds to an acute stressor. In most cases, acute stress responses are often diminished (Tsigos and Chrousos 2002, Generaal, Vogelzangs et al. 2014, Garafova, Penesova et al. 2015), indicating a decreased ability of the system to adapt to changes in the external environment. As stated previously, however, different stressors often result in different responses. One interesting response to chronic stress is the occurrence of homotypic and heterotypic stressors. Acute stressors that are similar to a chronic stressor, homotypic, result in a blunted stress response while acute stressors that are different from a chronic stressor, heterotypic, occasionally result in exaggerated stress responses (Dallman, Bhatnagar et al. 2000, Dronjak, Jezova et al. 2004).

Methods of Measurement

Cortisol

The assessment of unbound systemic cortisol can be assessed via blood samples or salivary samples. The use of salivary samples is a more recent methodology with few disadvantages (Kirschbaum and Hellhammer 1994). As cortisol is the effector of the HPA axis, this measurement is a good assessment of the activity of this system. Psychosocial stress has consistently been shown to increase circulating cortisol levels, indicating that this measure is sensitive to changes induced by stressors (Dickerson and Kemeny 2004).

Heart Rate Variability
HRV provides a non-invasive method of investigating ANS activity, providing partial information on the activity of the SAM system. Both heart rate and HRV are influenced by the SNS and PNS branches of the ANS. The SNS exerts its influence via sympathetic innervation of the sinoatrial node in the heart and systemic release of catecholamines from the adrenal medulla, acting to increase heart rate and decrease HRV. The PNS has the opposite effect, reducing heart rate and increasing HRV, via direct innervation of the sinoatrial node by the vagus nerve (Berntson, Bigger et al. 1997). The activity of both branches of the ANS is often assessed by frequency-domain measures of HRV, examining the spectral power within pre-defined frequency bands. The high frequency (HF) band ranges from 0.15 - 0.4 Hz and measures respiratory sinus arrhythmia, mainly attributed to the PNS (Piccirillo, Ogawa et al. 2009). Respiratory sinus arrhythmia is caused by the suppression of vagus nerve activity during inhalation, resulting in an increased heart rate, followed by a return of vagus activity during exhalation, slowing heart rate. This introduces variability into heart rate at the same frequency of respiration, and a decrease in vagus nerve activity will result in a reduced influence of respiratory sinus arrhythmia on HRV. The low frequency (LF) band ranges from 0.04 - 0.15 Hz and has a conflicted interpretation in the literature, though may represent modulation of the baroreflex, which is mediated by both the SNS and PNS branches of the ANS (Goldstein, Bentho et al. 2011). Finally, a dimension not assessed by frequency-domain measures is the complexity of HRV resulting from the interaction of multiple control systems. A decrease in complexity is thought to represent a decreased ability for the system to respond or adapt to environmental or physiologic changes (Manor and Lipsitz 2013). Sample entropy measures the regularity of the heart beat intervals, one aspect of
physiologic complexity (Richman and Moorman 2000) which has been shown to be responsive to psychosocial stress (Melillo, Bracale et al. 2011), and other alterations in ANS activity (Porta, G necchi-Ruscone et al. 2007).

**Interactions between the Stress and Motor Systems**

Even before Walter Cannon described the “fight-or-flight” response to stress, researchers have noticed a change in motor behavior with stress. However, most research on the stress response investigates how the body prepares for a motor response, but not always how that response is initiated or controlled. Both the HPA axis and SAM system have well-known mechanisms of energy mobilization, allowing the body to initiate and maintain an increased level of muscle activity in a stress response. The mechanisms behind the increased muscle activity itself, though, are less well-known. Given that increased muscle activity (“muscle tension”) is a diagnosing symptom of general anxiety disorder (APA 2013) and muscle relaxation is a highly utilized intervention for anxiety and related stress conditions (Rainforth, Schneider et al. 2007), these mechanisms must be better understood. The following gives a brief summary of how each stress system may directly influence motor behavior, and stress-induced changes in motor behavior in healthy humans.

**Effects of the HPA Axis**

Much of what is known of the HPA axis’ direct effects on motor behavior is based on animal studies and the influence of glucocorticoids (cortisol in humans and corticosterone in rats). Glucocorticoid receptors are found throughout the central nervous system, including on spinal motor neurons and in the motor cortex of the brain (Metz 2007). While the direct actions of these receptors are not fully understood, corticosterone
injections in rats and mice have been shown to reduce the ability to perform skilled reaching and coordination tasks (Howard and Granoff 1968, Metz, Jadavji et al. 2005).

**Effects of the SAM System**

The SAM system has more known mechanisms potentially contributing to increases in muscle activity. As discussed previously, the main effectors of the SAM system are catecholamines (epinephrine and norepinephrine). These catecholamines function along with circulating cortisol to increase energy mobilization for the stress response, but also affect muscle fibers themselves, primarily through binding to β2 receptors (Bowman 1980). Muscle fiber twitch force and half-relaxation time are decreased through an increased reuptake of calcium by the sarcoplasmic reticulum (Bowman 1980). This effect is primarily seen in Type I muscle fibers, so those recruited early on in a muscle contraction. While increased levels of catecholamines can have the opposite effect, increasing calcium outflow from the sarcoplasmic reticulum and potentiating muscle fiber twitch force, this level of catecholamines is likely non-physiologic and has not been observed in humans (Roatta and Farina 2013) while decreased twitch force and half-relaxation time have been observed in humans (Roatta, Arendt-Nielsen et al. 2008). While a decreased half-relaxation time would reduce force tetanus at a given input level, this could facilitate quickly switching tasks or the reciprocating agonist/antagonist muscle activity often required in a stress response (Roatta and Farina 2010). Decreased force tetanus would also contribute to increased force fluctuations. Catecholamines can act to facilitate the activity of sodium-potassium pumps, attenuating muscle fatigue (Roatta and Farina 2010), an effect seen by Cannon (Cannon 1915). Sympathetic fibers in the SAM system also directly innervate muscle
spindles (Radovanovic, Peikert et al. 2015), decreasing the response to a given muscle length change (Matsuo, Ikehara et al. 1995, Roatta, Windhorst et al. 2002). This decreased peripheral input could impair fine motor control and stability, while facilitating quick actions. Decreased peripheral control and twitch force could facilitate an increased level of central drive to the muscle in order to maintain a given level of control.

**Central Effects**

Little is known of the mechanisms of specific motor effects of stress at areas upstream of the HPA axis and SAM system. As discussed in previous sections, the locus coeruleus provides noradrenergic input to spinal motor neurons and is activated during periods of increased arousal (Berridge 2008). This could result in an increased neuromodulatory input to spinal motor neurons during periods of increased stress, promoting both self-sustained firing and increased amplification of synaptic input to the motor neurons (Heckman 2003). Both of these alterations could result in increased motor unit activity to a given stimulus or input. One central structure potentially involved in many changes in motor behavior in response stress is the amygdala. As stated in the previous section, different nuclei in the amygdala are activity by anxiety and fear stimuli. The amygdala has projections to both the HPA axis and SAM system initiators, but also to other central structures with potential impacts on motor behavior (Lang and Davis 2006). Some of these connections and their potential motor behavior implications are shown in Table 2.1.
### Table 2.1: Targets of amygdala projections and altered motor behavior (Adapted from Lang & Davis, 2006)

<table>
<thead>
<tr>
<th>Target</th>
<th>Motor behavior altered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbital Frontal Cortex</td>
<td>Choice behavior</td>
</tr>
<tr>
<td>Dorsal and Ventral Stratum</td>
<td>Avoidance behavior</td>
</tr>
<tr>
<td>Parabrachial Nucleus</td>
<td>Increased respiration</td>
</tr>
<tr>
<td>Central Grey</td>
<td>Increased freezing</td>
</tr>
<tr>
<td>Locus Coeruleus</td>
<td>Increased vigilance and motor unit activity</td>
</tr>
<tr>
<td>Nucleus Reticularis Pontis Caudalis</td>
<td>Increased startle</td>
</tr>
<tr>
<td>Trigeminal and facial motor nucleus</td>
<td>Facial expressions of stress/fear/anxiety</td>
</tr>
</tbody>
</table>

### Effects in Healthy Humans

Most of what is known of the mechanisms of stress-induced changes in motor behavior have been investigated in animal models, with little direct evidence in humans. Here, a summary is provided of what is known of stress-induced changes in motor behavior in humans. Several studies show increased corticospinal responsiveness to TMS in the presence of emotionally arousing or stressful stimuli (Hajcak, Molnar et al. 2007, Oathes, Bruce et al. 2008, Coombes, Higgins et al. 2009, Coelho, Lipp et al. 2010, van Loon, van den Wildenberg et al. 2010). These changes indicate an increased responsiveness somewhere along the corticospinal tract, either at the motor cortex itself, where TMS is applied, or at the spinal motor neuron, amplifying the input from the motor cortex. Reaction times have been shown to decrease with acute exposure to emotionally arousing images (Coombes, Tandonnet et al. 2009) while increased trait anxiety has been linked to increased reaction times (Coombes, Higgins et al. 2009). Changes in reaction...
time likely reflect central changes in response to stress, possibly due to increased attention from locus coeruleus and/or amygdala activation.

Stability of sustained force contractions has been shown to be decreased during exposure to an acute stressor (Noteboom, Barnholt et al. 2001, Christou, Jakobi et al. 2004, Christou 2005) and in relation to increased trait anxiety (Noteboom, Barnholt et al. 2001). Interestingly, this increased variability resulting in decreased stability is predominantly at a frequency thought to reflect descending input to the motor neurons (Christou, Jakobi et al. 2004) indicating an increased contribution of central inputs to force steadiness. Visual feedback can attenuate stress-induced variability increases (Christou 2005) likely through modification of central input to the motor neurons. This could be explained by the SAM system and central mechanisms of stress-induced motor changes. Activation of the SAM system results in decreased peripheral monitoring of muscle activity through decreased muscle spindle activity, and decreased twitch tetanus through actions at the muscle fiber. These changes would necessitate an increased central input to maintain a given contraction level, which would also be amplified via increased neuromodulatory input from the locus coeruleus, providing a potential explanation to the increased contribution of central input to force fluctuation. Finally, emotional arousal has been linked to increased force output (Coombes, Cauraugh et al. 2006), which is contradictory to a decreased twitch amplitude of muscle fibers, but possibly explained by an amplification of input to the spinal motor neuron.

These changes in motor output may contribute to or happen in conjunction with changes in more functional motor tasks, such as balance and posture. Stressful stimuli have been shown to both increase and decrease postural sway, depending on the task and
stimulus (Hainaut, Caillet et al. 2011, Staab, Balaban et al. 2013). Increased levels of trait anxiety interfering with sensorimotor processing may partially explain discrepancies, as individuals with increased trait anxiety show increased muscle coactivation and overall increases in stiffness strategies, while those with low trait anxiety do not (Hainaut, Caillet et al. 2011). Individuals with a fear of falling also show this increased coactivation of leg muscles, along with shorter strides and a wider base of support (Staab, Balaban et al. 2013). The amplitude of cervical muscle activity during computer use has also been shown to increase during acute increases in psychosocial stress (Bruflat, Balter et al. 2012, Shahidi, Haight et al. 2013). Finally, contradictory to what would be expected based on possible mechanisms of stress-induced motor changes, muscle fatigue has been shown to increase in the presence of an acute stressor (Yoon, Keller et al. 2009), but could possibly be accounted for by an increased central input to the motor neuron driving an increase in central fatigue.

The Upper Trapezius

This dissertation investigates psychosocial stress responses in the upper trapezius muscle and how they relate to chronic neck pain. The trapezius is an axial muscle involved in respiration, postural control, visual stabilization, and voluntary movements of the head, neck, and arm. The entire trapezius muscle spans from the base of the skull, spine of the scapula, and cervical and thoracic vertebra. It is subdivided into three function parts: the upper, middle, and lower trapezius. The upper trapezius, specifically has proximal attachments at the superior nuchal line on the occipital bone, nuchal ligament, and spinal process of the seventh cervical vertebra and distal attachments on the lateral clavicle and acromion process of the scapula. It acts to extend and laterally flex the
neck, contralaterally rotate the head, and upwardly rotate and elevate the scapula (Kendall, McCreary et al. 2005). The upper trapezius is unique in its innervations, as its motor innervation (cranial nerve XI, the accessory nerve) is mostly separate from its sensory innervation (C2-C4 nerve roots) (Vangsgaard, Norgaard et al. 2013), however some motor contribution from the spinal nerve roots is present in certain individuals (Pu, Tang et al. 2008). The upper trapezius is involved in many tasks that require bilateral activation and may have spinally mediated bilateral connections (Alexander and Harrison 2002), though the nature of this bilateral connection has been recently challenged (Vangsgaard, Norgaard et al. 2013). Bilateral control of the upper trapezius may be mediated by bilateral innervation from the reticulospinal tract (Davidson and Buford 2004, Davidson and Buford 2006). The upper trapezius also receives central input from the corticospinal tract (Alexander, Miley et al. 2007), though this input may be somewhat smaller than more distal muscles (Rothwell, Thompson et al. 1987).

**Upper Trapezius and Chronic Neck Pain**

Chronic neck pain is defined as pain in an anatomical location between the superior nuchal line and the spine of the scapula (Guzman, Hurwitz et al. 2009). It is one of the leading causes of disability in the world today (Hoy, March et al. 2014) with an annual prevalence of 30 – 50% (Hogg-Johnson, van der Velde et al. 2009). Trapezius myalgia (muscle pain) is often present in individuals with chronic neck pain (Sjøgaard, Søgaard et al. 2006), and patterns of upper trapezius muscle activity have long been investigated in relation to chronic neck pain. Hagg originally proposed the Cinderella Hypothesis (Hagg 1991), suggesting that sustained, low amplitude muscle activity can disproportionately activate low-threshold muscle fibers over high threshold fibers,
predisposing these fibers to tissue damage, possibly through ischemia (Jonsson 1982), and subsequently pain. These damaged fibers, referred to as moth eaten or ragged red fibers, are present to some degree in individuals with chronic neck pain, however findings are often inconclusive as these fibers are also found in healthy individuals (Hägg 2000).

Chronic neck pain has been showed to alter upper trapezius muscle activity in several ways which may predispose the muscle towards tissue damage. Patients with chronic neck pain demonstrate increased activity of superficial muscles, of which the upper trapezius is included (Nederhand, Jzerman et al. 2000, Falla and Farina 2008). These patients also show decreased specificity of cervical muscle activity, meaning that the upper trapezius becomes active during tasks that do not typically require high levels of upper trapezius muscle activity (Falla, Lindstrom et al. 2013). Finally, patients with chronic neck pain show a decreased ability to relax the upper trapezius during and after dynamic movements (Nederhand, Hermens et al. 2002, Falla, Bilenkij et al. 2004, Falla and Farina 2008). All of these changes could theoretically predispose the upper trapezius towards developing tissue damage and muscle pain, though this causal relationship has little supporting evidence (Veiersted and Westgaard 1993, Mork and Westgaard 2006). Even if these changes are not the initial cause of trapezius myalgia, they could contribute to the chronicity and recurrence of chronic neck pain via the mechanisms discussed. Once pain has developed, motor and neural adaptations to that pain may remain even if the initial painful stimulus has resolved, biasing acute neck pain to become chronic and recurrent (Hodges and Tucker 2011).
Psychosocial Stress and Chronic Neck Pain

A well investigated predisposing and related factor to chronic neck pain is psychosocial stress (Ariens, van Mechelen et al. 2001, Lang, Ochsmann et al. 2012). This relationship is often investigated in office workers, where the incidence of chronic neck is abnormally high, up to 34.4% annually (Côté, van der Velde et al. 2009), despite low physical loads on the upper trapezius and other cervical musculature. As discussed previously, increased stress can alter motor control, biasing muscles towards increased and excessive activity, and this stress-induced muscle activity is viewed as a potential contributor to chronic neck pain development (Lundberg 1999, Sjøgaard, Lundberg et al. 2000). The presence of stress-induced muscle activity has been validated in many studies showing increased cervical muscle activity during simulated stressful computer work (Lundberg, Forsman et al. 2002, Nilsen, Sand et al. 2007, Eijckelhof, Huysmans et al. 2013, Shahidi, Haight et al. 2013). However, the link between psychosocial stress and upper trapezius muscle activity in the workplace is lacking and the mechanism behind the contribution of psychosocial stress to chronic neck pain remains unknown. It is also interesting to note that stress may initiate similar changes to pain in cervical muscle activity. Evidence exists that pain centrally acts as a stressor, initiating the stress responses previously discussed (Heim, Ehlert et al. 2000, Pacak and McCarty 2000).

Overall, the possibility that stress alters upper trapezius muscle activity in such a way as to predispose it towards chronic pain development or recurrence is high, though little is known of the possible mechanisms linking these phenomenon. Mechanisms of stress-induced alterations in motor control are known but have not been investigated specifically in the upper trapezius muscle.
CHAPTER III

MODULATION OF INTRACORTICAL INHIBITION IN RESPONSE TO ACUTE PSYCHOSOCIAL STRESS IS IMPAIRED AMONG INDIVIDUALS WITH CHRONIC NECK PAIN

Abstract

Objective

Psychosocial stress has been associated with a variety of chronic pain disorders although the mechanisms responsible for this relationship are unknown. The purpose of this study was to compare the excitability of intracortical and corticospinal pathways to the trapezius muscle in individuals with and without chronic neck pain during exposure to low and high levels of psychosocial stress.

Methods

Single and paired-pulse transcranial magnetic stimulation was used to assess motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) during mental math performed in the presence and absence of social evaluative threat.

Results

All participants demonstrated higher amplitude MEPs in the high stress compared to the low stress condition (p<0.01). Participants with chronic neck pain had significantly greater SICI than healthy participants in the low stress condition (p=0.03). During exposure to the stressor, healthy participants showed an increase in SICI, whereas

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participants with neck pain showed no change (group difference for change in SICI, p<0.01).

**Conclusions**

These findings suggest that individuals with chronic neck pain inhibit motor output to the trapezius in the presence of minor stressors, and are unable to compensate for additional stress-evoked increases in corticospinal excitability through further modulation of SICI. This observation has potential implications for the management of patients who have difficulty relaxing painful muscles during times of stress.

**Introduction**

Psychosocial stress, defined as a disruption in homeostasis caused by actual or perceived adverse emotional threats (Chrousos 2009), is an established risk factor for a variety of chronic pain syndromes (Macfarlane, Pallewatte et al. 2009, Lang, Ochsmann et al. 2012) including neck pain (Ariëns, Mechelen et al. 2001, Bongers, Ijmker et al. 2006). Despite the high prevalence of co-morbid stress and pain, the mechanisms responsible for this association are not clear. Stress and anxiety have known modulatory effects on both pain perception (Bement, Weyer et al. 2010) and motor behavior (Noteboom, Fleshner et al. 2001, Christou, Jakobi et al. 2004). Transcranial magnetic stimulation (TMS) studies have shown that anxiety can increase corticospinal excitability in pain-free individuals (Oathes, Bruce et al. 2008). Similarly, a decrease in the responsiveness of GABA$_A$-mediated inhibitory circuits, assessed as short interval intracortical inhibition (SICI), has been associated with anxiety-related personality traits (Wassermann, Greenberg et al. 2001). The effects of psychosocial stress on excitability of motor pathways among individuals with chronic pain are currently unknown.
TMS studies have shown consistent evidence of reduced excitability of cortical neurons and corticospinal pathways to the muscle with experimentally induced acute pain in healthy adults (Le Pera, Graven-Nielsen et al. 2001, Svensson, Miles et al. 2003, Martin, Weerakkody et al. 2008, Dubé and Mercier 2011). Increased inhibition and reduced facilitation of intracortical motor circuits have also been observed during or shortly after resolution of acute pain (Schabrun and Hodges 2012), supporting central inhibition as one protective mechanism to restrict movement of painful areas within a muscle (Lund, Donga et al. 1991, Hodges and Tucker 2011). Neuromuscular adaptations to chronic pain, however, are less consistent. Corticospinal excitability is reduced in patients with chronic low back pain compared to healthy controls (Strutton, Theodorou et al. 2005), whereas patients with chronic knee pain show increased excitability of corticospinal pathways (On, Uludağ et al. 2004). Reductions in SICI have been demonstrated for patients with chronic regional pain syndrome (Schwenkres, Janssen et al. 2003, Eisenberg, Chistyakov et al. 2005), neuropathic pain (Lefaucheur, Drouot et al. 2006, Schwenkres, Scherens et al. 2010), and fibromyalgia (Mhalla, Andrade et al. 2010) compared to pain-free individuals. However, patients with chronic pain due to osteoarthritis show similar levels of SICI as healthy controls (Schwenkres, Scherens et al. 2010). Patterns of muscle activation are also highly variable among patients with chronic pain, and often differ from those observed in response to experimental pain (Falla and Farina 2008).

One possible explanation for the large variability in neural adaptations to chronic pain is the interaction between adaptations to pain and those associated with psychosocial stress. Therefore, the purpose of this study was to compare the excitability of intracortical
and corticospinal pathways to the upper trapezius muscle in individuals with and without chronic neck pain during exposure to low and high levels of psychosocial stress. Based on previous studies showing consistent inhibition of motor output in response to experimentally evoked pain (Le Pera, Graven-Nielsen et al. 2001, Svensson, Miles et al. 2003, Martin, Weerakkody et al. 2008, Dubé and Mercier 2011, Schabrun and Hodges 2012) and among patients with chronic spine pain (Strutton, Theodorou et al. 2005), we hypothesized that individuals with chronic neck pain would exhibit greater intracortical inhibition and reduced corticospinal excitability compared to healthy controls in the low stress condition. During an acute increase in stress, however, we expected to observe a greater increase in corticospinal excitability and reduction in intracortical inhibition in the neck pain group.

**Materials and Methods**

**Participants**

Participants with chronic neck pain and individuals without a history of chronic musculoskeletal pain were recruited through printed and electronic advertisements at a university medical campus and the surrounding community. All participants provided written informed consent in accordance with procedures approved by the Colorado Multiple Institutional Review Board, including additional protections for a partial waiver of consent required for the stress manipulation.

The neck pain group included participants with recurrent or persistent pain located between the superior nuchal line and the superior spine of the scapula for at least 1 year prior to enrollment, at least mild disability with a Neck Disability Index (NDI) score of ≥10% (En, Clair et al. 2009, MacDermid, Walton et al. 2009), and tender points
in the upper trapezius confirmed by manual palpation. Participants were asked to refrain from taking pain medication for at least 24 hours prior to the testing session. Individuals in the healthy control group reported a complete absence of neck pain in the year prior to enrollment, with no history of any neck pain lasting more than 12 weeks. These participants had no tender points in the upper trapezius with manual palpation on the day of testing.

Exclusion criteria for both groups included objective signs of structural pathology or neurologic impairment (e.g. radiculopathy), or a self-reported history of traumatic injury or surgery affecting the neck or shoulder region within 12 weeks of enrollment. Individuals with a history of widespread musculoskeletal pain were excluded. All participants were free from contraindications to TMS as outlined by the National Institute of Neurological Disorders and Stroke, denied any history of major cardiovascular, neurological, or psychiatric medical conditions, and were not taking centrally active medications. No participants reported a history of repetitive motor activity impairments.

**Electromyography**

Surface electromyography (EMG) was recorded from the dominant upper trapezius using bipolar Ag-AgCl surface electrodes. Electrodes were positioned with a 15 mm interelectrode distance, centered 20 mm lateral to the midpoint between C7 and the posterior lateral border of the acromion (Farina, Madeleine et al. 2002). A reference electrode was placed over a bony portion of the ipsilateral clavicle. EMG data were amplified (1000x), band-pass filtered (13–1000 Hz LabLinc V, Coulbourn Instruments, Whitehall, PA; 10-500Hz, MP150, Biopac Systems Inc, Goleta, CA), and sampled at 2000 Hz (Micro 1401, Cambridge Electronic Design, Cambridge, UK). Data were
collected during a 5–8 s time window surrounding the delivery of TMS stimulus to capture background levels of EMG and corresponding MEP.

**Transcranial Magnetic Stimulation**

TMS methods were performed and reported in accordance with Chipchase et al (Chipchase, Schabrun et al. 2012), unless otherwise noted. TMS was applied over the contralateral motor cortex using a standard 70 mm figure-of-eight coil to stimulate the cortical representation of the dominant upper trapezius. Monophasic stimuli were generated using a Magstim Bistim² (The Magstim Company, Whitland, UK). The coil was held with the handle pointing posterolaterally at approximately a 45° angle to induce a posterior-to-anterior current in the motor cortex. Stimuli were applied in a grid pattern centered around the previously reported locus for the cortical representation of the upper trapezius muscle (Alexander, Miley et al. 2007). The optimal coil position for evoking motor responses in the upper trapezius muscle was identified individually for each participant as the position inducing the largest MEP during low-intensity muscle contraction. This position was recorded with Brainsight neuronavigation software (Rogue Research Inc, Montreal, QC), and remained constant across experimental conditions. Stimulation intensities and EMG recordings were controlled using Signal software (Cambridge Electronic Design, Cambridge, UK).

Resting motor threshold (RMT) was determined as the lowest stimulation intensity that evoked an MEP with peak-to-peak amplitude of at least 50 μV in 50% of trials in a relaxed muscle. Active motor threshold (AMT) was determined as the lowest stimulation intensity that evoked an MEP with a peak-to-peak amplitude of at least 100 μV in 50% of trials during low-intensity contraction of the upper trapezius (Rothwell,
Net excitability of the corticospinal tract was assessed by the amplitude of MEPs produced by a series of 8–12 single stimuli delivered at 120% RMT with the muscle at rest (MEP_{120\%}).

SICI was assessed with a series of 8–12 paired stimuli delivered with the muscle at rest according to the paired-pulse stimulation paradigm developed by Kujirai et al. (Kujirai, Caramia et al. 1993). A suprathreshold test stimulus (120% RMT, MEP_{UNCOND}) was preceded by a subthreshold conditioning stimulus (70% AMT, MEP_{COND}), with an inter-stimulus interval of 2.5 ms. A conditioning stimulus intensity of 70% AMT was selected to isolate inhibitory GABA_{A} pathways, as conditioning stimuli at higher intensities may concurrently activate both inhibitory and facilitatory circuits (Ortu, Deriu et al. 2008). Intensity of the test stimulus was calculated based on RMT in the baseline condition. To assess intracortical adjustments to a given input under different psychoemotional states that were expected to alter corticospinal excitability, the intensity of both the conditioning and test stimuli remained constant across low and high stress conditions. Previous research indicates that the population of interneurons recruited by the test stimulus is dependent on absolute stimulator intensity, regardless of changes in corticospinal excitability (Garry and Thomson 2009). Therefore, a constant stimulation intensity allowed us to examine SICI for the same population of interneurons despite expected changes in the unconditioned MEP amplitude and motor threshold across stress conditions. These procedures contrast with recent recommendations to adjust the intensity of stimulation to maintain a constant MEP_{UNCOND} amplitude across experimental conditions (Chipchase, Schabrun et al. 2012); therefore, a correlation analysis was performed to assess whether changes in SICI were associated with changes in
MEP\textsubscript{UNCOND} amplitude across stress conditions. A similar analysis was performed for changes in SICI and changes in RMT. These analyses allowed us to identify any contribution of changes in corticospinal excitability to changes in SICI assessed at the same absolute intensity across stress conditions. SICI was calculated as one minus the ratio between the amplitude of the MEP\textsubscript{COND} and MEP\textsubscript{UNCOND} (illustrated in Figure 3.1). Thus, higher values of SICI indicated greater responsiveness of GABA\textsub{A}-mediated inhibitory circuits within the motor cortex.

\textbf{Figure 3.1}: Panel A shows representative EMG data during an unconditioned test stimulus at 120\% RMT (solid line), and the same test stimulus preceded by a subthreshold conditioning stimulus at 70\% AMT (dashed line). Peak-to-peak MEP amplitudes are shown for both the unconditioned (MEP\textsubscript{UNCOND}) and conditioned (MEP\textsubscript{COND}) stimuli. CS = conditioning stimulus artifact, TS = test stimulus artifact. Panel B shows a schematic of the experimental protocol. RMT = resting motor threshold, AMT = active motor threshold, SICI = short-interval intracortical inhibition, MEP\textsubscript{120\%RMT} = amplitude of motor evoked potential at 120\% RMT stimulus intensity.
**Perceived Anxiety and Physiologic Arousal**

Perceived anxiety was measured using the state anxiety portion of the Spielberger State-Trait Anxiety Inventory (STAI-S) (Spielberger 1983). Physiologic arousal was assessed based on cardiovascular responses collected with an automated monitor (Omron Healthcare, Inc., Bannockburn, IL) placed on the non-dominant arm to measure heart rate and blood pressure. Rate pressure product (RPP) was calculated as the product of systolic blood pressure and heart rate.

**Stress Manipulation**

Levels of psychosocial stress were manipulated using mental math (Noteboom, Flesher et al. 2001, Lundberg, Forsman et al. 2002) combined with social evaluative threat (Dickerson and Kemeny 2004, Stephenson and Maluf 2010). Participants were informed that the purpose of the study was to examine the effects of mental concentration on muscle activity, and therefore remained naïve to the stress manipulation. During both stress conditions, a four-digit number was displayed on the feedback monitor and participants were instructed to mentally count backwards from this number. A low stress condition controlled for attention effects by asking participants to count backwards by ten from a four-digit number that was evenly divisible by ten. Participants were told that these were practice trials in which their performance would not be monitored. Positive feedback was provided by a familiar examiner regardless of actual performance. This condition was followed by a high stress condition in which the difficulty of the counting task was increased by instructing participants to count backwards by a one- or two-digit number from a pseudorandomly selected four-digit number. Prior to the high stress condition, participants were informed that they would be videotaped and that it was
extremely important to perform the counting test as fast and accurately as possible. Time and accuracy scores were displayed on the feedback monitor, and monetary incentive was offered for high scores. The high stress condition was administered by the same unfamiliar and authoritative examiner for all participants, and no positive feedback was provided. Immediately after completing the high stress condition all participants were fully debriefed regarding the purpose of the stress manipulation. Participants were assured that the video of their performance had not been recorded, and that they would receive full monetary compensation regardless of their performance.

**Screening and Familiarization Session**

All participants completed a brief screening prior to the experimental session conducted on a separate day. During screening, participants completed the trait anxiety portion of the STAI, along with demographic and medical history questionnaires. Participants with chronic neck pain also completed the NDI. A brief physical exam was performed to assess the presence of tender points in the upper trapezius and screen for non-musculoskeletal sources of pain. Participants who met the study inclusion criteria were then exposed to varied intensities of TMS to become familiar with experimental procedures, followed by an assessment of RMT. Individuals whose RMT exceeded 83% of maximum stimulator output (MSO) were excluded from further participation due to an inability to deliver test stimuli of sufficient intensity (120% RMT) to assess corticospinal excitability (MEP$_{120\%}$) and SICI.

**Experimental Session**

The experimental protocol is illustrated in Figure 3.1B. Participants were seated with the trunk supported in an upright position and hip and knee joints flexed
approximately 90°. Participants were instructed to completely relax their neck and upper extremities, and were provided with head and arm rests as necessary to achieve an absence of muscle activity in EMG recordings from the dominant trapezius. The rectified and smoothed (200 ms window) EMG signal was displayed in real-time on a monitor located at eye level approximately 1 m in front of the participant during set up procedures and baseline measurements.

The optimal coil position for evoking MEPs in the trapezius was identified, and baseline measurements of RMT, AMT, SICI, and MEP_{120\%} were collected as participants viewed the feedback monitor. No cognitive task was performed during baseline measurements. After removing visual feedback, participants performed the low and then high stress experimental conditions in consecutive order. The order of experimental conditions was not randomized due to the length of time required to return to a stable baseline level of arousal after exposure to the high stress condition. Pilot experiments revealed no change in TMS outcomes when the low stress task was repeated twice in consecutive order (mean (SD) difference for SICI = -0.31(0.51), p = 0.12; RMT = 1.5(2.6), p = 0.15; MEP_{120\%} = -0.12(0.23), p = 0.19; N=8, one-sample t-tests to identify differences from zero), suggesting that any changes in the high stress condition were unlikely to be caused by time or order effects. SICI, RMT, and MEP_{120\%} were assessed during both stress conditions, with perceived anxiety (STAI-S) and cardiovascular (RPP) responses collected at the completion of each condition.

Counting blocks consisted of 90–120 s of mental calculation, after which participants were asked to verbalize the last number they had reached in the counting sequence and were provided with verbal and visual feedback regarding the number of
correct subtractions. Participants looked directly at the monitor while counting, and were asked not to speak or move their fingers. Specific instructions to completely relax the neck, shoulders, and arms were provided at the beginning of each counting block. Single or paired TMS stimuli were delivered in random order every 5–8 s during up to six counting blocks to assess SICI, RMT, and MEP\textsubscript{120\%}. In an attempt to keep the duration of the stress conditions consistent, and due to the variable number of stimulations required to determine RMT, the number of stimuli used to assess SICI and MEP\textsubscript{120\%} varied, with a minimum of 8 and maximum of 12 single and paired stimuli delivered for each condition. Counting blocks were separated by approximately 60 s rest, with a 5 min rest break between stress conditions.

**Data Analysis**

EMG data were processed using custom software written in Matlab (MathWorks Inc., Natick, MA, USA). Individual trials with any visible muscle activity in the 100 ms prior to stimulation were excluded from analysis to avoid any influence of background muscle activity on MEP amplitude (approximately 3\% of trials). Participants with visible muscle activity in greater than 50\% of trials in either stress condition were considered non-compliant with instructions to relax, and were also excluded from analysis (Figure 3.2). MEP amplitude was calculated as peak-to-peak amplitude of the EMG signal in a time window set 9–35 ms after the test stimulus. The amplitudes of MEP\textsubscript{COND} and MEP\textsubscript{UNCOND} were averaged separately within each stress condition for each participant, and SICI was calculated as described previously. Due to differences in baseline MEP\textsubscript{120\%}, changes in corticospinal excitability were assessed relative to baseline by expressing the
average MEP<sub>UNCOND</sub> in each stress condition as a percentage of the average MEP<sub>120%</sub> obtained at baseline.

**Figure 3.2:** Enrollment flow chart. The percentage of eligible participants excluded based on high resting motor thresholds (RMT) in the upper trapezius muscle upon initial screening or an inability to maintain a resting muscle during exposure to the psychosocial stressors was not significantly different between groups (p=0.25 and p=0.68, respectively).

Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC) and R (R Development Core Team, Vienna). Demographic characteristics and baseline TMS measures were compared between groups using an exact k-sample permutation test for continuous variables, and Fisher’s exact test for categorical variables. An exact k-sample permutation test was used for _a priori_ planned comparisons of 1) group differences within the baseline and low stress conditions, and 2) group differences in the change across low and high stress conditions for all experimental variables (STAI-S, RPP, SICI, RMT, and MEP<sub>120%</sub>). The baseline condition was analyzed separately from
both stress conditions due to substantial differences in attentional state and visual feedback that may independently affect TMS outcomes (Thomson, Garry et al. 2008). Variables with no group difference in the change between low and high stress conditions were averaged across groups to identify main effects of the stress condition. Due to the limited sample size, permutation methods were used because they do not rely on the assumptions of typical normal-theory methods (Curran-Everett 2012). Associations between changes in SICI and changes in MEP_{UNCOND} and RMT were quantified with Pearson's correlation analyses for the combined groups. Statistical significance was defined as p<0.05 for all analyses, with p≤0.10 described as trends.

Results

Participant Characteristics

Forty-two individuals with chronic neck pain and 45 healthy controls met the initial inclusion criteria, and underwent TMS screening (Figure 3.2). Sixty-four and 76% of participants with and without neck pain, respectively, were excluded from further participation based on high RMT values in the upper trapezius. An additional 14% of individuals with neck pain and 7% of healthy controls were unable to maintain a fully relaxed muscle during exposure to the psychosocial stressor, and were also excluded. The number of individuals excluded based on these criteria did not differ between groups (Chi-Square Test, p≥0.25). Table 1 reports demographic and clinical characteristics of the nine participants with chronic neck pain and eight healthy controls included in the final analysis. All participants with neck pain were right-hand dominant and reported pain on the dominant side (N=4 bilateral pain; N=5 unilateral pain). The neck pain and control groups were not significantly different in sex or trait anxiety, with a trend toward younger
participants in the healthy control group. Furthermore, participants who were excluded from the study did not differ from those who were included for any of the demographic or clinical characteristics reported in Table 1 (0.10 ≤ p ≤ 0.45).

**Table 3.1: Demographic and clinical characteristics of study participants.**

<table>
<thead>
<tr>
<th></th>
<th>Chronic Neck Pain (N = 9)</th>
<th>Healthy Controls (N = 8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.4 (11)</td>
<td>31.5 (14.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>77.8</td>
<td>50.0</td>
<td>0.45</td>
</tr>
<tr>
<td>STAI-trait score</td>
<td>32 (7.2)</td>
<td>29 (5)</td>
<td>0.37</td>
</tr>
<tr>
<td>NDI (%)</td>
<td>25.1 (16.6)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>VAS pain (mm)</td>
<td>17.1 (13.8)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

**Efficacy of the Stress Manipulation**

Figure 3.3 shows changes in STAI-S and RPP in response to the psychosocial stressor. RPP was not different between groups in the low stress condition (p=0.85), whereas STAI-S scores were initially higher in the neck pain group compared to healthy controls (p=0.01). RPP and STAI-S scores were significantly greater in the high stress compared to low stress condition (p<0.01), with both groups exhibiting similar increases in cardiovascular responses to the psychosocial stressor (between group difference for change in RPP, p=0.42). Changes in subjective responses to the stressor tended to be higher among healthy individuals due to initially low levels of anxiety in the low stress condition (between group difference for change in STAI-S, p=0.07).
Figure 3.3: Panel A shows Spielberger State Anxiety Inventory (STAI-S) scores for the chronic neck pain (white) and healthy control (black) groups in the low stress and high stress conditions (left), and the average change in STAI-S scores between conditions (right). Panel B shows rate pressure product (RPP) values using the same format. Values are mean (SE). * Significant difference from controls (p<0.05). ** Significant difference between stress conditions (p<0.01).

Intracortical Inhibition and Corticospinal Excitability

In the absence of cognitive demands, baseline measurements of RMT (72.3 (SD 8.8)% vs 71.5 (SD 9.9)% MSO, p=0.86) and AMT (51.6 (SD 13.2)% vs. 48.6 (SD 9.1)% MSO, p=0.62) did not differ for the neck pain and healthy control groups, respectively. A trend toward lower MEP120% was observed for the neck pain group at baseline (0.326 (SD 0.212) mV vs. 0.568 (SD 0.260) mV in the healthy control group, p=0.05). The neck pain group also demonstrated a trend toward higher SICI at baseline compared to healthy controls (0.51 (SD 0.40) vs. 0.08 (SD 0.58), p = 0.10).
Group averages for SICI within each stress condition, and the mean change in SICI between stress conditions are shown in Figure 3.4. SICI was significantly greater during the low stress condition in the neck pain group compared to the healthy control group (p=0.03). The change in SICI differed between groups (p<0.01), with healthy individuals showing increased SICI from the low stress to high stress condition and the neck pain group showing no change. Measures of corticospinal excitability are shown in Figure 3.5. Neither RMT (p=0.47) nor MEP$_{120\%}$ (p=0.83) differed between groups in the low stress condition. The change in RMT was significantly different between groups (p=0.02), with the chronic neck pain group showing a decreased RMT from the low to high stress condition and the healthy control group showing no change. The change in MEP$_{120\%}$ was not significantly different between groups (p=0.58). With the groups combined, MEP$_{120\%}$ was significantly greater in the high stress compared to the low stress condition (p<0.01). The change in SICI between stress conditions was not significantly correlated with changes in either MEP$_{\text{UNCOND}}$ (r = 0.072, p = 0.78) or RMT (r = 0.21, p = 0.42).

**Figure 3.4:** Panel A shows short-interval intracortical inhibition (SICI) for the chronic neck pain (white) and healthy control (black) groups in the low stress and high stress conditions (left), and the average change in SICI between conditions (right). Values are mean (SE). * Significant difference from controls (p<0.05).
Figure 3.5: Panel A shows resting motor threshold (RMT) for the chronic neck pain (white) and healthy control (black) groups in the low stress and high stress conditions (left), and the average change in RMT between conditions (right). MSO = Maximum Stimulator Output. Panel B shows the normalized peak-to-peak amplitude of motor evoked potentials at a stimulus intensity of 120% RMT (MEP_{120%}) using the same format. Values are mean (SE). * Significant difference from controls (p<0.05). ** Significant difference between stress conditions (p<0.01).

Discussion

This study compared intracortical inhibition and corticospinal excitability between individuals with and without chronic neck pain, and investigated how these measures change during exposure to a psychosocial stressor. The two groups showed similar increases in MEP amplitude in the high stress condition, indicating that psychosocial stress increases net excitability of the corticospinal tract to the trapezius both in the presence and absence of chronic pain. In contrast to healthy individuals who responded to heightened levels of stress with increased intracortical inhibition,
participants with chronic neck pain showed high levels of inhibition at rest and during exposure to the low stress condition, which did not change with further increases in psychosocial stress.

**Similar Group Effects of Psychosocial Stress on Corticospinal Excitability**

The stress manipulation evoked significant increases in physiologic arousal (RPP) and perceived anxiety (STAI-S) that were similar in magnitude for individuals with and without chronic neck pain, despite initially higher levels of perceived anxiety for the neck pain group in the low stress condition. There were no group differences in MEP amplitude or RMT in the upper trapezius during the low stress condition, and both groups showed increases in corticospinal excitability during an acute increase in psychosocial stress. This was observed as a similar increase in MEP amplitude for both groups, and a decrease in RMT in individuals with neck pain during the high stress condition. These findings are in agreement with a previous study showing that anticipatory anxiety causes a greater increase in MEP amplitude in the resting forearm muscles of healthy adults compared to a counting task performed without social evaluative threat (Oathes, Bruce et al. 2008). Although another study found no difference in MEP amplitude of the first dorsal interosseus muscle during a voluntary motor task performed under low and high stress (Tanaka, Funase et al. 2011), these assessments were made during voluntary movements of the index finger with different levels of background muscle activity, which can independently affect MEP size (Martin, Gandevia et al. 2006). Thus, findings from the present study support limited existing evidence that acute stress and anxiety increase corticospinal excitability in healthy humans (Oathes, Bruce et al. 2008), and further
indicate that this increase occurs both in the presence and absence of chronic muscle pain.

**Differential Group Effects of Psychosocial Stress on Intracortical Inhibition**

We found that individuals with chronic neck pain demonstrated higher levels of intracortical inhibition than pain-free individuals when exposed to nominal levels of stress. This observation is consistent with previous findings of increased SICI (Schabrun and Hodges 2012) and decreased EMG activity (Falla, Farina et al. 2007) following acute experimental pain in healthy adults, and supports central inhibition as one potential mechanism to protect a painful muscle from injury (Hodges and Tucker 2011). However, the finding of increased SICI among individuals with chronic neck pain contrasts with previous literature showing decreased (Schwenkreis, Janssen et al. 2003, Eisenberg, Chistyakov et al. 2005, Lefaucheur, Drouot et al. 2006, Mhalla, Andrade et al. 2010, Schwenkreis, Scherens et al. 2010) or unaltered (Schwenkreis, Scherens et al. 2010) SICI in other chronic pain populations. This discrepancy may be explained by differences in the etiology of various chronic pain syndromes, as the majority of studies have examined the role of SICI in widespread neuropathic pain syndromes as opposed to more localized muscle pain.

The observation of concurrent increases in corticospinal excitability and intracortical inhibition in response to psychosocial stress among healthy individuals is intriguing, and may be explained by a compensatory increase in cortical inhibition to offset stress-evoked increases in corticospinal excitability that occur downstream from the motor cortex. Potential sites of heightened corticospinal excitability in response to psychosocial stress have not yet been systematically investigated, but could include
stress-evoked increases in spinal reflex pathways to the trapezius (Alexander and Harrison 2002, Alexander and Harrison 2003) or enhanced neuromodulatory inputs to spinal motor neurons from brainstem structures that help regulate the stress response (Heckman, Johnson et al. 2008, Buharin, Butler et al. 2013). Findings from the present study suggest that stress-evoked increases in corticospinal output are not caused by reduced activation of GABA_A inhibitory circuits in the motor cortex, indicating a need for future investigations to identify other sources of increased corticospinal drive in the presence of psychosocial stress.

Interestingly, SICI increased for all eight of the healthy participants when exposed to an acute psychosocial stressor, but showed no consistent change for individuals with chronic neck pain despite similar increases in MEP amplitude across stress conditions for both groups. Furthermore, individuals with chronic neck pain showed significantly elevated levels of perceived anxiety and SICI even in the low stress condition, and a trend toward higher levels of SICI at baseline compared to healthy controls. These observations suggest that individuals with neck pain may inhibit motor output to chronically painful neck muscles in the presence of relatively minor stressors, and are unable to compensate for any additional stress-evoked increases in corticospinal excitability through further modulation of SICI. Values of SICI measured for the neck pain group fall within the normal range of SICI values reported for other muscles (Ortu, Deriu et al. 2008, Garry and Thomson 2009), whereas healthy individuals tended to exhibit smaller and more variable SICI in the trapezius. To our knowledge, SICI has not been previously measured in the trapezius, therefore the normal range of values for this muscle has not been established. It is possible that individuals with neck pain are unable to effectively
modulate SICI in response to stress because they have already reached their physiologic capacity to recruit intracortical pathways that inhibit the trapezius muscle in the presence of ongoing pain. The observation of similar and high values of SICI in the baseline, low stress, and high stress conditions for the neck pain group supports this interpretation.

**Study Limitations**

Due to technical limitations in maximum stimulator output, a large number of otherwise eligible participants were excluded because of high RMTs in the upper trapezius. This observation is likely explained by the relatively small cortical representation of the trapezius muscle, as proximal arm muscles are known to have higher motor thresholds than more distal arm muscles (Rothwell, Thompson et al. 1987). Interestingly, difficulties recruiting the trapezius at lower intensities of cortical stimulation may imply a lesser influence of corticospinal projections compared to subcortical inputs (Davidson and Buford 2006, Alexander, Miley et al. 2007, Stephenson, Christou et al. 2011) on the control of this postural muscle. Although the responsiveness of a muscle to cortical stimulation can be increased with a small voluntary contraction (Rothwell, Thompson et al. 1987, Thompson, Day et al. 1991, Ilić, Meintzschel et al. 2002), the present study aimed to examine intracortical and corticospinal responsiveness in a relaxed muscle to avoid the confounding influence of voluntary contraction on SICI (Garry and Thomson 2009). The potential sampling bias introduced by this study design is not known, however, a similar percentage of participants were excluded from both groups and no evidence of a sampling bias was found for any of the measured demographic characteristics. The relatively small sample size was sufficient to detect group differences in intracortical and corticospinal excitability across low and high stress.
conditions, but may have lacked power to identify more variable trends for group
differences in MEP$_{120\%}$ and SICI for the baseline condition in which attentional demands
were not systematically controlled. Future studies will investigate the effects of
psychosocial stress on modulation of intracortical and corticospinal inhibition during
voluntary contractions, facilitating the inclusion of a larger sample size.

A second limitation is that the order of the stress conditions was not
counterbalanced due to the amount of time required for participants to return to baseline
arousal after exposure to the psychosocial stressor, as well as the ethical obligation to
debrief participants immediately after the high stress condition. This does not appear to
have significantly affected the current results, as pilot experiments revealed no change in
TMS measures when the low stress condition was repeated twice. These findings are
consistent with the documented lack of time effects for other motor outcomes in previous
studies using a similar experimental protocol (Shahidi, Haight et al. 2013).

Finally, for reasons discussed previously, we chose to assess SICI using a
constant intensity of conditioning and test stimuli. Stimulation parameters were based on
motor thresholds measured in the baseline condition, and were not adjusted to control for
the observed changes in corticospinal excitability (e.g., MEP$_{\text{UNCOND}}$ and RMT) across
low and high stress conditions. Thus, it is possible that changes in the relative threshold
of stimulation across stress conditions may have influenced SICI outcomes. Two
observations suggest that such an influence, if present, was negligible. First, changes in
SICI were not significantly correlated with changes in MEP$_{\text{UNCOND}}$ amplitude or RMT
across low and high stress conditions. Second, MEP$_{\text{UNCOND}}$ increased similarly for both
groups in the high stress condition, whereas SICI increased only for healthy individuals.
Clinical Implications and Future Research

Psychosocial stress is a known risk factor for a variety of musculoskeletal pain syndromes (Macfarlane, Pallewatte et al. 2009, Lang, Ochsmann et al. 2012), including chronic neck pain (Ariëns, Mechelen et al. 2001, Bongers, Ijmker et al. 2006). Our findings suggest that individuals with neck pain may have tonically elevated levels of intracortical inhibition to help reduce activity of a chronically painful muscle. When these individuals encounter relatively minor stressors that increase overall excitability of the corticospinal tract, they appear to be unable to compensate with further increases in intracortical inhibition that would presumably help maintain a relaxed muscle under stress. An impaired ability to voluntarily inhibit increases in corticospinal excitability caused by exposure to daily stressors may thereby contribute to persistent muscle tension commonly observed in the superficial cervical muscles of patients with chronic neck pain (Falla, Jull et al. 2004, Falla and Farina 2008). This could prevent the typical redistribution of mechanical loads away from painful tissues and prolong healing.

Although it remains to be determined whether stress-evoked increases in muscle tension are an underlying cause or simply a correlate of other processes responsible for persistent neck pain (Mork and Westgaard 2006), the inability to relax a painful muscle during times of stress may indicate a need to address acute physiologic stress responses in chronic pain management.
Conclusions

Findings from this study are the first to demonstrate that psychosocial stress increases corticospinal excitability both in the presence and absence of chronic pain, and that individuals with neck pain exhibit heightened activation of inhibitory pathways within the motor cortex that fail to modulate in response to acute stressors.
CHAPTER IV

PSYCHOSOCIAL STRESS ALTERS THE STRENGTH OF RETICULOSPINAL INPUT TO THE HUMAN UPPER TRAPEZIUS MUSCLE

Abstract

Objective

Psychosocial stress has been shown to influence human motor control, with one presentation as increased muscle activity in the upper trapezius muscle. While several potential mechanisms contributing to this phenomenon have been investigated, it is not completely understood. This study investigated the potential contribution of reticulospinal input to stress-induced upper trapezius muscle activity.

Methods

Twenty-five healthy participants were exposed to startling acoustic stimuli (SAS) during periods of low and high psychosocial stress. Acoustic startle reflexes (ASRs) were investigated in the left upper trapezius during low-level contractions using both surface and intramuscular electromyography.

Results

The majority of participants demonstrated inhibitory ASRs. A small subgroup with significantly higher (p < 0.01) trait anxiety demonstrated excitatory ASRs. All ASRs decreased in magnitude during exposure to the acute psychosocial stressor. Single motor unit recordings exhibited decreased and increased discharge rates during inhibitory and excitatory ASRs, respectively.
Conclusions

These findings suggest that reticulospinal input to the upper trapezius is decreased during acute psychosocial stress, implicating an increased reliance on more central mechanisms of motor control during increased stress. Further research is required to investigate mechanisms behind the complex ASRs seen in this study.

Introduction

Psychosocial stress has been shown to influence human motor control (Noteboom, Fleshner et al. 2001, Christou, Jakobi et al. 2004, Stephenson and Maluf 2010, Staab, Balaban et al. 2013). Specifically, increases in upper trapezius muscle activity has been shown with a variety of stress-inducing tasks (Nilsen, Sand et al. 2007, Eijckelhof, Huysmans et al. 2013, Shahidi, Haight et al. 2013). Often thought of as a potential component of a “fight-or-flight” response, these stress-induced changes in motor control, especially in the upper trapezius muscle, are not fully understood.

Several potential mechanisms of this stress-induced change have been investigated, but current results are not conclusive. Sympathetic blockade does not eliminate the presence of stress-induced muscle activity (Nilsen, Sand et al. 2008), indicating that activation of the sympathetic nervous system during stress is not solely responsible for this excess muscle activity. Psychosocial stress has been shown to increase responsiveness of the corticospinal tract to transcranial magnetic stimulation (Oathes, Bruce et al. 2008), specifically when stimulating over the cortical representation of the upper trapezius (Marker, Stephenson et al. 2013), indicating some involvement of the motor cortex and corticospinal tract. However, despite known innervation from the reticular formation to the upper trapezius (Davidson and Buford 2004, Davidson and
Buford 2006) and the reticular formation’s association with arousal (Holstege 1992, Jones 2003), changes in the reticulospinal input to the upper trapezius during periods of psychosocial stress are not known.

Responsiveness of the reticulospinal tract can be assessed non-invasively in humans through investigation of the acoustic startle reflex (ASR) (Rothwell 2006). The ASR can be elicited in response to a large amplitude, unexpected, sudden onset sensory stimulus, most often in the form of an acoustic stimulus (Blumenthal, Cuthbert et al. 2005). The ASR is characterized as a generalized protective response proceeding from a rostral to caudal direction; observed movements associated with the ASR include an eye blink, cervical extension, superior and anterior movement of the shoulders, and a general shortening of body length (Yeomans and Frankland 1995). Little is known of the upper trapezius response to the ASR due to the rapid habituation of components other than the blink reflex (Aniss, Sachdev et al. 1998), which may be mediated by a separate pathway (Valls-Sole 2012). Recently it has been shown that habituation to an acoustic stimulus can be greatly reduced by the readiness to perform a motor task (Valls-Sole, Kumru et al. 2008, Carlsen, Maslovat et al. 2011). This StartReact phenomenon has been used to investigate the process of motor preparation (Carlsen, Maslovat et al. 2012), including that of head movements (Siegmund, Inglis et al. 2001), however specific investigations of cervical muscle activity during the ASR in these paradigms has not been performed.

This investigation utilizes the reduced habituation of the ASR in cervical muscles during a StartReact paradigm to investigate changes in reticulospinal input to the upper trapezius muscle during periods of low and high psychosocial stress. Due to the reticular formation’s involvement in increased arousal, it is hypothesized that the ASR in the
upper trapezius will increase during high psychosocial stress, indicating greater reticulospinal excitatory input. This finding would support the contribution of the reticular formation in stress-induced increases in upper trapezius muscle activity.

Methods

Participants

Healthy participants were recruited using electronic advertisements at a university medical campus and the surrounding community. All participants provided written informed consent in accordance with procedures approved by the Colorado Multiple Institutional Review Board, including additional protections for a partial waiver of consent required for the stress manipulation. Participants were screened for the absence of neck pain by a licensed physical therapist. Exclusion criteria included objective signs of structural or neurologic impairment (e.g. radiculopathy), self-reported history of traumatic injury or surgery affecting the neck or shoulder region within 12 weeks of enrollment, and history of any major cardiovascular, neurological, or psychiatric medical condition.

Electromyography

Surface electromyography (sEMG) was recorded from the non-dominant upper trapezius using bipolar Ag-AgCl surface electrodes. Electrodes were positioned with a 15 mm interelectrode distance, centered 20 mm lateral to the midpoint between C7 and the posterior lateral border of the acromion (Farina, Madeleine et al. 2002). A reference electrode was placed over a bony portion of the ipsilateral clavicle. sEMG data were amplified (1000x), band-pass filtered (13 – 1000 Hz LabLinc V, Coulbourn Instruments,
Intramuscular EMG recordings of single motor unit (SMU) activity were collected using fine wire intramuscular electrodes custom made from two Formvar-insulated stainless steel wires (50 μm diameter, California Fine Wire Co., Grover Beach, CA) with the cross-sectional area exposed for recording. Wire electrodes were placed with a 30-gauge needle approximately 1 cm anterior to the midpoint between surface electrodes. The needle was removed after electrode placement and small adjustments in wire position were made to enhance signal quality as necessary. A reference electrode was placed adjacent to the reference electrode for the sEMG. The intramuscular EMG signal was amplified (1000x), band-pass filtered (20 – 8000 Hz, LabLinc V, Coulbourn Instruments, Whitehall, PA), and sampled at 20,000 Hz (Power1401, Cambridge Electronic Design, Cambridge, UK).

**Motor Tasks and Startling Acoustic Stimulus**

Startling acoustic stimuli (SAS) were given in the context of a simple reaction time (RT) task to exploit the reduction in habituation seen with the StartReact effect (Carlsen, Maslovat et al. 2011). The RT task required participants to perform a lateral pinch grip on a force transducer (Lode, HDM-915, Groningen, Netherlands) in response to a non-startling auditory cue (76 dB tone, 50 ms duration) (LabLinc V, Model V85-05, Coulbourn Instruments, Whitehall, PA) presented binaurally via headphones (non-startle trials). The SAS consisted of a 124 dB, 50 ms burst of white noise with near instantaneous rise-time (LabLinc V, Model V85-05, Coulbourn Instruments, Whitehall, PA). The SAS replaced the RT task auditory cue in 33% of trials (startle trials) in a
pseudorandom order such that the SAS was never presented as the first auditory cue in each block, or during any two consecutive trials. Participants were instructed to perform the RT task as fast as possible in response to both the auditory cue and SAS. Continuous white noise (60 dB) was present in the background during tasks to minimize any effects of environmental noise. The amplitude of all auditory cues was confirmed before all investigations (Lutron SL-4001). RTs for both startle and non-startle trials were calculated.

In order to investigate the effect of the SAS on SMU behavior, participants performed a low intensity contraction with the non-dominant upper trapezius by shrugging up against a force transducer positioned over the acromion (1112 N range; 7.6 mV/N; P310, Cooper Instruments, Warrenton, VA). Real-time feedback of force production and a target contraction force was displayed on a monitor positioned approximately 1 m in front of the participant at eye level. The target level for the contraction was set individually for each participant at a level corresponding to the recruitment and steady discharge of 1 – 2 distinct motor units in the intramuscular EMG recording, and was held constant throughout the experimental protocol. Each RT trial began with a 3 s tone (76 dB) during which participants were instructed to slowly shrug up to the target force and maintain a steady contraction throughout the rest of the trial. The auditory cue or SAS was presented 3 – 8 s (random interval with uniform distribution) after this initial tone. Two seconds after the auditory cue or SAS, trials ended with a 3 s tone (76 dB) during which participants were instructed to slowly relax their shoulder. Figure 4.1A shows a diagram of an individual trial, including the timing of all auditory cues and motor tasks.
Figure 4.1: Diagrams of an individual trial (A) and the experimental protocol (B). The Habituation condition was composed of six SAS with no other task requirements. Task Practice contained eight individual trials with no SAS. Low Stress and High Stress conditions were composed of four blocks of 10 individual trials with the SAS replacing the 50 ms tone in 33% of trials.

RT = Reaction Time, LUT = Left Upper Trapezius, SAS = Startling Acoustic Stimulus

**Psychosocial Stressor**

The experimental stress protocol manipulated psychosocial stress using a numeric memorization task combined with social evaluative threat (Dickerson and Kemeny 2004). Participants were initially informed that the purpose of the study was to examine the effects of mental concentration on muscle activity, and therefore remained naïve to the stress manipulation until the end of the session. During both low and high stress conditions, series of five, two-digit numbers were presented on a video monitor for the participant to memorize and were removed after 10 – 15 s. Participants were asked to repeat the numbers in sequence after completing 10 trials of the RT task. Four blocks of 10 trials were completed in each stress condition, with a different sequence of numbers presented at the beginning of each block. Difficulty of the memorization task did not
differ between stress conditions to control for the effects of cognitive demand, and participants were instructed to perform the task as accurately as possible in both conditions. Participants were told that they were “only practicing” the task during the low stress condition. They were told that their performance was not being monitored and positive feedback was provided by a familiar investigator, regardless of actual performance. The high stress condition was administered by an unfamiliar, authoritative investigator who provided no positive feedback. Participants were told that they would be videotaped and paid based on their performance. Examiners during both stress conditions were the same for all participants. Immediately after completion of the high stress condition, all participants were fully debriefed on the details and purpose of the stress manipulation. They were assured that no video recordings were made and they would receive full monetary compensation regardless of performance.

Perceived anxiety was assessed by the state portion of the Spielberger State-Trait Anxiety Inventory (STAI-S) (Spielberger 1983), and physiologic arousal was assessed by mean arterial pressure (MAP) and heart rate (HR) collected with an automated oscillometric cuff (Coulbourn V series module) placed around the left arm. STAI-S was collected twice during baseline procedures and after each stress condition. MAP and HR were collected at the same two time points during baseline and after each block of RT trials in both stress conditions (see Figure 1B).

**Experimental Protocol**

The complete experimental protocol is shown in Figure 1B. Participants first completed a familiarization session, in which all details of the experiment (except stress manipulation) were explained. A brief physical screen was performed to rule out the
presence of neck pain, and participants completed a questionnaire containing demographic information and the trait portion of the STAI (STAI-T). Setup of sEMG and intramuscular electrodes was then performed, and participants transitioned to a custom designed experimental chair where they sat with both arms supported. Signal quality was verified for all channels, and a target contraction level was determined based on SMU recordings from the intramuscular EMG as described above. Participants were then exposed to six SAS, separated by 15 – 20 s, to familiarize them to the stimulus and to account for any initial habituation or orienting responses (Valls-Sole, Kumru et al. 2008). Finally, participants practiced 8 – 10 non-startle trials of the target matching and pinch tasks to become familiar with the motor tasks and minimize potential learning effects during the stress conditions. Participants then completed four blocks of 10 trials during both stress conditions, resulting in a total of 28 non-startle and 12 startle trials per stress condition. The order of SAS presentations was the same in both stress conditions, and the low stress condition was always presented first to avoid carry-over of stress responses. After debriefing, intramuscular electrodes were removed and participants performed 2 – 4 maximum voluntary contractions (MVCs) with the non-dominant upper trapezius by shrugging up against resistance.

**Control Session**

A subset of participants agreed to return for a control session to investigate the effects of time on the startle response. This session followed the same experimental protocol as that shown in Figure 1B, except participants performed a second low stress condition instead of the high stress condition. No intramuscular EMG was collected during the control session. The target contraction level for the left upper trapezius was set
to the same amplitude (%MVC) as the experimental session for each participant. The order of SAS presentations was also the same as during the experimental session.

**sEMG Data Processing**

sEMG data were pre-processed using custom Spike2 software (Cambridge Electronic Design, Cambridge, UK). All trials were visually inspected for the presence of cardiac artifacts, which were removed using a filtered template subtraction technique (Marker and Maluf 2014) (Appendix A). Trials where a cardiac artifact was present in the 150 ms after the SAS were discarded. DC offset was removed and trials were exported for processing with custom LabVIEW software (National Instruments, Austin, TX, USA).

A cumulative sum (CUSUM) analysis (Ellaway 1978) was performed on the sEMG from startle trials within each stress condition to characterize the ASR in the upper trapezius. This technique allows the characterization of inhibitory and excitatory components of a multi-phasic response. The analysis was performed according to recommendations from Brinkworth and Turker (2003). The 200 ms period prior to and after the SAS (400 ms total) was rectified and averaged across trials within each stress condition. The CUSUM calculation was then performed according to the following equation:

\[
CUSUM(t) = \sum_{t_p}^{t} \text{bw}(EMG(T) - \text{EMG}(T_0))
\]

Where t is the time of the current data point; \(t_p\) is the pre-SAS analysis time; \(\text{EMG}(T_0)\) is the pre-SAS EMG mean; and bw is the inverse of the sampling rate in ms (corrects for sampling rate).
Turning points (e.g. slope equal to zero) in the resulting CUSUM signal represent times when the averaged trial signal crosses the mean of the pre-SAS period and significant events (responses) are identified as vertical deviations between turning points greater than those seen in the pre-SAS period, representing periods above or below the pre-SAS mean sEMG for greater durations than those seen in the pre-SAS period. To reduce the occurrence of non-meaningful turning points in the CUSUM signal, it was passed through an additional zero-phase shift low pass filter (100 Hz). This filter was lower than published recommendations (200 Hz), but was necessitated by the low number of SAS trials used in creating trial averages, and results must be interpreted with the knowledge that response onset times may be slightly shifted by the selected filter setting (Brinkworth and Turker 2003). Response onset was identified as the initial turning point of a significant event, duration as the time between the turning points of a significant event, and amplitude as the vertical distance between turning points of a significant event. Response amplitudes were converted to response strength by expressing them as a percentage of response duration (representing maximum inhibition, see (Brinkworth and Turker 2003)). Figure 4.2 demonstrates the CUSUM procedure and response characterization with representative data. Finally, the root-mean-squared average of the sEMG signal in the 3 s period prior to each cue or SAS was averaged within each stress condition to quantify the amplitude of background muscle activity.
Figure 4.2: Shows the CUSUM processing technique with representative data. An individual response to a SAS in the left upper trapezius before (A) and after rectification (B). All rectified SAS responses in a given condition were averaged (C) (gray plots represent individual trials, black plot is averaged trial). The averaged trial after CUSUM processing is shown in D. A response when the vertical distance between two turning points (sign change in slope) was greater than those identified in the pre-stimulus period. The onset (On) was set as the first turning point, the amplitude (Amp) as the vertical distance between the two consecutive turning points, and the duration (Dur) as the horizontal distance between the two consecutive turning points. The strength (Str) of a response is calculated as amplitude divided by duration multiplied by 100, representing the percentage of maximum excitation or inhibition. This technique allows the identification of multiple excitatory or inhibitory responses. Response 1 (On1, Amp1, Dur1, Str1) is inhibitory and Response 2 (On2, Amp2, Dur2, Str2) is excitatory. Vertical dashed lines represent the timing of the SAS.

SAS = Startling Acoustic Stimulus

Intramuscular EMG Data Processing

Template matching software in Spike2 was used to discriminate SMUs in the intramuscular EMG signal in a 1 s window centered around the SAS. Discriminated SMUs were then converted to discharge rates (DRs) and exported for further analysis using MatLab software (MathWorks Inc., Natick, MA, USA). Large variability in SMU recruitment during the task led to few SMUs with an adequate number of SAS trials to
compare activity of the same SMU across stress conditions. SMU DRs were therefore combined within each stress condition by normalizing DRs to the mean DR from the 150 ms pre-SAS period in a given trial, and time-locking DRs to the ASR response onset for their respective condition. Normalized and time-locked DRs were combined and averaged in 10 ms bins to investigate changes in SMU DR during startle responses.

Data Analysis

All time points for STAI-S, MAP, and HR measurements were averaged within each experimental condition, and compared with a Repeated Measures Analysis of Variance (RM ANOVA) with one factor of Condition (baseline, low stress, high stress). RTs were compared with a 2x2 RM ANOVA, with factors of Trial (startle or non-startle) and Condition (low stress or high stress). Post-hoc analyses were performed with paired t-tests and Bonferroni corrected for multiple comparisons. Significance was set as p < 0.05. Mean pre-SAS sEMG values were compared between low stress and high stress conditions using a paired t-test. SMU mean DRs were calculated in the 500 ms pre-SAS period and compared between low stress and high stress conditions with an unpaired t-test.

To characterize inhibitory and excitatory response patterns of the upper trapezius ASR, reflexes were first visually inspected and categorized into subgroups based on similar response patterns in the low stress condition. Further analyses were performed with non-parametric statistics due to smaller sample sizes within subgroups. Response onsets, durations, and strengths were compared between conditions using rank-sum tests within subgroups. Differences between subgroups in demographic characteristics were investigated using exact k-sample permutation tests (Curran-Everett 2012). Significance
for these analyses was set as $p < 0.05$ and non-significant trends were identified as $p < 0.1$. All statistical analyses were performed using R software (R Development Core Team, Vienna).

**Results**

**Experimental Session**

Twenty-five participants were recruited for the study (mean age = 30 (range 24 – 49) years, 18 women). Mean (SD) STAI-T was 30 (6.6) out of a possible 80 points, with higher scores indicating higher trait anxiety (Spielberger 1983).

Results for STAI-S, MAP, and HR analyses are shown in Figure 4.3. The main effect of Condition was significant for STAI-S ($p < 0.01$), MAP ($p < 0.01$), and HR ($p < 0.01$). The high stress condition was significantly greater than the low stress and baseline conditions for all three measures ($p < 0.01$ for all). The low stress condition was significantly greater than the baseline condition for all three measures (STAI-S $p = 0.04$; Map and HR $p < 0.01$).

The target contraction for the upper trapezius contraction was set at a mean (SD) of 7.4 (9.5) %MVC force (range = 0.3 – 22 %MVC). Pre-SAS mean sEMG did not change between low stress and high stress conditions (8.3 (6.1) %MVC vs. 8.6 (6.8) %MVC, respectively, $p = 0.84$). RTs demonstrated a main effect of Trial ($p < 0.01$) but not Condition ($p = 0.41$). RTs were significantly faster during startle trials (236 (84) ms) compared to non-startle trials (340 (74) ms) ($p < 0.01$).
Figure 4.3: Changes in perceived anxiety (STAI-S) (A) and physiologic arousal (MAP (B) and HR (C)) during the experimental protocol. All time points collected for each measure are shown. Time points within each condition were averaged prior to statistical analysis, which compared Baseline (B1 and B2), Low Stress (L1, L2, L3, L4), and High Stress (H1, H2, H3, H4) only.

STAI-S = Spielberger State-Trait Anxiety Index, State Score, MAP = Mean arterial pressure, HR = Heart rate

Four characteristic response patterns were observed in the ASR during the low stress condition; representative examples of each response pattern are shown in Figure 4. One participant showed no identifiable ASR, and was not included in subsequent analyses. Observable startle response patterns included 1) a single period of inhibition, 2) inhibition followed by excitation, 3) two periods of inhibition, and 4) a single period of excitation. For subgroup comparisons, the three patterns demonstrating initial periods of inhibition were combined, creating an inhibitory ASR group (N = 19) and an excitatory ASR group (N = 5). No participants demonstrated a change from inhibition to excitation or vice versa between the low and high stress conditions. One participant in the inhibitory ASR group demonstrated no identifiable ASR in the high stress condition, and the
response strength stress was set to zero for this case. The two sub-groups did not differ in age (p = 0.34), however, the group with excitatory ASR responses showed significantly higher STAI-T compared to those with inhibitory ASR responses (36 (8) vs. 28 (4), p < 0.01).

**Figure 4.4:** Representative averaged traces from the four major response patterns identified in participants: a single period of inhibition (A), inhibition followed by excitation (B), two periods of inhibition (C), and a period of excitation (D). Participants were categorized based on the pattern seen during the low stress condition. Vertical dashed lines represent timing of the SAS. One participant showed no identifiable pattern or response.

SAS = Startling acoustic stimulus

ASR response onsets and durations for both sub-groups during each stress condition are shown in Table 4.1. Neither onset nor duration were significantly different between conditions (p > 0.22). Changes in ASR response strength between stress conditions for both groups are shown in Figure 4.5. The inhibitory ASR group demonstrated a significant reduction in the strength of inhibition in the high compared to low stress condition (-32 (12) % vs. -40 (9) % maximum inhibition, respectively, p <
The excitatory ASR group showed a trend for reduced strength of excitation in the high compared to low stress condition (61 (27)% vs. 32.3 (4)% maximum inhibition, respectively, p = 0.09).

**Table 4.1:** Response characteristics during the low and high stress conditions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Low Stress</th>
<th></th>
<th>High Stress</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset (ms)</td>
<td>Duration (ms)</td>
<td>Onset (ms)</td>
<td>Duration (ms)</td>
</tr>
<tr>
<td>Inhibition</td>
<td>57.0 (14)</td>
<td>57.0 (21)</td>
<td>54.8 (21)</td>
<td>52.4 (22)</td>
</tr>
<tr>
<td>(N = 19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excitation</td>
<td>60.2 (15)</td>
<td>37.6 (15)</td>
<td>49.2 (9)</td>
<td>30.9 (13)</td>
</tr>
<tr>
<td>(N = 5)</td>
<td></td>
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</tbody>
</table>

**Figure 4.5:** Changes in the strength (as calculated from the CUSUM analysis) of the initial response to the SAS between low and high stress. Participants who demonstrated two periods of inhibition or a period of excitation following inhibition were included in the initial inhibition analysis, and only the first period of inhibition was included in the analysis. Initial inhibition significantly decreased from low to high stress, and a trend for decreased excitation from low to high stress was seen.

SAS = Startling Acoustic Stimulus

A total of 37 SMUs in 17 participants were discriminated from 105 startle trials in the low stress condition, and 33 SMUs in 16 participants were discriminated from 81.
startle trials in the high stress condition. Mean (SD) DR of SMUs during 500 ms pre-SAS period was 11.2 (2.3) Hz in the low stress condition and 11.5 (1.9) Hz in the high stress condition and was not significantly different between conditions (p = 0.4). Representative examples of SMU recordings during inhibitory and excitatory ASR responses, and the group averaged DRs are shown in Figure 4.6. A sustained decrease in DR is seen during inhibitory ASRs in both low and high stress conditions, and a transient increase in DR is seen during excitatory ASRs in both low and high stress conditions.

Figure 4.6: Results from SMU recordings. Example SMU recordings are shown for participants with an initial (A) and excitatory (B) response. Vertical dashed lines represent timing of the SAS. Due to the small number of trials in each SMU, individual SMUs were combined to investigate changes in DR during startle responses. DRs were normalized to the average DR from a 150 ms pre-stimulus period and the average response onset for each participant was set as time zero due to the large variability in response latencies. DRs were then averaged within 10 ms bins. Periods of decreased and increased DR were noted in both the low and high stress conditions in the group with initial inhibition (C and D) and initial excitation (E and F), respectively. Note that several 10 ms bins in the group with initial excitation did not contain any SMU recordings due to the small number of SAS trials containing SMUs. The inhibitory ASR group contained 31 SMUs with 93 SAS trials in low stress, and 28 SMUs with 73 SAS trials in high stress. The excitatory ASR group contained 6 SMUs with 12 SAS trials in low stress, and 5 SMUs with 8 SAS trials in high stress.
Control Session

Twelve participants returned for the control session. No significant main effect for Condition was seen in STAI-S (p = 0.24) or HR (p = 0.05). A significant main effect for MAP (p = 0.04) was seen, with post-hoc analyses revealing an increase from the baseline condition to the high stress condition (p = 0.03). Results for RTs and pre-SAS sEMG means were the same as the experimental session.

Of the returning participants, one had no identifiable startle response in the experimental or control session. Nine participants from the inhibitory ASR group returned, though one had no identifiable response in the control session. Two participants from the excitatory ASR group returned. The excitatory group in the control session still demonstrated an increased STAI-T compared to the inhibition group (p = 0.04). In the inhibitory ASR group, no significant change in response strength was seen from the low stress to the high stress condition (-37 (11) % and -39 (13) % maximum inhibition, respectively, p = 0.25). No statistical analysis was performed on the returning excitation group due to low sample size, but a decrease in response strength from low stress to high stress was seen (36% and 20% maximum inhibition, respectively).

Discussion

This investigation is the first to use a StartReact paradigm to characterize stress-induced changes in the upper trapezius ASR. The upper trapezius showed predominantly inhibitory responses to the SAS, although excitatory responses were observed in some individuals with higher trait anxiety. Contrary to expectation, both types of responses decreased in the presence of an acute psychosocial stressor, indicating a generalized reduction in input from the reticulospinal tract under stress.
No Effects of Stress on RT

The StartReact paradigm is typically used to investigate preparation of a motor response (Carlsen, Maslovat et al. 2012), which results in a decreased habituation of the ASR in cervical muscles (Valls-Sole, Kumru et al. 2008, Carlsen, Maslovat et al. 2011). Although decreased habituation was achieved in the present investigation, changes in motor preparation, as assessed by pinch grip RTs, were unchanged in response to the acute psychosocial stressor. RTs were significantly shortened in SAS trials compared to non-SAS trials, but were still longer than those seen in traditional studies of the StartReact effect (Carlsen, Maslovat et al. 2012). Both these findings may be explained by the complexity of the task participants were performing in the present experiment. Participants were required to perform an ongoing motor task (upper trapezius contraction) with both visual (target matching) and auditory cues (contract and relax), a motor reaction time task with an auditory cue, and an ongoing mental concentration task. A recent study investigating the effects of dual-task performance on the StartReact effect showed similar results to those seen here, with longer RTs during dual-task performance (Maslovat, Drummond et al. 2015). This study also showed decreased sensitivity of RT change in response to a SAS when one task was cognitive and the other motor. The complex task in the current investigation likely increased motor RTs and potentially reduced their responsiveness to the psychosocial stressor.

Upper Trapezius ASR

The authors are aware of only one previous study attempting to quantify the ASR in the upper trapezius (Aniss, Sachdev et al. 1998). Results from the current study are similar to this previous study in that both found a mix of inhibitory and excitatory
components in the ASR, with response onsets averaging approximately 55 ms for the upper trapezius in both studies. This response latency in the upper trapezius is longer than those of responses produced by transcranial electrical and magnetic stimulation (Gandevia and Applegate 1988, Alexander, Miley et al. 2007). This could be due to a decreased conduction velocity within the reticulospinal track (Rothwell 2006) and/or mediation of the ASR through spinal inhibitory interneurons (Davidson and Buford 2004).

In the current study, an attempt was made to categorize participants based on two observed response patterns: those with an initial period of inhibition during the ASR (a combination of three different patterns showing this initial response) and those with an initial period of excitation during the ASR. The mechanisms underlying these different responses remain unclear. The reticulospinal tract is capable of coordinating complex tasks in primates (Rothwell 2006, Lemon, Landau et al. 2012). Specifically in non-human primates, the reticular formation has been shown to have both excitatory and inhibitory inputs to the upper trapezius (Davidson and Buford 2004) and this muscle has been shown to be highly responsive to reticular formation input (Davidson and Buford 2006).

The upper trapezius muscle was inhibited by reticulospinal input in the majority of participants. One purpose of the ASR is typically thought to facilitate a “fight-or-flight” response (Grillon 2008). Although potentially detrimental to the initiation of a "fight-or-flight" response, this inhibition was seen during an ongoing motor task. An inhibitory ASR may assist in the cessation of ongoing motor tasks, preparing the body for "fight-or-flight", or may be part of the freeze component of the more recent "freeze, flight, fight or fright" concept of a stress response (Bracha, Ralston et al. 2004). Group
analyses based on the initial response to the ASR suggest that individuals with higher trait anxiety show an initial excitatory response rather than inhibitory. This is similar to a previous study showing larger excitatory eye blink startle reflexes in women with higher trait anxiety (Poli and Angrilli 2015), though the current finding is interesting in that it involved an apparent reversal in direction of the response. Although some SMU recordings showed a combination of excitation and inhibition (see Figure 6B), this was not apparent in the analysis of group averages which demonstrated a transient increase in SMU DR during the ASR, and it is currently unclear whether some inhibition is still present during the excitatory responses.

The SMU data in current study supports that inhibitory and excitatory responses seen are a result of altered synaptic input to the spinal motor neurons and not a recording artifact or EMG crosstalk, which have been reported in upper trapezius reflexes previously (Vangsgaard, Norgaard et al. 2013). A decrease and increase in SMU DR was seen at response onset for both inhibitory and excitatory responses, respectively (Figure 6). This finding supports the ability of the reticulospinal tract to both facilitate and suppress upper trapezius muscle activity, consistent with previous findings from non-human primate studies (Davidson and Buford 2004, Davidson and Buford 2006). Although more studies are required to investigate mechanisms underlying the complex pattern of reticulospinal input during the ASR, the current and past studies (Aniss, Sachdev et al. 1998) support the idea that the ASR is more complex than the general protective response typically described (Yeomans and Frankland 1995), and is dependent on both participant characteristics and the task being performed.
Strength of the ASR Decreases with Increased Psychosocial Stress

The magnitude of both inhibitory and excitatory ASR response strength decreased in the presence of increased psychosocial stress (Figure 5). Although the magnitude of the response decreased, it was completely eliminated in only one participant and SMU data showed similar changes in DR in both stress conditions. The observed reduction in ASR response strength is contrary to the hypothesized stress-induced increase in reticulospinal input to the upper trapezius. The eye blink startle reflex, which is most often studied, has been shown to increase during periods of threat, fear, and anxiety (Dichter, Tomarken et al. 2002, Davis, Walker et al. 2010, Bublatzky, Guerra et al. 2013). However, the eye blink reflex may be mediated by a separate pathway than startle reflexes in other body regions (Valls-Sole 2012), and thus may show different changes in response to the environment. The decrease in strength of the ASRs could be explained by habituation to the SAS. Although participants in the control session were smaller in number, the results indicate that at least the decrease in inhibition during the acute stressor was not due to habituation. Excitatory responses in the control condition decreased, suggesting possible habituation, but the sample size in the control group was too small to make any statistical inferences.

When considering functional implications of the decreased startle magnitude during the psychosocial stressor, it is important to note that the psychosocial stressor in this study induces increased anxiety, or apprehension caused by unpredictable and symbolic threats (Grillon 2008). While the ASR may serve to facilitate a response to a sudden, immediate threat, when this threat is partially expected (as during anxiety), it may be more beneficial to facilitate voluntary action that is more specific to a given
threat. Functionally, this reduced reticulospinal input during increased anxiety may serve similar purposes as the reduced muscle spindle activity seen with sympathetic activation (Matsuo, Ikehara et al. 1995, Roatta, Windhorst et al. 2002). A reduction in peripheral and subcortical inputs during periods of increased anxiety may allow the body to make faster voluntary responses, via corticospinal mechanisms, to a partially anticipated threat. This idea is supported by the increased corticospinal responsiveness seen in response to acute psychosocial stressors (Oathes, Bruce et al. 2008, Marker, Stephenson et al. 2013). Reduced inhibitory input from the reticulospinal tract may also contribute to stress-induced increases in upper trapezius muscle activity (Eijckelhof, Huysmans et al. 2013, Shahidi, Haight et al. 2013), particularly in clinical pain populations where cortical inhibition may be impaired (Schwenkreis, Scherens et al. 2010, Marker, Stephenson et al. 2013).

**Limitations**

The major limitation of this study was the small number of startle trials available to quantify the ASR. Although the number of trials utilized for the CUSUM calculations was less than those previously recommended (Brinkworth and Turker 2003), it was necessary to limit the number of trials as physiologic arousal in response to the psychosocial stressor cannot be maintained indefinitely. Accordingly, a reduction in HR had already become evident near the end of the high stress condition (Fig 3C). The SAS was also presented at a slightly higher frequency (33% of trials) than previously recommended for StartReact paradigms (25% of trials) (Carlsen, Maslovat et al. 2011), but was similar to other studies investigating the StartReact in head movements (Siegmund, Inglis et al. 2001). Therefore, the number of SAS trials in the current study
was optimized for constraints of the stress protocol, and reliable responses were obtained. Sample sizes for sub-group analyses of inhibitory and excitatory ASR responses were also imbalanced and small. However, the statistical analysis used to compare groups makes no normality assumptions and is well suited for handling small, unbalanced groups (Curran-Everett 2012). The initial characterization of excitatory ASR responses, particularly regarding their presence and habituation among individuals with higher trait anxiety, should be confirmed in a larger sample.

**Conclusions**

This investigation was the first to quantify changes in the upper trapezius ASR in response to increased psychosocial stress. It was seen that the majority of participants demonstrated an inhibitory ASR, which may serve to cease ongoing motor tasks in preparation for an automatic protective response, and that all participants showed a decreased response strength magnitude during the acute psychosocial stressor, possibly serving to facilitate a fast voluntary response to an anticipated threat. Participants with higher trait anxiety also showed excitatory, instead of inhibitory ASRs. Future investigations are required to identify task determinants and physiologic mechanisms contributing to the complexity of the ASR response in humans.
CHAPTER V

UPPER TRAPEZIUS MUSCLE ACTIVITY INCREASES WITH PERCEIVED ANXIETY BUT IS NOT RELATED TO AUTONOMIC ACTIVITY IN HEALTHY OFFICE WORKERS DURING THE WORKDAY

Abstract

Objective

Chronic neck pain is leading cause of disability globally, especially in office workers. A well-established risk factor for developing chronic neck pain is psychosocial stress, though the mechanisms behind this link are unclear. The purpose of this investigation is to investigate the relationships between two potential mechanisms contributing to chronic neck pain, upper trapezius muscle activity and autonomic activity, with the other as well as perceived anxiety.

Methods

Upper trapezius electromyography (EMG) and electrocardiography (ECG) were recorded during 1 – 2 typical workdays in 77 healthy office workers. Upper trapezius muscle activity was quantified as muscular rest and median muscle activity, calculated from the EMG signal. Heart rate variability (HRV) measures were calculated from the ECG to assess autonomic activity. Participants reported trait anxiety at the start of the study and state anxiety at the end of each workday.

Results

Upper trapezius muscle activity and autonomic activity were not related to the other, either within or across participants. Neither were related to trait anxiety or absolute
levels of state anxiety. Within participants, median muscle activity was greater on days with higher perceived anxiety, but no changes were seen in muscular rest or HRV measures.

Conclusions

Amplitude of upper trapezius muscle activity, but not the amount of time it is active, increased with individual increases in state anxiety. Autonomic activity was not associated with upper trapezius motor activity or perceived anxiety, indicating that any contribution to the development of chronic neck pain is independent of these factors.

Introduction

Chronic neck pain is a leading cause of disability in the modern world (Hoy, March et al. 2014). Office workers have a disproportionately high incidence of neck pain compared to the general population, up to 63% annually (Côté, van der Velde et al. 2009). The primary cause of the high incidence of pain in this population is unknown. Given the significant increase in occupations in an office setting over the past several decades and the increased musculoskeletal problems associated with this setting (Andersen, Fallentin et al. 2011), investigating the cause or causes of pain in the office worker population is of utmost importance.

Well-established risk factors for work-related musculoskeletal pain development, including office work settings, are increased psychosocial stress, anxiety, and high perceived job stress (Ariens, van Mechelen et al. 2001, Bongers, Kremer et al. 2002, Lang, Ochsmann et al. 2012). Despite the known risk of high job stress, the mechanism behind the contribution it has to pain development is unknown. One possible mechanism linking stress and pain is through the influence of psychosocial stress on muscle activity
during the work day (Lundberg 1999). Psychosocial stress has been shown to increase cervical muscle activity during simulated computer work in a number of previous studies (Lundberg, Forsman et al. 2002, Nilsen, Sand et al. 2007, Eijckelhof, Huysmans et al. 2013, Shahidi, Haight et al. 2013) and increased muscle tension is associated increased musculoskeletal neck pain (Griffiths, Mackey et al. 2011). Originally proposed by Hagg in 1991, the Cinderella hypothesis proposes that sustained, low amplitude muscle activity caused by disproportionate activation of low-threshold motor units can lead to overuse and damage of Type I muscle fibers and the development pain. Increased psychosocial stress in the workplace may initiate or exaggerate this sustained, low amplitude muscle activity, increasing risk of tissue damage and pain development (Sjøgaard, Lundberg et al. 2000). Based on these prior observations and hypotheses, it is important to assess both muscle amplitude and muscular rest when investigating potential musculoskeletal disorder risk factors.

Altered activity of the autonomic nervous system (ANS) is present in individuals with chronic neck pain (Hallman and Lyskov 2012, Kang, Chen et al. 2012) and has been investigated in recent studies of musculoskeletal pain development (Maixner, Greenspan et al. 2011). ANS activity is also altered by actual and simulated workplace stressors (Vrijkotte, van Doornen et al. 2000, Shi, Hu et al. 2015) and can be measured continuously and non-invasively through assessment of heart rate variability (HRV) (Berntson, Bigger et al. 1997). ANS activity can directly influence (Bruehl and Chung 2004) and is influenced by the presence of pain (Maixner, Greenspan et al. 2011, Kulshreshtha, Gupta et al. 2012); however, increased activity of the sympathetic branch of the ANS can also alter muscle function (Roatta, Arendt-Nielsen et al. 2008, Roatta and
The relationship between cervical muscle activity and ANS activity in office workers, however, is unknown. It is possible that the contributions of psychosocial stress and ANS function to the development or chronicity of neck pain in office workers may be mediated through effects on cervical muscle activity. As such, it is important to determine the relationship between these factors in an office setting.

The main purpose of this study is to investigate relationships between measurements of upper trapezius muscle activity, ANS activity, and psychosocial stress during office work. It is hypothesized that an increased amplitude of upper trapezius muscle activity and a decreased amount of muscular rest will be associated with changes in HRV measures indicating increased sympathetic and decreased parasympathetic activity. It is also hypothesized increased muscle activity, decreased muscular rest, and HRV changes associated with increased sympathetic and decreased parasympathetic activity will be seen on days with high psychosocial stress when compared to days with low psychosocial stress.

Methods

Participants

Data represent the same population reported in Appendix B. A convenience sample of 77 healthy office workers was recruited through print and radio advertisements, new employee orientations, and employee bulletins and flyers posted at businesses employing a large number of office workers in the greater Denver area. Eligible participants were within three months of their date of hire and worked > 30 hours per week in an office setting that required the use of a computer for at least 75% of the workday. Participants were screened for the presence of neck pain or associated disorders
during the previous year. To avoid the potential for selection bias due to poor recall of pain symptoms, the Neck Disability Index (NDI) (Vernon and Mior 1991) was used to screen for activity limitations caused by pain which are more likely to be remembered than non-interfering neck pain. Participants were included in the study if they reported no neck pain or associated disorders during the previous year, and scored < 5 points on the NDI.

Exclusion criteria included: 1) objective signs of structural pathology upon physical examination by a licensed physical therapist, including but not limited to shoulder bursitis, impingement, tendonitis, fracture, and cervical nerve or disc impairment with radiculopathy or loss of sensory or motor function, 2) self-reported fibromyalgia diagnosis or musculoskeletal pain present in more than four body regions concurrently, 3) self-reported systemic illness including cancer, rheumatic, cardiovascular, or neurological disease, 4) prior surgery involving the cervical spine or shoulders, 5) acute (< 12 weeks prior to study) injury of the neck or shoulders, 6) untreated psychiatric condition, 7) uncontrolled hypertension, 8) pregnancy, and 9) an inability to type or comprehend written and oral instructions in English. All participants provided written informed consent according to study procedures approved by the Colorado Multiple Institutional Review Board.

Data Collection Protocol

Participants first completed a demographics questionnaire and the Spielberger State-Trait Anxiety Index, Trait Questionnaire (STAI-T) (Spielberger 1983) at a familiarization session. Bilateral upper trapezius electromyography (EMG) and electrocardiography (ECG) were recorded with a portable data monitor on a
representative workday selected by the participant based on convenience. A subset of participants (N = 63) completed a second day of worksite monitoring within two weeks of the first day. Participation in the second day of recording was based on participants’ willingness and availability to wear the monitor on a second workday. An investigator met the participant at a private location near his or her workplace 15 minutes prior to the start of the workday for equipment setup. After visual verification of signal quality, two submaximal reference voluntary efforts (RVE) and resting measurements were collected. Resting measurements were collected during quiet sitting with the shoulders relaxed and hands resting in the lap for 10 s.

Participants wore a portable data monitor continuously in a minimally obtrusive waist pack positioned on the anterolateral aspect of the waist throughout the workday as they performed their usual activities. Participants were instructed to ignore the device and not make any changes to their usual work routine. No observation of work activities was performed by study investigators to minimize observation bias. The portable data monitoring system was removed at the end of the workday by study investigators. In order to assess psychosocial stress for the workday, study participants then completed the Spielberger State-Trait Anxiety Index, State Anxiety questionnaire (STAI-S) (Spielberger 1983), with instructions to complete the questions based on how they felt during the workday.

**Data Recording and Processing**

The portable data monitor (Delsys Myomonitor IV; Delsys, Boston, MA, USA) recorded surface EMG bilaterally from the upper trapezius using surface electrodes (DE-2.3 Single Differential Surface EMG Sensor; Delsys) with two parallel 1 x 10mm silver
surface contacts with an inter-contact distance of 10 mm. Electrodes were placed on the upper trapezius muscle belly 2 cm lateral to the midline between the seventh cervical vertebra and the posterior acromion process (Farina, Madeleine et al. 2002). Electrode positions were marked and covered with a water-proof protective sealant to improve the reliability of sensor placement when multiple days were recorded. ECG was recorded with a bi-lead sensor (SP-X14 EKG Sensor for Myomonitor System; Delsys) with leads attached to 1.75 inch diameter foam and solid gel electrodes with 1 cm Ag/AgCl conducting snaps (Red Dot 9640; 3M, St. Paul, MN, USA) placed vertically on the flat portion of the sternum (Marker and Maluf 2014). EMG and ECG signals were referenced to a bony surface on the right clavicle with a self-adhesive electrode pad. EMG and ECG data were stored (sampled at 1000 Hz) on an internal 1GB memory card in consecutive one-hour data sets for offline processing. Each data set was 10 s short of an hour (3590 s) to allow for automated internal data storage.

EMG and ECG signals were preprocessed with custom scripts implemented in Spike2 (Cambridge Electronic Design, Cambridge, UK) and Matlab (MathWorks Inc., Natick, MA, USA) according to Appendix B. Each hour long data file was first visually inspected for EMG artifacts caused by wire movement, which were removed and replaced with the mean of the preceding 0.5 s of the EMG signal. ECG data was visually examined within each hour for recording artifacts and ectopic beats. Due to significant noise and interfering muscle activity present in the worksite ECG signals, 5 min periods clear of artifact and muscle activity were identified at end of each hour for HRV analysis. The ECG signal was also used to identify periods of ECG contamination in the EMG signal, which were subsequently removed using a validated filtered template subtraction
technique (Marker and Maluf 2014) (Appendix A). EMG was then root-mean-square (RMS) processed (100 ms window) and RVE normalized.

**Muscle and ANS Activity Outcomes**

All outcome measures were calculated for each hour of the workday. Participant averages were calculated by averaging each hour of the workday. Participant averages for participants with two days of recordings were calculated by averaging both days of recordings (Fethke, Gerr et al. 2012).

Muscle activity outcomes were calculated from the EMG signals according to Marker et al 2015. Muscular rest was calculated as the percent of recording time below a 5% RVE threshold (Hansson, Nordander et al. 2000). Periods where the EMG signal was below the threshold for less than 0.125 s were not included in this calculation due to lack of physiologic significance (Hansson, Nordander et al. 2000). Amplitude of muscle activity was assessed by calculating the median value (50th percentile) of the Active Amplitude Probability Distribution Function (Active APDF) (Appendix B), which removes all EMG values below 5% RVE prior to calculating the APDF. An example of the EMG processing and analysis procedure is illustrated in Figure 1.

ANS activity was assessed via HRV analysis. Detailed descriptions of HRV outcomes and interpretations can be found in Task Force Guidelines (1996) and Berntson et al 1997. R-waves were automatically detected by the software and then visually inspected and corrected for signal artifacts and ectopic beats. Mean HR was calculated for recording windows as the inverse of the mean R-R interval. Based on Task Force (1996) recommendations, no further time domain measures were calculated as these are more appropriate for extended (24 hour) analysis periods, whereas the current study
utilized multiple 5 min analysis periods. An equidistant, 4 Hz R-R interval time series was created from the original R-R interval time series via cubic spline interpolation. A fast fourier transform (FFT) was applied to these time series (300 s window) using Welch’s periodogram method to derive the power spectrum density. The LF (0.04 – 0.15 Hz) and HF (0.15 – 0.4 Hz) components of the power spectrum density were calculated for each 4 Hz R-R interval time series. Due to the short recording window, very low frequency (VLF) (0.0033 – 0.04 Hz) and hence total power of the R-R time series were unstable and not included in analyses. An illustration of ECG processing and HRV calculations is shown in Figure 5.1. Mean HR and the LF component of the power spectrum density represent overall ANS activity and are influenced by both the sympathetic and parasympathetic branches. The HF component of the power spectrum density is primarily influenced by the parasympathetic branch alone (Berntson, Bigger et al. 1997).

**Statistical Analysis**

Similar to previous studies (Maixner, Greenspan et al. 2011), HRV frequency components (LF and HF) were natural log transformed due to skewness in the data. Pearson correlation coefficients were calculated between EMG and HRV participant average outcomes to assess relationships between muscle and ANS activity across participants. To assess relationships between muscle and ANS activity during the workday, Spearman correlation coefficients were calculated between values of muscle and ANS outcomes calculated each hour within individual participants. Spearman correlation coefficients were utilized due to the low number of data points within each participant (5 – 7 hours). Correlation coefficients and their respective p-values were then
Figure 5.1: Representative example of processing and analysis of electromyography (EMG) signal and EMG outcomes (A-C), and electrocardiography (ECG) signals and heart rate variability (HRV) outcomes (D-F). Raw EMG (A) is smoothed with a 100ms root-mean-square (RMS) rolling window and normalized to reference voluntary efforts (RVE) (B). The percentage of signal below the 5% RVE threshold is quantified as Muscular Rest. Data below 5% RVE is removed prior to calculation of the Amplitude Probability Distribution Function (APDF) (C). Median Muscle Activity is defined as the 50th percentile of the APDF. R-waves are identified in the raw ECG signal (D) and used to create a 4 Hz time series of R-R intervals using cubic spline interpolation (E). Heart rate (HR) is defined as the inverse of the mean R-R interval for the time series. A fast-fourier transform is then applied to the R-R time series (F) and the Low Frequency (0.04 - 0.15 Hz) (LF) and High Frequency (0.15 - 0.4) (HF) components of the power spectrum are calculated.
averaged across participants, similar to previous studies assessing relationships between HRV and physical activity measures (Hautala, Karjalainen et al. 2010). To further test the significance of within participant correlations, a sign test was performed. In order to demonstrate a significant correlation, 46 out of the 74 participants would be required to have significant correlations between muscle and ANS outcomes during the workday.

Pearson correlation coefficients were calculated between STAI-T and STAI-S scores and muscle and ANS activity participant average outcomes to assess relationships to trait and state anxiety. To assess changes due to perceived anxiety during the workday, STAI-S scores were categorized as high or low within each participant with two days of recordings (with each participant having a high and low day. Participants with the same STAI-S score for both days of recording were excluded from the following analyses. Participant averages for EMG outcomes were compared with a 2x2 repeated measures analysis of variance (RM ANOVA) with Anxiety (high and low days) and Muscle (dominant and non-dominant) as factors. HRV participant averages were compared between days with high and low STAI-S using paired t-tests. Each analysis was Bonferroni corrected according to the number of tests performed. Significance for all analyses was set a p < 0.05.

Results

After visual inspection, three participants were excluded due to substantial baseline noise in the EMG signal (exceeding the 5% RVE muscular rest threshold) and six participants were excluded due to noise and ectopic beats in the ECG signal. The final analysis included 74 participants with EMG data and 71 participants with ECG data (age (SD) = 31 (7.6) years, 60 (78%) women, body mass index (BMI) (SD) = 24.4 (4.6)
kg·m², STAI-T (SD) 32 (7.6)/80 points). These groups did not differ in terms of demographic characteristics (p > 0.05) and 68 participants were in both groups. After exclusions, 56 participants had two days of EMG data and 51 participants had two days of ECG data, with 42 participants having both. Participants with two days of data did not differ significantly from the whole group (p > 0.05). The mean of all EMG and HRV outcomes during each hour of the workday are shown in Figure 5.2.

**Figure 5.2:** Mean of EMG and HRV outcomes during each hour of the workday. Error bars represent standard error.
RVE = Reference voluntary effort, HR = Heart rate, LF = Low Frequency, HF = High Frequency

Muscle and ANS activity outcomes were not significantly correlated across participants or within participants. Correlation coefficients and p-values are shown in Table 5.1. The number of participants with significant correlations between muscle and ANS outcomes within the workday ranged from 0 – 6, well below the 46 participants required for a significant sign test. Muscle and ANS activity outcomes were not significantly correlated with STAI-T or STAI-S. Correlation coefficients and p-values are shown in Table 5.2.
### Table 5.1: Correlations between muscle and ANS activity outcomes across and within participants (N = 68)

<table>
<thead>
<tr>
<th>Muscle Outcome</th>
<th>EMG Outcome</th>
<th>Across Participants</th>
<th>Within Participants</th>
<th>Across Participants</th>
<th>Within Participants</th>
<th>Across Participants</th>
<th>Within Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td>Median</td>
<td>r = 0.19, p &gt; 0.99</td>
<td>r = 0.17, p &gt; 0.99</td>
<td>r = 0.28, p 0.22</td>
<td>r = -0.01, p &gt; 0.99</td>
<td>r = 0.34, p 0.06</td>
<td>r = -0.12, p &gt; 0.99</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>r = 0.25, p 0.46</td>
<td>r = -0.04, p &gt; 0.99</td>
<td>r = -0.09, p &gt; 0.99</td>
<td>r = 0.03, p &gt; 0.99</td>
<td>r = -0.16, p &gt; 0.99</td>
<td>r = 0.01, p &gt; 0.99</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td>Median</td>
<td>r = -0.12, p &gt; 0.99</td>
<td>r = 0.12, p &gt; 0.99</td>
<td>r = 0.14, p &gt; 0.99</td>
<td>r = -0.01, p &gt; 0.99</td>
<td>r = 0.25, p 0.52</td>
<td>r = -0.04, p &gt; 0.99</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>r = 0.22, p 0.83</td>
<td>r = -0.11, p &gt; 0.99</td>
<td>r = -0.16, p &gt; 0.99</td>
<td>r = 0.08, p &gt; 0.99</td>
<td>r = -0.17, p &gt; 0.99</td>
<td>r = 0.05, p &gt; 0.99</td>
</tr>
</tbody>
</table>

ANS = Autonomic nervous system
Table 5.2: Correlations between ANS activity outcomes (N = 71) and muscle activity outcomes (N = 74) with STAI-T and STAI-S

<table>
<thead>
<tr>
<th>Muscle Activity</th>
<th>Outcome</th>
<th>STAI-T</th>
<th>STAI-S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Dominant Muscle</td>
<td>Median</td>
<td>0.09</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>-0.14</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td>Median</td>
<td>0.17</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>-0.08</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>ANS Activity</td>
<td>HR</td>
<td>-0.07</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>lnLF</td>
<td>-0.01</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>lnHF</td>
<td>0.07</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Nine participants with two days of EMG data and six participants with two days of ECG data had no change in STAI-S between days and were removed from subsequent analyses. The mean STAI-S on low days was 29.5 (7.5) and on high days was 34.3 (9.4). Results of the comparison of EMG outcomes between days of high and low STAI-S are shown in Figure 5.3. The RM ANOVA revealed significant main effects of muscle for both median EMG and rest (p < 0.01 and p = 0.01, respectively), with the dominant muscle showing higher median EMG and lower muscular rest. The main effect of STAI-S was significant for median EMG only (p = 0.03), with higher median EMG seen in days with high STAI-S. The main effect of STAI-S was not significant for muscular rest (p > 0.36). Interactions between muscle and STAI-S were not significant for median EMG or muscular rest (p = 0.24 and p = 0.10, respectively). Results from the comparison of ANS activity outcomes between days of high and low STAI-S are shown in Figure 5.4. No ANS activity outcomes were significantly different between days (p > 0.27).
Figure 5.3: Comparison of EMG outcomes between muscle (dominant and non-dominant) and workdays with lower and higher perceived stress as measured by Spielberger state anxiety scores (Low STAI-S and High STAI-S, respectively).

* Main effect of Muscle (p < 0.05)
† Main effect of STAI-S (p < 0.05)
Discussion

This study revealed that the amplitude of upper trapezius muscle activity is higher on average throughout the workday on days with higher perceived psychosocial stress, though no difference in muscular rest was apparent. ANS activity, assessed by HRV, showed no difference between workdays with high and low levels of perceived psychosocial stress, and was not associated with muscle activity between or within participants.

Muscle Activity and Perceived Psychosocial Stress

The main finding of this study was an increase in the amplitude of muscle activity in the upper trapezius on days with higher perceived stress. This change was only seen...
when comparing high to low stress days within participants. Correlations between muscle activity and state and trait anxiety were not significant. This indicates that no absolute level of stress is associated with increased muscle activity, and that only changes in perceived stress within an individual are associated with this increase. Practitioners attempting to lower muscle activity through stress-reduction interventions must establish an appropriate baseline for each individual and assess changes from that baseline at multiple time points to accurately assess the efficacy of the intervention. One measurement is not sufficient to assess whether stress is contributing to increased levels of muscle activity. This conclusion is supported by a previous study, where individuals who reported an increase in perceived tension in the neck and shoulders during busy computer work (Griffiths, Mackey et al. 2011) had the highest odds ratio for musculoskeletal pain during the preceding 12 months.

Only the amplitude of muscle activity was influenced by this change in stress. No changes in muscular rest were observed. Based on how amplitude was calculated, using an Active APDF calculation (Marker et al 2015), this indicates that differences between days only occurred during periods of muscle activity while the amount of time that the muscle was active did not change. Several biological mechanisms exist that could explain the pattern of increased muscle activity amplitude with no corresponding change in muscular rest.

Increased activation of the sympathetic branch of the ANS causes an increase in plasma concentrations of epinephrine (E) and norepinephrine (NE) via stimulation of the adrenal medulla (Pacak and Palkovits 2001, Chrousos 2009). While E and NE have many systemic effects to promote the redistribution of energy resources to active muscles, they
also have effects on the function of the muscle fibers themselves (Roatta, Arendt-Nielsen et al. 2008, Roatta and Farina 2010). Most of these effects are mediated through the activation of $\beta_2$ receptors (Bowman 1980). Contrary to the assumption that stress-induced increases in EMG increase muscle force production, at physiologic levels of E and NE, muscle fiber twitch force is likely decreased or unchanged and half-relaxation time is decreased, resulting in an increased motor unit discharge rate to maintain a constant force (Roatta, Arendt-Nielsen et al. 2008, Roatta and Farina 2013). Animal models of sympathetic activation also show a decrease in muscle spindle sensitivity (Matsuo, Ikehara et al. 1995, Roatta, Windhorst et al. 2002). Together, a decrease in muscle fiber twitch force, half-relaxation time, and muscle spindle sensitivity (all peripheral determinants of muscle force), would necessitate increased central drive to the muscle to perform a given task. This theory is supported by the increased motor unit discharge rate (Roatta, Arendt-Nielsen et al. 2008) and corticospinal excitability (Buharin, Butler et al. 2013) seen during sympathetic activation. Although sympathetic blockade does not eliminate the presence of stress-induced muscle activity (Nilsen, Sand et al. 2008), the evidence provided indicates that sympathetic activation precipitating increased central drive to the muscle during motor tasks may be an important component of increased muscle activity amplitude without a corresponding decrease in muscular rest.

Another possible mechanism contributing to increased amplitude of muscle activity during increased perceived stress is an increase in NE input to spinal motor neurons (Heckman, Lee et al. 2003). The locus coeruleus is a major source of NE input to both the brain and spinal cord, and is activated during periods of increased arousal, such as during stress (Berridge 2008). At the level of the spinal motor neuron, NE acts as a
neuromodulator, altering motor neuron excitability via activation of persistent inward currents, primarily located on motor neuron dendrites (Heckman, Mottram et al. 2009). In animal models, increased NE input to spinal motor neurons has two primary effects: promoting self-sustained firing of the motor neuron (continued discharge after removal of central input) and amplifying synaptic input to the motor neuron (Heckman 2003). Little direct evidence of these effects are available in humans (Walton, Kalmar et al. 2002, Udina, D'Amico et al. 2010). Amplification of synaptic input to the spinal motor neuron would result in an increase in muscle activity amplitude, however, an increase in self-sustained firing would be expected to result in a decrease muscular rest, not seen in the current study. However, the self-sustained firing behavior induced by increased NE input is very sensitive to inhibitory inputs (Johnson, Hyngstrom et al. 2012), and the upper trapezius receives many inhibitory inputs from brainstem regions (Aniss, Sachdev et al. 1998, Davidson and Buford 2004), which may diminish the effects of self-sustained firing. Self-sustained firing behavior, however, may have an increased effect in populations with chronic pain, where inhibition has been shown to be impaired (Schwenkreis, Janssen et al. 2003, Mhalla, de Andrade et al. 2010, Marker, Stephenson et al. 2014).

**ANS Activity Outcomes**

ANS activity, assessed by HRV, was not associated with muscle activity across or within participants, and did not differ on days with high and low perceived stress. Although previous studies have shown HRV to be responsive to mental stress (Maixner, Greenspan et al. 2011, Matthews, Jelinek et al. 2012, Visnovcova, Mestanik et al. 2014, Garafova, Penesova et al. 2015), these changes have been reported to occur in different
directions depending on the protocol used (Visnovcova, Mestanik et al. 2014). It is possible that variable responses to acute stressors throughout the workday may diminish any measurable effect of more global changes in HRV in response to workplace stressors. This issue may be especially apparent in the current study, where only 5 min of ECG data were used to determine HRV for each hour of the workday. HRV could be disproportionately influenced by specific activities occurring during the time of assessment unrelated to the overall perceived stress reported for the workday. Indeed, previous studies have shown associations between HRV and workplace stressors (Vrijkotte, van Doornen et al. 2000, Togo and Takahashi 2009, Tonello, Rodrigues et al. 2014), but these studies utilized long-term HRV recordings and analyses, often 24 hours. The findings from the current study may indicate that short-term analyses, even performed at various time points throughout the full workday, may be insufficient to accurately assess the influence of workplace stress on ANS activity, necessitating the use of long-term recordings and analyses.

It is possible that the lack of association between muscle and ANS activity may be due to the limitations discussed above. Also, no HRV measure is a pure assessment of sympathetic activity. Most available measures are influenced by the activity of both the sympathetic and parasympathetic branches of the ANS (HR and LF), whereas HF is a more specific measure of parasympathetic activity. Although sympathetic and parasympathetic activity often change in opposite directions in response to a given stimulus, this is not always the case (Berntson, Cacioppo et al. 1994). If associations between muscle and ANS activity are mediated solely through the sympathetic branch of the ANS, then HRV measures currently are not sufficient to assess this relationship. It is
also possible that HRV measurements may be more sensitive to other events throughout the workday, such as physical activity (Perini and Veicsteinas 2003), which do not directly involve the upper trapezius, masking any association between the two.

**Limitations**

One main limitation of the current study is the use of intermittent 5 min periods for HRV analysis, and the implications of this limitation are discussed above. Another limitation is the lack of activity and task monitoring throughout the workday. Specific tasks throughout the workday may have disproportionately large or very brief influences on measures of muscle and autonomic activity. No observations of workday tasks were made to minimize the effect of observation in order to accurately assess normal workplace behavior. This also allowed the current study to assess global measures of muscle and ANS activity, which may not be assessed in studies of specific office work tasks. Finally, the assessment of perceived stress for the workday was made at the end of the workday. While the STAI-S has been shown to be valid and reliable (Spielberger 1983), it may be biased towards perceived stress at the end of the workday. It is also possible that increased amplitudes of upper trapezius muscle activity throughout workday may result in an increased perceived stress at the end of the day, and thus, causation between the two measures cannot be inferred.

**Conclusions**

The current study demonstrated that upper trapezius muscle activity amplitude increased during workdays with higher perceived stress within an individual, whereas no changes were seen in muscular rest and no associations were seen with trait anxiety or absolute levels of state anxiety. ANS activity was not associated with perceived stress or
muscle activity. Currently, contributions of altered muscle and ANS activity to musculoskeletal pain would seem to occur through independent mechanisms. Future studies should investigate the relationship between muscle and ANS activity with long-term HRV recordings and analyses (24 hours) and clinicians assessing the influence of workplace stress on autonomic activity should also utilize these long term analyses.
CHAPTER VI
HEART RATE VARIABILITY DOES NOT PREDICT THE DEVELOPMENT OF CHRONIC NECK PAIN IN HEALTHY OFFICE WORKERS

Abstract

Objective

Chronic neck pain is a leading cause of global disability, with a particularly high prevalence in office workers, despite low physical demands. Altered autonomic nervous system (ANS) activity has been seen in chronic pain populations, including chronic neck pain, but no studies have investigated whether this altered ANS activity contributes to the development of pain. This study investigates whether ANS response to an acute psychosocial stressor, or ANS activity during the workday, predicts the development of chronic neck pain in healthy office workers.

Methods

Electrocardiography (ECG) was recorded during exposure to an acute psychosocial stressor in healthy office workers. ECG was recorded during 1 – 2 typical workdays in a subset of the population. Mean heart rate (HR) and heart rate variability (HRV) measures were calculated from the ECG signal to assess ANS activity. Participants were tracked for 12 months to identify the development of chronic neck pain. Logistic regressions were run to test whether the acute stress response or workday assessments of any variables were predictive of developing chronic neck pain.
Results

All measures exhibited significant changes to the acute psychosocial stressor. No measures were significantly predictive of the development of chronic neck pain (p > 0.05).

Conclusions

Given the presence of altered ANS function in chronic pain populations, results from this study suggest this alteration arises in response to the development of pain. Therefore, interventions targeting altered ANS function should be utilized in patients already experiencing pain, and not as preventive measures.

Introduction

Chronic neck pain is one of the leading causes of disability in the world today (Hoy, March et al. 2014), having an annual prevalence of 30 - 50% (Hogg-Johnson, van der Velde et al. 2009). Office workers have a disproportionately high incidence of neck pain, up to 63% annually (Côté, van der Velde et al. 2009), despite low physical demand on cervical muscles (Marker et al. 2015). Psychosocial stress and related constructs, such as high job demand, have been implicated as significant risk factors for developing chronic neck pain and other chronic pain conditions. Many investigations of potential mechanisms linking psychosocial stress to the development of neck pain have been explored (Knardahl 2005), including excessive muscle activity (Lundberg, Forsman et al. 2002, Thorn, Sogaard et al. 2007) and altered blood flow (Strom, Roe et al. 2009). One less explored mechanism contributing to the association between psychosocial stress and the development of chronic neck pain is altered activity of the autonomic nervous system (ANS).
Heart rate variability (HRV) provides a non-invasive method of investigating ANS activity. Heart rate (HR) and HRV are influenced by both the parasympathetic and sympathetic branches of the ANS. The sympathetic nervous system increases HR and reduces HRV through the release of norepinephrine at the sinoatrial (SA) node and systemic release of catecholamines from the adrenal medulla, whereas the parasympathetic nervous system reduces HR and increases HRV through acetylcholine release at the SA node from direct innervation of the vagal nerve (Berntson, Bigger et al. 1997). The activity of both branches of the ANS is often assessed by frequency-domain measures of HRV, examining the spectral power within pre-defined frequency bands. The high frequency (HF) band ranges from 0.15 - 0.4 Hz and measures respiratory sinus arrhythmia, mainly attributed to vagal, or parasympathetic tone (Piccirillo, Ogawa et al. 2009), though this band can be influenced by sympathetic activity (Cohen and Taylor 2002). The low frequency (LF) band ranges from 0.04 - 0.15 Hz and has a controversial interpretation in the literature, though may reflect modulation of the baroreflex, which is mediated by both the sympathetic and parasympathetic branches of the ANS (Goldstein, Benthø et al. 2011). Finally, a dimension not assessed by frequency-domain measures is the complexity of HRV resulting from the interaction of multiple control systems. A decrease in complexity is thought to represent a decreased ability for the system to respond or adapt to environmental or physiologic changes (Manor and Lipsitz 2013).

Sample entropy (SampEnt) measures the regularity of the heart beat intervals, one aspect of physiologic complexity (Richman and Moorman 2000), which has been shown to be responsive to psychosocial stress (Melillo, Bracale et al. 2011), and other alterations in ANS activity (Porta, Gneccchi-Ruscone et al. 2007).
The ANS is involved in both acute and chronic pain modulation (Bruehl and Chung 2004). Altered function of the ANS, assessed by changes in HRV, has been shown in multiple chronic pain populations (Maixner, Greenspan et al. 2011, Kulshreshtha, Gupta et al. 2012), including chronic neck pain (Hallman and Lyskov 2012), and decreased HRV has been correlated with increased disability in patients with chronic neck pain (Kang, Chen et al. 2012). Acute psychosocial stress has also been shown to decrease HRV (Melillo, Bracale et al. 2011, Matthews, Jelinek et al. 2012, Visnovcova, Mestanik et al. 2014), indicating decreased parasympathetic tone and/or increased sympathetic tone. Anxiety conditions (Chalmers, Quintana et al. 2014) and job-related stress (Vrijkotte, van Doornen et al. 2000, Togo and Takahashi 2009, Jarczok, Jarczok et al. 2013, Tonello, Rodrigues et al. 2014) are also associated with decreased HRV and decreased responses to acute stressors. Despite these associations, few studies have investigated whether alterations in ANS activity predict the development of pain (Greenspan, Slade et al. 2013), and none have investigated chronic neck pain specifically. These investigations are critical in the prevention of stress-related chronic pain, as ANS activity is a modifiable risk factor (Hallman, Olsson et al. 2011, Hallman, Ekman et al. 2013).

The purpose of this investigation was to determine if changes in HRV in response to acute psychosocial stress or HRV recorded during a typical workday in healthy office workers predict the development of chronic neck pain. Associations between HRV responses to acute stress and workday recordings were also investigated to determine the contribution of an acute stress response to ambulatory measures. It was hypothesized that a reduction in HRV in response to acute psychosocial stress would be associated with
reduced HRV throughout the workday, and that both measures would predict the development of chronic neck pain.

Methods

Participants

A convenience sample of 227 healthy office workers were recruited from the greater Denver area through print and radio advertisements, new employee orientations, and employee bulletins and flyers posted at businesses employing a large number of office workers. These participants were the same population reported elsewhere (Shahidi et al 2015). All participated in the laboratory protocol and a subset participated in the worksite protocol (described below). Eligible participants were 18 – 65 years of age and within three months of their date of hire working > 30 hours per week in an office setting that required the use of a computer for at least 75% of the workday. Participants were screened by a licensed physical therapist for the absence of neck pain or associated disorders during the previous year. To avoid the potential for selection bias due to poor recall of pain symptoms, the Neck Disability Index (NDI) (Vernon and Mior 1991) was used to screen for activity limitations caused by pain which are more likely to be remembered than non-interfering neck pain. Participants were included in the study if they reported no neck pain or associated disorders during the previous year, and scored < 5 points on the NDI.

Exclusion criteria included: 1) objective signs of structural pathology upon physical examination, including but not limited to shoulder bursitis, impingement, tendonitis, fracture, and cervical nerve or disc impairment with radiculopathy or loss of sensory or motor function, 2) self-reported fibromyalgia diagnosis or musculoskeletal
pain present in more than four body regions concurrently, 3) self-reported systemic illness including cancer, rheumatic, cardiovascular, or neurological disease, 4) prior surgery involving the cervical spine or shoulders, 5) acute (< 12 weeks prior to study) injury of the neck or shoulders, 6) untreated psychiatric condition, 7) uncontrolled hypertension, 8) pregnancy, and 9) an inability to type or comprehend written and oral instructions in English. All participants provided written informed consent according to study procedures approved by the Colorado Multiple Institutional Review Board.

**Electrocardiography Recordings**

Laboratory electrocardiography (ECG) recordings were collected using a Power 1401 16-bit A/D board (Cambridge Electronic Design, Cambridge, UK) with bipolar Ag/AgCl surface electrodes (8 mm diameter) placed on a bony aspect of the superior sternum with an inter-electrode distance of 2 cm and a reference electrode placed on the right clavicle (Marker and Maluf 2014). Recordings were sampled at 2000 Hz and amplified (x1000). ECG recordings were bandpass filtered online at 13-1000 Hz (Coulbourn V-series modules, Allentown, PA, USA) and then low-pass filtered (30 Hz) offline (Matlab, MathWorks Inc., Natick, MA, USA) to reduce noise and isolate the ECG signal by removing interfering muscle activity (Christov and Daskalov 1999).

Worksite ECG was collected using a portable data logger (Delsys Myomonitor IV; Delsys, Boston, MA, USA) with a bi-lead sensor (SP-X14 EKG Sensor for Myomonitor System; Delsys) attached to 1.75 inch diameter foam and solid gel electrodes with 1 cm Ag/AgCl conducting snaps (Red Dot 9640; 3M, St. Paul, MN, USA) which were placed vertically on the flat portion of the sternum with a reference electrode on the right clavicle. Recordings were sampled at 1000 Hz, amplified (x1000),
and band-pass filtered (0.5 – 30 Hz) online. Worksite ECG data were stored on an internal 1GB memory card in consecutive one-hour data sets (minus 10 s for automated storage processing) for offline processing.

**Laboratory Protocol**

The intention of the laboratory protocol was to expose participants to a standardized, acute psychosocial stressor that combined cognitive demands with social evaluative threat to simulate stressors often encountered in the workplace. The full laboratory protocol can be found in Shahidi et al 2015. Low stress (LS) and high stress (HS) conditions were performed with each participant. In both, participants were seated at a computer desk in a standardized seated posture based on accepted ergonomic guidelines. Participants used their dominant hand to maneuver a computer mouse and complete a mental concentration task at the same time (Shahidi, Haight et al. 2013). The mental concentration task was a computerized version of the Operation Span (OpSpan) test (Conway, Kane et al. 2005) requiring participants to solve arithmetic problems while memorizing and selecting 2-8 word lists in sequential order. During the LS condition, participants were told that they were "practicing" the task and would not be judged on their performance. Positive feedback was provided by a familiar and friendly investigator. The HS condition was performed after a 5-10 rest period following the LS condition, and previous studies have shown no order or time effects using a similar protocol (Shahidi, Haight et al. 2013). During the HS condition, participants were instructed by an unfamiliar and authoritative investigator that speed and accuracy on the task were important and they would be paid based on their performance. No positive feedback was provided. All participants were immediately debriefed on the purpose of
the study following the HS condition. Mean arterial pressure (MAP), HR, and ECG recordings for HRV analysis were collected during both the LS and HS conditions. The state anxiety portion of the STAI (STAI-S) was administered immediately after each stress condition (Spielberger 1983).

**Worksite Protocol**

Worksite ECG recordings were collected for 1 – 2 full workdays within 3 weeks of the laboratory protocol in consenting participants. Participants completing 2 days of ECG recordings completed the second day within 2 weeks of the first. An investigator met participants at a private location near his or her worksite 15 minutes prior to the start of the workday for equipment setup. After visual inspection of the ECG signal at setup, participants wore the portable data monitor continuously in a minimally obtrusive waist pack positioned on the anterolateral aspect of the waist as they performed their usual activities throughout the workday. They were instructed to ignore the device and not make changes to their daily routine. The portable data monitoring system was removed at the end of the workday by study investigators.

**Follow Up Assessment**

All participants were followed prospectively for 12 months after the laboratory protocol via a monthly online survey requiring 5 – 10 min to complete (REDCap Software, v 5.5.9, Vanderbilt University 2014). A link to a secure, personally identified survey was electronically mailed to participants on the same day of the month for 12 consecutive months; if not completed within 7 days, one additional electronic reminder was sent. The survey contained all questions from the NDI, with additional questions related to health care utilization and filing of an insurance claim due to neck pain during
the previous month. Questionnaires were used to identify participants who developed chronic interfering neck pain, as defined by the Task Force on Neck Pain (Guzman, Hurwitz et al. 2009). This defines interfering neck pain as self-reported restriction of participation in usual activities, seeking health care, and/or filing an insurance claim. Participation restriction was defined as a score ≥ 5 points on the NDI (Vernon and Mior 1991), seeking health care as spending healthcare dollars in traditional (e.g. physician, physical therapist) or complementary (e.g. massage therapist, chiropractor) health care settings, and filing an insurance claim as filing a health insurance, auto insurance, workers’ compensation, or personal injury claim related to neck pain.

Individuals who met the definition of interfering neck pain for 3 or more months (consecutive or non-consecutive) were classified as having developed chronic neck pain (van Tulder, Furlan et al. 2003). Participants were considered lost to follow up if they completed fewer than 8 of the 12 monthly surveys, or failed to respond to the surveys for 3 consecutive months. Participants were excluded if they moved to a new job that no longer met the inclusion criteria for office work or developed pain from a known mechanism of injury not meeting the criteria for non-specific neck pain (e.g. motor vehicle accident).

**ECG and Heart Rate Variability Processing**

Continuous 3-min periods of laboratory ECG recordings and 5-min periods of worksite ECG recordings were imported into Kubios HRV software (version 2.2, http://kubios.uef.fi/KubiosHRV/) (Niskanen, Tarvainen et al. 2004) for analysis. All ECG recordings were visually inspected and selected to be clear of artifact and muscle activity. Selected periods of laboratory ECG recordings were made while participants completed
the combined concentration and computer task, after receiving instructions. Three minute periods were used as few participants required a full five minutes to complete the task. Selected periods of worksite ECG recordings identified near the end of each 1-hr file to provide a representative sample of HRV throughout the workday.

Detailed descriptions of HRV outcomes and interpretations can be found in Task Force Guidelines (Force 1996) and Berntson et al 1997. R-waves were automatically detected by the software and then visually inspected and corrected for signal artifacts and ectopic beats. Mean HR was calculated for recording windows as the inverse of the mean R-R interval. Based on Task Force (Force 1996) recommendations, no further time domain measures were calculated as these are more appropriate for extended (24 hour) recordings. An equidistant, 4 Hz R-R time series was created from the original R-R time series via cubic spline interpolation. A fast fourier transform (FFT) was applied to these time series (180 s for laboratory ECG, and 300 s for worksite ECG) using Welch’s periodogram method to derive the power spectrum density. The LF (0.04 – 0.15 Hz) and HF (0.15 – 0.4 Hz) components of the power spectrum density were calculated for each 4 Hz R-R time series. Due to the short recording window, very low frequency (VLF) (0.0033 – 0.04 Hz) and hence total power of the R-R time series were unstable and not included in analyses. The non-linear measure sample entropy (SampEnt) (Richman and Moorman 2000) was calculated for each R-R time series. The calculation and analysis of sample entropy is described by (Mayer, Bachler et al. 2014).

Data Analysis

Similar to previous studies (Maixner, Greenspan et al. 2011), HRV frequency measures were natural log-transformed prior to analysis due to skewness. HRV measures
were calculated for both the LS and HS condition in order to investigate changes in response to the acute stressor, and were expressed as change scores from the LS to HS condition (ΔMAP, ΔHR, Δln(HF), Δln(LF), ΔSampEnt) for subsequent regression and correlation analyses. Worksite HRV measures for each hour of data were averaged across the workday, and then averaged across days for participants with 2 days of worksite recordings. Participant demographic data were summarized as mean (SD). Participant characteristics were compared between those who developed chronic neck pain and those who did not using t-tests for continuous variables and chi-square for categorical. The purpose of this report is to characterize changes and associations with HRV measures; detailed analyses of demographic characteristics as they relate to the chronic pain group are reported in Shahidi et al 2015.

Laboratory measures were compared between the LS and HS condition with paired t-tests. P-values were Bonferroni corrected for the number of tests performed. Univariate logistic regressions were performed for each laboratory and worksite measure to determine potential significant predictors of developing chronic neck pain. Finally, Pearson correlation coefficients were calculated between demographic measures and HRV measures to assess the relationship of these measures to participant characteristics and self-reported psychological measures, and between laboratory and worksite HRV measures to assess the contribution of an acute stress response to ambulatory measures. Significance for all analyses was defined as p ≤ 0.05.
Results

Figure 6.1 shows the flow of participants from initial enrollment through follow up. Sixty-four participants were included in both the final laboratory and worksite analyses. Descriptive statistics of both samples are shown in Table 6.1. Participant characteristics did not differ between those who developed chronic neck pain and those who did not.

Figure 6.1: Flow diagram of participant enrollment, screening, testing, and follow-up.

Results of changes in laboratory measures from the LS to HS condition are presented in Figure 6.2. STAI-S, HR, and MAP all increased significantly during the HS condition (p < 0.01), while all measures of HRV significantly decreased (p < 0.01). Results of the laboratory measure logistic regressions are shown in Table 6.2. No measures were significant predictors of developing chronic neck pain.
Table 6.1: Descriptive statistics for participants in both laboratory and worksite protocols, separated by those who did (CNP+) and did not (CNP-) develop chronic neck pain

<table>
<thead>
<tr>
<th></th>
<th>Laboratory (N=147)</th>
<th></th>
<th>Worksite (N=71)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNP+</td>
<td>CNP-</td>
<td>p-value</td>
<td>CNP+</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.4 (7.2)</td>
<td>29.6 (7.0)</td>
<td>0.58</td>
<td>30.4 (6.0)</td>
</tr>
<tr>
<td>No. Women (%)</td>
<td>25 (86%)</td>
<td>90 (76%)</td>
<td>0.36</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Body Mass Index (kg*m²)</td>
<td>23.8 (3.7)</td>
<td>23.7 (3.4)</td>
<td>0.96</td>
<td>23.5 (4.3)</td>
</tr>
</tbody>
</table>

**Figure 6.2:** Indices of perceived and physiologic arousal (top row) and heart rate variability (bottom row) during Low Stress (LS) and High Stress (HS) conditions in the laboratory protocol.
* p < 0.01
STAI-S = Spielberger State-Trait Anxiety Index State Score, HR = Heart rate, MAP = Mean arterial pressure, HF = High frequency, LF = Low frequency, SampEnt = Sample entropy
<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNP+</td>
<td>CNP-</td>
<td></td>
</tr>
<tr>
<td>ΔSTAI-S (points)</td>
<td>10.2 (9.3)</td>
<td>11.0 (7.6)</td>
<td>0.98 (0.93 - 1.04)</td>
</tr>
<tr>
<td>ΔMAP (mmHg)</td>
<td>3.8 (7.7)</td>
<td>3.5 (6.3)</td>
<td>1.01 (0.95 - 1.07)</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>3.9 (5.0)</td>
<td>5.1 (6.4)</td>
<td>0.96 (0.89 - 1.04)</td>
</tr>
<tr>
<td>Δln[HF (ms²)]</td>
<td>-0.08 (0.6)</td>
<td>-0.15 (0.6)</td>
<td>1.23 (0.62 - 2.42)</td>
</tr>
<tr>
<td>Δln[LF (ms²)]</td>
<td>-0.20 (0.7)</td>
<td>-0.27 (0.8)</td>
<td>1.13 (0.65 - 1.96)</td>
</tr>
<tr>
<td>ΔSampEnt</td>
<td>-0.03 (0.3)</td>
<td>-0.10 (0.3)</td>
<td>2.39 (0.58 - 9.93)</td>
</tr>
</tbody>
</table>

CNP+ = Participants who developed chronic neck pain, CNP- = Participants who did not develop chronic neck pain, STAI-S = Spielberger State Anxiety Index, MAP = Mean arterial pressure, HR = Heart rate, HF = High frequency, LF = Low frequency, SampEnt = Sample entropy.

In the 71 participants with worksite HRV measures, 122 days of data were recorded with 6.8 (0.7) hours of data recorded per day on average. Descriptive statistics and results of logistic regressions are reported in Table 6.3. No measures were significant predictors of developing chronic neck pain.

Age was significantly correlated with worksite ln(HF) (N = 64, r = -0.34, p < 0.01) and worksite ln(LF) (N = 64, r = -0.25, p = 0.04). No other descriptive characteristics were correlated with laboratory (r = -0.16 – 0.15, p = 0.06 – 0.85) or worksite measures (r = -0.22 – 0.18, p = 0.09 – 0.97). Laboratory change measures (ΔMAP, ΔHR, Δln(HF), Δln(LF), ΔSampEnt) were not correlated with their corresponding worksite measures (r = -0.01 – -0.07, p = 0.57 – 0.92).
Table 6.3: Descriptive statistics and logistic regression analysis of worksite heart rate variability in the development of chronic neck pain

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNP+</td>
<td>CNP-</td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>78 (6.3)</td>
<td>78 (8.2)</td>
<td>0.99 (0.92 - 1.07)</td>
</tr>
<tr>
<td>ln[HF (ms²)]</td>
<td>6.3 (0.7)</td>
<td>6.1 (1.0)</td>
<td>1.23 (0.61 - 2.5)</td>
</tr>
<tr>
<td>ln[LF (ms²)]</td>
<td>7.0 (0.5)</td>
<td>7.0 (0.8)</td>
<td>1.04 (0.43 - 2.52)</td>
</tr>
<tr>
<td>SampEnt</td>
<td>1.3 (0.1)</td>
<td>1.4 (0.2)</td>
<td>0.46 (0.02 - 13.3)</td>
</tr>
</tbody>
</table>

CNP+ = Participants who developed chronic neck pain, CNP- = Participants who did not develop chronic neck pain, HR = Heart rate, HF = High frequency, LF = Low frequency, SampEnt = Sample entropy

Discussion

This investigation showed that perceived anxiety, HR, and MAP increased while HRV measures decreased during exposure to a laboratory protocol designed to simulate a stressful office environment. These changes, however, were not associated with worksite recordings of HRV. No measures of HRV, in response to the laboratory stressor or during worksite recordings, predicted the development of chronic neck pain. The annual incidence of neck pain was 20% and 17% in the laboratory protocol and worksite protocol, respectively. A detailed analysis of participant characteristics and their ability to predict the development of chronic neck pain can be found in Shahidi et al 2015.

Response to Laboratory Stressor

In response to the laboratory stress protocol, perceived anxiety, as assessed by the STAI-S, increased, as did physiologic arousal, assessed by MAP and HR. All measures
of HRV decreased. A decrease in the HF component of HRV in response to mental stress is in agreement with previous studies, indicating a decrease in parasympathetic tone (Matthews, Jelinek et al. 2012, Visnovcova, Mestanik et al. 2014). The increase in HR and arterial pressure may indicate an increase in sympathetic tone in response to the stressor. The decrease in sample entropy indicates an increase in the regularity of the R-R intervals and also may reflect a shift towards increased sympathetic activity in the ANS (Porta, G Necchi-Ruscone et al. 2007, Melillo, Bracale et al. 2011). The decrease in the LF component of HRV is more difficult to interpret. This measure has been shown to both increase (Matthews, Jelinek et al. 2012, Visnovcova, Mestanik et al. 2014, Garafova, Penesova et al. 2015) and decrease (Maixner, Greenspan et al. 2011, Visnovcova, Mestanik et al. 2014) with different protocols for mental stress. Our finding of a decreased LF response to an acute stressor mimicking psychosocial stressors found in a workplace may indicate reduced modulation of the baroreceptor reflex during these situations (Goldstein, B entho et al. 2011).

**Worksite Measures**

Worksite frequency-domain HRV measures were similar to normative values presented by the HRV Task Force (1996), but lower than those reported in a more recent systematic review, though the ranges of values presented in this review study were large (Nunan, Sandercock et al. 2010). Age was also negatively correlated with both the HF and LF components of HRV, consistent with other studies indicating decreased HRV with age (Voss, Heitmann et al. 2012, Abhishekh, Nisarga et al. 2013). Thus, the higher HRV values in the current population compared to the study by Nunan et al. (2010) could reflect a younger, healthier demographic.
Worksite measures were not correlated with changes in response to the laboratory stress protocol. Prior studies have found associations between worksite HRV and worksite stressors (Vrijkotte, van Doornen et al. 2000, Togo and Takahashi 2009, Tonello, Rodrigues et al. 2014). In the current study, no direct measures of worksite activities or stressors were collected, and worksite stressors were only mirrored in the laboratory protocol. Given the sensitivity of our HRV measures to detect autonomic responses to the acute laboratory stressor, it is likely that HRV changes in response to actual worksite stressors are best observed specifically during stressful work events, which may be masked or missed by sampling HRV at standardized time points across the full workday. Such averages should be considered global, rather than stress-related, indices of autonomic activity in the workplace.

**Prediction of Chronic Neck Pain**

No HRV measures in the current study, either in response to the laboratory stressor or recorded at the worksite, were predictive of participants developing chronic neck pain in the one year follow-up. This is in line with a recent study from Greenspan et al (2013) demonstrating that baseline HR, but not HRV, was predictive of developing first-onset temporomandibular disorder (TMD). The current study validates the lack of association between HRV and new onset pain in a chronic neck pain population. HR was not predictive of neck pain development, but was assessed in response to the laboratory stressor and during the workday rather than a resting baseline period.

These findings, together with findings of altered autonomic function and reduced HRV in cross-sectional studies of chronic neck pain and TMD (Maixner, Greenspan et al. 2011, Hallman and Lyskov 2012), indicate that impairments in autonomic function are
likely caused by the onset or presence of pain, rather than predisposing individuals to the future development of pain. The relationship between pain and the cardiovascular system is reviewed by Bruehl and Chung (2004) and may offer insights into the development of altered ANS function with pain. An elevation in blood pressure, indicative of increased sympathetic activity, is associated with elevated nociception reflex thresholds (Umeda, Corbin et al. 2013) and diminished pain sensitivity (Bruehl, Carlson et al. 1992), part of a proposed feedback loop to restore resting arousal levels in the presence of a painful stimulus (Ghione 1996). Chronic painful stimulation may lead to a shift towards decreased baseline sensitivity of the baroreceptors responsible for this feedback loop (Randich and Maixner 1984), which could impair inhibition of sympathetic activation in the presence of pain. The relationship between blood pressure and pain sensitivity is actually reversed in some populations with chronic pain (Bruehl, Chung et al. 2002), indicating dysfunction in this system. These alterations induced by pain could lead to the reduced HRV seen in chronic pain populations. It is interesting to note that chronic stress has similar effects as pain on baroreceptor sensitivity (Steptoe and Sawada 1989), which could lead to similar ANS dysfunction in anxiety disorders. Longitudinal studies are needed to determine the time course of changes in ANS function and its relationship to changes in pain sensitivity during the transition from acute to chronic pain.

**Limitations**

A limitation of this study is that observations were not made during worksite recordings, so the influence of factors such as exposure to specific psychosocial stressors or changes in physical activity on HRV (Hautala, Karjalainen et al. 2010) could not be assessed. Finally, the low number of participants developing chronic neck pain,
especially in participants with workplace recordings, reduces the power of the current study to find significant predictors of developing chronic pain.

Conclusions

This study showed that although measures of HRV were sensitive to simulated worksite stressors in a laboratory setting, these changes were not associated with worksite recordings of HRV. Changes in HRV in response to the laboratory stressor and worksite HRV recordings were not predictive of chronic neck pain development. Given the presence of ANS dysfunction in chronic pain populations, this study suggests that these impairments develop in response to pain, rather than serving as a predisposing factor for new onset musculoskeletal pain.
CHAPTER VII

SUMMARY

The primary purpose of this thesis was to investigate different neurophysiologic mechanisms that may contribute to psychosocial stress-induced increases in muscle activity in the upper trapezius. This stress-induced muscle activity is thought to contribute to the development or persistence of chronic neck pain, a leading cause of global disability. Despite this potential association, the actual mechanisms linking psychosocial stress to upper trapezius muscle activity are largely unknown. The studies presented in this thesis investigate potential mechanisms in both laboratory and ecologically valid, or real-world, settings, as well as the relationships of some of these measures to chronic neck pain.

Chapters III and IV investigate potential neurophysiologic mechanisms to stress-induced upper trapezius muscle activity. In both chapters, a standardized protocol was utilized to manipulate levels of psychosocial stress. Chapter III examined changes in corticospinal responsiveness and intracortical inhibition in response to the acute psychosocial stressor using transcranial magnetic stimulation (TMS). These changes were assessed in both healthy individuals and individuals with chronic neck pain. All individuals showed an increased responsiveness of the corticospinal tract. Individuals with chronic neck pain exhibited increased levels of intracortical inhibition initially, compared to healthy individuals, which did not change in response to the acute psychosocial stressor, while healthy individuals exhibited an increase in intracortical inhibition during the stressor. Chapter IV examined reticulospinal input to the upper
trapezius and how it changed with the acute psychosocial stressor through investigation of the acoustic startle reflex (ASR). It was found that the reticulospinal tract provides primarily inhibitory input to the upper trapezius and the strength of reticulospinal input to the upper trapezius is reduced during the acute psychosocial stressor.

The results of these investigations support previous literature suggesting that peripheral and subcortical mechanisms of motor control may be reduced during psychosocial stress, necessitating an increased corticospinal drive to maintain adequate motor control. In healthy individuals, this increased corticospinal drive may be regulated and finely-tuned via increases in intracortical inhibition. Chronic activation of this intracortical inhibitory control system in individuals with chronic neck pain may lead to a decreased responsiveness of this system, and subsequently increased upper trapezius muscle activity through unregulated increases in corticospinal responsiveness.

Chapters V and VI investigated upper trapezius muscle activity and autonomic nervous system (ANS) activity, another potential contributor to chronic neck pain, in a population at high risk for developing chronic neck pain, office workers. The investigation in Chapter V demonstrated the presence of stress-induced increases in upper trapezius muscle activity in a real-world setting, though this activity was not associated with ANS activity. This finding is in agreement with the results presented in Chapters III and IV, which indicate that more central mechanisms are likely greater contributors to stress-induced upper trapezius muscle activity. Finally, Chapter VI investigated the use of ANS activity, from a real-world setting as well as in response to an acute psychosocial stressor, in predicting the development of chronic neck pain. No measures of ANS activity were able to predict the development of chronic neck pain, indicating that ANS
interventions should focus on individuals with current pain who have developed altered ANS activity, rather than focusing on prevention in healthy individuals.

Overall, the investigations in this thesis provided information on the neurophysiologic mechanisms contributing to stress-induced increases in upper trapezius muscle activity. These mechanisms could serve as targets in future interventions aiming to decrease this muscle activity. Central mechanisms, such as corticospinal responsiveness or intracortical inhibition, would likely be the most suitable targets for interventions, though more research is required to see how these, or reticulospinal responsiveness, can be modified. In a healthy population, ANS activity, at least as assessed by heart rate variability measures, is not associated with stress-induced upper trapezius muscle activity, and is not a promising mechanism to intervene on. Finally, while populations with chronic pain do show alterations of ANS activity, these changes likely develop in response to pain, making ANS activity a poor target for preventative interventions. The work presented here provides evidence that stress-induced changes in motor control, particularly in the upper trapezius muscle, are a complex process involving multiple systems, and requires further work to completely understand.
REFERENCES


work tasks - influence of gap definition and normalisation methods." J Electromyogr
Kinesiol 10(2): 103-115.

Hautala, A. J., J. Karjalainen, A. M. Kiviniemi, H. Kinnunen, T. H. Makikallio, H. V.
Huikuri and M. P. Tulppo (2010). "Physical activity and heart rate variability measured
simultaneously during waking hours." Am J Physiol Heart Circ Physiol 298(3): H874-
880.


motoneurons and their neuromodulatory control during motor behavior." Trends
Neurosci 26(12): 688-695.

excitability: the importance of neuromodulatory inputs." Clin Neurophysiol 120(12):
2040-2054.

in the pathophysiology of stress-related bodily disorders." Psychoneuroendocrinology

Henneman, E. (1957). "Relation between size of neurons and their susceptibility to


explain the adaptation to pain." Pain 152(3 Suppl): S90-98.

explain the adaptation to pain." Pain 152(3 Suppl): S90-98.

Hogg-Johnson, S., G. van der Velde, L. J. Carroll, L. W. Holm, J. D. Cassidy, J. Guzman,
P. Cote, S. Haldeman, C. Ammendolia, E. Carragee, E. Hurwitz, M. Nordin and P. Peloso
(2009). "The burden and determinants of neck pain in the general population: results of
the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated


Lemon, R. N., W. Landau, D. Tutssel and D. G. Lawrence (2012). "Lawrence and Kuypers (1968a, b) revisited: copies of the original filmed material from their classic papers in Brain." Brain 135(Pt 7): 2290-2295.


APPENDIX A

EFFECTS OF ELECTROCARDIOGRAPHY CONTAMINATION AND COMPARISON OF ECG REMOVAL METHODS ON UPPER TRAPEZIUS ELECTROMYOGRAPHY RECORDINGS¹

Abstract

Electromyography (EMG) recordings from the trapezius are often contaminated by the electrocardiography (ECG) signal, making it difficult to distinguish low-level muscle activity from muscular rest. This study investigates the influence of ECG contamination on EMG amplitude and frequency estimations in the upper trapezius during muscular rest and low-level contractions. A new method of ECG contamination removal, the filtered template subtraction (FTS) method, is described and compared to 30 Hz high-pass filtering (HPF) and averaged template subtraction (ATS). The FTS method creates a unique template of each ECG artifact using a low-pass filtered copy of the contaminated signal, which is subtracted from contaminated periods in the original signal. ECG contamination results in an over-estimation of EMG amplitude during rest in the upper trapezius, with negligible effects on amplitude and frequency estimations during low-intensity isometric contractions. The FTS and HPF methods successfully removed ECG contamination from periods of muscular rest while the ATS method did not, yet introduced errors during muscle contraction, which the ATS method did not. Results suggest ECG contamination removal may only be necessary during periods of muscular

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rest. Advantages and disadvantages of contamination removal methods should be considered in the context of the specific tasks that require analysis.

**Introduction**

Electromyography (EMG) is a prominent method of recording and quantifying muscle activity. These recordings can be contaminated by environmental noise, as well as by other electrical signals within the body. Specifically, the electrical signal from the heart can cause electrocardiography (ECG) contamination. EMG recordings from trunk muscles are especially vulnerable to contamination by the ECG signal, due to their proximity to the heart (Butler, Newell et al. 2009).

The upper trapezius is particularly susceptible to ECG contamination, and is often investigated with EMG. For example, occupational exposure studies commonly investigate how insufficient muscular rest and sustained periods of low-level muscle activity may contribute to overuse injury and pain in the upper trapezius muscle (Veiersted, Forsman et al. 2013). The validity of these investigations depends on the ability to distinguish between a fully resting muscle and low-level muscle contractions in the EMG signal. The presence of ECG contamination may result in errors in the determination of muscular rest, and may overestimate the amplitude of muscle activity during low intensity contractions. However, the issue of ECG contamination and its removal is inconsistently addressed in studies of low amplitude postural activity and muscular rest. Few studies have investigated the influence of ECG contamination on EMG amplitude and frequency estimates in the upper trapezius, or the effectiveness of existing ECG artifact removal methods for this muscle (Spalding, Schleifer et al. 2003, Lu, Brittain et al. 2009).

Furthermore, most methods are investigated during periods of muscle activity for varying tasks (Mak, Hu et al. 2010, von Tscharner, Eskofier et al. 2011, Willigenburg, Daffertshofer et al. 2012), rather than during muscular rest (Spalding, Schleifer et al. 2003, Drake and Callaghan 2006).

Several proposed ECG contamination removal methods, such as by independent component analysis (ICA), require significant processing time and power, as well as input signals from multiple muscles to successfully isolate the ECG signal component (Mak, Hu et al. 2010, Willigenburg, Daffertshofer et al. 2012). Thus, ICA and similar methods may not be feasible for many EMG applications, including occupational exposure studies that collect prolonged EMG recordings from a limited number of muscles. ICA also requires the skill of personnel trained specifically in that method (Mak, Hu et al. 2010), making it less practical for widespread application.

The most recommended method to efficiently remove ECG contamination from the EMG signal is a 30 Hz high-pass filter (Drake and Callaghan 2006). This method, however, may remove important EMG data arising from muscle activity along with ECG contamination (von Tscharner, Eskofier et al. 2011, Willigenburg, Daffertshofer et al. 2012). The creation and subsequent subtraction of an averaged template derived from
multiple ECG artifacts has been investigated in the upper trapezius at rest (Spalding, Schleifer et al. 2003), but this method may not adequately account for variability in the shape of individual ECG artifacts. Finally, gating completely removes all time intervals containing ECG contamination from the EMG recording (Bartolo, Roberts et al. 1996), but may also result in a significant loss of data, as multiple time intervals are deleted from the signal.

The purpose of the current investigation is to quantify the effects of ECG contamination on EMG amplitude and frequency estimates during muscle rest and low intensity isometric contractions, and to compare new and existing methods of ECG removal on reducing errors introduced by ECG contamination when present. The Filtered Template Subtraction (FTS) method is proposed as a modification of the averaged template subtraction technique to account for variability in the shape of individual ECG artifacts while minimizing loss of the actual EMG signal.

**Methods**

**EMG and ECG Data Collection**

Surface EMG was collected from a total of 11 participants (age = 26.1 (range 22 – 34) years, body mass index = 22.5 (range 19.1 – 29.8) kg/m\(^2\), 9 women). Not every task was completed by all participants, and technical complications resulted in the loss of EMG data from the right upper trapezius in one participant. Sample sizes for all calculations are reported. EMG was collected using bipolar surface electrodes (silver-silver chloride; 8 mm electrode diameter; In Vivo Metric, Healdsburg, CA, USA) placed over the left upper trapezius (LUT) and right upper trapezius (RUT). Electrodes were placed with a 10 mm inter-electrode distance, 20 mm lateral to the midpoint between C7
and the posterior lateral acromion. Reference electrodes were placed over a bony portion of the ipsilateral clavicle. ECG signals were collected by two electrodes, separated by a 10 mm inter-electrode distance and positioned along the midsagittal line on bony aspects of the manubrium and upper sternum, with a ground placed on the right ulnar styloid process. This position was chosen to minimize the influence of muscle activity on the ECG signal.

EMG and ECG data were amplified (1000x), band-pass filtered (13 – 1000 Hz LabLinc V, Coulbourn Instruments, Whitehall, PA), and sampled at 2000 Hz (Power1401, Cambridge Electronic Design, Cambridge, UK). Data were collected online with Spike2 software (Cambridge Electronic Design, Cambridge, UK), and all offline data processing was performed with custom software (LabVIEW, National Instruments, Austin, TX, USA).

**Experimental Protocol**

Participants were informed of the purpose of the study before providing written consent in accordance with procedures approved by the Colorado Multiple Institutional Review Board. After placement of surface electrodes, participants were instructed to lie supine on a cushioned table, with arms by their sides and palms facing up, a position placing no postural demand on the upper trapezius. They were told to completely relax their neck, shoulder, and arm muscles in this position for five minutes, and the complete absence of muscle activity was confirmed by online inspection of all EMG signals throughout the rest trial. Participants were visually monitored by study personnel, and any periods of voluntary movement or changes in the EMG signal indicating possible muscle activity were marked for offline removal.
Participants transitioned to a custom experimental chair with adjustable shoulder restraints positioned bilaterally over the acromion process to restrict shoulder elevation. Visual feedback of smoothed muscle activity from the LUT and RUT was presented on a computer monitor. Participants performed 2 – 4 isometric maximal voluntary contractions (MVCs) by elevating both shoulders against resistance while receiving loud verbal encouragement. The maximal EMG value recorded from each muscle during these trials was designated as the MVC. Participants then were presented with concurrent visual targets for sub-maximal contractions of the LUT and RUT, set at 2, 5, 10, and 15% of the MVC for each respective muscle. This range of contraction intensities reflects those typically observed with routine postural tasks. Participants practiced matching the target contractions for approximately 3 – 5 minutes, and then maintained a stable isometric contraction at each target level for one minute. Sub-maximal contractions were performed in random order, with at least two minutes rest between contractions.

**ECG Contamination Removal**

**ECG artifact detection**

ECG contamination is primarily comprised of QRS complexes in the EMG signal, referred to here as ECG artifacts. With the exception of 30 Hz high-pass filtering, all ECG contamination removal methods investigated in the present study first required identification of the time intervals corresponding to each ECG artifact in the EMG recordings. ECG artifacts were identified using a semi-automated threshold detection procedure, similar to that described by Mak et al (2010). Briefly, an envelope of the ECG signal was created by obtaining the instantaneous amplitude via a Hilbert Transform, which was then passed through a 50th order median filter to magnify the QRS complex.
An amplitude threshold was manually selected for each participant, such that only the QRS complexes exceeded threshold. ECG artifact time intervals were automatically defined as a 0.2 s window surrounding the peak of each enveloped QRS complex in the corresponding EMG signal. To account for transmission delays between the ECG and EMG recording sites, this interval was manually adjusted for each participant to include only the QRS complex, based on visual inspection of the EMG signal.

**Gating**

Figure 1 illustrates three existing methods of ECG artifact removal on a contaminated EMG recording from the LUT during a five minute rest period for a representative participant. Gating (Figure 1C) involves the complete removal of ECG artifact time intervals from the contaminated EMG signal (Bartolo, Roberts et al. 1996). This process assumes that any 'true' EMG signal contained within the ECG time intervals selected for removal is redundant of EMG activity occurring outside of these intervals; therefore, calculations made on the gated signal will accurately represent calculations made on an uncontaminated signal.
Figure 1: Contaminated electromyography (EMG) signal recorded from the left upper trapezius is shown for one minute of a five minute rest period in a representative participant (A). The first 4.5 s of the contaminated EMG signal are enlarged (B) to illustrate three existing methods of electrocardiography (ECG) contamination removal: Gating (C), 30 Hz high pass filter (D), and average template subtraction (E). The averaged ECG template created from all artifacts present in the five minute EMG recording is shown to the right of (E).

The primary assumption of the gating method is often violated during dynamic motor tasks, which are typical of most functional activities. Furthermore, the loss of data resulting from removal of multiple time intervals surrounding each QRS complex can be substantial for brief recording times. Although these methodological issues limit the widespread application of gating for ECG contamination removal, the longer duration recordings of resting and static tasks in the current study were designed to meet the primary assumption of the gating method. Thus, the gated and concatenated EMG signal was used as the gold standard reference for comparison of all other ECG contamination
removal methods. It is important to recognize that in studies utilizing EMG to assess dynamic muscle activity, this primary assumption is violated and the gating method would not be appropriate.

30 Hz high-pass filter (HPF)

The removal of ECG contamination by passing the EMG signal through a 30 Hz HPF has been recommended as the most efficient and practical of several reviewed methods (Drake and Callaghan 2006). This method of ECG removal was implemented in the present study with a 30 Hz, zero-phase shift, high-pass 4th order Butterworth filter (Figure 1D).

Average template subtraction (ATS)

The ATS method (Figure 1E) has been utilized in wire recordings of diaphragm muscle activity (Bartolo, Roberts et al. 1996), as well as surface EMG recordings in the upper trapezius (Spalding, Schleifer et al. 2003). All ECG artifacts present in the EMG recording are aligned along a common distinguishing feature such as the R-wave, and are averaged together to create an averaged template of the ECG artifact. This template is then subtracted out of the EMG signal at each ECG artifact time interval. This method assumes that uncontaminated EMG data contained within the ECG artifacts will be reduced to zero during the averaging process and thus are not removed during subtraction. It also assumes that the ECG artifact has a constant shape across the duration of the EMG recording. In the present study, ECG artifact time intervals were aligned on the peak of the ECG envelope corresponding to the R-wave and averaged across all intervals within each task.

Filtered template subtraction (FTS)
The FTS method (Figure 2) is a proposed modification of the ATS method, which both subtract a template of the ECG artifact out of the contaminated EMG signal; however, instead of an averaged template, FTS creates a template specific to the individual shape of each artifact. First, a copy of the contaminated EMG signal (Figure 2A) is passed through a zero-phase shift, low-pass 4th order Butterworth filter (Figure 2B). ECG artifact time intervals are identified, and the filtered signal is subtracted from the contaminated EMG signal only within time intervals corresponding to the ECG artifact (Figure 2C). This method uses a zero-phase shift filter to create a unique template for each ECG artifact present in the EMG signal that is not dependent on the presence or characteristics of other artifacts; thus, it is not necessary to align artifacts based on any distinguishing characteristic.

Influence of ECG Contamination on Trapezius EMG

Contaminated EMG signals recorded from the LUT and RUT during rest and low-intensity isometric contractions were processed with the gating method to remove ECG artifacts, and then compared to the original contaminated signals to quantify the influence of ECG contamination on EMG amplitude and frequency estimates during varying levels of muscle activation. Contaminated and gated signals were RMS processed using a rolling 100 ms window, and mean RMS values were calculated for the five minute rest period and one minute sub-maximal contractions. Raw values were expressed as a percentage of MVC to facilitate interpretation of the magnitude of ECG contamination errors. Median frequency (MDF) was also calculated for contaminated and gated signals during low-intensity contractions. Mean RMS and MDF values for the contaminated and
gated signals were compared using paired t-tests, with significance set at \( p < 0.05 \) (Bonferroni-corrected for the number of tests performed).

**Figure 2**: Demonstration of the proposed filtered template subtraction (FTS) method for the same 4.5 s period of contaminated EMG recorded from the left upper trapezius (A) as presented in Figure 1B. A copy of the contaminated EMG signal is passed through a zero phase-shift, 30 Hz low-pass filter, and periods of ECG contamination (indicated as black signal between dotted lines) are identified using a semi-automated threshold detection procedure (B). Each filtered ECG template is then subtracted from its corresponding time interval in the contaminated EMG signal, thereby removing ECG artifact contamination (C).

**FTS Filter Optimization**

The first step in developing the FTS method was to determine the optimum low-pass filter frequency for creating filtered ECG templates. To accomplish this, the FTS procedure was implemented a total of 7 times for each participant, utilizing 10, 15, 20, 25, 30, 35, and 40 Hz low-pass filter settings to create a filtered template of the
contaminated LUT EMG signal during rest and low-intensity contractions prior to ECG removal. These filter settings were selected to isolate the ECG component from the EMG signal, where the majority of power lies below 35 Hz for ECG (Christov and Daskalov 1999) and between 20 and 200 Hz for EMG (Winter 1990).

Mean RMS and MDF values were calculated for the FTS processed signals at each filter setting as described previously. The difference between these values and the gated values (Δ RMS and Δ MDF) were plotted as a function of filter setting to model the effects of varying the filters used to generate ECG templates on amplitude and frequency estimates in the resting and active muscle.

**Comparison of Methods for ECG Contamination Removal**

Contaminated EMG signals recorded from the LUT and RUT during the five minute rest period and all isometric contractions were processed with the 30 Hz HPF, ATS, and FTS methods. Based on results of the FTS filter optimization analysis, a 30 Hz low-pass filter was used for FTS processing. EMG signals were processed as described previously, resulting in a mean RMS value for resting signals, and mean RMS and MDF values for isometric contractions. Differences in these values between the processed signals and the gated (i.e. uncontaminated) signal (ΔRMS and ΔMDF) were calculated to assess the extent to which ECG artifact was removed. One-sample t-tests were used to assess whether the mean ΔRMS and ΔMDF values for each ECG processing method were significantly different from zero, as a value of zero represents no difference from the gated signal and complete removal of ECG contamination. Significance was set at p < 0.05 (Bonferroni-corrected for the number of tests performed).
Results

The influence of ECG contamination on EMG amplitude estimations (mean RMS) at rest is presented in Figure 3. ECG contamination resulted in a significant overestimation of EMG amplitude in both the LUT and RUT, though the difference was much smaller in the latter (0.59 µV (0.07 %MVC) vs. 0.08 µV (0.01 %MVC), respectively). The influence of ECG contamination on EMG amplitude and frequency (mean MDF) estimations during sub-maximal levels of muscle contraction are reported in Table 1. Amplitude and frequency estimations differed negligibly between the contaminated and gated signals at all contraction intensities, with no significant differences (p > 0.05 for all paired comparisons).

Figure 3: The mean root mean square (RMS) of the recorded signal contaminated by electrocardiography (ECG) artifact, and the gated signal are shown for the left and right upper trapezius muscles during the five minute period of muscular rest. The gated signal represents a signal with no ECG contamination. Error bars represent standard deviation. * p< 0.05
Table 1: Values are mean (SD). Root mean square (RMS), expressed as both raw values (µV) and percent maximum voluntary contraction (%MVC), and median frequency (MDF) during isometric contractions of the left upper trapezius (LUT) and right upper trapezius (RUT) muscles at sub-maximal target levels ranging from 2 – 15 %MVC. Sample sizes (N) for each analysis are indicated. The gated signal was used as the gold-standard reference for estimating the amplitude of the uncontaminated electromyography (EMG) signal. There were no significant differences in amplitude or frequency estimations between the contaminated and gated signals at any contraction intensity for either muscle.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Target Contraction</th>
<th>Contaminated Signal</th>
<th>Gated Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RMS (µV)</td>
<td>RMS (%MVC)</td>
</tr>
<tr>
<td>LUT</td>
<td>2% MVC (N=11)</td>
<td>18 (7.3)</td>
<td>1.8 (0.81)</td>
</tr>
<tr>
<td></td>
<td>5% MVC (N=7)</td>
<td>43 (17)</td>
<td>4.0 (0.71)</td>
</tr>
<tr>
<td></td>
<td>10% MVC (N=7)</td>
<td>82 (44)</td>
<td>7.3 (1.4)</td>
</tr>
<tr>
<td></td>
<td>15% MVC (N=7)</td>
<td>120 (45)</td>
<td>11 (1.2)</td>
</tr>
<tr>
<td>RUT</td>
<td>2% MVC (N=10)</td>
<td>21 (4.1)</td>
<td>2.0 (0.54)</td>
</tr>
<tr>
<td></td>
<td>5% MVC (N=7)</td>
<td>48 (13)</td>
<td>4.5 (0.43)</td>
</tr>
<tr>
<td></td>
<td>10% MVC (N=7)</td>
<td>85 (23)</td>
<td>7.9 (0.76)</td>
</tr>
<tr>
<td></td>
<td>15% MVC (N=7)</td>
<td>130 (35)</td>
<td>12 (1.2)</td>
</tr>
</tbody>
</table>
Results from the FTS filter optimization analysis are presented in Figure 4. ∆RMS approached zero (complete ECG artifact removal) as the FTS template filter increased to 40 Hz in a resting muscle; however, ∆RMS decreased and ∆MDF increased as the template filter increased for low intensity contractions, indicating an introduction of estimation error. For comparison to other methods of ECG contamination removal, the lowest filter setting that resulted in no significant difference from the gated signal in a resting muscle (30 Hz low-pass filter) was selected for FTS processing.

Figure 4: Mean root mean square (RMS) and median frequency (MDF) differences between contaminated and gated (uncontaminated) signals before (None) and after removing electrocardiography artifact using the filtered template subtraction (FTS) method with low-pass filters ranging from 10 – 40 Hz. A) RMS difference (∆RMS) for a resting muscle. B) ∆RMS for isometric contractions ranging from 2 – 15% of maximal voluntary contraction (MVC), and C) MDF difference (∆MDF) during the same isometric contractions.
Figure 5 compares the efficacy of ECG contamination removal (relative to the gated signal) for the HPF, ATS, and FTS signal processing methods in a resting muscle. As indicated by ∆RMS values that did not significantly differ from zero, ECG contamination was successfully removed by the FTS and HPF methods in the LUT and by all three methods in the RUT. The ATS method resulted in a significant overestimation (0.13±0.12 µV, p = 0.005) of LUT muscle activity during rest. The effects of ECG removal on ∆RMS and ∆MDF during low-intensity muscle contractions are shown in Table 2. With one exception that did not reach statistical significance, the HPF and FTS methods significantly underestimated RMS at all contraction intensities for both muscles. MDF values were significantly overestimated by the HPF method at all contraction intensities, and by the FTS method at lower contraction intensities (2-5% for LUT and 2-10% for RUT). The magnitude of errors produced by the HPF method was consistently greater than that of the FTS method. The ATS method produced no estimations that differed significantly from the gated (uncontaminated) signal in either muscle at any contraction level.
Figure 5: Comparison of the filtered template subtraction (FTS), 30 Hz high pass filter (HPF), and averaged template subtraction (ATS) methods of electrocardiography (ECG) contamination removal in the left and right upper trapezius at rest. The root mean square differences (ΔRMS) from the gated signal are shown. Error bars represent standard deviation. A significant difference from zero (* p < 0.05) indicates that ECG contamination was not completely removed from the processed signal. Negative values indicate an underestimation of RMS amplitude relative to the gated signal, whereas positive values indicate an overestimation.
Table 2: Differences in root mean square amplitude (ΔRMS) and median frequency (ΔMDF) estimations between signals processed with the specified ECG removal method and the gated signal. Differences were calculated for isometric contractions ranging from 2-15%MVC. The gated signal represented an absence of ECG contamination, therefore differences closer to zero indicate more complete ECG contamination removal. Values are mean (SD). Sample sizes (N) for each analysis are indicated. (HPF = High Pass Filter, ATS = Averaged Template Subtraction, FTS = Filtered Template Subtraction; RUT = right upper trapezius; LUT = left upper trapezius)

<table>
<thead>
<tr>
<th>Method</th>
<th>Target Contraction</th>
<th>LUT</th>
<th></th>
<th>RUT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔRMS (µV)</td>
<td>ΔMDF (Hz)</td>
<td>ΔRMS (µV)</td>
<td>ΔMDF (Hz)</td>
<td></td>
</tr>
<tr>
<td>HPF</td>
<td>2% MVC (N=11 LUT, N=10 RUT)</td>
<td>-2.4 (1.3)*</td>
<td>9.9 (3.0)*</td>
<td>-2.9 (1.1)*</td>
<td>11 (2.3)*</td>
</tr>
<tr>
<td></td>
<td>5% MVC (N=7)</td>
<td>-5.7 (1.3)*</td>
<td>11 (2.4)*</td>
<td>-6.7 (2.7)*</td>
<td>10 (1.9)*</td>
</tr>
<tr>
<td></td>
<td>10% MVC (N=7)</td>
<td>-9.5 (3.6)*</td>
<td>10 (2.1)*</td>
<td>-10 (3.2)*</td>
<td>8.9 (1.9)*</td>
</tr>
<tr>
<td></td>
<td>15% MVC (N=7)</td>
<td>-13 (3.4)*</td>
<td>8.6 (2.7)*</td>
<td>-13 (4.1)*</td>
<td>8.4 (2.2)*</td>
</tr>
<tr>
<td>ATS</td>
<td>2% MVC (N=11 LUT, N=10 RUT)</td>
<td>-0.032 (0.16)</td>
<td>-0.18 (1.4)</td>
<td>-0.055 (0.12)</td>
<td>-0.20 (0.62)</td>
</tr>
<tr>
<td></td>
<td>5% MVC (N=7)</td>
<td>-0.24 (0.15)</td>
<td>0.56 (0.95)</td>
<td>-0.27 (0.13)</td>
<td>0.28 (0.74)</td>
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<td>10% MVC (N=7)</td>
<td>-0.33 (0.74)</td>
<td>-0.28 (0.74)</td>
<td>-0.18 (0.21)</td>
<td>0 (1.1)</td>
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<tr>
<td></td>
<td>15% MVC (N=7)</td>
<td>-0.41 (0.69)</td>
<td>-0.28 (1.3)</td>
<td>-0.030 (0.55)</td>
<td>0 (1.1)</td>
</tr>
<tr>
<td>FTS</td>
<td>2% MVC (N=11 LUT, N=10 RUT)</td>
<td>-0.67 (0.41)*</td>
<td>2.7 (1.6)*</td>
<td>-0.75 (0.27)*</td>
<td>2.5 (0.94)*</td>
</tr>
<tr>
<td></td>
<td>5% MVC (N=7)</td>
<td>-1.5 (0.36)*</td>
<td>2.8 (1.0)*</td>
<td>-1.8 (0.65)*</td>
<td>3.1 (1.0)*</td>
</tr>
<tr>
<td></td>
<td>10% MVC (N=7)</td>
<td>-2.7 (1.4)</td>
<td>2.5 (1.5)</td>
<td>-2.7 (0.61)*</td>
<td>2.2 (0.74)*</td>
</tr>
<tr>
<td></td>
<td>15% MVC (N=7)</td>
<td>-3.4 (1.4)*</td>
<td>1.7 (1.3)</td>
<td>-3.3 (1.1)*</td>
<td>2.0 (1.1)</td>
</tr>
</tbody>
</table>

* Difference from gated signal significantly different from zero (p < 0.05)


Discussion

Influence of ECG Contamination on Upper Trapezius EMG Recordings

One interesting observation from this study was the differential influence of ECG contamination on EMG recordings from the trapezius muscle at rest and during low-intensity contractions. At rest, ECG contamination resulted in an over-estimation of mean RMS amplitude in the LUT. Once the muscle was activated, even at low levels, ECG contamination had a negligible effect on the amplitude of the EMG signal. Similarly, ECG contamination had minimal to no influence on the EMG signal from the RUT at rest or during muscle contraction.

The overestimation of LUT activation during periods of muscular rest demonstrates the importance of removing ECG contamination from such recordings prior to subsequent analysis. For example, ECG contamination may be falsely interpreted as muscle activity during periods of muscular rest that are interspersed with low-intensity contractions of the LUT in occupational exposure studies (Veiersted, Forsman et al. 2013). ECG contamination also has implications for the detection of low-level muscle activity during instructed rest periods, commonly reported by investigations of psychosocial stress and increased muscle tension (Stephenson, Christou et al. 2011). The negligible influence of ECG contamination during muscle contraction, combined with errors introduced into the EMG signal of an active muscle by the FTS and HPF processing methods, does not support the removal of ECG contamination from periods other than muscular rest.
Accuracy of EMG Amplitude Estimates with the FTS Method

Application of FTS processing with a 30 Hz low-pass filter in a resting muscle successfully removed the overestimation errors in RMS amplitude caused by the ECG artifact. During low-intensity contractions, the FTS method underestimated RMS amplitude and overestimated MDF in both the LUT and RUT for most, but not all contraction intensities. This error was small in magnitude, though may still represent a loss of ‘true’ muscle activity that must be considered when applying the FTS method in EMG processing. As illustrated in Figure 4, reducing the low-pass filter used to create the filtered template decreased the magnitude of error introduced by the FTS method in an active muscle, yet also reduced the efficiency of removing ECG contamination from a resting muscle. This trade off should be considered when selecting the optimal filter for FTS processing, which will depend on both the research question and the specific characteristics of the motor task.

Comparison of FTS to Existing Methods of ECG Removal

The HPF method produced a similar pattern of results as FTS processing, however, errors were larger and more consistent than those observed for the FTS method. The ATS method produced no significant error when processing signals from low-intensity contractions, which were negligibly affected by ECG artifacts prior to processing. During periods of muscular rest, however, the ATS method overestimated the amplitude of EMG activity in the LUT.

Differences in outcomes for the ATS method compared to the FTS and HPF methods are likely due to two reasons. First, individual ECG artifacts recorded in the LUT showed relatively large variability in shape, as illustrated in Figure 1A. This
variability could be influenced by multiple factors, including changes in the distance between the recording electrodes and the source of the ECG signal with movements of the shoulder girdle during respiration. Thus, an averaged template with a fixed waveform shape may not be sufficient to completely remove ECG contamination from the trapezius muscle. Second, the ATS method assumes that any value not contained within the ECG artifact will be reduced to zero in the averaged template. Due to the presence of undetectable ECG artifacts during periods of muscle activity, most values within the averaged template likely tended towards zero. Subtraction of this template would then remove little to none of the recorded signal, causing no underestimation errors. This contrasts with the FTS and HPF methods, which both remove some components of the EMG signal even when no ECG artifacts are detectable. This advantage of the ATS method is of limited benefit in a continuously active muscle, as RMS and MDF estimates are unaffected by ECG artifact during even low intensity muscle contraction. An important consideration for occupational exposure studies is that the shape of the averaged template produced when the ATF method is applied to EMG recordings of muscle contraction interspersed with periods of muscle rest will not accurately reflect and remove ECG artifacts present during the rest periods.

**Advantages and Limitations of the FTS Method**

The FTS method offers several advantages compared to existing methods of ECG contamination removal. Similar to HPF, the FTS method is easily implemented but consistently introduces less error. This is likely due to restricting periods of data removal only to time intervals containing the ECG artifact in the FTS method, whereas HPF affects the entire EMG signal. This finding is consistent with previous observations that
the commonly recommended HPF method of ECG removal does not perform as well as other methods (Willigenburg, Daffertshofer et al. 2012). Implementation of the FTS method requires only 1) identification of time intervals corresponding to ECG artifacts, 2) low-pass filtering a copy of the contaminated EMG signal, and 3) subtraction of the filtered signal from the contaminated signal at time intervals containing ECG artifacts. A zero phase-shift filter is used to create individual ECG templates; therefore, no further alignment is necessary prior to subtraction, as any variability in the shape or temporal distribution of the artifact will be reflected in the filtered signal. The ATS method requires an additional step of aligning each ECG artifact along a distinguishing attribute, such as the R-wave. This step can be time consuming to implement, decreasing the efficiency of the ATS method in larger data sets. Given that each ECG artifact is removed using its own corresponding template in the filtered signal, the FTS method can be applied to a single ECG artifact or an entire day of work activities with the same efficiency.

Second, a separate ECG signal was collected to identify ECG artifact time intervals in the present study; however, this additional channel is not necessary if ECG artifacts can be reliably identified in the contaminated EMG signal. This advantage also applies to the ATS method. Given the negligible influence of ECG contamination during even low levels of muscle contraction, removal of only those ECG artifacts that can be visually distinguished from background noise or muscle activity in the EMG signal may be sufficient to minimize ECG contamination errors in the upper trapezius.

One limitation of the FTS method is the assumption that the low-frequency components of the EMG signal and the high-frequency components of the ECG artifact
are both negligible. If these assumptions are not valid, a significant portion of the EMG signal may be lost, or the ECG artifact may not be sufficiently removed. Our results suggest that the latter assumption is valid for ECG templates created using a 30 Hz low pass filter, as ECG contamination was completely removed during periods of muscular rest. In contrast, the shift toward lower RMS and higher MDF values with increases in the FTS filter setting during periods of muscle contraction likely indicates a loss of actual EMG signal due to the removal of low frequency signal components. Finally, the shape of an ECG artifact can be altered by several experimental factors, such as the location of the reference electrode. Alterations in ECG artifact shape resulting from different experimental setups may change the magnitude, but not the general pattern, of effects reported for the FTS filters in the current study.

**Study Limitations**

The major limitation of this study was the lack of a known gold standard for comparing each ECG removal method to an uncontaminated EMG signal. The gated EMG signal, in which contaminated time intervals were completely removed, was selected as the reference standard. The resting and isometric tasks investigated in the present study were designed to meet the assumption that non-ECG signal components contained within the ECG artifact window are redundant of the remaining EMG signal, and can be removed without a loss of information. Gating may also create errors in frequency analyses of the concatenated signal. However, these errors are expected to be greatest around the frequency of the gated ECG artifact (1 – 1.7 Hz, or 60 – 100 beats per minute), with minimal influence on median frequency values which were comparable between this and previous studies (Kumar, Narayan et al. 2001). Discontinuities in the
concatenated gated signal may also introduce high frequency artifacts, though little evidence of this was seen in the current data set with minimal to no difference in MDF after gating (Table 1). Moreover, MDF is robust to changes at extreme values as a measure of frequency. Our approach using the gated signal as the reference standard for an uncontaminated signal allowed us to investigate the effects of ECG removal on recorded EMG signals, which included confounding experimental factors such as large variability in the shape of ECG artifacts that may not be present in artificially contaminated signals.

**Recommendations**

This is the first study to quantify the effects of ECG artifact on the amplitude and frequency of EMG recordings from the upper trapezius muscle, and provides important information to help investigators decide under what task-specific conditions ECG removal should be applied for a wide range of EMG applications. Our results suggest that ECG artifact removal, by any method, may not be necessary during periods of known muscle contractions due to the negligible influence of ECG contamination on both RMS and MDF estimations in these periods. In contrast, the presence of ECG artifact significantly overestimates the amplitude of muscle activity in a resting muscle. Our results support both HPF and FTS processing with a 30 Hz filter for ECG artifact removal during periods of muscular rest, but suggest that these methods may underestimate the true amplitude and overestimate the true frequency of trapezius muscle activity during low-intensity contractions, with greater errors introduced by the HPF method. The relative advantages and disadvantages of available methods of ECG artifact removal should be carefully considered in the context of the specific motor tasks that
require analysis, particularly those that involve intermittent periods of muscle activity and rest.
References


APPENDIX B

MUSCLE DOMINANCE, SEX DIFFERENCES, AND RELIABILITY OF WORKSITE UPPER TRAPEZIUS MOTOR PATTERNS IN HEALTHY OFFICE WORKERS

Abstract

Patterns of cervical muscle activity may contribute to muscle overuse injuries in office workers. The purpose of this investigation was to characterize patterns of upper trapezius muscle activity in pain-free office workers using traditional occupational exposure measures and a modified Active Amplitude Probability Distribution Function (APDF), which analyzes only periods of active muscle contraction. Bilateral trapezius muscle activity was recorded in 77 pain-free office workers for 1-2 full days in their natural work environment. Mean amplitude, gap frequency, muscular rest, and Active APDF amplitudes were calculated. All measures demonstrated moderate to almost perfect reliability. The dominant muscle demonstrated higher amplitudes of activity and less muscular rest compared to the non-dominant, and women demonstrated less rest with no difference in amplitude assessed by Active APDF compared to men. These findings provide normative data to identify abnormal motor patterns that may contribute to persistence or recurrence of neck pain in office workers.

Introduction

Neck pain, defined as pain in an anatomical location between the superior nuchal line and the spine of the scapula (Guzman, Hurwitz et al. 2009), is a significant health

1 This appendix has been submitted to the journal Ergonomics.
problem, ranking as the fourth greatest contributor to global disability (Hoy, March et al. 2014) with 6-12 month incidence rates ranging from 6% – 17.4% in the general working population (Côté, van der Velde et al. 2009). Compared to other occupational groups, office workers experience particularly high incidence rates ranging from 15.4% - 34.4% annually (Côté, van der Velde et al. 2009). The high incidence of chronic neck pain in office workers, despite the relatively low physical demand of this occupation, has led researchers to investigate potential etiologic factors associated with this condition (Christensen and Knardahl 2010). Many studies have explored the potential contribution of sustained, low amplitude cervical muscle activity to muscle overuse injuries (Hägg 2000, Sjøgaard, Lundberg et al. 2000) and the development of pain in this population (Blangsted, Hansen et al. 2003, Dennerlein and Johnson 2006, Richter, Mathiassen et al. 2009, Szeto, Straker et al. 2009).

Trapezius myalgia is present in one third of office workers with chronic neck pain (Sjøgaard, Søgaard et al. 2006); therefore, the trapezius muscle is a common target of electromyographic (EMG) studies of physical workload (Hansson, Nordander et al. 2000, Nordander, Hansson et al. 2000, Østensvik, Veiersted et al. 2009). There is no gold standard for assessing patterns of muscle activation from prolonged (i.e., whole work day) EMG recordings and their relation to musculoskeletal pain; however, several methods have been proposed. For example, muscle activation patterns have been characterized as both the frequency and total duration of continuous periods during which EMG amplitude is greater than (Østensvik, Veiersted et al. 2009, Østensvik, Veiersted et al. 2009) or less than a predefined level of muscular rest (Veiersted, Westgaard et al. 1993, Hansson, Nordander et al. 2000, Veiersted, Forsman et al. 2013). These measures
focus on the temporal aspects of muscle activation, but do not provide estimates for the cumulative magnitude of physical exposures (i.e. EMG amplitude). A more common approach has been to use the Amplitude Probability Distribution Function (APDF) to evaluate static, median, and peak amplitudes of muscle activity that occur over prolonged recording periods (Jonsson 1982, Hansson, Nordander et al. 2000, Nordander, Hansson et al. 2000). Static muscle activity is defined as the EMG amplitude exceeded for 90% of the sampled workday (calculated as the 10th percentile of the APDF). Static muscle activity is thought to represent the level of sustained, low-amplitude muscle activity required for biomechanical functions such as maintaining postural control. Median and peak values are calculated as the 50th and 90th percentiles of the APDF, respectively, and are thought to represent briefer and less frequent bouts of higher amplitude muscle activity required for more dynamic tasks (Jonsson 1982, Hansson, Nordander et al. 2000).

APDF values are traditionally calculated using the EMG signal from the entire recording period, including periods with EMG amplitudes below the defined level of muscular rest, to estimate the cumulative load placed on the muscle throughout the workday. However, the inclusion of EMG amplitudes recorded during muscular rest reduces the sensitivity of this analysis to differences in the amplitude of static and dynamic loads placed on the muscle only after it becomes active. By definition, the APDF is more influenced by low resting EMG values as the amount of muscular rest increases, thereby reducing the estimated amplitude of muscle activity during periods of active muscle contraction. Furthermore, large amounts of muscular rest reduce the amplitude of static, median, and peak EMG derived from the APDF in a non-linear fashion due to the logarithmic shape of the function. Several studies of office workers
have reported large amounts of muscular rest (Aarås, Veierød et al. 1996, Nordander, Hansson et al. 2000, Blangsted, Hansen et al. 2003) during prolonged recordings, thereby having a large influence on reported levels of static, median, and peak muscle activity. Although such studies provide an estimate of cumulative workload, it may also be informative to consider the amplitude of active muscle contraction independently from the amount of muscle rest when assessing physical risk factors for musculoskeletal pain. For example, this approach could help identify whether the magnitude of muscle contraction required to perform job duties or the duration of rest breaks throughout the workday should be prioritized by injury prevention programs. The current study proposes an *Active APDF* methodology in which periods of muscular rest are removed prior to constructing the amplitude probability distribution function to insure that static, median, and peak values reflect the amplitude of muscle contraction when the muscle is active, independent of muscular rest. Importantly, the reliability and sensitivity of this approach to detect distinct patterns of muscle activity throughout the workday is also assessed.

Most previous investigations of muscle activity in the workplace have included populations with diverse occupations (Hansson, Nordander et al. 2000, Nordander, Hansson et al. 2000, Østensvik, Veiersted et al. 2009). Studies that have focused specifically on office workers often examine standardized tasks performed in a controlled laboratory environment (Dennerlein and Johnson 2006, Szeto, Straker et al. 2009), or analyze isolated office tasks such as keyboarding in the workplace (Nordander, Hansson et al. 2000). Characterization of more global patterns of muscle activity that occur throughout the workday, including task transitions and extraneous activities that are not performed during typical computer work, may provide additional insight into activity
patterns that contribute to overuse injuries in an office setting where physical demands are generally low. Furthermore, differences in cervical muscle activity associated with hand dominance (Nordander, Hansson et al. 2000, Richter, Mathiassen et al. 2009) have rarely been addressed in previous literature despite asymmetrical demands typical of office work, such as using a computer mouse. Similarly, sex differences in work-related cervical muscle activity are not well understood despite a consistently greater prevalence of neck pain in women compared to men (Hoy, March et al. 2014).

The purpose of this investigation was to characterize patterns of upper trapezius muscle activity in a large cohort of pain-free office workers using the Active APDF calculation, in addition to more traditional occupational exposure summary measures. The day-to-day reliability and sensitivity of these measures to intra- and inter-individual differences in hand dominance and sex were examined to determine their utility for future investigations in the workplace. We expected all occupational exposure measures to demonstrate acceptable reliability for research applications, with a greater magnitude of static activity and less muscle rest observed in the dominant compared to non-dominant upper trapezius and in women compared to men.

Methods

Participants

A convenience sample of 77 healthy office workers was recruited through print and radio advertisements, new employee orientations, and employee bulletins and flyers posted at businesses employing a large number of office workers in the greater Denver area. Eligible participants were within three months of their date of hire and worked > 30 hours per week in an office setting that required the use of a computer for at least 75% of
the workday. Participants were screened for the presence of neck pain or associated disorders during the previous year. To avoid the potential for selection bias due to poor recall of pain symptoms, the Neck Disability Index (NDI) (Vernon and Mior 1991) was used to screen for activity limitations caused by pain which are more likely to be remembered than non-interfering neck pain. Participants were included in the study if they reported no neck pain or associated disorders during the previous year, and scored < 5 points on the NDI.

Exclusion criteria included: 1) objective signs of structural pathology upon physical examination by a licensed physical therapist, including but not limited to shoulder bursitis, impingement, tendonitis, fracture, and cervical nerve or disc impairment with radiculopathy or loss of sensory or motor function, 2) self-reported fibromyalgia diagnosis or musculoskeletal pain present in more than four body regions concurrently, 3) self-reported systemic illness including cancer, rheumatic, cardiovascular, or neurological disease, 4) prior surgery involving the cervical spine or shoulders, 5) acute (< 12 weeks prior to study) injury of the neck or shoulders, 6) untreated psychiatric condition, 7) uncontrolled hypertension, 8) pregnancy, and 9) an inability to type or comprehend written and oral instructions in English. All participants provided written informed consent according to study procedures approved by the Colorado Multiple Institutional Review Board.

Data Collection Protocol

Participants first completed a demographics questionnaire at a familiarization session. Bilateral upper trapezius muscle activity and electrocardiography (ECG) were recorded with a portable data monitor on a representative workday selected by the
participant based on convenience. A subset of participants (N = 63) completed a second day of worksite monitoring within two weeks of the first to examine the reliability of occupational exposure summary measures. Participation in the second day of recording was based on participants’ willingness and availability to wear the monitor on a second workday. An investigator met the participant at a private location near his or her workplace 15 minutes prior to the start of the workday for equipment setup. After visual verification of signal quality, two 10 s submaximal reference voluntary efforts (RVE) and resting measurements were collected. RVEs were measured with both arms held at 90 degrees of flexion in the sagittal plane and 45 degrees of abduction in the horizontal plane, while wearing 1 kg wrist weights with the forearms in full supination. This position was slightly modified from Hansson et al., (2000) to reduce the setup time required in the workplace, and for comparison with ongoing laboratory studies using the same reference position. Resting measurements were collected during quiet sitting with the shoulders relaxed and hands resting in the lap for 10 s. Trained laboratory personnel monitored the EMG signal online to confirm the absence of muscle activity during resting measurements, and provided verbal feedback as necessary to insure complete muscle relaxation.

Participants wore a portable data monitor continuously in a minimally obtrusive waist pack positioned on the anterolateral aspect of the waist throughout the workday as they performed their usual activities. Participants were instructed to ignore the device and not make any changes to their usual work routine. No observation of work activities was performed by study investigators to minimize observation bias. The portable data
monitoring system was removed at the end of the workday by study investigators after repeating resting EMG measurements at the workplace.

**Data Recording and Processing**

The portable data monitor (Delsys Myomonitor IV; Delsys, Boston, MA, USA) recorded surface EMG bilaterally from the upper trapezius. Surface electrodes (DE-2.3 Single Differential Surface EMG Sensor; Delsys) with two parallel 1 x 10mm silver surface contacts with an inter-contact distance of 10 mm were used. Signals were amplified (x1000 V/V) and band-pass filtered using hardwired settings (20-450 Hz) prior to sampling (1000 Hz). Electrodes were placed on the upper trapezius muscle belly 2 cm lateral to the midline between the seventh cervical vertebra and the posterior acromion process (Farina, Madeleine et al. 2002). Electrode positions were marked and covered with a water-proof protective sealant to improve the reliability of sensor placement when multiple days were recorded. ECG was recorded with a bi-lead sensor (SP-X14 EKG Sensor for Myomonitor System; Delsys) which amplified (x1000 V/V) and band-pass filtered (0.5 – 30 Hz) the signal prior to sampling (1000 Hz). ECG leads were attached to 1.75 inch diameter foam and solid gel electrodes with 1 cm Ag/AgCl conducting snaps (Red Dot 9640; 3M, St. Paul, MN, USA) which were placed vertically on the flat portion of the sternum (Marker and Maluf 2014). EMG and ECG signals were referenced to a bony surface on the right clavicle with a self-adhesive electrode pad. EMG and ECG data were stored on an internal 1GB memory card in consecutive one-hour data sets for offline processing. Each data set was 10 s short of an hour (3590 s) to allow for automated internal data storage.
EMG signals were pre-processed with custom scripts implemented in Spike2 (Cambridge Electronic Design, Cambridge, UK) and Matlab (MathWorks Inc., Natick, MA, USA) prior to occupational exposure analyses. Each hour long data file was first visually inspected for artifacts caused by wire movement, which were removed and replaced with the mean of the preceding 0.5 s of the EMG signal. The ECG signal was used to identify periods of ECG contamination in the EMG signal, which were subsequently removed using a validated filtered template subtraction technique to minimize the loss of EMG signal in the process of ECG contamination removal (Marker and Maluf 2014) (Appendix A). EMG was then root-mean-square (RMS) processed with a 100 ms window, updating every 10 samples. System noise was removed by linear subtraction of the lowest RMS value recorded in the baseline resting period from the RMS processed EMG signal. RVE values were computed as the mean of the RMS processed signal for a stable 8 s period within each RVE trial and then averaged across the two trials (Jackson, Mathiassen et al. 2009). EMG was normalized to RVE prior to computation of occupational exposure summary measures. Figure 1 shows representative EMG data before and after processing and normalization.
Figure 1: Electromyographic (EMG) data for a representative 100 s sample prior to processing (A) and after root mean square (RMS) processing and normalization to a reference voluntary effort (RVE) (B). The dashed line represents the 5% RVE muscular rest threshold. Solid lines above (B) indicate periods of muscle activity above rest and the respective contraction durations. The traditional Amplitude Probability Distribution Function (APDF) derived from normalized RMS data is shown in (C) and the Active APDF in (D) for visualization of the 10th (static), 50th (median), and 90th (peak) percentiles (dotted lines). Note that no data below the 5% RVE muscular rest threshold in (B) is included in the calculation of the Active APDF or percentile values in D, resulting in a leftward shift of all percentile values when compared to the traditional APDF (C) which includes all data below 5% RVE.

Data Analysis and Occupational Exposure Summary Measures

All occupational exposure analyses were performed using custom software written in LabVIEW (National Instruments, Austin, TX, USA). Occupational exposure summary measures were calculated for each complete hour of data collected, and then averaged across the workday for each participant. Following reliability analyses, summary measures were also averaged across days (Fethke, Gerr et al. 2012) for participants who completed two days of data collection. Summary measures were calculated separately for the dominant and non-dominant upper trapezius muscles.
Mean amplitude across the entire recording period was calculated as a global index of muscular load. Gaps in muscular activity were defined as any periods in which muscle activity fell below 5% RVE for at least 0.125 s (Hansson, Nordander et al. 2000). Gap frequency was expressed as the number of gaps/min and muscular rest was defined as the summed duration of all gaps expressed as a percentage of total recording time.

Static, median, and peak amplitudes of muscle activity were calculated as the 10th, 50th, and 90th percentiles of the RMS processed signal, respectively. These values are equivalent to the static, median, and peak percentile rankings of the APDF (Jonsson 1982), as illustrated in Fig 1. In contrast with prior studies utilizing traditional APDF methodology (Figure 1C), all values below the 5% RVE muscular rest threshold were removed prior to calculating percentiles to assess the amplitude of muscle activity only during periods of active muscle contraction (Figure 1D). The resulting values from this modified analysis will be referred to as Active APDF values. Comparing Figures 1C and 1D illustrates how large periods of muscle rest cause a leftward shift and steep initial increase in the ADPF function that reduce estimates of static, median, and peak muscle activity.

Finally, muscular contraction durations were calculated similar to other studies (Østensvik, Veiersted et al. 2009) to characterize temporal aspects of muscle activation patterns throughout the workday. Epochs of time in which muscle activity was above the muscular rest threshold of 5% RVE were determined sequentially for each recording period. The end of each epoch was identified when EMG activity dropped below the muscular rest threshold for > 0.125 s, based on the same criterion used to identify gaps in muscle activity. An example of muscular contraction epochs of varied duration is shown.
in Figure 1B. Based on the distribution of contraction durations observed for dynamic and postural activity of the upper trapezius muscle in preliminary analyses, epochs were empirically categorized as having short (≤ 3 s), medium (3.01–120 s), or prolonged (> 120 s) durations. The durations of all epochs within each category were then summed and expressed as a percentage of total recording time.

**Statistical Analysis**

All statistical analyses were performed using R (R Development Core Team, Vienna) and SAS (SAS Institute, Cary, N.C.). Between day reliability was calculated for all occupational exposure summary measures among participants who completed two days of data collection, using intraclass correlations (ICC(2,1)). Variance components associated with participant ($S_P^2$), day ($S_D^2$, within participant), and hour ($S_H^2$, within day) were calculated with a mixed model Analysis of Variance (ANOVA), entering participant, day, and hour as random effects. Variance components were computed separately for each muscle (dominant and non-dominant), and the first-order autoregressive covariance structure was applied to account for correlation between consecutive hours of data collection. Given the large imbalance between men and women in the sample, sex differences were calculated using non-parametric comparisons (Wilcoxon Rank-Sum Test) for age, body mass index (BMI), mean amplitude, gap frequency, muscular rest, and Active APDF variables. Occupational exposure summary measures for the dominant and non-dominant muscles were averaged when investigating sex differences. Occupational exposure summary measures were also compared between the dominant and non-dominant upper trapezius using paired t-tests. Differences in muscular contraction duration were analyzed with a 2x3 repeated measures ANOVA,
including a Muscle factor (dominant vs non-dominant) and Duration factor (short, medium, or prolonged). Post-hoc paired t-tests utilizing a Bonferroni correction were used to identify the source of significant main or interaction effects.

**Results**

A total of 60 women and 17 men participated. Poor signal quality identified during visual inspection of the data led to the exclusion of 10 days of data (unstable system noise periodically exceeding 5% RVE), completely removing all days of data collection for three participants. Seventy-four participants remained in the final analyses (59 women, 15 men). Mean age (SD) was 31 (7.6) years (women: 31 (7.8), men: 31 (7.1)) and mean BMI (SD) was 24 (4.7) kg/m$^2$ (women: 24 (5.0), men: 25 (3.1)). Two days of EMG recordings remained for 56 participants in the final analysis, who did not differ from the full sample of participants with regard to demographic characteristics ($p \geq 0.05$). On average, 6.65 hours of data were analyzed per workday (range: 4 – 8 hours). One participant was left-hand dominant. There were no significant differences between men and women in age and BMI.

Table 1 reports ICC values and 95% confidence intervals for between-day reliability of all occupational exposure summary measures in the dominant and non-dominant upper trapezius muscles. Reliability was qualitatively described as recommended by Landis and Koch (Landis and Koch 1977), with ICC values interpreted as: 0–0.20 – slight agreement, 0.21–0.40 – fair agreement, 0.41–0.60 – moderate agreement, 0.61–0.80 – substantial agreement, and 0.81–1.00 – almost perfect agreement. Values ranged from 0.51 to 0.86, demonstrating moderate to almost perfect agreement for all occupational exposure summary measures. Reliability was higher for the non-
dominant compared to the dominant trapezius muscle for the majority of summary measures.

Table 1: Between-day reliability of occupational exposure outcomes

<table>
<thead>
<tr>
<th></th>
<th>Dominant Trapezius</th>
<th></th>
<th>Non-Dominant Trapezius</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICC (2,1)</td>
<td>95% Confidence Interval</td>
<td>ICC (2,1)</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>Mean Amplitude</td>
<td>0.73</td>
<td>0.58 – 0.83</td>
<td>0.80</td>
<td>0.68 – 0.88</td>
</tr>
<tr>
<td>Muscular Rest</td>
<td>0.64</td>
<td>0.46 – 0.77</td>
<td>0.77</td>
<td>0.64 – 0.86</td>
</tr>
<tr>
<td>Gap frequency</td>
<td>0.52</td>
<td>0.29 – 0.68</td>
<td>0.53</td>
<td>0.31 – 0.70</td>
</tr>
<tr>
<td>Active APDF:</td>
<td>Static</td>
<td>0.77</td>
<td>0.63 – 0.86</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.77</td>
<td>0.63 – 0.86</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>0.69</td>
<td>0.52 – 0.80</td>
<td>0.74</td>
</tr>
<tr>
<td>Contraction Duration:</td>
<td>Short (≤ 3 s)</td>
<td>0.54</td>
<td>0.33 – 0.71</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Medium (3.001-120 s)</td>
<td>0.51</td>
<td>0.28 – 0.68</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Prolonged (&gt; 120 s)</td>
<td>0.64</td>
<td>0.45 – 0.77</td>
<td>0.58</td>
</tr>
</tbody>
</table>

ICC – Intraclass Correlation Coefficient
APDF – Amplitude probability distribution function

Mean amplitude, muscular rest, gap frequency, and Active APDF values for the dominant and non-dominant upper trapezius, along with variance components due to participant, day, and hour, are presented in Table 2. All summary measures were significantly different between the two muscles. The largest difference was observed in the amount of muscular rest, with the non-dominant muscle being inactive for approximately 10% more of the total recording time than the dominant muscle. All active
APDF values (static, median, and peak) were greater in the dominant compared to the non-dominant upper trapezius. Relative variance components were similar to previous studies (Fethke, Gerr et al. 2012), and variance due to day of recording was consistently the smallest component. It should be noted that variance due to hour (within day) is dependent on the length of the sampling window, and would be expected to differ for sampling windows other than 1 hour in length. Results of these comparisons did not meaningfully change when men were removed from the analysis.

Figure 2 illustrates the results of the muscular contraction duration analysis. Main effects for Muscle (p < 0.001) and Duration (p < 0.001) were observed, as well as a significant interaction between these factors (p < 0.001). No difference was observed between muscles for short duration contractions (9.3 vs 9.5% recording time, dominant vs non-dominant, p > 0.99). However, medium (46.1 vs 41.0% recording time, p < 0.001) and prolonged (12.8 vs 9.1% recording time, p = 0.006) duration contractions were significantly greater for the dominant upper trapezius. Both muscles exhibited significantly greater time spent performing contractions of medium duration compared to both short and prolonged duration contractions (p < 0.001). Short and prolonged durations were not significantly different from each other in either muscle (p > 0.45).
Table 2: Comparison of occupational exposure outcomes for the dominant and non-dominant trapezius muscles.

<table>
<thead>
<tr>
<th></th>
<th>Dominant Trapezius</th>
<th></th>
<th>Non-Dominant Trapezius</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>$S^2_{p}$ (%)</td>
<td>$S^2_{d}$ (%)</td>
<td>$S^2_{h}$ (%)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Mean Amplitude</td>
<td>25.8 (10)</td>
<td>82.9 (47)</td>
<td>24.6 (14)</td>
<td>67.3 (39)</td>
<td>21.2 (9.3)</td>
</tr>
<tr>
<td>Muscular Rest</td>
<td>31.9 (13)</td>
<td>120.9 (38)</td>
<td>46.6 (15)</td>
<td>147.4 (47)</td>
<td>40.4 (15)</td>
</tr>
<tr>
<td>(% recording time)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gap Frequency</td>
<td>13.7 (4.0)</td>
<td>10.0 (26)</td>
<td>4.5 (12)</td>
<td>23.2 (62)</td>
<td>15.1 (5.0)</td>
</tr>
<tr>
<td>(gaps/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active APDF:</td>
<td>9.3 (2.5)</td>
<td>5.5 (49)</td>
<td>1.2 (11)</td>
<td>4.4 (40)</td>
<td>8.6 (2.2)</td>
</tr>
<tr>
<td>Static (%RVE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>27.9 (8.7)</td>
<td>64.6 (49)</td>
<td>15 (11)</td>
<td>52.4 (40)</td>
<td>25.7 (8.7)</td>
</tr>
<tr>
<td>(%RVE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>74.7 (21)</td>
<td>344.1 (45)</td>
<td>123.8 (16)</td>
<td>294.5 (39)</td>
<td>69.5 (18)</td>
</tr>
<tr>
<td>(%RVE)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

RVE – Reference Voluntary Effort
APDF – Amplitude probability distribution function
$S^2_{p}$ – Variance due to participant
$S^2_{d}$ – Variance due to day (within participant)
$S^2_{h}$ – Variance due to hour (within day)
Variance components also expressed as percent of total variance.
Figure 2: Percent of recording time above rest (5% RVE) categorized as short (0-3 s), medium (3-120 s), and prolonged (> 120 s) contraction durations for the dominant and non-dominant upper trapezius muscles.

* p < 0.05

Comparisons between women and men for mean amplitude, gap frequency, muscular rest, and active APDF values (averaged across dominant and non-dominant muscles) are reported in Figure 3. Women showed a significantly greater mean amplitude of muscle activity and less muscular rest compared to men. No significant sex differences were observed for active APDF values or gap frequency.

To insure that the results were not biased by combining data from participants with either one or two days of data collection, all analyses were repeated using only mean values from participants with two days of recording. No significant changes in the results were observed.
**Discussion**

This study characterized patterns of muscle activity in the dominant and non-dominant upper trapezius muscles in the largest studied cohort of pain-free office workers to date. All occupational exposure measures demonstrated moderate to almost perfect reliability between days and low relative variance due to day of recording, supporting their use in clinical and research applications. Both muscles demonstrated large amounts of muscular rest (greater than 30% of the workday), with the non-dominant muscle spending significantly more time at rest than the dominant. Active APDF amplitude measures were all lower in the non-dominant muscle compared to the dominant. Both
muscles spent a similar amount of time performing short duration (0–3 s) contractions, whereas the dominant muscle spent greater time performing contractions of medium (3–120 s) and long (> 120 s) duration. Both muscles spent the majority of the workday performing contractions of medium duration. Women demonstrated significantly less muscular rest with no difference in EMG amplitude assessed by the Active APDF method compared to men.

**Muscular Rest, Mean Muscle Activity, and Gap Frequency**

Muscular rest was significantly less and mean muscle activity was significantly greater in the dominant trapezius muscle. This pattern is to be expected, given that periods of muscular rest are included in the calculation of mean muscle activity thereby decreasing its mean amplitude. Mean muscle activity is often used as a global measure of muscular load (Richter, Mathiassen et al. 2009), and is influenced by long periods of rest during which there is no muscular load. This relation between mean activity and rest necessitates a measure of the amplitude of muscle activity independent of rest, which is provided by the Active APDF calculation. Differences between the dominant and non-dominant muscles indicate that the non-dominant trapezius is utilized less by healthy office workers, likely decreasing the cumulative load placed on this muscle throughout the workday. Gap frequency was also greater in the non-dominant muscle. This difference is not solely due to the increased amount of muscular rest, as muscular rest and gap frequency reflect distinct motor patterns (Hansson, Nordander et al. 2000), in which the maximum amount of muscular rest possible would be obtained with fewer rather than more periods of muscle activity separating periods of rest. The difference in gap frequency between muscles may represent differences in work demands and individual
neuromuscular control of the dominant and non-dominant upper extremities (Hansson, Nordander et al. 2000).

**Active APDF**

The office workers included in the study sample were similar to those of previous studies (Aarås, Veierød et al. 1996, Nordander, Hansson et al. 2000, Blangsted, Hansen et al. 2003), showing large periods of muscular rest throughout the workday. To estimate the amplitude of muscle activity only during periods of active muscle contraction, muscular rest periods were removed prior to calculating percentile values for the remaining signal, thereby creating the Active APDF from which static, median, and peak amplitudes of muscle activity were computed. Previous studies using the traditional APDF method have shown static values as low as 0.1% MVC (Holte and Westgaard 2002) or 2.3% RVE (Nordander, Hansson et al. 2000). These estimates are likely influenced by large amounts of rest, and may underestimate the true amplitude of muscle contraction while the muscle is active (as shown in Figure 1C-D). The low values previously reported for static muscle activity may also be insensitive to subtle differences in the magnitude of muscle activity required to maintain postural control of the dominant (more active) and non-dominant (less active) limbs, resulting in few and conflicting reports of hand dominance effects on muscle activity (Nordander, Hansson et al. 2000). In contrast, the current study demonstrated consistently larger values in all three (static, median, and peak) exposure measures for the dominant compared to the non-dominant muscle during periods of active contraction. Consistent differences in static, median, and peak levels of muscle activity between dominant and non-dominant limbs suggest that the proposed method of calculating Active APDF values may be more appropriate for this
population and provide greater sensitivity to detect differences in motor control of the active muscle.

Overall, the results of the Active APDF analyses indicate that the amplitude of muscle activity in office workers typically remains low. In the current study, muscle activity was normalized to a submaximal reference instead of a maximal contraction, and peak activity (90th percentile) was still below 100%. Although submaximal reference contractions are reliable (Bao, Mathiassen et al. 1995, Delisle, Larivière et al. 2009), normalized EMG values are more difficult to interpret than those normalized to maximal contractions. However, 100% RVE recorded while holding a 1 kg weight with the arm extended would likely not represent a very demanding load on the muscle. Previous research utilizing a similar RVE method reported RVE to be approximately 20% of a maximum contraction (Balogh, Hansson et al. 1999). This indicates low levels of muscle activity in the current population, as peak values were consistently less than 100% RVE.

**Contraction Duration**

The contraction duration analyses revealed that when both the dominant and non-dominant muscles are active, they predominantly perform contractions of medium duration (3 – 120 s), and spend less time performing short (0 – 3 s) or prolonged contractions (> 120 s). Differences between the dominant and non-dominant sides were only observed for contractions of medium and prolonged durations, with both muscles spending a similar amount of time performing short duration contractions. Several studies have indicated that sustained periods of muscle activity lasting greater than 4 - 8 min may be a risk factor for developing or worsening neck pain (Østensvik, Veiersted et al. 2009, Østensvik, Veiersted et al. 2009). These studies, however, were performed in forestry.
workers rather than office workers. The small percentage of time observed for prolonged contractions lasting more than two minutes in the current population indicate that previously identified risk thresholds for neck pain in more physically demanding occupations such as forestry work likely do not generalize to office work. Given that prolonged muscle activity is commonly thought to contribute to trapezius myalgia in office workers (Hägg 2000, Sjøgaard, Lundberg et al. 2000), it will be important for future investigations to establish thresholds of risk specific to this population. It should be noted that the contraction duration categories in this study represent a preliminary attempt to characterize the typical duration of sustained contractions observed in the trapezius muscle during pain-free office work. Given that the majority of trapezius contractions were categorized as medium duration, future research should investigate whether additional categories with a higher temporal resolution within this category can provide meaningful information for risk assessment.

**Sex Differences**

Women demonstrated significantly less muscular rest and a greater amplitude of mean muscle activity than men. In contrast, gap frequency and Active APDF values were not different between sexes. This indicates that although the upper trapezius muscle in women may be at rest for a smaller proportion of the workday compared to men, its pattern of activation when not at rest is similar between sexes, contrary to what was hypothesized. These findings highlight the ability of the Active APDF to distinguish differences in the amplitude of muscle activity from differences in muscular rest, as we show for the first time that the greater muscular load (mean muscle activity) observed in women is due to a decrease in muscular rest but not higher levels of activity while the
muscle is active. Future studies should determine whether reduced amounts of muscular rest contribute to the higher prevalence of neck pain in female compared to male office workers (Paksaichol, Janwantanakul et al. 2012).

Limitations

It should be emphasized that Active APDF values provide information on the amplitude of muscle activity during known periods of active muscle contraction. As these values are independent of rest periods, they must be interpreted alongside other measures such as muscular rest and gap frequency to provide a more comprehensive assessment of work-rest patterns in the workplace. This may provide important information on rest breaks throughout the workday, the benefits of which have been examined by previous studies with conflicting results (Hoe, Urquhart et al. 2012). Similarly, the current study did not consider all available occupational exposure summary measures as each provides different information, and no single or standard set of measures is currently accepted as the gold standard (Van Eerd, Hogg-Johnson et al. 2012). Other methods such as Exposure Variation Analysis (EVA)(Mathiassen and Winkel 1991) may provide additional insight regarding temporal aspects (e.g. repetitiveness) and individual variability of physical workload (Srinivasan and Mathiassen 2012) not provided by the Active APDF method. Thus, the Active APDF is proposed as an independent measure of EMG amplitude during active muscle contraction, which is sensitive to individual differences in hand dominance and sex that can complement rather than replace existing methods of exposure analysis.

As for all studies of this type, it can be difficult to distinguish muscular rest from low-amplitude muscular activity (Veiersted, Forsman et al. 2013). The use of a 5% RVE
threshold for muscular rest in this investigation may be considered conservative, potentially resulting in the misinterpretation of low-amplitude muscle activity as muscular rest for some participants. This was a large field study, in which participants performed a multitude of tasks in many different worksites without direct surveillance of factors that can affect signal quality, and factors such as environmental electrical noise and movement artifacts varied widely between participants. Therefore, a conservative 5% RVE threshold was selected to insure that the rest threshold would only be breached by true increases in muscle activity. Baseline noise subtraction was also linear, instead of the power-wise subtraction method recommended by other studies (Hansson 2011). This difference in the subtraction process likely has a negligible effect on amplitude estimates above the 5% RVE threshold for rest, though future studies should consider utilizing the power-wise subtraction method for removal of baseline noise.

Normalization to a submaximal reference contraction does not permit direct comparisons of the amplitude of muscle activity to a physiologic maximum or to previous studies using different normalization methods. However, RVE normalization methods are reliable (Bao, Mathiassen et al. 1995, Delisle, Larivièrè et al. 2009) and have been recommended in previous studies (Hansson, Nordander et al. 2000). This method was chosen based on the results of a pilot study demonstrating greater reliability of submaximal compared to maximal voluntary exertions when performed within the constraints of the participants’ worksites. RVE normalization was also selected to facilitate comparison of the present results to future studies of patients with chronic neck pain, in which maximal effort is inhibited by pain and not considered a valid normalization method (Burden 2010).
Another limitation is that participants were not observed throughout the work day to synchronize periods of muscle activity with specific tasks, as has been done previously (Hansson, Nordander et al. 2000, Nordander, Hansson et al. 2000). This was done to minimize the influence of study observers on the behavior of participants to insure externally valid measurements. It is possible, however, that significant associations with self-reported activity in the workplace may exist for specific office tasks that are masked by more global measures of muscle activity across the full workday. Finally, the proportion of men in the present sample was low, although representative of the lower proportion of male office workers in the general population (Gjerdingen, McGovern et al. 2001).

Conclusions

This is the largest study to date describing upper trapezius motor patterns across the full workday for pain-free office workers in their natural work environment. A modification to the traditional APDF calculation was proposed in which periods of muscular rest are removed prior to calculating amplitude distribution percentiles for the active muscle. These analyses were both reliable and sensitive to differences in motor patterns associated with hand dominance and sex. Findings from the present study provide normative data that may be useful in identifying abnormal motor patterns that contribute to the persistence or recurrence of chronic neck pain in office workers.
References


