9-13-2019 1:30 PM

Investigating the simulated diving reflex in professional divers

Sydney O. Smith
The University of Western Ontario

Supervisor
Shoemaker, J Kevin
The University of Western Ontario

Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

Abstract

Background: Submergence underwater activates the diving response (DR) in both diving mammals and humans in order to preserve oxygen. Some evidence suggests that the DR can be trained in humans to provide a larger magnitude of bradycardia during submergence.

Hypothesis: Individuals with breath holding experience will have a greater magnitude of bradycardia during the DR than untrained, age-matched controls.

Methods: Participants performed three different protocols: 1) an apnea to volitional end point, or until 30 seconds of involuntary breathing movements were observed (APNEA), 2) one minute of cold pack to the forehead (COLD), and 3) one minute of cold pack contact on forehead during an apnea (COLD+APNEA).

Results: BHD had a larger magnitude of bradycardia during COLD+APNEA compared to controls (p=0.099).

Conclusions: The greater magnitude of bradycardia and MAP response in BHD shows that there is a physiological difference between trained and untrained individuals in breath hold diving.

Keywords

Breath hold diving; apnea; diving response; bradycardia; heart rate; total peripheral resistance; mean arterial blood pressure

Summary for Lay Audience

When mammals dive underwater the activate a reflex that allows them to maintain as much oxygen in their blood as possible. This reflex is called the diving response (DR). The characteristics of the DR are a reduction in heart rate (HR), an increase in blood pressure, and an increase in the blood vessels throughout the body constricting. In order to measure how extreme the DR is you measure how much HR decreases. Some researchers have found that some individuals can actually train the DR to be more extreme by practicing diving and breath holding.

This study investigated whether or not the DR can actually be trained by observing whether professional breath hold divers had a more extreme response than naïve individuals.

Professional breath hold divers (BHD) and naïve individuals who have never completed breath hold training participated in this study. They completed three different trials: 1) all participants held their breath for as long as possible; 2) an ice pack was placed on their forehead; 3) they held their breath for as long as possible while an ice pack was placed on their forehead.

This study found that BHD recovered their HR faster than naïve individuals. This means that their HR recovered to normal resting levels at a faster rate than naïve individuals after just a cold pack was placed on their forehead.

These results indicate that the BHD have developed some adaptation in recovery to cold exposure. Otherwise, there were no adaptations observed that related directly to the extremity of the DR.

Acknowledgments

To Dr. Kevin Shoemaker, thank you for your amazing support and guidance throughout this whole process. I would not be where I am today without your acceptance into your lab not only to complete my Master's but truly as part of a big family. Thank you for supporting my decisions to try out a different path in business. Your acceptance of my choices helped me to excel not only in my research but into another path. You are an inspiration to many, and I wish you continued success.

To my Serbia labmates. Emilie and Erin, thank you for pushing with me through the long hours in Serbia testing but also for emotional support through that busy time. You both were there to help when we all needed a shoulder to lean on.

Emilie and Jenna, I treasure our friendship and I thank you for your hard work throughout these two years because you pushed me to be the best I could be in my work. I will never forget the times we shared and the fun we had. I know there will be many more adventures in the future.

Baraa, I truly would not have made it through these two years without you. You are an amazing friend and mentor and I wish you all the best in your time at McMaster. I know you will inspire the lives of many others in your time there just as you did at Western.

To the Neurovascular Research Laboratory, I thank you. You all welcomed me into your lab and helped teach me the ways! The mentorship and learning that occurred for me are unmatched and I thank you all for your contributions to my success.

To Dr. Otto Barak, Enis, Nebojsha and other Serbian lab members, you made our journey to Serbia so amazing. We could not have completed our work without your constant support. Enis, we would not have been able to do what we did without your running around the streets of Novi Sad to retrieve things such as ice or power converters. Your commitment to our success made a difference in the outcomes of our projects.

To my Mother, Father, Brother, Grandparents, and all other family members, thank you for supporting me through all the good and bad times. Your love helped me push through times when I was questioning myself and my abilities. Thank you for accepting and loving me for me! I love you all and I know you will continue to support me through all the chapters in my life. I treasure how close our family is and I love you all for the supportive and accepting environment we have fostered.

To my roommates, Cadence and Steven, thank you for going through this process a year in advance. It helped me see the hard work it took to be successful. Thank you for supporting me through this process. I know you both will do great things and I wish you all the best.

To Western track and field, thank you for helping me reach my potential again. I never realized how such a large team can come together as one and become a family. I have never experienced such an amazing team atmosphere and I thank you for accepting me as you did when I came from another school. The coaching, team, and support helped me to finally reach my potential again and make me love the sport of track and field. I will never forget the amazing times with my sprint crew and the rest of the team.

To Dr. Derek Kimmerly and Dr. Scott Grandy, thank you for showing me physiology and helping me get to my Masters at Western. Both of you introduced me to the field and showed me research before I committed to a Master's degree. You inspired me to begin this chapter in my life and helped facilitate my acceptance.

To Pam Gray, I would not be the person I am today without your willingness to work with me through various life and career choices. You have been there for me no matter what the issue to support me and provide unparalleled guidance in all my endeavours.

To everyone else who took a part in the completion of this degree, I thank you. There were so many individuals who helped me through this process and I will never forget your contributions. I cannot believe this chapter of my life is now closing and I know I could not have done it on my own.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

ANS – Autonomic Nervous System
APNEA – apnea protocol
BHD – Breath hold divers
bpm – beats per minute
CNS – central nervous system
COLD – Cold protocol
COLD+APNEA – cold and apnea protocol
CVLM – caudal ventrolateral medulla
DR – Diving response
HR – Heart rate
HRV – Heart rate variability
IML – intermediolateral cell column
MAP – mean arterial pressure
NA – Nucleus Ambiguous
NTS – Nucleus tractus solitarius
PFC – Prefrontal cortex
PNS – parasympathetic nervous system
RVLM – rostral ventrolateral medulla
SNS – sympathetic nervous system
TPR – total peripheral resistance

Chapter 1

1 Introduction

Oxygen deprivation even for a short period of time can be detrimental to many species. Thus, animals have developed and adapted to be able to survive in such hypoxic conditions. The main adaptation found in animals to maintain consciousness and blood distribution during diving is the “diving response”. It is characterized by bradycardia, peripheral vasoconstriction and subsequent increase in mean arterial pressure. This response allows the animal to redistribute blood flow to areas such as the heart and brain and restriction of the peripheral vascular beds and vasculature in the digestive organs. Although these responses are reserved for mammals who dive frequently (e.g. seals, ducks etc.) humans also possess some similar mechanisms. The magnitude of the DR may be characterized by the magnitude of the bradycardia the mammal experiences.

The DR demonstrates the complicated balance of the two divisions of the autonomic nervous system. It is activated by simultaneous activation of the cold receptors of the face along with cessation of breathing, called apnea (Foster & Sheel, 2005). Submersion of the face activates a vagal response to reduce heart rate (HR) whereas apnea elicits a sympathetic response that causes an increase in blood pressure through peripheral vasoconstriction (Buchholz, Kelly, Bernatene, Diodati, & Gelpi, 2017). In humans peripheral resistance increases dramatically to raise blood pressure higher than normal physiological levels. Unlike humans, other mammals (e.g. seals, whales, dolphins) that have adapted to diving appear to maintain their BP within normal ranges despite a large increase in sympathetic tone (Buchholz et al., 2017). This dissertation

examined the autonomic and cardiovascular physiology of the DR in humans with particular focus on the adaptations that may occur in elite breath-hold divers.

Chapter 2

2 Literature Review

2.1 Autonomic Nervous System Characteristics and Divisions

The autonomic nervous system (ANS) can function without conscious awareness and is responsible for many visceral responses and the preservation of homeostasis in the human body. The ANS is connected with functions associated with the cardiorespiratory system, metabolism, and immune system (Wehrwein, Orer, & Barman, 2016). These functions are regulated through activation of various visceral sensors, which provide information to the ANS sections of the brain. In the ANS sections of the brain information is refined and specific motor responses are transferred throughout the body (Shields, 1993).

The ANS comprises of three primary divisions: the sympathetic nervous system (SNS), the parasympathetic nervous system (PNS) and the enteric nervous system (ENS) and has an influence on most functions in the human body. Specifically, this paper will focus on the SNS and PNS.

The SNS and PNS can function separately, together (synergistically) or against one another (antagonistically). The SNS is specifically known to cause “fight or flight” reactions such as, increasing heart rate, and mean arterial pressure during a stressful situation. Thus, it is thought that the SNS responds to threats, danger and other emergency situations.

The PNS is responsible for “rest and digest” reactions because of its role during rest of saving energy and encouraging resting metabolism. The PNS also plays an integral part in the normal movements of the eye, sphincter muscles and tear production. Although the PNS and SNS are generally thought in the simple terms of “fight or flight” and “rest and digest” they do allow for automatic actions that are outside those scopes of understanding.

2.2 Resting Physiological Variables

2.2.1 Resting Heart Rate

The dynamic interplay of both the sympathetic and parasympathetic nervous systems on the sinoatrial node in the heart are what determine heart rate in a given physiological state (Jose & Collison, 1970). Vagal tone is dominant over sympathetic tone in healthy individuals in a resting state, allowing heart rate to be lower compared to some states where sympathetic tone would be increased. This was shown by Jose and Collison, (1970) where they injected propranolol and atropine for autonomic blockade, which allowed them to measure intrinsic HR. Their results showed that intrinsic HR was higher than resting HR demonstrating that vagal tone influences resting HR.

2.2.2 Heart Rate Variability

Heart rate variability (HRV) is a measure of the variation in R-R intervals over a certain time period. R-R intervals are the measure of time between successive heart beats. This normal irregularity of heart rate can be used to characterize and measure the status of the ANS and is a valuable tool in investigating the interplay of both the SNS and PNS (Acharya, Joseph, Kannathal, Lim, & Suri, 2006).

HRV can be measured using several different parameters. HRV is quantified in the frequency domain and in the time domain through the use of signal processing techniques. For the purposes of this paper we will focus on time domain measures, soecifically, root mean square of successive differences (RMSSD).

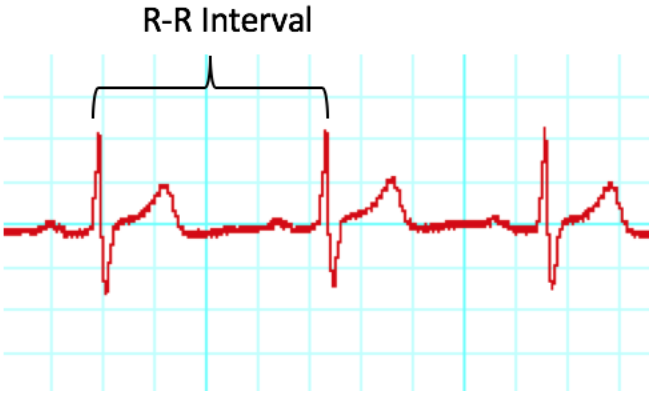


Figure 1: Depiction of how R-R intervals are calculated using an electrocardiogram (ECG) tracing. R-R intervals are calculated by measuring the time between subsequent R peaks.

RMSSD is a time domain measure that is calculated using the time difference between successive heartbeats. RMSSD can also be used in very short term bouts (10s) of recording, which makes it ideal for protocols with short condition durations (Munoz et al., 2015). It is the primary time domain analysis used to assess vagal function (McCraty & Shaffer, 2015). This means RMSSD is ideal to measure short term bouts of data while still being able to assess vagal tone. RMSSD is calculated using Equation 1.

$$RMSSD = \sqrt{\frac{1}{N-1}(\sum_{i-1}^{N-1}((R-R)_{i+1} - (R-R)_i)^2)}$$

Equation 1: Formula used to calculate RMSSD. N=number of RR intervals.

2.2.3 Respiratory Sinus Arrhythmia

The ANS plays an important role in the integration and connections between the respiratory and circulatory systems. One of the interactions seen between the respiratory and circulatory systems is respiratory sinus arrhythmia (RSA). This is when heart rate changes synchronously with an individual's breaths. R-R intervals are shortened during inspiration and increased during expiration. The cardiac vagal nerve activity is thought to be determined by respiratory rhythm therefore, heart rate is ultimately controlled by respiratory rhythm. It is thought that both the respiratory centres and circulatory centres of the brainstem control RSA (Yasuma & Hayano, 2004).

Two major physiological mechanisms have been recognized in developing RSA. The first is the control of cardiac vagal neurons by respiratory pattern and the second is the inhibition of cardiac vagal activity during lung expansion. During expiration the vagal efferent fibres are excited leading to bradycardia whereas, during inspiration inflation of the lungs occurs causing inhibition of the vagal efferent fibres. This then leads to tachycardia during inspiration due to almost complete abolishment of efferent cardiac vagal activity. To measure magnitude of RSA the difference in R-R interval from inspiration and expiration may be taken, which also can be a reflection of an individual's cardiac vagal function (Yasuma & Hayano, 2004).

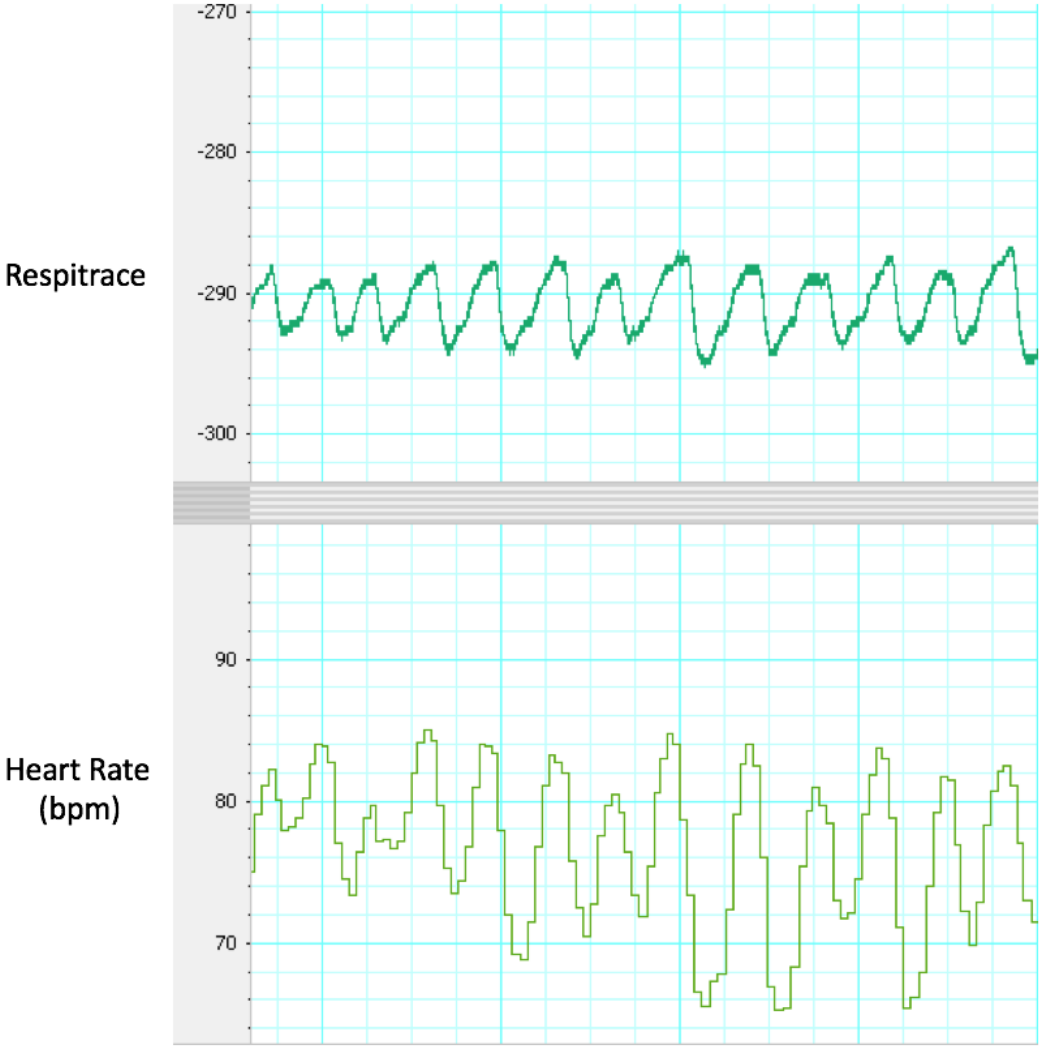


Figure 1: Example of RSA on strain gauge tracing (Respitrace) of breathing movements and calculated HR from ECG. Peaks on Respitrace show peak inspiration which match up in time with peaks in HR. Nadir values on Respitrace show peak of expiration. This participant was a control subject. Bpm, beats per minute; HR, heart rate; RSA, respiratory sinus arrhythmia.

2.3 Anatomy of the Autonomic Nervous System

Various neural sites contribute to ANS modulation, including higher brain areas such as the prefrontal cortex (PFC). The PFC has projections to the central nucleus of the amygdala and then onto the hypothalamus and midbrain which then project to the

medulla. Primary baroreceptor afferent fibres run through the glossopharyngeal and vagus nerves synapse onto the NTS. Neurons from the NTS project to the nucleus ambiguous and the caudal ventrolateral medulla (CVLM). Neurons from the CVLM then inhibit the rostral ventral lateral medulla (RVLM) that normally emits high levels of sympathetic nerve activity (Dampney, 2016). The NA is a source of vagal outflow to the heart. The cell bodies of both the SNS and the PNS originate in the spinal cord and the brain stem. Axons then leave through ventral roots and cranial nerves to synapse onto second order neurons which innervate smooth muscle, cardiac muscle, and other autonomic targets. Autonomic sensory fibres are the specific fibres which relay information to the brain stem and spinal cord and thus, the ANS centres. Some ANS organs and viscera are innervated by both the SNS and PNS and can be antagonistically activated (Shields, 1993).

2.3.1 Sympathetic Nervous System

Some of the cell bodies of the SNS are located in the intermediolateral (IML) cell column, of the spinal cord, between T1 and L2. There are two components to the efferent SNS. The first includes the neurons within the spinal cord and brain whereas the second involves the neurons projecting beyond the paravertebral ganglia. Distribution of postganglionic sympathetic axons exiting the spinal cord is organized in segments. T1 and T2 supply the face and neck, T2-T8 supply the upper extremities, T5-T10 the abdominal viscera, T11-L1 the colon up to the splenic flexure, and T6-L2 the adrenal glands (Shields, 1993).

2.3.2 Parasympathetic Nervous System

Some of the cell bodies of the PNS lie in specific cranial nerve nuclei and the IML cell column of the sacral spinal cord. Like the SNS, the PNS efferent system contains two different neurons, one in the CNS and one that projects from the ganglia to the organ. However, in the PNS case, the ganglia exist distant from the spinal cord and are normally in the organ being innervated. The cranial preganglionic nerve fibres of the PNS include: the oculomotor (III) nerve, the facial (VII) nerve, the glossopharyngeal (IX) nerve, and the vagus (X) nerve (Shields, 1993).

2.4 Diving Response

Lack of oxygen is detsrimental to all mammals and birds. Therefore, animals have developed various mechanisms to increase their survival capabilities to help budget the usage of oxygen during diving (Foster & Sheel, 2005). Together, these mechanisms form the "DR". The DR is characterized by bradycardia and an increase in total peripheral resistance (TPR). One prime example of the magnitude of the DR is seen in diving seals. Their baseline HR can start at 100 bpm and dramatically decrease to approximately 10 bpm immediately after submersion in water (Hurwitz & Furedy, 1986). This is viewed as beneficial due to the reduction in oxygen needed for cardiac metabolism.

Also, during the DR an impressive change in blood flow distribution is seen in many animals. Blood is redistributed to essential organs such as the brain and is restricted from the periphery and non-essential organs such as the kidneys (Hurwitz & Furedy, 1986).

Some evidence has also shown a change in splenic contraction volumes during a breath hold. Schagatay and colleagues (2012) has reported a correlation between breath hold duration and spleen size (Erika Schagatay, Richardson, & Lodin-Sundström, 2012). Also, trained apneists have been reported to have larger spleens (E. Schagatay, 2014). Splenic contraction is important in the context of diving because the spleen can act as a red blood cell reservoir that can contract and dispense red blood cells during hypoxia (Foster & Sheel, 2005).

The mechanisms that mediate the DR are known. Contact of the face and water excites the areas of the face supplied by the trigeminal nerve (TGN). Excitation of the TGN causes “inhibition and excitation of vasomotor centers and cardiac vagal motorneurones” in the brainstem (Foster & Sheel, 2005). However, some factors determine the magnitude of the DR. For example, temperature of the water during submergence has been shown to affect the magnitude of the DR. Schagatay and Holm (1996) found that an increase in air temperature generally increased the amount of bradycardia seen during facial immersion and an increase in water temperature saw a general decline in HR during immersion. This suggests that the change in temperature from room air to the water during submergence has an effect on the DR (Erika Schagatay & Holm, 1996).

2.4.1 Specific Cardiovascular Changes During the Diving Response

During the DR some specific physiological and cardiovascular changes occur. The dynamic interplay of both the sympathetic and vagal nervous systems cause changes in heart rate, blood pressure and peripheral resistance. More specifically, heart rate tends to

follow a pattern based on the length of the breath hold and response in general. During a prolonged breath hold in cold water, the HR of trained apneists may dip as low as 20-30 beats per minute. This reduction in HR allows the body to reduce oxygen consumption that can, in turn, lead to a longer duration maximal apnea (Bain, Drvis, Dujic, MacLeod, & Ainslie, 2018). The bradycardia response to apnea can be largely influence by the duration of the breath hold and the level of facial cooling (Bain et al., 2018).

Also, during a prolonged apnea, elevation of sympathetic nerve activity occurs. This then causes an increase in peripheral vasoconstriction leading to elevated TPR and MAP. These increases in blood pressure may also allow the baroreflex to cause reductions in HR (Bain et al., 2018).

2.5 Apnea Reflex

The DR is activated by the submergence underwater along with apnea. The latter portion of this response also has its own reflex, the apnea reflex. When an individual performs a static apnea without submergence underwater or facial cooling there is still a decrease in HR as reported by Perini et al., (2008). Also, an increase in both SBP and DBP was seen at the end of maximal dry static apnea. This increase is due to a corresponding increase in vasoconstriction of vascular beds elicited by the sympathetic response (Perini et al., 2008).

2.6 Overview of Reflexes

Reflexes are automatic responses generally concerned with protective and regulatory functions of the body. The simplest reflex pathways include, at the very least,

a receptor, an afferent neuron, synapses, an integrating centre or ganglion, an efferent neuron or effector organ (Hunyor, 1994).

2.6.1 Monosynaptic reflexes

Monosynaptic reflexes are the simplest of the reflex arcs and they contain one synapse. One example of a monosynaptic reflex is the stretch reflex (Hunyor, 1994).

2.6.2 Polysynaptic Reflexes

Polysynaptic reflexes are more complicated in that they contain multiple synapses and more extensive afferent and efferent pathways. These pathways contain various numbers of interneurons and synaptic connections. One example of a polysynaptic reflex is the flexor reflex, which is a protective reflex by withdrawing a limb away from a noxious stimulus by activating the flexor muscles of a limb (Hunyor, 1994).

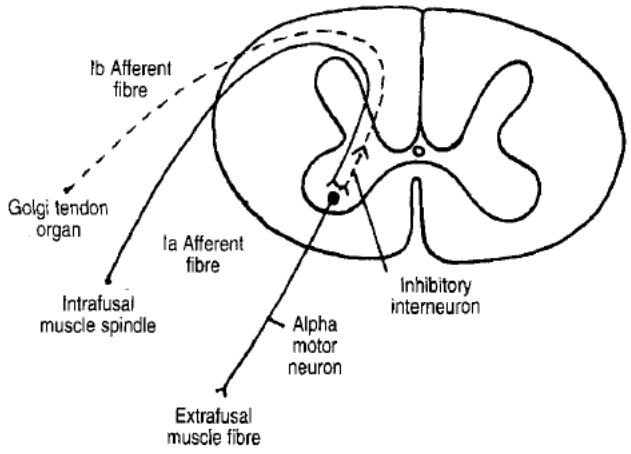


Figure 2: Schematic diagram of monosynaptic (solid line) and polysynaptic reflexes; used with permission (Hunyor, 1994).

2.6.3 Autonomic Reflexes

Autonomic reflex pathways occur at different levels. Some local reflexes relay through the spinal cord whereas, others relay through the brainstem and higher brain centres.

2.7 Trigeminal Nerve Reflexes

Part of the DR occurs due to activation of receptors in the TGN. Along with the DR there are other reflexes that occur by activation of receptors in the TGN.

2.7.1 Anatomy of the Trigeminal Nerve

The trigeminal nerve, also known as the fifth cranial nerve, is made up of three different branches: the ophthalmic (V1), the maxillary (V2), and the mandibular (V3). It is the largest cranial nerve and is primarily distributed throughout the suprahyoid neck. The ophthalmic and maxillary nerve are both sensory nerves whereas, the mandibular nerve contains both sensory and motor components. V1 is the smallest division of the TGN and V3 is the largest. All the divisions converge within Meckel's cave where they form the trigeminal (semilunar) ganglion. This ganglion contains the cell bodies of first order sensory neurons. In contrast, the first order motor neurons from V3 are located in the mesencephalic nucleus. The semilunar ganglion separates and continues through the prepontine cistern to the brainstem (Bathla & Hegde, 2013).

In the brainstem the divisions diverge to three different sensory nuclei: principal sensory nucleus located in the pontine tegmentum, the mesencephalic nucleus, and the spinal trigeminal nucleus (Bathla & Hegde, 2013).

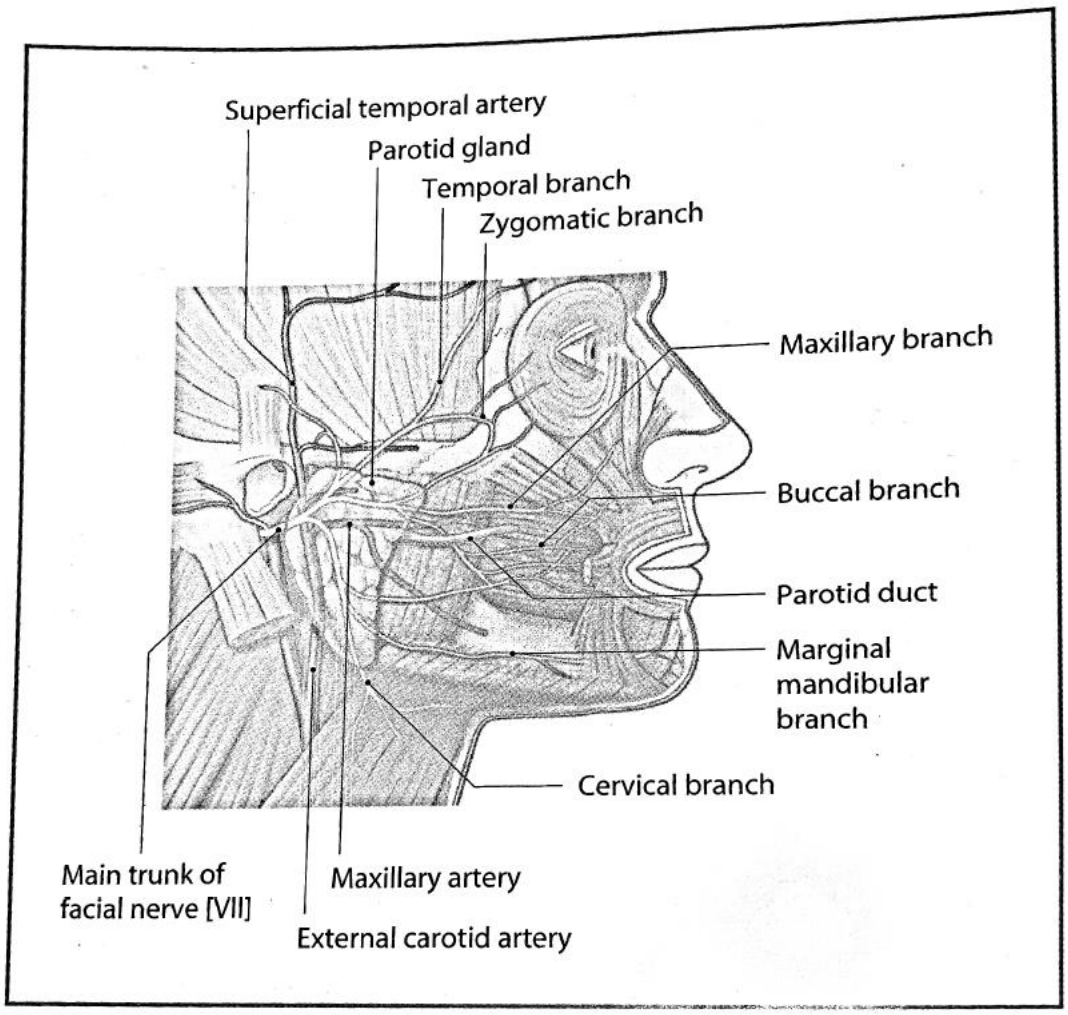


Figure 3: Schematic diagram of the trigeminal nerve and its branches; used with permission (Moses, Banks, Nava, & Petersen, 2005)

2.7.2 The Trigeminocardiac Reflex

The trigeminocardiac reflex (TCR) is a brainstem reflex known to cause the rapid onset of increase vagal activity. The onset of vagal activity is displayed by the occurrence of "cardiac dysrhythmia to asystole, arterial hypotension, apnea, and gastric hypermotility". The suggested theoretical explanation for this response is the activated sensory nerve endings of the TGN signal the sensory nucleus of the TGN through the Gasserian, or trigeminal, ganglion. This pathway is the afferent pathway of the trigeminal

reflex arc. The afferent pathway then extends through the internuncial nerve fibers in the reticular formation which then synapses on with the motor nucleus, of the vagus nerve, which starts the efferent pathway of the trigeminal reflex arc (Schaller et al., 2009).

The activation of the TCR is generally known to cause bradycardia. This bradycardia occurs via stimulation of the TGN's afferent receptors. These receptors can be from the main branches of the TGN or even the peripheral branches. Although the pathway of the nerves of the TCR has been studied extensively there is still no sufficient evidence to say exactly the pathway of this reflex. Some studies have tried blocking certain areas of the brain stem but there have been no conclusions made about the connections of the TGN and the brainstem autonomic system (Schaller et al., 2009).

2.7.3 Antagonistic and Synergistic Activation of Vagal and Sympathetic Outflow in Trigeminal Reflexes

Many of the trigeminal reflexes have similar characteristics but ultimately their outcomes are different. These different outcomes can occur from contrasting sympathetic and parasympathetic reactions. Specifically, the DR occurs through co-activation, or synergistic, outflow of both the vagal and sympathetic branches of the ANS. This activation occurs through stimulation of facial thermoreceptors and apnea. The competing branches of the ANS allow for simultaneous reduction in heart rate as controlled by the vagal system and maintenance of peripheral constriction and in turn, increase in mean arterial pressure (MAP), via sympathetic activation. Also, there is evidence that shows the DR also causes an increase in splenic contraction (Buchholz et al., 2017).

Two other trigeminal reflexes, nasopharyngeal and oculocardiac, occur from nasal mucosa irritation and physical arousal of the eye, respectively. Both these reflexes, like the DR, cause synergistic co-activation of the PNS and SNS (Buchholz et al., 2017).

Conversely, the TCR occurs through stronger vagal activation and sympathetic inhibition decreases in HR, peripheral resistance, MAP, and an increase in gastric motility. This activation occurs through apnea and the direct stimulation of the TGN (Buchholz et al., 2017).

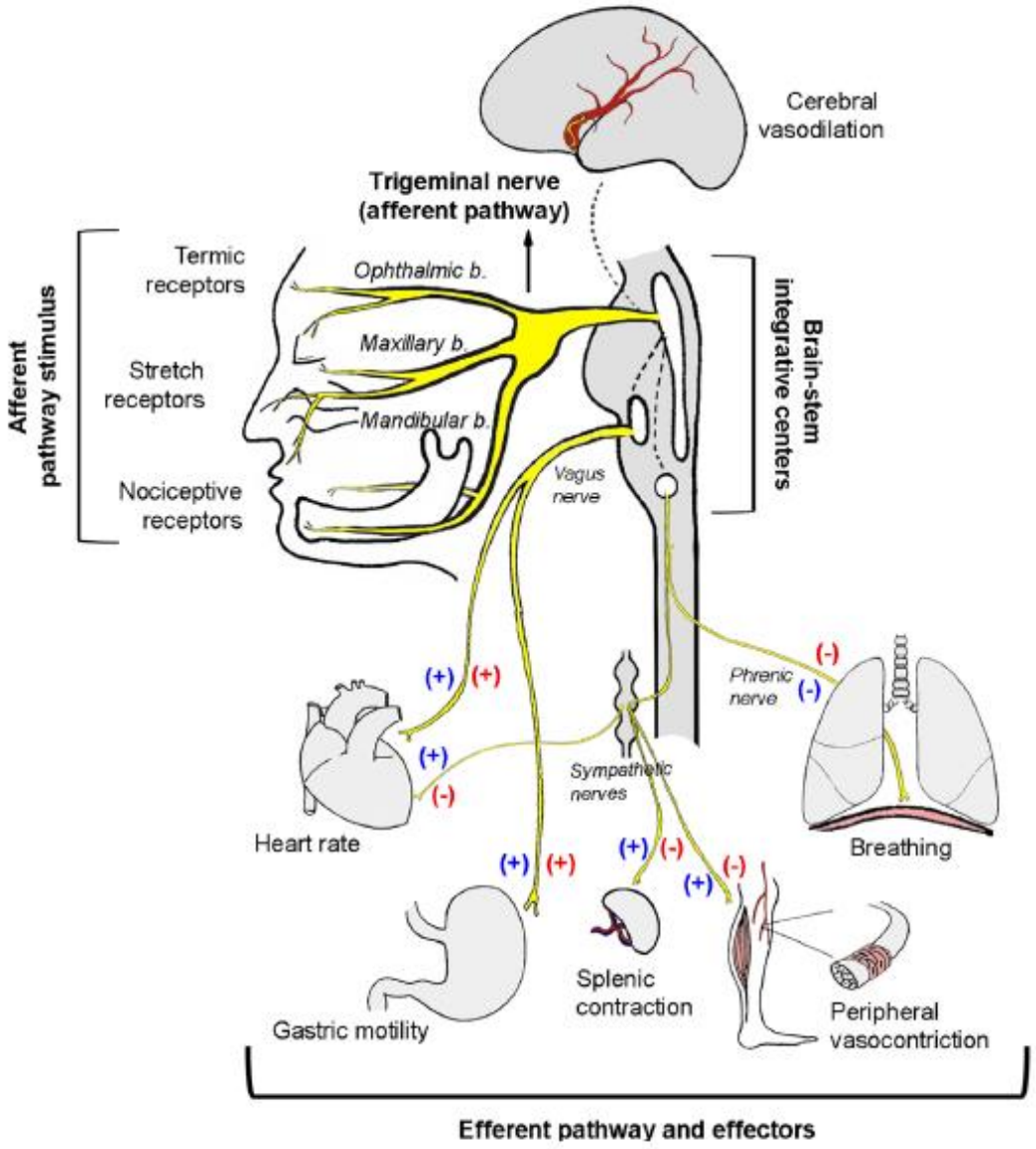


Figure 4: Schematic diagram of the pathways and effected organs activated by the trigeminal nerve; used with permission (Buchholz et al., 2017)

2.8 History of the Sport of Breath Holding

The act of breath hold diving can be traced back ~2000 years to when the Japanese Ama would dive to retrieve shellfish and edible seaweed. The Ama divers were generally women who used little equipment, which included merely glass facemasks or goggles.

They would perform repeated dives, continuously every day, of up to 80 feet requiring breath holds of up to two minutes (Hong & Rahn, 1967).

Although historically, breath hold diving has not been a source of competition but rather a source of income, recently in the past ~30 years apnea competition has become an organized sport. There are two governing bodies in the competitive sport of apnea diving, The Association Internationale pour le Développement de l'Apnée (AIDA) and the World Confederation of Underwater Activities (CMAS).

Competitive apnea diving can be divided into different disciplines based on breath hold time, distance or depth swam during breath hold. The two main streams of breath hold type are static, where the individual does not perform exercise during apnea and simply lies facedown in a body of water and dynamic, where the individual will swim either to a maximum depth of water or maximum distance of water. No matter the type of breath hold, the competition relies on the individual's ability to conserve oxygen during apnea. The sport also includes spearfishing, underwater hockey and rugby, synchronized swimming among others (Bain et al., 2018).

2.9 Apnea Overview

2.9.1 Phases of Apnea

During a breath hold the human body undergoes various physiological stresses, which cause discomfort. During the apnea respiratory signals cause the body to contract the breathing muscles involuntarily. These contractions are called involuntary breathing movements (IBM) (Bain et al., 2018).

There are two phases in breath holds. The first phase is coined the “easy going” phase, which is before the first IBM occurs. Then the time when the first IBM occurs is called the “physiological breaking point” where most untrained apneists will stop as they are unfamiliar with feeling and process of breath holds. Then, the phase after the “physiological breaking point” is called the “struggle” phase where apneists will experience IBMs until they reach a critical level of hypoxemia before losing consciousness (Bain et al., 2018).

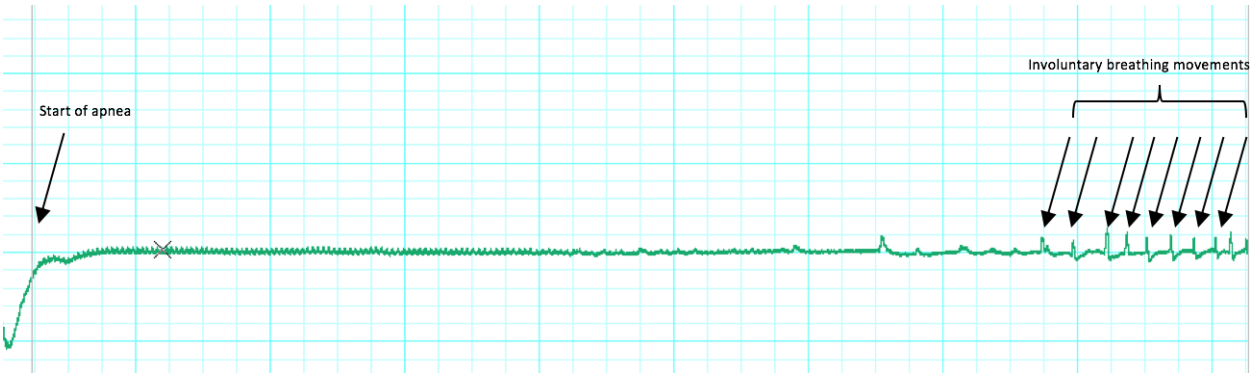


Figure 5: Diagram of strain gauge tracing around abdomen during an elite prolonged breath hold.

2.9.2 Apnea Training

To be an elite apneist and perform breath holds that are longer than 5 minutes requires a combination of physiological, psychological strengths and training as well as natural genetic talent. Some ways to increase apnea duration are to increase lung volume, thus increasing oxygen storing capabilities; cardiovascular adaptations; DR adaptations and chemoreflex adaptations (Bain et al., 2018). Specific training can help to increase these adaptations.

2.10 The Effect of Training on the Diving Response

The idea that trained apneists have a greater magnitude of DR is generally accepted, although there is some speculation whether this difference could be due largely to an increased duration of apnea. Evidence from Perini and colleagues (2008) shows that after the first 30 seconds of dry apnea there is a small decrease in HR but those who were able to sustain apnea for longer than 100 seconds saw an even greater decrease in HR (Perini et al., 2008). This shows that duration of apnea needs to be taken into consideration when comparing DR magnitude and changes in HR.

Although there has been some exploration of the effect that apneic training can have on the magnitude of the DR a true reflection of this relationship has yet to be determined. The closest any study has come to fully understanding the relationship between training and DR responses was done by Schagatay and colleagues (2000). After a two week training program, previously untrained individuals significantly increased the magnitude of their bradycardia during a simulated DR where apnea duration was also longer (Erika Schagatay, Van Kampen, Emanuelsson, & Holm, 2000). It remains uncertain whether the greater bradycardia response is due to an adaptation in the actual reflex or simply to a longer apnea. Although DR effects emerge after a short training program of two weeks, the question still arises as to whether there would be an effect of long-term apnea training on the magnitude of the reflex. Some cross sectional and case studies have attempted to determine the magnitude of the response in professional and lifetime diving populations. One such investigation by Schagatay and Andersson (1988) observed different populations, who are known to participate in breath hold training, and their heart rate reduction and apnea times. They found that only young divers had a more

pronounced DR compared to young controls and older divers and controls (Erika Schagatay & Andersson, 1998).

Also, one specific population that has been studied in regard to the DR are the Japanese Ama divers. This specific population of women has been diving for seaweed for over 1500 years. The demands of these dives require the women to perform 30-120 second dives multiple times per day (Hong & Rahn, 1967). Nonetheless, the divers have extreme abilities to hold their breath but evidence to support an extreme DR is scarce: According to Schagatay and Andersson (1988) there is a linear relationship between duration of maximal apnea and magnitude of bradycardia response.

This leaves the questions of whether apneic training for long periods of an individual's life can cause a greater DR.

2.11 Purposes and Hypotheses

The purpose of the current study was to investigate whether there is a training effect of the DR. This study tested the hypothesis that BHD will have a larger magnitude of DR than CTL as shown by BHD divers having lower HR and greater TPR and MAP during the DR.

Chapter 3

3 Methods

3.1 Participants

A total of 20 BHD (17 males, 3 females) and 20 controls (CTL; 15 males, 5 females) were included in this study. Of these, two participants were excluded: one BHD due to inability to maintain a sufficient Finometer signal and one CTL was excluded because he could not complete the cold protocols due to discomfort. Therefore, a total of 19 BHD and 19 CTL completed the study (Table 1). All participants provided informed written consent to the experimental procedures that were approved by the Health Science Research Ethics Board at Novi Sad University and Western University.

Table 1: Participant Characteristics

	Breath Hold Divers	Controls
Physical Characteristics		
n, males/females	19, 16/3	19, 14/5
Age (y)	30±9	31±9
Height (cm)	180±10	177±6
Mass (kg)	80±12	78±15
BMI (kg/m^2)	24.4±3.1	24.7±4.0
Clinical Characteristics		
Systolic BP (mmHg)	117±11	112±12
Diastolic BP (mmHg)	71±7	68±9

Y, years; cm, centimeters; kg, kilograms; BMI, body mass index; kg/m^2, kilograms per meter squared; BP, blood pressure; mmHg, millimeters of mercury

3.2 Experimental Protocol

The BHD were tested at the Faculty of Medicine at Novi Sad University, Novi Sad, Serbia. Evaluation of the CTL group occurred at the Laboratory for Brain and Heart Health at Western University, London, Ontario, Canada. Identical protocols were used in each site. Specifically, data were collected from each participant during a 10-minute baseline period (10 min-BL) and then three different protocols: 1) one functional residual capacity (FRC) apnea (APNEA) to volitional maximum or until 30 seconds of involuntary breathing movements (see Figure 8 above) were observed; 2) one FRC apnea with an ice pack held on the participant's forehead (APNEA+COLD); 3) and one trial where a cold pack was applied to the participant's forehead while the participant continued to breathe. In these protocols, the separate contributions of cold or apnea alone were studied. The DR was mimicked in the APNEA + COLD protocol.

Due to variations in the expected duration of apnea tolerance for each individual, particularly the BHD group, the APNEA protocol was always performed first to determine the durations and timing of the COLD and COLD+APNEA protocols. In the case the BHD could not continue the FRC apnea until 30 sec post IBM the duration of their APNEA was recorded and subsequent tests were standardized to one minute in order to observe cardiovascular changes associated with the DR (bradycardia, hypertension). For example if a BHD started experiencing IBMs after 35 seconds but could only continue their breath hold until 50 seconds then the APNEA stopped at that time. Then, their subsequent COLD and COLD+APNEA tests would be standardized to 1 minute. This meant that for COLD+APNEA they would start the condition with 10 seconds of just an ice pack on their forehead after which, we would instruct them to

complete their APNEA for 50 seconds. This would total 1 minute of testing for COLD+APNEA. The order of COLD and COLD+APNEA were randomized.

3.2.1 Protocol 1 – APNEA

Participants were instructed to perform an APNEA by taking a normal tidal inhalation, normal tidal exhalation, and then at the end of the exhale to start their breath hold. Participants were instructed to hold their breath until 30 seconds after the first IBM was observed (BHD) or until their volitional maximum (CTL). The total time of the APNEA was recorded and then used for subsequent protocols. In the case that IBMs were not observed, or the participant could not hold their breath for 30 seconds after the first IBM, the participant was instructed to start breathing when they could no longer perform the apnea (volitional maximum). Participants then rested until heart rate and blood pressure returned to resting values before the subsequent trial.

3.2.2 Protocol 1 – COLD

Participants laid still while an ice pack was placed on their forehead. This ice pack was made of a mixture of ethanol and water mixture to form a slurry that would mold to the participant's forehead contours. The ice pack remained on the participant's forehead for the duration of the apnea that was determined during the APNEA protocol. Participants were instructed to lie still and quiet while ice was applied. The COLD protocol was included as a control to determine the separate effect of COLD on the trigeminal nerve.

3.2.3 Protocol 1 – COLD + APNEA

Participants were instructed to perform an APNEA the same way that was performed during the normal APNEA protocol. When the participant started the breath hold the ice pack was placed on the participant's forehead. The ice pack remained on the forehead for the duration of the APNEA and they were instructed to hold the apnea for the same amount of time as the first APNEA protocol.

3.3 Physiological Recordings

Three adhesive electrodes were placed on the participant's chest (one just below each collar bone and one just below the left 12^{th} intercostal) to record the electrocardiogram (lead 2). ECG was sampled at 1000Hz. An automated sphygmomanometer was used to perform 3 resting blood pressure measurements. Blood pressure was also measured continuously throughout all protocols using ADInstruments Human NIBP Nano system at University of Novi Sad, and Finometer at Western University. A respiratory belt transducer (ADInstruments, MLT1132 Piezo Respiratory Belt Transducer, Colorado Springs, CO, USA) placed around each participants waist was used to record breathing movements. All measurements were recorded and stored for later analysis (PowerLab, ADInstruments, Colorado Springs, CO).

3.4 Physiological Data Analysis

An illustration of data from a representative participant is provided in Figure 9. Baseline data were averaged over 10 min at the beginning of each subject's test session. Subsequently, HR, MAP, and TPR were averaged for 30 seconds of baseline that occurred prior to each protocol (Baseline), for 10 seconds at the end of each protocol

(average during), and 30 seconds after the protocol was complete (recovery). The peak bradycardia, MAP, and TPR during each protocol were also recorded (peak during).

Heart rate variability (HRV) was used as a measure of vagal influence over heart rate. The root mean squared standard deviation (RMSSD) approach was used which provides a reliable approach to reporting short-term data samples (Shaffer & Ginsberg, 2017). Thirty second epochs of RMSSD were recorded for baseline and recovery measures for each protocol. Twenty second epochs of RMSSD were recorded during the end of each protocol. The change in HR, RMSSD, MAP and TPR (ΔHR, ΔRMSSD, ΔMAP, ΔTPR) values from baseline to the peak response were calculated.

For all participants, HR, MAP, and TPR were averaged for 10 min-BL. Cardiac output was calculated using the ModelFlow calculation(Sugawara et al., 2003). TPR was calculated in LabChart using the formula, TPR=MAP/(SVxHR) (Dyson, Shoemaker, Arbeille, & Hughson, 2010). In addition to the time domain measures of heart rate (bpm), frequency domain measurements of HRV were also calculated. This approach quantified spectral power at the low frequency (LF; 0.04-0.15 Hz), high frequency (HF; 0.15-0.40 Hz) and the LF/HF ratio using a Fast Fourier analysis model within ADInstruments post-processing programs (Shaffer & Ginsberg, 2017).

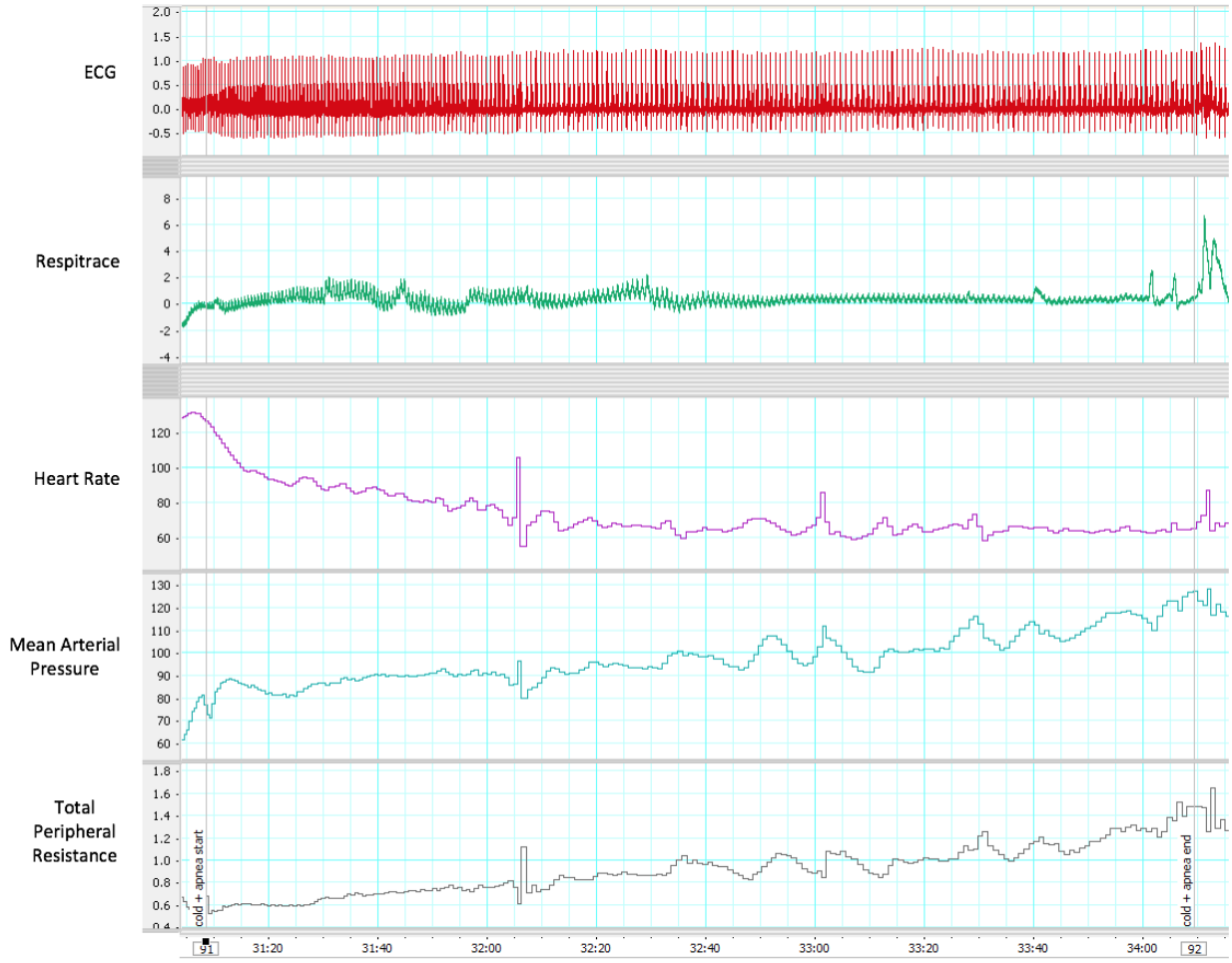


Figure 6: Depiction of ECG, Respitrace, HR, MAP, and TPR tracings during one APNEA+COLD protocol.

3.5 Statistical Analyses

Two-tailed unpaired t-tests were used to assess the difference between BHD and CTL during 10 min-BL period for HR, HRV [(RMSSD, LF(nu), HF(nu)], MAP, and TPR. Significance level was set at 0.05. Two-tailed unpaired t-tests were also used to assess the difference in apnea duration between BHD and CTL.

A two-way mixed analysis of variance (ANOVA) was used to determine two-way interactions for ΔHR, ΔRMSSD, ΔMAP, and ΔTPR between all three conditions (COLD, COLD+APNEA, and APNEA). When there was a significant one tailed group*condition interaction ($p<0.1$) separate one-way ANOVA's were run to determine if there were any simple main effects present.

A three-way mixed model ANOVA was used to determine if there was a condition*time*group interaction for HR for all three conditions (COLD+APNEA, COLD, and APNEA) and all three time periods (baseline, during, and recovery). The directional hypothesis justified the significance level to be set at $p<0.1$.

Chapter 4

4 Results

4.1 Physiological Results

4.1.1 Baseline Results

Average baseline HR; HRV, RMSSD, LF (nu) and HF (nu); MAP, and TPR did not differ between Control and BHD groups (Table 2; $p>0.05$).

Table 2: Resting Values of HR, MAP, TPR, RMSSD, LF, HF

	Diver	Control	p-value
HR (bpm)	62±9	64±9	0.30
MAP (mmHg)	86±10	84±7	0.45
TPR (mmHg*min/L)	13±3	13±4	0.92
RMSSD (ms)	71±48	71±59	0.10
CO (L/min)	7±3	6±2	0.66

Values are Mean ± SD. HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; mmHg, millimeters of mercury; TPR, total peripheral resistance; min, minutes; L, liters; RMSSD, root mean square of successive differences; ms, milliseconds; LF, low frequency; HF, high frequency.

4.1.2 Heart Rate

A three-way mixed model ANOVA determined that there was a condition*time*group interaction for HR for all three conditions (COLD+APNEA, COLD, and APNEA) and all three time periods (baseline, during, and recovery; $p=0.004$). Further analysis determined there was a time*group interaction the COLD

condition where the HR of the divers (HR=63±7 bpm) during the recovery period was significantly higher than that of the controls (HR=49±11; $p<0.001$).

A two-way mixed model ANOVA determined there was a condition*group interaction for ΔHR ($p=0.08$). Further one-way ANOVA's showed divers had a larger magnitude of bradycardia during COLD+APNEA compared to controls ($p=0.099$). There were no differences between divers and controls ΔHR observed during COLD or APNEA (Fig 10). All HR data was normally distributed as determined by a Shapiro-Wilk test ($p>0.05$). Mauchly's test of sphericity was also not significant ($p>0.05$).

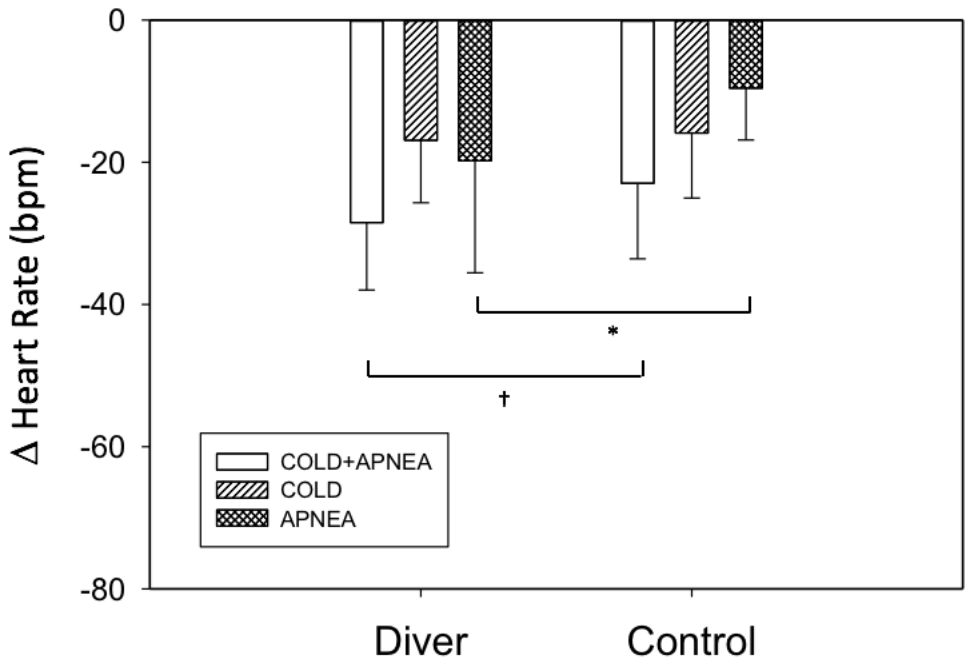


Figure 7: ΔHeart Rate values for all three conditions, BHD and CTL. Condition*Group interaction=0.08; * = p < 0.05, † = p < 0.1; bpm=beats per minute

4.1.3 Heart Rate Variability

A three-way mixed model ANOVA determined there was no condition*time*group interaction for RMSSD for all three conditions (COLD+APNEA, COLD, and APNEA) and all three time periods (baseline, during, and recovery; $p>0.1$).

A two-way mixed model ANOVA determined there was no condition*group interaction found for ΔRMSSD for all three conditions ($p>0.1$; Fig 11).

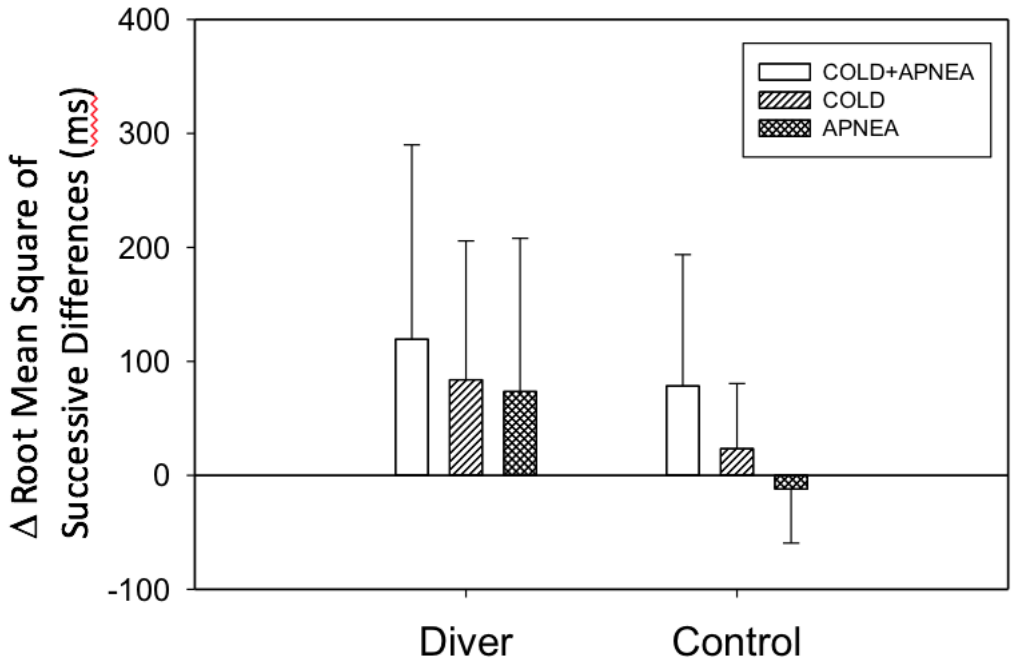


Figure 8: ΔRoot mean square of successive differences values for all three conditions, BHD and CTL. Condition*Group interaction= 0.71; ms=milliseconds

4.1.4 Mean Arterial Pressure (MAP)

A three-way mixed model ANOVA determined there was a condition*time*group interaction for MAP for all three conditions (COLD+APNEA, COLD, and APNEA) and all three time periods (baseline, during, and recovery; $p<0.001$). Further analysis showed

a time*group interaction during the APNEA condition where divers had a higher MAP during (122±19 mmHg) than controls (106±19 mmHg; $p<0.001$).

A two-way mixed model ANOVA determined there was a condition*group interaction found for ΔMAP for all three conditions ($p=0.001$). Further one-way ANOVAs showed divers had a larger magnitude of ΔMAP during APNEA compared to controls ($p<0.001$). There were no differences between divers and controls ΔMAP observed during COLD+APNEA or COLD (Fig 12). All MAP data was normally distributed as determined by a Shapiro-Wilk test ($p>0.05$). Mauchly's test of sphericity was also not significant for all conditions ($p>0.05$) except for time ($p<0.000$). This is acceptable due to the fact that ANOVA's are robust and are not significantly affected by data that violated the sphericity assumption (Kirk, 2005).

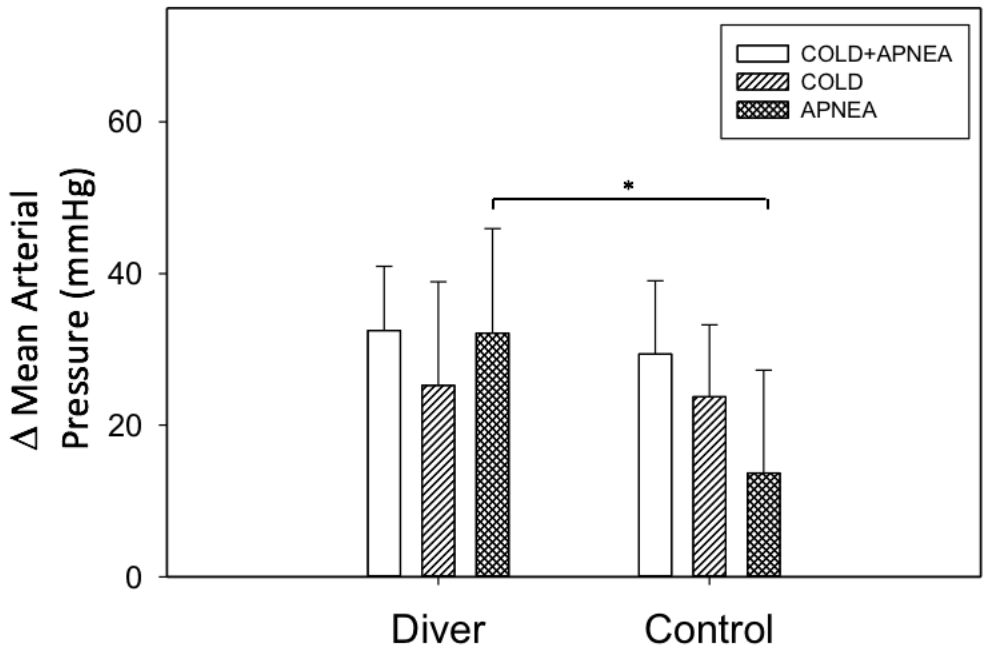


Figure 9: ΔMean Arterial Pressure values for all three conditions, BHD and CTL. Condition*Group interaction= 0.001; * = p < 0.05; mmHg=millimeters of mercury

4.1.5 Total Peripheral Resistance

A three-way mixed model ANOVA determined there was no condition*time*group interaction for TPR for all three conditions (COLD+APNEA, COLD, and APNEA) and all three time periods (baseline, during, and recovery; $p>0.1$).

A two-way mixed model ANOVA determined there was no condition*group interaction found for ΔTPR for all three conditions ($p>0.1$; Fig 13). There was a time*group interaction for the apnea condition ($p<0.05$; Fig. 13)

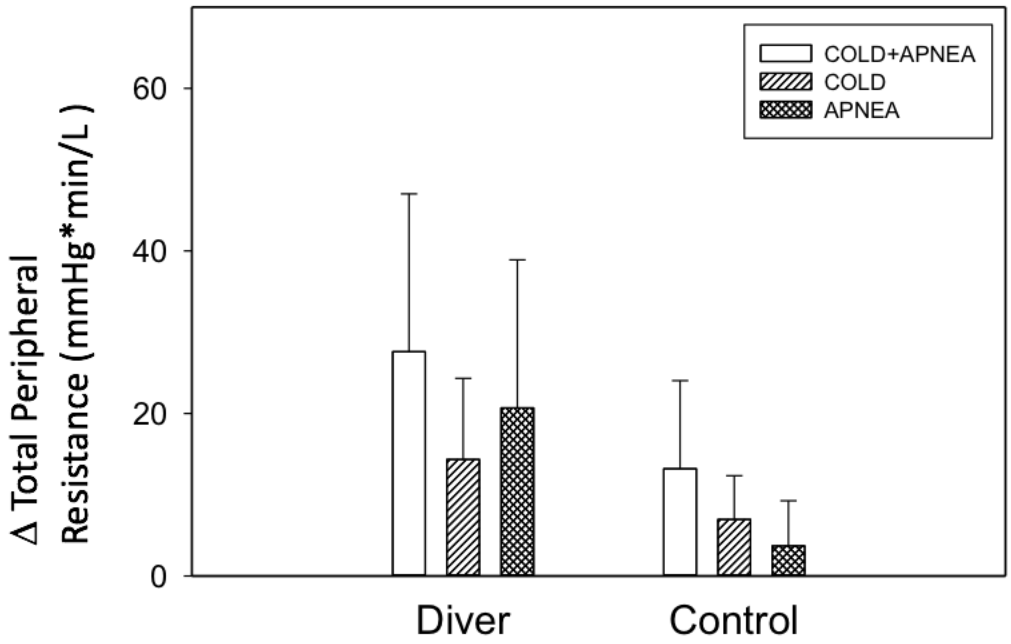


Figure 10: ΔTotal Peripheral Resistance values for all three conditions, BHD and CTL. Condition*Group interaction= 0.13; mmHg=millimeters of mercury; min=minutes; L=liters.

4.1.6 Cardiac Output

Table 3: ΔCardiac Output (L/min) Values for divers and controls during all 3 conditions

	Diver	Control
COLD+APNEA	2.2±2.2	3.6±3.5
COLD	2.7±2.5	6.0±4.9
APNEA	2.4±1.4	1.4±7.8

Cardiac output data for all three conditions is displayed in Table 3.

4.1.7 Duration of Apnea

A one-tailed, paired t-test showed BHD (80 ± 43 seconds) had longer duration apneas compared to CTL (30 ± 10 seconds; $p<0.001$).

4.1.8 Heart Rate at Apnea Duration Match

It is possible that any differences between BHD and CTL groups was due simply to the fact that BHD can hold their breath longer than CTL. To adjust for variations in the ability in apnea duration between BHD and CTL each BHD and CTL were matched based on duration of apnea performed by the CTL. Specifically, in matched pairs the HR at the end of an apnea performed by a CTL participant provided the time at which the HR was also derived from the BHD's performance. A scatterplot of the ΔHR data of BHD and CTL at the matched CTL's break point time are plotted in (Fig. 14).

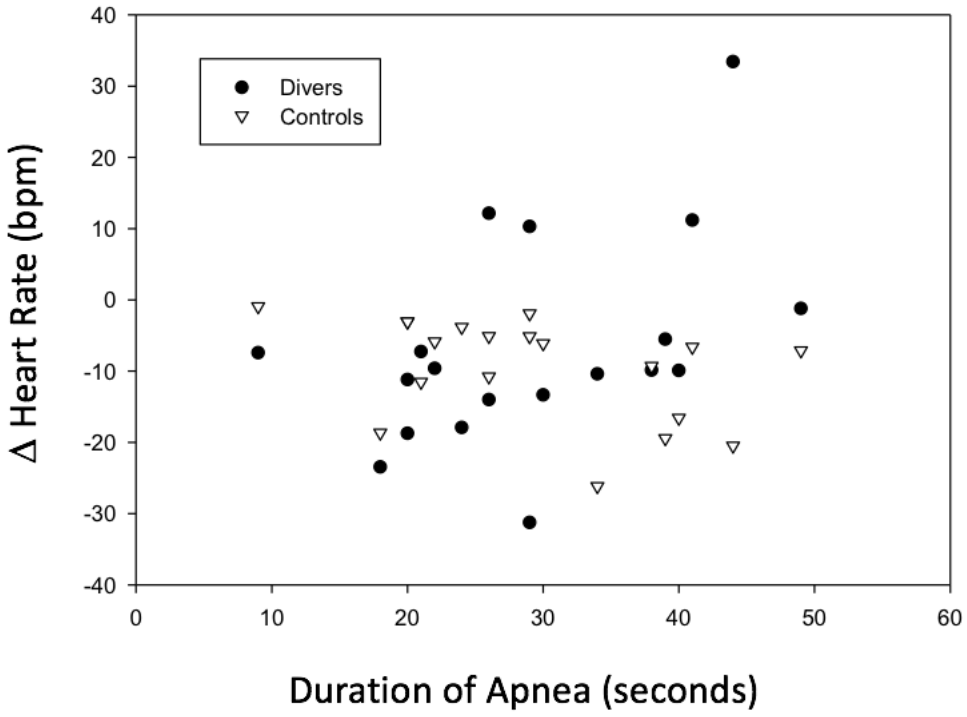


Figure 11: Depiction of ΔHeart Rate of BHD and CTL at the same time each CTL completed their apnea. Relationship between ΔHR and duration of apnea for controls is y=-0.3603x+1.1796 with an R^2 value of 0.2082. Relationship between ΔHR and duration of apnea for BHD is y=0.6585x-25.293 with an R^2 value of 0.2028.

Chapter 5

5 Discussion

This study demonstrated four important findings. First, divers had a larger magnitude of bradycardia during COLD+APNEA compared to controls. Second, divers had higher heart rate on average during the recovery portion of the COLD condition. Third, divers had a higher MAP during the APNEA+COLD protocol than controls. Last, divers had a larger ΔMAP during APNEA than the controls. These data suggest that divers have developed some adaptations through apneic training that untrained naïve individuals would not possess. However, when BHD were studied at the same duration of apnea as the CTLs, the differences between groups was no longer present. Thus, apnea duration appears to determine the magnitude of bradycardia rather than adaptations from training. The first major observation above supports the hypothesis that divers have a larger magnitude of DR.

5.1 Heart Rate

Some results from the current study are not in accordance with those of Schagatay and Andersson (1998) where the experienced breath holders had a greater reduction in HR during the simulated DR compared to untrained controls. In contrast to Schagatay and Andersson (1998) our results did not show a difference between BHD and CTL during a simulated DR, but rather showed that BHD had higher HR during recovery of COLD. This suggests that BHD have developed the ability to recover faster after a COLD stimulus. Although this COLD stimulus was used as a control to separate the competing portions of the DR some unique results were observed. After multiple

literature searches we determined that there is no similar research investigating the HR responses during the recovery period of a DR.

One possible adaptation that the BHD could have developed is the augmented activity of the enzyme acetylcholine (ACh) esterase. The activity of this enzyme is the main mechanism for removing ACh, which is a neurotransmitter released during vagal activity. Thus, by having augmented ability of ACh esterase or a greater number of these enzymes would allow divers to recover faster from vagal activity during the COLD protocol. More investigation is necessary to determine the how this mechanism differs between the full simulated DR and just the COLD stimulation (Gorini, Philbin, Bateman, & Mendelowitz, 2010).

Another possible adaptation that the BHD could have developed is modified brain stem trigeminally evoked neurotransmission to cardiac vagal neurons. It is possible that the inhibition of the vagal efferent pathway could be faster in the divers (Michael Panneton, 2013).

One uncertainty with the current observations that BHD exhibit a greater bradycardia is the relationship between duration of a breath hold and the ensuing reduction in heart rate. Specifically, our results indicated that BHD had a significantly longer breath hold than CTL, a finding that is in accordance with Schagatay and Andersson (1998). Further, the magnitude of the DR can be affected by the length of the apnea. Perini et al., (2008) found three phases of cardiovascular changes occur during an apnea based on the duration. The first phase usually lasts approximately 30 seconds during which HR is reduced. In phase 2 HR remained unchanged for approximately 150

seconds. However, in the third phase more bradycardia occurs. In the current study, the BHD breath holds during the APNEA+COLD were one minute or longer. However, the apneas performed by the CTL group in this protocol were only 30 seconds, corresponding to the first phase. This means the bradycardia exhibited by the CTL participants may have been limited by the shorter apnea duration. Nonetheless, each group performed the apneas to their own personal physiological end point. In secondary analysis, the bradycardia for the BHD group were not different from CTL when obtained at the same absolute time.

Another important factor to note in this bradycardic response is the possible role of the baroreflex. The baroreflex responds to increased MAP, which was also observed during the simulated DR. When MAP is increased the baroreflex relays signals throughout the brainstem to ultimately increase vagal output and decrease sympathetic output, leading to bradycardia (Dampney, 2016). To determine whether this occurs in the simulated DR more investigation would have to observe the time course where bradycardia starts occurring in the DR. Further studies should investigate baroreflex function in this specific population to determine its role in the DR.

5.2 Heart Rate Variability

In the current study we observed no between-group differences in RMSSD across the APNEA, COLD, and COLD+APNEA protocols. This contradicts the previous research reported by Lemaître, Buchheit, Joulia, Fontanari, & Tourny-Chollet (2008), who described an increase in RMSSD in BHD during static apnea. Their research also showed a correlation between increase in RMSSD and years of practice with apnea training. Although the present study measured RMSSD during apnea, there were some

differences in methodology that could also explain the discrepancies in results. In the present study participants were lying supine for all tests, with an ice pack on the forehead whereas Lemaître et al. (2008) had participants lying prone with their whole face immersed in water. This could cause changes in results due to during full face submersion all areas of the trigeminal branches are activated whereas, the ice pack will only cover some parts of the trigeminal branches (Vybiral, Bryg, Maddens, Michael, & Boden, William, 1989). Participants in the previous study also performed apneas while face down in water whereas the current participants simulated the DR with a cold pack on their forehead. Although the cold pack method is commonly used to provide simulation of the DR, there may be variations in the temperature of the water/cold pack (Al-Ani, Powell, West, Townend, & Coote, 1995; Chapleau & Sabharwal, 2011; Fisher et al., 2015). The temperature of the water or surface that is activating the trigeminal nerve also plays a role in the magnitude of DR (Gooden, 1992). If there is no standardization between studies with the temperature of the ice pack or water then there may be discrepancies in HRV data due to this difference.

5.3 Mean Arterial Pressure

Our study showed that BHD divers had a higher MAP during the APNEA than the CTL. This differs from the results of Schagatay, Van Kampen, Emanuelsson, & Holm, (2000) where individuals completed apnea training for 2 weeks. After the subjects finished apnea training, their MAP response during a simulated dive increased compared to their untrained values. In the current study, we did not see any difference between controls and BHD during the simulated DR (APNEA+COLD). Similarly, Engan, Richardson, Lodin-Sundström, van Beekvelt, & Schagatay, (2013) found a similar MAP

response to apnea alone (no DR test) after two weeks of apnea training in naïve individuals.

The discrepancies in results between our study and that of Engan et al., (2013) and Schagatay et al., (2000) could be explained by some differences in study design and methodology. Both of the previous studies used a longitudinal design which compared the same subjects before and after a period of training, whereas our study compared two different populations with varying levels of apnea training. Despite these differences, the increase in MAP that occurs during the DR is prominent, it is generally thought not to play a role in the conservation of oxygen. Due to this fact, there is minimal past research on the changes in MAP during the DR in untrained individuals and trained divers.

5.4 Total Peripheral Resistance

In the context of severe bradycardia during the DR, the rise in blood pressure must be due to a potent vasoconstrictor responses. In fact, the TPR response might be considered to be a critical element of the response as it performs a critical role in the large increase in blood flow to the brain in divers (Willie et al., 2015). In the current study, such an increase in TPR was observed but this response was not different across groups or between the three conditions despite a greater bradycardia in the BHD. As far as we know, this is the first study to investigate TPR during the APNEA+COLD response. For example, Schagatay et al., (2000) found two weeks of apnea training did not change the amount of skin capillary blood flow found during the DR. The authors indicate that constant blood flow coupled with the increase in MAP that was observed implies there was an increase in TPR after the two weeks of apnea training.

The current study is novel, in that it is the first study to investigate how TPR is affected after years of apnea training. Further, we know that skin vascular control contributes little to overall TPR. Therefore, while it is difficult to make direct comparisons from skin capillary blood flow to TPR, we believe the current study to incorporate systemic vasoconstrictor responses to DR in controls or trained divers. Based on our data there does not appear to be any training effect of apnea training on TPR.

It is also imperative to understand that TPR is calculated based on other variables determined by the Finometer. This device uses information regarding the height, weight and age of the subject to calculate stroke volume based on assumptions related that aortic compliance is the same for people of similar age, weight and age. Therefore, the calculations may not be completely accurate. These inaccuracies could be the cause of discrepancies between the current study and past studies, which use other techniques to calculate TPR. Although, BHD have a higher MAP than CTL during apnea, with no difference in HR, there appears to be some change in vascular resistance in the body.

5.5 Perspectives

The greater bradycardia and MAP response seen in the BHD shows that there is a physiological difference related to the DR between BHD and CTL. This difference is important because it allows the BHD to 1) perform longer duration apneas; and 2) allows them to push their body past the “physiological breaking point”. The bradycardia and increase in TPR that are seen during that DR are what causes the changes in blood flow. Vasoconstriction that occurs in the periphery allows oxygen to be distributed towards the brain whereas, bradycardia allows a reduction in myocardial oxygen usage. The ability of the BHD to push their bodies to these points come from a greater ability to perfuse

oxygen away from organs such as, skin, skeletal muscle, spleen, heart and lungs (Bain et al., 2018) allowing increased blood flow towards the brain.

5.6 Assumptions and Limitations

There were a few limitations in this study. To truly understand the trainability of the DR we would need to have a different study design that was longitudinal, instead of the current cross-sectional design. This would allow us to observe the same participants as they completed apnea training over a number of years, and we could see how this affected their cardiovascular variables. Another limitation was the differences in ability of BHD compared to CTL in duration of apnea as well as the ability of the BHD to complete the apnea past the "physiological breaking point". This can cause differences in the results because the bradycardia and MAP outcomes will be greater with longer apnea (Perini et al., 2008). To try to combat this discrepancy, comparisons were made of the BHD's cardiovascular variables at the same time points as CTL's were completing their apneas. This allowed us to investigate whether duration did have an effect on their results.

Another limitation of this study was some of the BHD had not completed apnea training for a number of years. It is possible that due to their lack of recent training, their bodies no longer had the adaptations from the intense training they used to participate in. This could cause their cardiovascular changes to not have the same magnitude as they did when they were training.

One assumption of the current study is the calculations of certain values, such as cardiac output and with it, TPR, from the Finometer. An assumption of cardiac output is

based on the height, weight and age of the individual (Bogert & Van Lieshout, 2005). With this assumption we were able to estimate continuous MAP, SBP, DBP and TPR. Although this calculation has been validated for young healthy population it has not been directly validated for this specific population of divers and those performing apneas. Although this is a different population of individuals performing unique maneuvers there does not appear to be a large discrepancy in the TPR calculations.

5.7 Conclusions

This study investigated the trainability of the DR by measuring HR, HRV, MAP and TPR in BHD and naïve untrained age-matched CTL. It demonstrated that there might be a training effect from apnea training on the DR but only for some portions of the DR. For example, when just COLD was applied to subject's foreheads, BHD were able to recover faster after the stimulus compared to CTL. Also, during APNEA, BHD had higher MAP than CTL. This could partially be due to the longer duration of the APNEA that the BHD were able to complete compared to the CTL. Also, it could be due to apnea training causing a more pronounced DR and in turn, greater peripheral constriction as indicated by the TPR response. As few studies have explored the DR in elite breath hold divers, the current data need to be replicated. Nonetheless, although cross-sectional in design, the current observations support the overall hypothesis that BHD improves the DR with greater bradycardia and TPR. In the absence of previous studies in the area of DR trainability, additional data to replicate the current study and, more importantly, a prospective BHD training study is required to study directly the trainability of this reflex. Future research should focus on more studies to determine if this reflex, specifically the magnitude of bradycardia, can be increased through apnea or some other kind of training.

This would allow professional diving athletes to maximize their training ability and to train in more efficient manners.

References

Acharya, U. R., Joseph, K. P., Kannathal, N., Lim, C. M., & Suri, J. S. (2006). Heart rate variability: A review. *Medical and Biological Engineering and Computing*, *44*(12),

1031–1051. https://doi.org/10.1007/s11517-006-0119-0

Al-Ani, M., Powell, L., West, J., Townend, J., & Coote, J. H. (1995). Exercise and diving, two conflicting stimuli influencing cardiac vagal tone in man. *The Journal of Physiology*, *489*(2), 603–612. https://doi.org/10.1113/jphysiol.1995.sp021076

Bain, A. R., Drvis, I., Dujic, Z., MacLeod, D. B., & Ainslie, P. N. (2018). Physiology of static breath holding in elite apneists. *Experimental Physiology*, *103*(5), 635–651. https://doi.org/10.1113/EP086269

Bathla, G., & Hegde, A. N. (2013). The trigeminal nerve: An illustrated review of its imaging anatomy and pathology. *Clinical Radiology*, *68*(2), 203–213. https://doi.org/10.1016/j.crad.2012.05.019

Bogert, L. W. J., & Van Lieshout, J. J. (2005). Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Experimental Physiology*, *90*(4), 437–446. https://doi.org/10.1113/expphysiol.2005.030262

Buchholz, B., Kelly, J., Bernatene, E. A., Diodati, N. M., & Gelpi, R. J. (2017). Antagonistic and synergistic activation of cardiovascular vagal and sympathetic motor outflows in trigeminal reflexes. *Frontiers in Neurology*, *8*(FEB), 1–6. https://doi.org/10.3389/fneur.2017.00052

Chapleau, M. W., & Sabharwal, R. (2011). Methods of assessing vagus nerve activity and reflexes. *Heart Failure Reviews*, *16*(2), 109–127. https://doi.org/10.1007/s10741-010-9174-6

Dampney, R. A. L. (2016). Central neural control of the cardiovascular system: current perspectives. *Advances in Physiology Education*, *40*(3), 283–296. https://doi.org/10.1152/advan.00027.2016

Dyson, K. S., Shoemaker, J. K., Arbeille, P., & Hughson, R. L. (2010). Modelflow estimates of cardiac output compared with Doppler ultrasound during acute changes in vascular resistance in women. *Experimental Physiology*, *95*(4), 561–568. https://doi.org/10.1113/expphysiol.2009.050815

Engan, H., Richardson, M. X., Lodin-Sundström, A., van Beekvelt, M., & Schagatay, E. (2013). Effects of two weeks of daily apnea training on diving response, spleen contraction, and erythropoiesis in novel subjects. *Scandinavian Journal of Medicine and Science in Sports*, *23*(3), 340–348. https://doi.org/10.1111/j.1600-0838.2011.01391.x

Fisher, J. P., Fernandes, I. A., Barbosa, T. C., Prodel, E., Coote, J. H., Nóbrega, A. C. L., & Vianna, L. C. (2015). Diving and exercise: The interaction of trigeminal receptors and muscle metaboreceptors on muscle sympathetic nerve activity in humans. *American Journal of Physiology-Heart and Circulatory Physiology*, *308*(5), H367–H375. https://doi.org/10.1152/ajpheart.00728.2014

Foster, G. E., & Sheel, A. W. (2005). The human diving response, its function, and its control. *Scandinavian Journal of Medicine and Science in Sports*, *15*(1), 3–12. https://doi.org/10.1111/j.1600-0838.2005.00440.x

Gooden, B. A. (1992). Why some people do not drown. *The Medical Journal of Australia*, *84*(9), 629–532.

Gorini, C., Philbin, K., Bateman, R., & Mendelowitz, D. (2010). Endogenous inhibition of the trigeminally evoked neurotransmission to cardiac vagal neurons by muscarinic acetylcholine receptors. *Journal of Neurophysiology*, *104*(4), 1841–1848. https://doi.org/10.1152/jn.00442.2010

Hong, S. K., & Rahn, H. (1967). The diving women of Korea and Japan. *Scientific American*, *216*(5), 34–43. https://doi.org/10.1038/scientificamerican0567-34

Hunyor, A. P. (1994). Reflexes and the eye. *Australian and New Zealand Journal of Ophthalmology*, *22*(3), 155–159. https://doi.org/10.1111/j.1442-9071.1994.tb01710.x

Hurwitz, B. E., & Furedy, J. F. (1986). The Human Dive Reflex : An Experimental , Topographical and Physiological Analysis I. *Physiology and Behavior*, *36*, 287–294.

Jose, A. D., & Collison, D. (1970). The normal range and determinants of the intrinsic

heart rate in man. *Cardiovascular Research*, *4*(2), 160–167. https://doi.org/10.1093/cvr/4.2.160

Kirk, R. E. (2005). Analysis of Variance: Classification. *Encyclopedia of Statistics in Behavioural Science*.

Lemaître, F., Buchheit, M., Joulia, F., Fontanari, P., & Tourny-Chollet, C. (2008). Static apnea effect on heart rate and its variability in elite breath-hold divers. *Aviation Space and Environmental Medicine*, *79*(2), 99–104. https://doi.org/10.3357/ASEM.2142.2008

McCraty, R., & Shaffer, F. (2015). Heart rate variability: New perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Global Advances In Health and Medicine*, *4*(1), 46–61. https://doi.org/10.7453/gahmj.2014.073

Michael Panneton, W. (2013). The Mammalian Diving Response: An Enigmatic Reflex to Preserve Life? *Physiology*, *28*(5), 284–297. https://doi.org/10.1152/physiol.00020.2013

Munoz, M. L., Van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., … Snieder, H. (2015). Validity of (Ultra-)Short recordings for heart rate variability measurements. *PLoS ONE*, *10*(9), 1–15. https://doi.org/10.1371/journal.pone.0138921

Perini, R., Tironi, A., Gheza, A., Butti, F., Moia, C., & Ferretti, G. (2008). Heart rate and blood pressure time courses during prolonged dry apnoea in breath-hold divers. *European Journal of Applied Physiology*, *104*(1), 1–7. https://doi.org/10.1007/s00421-008-0771-1

Schagatay, E. (2014). Human breath-hold diving ability and the underlying physiology. *Human Evolution*, *29*(1–3), 125–140.

Schagatay, Erika, & Andersson, J. P. A. (1998). Diving response and apneic time in humans. *Undersea & Hyperbaric Medicine: Journal of the Undersea and*

Hyperbaric Medical Society, (March).

Schagatay, Erika, & Holm, B. (1996). Effects of water and ambient air temperatures on human diving bradycardia. *European Journal of Applied Physiology*, *73*, 1–6.

Schagatay, Erika, Richardson, M. X., & Lodin-Sundström, A. (2012). Size matters: Spleen and lung volumes predict performance in human apneic divers. *Frontiers in Physiology*, *3 JUN*(June), 1–8. https://doi.org/10.3389/fphys.2012.00173

Schagatay, Erika, Van Kampen, M., Emanuelsson, S., & Holm, B. (2000). Effects of physical and apnea training on apneic time and the diving response in humans. *European Journal of Applied Physiology*, *82*(3), 161–169. https://doi.org/10.1007/s004210050668

Schaller, B., Cornelius, J. F., Prabhakar, H., Koerbel, A., Gnanalingham, K., Sandu, N., … Buchfelder, M. (2009). The trigemino-cardiac reflex: An update of the current knowledge. *Journal of Neurosurgical Anesthesiology*, *21*(3), 187–195. https://doi.org/10.1097/ANA.0b013e3181a2bf22

Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*(September), 1–17. https://doi.org/10.3389/fpubh.2017.00258

Shields, R. W. W. (1993). Functional anatomy of the autonomic nervous system. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*. Retrieved from http://journals.lww.com/clinicalneurophys/Abstract/1993/01000/Functional_Anatomy_of_the_Autonomic_Nervous_System.2.aspx%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/8458993

Sugawara, J., Tanabe, T., Miyachi, M., Yamamoto, K., Takahashi, K., Iemitsu, M., … Matsuda, M. (2003). Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiologica Scandinavica*, *179*(4), 361.

https://doi.org/10.1046/j.0001-6772.2003.01211.x

Vybiral, T., Bryg, R. J., Maddens, Michael, E., & Boden, William, E. (1989). 9485180.pdf. *The American Journal of Cardiology*, *63*, 1117–1120.

Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2016). Overview of the Anatomy, Physiology, and Pharmacology of the Autonomic Nervous System. *Comprehensive Physiology*, *6*(3), 1239–1278. https://doi.org/10.1002/cphy.c150037

Willie, C. K., Ainslie, P. N., Drvis, I., MacLeod, D. B., Bain, A. R., Madden, D., … Dujic, Z. (2015). Regulation of brain blood flow and oxygen delivery in elite breath-hold divers. *Journal of Cerebral Blood Flow and Metabolism*, *35*(1), 66–73. https://doi.org/10.1038/jcbfm.2014.170

Yasuma, F., & Hayano, J. (2004). Why does the heartbeat synchronize with respiratory rhythm? *Chest*.

Appendices

Appendix A: Permissions for Figure 1

BMJ PUBLISHING GROUP LTD. LICENSE TERMS AND CONDITIONS

Aug 09, 2019

This Agreement between Sydney Smith ("You") and BMJ Publishing Group Ltd. ("BMJ Publishing Group Ltd.") consists of your license details and the terms and conditions provided by BMJ Publishing Group Ltd. and Copyright Clearance Center.

License Number	4644870582460
License date	Aug 09, 2019
Licensed Content Publisher	BMJ Publishing Group Ltd.
Licensed Content Publication	Heart
Licensed Content Title	Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms.
Licensed Content Author	A. Malliani,F. Lombardi,M. Pagani
Licensed Content Date	Jan 1, 1994
Licensed Content Volume	71
Licensed Content Issue	1
Type of Use	Dissertation/Thesis
Requestor type	Individual
Format	Print and electronic
Portion	Figure/table/extract
Number of figure/table/extracts	1
Descriptionof figure/table/extracts	figure 1
Will you be translating?	No
Circulation/distribution	1
Title of your thesis / dissertation	Investigating the diving reflex in professional divers
Expected completion date	Sep 2019
Estimated size(pages)	100
Requestor Location	[redacted]
Publisher Tax ID	
Total	
Terms and Conditions	

BMJ Group Terms and Conditions for Permissions

When you submit your orderyou are subject to the terms and conditions set out below. You will also haveagreed to the Copyright Clearance Center's ("CCC") terms and conditionsregarding billing and

payment https://s100.copyright.com/App/PaymentTermsAndConditions.jsp. CCC are acting as the BMJ Publishing Group Limited's ("BMJ Group's") agent.

Subject to the terms set outherein, the BMJ Group hereby grants to you (the Licensee) a non-exclusive,non-transferable licence to re-use material as detailed in your request forthis/those purpose(s) only and in accordance with the following conditions:

1) **Scope of Licence:** Useof the Licensed Material(s) is restricted to the ways specified by you duringthe order process and any additional use(s) outside of those specified in thatrequest, require a further grant of permission.

2) **Acknowledgement:** Inall cases, due acknowledgement to the original publication with permission fromthe BMJ Group should be stated adjacent to the reproduced Licensed Material.The format of such acknowledgement should read as follows:

"Reproduced from [publicationtitle, author(s), volume number, page numbers, copyright notice year] withpermission from BMJ Publishing Group Ltd."

3) **Third Party Material**:BMJ Group acknowledges to the best of its knowledge, it has the rights to licenceyour reuse of the Licensed Material, subject always to the caveat thatimages/diagrams, tables and other illustrative material included within, whichhave a separate copyright notice, are presumed as excluded from the licence. Therefore,you should ensure that the Licensed Material you are requesting is original toBMJ Group and does not carry the copyright of another entity (as credited inthe published version). If the credit line on any part of the material you haverequested in any way indicates that it was reprinted or adapted by BMJ Groupwith permission from another source, then you should seek permission from thatsource directly to re-use the Licensed Material, as this is outside of thelicence granted herein.

4) **Altering/ModifyingMaterial:** The text of any material for which a licence is granted may notbe altered in any way without the prior express permission of the BMJ Group. Subject to Clause 3 above however, single figure adaptations do not require BMJGroup's approval; however, the adaptation should be credited as follows:

"Adapted by permission fromBMJ Publishing Group Limited. [publication title, author, volume number, pagenumbers, copyright notice year]

5) **Reservation of Rights:**The BMJ Group reserves all rights not specifically granted in the combinationof (i) the licence details provided by you and accepted in the course of thislicensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment Terms and Conditions.

6) **Timing of Use:** Firstuse of the Licensed Material must take place within 12 months of the grant ofpermission.

7). **Creation of Contractand Termination:** Once you have submitted an order via Rightslink and thisis received by CCC, and subject to you completing accurate details of yourproposed use, this is when a binding contract is in effect and our acceptanceoccurs. As you are ordering rights from a periodical, to the fullest extentpermitted by law, you will have no right to cancel the contract from this pointother than for BMJ Group's material breach or fraudulent misrepresentation oras otherwise permitted under a statutory right. Payment must be made inaccordance with CCC's Billing and Payment Terms and conditions. In the eventthat you breach any material condition of these terms and condition or any of CCC's Billing and Payment Terms and Conditions, the license is automatically terminated uponwritten notice from the BMJ Group or CCC or as otherwise provided for in CCC's Billing and Payment Terms and Conditions, where these apply.. Continued use of materials wherea licence has been terminated, as well as any use of the Licensed Materialsbeyond the scope of an unrevoked licence, may constitute intellectual propertyrights infringement and BMJ Group reserves the right to take any and all actionto protect its intellectual property rights in the Licensed Materials.

8. **Warranties:** BMJGroup makes no express or implied representations or warranties with respect tothe Licensed Material and to the fullest extent permitted by law this isprovided on an "as is" basis. For the avoidance of doubt BMJ Group does notwarrant that the Licensed Material is accurate or fit for any particularpurpose.

9. **Limitation ofLiability:** To the fullest extent permitted by law, the BMJ Group disclaimsall liability for any indirect, consequential or incidental damages (includingwithout limitation, damages for loss of profits, information or interruption)arising out of the use or inability to use the Licensed Material or theinability to obtain additional rights to use the Licensed Material. To thefullest extent permitted by law, the maximum aggregate liability of the BMJGroup for any claims, costs, proceedings and demands for direct losses causedby BMJ Group's breaches of its obligations herein shall be limited to twice theamount paid by you to CCC for the licence granted herein.

10. **Indemnity:** You hereby indemnify and holdharmless the BMJ Group and their respective officers, directors, employees andagents, from and against any and all claims, costs, proceeding or demands arisingout of your unauthorised use of the Licensed Material.

11. **No Transfer of License:** This licence ispersonal to you, and may not be assigned or transferred by you without priorwritten consent from the BMJ Group or its authorised agent(s). BMJ Groupmay assign or transfer any of its rights and obligations under this Agreement,upon written notice to you.

12. **No Amendment Except inWriting:** This licence may not be amended except in a writing signed by bothparties (or, in the case of BMJ Group, by CCC on the BMJ Group's behalf).

13. **Objection to Contraryterms:** BMJ Group hereby objects to any terms contained in any purchaseorder, acknowledgment, check endorsement or other writing prepared by you,which terms are inconsistent with these terms and conditions or CCC's Billing and Payment Terms and Conditions. These terms and conditions, together with CCC's Billing and Payment Terms and Conditions (which to the extent they are consistent areincorporated herein), comprise the entire agreement between you and BMJ Group (and CCC) and the Licensee concerning this licensing transaction. In the event ofany conflict between your obligations established by these terms and conditionsand those established by CCC's Billing and Payment Terms and Conditions, theseterms and conditions shall control.

14. **Revocation:** BMJGroup or CCC may, within 30 days of issuance of this licence, deny thepermissions described in this licence at their sole discretion, for any reasonor no reason, with a full refund payable to you should you have not been ableto exercise your rights in full. Notice of such denial will be made using thecontact information provided by you. Failure to receive such notice from BMJGroup or CCC will not, to the fullest extent permitted by law alter orinvalidate the denial. For the fullest extent permitted by law in no event willBMJ Group or CCC be responsible or liable for any costs, expenses or damageincurred by you as a result of a denial of your permission request, other thana refund of the amount(s) paid by you to BMJ Group and/or CCC for denied permissions.

15. **Restrictions to thelicense**:

15.1 Promotion: BMJ Group will not give permission to reproduce infull or in part any Licensed Material for use in the promotion of thefollowing:

a) non-medical products thatare harmful or potentially harmful to health: alcohol, baby milks and/or, sunbeds

b) medical products that donot have a product license granted by the Medicines and Healthcare productsRegulatory Agency (MHRA) or its international equivalents. Marketing of theproduct may start only after data sheets have been released to members of themedical profession and must conform to the marketing authorization contained inthe product license.

16. **Translation**: This permission is granted for non-exclusive worldEnglish language rights only unless explicitly stated in your licence. Iftranslation rights are granted, a professional translator should be employedand the content should be reproduced word for word preserving the integrity ofthe content.

17. **General:**Neither party shall be liable for failure, default or delay in performingits obligations under this Licence, caused by a Force Majeure event which shallinclude any act of God, war, or

threatened war, act or threatened act ofterrorism, riot, strike, lockout, individual action, fire, flood, drought,tempest or other event beyond the reasonable control of either party.

17.1 Inthe event that any provision of this Agreement is held to be invalid, theremainder of the provisions shall continue in full force and effect.

17.2 Thereshall be no right whatsoever for any third party to enforce the terms andconditions of this Agreement. The Parties hereby expressly wish to exclude theoperation of the Contracts (Rights of Third Parties) Act 1999 and any otherlegislation which has this effect and is binding on this agreement.

17.3 Tothe fullest extent permitted by law, this Licence will be governed by the lawsof England and shall be governed and construed in accordance with the laws of England. Any action arising out of or relating to this agreement shall be brought in courtssituated in England save where it is necessary for BMJ Group for enforcement tobring proceedings to bring an action in an alternative jurisdiction.

Appendix B: Permissions for Figures 4 and 5

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Aug 09, 2019

This Agreement between [redacted] John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4644870991901
License date	Aug 09, 2019
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Clinical & Experimental Ophthalmology
Licensed Content Title	Reflexes and the eye
Licensed Content Author	Alex Paul Hunyor
Licensed Content Date	Dec 7, 2009
Licensed Content Volume	22
Licensed Content Issue	3
Licensed Content Pages	5
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	2
Original Wiley figure/table number(s)	Figure 1, Figure 2
Will you be translating?	No
Title of your thesis / dissertation	Investigating the diving reflex in professional divers
Expected completion date	Sep 2019
Expected size (number of pages)	100
Requestor Location	[redacted]
Publisher Tax ID	
Total	
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work

(collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Appendix C: BREATHE Research Ethics Board Approval #111375

Western Research

Date: 14 May 2018

To: Dr. Kevin Shoemaker

Project ID: 111375

Study Title: BREATHE: Breath-Hold Diving and Brain Health

Application Type: HSREB Initial Application

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 05/Jun/2018

Date Approval Issued: 14/May/2018

REB Approval Expiry Date: 14/May/2019

Dear Dr. Kevin Shoemaker

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Acknowledged:

Document Name	Document Type	Document Date
Croatia Ethics Certification	Translation Certificate	03/May/2018
Reference List	References	
Translation Ethics Kamenica Novi Sad	Translation Certificate	03/May/2018

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Patricia Sargeant, Ethics Officer (ext. 85990) on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix D: Somatosensory Nerve Stimulation Study Research Ethics Board Approval #108026

Western Research

Date: 20 November 2017

To: Kevin Shoemaker

Project ID: 108026

Study Title: Exploring the Impact of Somatosensory Nerve Stimulation on Autonomic Regulation (SSNS)

Application Type: HSREB Amendment Form

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 05/Dec/2017

Date Approval Issued: 20/Nov/2017

REB Approval Expiry Date: 13/Jul/2018

Dear Kevin Shoemaker ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
HSREB_108026_SSNS_Screening_Form_19_09_2016_clean	Recruitment Materials	19/Sep/2017	2
REB_108026_SSNS_LOI_6_11_2017_clean	Consent Form	06/Nov/2017	3
REB_108026_SSNS_Newspaper_Advertisement_9_09_2017_clean	Recruitment Materials	19/Sep/2017	2
REB_108026_SSNS_Recruitment_Classroom_Script_6_11_2017_clean	Recruitment Materials	06/Nov/2017	3
REB_108026_SSNS_Recruitment_Email_6_11_2017_clean	Recruitment Materials	06/Nov/2017	3
REB_108026_SSNS_Recruitment_Handout_9_09_2017_clean	Recruitment Materials	19/Sep/2017	2
REB_108026_SSNS_Recruitment_Phone_Script_9_09_2017_clean	Recruitment Materials	19/Sep/2017	2
REB_108026_SSNS_Recruitment_Poster_9_09_2017_clean	Recruitment Materials	19/Sep/2017	2
REB_108026_Western_Protocol_Edited_6_11_2017_clean	Protocol	06/Nov/2017	3

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Patricia Sargeant, Ethics Officer on behalf of Dr. Marcelo Kremenchutzky, HSREB Vice-Chair

Appendix E: Ischemic Heart Disease Research Ethics Board Approval #107620

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology,Western University

Review Type: Full Board
HSREB File Number: 107620
Study Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation
Sponsor: Canadian Institutes of Health Research

HSREB Initial Approval Date: April 29, 2016
HSREB Expiry Date: April 29, 2017

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	Neuropsych test 1 (Received 30Jan16)	
Instruments	Neuropsych test 2 HADs (Received 30Jan16)	
Instruments	Neuropsych test 3 MOCA (Received 30Jan16)	
Instruments	Neuropsych test 4 RA (Received 30Jan16)	
Instruments	Neuropsych test RAVLT (Received 30Jan16)	
Instruments	Neuropsych test SF12 (Received 30Jan16)	
Instruments	Neuropsych test Trail making (Received 30Jan16)	
Instruments	Neuropsych test WAIS (Received 30Jan16)	
Advertisement	newspaper advert METS (Received 30Jan16)	
Advertisement	Newspaper Advertisement CTL (Received 30Jan16)	
Recruitment Items	Poster CTL CLEAN (Received 30Jan16)	
Recruitment Items	CAD Recruitment presentation (Received 30Jan16)	
Recruitment Items	Mail CAD NonCR CLEAN (Received 30Jan16)	
Other	Screening Forms (medical questionnaires, contact info, etc) (Received 06Jan16)	
Letter of Information & Consent	Revised LOI, CTL Group, CLEAN	2016/03/29
Letter of Information & Consent	Revised LOI, CAD group, CLEAN	2016/03/29
Western University Protocol	Revised HSREB Protocol form, CLEAN (Received 29Mar16)	
Recruitment Items	Poster CAD CLEAN (Received 30Jan16)	
Instruments	PAR-Q form	2016/03/29
Recruitment Items	Screening Forms (Received 6Jan16)	
Other	Doctor Notification Letter (Received 27Apr16)	
Other	Telephone Script	2016/04/24
Letter of Information & Consent	Revised LOI, Metabolic Syndrome Group, CLEAN	2016/03/29

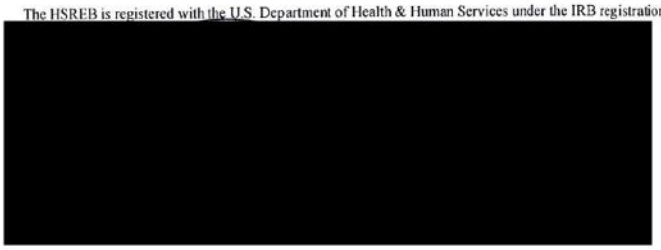
The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.



Appendix F: Ischemic Heart Disease Letter of Information #107620

Western HealthSciences

School of Kinesiology

Letter of Information

TITLE: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: Control Group

Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Jen Vording, Mark Badrov, Arlene Fleischbauer, Jeff Risdon, Baraa Al-Khazraji, Peter Prior, Neville Suskin.
Sponsor: Canadian Institutes of Health Research

INTRODUCTION AND PURPOSE

You are being invited to participate in a research study that will examine the role of vascular disease on the size and function of the brain and the health of blood vessels in the brain. We are particularly interested in how vascular disease affects brain blood flow as well as whether or not exercise training improves brain blood flow in individuals with, or at risk for, cardiovascular disease. The study will consist of three visits to our lab, which may be repeated before and following a period of exercise rehabilitation or training. The experiments on each visit day will last anywhere from two to three hours depending on the tasks being performed. A total of 270 participants will be recruited in this study.

Before agreeing to participate, please read this LETTER OF INFORMATION and ask any questions you wish.

PARTICIPANT INCLUSION/EXCLUSION CRITERIA

Overall, this investigation will study three groups of individuals: 1) healthy participants (Control Group), 2) participants with risk for cardiovascular disease, and 3) those with Coronary Artery Disease (CAD) who have recently had a cardiac event. You are invited to participate in the **Control group**.

Inclusion Criteria:
You may be included in this Control group if you are between the ages of 18 and 80 years, and if you are normally physically active and have not been diagnosed with any medical concern. Your inclusion in the Control group will be confirmed following measures of levels of glucose and triglycerides in your blood, as well as blood pressure, body size, and waist circumference. These measures will be made during your first visit (see Visit 1 below).

Exclusion Criteria:
You will not be included in the study if you smoke or have any of the following: Raynaud's disease, respiratory illnesses, diabetes, claustrophobia, history of psychosis, eating disorders, manic or bipolar disorder, major psychiatric conditions, dependence on alcohol or drugs within the past year. In addition, you will not be included in the study if you are, or think you might be, pregnant. A routine pregnancy test may be performed on women of child-bearing potential. If you are a woman of child-bearing potential you must be using an effective method of contraception.

Magnetic resonance imaging (MRI) will be used to examine the brain's vascular system in this experiment. You will not be included in this study if you have any history of head or eye injury involving metal fragments, if you have some type of implanted electrical device (such as a cardiac pacemaker). If you have severe heart disease (including susceptibility to heart rhythm abnormalities) you should not have an MRI scan

unless supervised by a physician. Additionally, you should not have an MRI scan if you have conductive implants or devices such as skin patches, body piercing or tattoos containing metallic inks because there is a risk of heating or induction of electrical currents within the metal elemental causing burns to adjacent tissue.

Finally, participants will be excluded if they are unable to provide written informed consent, or to complete questionnaires or health history forms due to language or cognitive difficulties.

STUDY DESIGN and PROCEDURE

If you agree to participate you will be assigned in a random manner (by the tossing of a coin) to one of two groups. Each group will be tested at three stages corresponding to 0, 6 and 12 months. Group 1 will begin the exercise training immediately whereas Group 2 will wait for six months before beginning the training. At each test period (i.e., 0, 6 and 12 months) you will be asked to come to the laboratory for a series of visits (see below).

Training will occur at the Laboratory for Brain and Heart Health, Room 402, Labatt Health Sciences Building. We ask you to commit to exercising at the designated site three times each week as per a program provided to you by the staff. The exercise staff include a Nurse and a Certified Exercise Physiologist. The exercise program will include approximately 30 minutes of aerobic exercise (on a bicycle ergometer or treadmill for example) and 30 minutes of strength training. The levels of exercise will be determined by a pre-study examination of your fitness level. The exercise will be progressive in the sense that as you improve, the exercise loads will increase accordingly. Your blood pressure and heart rate will be measured at each visit. Emergency equipment includes a defibrillator.

Tests: All testing and training will occur in the Laboratory for Brain and Heart Health at Western University (Room 402 Labatt Health Sciences Building) We try to schedule your testing to fit into three visits. Here is a sample of what those visits include (the order of testing may vary depending on schedules):

Visit 1 - Laboratory Testing 1 (Consent, Neurovascular Health) (3-4 hours)

Read LOI Consent Enrolment	30min supine rest	Blood Draw	Snack	Instrumentation ECG, Blood Pressure, Respiration, Waist Circumference,	Cerebrovascular Reactivity (supine 5% CO2 inhalation; Sit-to-Stand protocol).
(15min)	(30m)	(10m)	(15m)	(1 hour)	(1 hour)

Visit 2 - Laboratory Testing 2 (Vascular Imaging, Exercise Capacity, Brain blood flow) (1-2 hours)

Psychology Tests	Vascular Imaging	6-min walking test
(45 min)	(30 min)	(10 min)

Visit 3 - MRI Testing (1-2 hours)

MR Safety Screen	Instrumentation: Finger pulse oximeter Respiration, blood pressure, MoCA test	MRI brain imaging of brain blood vessels and blood flow
(15min)	(15min)	(1 hour)

Pre-Visit Preparation: We will ask that you abstain from exercise for 24 hours, and fast for 12 hours before Visit 1. Also, we ask that you abstain from exercise, and that you do not consume alcohol, nicorette gum (or any source of nicotine), coffee, tea, caffeinated soft drinks and chocolate for at least 12 hours before each Visit. At each visit, the testing will require approximately 2-4 hours of your time depending on the test sequence.

Visit 1 - Laboratory Testing 1 - Laboratory for Brain and Heart Health, HSB 402
On arrival for your first visit, you will be given opportunity to read this information letter as you decide whether or not to participate in the study. You may wish to take more time to ponder a decision about whether or not to participate in this study. If so, please feel free to take this form with you and take your time in deciding. After signing the form (and returning to the lab at the scheduled appointment), you will rest quietly for 30 minutes after which we will take a resting, fasted blood sample. A venous catheter will be inserted into a large vein near your elbow through which the research nurse (Arlene Fleischhauer) will take a blood sample. This is similar to the blood sample you would give for your annual physical, but we analyse your blood for a number of additional markers of health. We will not take more than 12 tablespoons of blood at each visit. Your blood will be analyzed for general health markers (glucose, cholesterol) as well as markers of inflammation, hormones and markers that reflect blood vessel health. One of the markers we analyze your blood for is a genetic marker called apolipoprotein (APOE). The APOE is present in 15-20% of Caucasians and has been associated with the risk of changes to brain blood vessels and cortical thickness in the brain of aging individuals.

After the blood draw is finished, we will provide you with a light snack and something to drink. You will be asked to fill in some questionnaires about your medical history while you eat and rest. You may then wish to go to the bathroom, before we measure your height and weight, and abdominal girth.

During the subsequent tests we will measure your heart rate using an electrocardiogram (ECG) and a pulse monitor attached to one of your toes. We will measure your blood pressure with a cuff around your finger, and also with a larger cuff placed around the upper part of your arm, just like it is done in a doctor's office. The arm cuff will be inflated to a high pressure for about 30 seconds to measure your blood pressure. Your rate and depth of breathing will be measured by placing a respiratory belt around your ribcage.

Brain Blood Flow Stimulation: For this task, we will have you breathe through a mask. For the first few minutes, you will breathe normal room air. You will then breathe a gas mixture that contains a higher (5%) level of carbon dioxide but the normal level of oxygen (21%) and nitrogen (74%). Carbon dioxide is a gas that your body normally produces and it increases brain blood flow. We will examine the reaction of your blood vessels and nerves to the increased level of carbon dioxide. Breathing the carbon dioxide will last up to five minutes, and will be followed by a five minute recovery period. You may be asked to breathe more frequently (in time with a rhythmic tone) for up to five minutes in order to reduce levels of carbon dioxide.

While measuring your brain blood flow, and breathing room air through this mask (or mouth piece), you will be asked to perform a series of "sit-to-stand" tasks where you will sit quietly in a chair for up to 3 minutes, stand for two minutes, and then sit again. This would be repeated up to 5 times. This task may be repeated while you are asked to either breathe a little faster or while you are breathing the 5% carbon dioxide gas mixture outlined above.

Visit 2 - Laboratory Testing 2 - Laboratory for Brain and Heart Health, HSB 402
Your second visit to the lab will involve completion of some psychological tests, characteristics of your blood vessel health, and a 6-minute walking test of your overall physical fitness.

Psychological measurements: You will undergo brief standardized testing to measure cognition, (certain features of your brain's information processing) and emotional state (mood and anxiety). This will take about 45 minutes and will consist of a series of paper-and-pencil measures and a test on the computer. In

addition, the Stroop Test is a psychological test of your mental vitality and flexibility. The task takes advantage of our ability to read words more quickly and automatically than we can name colors. If a word is printed or displayed in a color different from the color it actually names; for example, if the word "green" is written in blue ink we will say the word "green" more readily than we can name the color in which it is displayed, which in this case is "blue."

Vascular Properties: You will be asked to rest quietly on the bed as we collect 10 minutes of baseline data. The ECG and blood pressure systems outlined above for Visit 1 will be used again. Then, images of your blood vessels and blood flow will be measured using ultrasound probes placed on the skin over the arteries in your neck, arm and/or leg and brain. We will also measure the blood ejected from your heart using ultrasound. Then, we will measure the change in vessel diameter at your elbow before and for 3 minutes following, a brief (e.g., 5 minute) period where blood flow to your arm will be stopped by a cuff placed around your forearm. These ultrasound measures will be repeated before and for 4 minutes following a small dose of sodium nitroglycerine sprayed just under your tongue.

Six-minute walking test: The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in a hallway. Six minutes is a long time to walk, so you will be exerting yourself. You may become out of breath or tired. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.

Visit 3 - MRI

The MRI visit will occur at Robarts Research Institute, 100 Perth Drive, Western University Campus.
At the beginning of your visit to the MRI we will confirm whether or not it is safe for you to enter the MRI suite by completing an MRI Safety Screen. We will put a small cuff around your finger to monitor your heart rate, and a respiratory belt around your chest to measure the depth and rate of your breathing. You will lie on a bed for about two hours while the MRI machine gathers data. MRI makes images of the interior of your body using strong magnetic and radio waves. You will not feel either. You will, however, hear loud, repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones to wear which will minimize the sound and protect your hearing.

You will rest lying down on a padded bed quietly for 10 minutes for measurement of resting heart rate. During the MRI visit we will measure the structural properties of your brain and its blood vessels while you breathe room air. Your brain's blood vessels will be measured again when you breathe the 5% carbon dioxide gas mixture outlined above for up to 5 minutes.

STUDY BENEFITS

There is the possibility that you will receive no personal benefit from this study. However, it is likely that you may be able to lower your risk for heart disease from participating. Your participation may also increase your awareness of new health habits. In reports about the study, your contributions will be grouped with those of other participants to develop conclusions that could be used to improve the education and support available for people with heart disease or who are at high risk of heart disease or stroke.

STUDY RISKS

Laboratory Test Procedures Risk

There is a small risk of bruising or infection when collecting blood from your vein. Some people may experience mild pain and discomfort and some may feel nauseous or dizzy when blood is taken. To avoid this, we will be collecting blood from you while you are lying down.

The adhesive on the electrodes used to measure your heart rate may lead to temporary redness of the skin on your chest.

There are no known harmful effects with the measures of blood vessels or blood flow using ultrasound imaging, or blood pressure, as used in this study.

The sodium nitroglycerine is a common self-administered treatment for angina pain. It may give you a mild headache for a few minutes.

Breathing a slightly higher level of carbon dioxide may give you a small headache and it may make you feel breathless. These feelings vanish quickly when you start breathing room air again.

Exercise Tests: Walking or running can be a physically challenging activity. You will breathe harder and may begin to sweat. This is part of your body's normal response to exercise. There is minimal risk associated with the self-paced six-minute walking test.

Psychology Tests
There are no risks with the psychology tests.

MRI Risk
This MRI machine uses a strong magnet and radio waves to make images of the body interior. You will be asked to lie on a long narrow couch for about 1.5 hours in each MRI session while the machine gathers data. During this time you will be exposed to magnetic fields and radio waves. You will not feel either. You will, however, hear repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones that you will be required to wear to minimize the sound and protect your hearing. The space within the large magnet in which you lie is somewhat confined, although we have taken many steps to relieve the "claustrophobic" feeling. There are no known significant risks with this procedure at this time because the radio waves and magnetic fields at the strengths used, are thought to be without harm. The exception is if you have a cardiac pacemaker, or a metallic clip in your body (e.g., an aneurysm clip in your brain), have severe heart disease, body piercings, tattoos containing metallic ink or slow release pharmaceutical skin patches.

There is the possibility that you will experience a localized twitching sensation or perhaps a little dizzy due to the magnetic field changes during the scan. These responses are not unexpected and should not be painful. However, you can stop the exam at any time. The magnetism and radio waves do not cause harmful effects at the levels used in the MRI machine. However, because the MR scanner uses a very strong magnet that will attract metal, all metallic objects must be removed from your person before you approach the scanner. In addition, watches and credit cards should be removed as these could be damaged (these items will be watched for you).

As with any technology there is a risk of death or injury. For MRI the risk of death is less than 1 in 10 million and the risk of injury is less than 1 in 100,000. These risks do not arise from the MRI process itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the participant or the scanner room. It is therefore very important that you answer all the questions honestly and fully on the MRI screening questionnaire. Almost all the deaths and injuries related to MRI scans have occurred because the MRI operator did not know that surgically implanted metal hardware (such as a cardiac pacemaker) was present inside the participant during the MRI scan. For comparison, the risk of death in an MRI is similar to travelling 10 miles by car, while the risk of injury during an MRI is much less than the risks associated with normal daily activities for 1 hour. Any unusual findings from the MRI images will be provided to you so that you can seek further medical attention.

Exercise training Risk

All participants will obtain their family physician's signed permission to participate in exercise before they may participate in the exercise training segment.

YOUR PARTICIPATION

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care, academic status, or employment. If you withdraw from the study before its completion then you may decide whether to also withdraw your data. Participation in this study will be brought to the attention of your family doctor.

The blood specimens will be discarded or destroyed once they have been used for the purposes described in the protocol. All other study data (e.g., paper files, digital files) will be kept for a minimum of 20 years.

If you are participating in another study at this time, please inform the study coordinator right away to determine if it is appropriate for you to participate in this study.

Whether you agree to participate in this study or not, you will be asked if you consent to having your name and contact information added to a master database of individuals who would be willing to be contacted in the future regarding your interest in other research studies.

Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. Representatives of Lawson Quality Assurance (QA) Education Program may look at study data for quality assurance purposes.

CONFIDENTIALITY

Your research records will be stored in a secure office at Western University. To further protect your confidentiality, your name will be replaced with a participant ID number on all documents. The master list linking your identity and participant ID number and your contact information will be stored separately in a secure office at Western University. Your contact information will be securely maintained at Western University to allow for setting up follow up visits. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published. No information that could reveal your identity will be released to anyone with the exception of your Family Doctor if you give permission for this.

If we find information we are required by law to disclose, we cannot guarantee confidentiality.

ALTERNATIVES TO STUDY PARTICIPATION

You may choose not to participate in this study.

REIMBURSEMENT

You will be reimbursed for travel costs up to $60 for your participation in each of the pre and post series of tests outlined above. For coronary artery disease participants, the costs associated with your participation in the cardiac rehabilitation program are provided for you. However, we cannot provide support for the travel associated with your participation in the cardiac rehabilitation program. For all participants, your parking and exercise costs will be covered for six months if you perform the exercise training in the Laboratory for Brain and Heart Health.

CONTACT PERSONS

[redacted]

Western
HealthSciences

Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: CONTROL GROUP
Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Jen Vording, Mark Badrov & Arlene Fleischhauer

CONSENT

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

☐ I consent to be contacted for future research

SIGNATURES

Signature of Participant	Date
Print	
Signature of Person Obtaining Informed Consent	Date
Print	

Appendix G: Somatosensory Nerve Stimulation Study Letter of Information #108026

Western HealthSciences

School of Kinesiology

Letter of Information

TITLE: Exploring the Impact of Somatosensory Nerve Stimulation on Autonomic Regulation

Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Stephen Klassen, Sydney Smith, Arlene Fleischhauer
Sponsor: The *Natural Sciences and Engineering Research Council* of Canada

INTRODUCTION AND PURPOSE

You are being invited to participate in a research study that will examine how different nervous systems work together to affect blood pressure. The study will consist of two visits to our lab (The Laboratory for Brain and Heart Health) as well as one visit to Robarts Research Institute. The experiments on each visit day will last approximately two to three hours. A total of 100 participants will be recruited in this study. Before agreeing to participate, please read this LETTER OF INFORMATION and ask any questions you wish.

PARTICIPANT INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria:

You may be included in this study if you are 1) a male or female between the ages of 18 and 80 years or 2) a male or female between the ages of 18 and 80 years, and have been diagnosed with a sensory ganglionopathy.

Exclusion Criteria:

You will not be included in the study if you smoke, have diabetes, impaired glucose tolerance, autonomic neuropathy, Parkinson's disease, history of psychosis, eating disorders, manic or bipolar disorder, major psychiatric conditions, or dependence on alcohol or drugs within the past year. Individuals who have suffered severe traumatic brain injuries will be excluded. Women who are pregnant or trying to become pregnant will also be excluded.

Magnetic resonance imaging (MRI) will be used to examine brain activity in this experiment. You will not be included in this study if you have any history of head or eye injury involving metal fragments, or if you have some type of implanted electrical device (such as a cardiac pacemaker). If you have severe heart disease (including if you may suffer from altered heart rhythms) you should not have an MRI scan unless supervised by a physician. Additionally, you should not have an MRI scan if you have conductive implants or devices such as skin patches, body piercing or tattoos containing metallic inks because there is a risk of heating or induction of electrical currents within the metal elemental causing burns to adjacent tissue. Individuals with claustrophobia will be excluded.

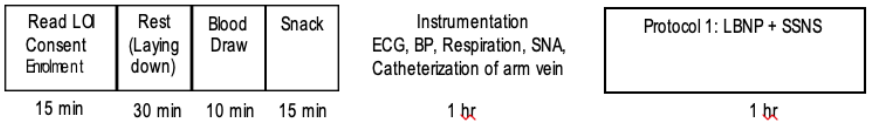
Finally, participants will be excluded if they are unable to provide written informed consent, or to complete questionnaires or health history forms due to language or cognitive difficulties.

STUDY DESIGN and PROCEDURE

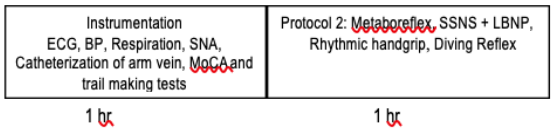
If you agree to participate you will be asked to attend three testing visits. Two testing visits will occur in the Laboratory for Brain and Heart Health at Western University (Room 402 Labatt Health Sciences Building)

and one testing visit will occur at the Robarts Research Institute (100 Perth Drive, Western University Campus). Here is a sample of what your testing visits include (the order of testing may vary depending on schedules):

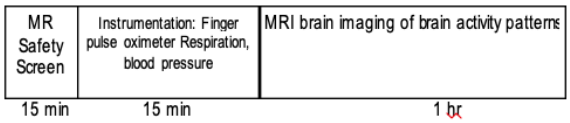
Visit 1 - Laboratory Testing 1 (~ 3 hrs)



Visit 2 - Laboratory Testing 2 (1-2 hours)



Visit 3 - MRI Testing (1-2 hours)



Pre-Visit Preparation: We will ask that you abstain from exercise for 24 hours, and fast for 12 hours, before each visit. Also, we ask that you abstain from exercise, and that you do not consume alcohol, nicorette gum (or any source of nicotine), coffee, tea, caffeinated soft drinks and chocolate for at least 12 hours before each visit. At each visit, the testing will require approximately 2-3 hours of your time depending on the test sequence. A small snack and water will be provided after the blood draw in visit 1, otherwise food will not be provided. Water will be available to participants throughout this study.

Visit 1 - Laboratory Testing 1 - Laboratory for Brain and Heart Health, HSB 402

On arrival for your first visit, you will be given the opportunity to read this information letter as you decide whether or not to participate in the study. You may wish to take more time to ponder a decision about whether or not to participate in this study. If so, please feel free to take this form with you and take your time in deciding. After signing the form (and returning to the lab at the scheduled appointment), you will be asked to fill in some questionnaires about your medical history. You may go to the bathroom if you wish, before we measure your height and weight. The following procedures will prepare you for the actual testing.

First, a catheter will be inserted into a large vein near your elbow by a research nurse (Arlene Fleischhauer) in order to measure the pressure of blood in this vein. We will also be taking a blood sample. We will not

take more than 60 mL (approximately ¼ cup) of blood at each visit. Your blood will be analyzed for general health markers (glucose, cholesterol) as well as markers of inflammation, hormones and markers that reflect blood vessel health. One of the markers we analyze your blood for is a genetic marker called apolipoprotein (APOE). The APOE is present in 15-20% of Caucasians and has been associated with the risk of changes to brain blood vessels and the thickness of the outer layer of the brain in aging individuals. Following the blood sample you will be provided with a small snack and a bottle of water.

Microneurography: The nervous system that regulates your blood pressure will be examined with a technique called "microneurography". This procedure has two phases. First, the position of a nerve that runs very close to your skin just on the outside of the knee will be located using a small electrical pulse that will cause your foot to twitch. This will be a strange sensation but will not be uncomfortable and carries no risk at the levels of electrical current that are being used. Second, a thin needle-like electrode (made out of tungsten and about the size of a large human hair, 200 microns) will be inserted through the intact skin and positioned just under the skin about 2-3 cm from the nerve site. You may feel a pin-prick sensation when the electrode is passed through the skin, much like the feeling you get when your blood is being taken. This will be followed by the placement of a second electrode through the intact skin into this nerve (called the peroneal nerve). This second electrode will be manipulated by the researcher until the appropriate recording site is found; this search will not last longer than 60-min. The microelectrodes are sterilized before use and the area of skin around the knee is cleaned with alcohol before and after the procedure. To confirm an acceptable recording of nervous system activity we will perform a series of breath holds and maneuvers that require you exhale while your mouth is blocked (the Valsalva manoeuvre) for about 20 sec.

Muscle Sensory Nerve Stimulation (SSNS): For SSNS, we will stimulate your muscles with an electrical stimulator and adhesive pads on the skin. Normally used to control pain, the amount of stimulation will be set just below that needed to cause a very small muscle contraction. Therefore, this type of muscle stimulation is painless and you may not feel anything. Before placement of the stimulation pads, the skin is numbed using a lidocaine cream. This SSNS will be applied in 30-60 sec intervals.

During the subsequent tests we will measure your heart rate using an electrocardiogram (ECG) and a pulse monitor attached to one of your toes. We will measure your blood pressure with a cuff around your finger, and also with a larger cuff placed around the upper part of your arm, just like in a doctor's office. The arm cuff will be inflated to a high pressure for about 30-sec to measure your blood pressure. Your rate and depth of breathing will be measured by placing a respiratory belt around your ribcage. Also, we will have you breathe through a mask to record the levels of gases that you breathe out.

Tasks:

Baseline: You will be asked to rest quietly on the bed for 10 minutes. The SSNS protocol will be applied 2-3 times while you rest quietly.

Lower body negative pressure (LBNP): You will be sealed in a box from the waist down and suction will be applied within this box which will cause blood to move to the lower body in the same way that standing upright does. Repeated sessions of lower body suction will be applied, each lasting up to 5-min. Different levels of suction will be applied with the most severe (-80 mmHg) being used last; this last level represents the level of stress on your body that occurs when you stand upright. During each level of suction, up to 3 periods of SSNS will be applied, each lasting up to 1-min in duration. You likely will not know if the nerve stimulation is on or not (i.e. a sham-SSNS period may be used). During this task we will measure blood flow in arteries in your neck and arms or leaving the heart using ultrasound imaging probes placed against your skin.

Visit 2 - Laboratory Testing 2 - Laboratory for Brain and Heart Health, HSB 402

You will be instrumented with the same equipment as the first visit (i.e., catheter in a vein, ECG, pulse monitor, finger and arm blood pressure cuffs, respiratory belt). We will also be performing microneurography, imaging the arteries, measuring the pressure inside the arteries of the neck, measuring the speed of blood flow and measuring exhaled gases. Prior to instrumentation you will be asked to perform two brief assessments of mental functioning. The Montreal Cognitive Assessment (MoCA) requires you to perform several tasks including drawing, image naming and memory tests. The Trail Making Test involves drawing lines to connect consecutive circles containing numbers or letters, as quickly as possible.

Baseline: Your first task will be to rest quietly on the bed as we collect 10-min of baseline data. During this time we ask that you remain as still as possible. The SSNS protocol will be applied 2-3 times while you rest quietly.

Metaboreflex Stress, SSNS and LBNP: You will squeeze a device as hard as you can so we can record your maximum handgrip strength. The protocol will begin by inflating a blood pressure cuff around your arm for 1-min of baseline rest. You will then be asked to perform mild-to-moderate-intensity handgrip contractions with the cuff inflated until you feel you can no longer continue (duration will be different for each participant). The inflated cuff restricts blood flow from entering or leaving your arm, trapping products of exercise in your arm and causing an increase in blood pressure: this is known as metaboreflex stress. After the exercise is complete you will be asked to rest with the cuff inflated for up to 5-min. During each phase of this trial (i.e., baseline, exercise, post-exercise recovery) trial, up to 3 periods of SSNS or sham-SSNS will be applied each up to 1-min in duration (see above for description of SSNS). The entire protocol may be repeated up to three times. In a second version of this test, you will simply rest quietly as the cuff remains continuously inflated for the 11-min protocol, without any exercise. In a third version, the handgrip exercise will be performed while suction is applied in the box around your lower body, as described above. The level of suction used will be -35 mmHg, and is used to mimic the effects of the seated posture on your cardiovascular system. The order of exercise or no-exercise versions will be assigned randomly through the flipping of a coin. At each minute of each trial you will tell the investigator how tired your arm feels using a scale of 6-20 (20 = maximal effort and discomfort). Prior to and during the hand-grip protocols and post-exercise ischaemic period, we may use the venous catheter in the exercising arm to take small blood samples (only a drop each time, up to 10 samples). These blood samples will be analyzed for lactate, a marker of muscle fatigue. Also, during this procedure, we may measure the electrical signals going from your nerves to your forearm muscles by applying electrodes to the skin on your forearm. During this task we may perform ultrasound imaging of the arteries in your neck, arms and heart. We may use a pen-like device to measure blood pressure of the arteries in your neck and arms.

Rhythmic handgrip exercise: First, you will squeeze a device as hard as you can so we can record your maximum handgrip strength. You will then perform a series of lighter squeezes that will last up to 30-sec. Each squeeze will be followed by up to 1-min of rest. This pattern of squeezing and resting will be repeated up to 10 times. Up to 5 of these will be performed together with SSNS. You will be asked periodically how tired your arm is using a Borg scale that ranges from 6 (no tiredness) to 20 (completely tired).

Diving Reflex: The nerves in your forehead skin elicit important control over your heart rate and blood pressure. To study this reflex you are asked to perform up to three breath-holds (no longer than 60 seconds) that will be performed with, or without, a cold pack placed on your forehead. The effect of the cold pack will also be studied without a breath hold, by placing the pack on your forehead and by immersing your foot in ice water for up to 3 minutes, similar to what a physical therapist might ask you to do for a sprained ankle.

Visit 3 - MRI

The MRI visit will occur at Robarts Research Institute, 100 Perth Drive, Western University Campus. At the beginning of your visit to the MRI we will confirm whether or not it is safe for you to enter the MRI suite by completing an MRI Safety Screen. We will put a small cuff around your finger to monitor your heart rate, and a respiratory belt around your chest to measure the depth and rate of your breathing. You will lie on a bed for about two hours while the MRI machine gathers data. MRI makes images of the interior of your body using strong magnetic and radio waves. You will not feel either. You will, however, hear loud, repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones to wear which will minimize the sound and protect your hearing.

You will rest lying down on a padded bed quietly for 10-min for measurement of resting heart rate. During the MRI visit we will measure brain activation during baseline and up to 3 periods of lower body suction (-35 mmHg) each lasting up to 5-min. Two minutes of rest will be provided between each period of lower body suction. During each level of suction, up to 3 periods of SSNS will be applied, each lasting up to 1-min in duration. Also, the metaboreflex stress protocol will be performed as described above, with the exception of the non-exercising version.

STUDY BENEFITS

You will receive no personal benefit from this study.

STUDY RISKS

Laboratory Test Procedures Risk

Catheterization of an arm vein carries a small risk of discomfort, bruising, light-headedness, dizziness, fainting and rarely infection. Some people may experience mild pain and discomfort and some may feel nauseous or dizzy when blood is taken. The risks of catheterization and blood sample collection are minimal because these procedures will be performed while you are lying down. Also, an experienced nurse will be performing the blood sample and catheterization with sterile equipment. Moreover, you will be told to apply pressure to vein for several minutes following its removal.

There are no risks associated with the use of a finger or arm cuffs to examine blood pressure. With the finger cuff, the finger tip may turn a little blue and feel numb during the tests but this vanishes immediately when the cuff is turned off.

There are no known harmful effects with the measures of blood vessels or blood flow using ultrasound imaging.

The handgrip exercise will produce forearm fatigue. Also, the post-exercise period of forearm occlusion may be uncomfortable. This fatigue and discomfort vanish quickly following the protocol. No major risks of continuous cuff inflation have been reported. About 30 min will occur between repeated sessions of exercise to allow your arm to recover.

During microneurography you may experience a small pinch similar to that of a small needle when the electrode crosses the skin. You may feel transient paresthesias (pins and needles) or involuntary muscle twitches during the process of locating an adequate recording site. There are no residual consequences of microneurography. Our laboratory has performed over 1200 such procedures during the past 17 years. In

approximately 0.5% of cases, tenderness over the microelectrode insertion site was reported for ~7 days following the microneurographic procedure. However, this tenderness resolved thereafter. This risk will be minimized by limiting the search for an adequate signal to 60-min. We advise that you restrain from heavy physical exercise with the involved limb for 24-hours after the test session. Also we recommend that no pressure be placed over the insertion site for the 24-hour post-test period. Repeated microneurography experiments on the same nerve location are not performed within 4 weeks of each other.

Somatosensory nerve stimulation is a commonly applied technique for pain management and carries no risk as performed in the current study. You may feel a slight tingling underneath the electrodes and a muscle twitch while the intensity level is adjusted; however, these are not painful and become unnoticeable when the stimulation parameters are determined. In fact, you will likely not feel the stimulation during the trial. The skin will be anesthetized with numbing cream. Therefore, once the stimulation levels are adjusted, very little sensation is present. Additional precautions will be taken for wire shielding when conducted during MRI sessions.

Holding one's breath too long can cause fainting or blackouts if the breath hold begins following a period of excessive breathing (hyperventilation) and if it is held too long. The breath hold tests in this study carry very little risk because of the following: 1) it will follow a period normal breathing, 2) a breath hold of less than 60 seconds does not cause any important changes in your body's oxygen supply, 3) you control when you start breathing again, and 4) the breath hold will be stopped if you reach 60 sec and/or if we detect more than a 5% drop in your blood oxygen levels, as measured by a monitor placed on your ear lobe or finger.

The use of a cold ice pack to stimulate the nerves in your forehead or foot can be uncomfortable but only as long as the cold pack is on your skin.

The lower body negative pressure manoeuvre carries the risk of brief light headedness, and possibly fainting. However, your blood pressure and heart rate are monitored continuously so that the test will be stopped if we think you might faint. Also, we ask that you tell the investigators if you feel light headed, sweaty, or have "tunnel" vision so that the test can be stopped after which you will feel better.

MRI Risk

This MRI machine uses a strong magnet and radio waves to make images of the body interior. You will be asked to lie on a long narrow couch for about 1.5 hours in the MRI session while the machine gathers data. During this time you will be exposed to magnetic fields and radio waves. You will not feel either. You will, however, hear repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones that you will be required to wear to minimize the sound and protect your hearing. The space within the large magnet in which you lie is somewhat confined, although we have taken many steps to relieve the "claustrophobic" feeling. There are no known significant risks with this procedure at this time because the radio waves and magnetic fields at the strengths used, are thought to be without harm. The exception is if you have a cardiac pacemaker, or a metallic clip in your body (e.g., an aneurysm clip in your brain), have severe heart disease, body piercings, tattoos containing metallic ink or slow release pharmaceutical skin patches.

There is the possibility that you will experience a localized twitching sensation or perhaps a little dizzy due to the magnetic field changes during the scan. These responses are not unexpected and should not be painful. However, you can stop the exam at any time. The magnetism and radio waves do not cause harmful effects at the levels used in the MRI machine. However, because the MR scanner uses a very

strong magnet that will attract metal, all metallic objects must be removed from your person before you approach the scanner. In addition, watches and credit cards should be removed as these could be damaged (these items will be watched for you).

As with any technology there is a risk of death or injury. For MRI the risk of death is less than 1 in 10 million and the risk of injury is less than 1 in 100,000. These risks do not arise from the MRI process itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the participant or the scanner room. It is therefore very important that you answer all the questions honestly and fully on the MRI screening questionnaire. Almost all the deaths and injuries related to MRI scans have occurred because the MRI operator did not know that surgically implanted metal hardware (such as a cardiac pacemaker) was present inside the participant during the MRI scan. For comparison, the risk of death in an MRI is similar to travelling 10 miles by car, while the risk of injury during an MRI is much less than the risks associated with normal daily activities for 1 hour. Any unusual findings from the MRI images will be provided to you so that you can seek further medical attention.

YOUR PARTICIPATION

Voluntary Participation:
Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care, academic status, or employment. If you withdraw from the study before its completion then you may decide whether to also withdraw your data.

Study data (e.g., paper files, digital files) will be kept for a minimum of 10 years.

If you are participating in another study at this time, please inform the study coordinator right away to determine if it is appropriate for you to participate in this study.

Whether you agree to participate in this study or not, you will be asked if you consent to having your name and contact information added to a master database of individuals who would be willing to be contacted in the future regarding your interest in other research studies.

We will communicate to your family physician or health team that you are participating in this study.

Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. Representatives of the Lawson Quality Assurance Education Program may look at study data for quality assurance purposes.

CONFIDENTIALITY

Your research records will be stored in a secure office at Western University. To further protect your confidentiality, your name will be replaced with a participant ID number on all documents. The master list linking your identity and participant ID number and your contact information will be stored separately in a secure office at Western University. Your contact information will be securely maintained at Western University to allow for setting up follow up visits. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published.

If we find information we are required by law to disclose, we cannot guarantee confidentiality.

ALTERNATIVES TO STUDY PARTICIPATION
You may choose not to participate in this study.

BENEFITS TO SOCIETY

This study will generate knowledge regarding the nervous systems that work together to regulate your blood pressure and as a result this study may not directly benefit you or other members of society.

REIMBURSEMENT

You will be reimbursed for travel costs up to $30 total for your participation in the tests outlined above.

CONTACT PERSONS

If you have any questions about the study please contact:

Research Staff: Stephen Klassen, [redacted]
Principal Investigator: Dr. Kevin Shoemaker, [redacted]

Please note that email is not considered a secure method of communication and you should not send any personal health information via email.

If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics [redacted]

You will receive a copy of the fully signed informed consent document for your records. You do not waive any legal rights by signing the consent.

Western HealthSciences

Title: Exploring the Impact of Somatosensory Nerve Stimulation on Autonomic Regulation
Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Stephen Klassen, Sydney Smith, Arlene Fleischbauer

CONSENT

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

CONTACT FOR FUTURE STUDIES

Please check the appropriate box below and initial:

☐ I agree to be contacted for future research studies _____

☐ I do NOT agree to be contacted for future research studies _____

SIGNATURES

_____ _____
Signature of Participant Date

_____
Print

_____ _____
Signature of Person Obtaining Informed Consent Date

_____
Print

Appendix H: BREATHE Study Ethics Letter of Information #111375

[redacted]

CONSENT FORM FOR POTENTIAL PARTICIPANTS IN SCIENTIFIC RESEARCH

Project title: "Morpho-functional changes of the brain in extreme apnea"

Duration of research: three years

Principal researcher: Prof. Dr. Otto Barak, Department of Physiology, Faculty of Medicine, Novi Sad

Research location: Oncology Institute of Vojvodina, Center for Imaging Diagnostics

Invitation to participate

Dear Sir / Madam, We invite you to participate in a scientific research that will explore the impact of diving on your blood flow and morpho-functional changes in the brain. During long-term breath holding, there is an increase in arterial blood pressure and brain blood flow, which can result in minor changes in the brain, which may have transient functional consequences. Magnetic resonance is an imaging method by which these small changes can be registered.

The aim of the research

The aim of the study is to register acute changes in the brain after long-term breath hold and to correlate them with the existence of functional changes that occur after cesation of apnea.

Initials

[redacted]

Participants

We intend to involve about 15 young divers with short experience and 15 older divers with longer experience in breath hold diving.

Procedure

During the research, you will be subjected to magnetic resonance imaging. When arriving at the lab, you will be informed in detail about the conditions under which the test will be carried out. At the beginning, you will lie steadily in the magnetic resonance device, and during that period doctors will take the images of your brain. You will then be asked to keep your breath as long as you can (as during your training), and images of your brain will be acquired after the cessation of the apnea.

What are the benefits of participation for you as respondents?

Participation in the research does not bring you any financial gain. The results of this study will additionally increase the knowledge of processes occurring in the brain during extreme apnea. In this way, we want to increase safety during diving for you and everyone involved in this sport. Upon completion of the research, you will be given insight into your findings and all new knowledge gained during the research.

What are the possible risks of participating in this research?

Magnetic resonance is a non-invasive method that does not impose any health risks for participant. During the recording, you will find yourself in a cramped space, which may cause discomfort to some.

Is participation compulsory?

You decide wether you want to participate in the research. If you choose to participate, sign your initials on each page of this consent form. Participation in this research is voluntary and you can withdraw from it at any time without the need to state the reasons. For any additional questions, you can contact the Principal Researcher, both before and during the course of the research.

Confidentiality and insight into documentation

When accessing this scientific research, we will give you an identification number that will protect your identity. The documentation may be reviewed by the Principal investigator, lead researchers and members of the research team as well as representatives of the relevant Ethics Committee.

[redacted]

COMPLIANCE – INFORMED CONSENT OF PARTICIPANT

Project title: "Morpho-functional changes of the brain in extreme apnea"

Principal researcher: Prof. Dr. Otto Barak, Department of Physiology, Faculty of Medicine, Novi Sad

1. I confirm that I have received, read, and understood the document on the above stated research.
2. I confirm that the research has been explained to me in detail both in written form and verbally. I know to whom to address in case of possible problems.
3. I know that my participation in this scientific research is voluntary and that I can retret from it at any time without any consequences to my health or any legal consequences.
4. I understand that my documentation is under open access to responsible individuals, i.e. Principal investigator, lead researchers and members of the research team, as well as representatives of the relevant Ethics Committee. I give permission to these individuals to access my documentation.
5. I want to participate in the above stated scientific research.

Name and last name of participant	Signature	Date

Name and last name of researcher	Signature	Date

Appendix I: Raw Data

	Apnea Duration	Training Volume	Competitive Training Volume	% HR decline to absolute min	% HR decline to 10 sec avg
007-SRB-1	180.8	60.0	42.0	37.29	26.89
008-SRB-2	86.4			48.68	40.96
012-SRB-1	34.7	36.0	36.0	41.12	28.40
002-SRB-1	72.1	60	49	46.75	34.50
014-SRB-1	33.3	56	36	40.76	26.74
017-SRB-1	60.4	90.0	90.0	63.10	26.75
020-SRB-2	59.4	12.5	0.0	38.34	25.51
015-SRB-1	111.2			52.48	3.04
018-SRB-2	66.3	56.0	0.0	38.28	25.53
016-SRB-1	45.0	70.0	36.0	38.02	32.91
001-SRB-1	66.9			37.67	32.63
009-SRB-1	146.4	72.0	56.0	19.02	-6.73
011-SRB-1	140.3	45.0	36.0	41.12	24.17
019-SRB-2	53.0	1.5	0.0	26.69	18.87
006-SRB-1	57.9	20.0	0.0	42.75	28.88
013-SRB-2	39.4	90.0	42.0	30.32	18.67
003-SRB-1	75.8			71.78	37.88
010-SRB-1	133.8	31.5	27.0	58.71	34.01
005-SRB-1	52.7	70.0	70.0	28.57	17.76
021-SRB-3	24.3	0	0	40.96	39.20
022-SRB-3	38.1	0	0	33.79	15.66
023-SRB-3	.	.	.	.	.
024-SRB-3	29.2	0	0	48.81	18.78
025-SRB-3	22.1	0	0	12.00	3.88
026-SRB-3	34.3	0	0	41.74	-8.36
027-SRB-3	25.6	0	0	25.66	-15.97
028-SRB-3	.	.	.	.	.
029-SRB-3	28.9	0	0	43.61	10.35
030-SRB-3	20.3	0	0	27.97	19.05
031-SRB-3	30.2	0.0	0.0	39.7	36.9
032-SRB-3	20.5367	0	0	49.31	35.04
033-SRB-3	44.3163	0	0	40.89	-10.83
034-SRB-3	19.9869	0	0	10.17	5.85
035-SRB-3	25.7616	0	0	34.77	22.95
036-SRB-3	38.7617	0	0	55.44	44.83
037-SRB-3	49.479	0	0	28.93	18.39
038-SRB-3		0	0	39.27	22.51
039-SRB-3		0.0	0.0	1.1	-9.0
040-SRB-3		0.0	0.0	11.8	7.7
041-SRB-3				39.88	14.51
042-SRB-3				#DIV/0!	#DIV/0!
043-SRB-3				#DIV/0!	#DIV/0!

Heart Rate						
BL before (30s)	min during	recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)
87.7	55.0	67.2	64.1	32.7	23.6	92.2
60.5	31.0	49.9	35.7	29.4	24.8	104.7
71.3	42.0	56.9	51.0	29.3	20.2	94.2
80.6	42.9	59.5	52.8	37.7	27.8	70.7
58.0	34.4	46.0	42.5	23.7	15.5	71.1
62.3	23.0	59.4	45.7	39.3	16.7	106.2
62.9	38.8	60.3	46.9	24.1	16.1	102.1
58.6	27.8	66.5	56.8	30.7	1.8	94.0
75.2	46.4	71.8	56.0	28.8	19.2	94.1
68.7	42.6	62.8	46.1	26.1	22.6	97.6
66.8	41.6	62.8	45.0	25.2	21.8	104.6
63.4	51.4	70.6	67.7	12.1	-4.3	80.4
53.9	31.8	62.1	40.9	22.2	13.0	84.1
59.4	43.6	66.6	48.2	15.9	11.2	83.7
82.2	47.1	67.3	58.5	35.2	23.7	82.4
73.1	51.0	65.9	59.5	22.2	13.7	104.7
69.8	19.7	52.9	43.4	50.1	26.5	58.7
69.3	28.6	78.3	45.7	40.7	23.6	101.8
54.5	38.9	53.5	44.8	15.6	9.7	81.1
67.5	39.9	63.0	41.0	3.8	-3.0	71.3
44.9	29.7	39.6	37.9	15.2	7.0	100.2
.	.	.	.	.	.	.
76.4	39.1	52.7	62.1	37.3	14.3	96.5
69.5	61.1	69.0	66.8	8.3	2.7	91.3
81.1	47.2	79.2	87.8	33.8	-6.8	82.3
66.3	49.3	64.9	76.9	17.0	-10.6	86.9
.	.	.	.	.	.	.
64.1	36.2	55.2	57.5	28.0	6.6	89.3
71.5	51.5	74.3	57.9	20.0	13.6	97.5
76.6	46.2	77.9	48.4	30.4	28.3	98.4
66.4	33.7	19.8	43.2	32.8	23.3	104.5
52.6	31.1	48.3	58.3	21.5	-5.7	108.0
62.4	56.0	60.3	58.7	6.3	3.6	93.3
69.2	45.1	62.4	53.3	24.0	15.9	102.5
70.0	31.2	58.2	38.6	38.8	31.4	98.4
65.3	46.4	84.0	53.3	18.9	12.0	86.4
83.7	50.8	72.3	64.9	32.9	18.8	82.0
70.7	69.9	69.3	77.0	0.8	-6.4	90.2
56.1	39.7	54.0	42.4	16.3	13.6	90.0
64.8	39.0	53.5	55.4	25.8	9.4	85.5
				0.0	0.0	
				0.0	0.0	

COLD + APNEA

MAP						
max during	recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)	max during
127.2	91.0	119.5	-35.0	-27.3	8.5	15.5
138.1	111.5	124.0	-33.5	-19.3	24.5	71.6
136.2	98.9	128.5	-41.9	-34.3	15.1	43.6
96.4	74.0	88.6	-25.7	-17.9	8.5	20.7
100.3	87.7	94.5	-29.2	-23.4	13.4	24.1
155.5	113.3	138.5	-49.3	-32.3	25.2	87.7
139.7	110.6	135.3	-37.6	-33.2	19.7	56.5
139.7	78.0	132.4	-45.7	-38.4	17.6	54.9
117.3	92.4	112.1	-23.2	-18.0	15.2	67.1
124.3	103.2	118.8	-26.7	-21.2	14.0	27.0
138.2	117.4	118.3	-33.6	-13.6	17.3	45.4
117.4	84.2	111.5	-37.0	-31.1	12.9	64.8
120.5	97.0	113.5	-36.4	-29.4	16.3	64.3
110.8	90.0	108.2	-27.2	-24.5	17.2	24.0
96.5	84.2	92.3	-14.1	-9.9	10.2	17.4
133.3	111.2	128.1	-28.7	-23.5	16.9	26.4
96.3	114.4	80.8	-37.6	-22.2	5.2	9.0
133.6	106.8	122.0	-31.8	-20.2	14.7	61.5
104.5	87.7	93.0	-23.3	-11.8	18.0	33.1
91.5	82.7	89.5	-20.3	-18.2	25.0	36.7
149.4	113.8	138.4	-49.2	-38.1	24.5	65.3
.	.	.	.	.	.	.
137.3	100.2	116.4	-40.8	-19.9	13.7	34.9
133.9	89.1	127.2	-42.6	-35.9	11.9	37.0
121.9	84.5	85.2	-39.6	-2.8	8.9	12.3
107.2	87.3	94.1	-20.3	-7.2	10.0	12.0
.	.	.	.	.	.	.
119.2	80.9	124.1	-29.9	-34.9	10.3	14.5
125.7	98.3	119.5	-28.3	-22.0	15.1	24.1
102.7	94.5	102.8	-4.3	-4.5	12.7	8.8
129.3	106.6	119.3	-24.7	-14.8	19.6	38.7
136.2	101.2	129.7	-28.2	-21.7	25.9	50.1
121.2	93.4	116.0	-27.9	-22.7	13.9	17.0
132.0	103.7	127.5	-29.5	-25.0	17.6	35.8
127.8	104.2	121.4	-29.5	-23.0	9.9	23.8
114.4	88.4	109.4	-28.0	-23.0	21.8	46.4
116.3	77.9	99.7	-34.3	-17.7	8.6	16.0
114.3	91.2	107.4	-24.2	-17.2	17.1	22.0
121.1	90.0	117.5	-31.0	-27.5	14.2	22.8
111.0	103.2	85.7	-25.5	-0.2	13.8	27.5
			0.0	0.0		
			0.0	0.0		

TPR						Heart
recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)	min during	recovery average
9.3	14.2	-7.0	-5.7	70.6	60.1	63.7
29.5	64.3	-47.1	-39.8	48.9	41.0	55.1
18.2	35.6	-28.5	-20.5	59.7	38.7	72.3
13.3	14.7	-12.2	-6.3	63.9	53.4	62.2
20.2	20.9	-10.7	-7.5	50.2	38.5	53.6
26.6	60.1	-62.6	-34.9	58.0	45.7	57.7
19.4	47.6	-36.7	-27.9	61.5	43.1	62.5
13.2	31.4	-37.3	-13.8	56.2	44.6	57.6
14.0	31.2	-51.8	-16.0	70.4	51.0	69.9
15.6	24.9	-12.9	-10.9	65.1	52.8	69.7
21.0	34.5	-28.1	-17.2	55.3	40.9	57.9
11.6	32.1	-51.9	-19.3	52.2	47.1	47.9
16.6	49.0	-48.0	-32.7	53.0	27.7	62.8
14.7	22.4	-6.8	-5.2	61.1	45.6	62.5
11.3	12.2	-7.2	-2.0	72.0	58.6	74.7
18.7	23.2	-9.5	-6.3	62.9	41.1	69.6
24.1	7.3	-3.8	-2.2	62.3	28.6	66.8
11.3	30.9	-46.8	-16.2	65.3	24.7	70.6
16.8	27.3	-15.1	-9.4	54.5	38.3	55.4
24.7	34.3	-11.7	-9.3	60.4	36.5	36.5
25.5	53.4	-40.8	-28.9	40.5	31.2	31.2
.	.	.		.	.	.
18.5	18.1	-21.1	-4.4	65.8	39.1	39.1
10.8	30.2	-25.1	-18.2	67.9	56.9	56.9
9.0	8.8	-3.4	0.0	76.9	49.6	49.6
11.1	10.4	-2.0	-0.4	65.8	61.7	61.7
.	.	.		.	.	.
10.4	14.6	-4.2	-4.3	54.1	40.2	40.2
15.2	21.3	-8.9	-6.2	66.0	52.7	52.7
13.6	12.8	3.9	-0.1	74.3	46.0	46.0
19.8	32.2	-19.0	-12.6	61.3	54.2	54.2
22.6	29.2	-24.2	-3.3	52.0	30.4	30.4
13.5	15.4	-3.1	-1.5	65.3	57.7	57.7
18.6	29.7	-18.2	-12.1	69.5	58.3	58.3
11.4	19.2	-13.9	-9.4	72.2	34.9	34.9
14.7	25.0	-24.5	-3.2	60.6	52.1	52.1
9.7	11.6	-7.4	-3.1	79.6	60.6	60.6
16.7	18.8	-4.9	-1.7	68.8	60.7	60.7
13.0	21.2	-8.6	-7.0	57.6	45.3	45.3
19.8	16.4	-13.7	-2.6	48.8	37.5	60.7
		0.0	0.0			
		0.0	0.0			

						COLD
: Rate			MAP			
Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)	max during	recovery average	Average last 10 s
66.2	10.4	4.3	74.2	104.8	90.8	86.7
43.6	7.9	5.3	97.0	130.9	102.9	127.3
55.8	21.0	3.8	100.9	126.4	92.0	116.0
60.0	10.5	3.9	71.8	75.8	66.2	50.7
64.0	11.7	-13.9	67.1	77.0	68.5	66.1
52.3	12.3	5.7	100.2	140.8	109.3	134.5
50.4	18.3	11.1	97.2	129.4	107.5	123.3
55.6	11.6	0.5	96.6	129.8	112.7	119.7
63.1	19.4	7.3	89.3	114.7	90.9	107.0
65.6	12.3	-0.5	93.4	121.7	99.7	115.1
44.2	14.4	11.0	96.9	129.6	115.5	120.1
51.7	5.1	0.5	65.9	79.0	78.4	66.3
44.6	25.3	8.4	82.1	98.9	76.6	83.8
52.6	15.5	8.5	85.3	106.1	87.3	104.1
65.8	13.4	6.2	83.1	85.1	78.6	81.6
57.2	21.7	5.6	108.6	138.5	110.1	129.3
43.2	33.7	19.1	90.1	142.2	101.2	115.9
54.3	40.5	11.0	100.3	142.6	104.7	122.4
42.0	16.1	12.5	82.0	88.1	83.2	84.1
42.3	23.9	18.1	81.5	108.9	88.2	96.1
34.5	9.4	6.0	102.2	119.2	99.2	116.9
.	.	.	.	.	.	.
46.6	26.7	19.1	104.5	132.6	100.3	126.2
64.9	11.0	3.0	93.6	111.9	93.7	105.1
69.6	27.3	7.3	84.9	110.6	87.1	101.0
76.9	4.1	-11.1	93.2	122.6	91.8	115.9
.	.	.	.	.	.	.
55.9	13.8	-1.8	80.6	129.9	83.7	123.5
65.4	13.3	0.6	94.3	126.5	97.0	120.9
70.5	28.3	3.8	101.6	115.3	99.3	107.2
66.9	7.1	-5.7	106.7	135.0	112.9	123.3
47.8	21.6	4.1	103.7	124.1	107.0	115.6
63.0	7.7	2.4	95.2	122.8	97.2	113.8
64.1	11.2	5.4	103.2	125.7	101.2	119.7
40.3	37.3	31.9	95.5	131.3	107.7	121.0
55.1	8.5	5.5	91.6	102.3	84.0	100.3
75.1	19.0	4.4	85.1	95.6	83.8	88.1
68.5	8.1	0.3	89.3	110.3	92.0	98.8
51.1	12.3	6.5	96.9	113.3	95.3	109.4
53.8	11.3	-5.0	90.8	107.5	100.7	90.3
	0.0	0.0				
	0.0	0.0				

		TPR				
Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)	max during	recovery average	Average last 10 s	Delta before vs min during
-30.6	-12.5	9.2	13.0	11.6	10.2	-3.8
-33.9	-30.3	24.8	52.5	23.5	46.3	-27.7
-25.5	-15.2	19.7	40.5	12.9	25.6	-20.8
-4.0	21.1	11.0	11.4	11.0	9.9	-0.4
-9.8	1.1	15.9	24.1	15.9	17.2	-8.1
-40.6	-34.3	23.2	45.8	27.9	41.4	-22.6
-32.1	-26.1	19.0	36.5	19.6	31.8	-17.5
-33.1	-23.0	21.9	36.7	23.2	26.0	-14.7
-25.5	-17.8	14.9	25.6	15.1	21.9	-10.7
-28.3	-21.7	13.8	21.3	13.7	16.9	-7.5
-32.7	-23.2	20.6	44.3	23.1	40.1	-23.7
-13.1	-0.4	12.4	17.3	13.5	12.7	-4.9
-16.8	-1.7	15.6	29.0	11.4	16.9	-13.4
-20.7	-18.8	16.1	22.1	14.6	20.8	-6.0
-2.0	1.6	10.9	12.0	10.7	11.2	-1.0
-29.9	-20.7	19.5	38.5	16.3	24.0	-19.0
-52.1	-25.8	15.8	52.8	15.9	33.5	-37.0
-42.2	-22.0	14.5	39.0	13.3	20.4	-24.4
-6.1	-2.1	17.0	26.0	17.1	24.3	-9.0
-27.4	-14.6	23.3	37.8	24.8	31.7	-14.5
-17.1	-14.8	28.2	44.6	25.8	38.0	-16.4
.	.	.	.	.	.	.
-28.1	-21.8	17.0	31.3	18.6	27.6	-14.3
-18.3	-11.5	11.5	12.6	12.2	12.1	-1.0
-25.7	-16.1	9.9	12.4	10.3	11.2	-2.5
-29.5	-22.8	9.1	11.3	9.3	10.2	-2.2
.	.	.	.	.	.	.
-49.4	-42.9	13.1	18.3	10.3	15.1	-5.2
-32.3	-26.7	16.4	23.4	15.1	19.5	-7.0
-13.7	-5.6	14.4	16.1	12.1	12.2	-1.7
-28.3	-16.6	24.0	30.7	22.8	27.2	-6.7
-20.4	-11.9	24.9	43.5	23.8	25.3	-18.5
-27.5	-18.5	13.4	15.3	14.3	13.4	-1.9
-22.5	-16.5	17.5	23.8	17.6	22.3	-6.3
-35.8	-25.5	9.4	19.1	12.5	17.6	-9.7
-10.7	-8.7	21.5	28.8	18.8	24.8	-7.3
-10.5	-3.0	9.6	11.6	10.0	9.9	-1.9
-21.0	-9.5	17.4	21.0	18.0	18.8	-3.6
-16.4	-12.5	14.1	18.8	13.9	16.9	-4.7
-16.7	0.5	19.4	26.0	16.1	17.1	-6.6
0.0	0.0					0.0
0.0	0.0					0.0

	Heart Rate					
Delta before vs 10 sec avg	BL before (30s)	min during	recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg
-1.1	77.6	33.7	66.4	57.4	43.9	20.2
-21.6	62.2	30.4	52.2	54.8	31.8	7.4
-6.0	73.3	48.3	54.1	50.3	25.0	23.0
1.1	79.7	52.5	68.1	55.8	27.2	24.0
-1.3	74.3	37.0	46.7	48.0	37.3	26.3
-18.2	66.1	37.7	65.4	46.6	28.4	19.5
-12.8	66.0	92.8	57.4	84.4	-26.8	-18.4
-4.1	67.0	54.8	59.7	69.6	12.3	-2.5
-7.0	71.5	54.7	76.7	70.8	16.8	0.7
-3.1	80.3	58.8	70.5	60.2	21.4	20.1
-19.5	74.2	41.6	58.8	50.0	32.6	24.2
-0.3	56.0	60.2	64.5	71.8	-4.2	-15.8
-1.3	54.7	44.8	55.7	53.1	9.9	1.7
-4.7	68.0	51.8	70.0	60.4	16.2	7.6
-0.3	80.3	50.3	84.8	54.5	30.1	25.8
-4.6	76.2	53.0	69.7	65.2	23.2	11.0
-17.7	74.1	56.8	63.7	68.8	17.3	5.3
-5.8	64.2	43.2	75.6	48.4	21.0	15.8
-7.3	54.6	42.1	50.3	52.4	12.5	2.2
-8.4	62.5	58.7	63.7	65.5	3.8	-3.0
-9.8	47.9	38.6	35.9	46.1	9.3	1.8
.	.	.	.	.	.	.
-10.6	52.3	50.4	63.0	66.7	1.9	-14.4
-0.6	68.5	62.6	73.5	76.3	5.9	-7.8
-1.3	79.4	53.2	81.1	73.4	26.2	6.0
-1.1	69.2	64.1	65.2	67.7	5.1	1.5
.	.	.	.	.	.	.
-2.0	57.8	52.7	57.2	57.8	5.1	0.0
-3.0	67.9	64.9	69.5	67.9	3.1	0.0
2.3	71.3	65.2	74.8	76.3	6.1	-4.9
-3.2	65.4	53.8	60.8	61.9	11.5	3.5
-0.3	53.2	32.7	53.5	60.7	20.5	-7.4
0.0	65.7	62.7	59.5	70.1	3.0	-4.4
-4.8	72.3	61.5	62.6	69.2	10.8	3.0
-8.2	63.7	44.2	57.2	48.5	19.5	15.1
-3.3	62.7	55.5	66.4	58.3	7.1	4.4
-0.3	75.0	56.4	75.7	73.5	18.7	1.5
-1.4	86.1	85.2	71.1	88.7	0.9	-2.6
-2.7	56.0	49.3	59.2	51.7	6.6	4.3
2.3	51.8334692	35.2576	47.0243889	50.7787192	16.6	1.1
0.0					0.0	0.0
0.0					0.0	0.0

Apnea

MAP

BL before (30s)	min during	recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg	BL before (30s)
84.2	154.5	109.8	137.8	-70.4	-53.6	9.0
94.7	147.5	99.2	132.9	-52.8	-38.2	22.0
88.0	116.6	94.9	109.4	-28.6	-21.4	13.2
72.9	100.5	71.5	93.8	-27.6	-20.9	9.1
77.5	106.0	81.6	94.9	-28.5	-17.4	12.7
109.4	147.4	112.4	140.1	-38.0	-30.6	22.8
96.3	142.6	97.3	137.4	-46.3	-41.1	18.0
96.4	126.0	90.8	122.4	-29.6	-26.0	17.4
88.9	107.1	83.4	99.9	-18.2	-10.9	15.5
95.7	108.6	92.3	106.0	-12.9	-10.3	12.6
99.5	129.7	107.8	123.3	-30.2	-23.8	16.3
65.1	80.0	60.0	77.1	-15.0	-12.0	15.8
81.9	115.6	87.6	111.7	-33.7	-29.8	17.0
88.3	114.2	80.3	107.5	-26.0	-19.3	16.4
86.7	122.9	85.0	111.8	-36.2	-25.1	10.7
102.1	131.5	102.7	119.2	-29.4	-17.0	14.0
92.7	131.3	96.1	123.0	-38.6	-30.3	16.0
97.7	131.2	91.1	119.3	-33.5	-21.6	15.1
84.1	99.8	86.6	97.4	-15.7	-13.3	18.7
71.6	67.5	70.8	71.2	4.2	0.4	26.4
95.4	94.7	105.3	121.2	0.6	-25.8	24.4
.	.	.	.	.	.	.
97.0	103.6	95.0	122.5	-6.6	-25.5	21.6
92.8	85.5	81.0	114.4	7.3	-21.6	13.2
81.9	78.0	80.1	101.8	3.9	-19.9	8.7
90.0	88.4	79.3	96.3	1.5	-6.4	9.8
.	.	.	.	.	.	.
85.7	110.2	79.1	99.4	-24.6	-13.8	12.6
92.4	105.0	92.7	96.9	-12.6	-4.5	15.6
99.7	110.9	96.6	97.4	-11.2	2.3	13.8
115.5	132.8	111.2	117.4	-17.3	-1.9	27.6
108.6	138.0	99.7	130.2	-29.4	-21.6	24.2
95.8	101.3	90.8	93.8	-5.5	2.0	13.9
99.2	126.9	95.4	114.7	-27.6	-15.5	16.2
93.4	130.9	100.4	127.3	-37.5	-33.9	10.4
92.0	106.6	91.8	96.6	-14.5	-4.5	22.3
82.5	100.8	81.9	89.6	-18.3	-7.1	10.0
86.9	116.1	94.0	113.7	-29.2	-26.8	12.9
90.4	121.0	91.4	116.1	-30.6	-25.7	14.0
84.7921346	97.2292	92.5076889	81.6971	-12.4	3.1	18.1410577
				0.0	0.0	
				0.0	0.0	

TPR				
max during	recovery average	Average last 10 s	Delta before vs min during	Delta before vs 10 sec avg
44.4	14.4	32.2	-35.3	-23.2
59.5	23.0	49.4	-37.5	-27.4
30.5	19.5	26.9	-17.3	-13.6
16.4	11.7	15.0	-7.3	-5.9
22.6	18.8	19.4	-9.9	-6.7
63.3	23.6	53.1	-40.5	-30.4
27.1	18.1	23.8	-9.0	-5.7
29.2	18.6	27.1	-11.9	-9.8
22.1	12.6	17.4	-6.7	-1.9
18.4	12.6	17.2	-5.7	-4.6
42.3	19.3	29.8	-26.0	-13.5
94.9	11.5	30.6	-79.1	-14.8
43.2	16.0	37.1	-26.2	-20.0
24.7	14.5	20.0	-8.3	-3.6
17.1	9.2	14.9	-6.4	-4.1
23.7	16.6	18.9	-9.6	-4.8
37.6	18.1	31.8	-21.7	-15.8
41.5	10.5	30.2	-26.4	-15.2
26.7	19.3	21.6	-7.9	-2.8
22.2	26.4	25.4	4.2	1.0
22.1	29.3	34.7	2.3	-10.3
.	.	.	.	.	.
19.9	17.0	22.4	1.7	-0.8
12.4	9.6	21.9	0.9	-8.7
7.6	8.0	8.9	1.1	-0.2
9.9	10.0	10.6	-0.1	-0.8
.	.	.	.	.	.
16.3	12.0	13.8	-3.7	-1.2
18.2	15.0	17.1	-2.6	-1.5
15.1	13.3	12.5	-1.3	1.3
38.3	25.4	29.4	-10.7	-1.8
42.3	22.3	29.3	-18.1	-5.1
13.6	14.4	12.6	0.3	1.3
25.7	17.4	21.2	-9.4	-4.9
18.8	11.5	16.4	-8.4	-6.0
25.4	20.7	21.1	-3.1	1.1
14.5	10.0	10.4	-4.5	-0.4
17.7	16.7	17.3	-4.8	-4.4
18.2	12.7	16.7	-4.1	-2.7
27.7956	20.9806889	16.8424	-9.7	1.3
			0.0	0.0
			0.0	0.0

Curriculum Vitae

Name:	Sydney Smith
Post-secondary Education and Degrees:	Dalhousie University Halifax, Nova Scotia, Canada 2012-2017 B.Sc. (Hons) Western University London, Ontario, Canada MSc in Kinesiology, Integrative Biosciences, 2017-2019 Queen's University Kingston, Ontario, Canada Graduate Diploma in Business, 2019
Honours and Awards:	Dalhousie University Undergraduate Entrance Scholarship 2012 Academic All-Canadian 2014-2019 Dean's Honour List 2014-2019 Western Graduate Research Scholarship, 2017-2019 Province of Ontario Graduate Scholarship 2018-2019
Related Work Experience	Teaching Assistant The University of Western Ontario 2017-2018

Publications:

1. Moir ME, Klassen SA, Al-Khazraji BA, Woehrle E, Smith SO, Matushewski BJ, KozicD, Dujic Z, Barak OF, Shoemaker JK. Impaired dynamic cerebral autoregulation in trained breath-hold divers. J Appl Physiol. (In Press).

2. Moir ME, Woehrle E, Smith SO, Klassen SA, Matushewski BJ, Barak OF, Dujic Z, Kozic D, Shoemaker JK. Cerebrovascular regulation in breath-hold divers with chronic exposure to long-duration apneas. Experimental Biology, April 2019.

3. Smith, SO, Woehrle, E., Klassen, SK, Jacobs, KG, Knetsch, RJ, Shoemaker, JK. Effects of Contralateral Forearm Somatosensory Stimulation on Heart Rate Responses to Isometric Hand Grip Exercise. Experimental Biology, April 2018.

4. Jacobs KG, Woehrle E, Smith SO, Klassen SA, Knetsch RJ, Barker AL, Shoemaker JK. Sex differences in heart rate response to isometric handgrip exercise with concurrent contralateral forearm somatosensory stimulation. Experimental Biology, April 2018.

5. Smith, S., Grandy, S., (2017). Investigating the necessity for cardiovascular screening for college aged cross country runners. Atlantic Provinces Exercise Scientist and Socioculturalists (APES+) March 2017; Crossroads Conference, March 2017.