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The impact of primary motor cortex, spinal cord, and sciatic nerve cooling on spinal reflex activity in the rat: a reversible deactivation study

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THE IMPACT OF PRIMARY MOTOR CORTEX, SPINAL CORD, AND
SCIATIC NERVE COOLING ON SPINAL REFLEX ACTIVITY IN THE RAT: A
REVERSIBLE DEACTIVATION STUDY

by

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Most Sincerely,

DJO
THE IMPACT OF PRIMARY MOTOR CORTEX, SPINAL CORD, AND SCIATIC NERVE COOLING ON SPINAL REFLEX ACTIVITY IN THE RAT: A REVERSIBLE DEACTIVATION STUDY

DANIEL JAMES OLIx

ABSTRACT

The influence of spinal reflex arcs on lower limb movement cannot be understated, but the individual contribution of various parts of the reflex pathway, namely the primary motor cortex, spinal cord, and sciatic nerve, are incompletely known. This study aims to consider each of these to develop a better understanding of how spinal cord reflexes and the relationship between the central and peripheral nervous systems, particularly in terms of motor control. In the anesthetized rat, recording electrodes were placed in the tibialis anterior muscle of the hindlimb to record both the direct muscle response (M-wave) and the muscle reflex response (H-wave) in response to electrical stimulation of the sciatic nerve. After baseline recordings, thermal deactivation was used to selectively silence the primary motor cortex, spinal cord, or sciatic nerve in the rat and test the hypothesis that different locations exerted different effects on the excitability and timing of the spinal cord reflexes. Deactivation of motor cortex produced a faster or more excitable spinal cord reflex, whereas sciatic nerve deactivation produced a profound attenuation of both the M and the H waves. This study strongly supports the contention that the motor cortex, through pathways that travel through the spinal cord, normally serves to inhibit the excitability of spinal cord reflexes.
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LIST OF ABBREVIATIONS

CNS ................................................................. central nervous system
CSF ................................................................. cerebrospinal fluid
DRG ................................................................. dorsal root ganglion
MEPs ............................................................... motor evoked potentials
PNS ................................................................. peripheral nervous system
SSEPs .............................................................. somatosensory evoked potentials
INTRODUCTION

Spinal Cord Anatomy

The spinal cord is an integral part of the central nervous system and serves as the vital link between the body and the brain, extending from the base of the brain to the level of the lumbar vertebrae within the vertebral canal (Knierim, 2015). The spinal cord is enclosed and protected within the bony vertebral column and is about 45 cm long in men and 43 cm long in women, and is about 1.5 cm in diameter (Bican, Minagar, & Pruitt, 2013). The spinal cord is divided into five different regions, each of which corresponds to the five vertebral regions: cervical, thoracic, lumbar, sacral, and coccygeal (Knierim, 2015). Within each of the major spinal cord regions, the spinal cord is divided into segments; the numbers of segments vary according to the length of the division. Each segment is associated with two nerve roots on each side; in total there are 31 nerve roots that extend from each side and form the spinal nerves (Marieb, 2011); these nerves are divided into 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal nerves. It is important to note that there is a mismatch between the length of the vertebral canal and the length of the spinal cord; namely, the spinal cord itself ends at the lumbar vertebral level (Boonpirak & Apinhasmit, 1994). Therefore, the segments of the spinal cord that receive and emit nerves that come from or terminate in the lumbar regions exist at the thoracic level of the vertebral canal. This mismatch creates a large number of nerves that travel inferiorly to go through foramina between the vertebra (intervertebral foramina)
and together form a bundle of nerves that is present below the 2\textsuperscript{nd} lumbar level (Levy, 2014). This is referred to as the cauda equina ("horse’s tail") (Marieb, 2011).

There are also two enlargements found on the spinal cord, the first of which is between the third cervical and first thoracic vertebrae and the second between the first lumbar and second sacral vertebrae (Knierim, 2015). These two enlargements correspond to a larger number of neuronal cell bodies (particularly the neurons controlling muscles) associated with an increased number of voluntary muscles in the upper and lower limb relative to the thoracic area, which contains only appendicular muscles (Levy, 2014).

The spinal cord is covered by three connective tissue layers: the pia mater, the arachnoid mater, and the dura mater (Decimo, Fumagalli, Berton, Krampera, & Bifari, 2012). The dura mater is the outermost layer of the meninges, and it is a tough and inflexible layer of dense collagenous tissue (McCaffrey, 2014). The arachnoid mater is the middle layer and the pia mater is the layer closest to the spinal cord and the brain. Between the arachnoid and pia mater layers is the subarachnoid space, which is filled with cerebrospinal fluid. Additionally, all blood vessels and cranial nerves pass through the subarachnoid space (Decimo et al., 2012). The cerebrospinal fluid, or CSF, is produced in the ventricles in the brain, and is a source of protection for the spinal cord and brain as well as a nutrient and waste exchanger (Brinker, Stopa, Morrison, & Klinge, 2014).
The structure of transversely-sectioned spinal cord is divided into a gray matter interior, which consists largely of neuronal cell bodies, and a white matter exterior, which contains large bundles of nerve fibers (Marieb, 2011). The gray matter is shaped like a butterfly, with the two butterfly “wings” connected in the middle, and it contains the neuronal cell bodies and glia (Knierim, 2015). The gray matter is divided into three main parts: the dorsal horn, the lateral horn, and the ventral horn (Marieb, 2011). The dorsal horn, or dorsal root, is found throughout the entire spinal cord and it receives incoming sensory information from the periphery (Knierim, 2015). From the dorsal horn, sensory information is transmitted to the brain or to neurons in the spinal cord (Takahashi, Ohtori, & Takahashi, 2010). The lateral horn contains autonomic neurons for sympathetic and parasympathetic innervation of the internal organs. The ventral horn is present at every level of the spinal cord and is where the motor neurons’ cell bodies that innervate skeletal muscle are located (Rezania & Roos, 2013).

The white matter encapsulation of the spinal cord consists of axon bundles and neuronal cell body processes that relay signals from brain to the spinal cord motor neurons, or from the sensory neurons to the brain (Marieb, 2011). Many of the sensory axon parent neurons in the white matter are spinal cord sensory neurons in the dorsal horns, but many sensory axons that travel to brain have their cell bodies in a grouping in the dorsal root. The dorsal roots are branches of the incoming sensory component of the spinal nerves (Levy, 2014). The cluster of cells in the center of the root is called a dorsal root ganglion (DRG), and each DRG contains neurons that relay all sensation from a specific region of
the body surface to the brain (Levy, 2014). Some of the DRG cells axons enter the spinal
cord and synapse with neurons in the dorsal horn, but some go directly to the brain
without synapse (Don Fitz-Ritson, 1979).

Sensory neurons carry three main types of sensation: somatosensation, pain, and
proprioception. Each of these sensation modalities is transduced from a physical or
chemical signal to a neural (electrical) signal via dedicated receptors (Barabas, Mattson,
Aboualizadeh, Hirschmugl, & Stucky, 2014). Somatosensation receptors are present both
in and nearby the skin and mucosal surfaces and include hair follicles, Merkel cells,
Pacinian corpuscles, and Meissner’s corpuscles (Reed-Geaghan & Maricich, 2011).
Receptors for pain and temperature receive signals from free nerve endings and bulbs of
Krause (Swieboda, Filip, Prystupa, & Drozd, 2013). Receptors for proprioception include
two types of receptors: the muscle spindle, which senses changes in muscle length, and
the Golgi tendon organ, which senses changes in muscle tension (Linder & Melby, n.d.).
Muscle spindles run parallel to normal muscle fibers and are encased in a capsule that is
surrounded by the sensory end of a primary (Ia), muscle afferent neuron (Banks, 1994).
When the muscle is stretched, this activates the afferent sensory neuron and it triggers a
signal to the DRG (Linder & Melby, n.d.). When the muscle’s length increases, the Ia
afferent firing frequency also increases, causing the muscle to contract and regulate its
length via the monosynaptic stretch reflex (Vilis, n.d.). The Golgi tendon organs are
located in tendons, which attach muscle to bone. When a muscle contracts, tension is put
on the tendon, which activates the Golgi tendon organs, which in turn send a signal via a Ib sensory afferent neuron to the DRG (Linder & Melby).

Spinal cord output occurs through neurons in the ventral horn. The ventral roots of the spinal cord are collections of efferent motor axons, and the ventral roots merge with the spinal nerve, then travel to, synapse with, and control muscle activation (Marieb, 2011). There are two different types of efferent motor neurons from the ventral root. The first type is the alpha motor neuron, which innervates extrafusal muscle fibers (normal skeletal muscle fibers), and the second is the gamma motor neuron, which innervates the intrafusal muscle fibers (Bessou, Emonet-Denand, & Laporte, 1965). Intrafusal muscle fibers, which are encapsulated in a collagen sheath, are specialized skeletal muscle fibers that are part of the muscle spindles (Rumsey, Das, Bhalkikar, Stancescu, & Hickman, 2010). These efferent motor neurons innervate muscles fibers, and the neuron and the muscle fibers a single neuron innervates are collectively known as a motor unit (Linder & Melby). One efferent motor neuron can synapse with multiple muscle fibers, but each muscle fiber synapses with only one motor neuron.

**Reflexes**

The circuit whereby a sensory input is paired with a motor output at the level of the spinal cord is called a spinal cord reflex, or a reflex arc. The simplest reflex is the direct connection between the DRG neuronal process and the alpha motor neurons (Marieb, 2011). Reflexes are rapid, involuntary responses to stimuli, and can be somatic, resulting
in the contraction of skeletal muscle, or visceral, which activate smooth muscle, cardiac muscle, and glands. Every reflex has five basic components, which happen in the following order: 1) The receptor, located at the end of a peripheral sensory neuron is stimulated; 2) The sensory neuron transmits the afferent impulse data to the central nervous system; 3) The afferent neuron data goes to an integration center within the central nervous system (CNS), usually the spinal cord. The integration center can either be a simple, single synapse between the afferent sensory neuron and efferent motor neuron, or it can be more complex and involve one or more interneurons between the afferent and efferent neurons; 4) The efferent motor neuron receives a signal and transmits the impulse to some effector, which can be a muscle or gland; 5) The effector responds accordingly, either contracting if a muscle, or secreting something if a gland (Marieb, 2011).

Simple reflexes, also known as monosynaptic reflexes, do not have an interneuron between the afferent sensory and efferent motor neurons. These reflexes are fast and automatic: the sensory neuron directly activates the motor neuron. Stretch reflexes are examples of monosynaptic reflexes, and they help to maintain body equilibrium and posture (Marieb, 2011). More complex reflexes, known as polysynaptic reflexes, include one or more interneurons between the sensory and motor neurons. The most simple of these polysynaptic reflexes is the withdrawal reflex, which includes only one interneuron (Marieb, 2011). Interneurons have multiple functions, and therefore polysynaptic reflexes are not quite as fast as monosynaptic ones. Polysynaptic reflex interneurons have to
integrate all the sensory input, then initiate the motor output. The integration includes processing the nerve impulses to locate the stimulus on the body, identify its source, and then plan the appropriate motor response (Marieb, 2011).

Electrophysiology

There are various ways to record and analyze reflex responses, including somatosensory evoked potentials (SSEPs) and motor evoked potentials (MEPs). An SSEP shows how well a stimulus impulse is transmitted through the spinal cord and peripheral nervous system, or basically determines the integrity of the nerve fibers themselves (Curt & Dietz, 1999). MEPs on the other hand, are recorded from muscles of the upper and lower limbs, and can be used to assess the stability of cortical and spinal motor tracts, particularly following spinal cord injury (Curt & Dietz, 1999). These electrophysiological recordings provide valuable information about the underlying physiological mechanisms that allow for functional evaluations of the neuronal circuits in the CNS and peripheral nervous system (PNS) (Navarro et al., 1996). This study focuses on rat spinal cord reflex circuits evaluated via recorded MEPs.

Motor evoked potentials generate a measurable contraction in the muscle that can be recorded via needle electrodes placed in the muscle (Jameson, 2012). MEPs can then be evaluated based on the H-reflex. The H-reflex, first described by Johann Hoffman in 1918, is an electrically induced monosynaptic reflex that outlines the functionality of the afferent, spinal-segmental, and efferent pathways (Curt & Dietz, 1999). The H-reflex
gives two wave peaks: the M-wave and the H-wave. The M-wave, or direct muscle response, is an early response from the muscle that occurs shortly after direct stimulation of the motor axon (Palmieri, Ingersoll, & Hoffman, 2004). However, the M-wave is not a reflex response, as the H-wave is, because it is not elicited from the spinal cord, but rather just from direct stimulation of the motor axon, and therefore is not influenced by excitability changes within the CNS (Frigon, Carroll, Jones, Zehr, & Collins, 2007). The M-wave maximum value represents the total number of motoneurons that can be recruited during a stimulus (Palmieri et al., 2004). The second peak on the MEP is the H-wave, which reflects the H-reflex, which is the electrical manifestation of a monosynaptic reflex where the stimulus travels up the afferent (Ia sensory) fibers to a spinal motoneuron, then back down the efferent motor neuron to the muscle. The H-wave magnitude can be used to estimate the number of motor units that are being recruited during that stimulus (Palmieri et al., 2004). The delayed latency is due to the fact that it is a spinal cord reflex and must travel the length of the limb in question to the spinal cord and then back down to illicit a muscle response (Palmieri et al., 2004). To normalize the H-reflex, the H/M ratio is often used. This ratio can be used as a diagnostic tool to determine the proportion of motor units being recruited and used to the total number available (Palmieri et al., 2004).

What is not well understood, is the individual contributions of the motor cortex, spinal cord, and peripheral nerves to this withdrawal reflex arc, and that is the focus of this study.
Reversible Deactivation

One of the major deterrents to studying the roles that each part of the reflex arc plays has been the lack of an efficient and consistent procedure to reversibly deactivate the different regions in the system without damage. There are various techniques that can be used for neural deactivation, the most common of which is nerve transection, as well as drug injection and, more recently, cooling (Cooke et al., 2012). However, all lesion techniques, both reversible and not, have played an essential role in revealing the complex neural systems that control movement (Martin & Ghez, 1999). However, reversible deactivation is preferable to irreversible deactivation for several reasons. It can be repeated during multiple sessions with the same animal, which allows the animal to act as its own control (Martin & Ghez, 1999). It also allows for different combinations of sites to be deactivated at once, which can provide information on functional localization and cannot be reproduced in nonreversible deactivation studies (Cooke et al., 2012). The major advantage of neuronal reversible deactivation is that it has the capacity to eliminate transmission of signals without incurring damage to the circuits (Orton, Poon, & Rees, 2012). This latter aspect is important because damage of the circuit induces an inflammatory process that may obscure the effect of simply turning off a node of the network.

Neuronal cooling via a cryoloop is one way to achieve temporary, reversible deactivation. The cryoloop device can be permanently or temporarily implanted to an area of the CNS or PNS as an alternative method to creating a physical lesion. A cryoloop is a custom-
made device made of hypodermic tubing and cooling is achieved by passing cooled methanol through the tube (Lomber, Payne, & Horel, 1999). There are many advantages to the cryoloop, including increased selectivity and reversibility of the deactivated areas, the formation of stable and reversible effects and lastly, minimal neuronal degeneration following repeat coolings - all in a reproducible way (Lomber et al., 1999).

In this project, cooling devices were used in different areas in rats to study the impact of cooling deactivation on spinal reflex activity. Rodents and rats in particular, are the most commonly used study model in cerebral and spinal cord injury research. This is because they can be used in relatively large numbers and their larger body size allows for more precise evaluation of cortical and spinal motor networks (Iannaccone & Jacob, 2009). Additionally, although there are certainly differences between the rat and human motor systems, they share very similar signs and symptoms when reacting to cortical and spinal injuries, which are not found in other species (Onifer, Rabchevsky, & Scheff, 2007).

The H-reflex can be used to study the excitability of spinal motor neuronal circuitry, particularly in those patients that have disease or suffered a spinal cord injury, as many signs and symptoms of spinal cord injury are associated with changes in the H-reflex (Kumru et al., 2015). These recordings are of particular importance because changes are indicative of changes within the central nervous system (Frigon et al., 2007). These changes, which using these techniques can be found in noninvasive ways, can then be applied in a clinical setting.
The information from this study is particularly relevant to further understanding the interactions between the cortical and spinal systems and their role as a holistic network during motor execution, control, and learning. Clinically, the information could be used to improve rehabilitation and recovery following cortical or spinal injury. The study could be further expanded upon to address the specific impact or descending projections to populations or interneurons within the spinal cord through electrophysiology to intact subjects with incomplete spinal lesions.

**Specific Aims**

In order to determine interactions of the primary motor cortex, spinal cord, and sciatic nerve with spinal cord reflexes a rat model was utilized. Stimulating electrodes were places at the sciatic notch and recording electrodes were placed in the tibialis anterior muscle of the hindlimb. Each part of the pathway (primary motor cortex, spinal cord, sciatic nerve) was then individually cooled via a cryoloop and the sensory nerve was stimulated and the resulting H-reflex was recorded. Data was recorded before cooling, during cooling, and after cooling, and was then analyzed for significance. The expected results were as follows:

1) Cooling of the primary motor cortex or the spinal cord would not reduce the excitability of the spinal cord reflex and would delay the H wave.
2) Cooling of the sciatic nerve would cause the amplitude of both the M-wave and the H-wave to decrease and would cause both responses’ latencies to increase.
METHODS

Study Plan

Sprague-Dawley rats (n=5) were used in this study.

Rats were anesthetized with sodium pentobarbital (40mg/kg) and fixed in a rodent stereotaxic apparatus. Under aseptic conditions, surgery was performed to access the primary motor cortex, spinal cord, or sciatic nerve to implant the cryoloop cooling devices, which were built in advance to optimally fit the target regions. Additional doses of anesthesia were delivered as necessary (10 ml/kg) to maintain the anesthetic level. The rats were kept at a constant 38-39 °C rectal temperature through a warming pad.

Needle stimulation electrodes were placed at the sciatic notch and recording electrodes were placed in the belly of the tibialis anterior muscle of the rat’s hindlimb. A reference electrode was placed in the tip of the 4th finger and a ground electrode was placed at the base of the 5th finger. Recordings were carried out with EMG equipment (Zaphire Medelec) and stored online for further analysis (Powerlab). The temperature of deactivation was monitored through a thermocouple inserted at the union of cooling device.

Electrical stimulation was applied to the stimulation electrode at the sciatic notch. This produced activation of axons in the sciatic nerve, and led to a characteristic waveform
recorded from the muscle: the axons stimulated by the electric current that innervated the
muscle caused the muscle to move. This movement caused an electrophysiologically-
recorded wave called the M wave. The stimulation also produced activation of sensory
axons coming from the muscle – this activation was sent to the spinal cord, which then
activated the motor neurons that cause the muscle to contract: this phenomenon is called
the H wave.

Cooling of the different regions caused deactivation of the underlying axons and/or
neurons. Cold (-80 degrees C) methanol was circulated through the deactivation device.
The rate at which the methanol was circulated corresponded to the degree of deactivation:
a higher rate produced more deactivation while a slower rate caused less cooling. Data
were recorded before cooling (baseline), during cooling (at the subthreshold temperature
level), and 30 minutes after the cooling device had been turned off.

Data Analysis

For each rat, several parameters were measured from the pre-cooling (baseline) period,
the cooling period, and the post-baseline period. These parameters were:

1. M-wave magnitude
2. M-wave area under the curve
3. M-wave slope
4. M-wave latencies (beginning, positive peak, negative peak)
5. H-wave magnitude
6. H-wave area under the curve
7. H-wave slope
8. H-wave latencies (beginning, positive peak)
9. H/M magnitude ratio
10. H/M area under the curve ratio

Based on previously published literature on how to evaluate MEP curves, it was decided to focus on the data for the M-wave magnitude and beginning latency, the H-wave magnitude and peak latency, and the H/M magnitude ratio to complete the data analysis (Figure 1).

Data were analyzed to test the a priori hypothesis that cooling of these regions would have a significant effect on the amplitude and timing of the M and H wave. As a result, the baseline data were compared to the subsequent cooling data. In addition, the baseline data was compared to the post-cooling data to determine whether the measures had come back to baseline after cooling. Differences were determined using a Student’s t-test in Microsoft Excel, with significance set to $\alpha = 0.05$.

![Figure 1. Example of an H-reflex.](image_url)

**Figure 1. Example of an H-reflex.** An H-reflex curve showing the M-wave and H-wave. Also labeled are the locations of the four specific values that were used for data analysis. The fifth data point, the H/M Magnitude ratio, was determined from the M-wave magnitude and H-wave magnitude.
RESULTS

R1 (M1 COOLING)

R1 (Experiment 1)

The goal of this experiment was to determine the impact of deactivation of the primary motor cortex (M1) on the spinal cord reflexes. To generate the reflex, stimulation electrodes were placed at the sciatic notch, and recording electrodes were placed in the tibialis anterior muscle of the hindlimb. The M-wave represents the direct activation of the muscle from the stimulation. Since the sciatic nerve also contains sensory axons that carry signals from the muscle itself (e.g., muscle spindle fibers), stimulation of these fibers will produce a volley of action potentials that then enter the dorsal aspect of the spinal cord, and then monosynaptically activate the anterior horn neurons that control the muscle. This is a delayed response that produces an activation of the muscle and is registered as an H-wave. As a result, the H-wave reflects the electrophysiological correlate of the deep tendon reflex.

Before cooling, two baseline experiments were performed. In the first, intensity was set to generate a maximum response. In the second, the intensity was set to be at 150% of the H-wave threshold (Table 1, Figure 1). The responses were elicited when the cortex was warm (26 °C) and when the cortex was cooled slightly (21 °C). The data show that when the cortex was cooled by five degrees, there was a significant reduction in the M-
and the H-wave amplitudes and an increase in the latency of the M-wave when the stimulation was maximal. Importantly, cooling did not significantly change the H/M ratio in either experiment. Since the cortex was cooled to a value that did not produce deactivation of the layer 5 pyramidal neurons that contact the spinal cord, and since the H/M amplitude ratio is taken as the principal measure of reflex excitability, these data show that minor cooling of the motor cortex does not significantly change the excitability of the lumbar spinal cord reflex. Changes in the amplitudes of the M and the H wave may vary considerably along the course of the experiment, but the ratio normalizes the reflex to that variability. There did appear to be some change in the latency of the direct (M) response, but it is unclear whether this latency shift was due to normal variations in the elicited wave or some other factor.

Table 1. Data from R1 (M1 Cooling), Experiment 1. This table illustrates the differences between precooling and cooling data and precooling and postcooling data from two different stimulation intensities. The first set of data was at a stimulation intensity set to the maximum H-wave threshold, and the second at 150% of the H-wave threshold. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>preMax (29.6°C)</td>
<td>1.7614</td>
<td>0.08945</td>
<td>0.06725978</td>
<td>2.4525</td>
<td>10.1075</td>
</tr>
<tr>
<td>postMax (21°C)</td>
<td>1.1848535</td>
<td>0.0585235</td>
<td>0.067702666</td>
<td>2.54</td>
<td>9.8525</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>9.7212E-08</td>
<td>1.1461E-05</td>
<td>0.771444632</td>
<td>0.032985635</td>
<td>0.186711956</td>
</tr>
<tr>
<td>pre150% (26°C)</td>
<td>1.761219512</td>
<td>0.082243902</td>
<td>0.062922067</td>
<td>2.429268293</td>
<td>9.987195122</td>
</tr>
<tr>
<td>post150% (21°C)</td>
<td>1.452157895</td>
<td>0.06</td>
<td>0.051194002</td>
<td>2.460526316</td>
<td>10.61428571</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>1.2364E-07</td>
<td>0.042781441</td>
<td>0.181731318</td>
<td>0.056019287</td>
<td>0.1928161</td>
</tr>
</tbody>
</table>
Figure 2. Data from R1 (M1 Cooling), Experiment 1, Precooling vs. Post-cooling data, Maximum stimulation. This figure shows the differences between the precooling and cooling data for when the nerve was stimulated to generate a maximum response for the five data points considered. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.

Figure 3. Data from R1 (M1 Cooling), Experiment 1, Precooling vs. Post-cooling data, 150% stimulation. This figure shows the differences between the precooling and cooling data for when the nerve was stimulated at 150% of the H-wave threshold for the five data points we considered. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
RI (Experiment 2)

For this experiment, three different serial conditions were performed to determine the effect of M1 cooling on spinal cord reflexes, all with intensities set to produce a maximal response. The first was performed at 29.6 °C, before cooling of the primary motor cortex (M1) occurred. Data collection continued after M1 had been cooled, with the temperature being held between 0.5 and 2.0 °C. The last set of data was collected after M1 had been rewarmed to 29.1 °C (Table 2, Figure 4).

Following deactivation of the primary motor cortex, the M-wave magnitude increased significantly and its latency decreased significantly with maximal stimulation of the nerve. This indicates that M1 cooling creates a stronger and faster direct muscle response (M-wave). M1 also significantly increased the magnitude of the H-wave and significantly decreased its latency, also indicating that M1 cooling produces a stronger and faster H-wave response. Deactivation of the primary motor cortex did not significantly alter the H/M ratio, indicating that the motor cortex had no effect on the reflex excitability of the lumbar spinal cord. However, it does appear that the motor cortex may in fact cause the reflex to be delayed, since both components of the reflex were faster following motor cortex deactivation. The M and H waves recovered to normal levels after the cessation of the deactivation. The increase in speed of response did persist after cooling, indicating that there was a lasting effect from the deactivation.
Table 2. Data from R1 (M1 Cooling), Experiment 2. Precooling vs. Cooling and Precooling vs. Postcooling data, Maximum stimulation. This table shows the differences between precooling and cooling data and precooling and post-cooling data when the stimulation intensity was set to produce a maximum response. Experimental temperatures are given in the parentheses. It also shows significance between the two values, with α = 0.05.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PreMax (29.6°C)</td>
<td>1.320238095</td>
<td>0.051142857</td>
<td>0.04386343</td>
<td>3.0921875</td>
<td>9.780952381</td>
</tr>
<tr>
<td>CoolingMax (0.5-2°C)</td>
<td>1.50047619</td>
<td>0.05847619</td>
<td>0.03896196</td>
<td>2.427380952</td>
<td>8.973809524</td>
</tr>
<tr>
<td>Significance (α = 0.05)</td>
<td>0.023956684</td>
<td>0.015039729</td>
<td>0.491298993</td>
<td>5.63793E-22</td>
<td>0.013534541</td>
</tr>
<tr>
<td>PreMax (29.6°C)</td>
<td>1.320238095</td>
<td>0.051142857</td>
<td>0.04386343</td>
<td>3.0921875</td>
<td>9.780952381</td>
</tr>
<tr>
<td>PostMax (29.1°C)</td>
<td>1.475</td>
<td>0.059695652</td>
<td>0.040470518</td>
<td>2.929347826</td>
<td>8.916304348</td>
</tr>
<tr>
<td>Significance (α = 0.05)</td>
<td>0.125893611</td>
<td>0.06961156</td>
<td>0.971241723</td>
<td>0.0003047</td>
<td>0.001736003</td>
</tr>
</tbody>
</table>

Figure 4. Data from R1 (M1 Cooling), Experiment 2. Precooling vs. Cooling data, Maximum stimulation. This figure illustrates the differences between precooling and cooling data from when the stimulation intensity was set to produce a maximum response. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
RI (Experiment 3)

In this experiment, three sequential recordings were performed to determine the impact of M1 cooling on the M-wave and H-wave responses; each trial set at an intensity of 110% of the maximum H-wave threshold. This intensity was smaller than the maximum response and was performed to avoid any ceiling effects that may have been observed with the previously described maximal intensity experiments. As in previous experiments, the first set of recordings were performed before cooling, the second set during cooling, and the last after the cooling had been stopped (Table 3, Figure 5).

These data show that, at a stimulus intensity set to 110% of the H-wave threshold, there was no significant difference for the M-wave or H-wave magnitudes between the precooling and cooling data, nor was there a significant difference between the H-wave latencies between the precooling and cooling data. However, there was a difference between the M-wave latencies before cooling and during cooling, as during cooling it became significantly quicker. These data support the idea that the role of the primary motor cortex is not to adjust the magnitude of the reflex, its timing. At this intensity level, the H-wave was unaffected, but the M-wave was affected. After the cooling was stopped, the magnitude of the H-wave increased. However, this increase was not sufficient to adjust the H/M ratio.
Table 3. Data from R1 (M1 Cooling), Experiment 3, Precooling vs. Cooling and Precooling vs. Postcooling data, 110% stimulation. This table shows the differences between precooling and cooling data and precooling and post-cooling data from when the stimulation intensity was set to 110% of the H-wave threshold. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (110%)</td>
<td>2.126838636</td>
<td>0.045465909</td>
<td>0.021294498</td>
<td>3.178977273</td>
<td>9.54375</td>
</tr>
<tr>
<td>Cooling (110%)</td>
<td>2.052943662</td>
<td>0.043098592</td>
<td>0.020992074</td>
<td>2.279929577</td>
<td>9.588380282</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>0.097851461</td>
<td>0.312529289</td>
<td>0.770002968</td>
<td>5.33015E-081</td>
<td>0.767636464</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
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<tr>
<td>Precooling (110%)</td>
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<td>0.045465909</td>
<td>0.021294498</td>
<td>3.178977273</td>
<td>9.54375</td>
</tr>
<tr>
<td>Post-cooling (110%)</td>
<td>2.177785</td>
<td>0.051405</td>
<td>0.023548117</td>
<td>3.06625</td>
<td>9.342125</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>0.365689172</td>
<td><strong>0.04609733</strong></td>
<td>0.075113884</td>
<td>0.063049193</td>
<td>0.336423198</td>
</tr>
</tbody>
</table>

Figure 5. Data from R1 (M1 Cooling), Experiment 3, Precooling vs. Cooling data, 110% stimulation. This figure illustrates the differences between precooling and cooling data from when the stimulation intensity was set to 110% of the H-wave maximum threshold. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
RI (Experiment 4)

In this experiment, three different serial conditions were performed to determine the impact of M1 cooling on the M-wave and H-wave responses, all at an intensity of 6.5mA, which is an intermediate intensity level. The first recording was done before cooling, the second during cooling, and the last after M1 had been rewarmed (Table 4, Figure 6).

At this stimulus intensity and as in previous experiments, M1 cooling was found to have a significant influence over the M-wave latency. There were no lasting effects from the cooling, although the M-wave magnitude was significantly smaller in the post-cooling data. This effect did not translate to an appreciable change in the H/M magnitude, showing that the reflex excitability was unchanged after cooling deactivation of the primary motor cortex.
Table 4. Data from R1 (M1 Cooling), Experiment 4, Precooling vs. Cooling and Precooling vs. Post-cooling data, 6.5mA stimulation. This table shows the differences between precooling and cooling data and precooling and post-cooling data from when the stimulation intensity was set to 6.5mA. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (6.5mA)</td>
<td>1.562782857</td>
<td>0.05756</td>
<td>0.040106206</td>
<td>3.182857143</td>
<td>8.827857143</td>
</tr>
<tr>
<td>Cooling (6.5mA)</td>
<td>1.503008</td>
<td>0.065461333</td>
<td>0.04355863</td>
<td>2.760666667</td>
<td>8.682133333</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>0.157069921</td>
<td>0.479820544</td>
<td>0.157823621</td>
<td>3.18559E-52</td>
<td>0.15407045</td>
</tr>
</tbody>
</table>

| Precooling (6.5mA)       | 1.562782857      | 0.05756          | 0.040106206         | 3.182857143    | 8.827857143         |
| Post-cooling (6.5mA)     | 1.548462745      | 0.06547451       | 0.042348328         | 3.106372549    | 8.915196078         |
| Significance ($\alpha = 0.05$) | 5.95009E-07   | 0.485112637      | 0.404775933         | 0.207225948    | 0.496618494         |

Figure 6. Data from R1 (M1 Cooling), Experiment 4, Precooling vs. Cooling data, 6.5mA stimulation. This figure illustrates the differences between precooling and cooling data from when the stimulation intensity was set to 6.5mA. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
R2 (M1 COOLING)

R2 (Experiment 1)

These deactivation experiments were similar to the experiments described above. In this instance, all recordings were performed with a stimulus intensity set to 110% of the H-wave threshold value (Table 5, Figure 7).

In this animal, M1 cooling had a significant effect on every value measured. Interestingly, the magnitude of the H and M waves increased when M1 was deactivated, but both waves were delayed with respect to baseline values. In this instance, the H/M ratio was significantly affected, indicating that deactivation of the cortex produced a significant increase in spinal cord reflex excitability. These changes persisted in the follow up period, indicated a lasting effect of M1 deactivation on these measures.
Table 5. Data from R2 (M1 Cooling), Experiment 1, Precooling vs. Cooling and Precooling vs. Postcooling data, 110% stimulation. This table shows the differences between precooling and cooling data and precooling and post-cooling data from when the stimulation intensity was set to 110% of the H-wave threshold. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (110%)</td>
<td>3.671503448</td>
<td>0.169813793</td>
<td>0.046100373</td>
<td>3.125517241</td>
<td>8.939482759</td>
</tr>
<tr>
<td>Cooling (110%)</td>
<td>3.814568852</td>
<td>0.235562295</td>
<td>0.0617213</td>
<td>3.250983607</td>
<td>9.215245902</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>7.69064E-08</td>
<td>1.58595E-13</td>
<td>2.44131E-12</td>
<td>1.04482E-09</td>
<td>8.47438E-17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (110%)</td>
<td>3.671503448</td>
<td>0.169813793</td>
<td>0.046100373</td>
<td>3.125517241</td>
<td>8.939482759</td>
</tr>
<tr>
<td>Post-cooling (110%)</td>
<td>3.64627</td>
<td>0.277973333</td>
<td>0.076401421</td>
<td>3.365166667</td>
<td>9.458</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>0.26218655</td>
<td>6.14045E-24</td>
<td>1.0236E-24</td>
<td>8.59325E-21</td>
<td>5.37564E-96</td>
</tr>
</tbody>
</table>

Figure 7. Data from R2 (M1 Cooling), Experiment 1, Precooling vs. Cooling data, 110% stimulation. This figure illustrates the differences between precooling and cooling data from when the stimulation intensity was set to 110% of the H-wave threshold. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
R2 (Experiment 2)

For this data set, three experiments were performed during primary motor cortex cooling. The first was done before cooling, the second during cooling, and the third after M1 had been rewarmed. All these data were taken at a stimulus intensity set to 110% of the H-wave threshold (Table 6, Figure 8).

In this experiment, M1 cooling produced a significant decrease in the M-wave magnitude, an increase in the M-wave latency, as well as a decrease in the H-wave latency, indicating that these changes increased the excitability of the spinal cord reflex. These changes were lasting and were also observed in the follow-up recording. Additionally, there was a significant increase in the H/M ratio both during cooling and following cooling when compared to precooling values.
**Table 6. Data from R2 (M1 Cooling), Experiment 2, Precooling vs. Cooling and Precooling vs. Postcooling data, 110% stimulation.** This table shows the differences between precooling and cooling data and precooling and post-cooling data from when the stimulation intensity was set to 110% of the H-wave threshold. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (110%)</td>
<td>3.18766875</td>
<td>0.363825</td>
<td>0.114883707</td>
<td>3.47640625</td>
<td>9.5946875</td>
</tr>
<tr>
<td>Cooling (110%)</td>
<td>2.59034</td>
<td>0.373362</td>
<td>0.144181699</td>
<td>3.518</td>
<td>9.5564</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>6.84563E-20</td>
<td>0.549034</td>
<td>4.83805E-05</td>
<td>0.029531732</td>
<td>0.04551422</td>
</tr>
<tr>
<td>Precooling (110%)</td>
<td>3.18766875</td>
<td>0.363825</td>
<td>0.114883707</td>
<td>3.47640625</td>
<td>9.5946875</td>
</tr>
<tr>
<td>Post-cooling (110%)</td>
<td>2.229440506</td>
<td>0.340112658</td>
<td>0.152175417</td>
<td>3.524556962</td>
<td>9.549113924</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>1.00856E-40</td>
<td>0.193206876</td>
<td>9.02542E-07</td>
<td>0.003685375</td>
<td>0.013051093</td>
</tr>
</tbody>
</table>

**Figure 8. Data from R2 (M1 Cooling), Experiment 2, Precooling vs. Cooling data, 110% stimulation.**
This figure illustrates the differences between precooling and cooling data from when the stimulation intensity was set to 110% of the H-wave threshold. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
R3 (T8 COOLING)

In this experiment, the spinal cord was cooled at the level of the eighth thoracic vertebrae, and its goal was to determine the impact of spinal cord deactivation on lower lumbar spinal reflexes. The rat setup was the same as the first two experiments on the impact of primary motor cortex cooling, but in this experiment the spinal cord, not the motor cortex, was cooled.

Cooling the spinal cord significantly decreased the magnitude of the M-wave and delayed the H-Wave. The decrease in the M-wave without change in the H-wave amplitude produced a commensurate increase in the H/M ratio, indicating that T8 cooling produced an increase in the reflex excitability. This increase in excitability lasted past the duration of the deactivation; however, this time point showed a slowed M-wave, but no significant change in the H-wave latency.
Table 7. Data from R3 (T8 Cooling), Precooling vs. Cooling and Precooling vs. Post-cooling data.
This table shows the differences between precooling and cooling data and precooling and post-cooling data. Precooling data was taken at a stimulus of 130% of the H-wave threshold, cooling data was taken when the spinal cord was kept between 0 and -3 °C, and post-cooling data was taken when the spinal cord was rewarmed to 33 °C. It also shows significance between the two values, with \( \alpha = 0.05 \).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Peak Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precooling (130%)</td>
<td>2.785645161</td>
<td>0.089419355</td>
<td>0.032105201</td>
<td>2.028548387</td>
<td>9.789516129</td>
</tr>
<tr>
<td>Cooling (0—3°C)</td>
<td>2.22657971</td>
<td>0.093507246</td>
<td>0.042042893</td>
<td>2.033623188</td>
<td>9.906666667</td>
</tr>
<tr>
<td>Significance (( \alpha = 0.05 ))</td>
<td>6.82498E-82</td>
<td>0.220475331</td>
<td>5.3183E-11</td>
<td>0.795294123</td>
<td>0.011063906</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
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<tr>
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<td>0.089419355</td>
<td>0.032105201</td>
<td>2.028548387</td>
<td>9.789516129</td>
</tr>
<tr>
<td>Post-cooling (33°C)</td>
<td>2.325470588</td>
<td>0.084044118</td>
<td>0.036121392</td>
<td>2.070441176</td>
<td>9.807647059</td>
</tr>
<tr>
<td>Significance (( \alpha = 0.05 ))</td>
<td>1.0968E-127</td>
<td>0.073619824</td>
<td>0.000925447</td>
<td>0.023737736</td>
<td>0.682780756</td>
</tr>
</tbody>
</table>

Figure 9. Data from R3 (T8 Cooling), Precooling vs. Cooling and Precooling vs. Post-cooling data.
This figure illustrates the differences between precooling and cooling data. Precooling data was taken at a stimulus of 130% of the H-wave threshold, cooling data was taken when the spinal cord was kept between 0 and -3 °C. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation.
In this experiment, the sciatic nerve was cooled to determine the impact of deactivation on spinal reflexes. The rat setup was the same as the first three experiments, but in this experiment the sciatic nerve was cooled and recordings were taken from the plantar muscle (Tables 8 & 9, Figures 10 & 11).

To examine the effect of sciatic nerve deactivation via cooling on spinal cord reflexes, two identical experiments were carried out in two different rats (R8 and R12). In these rats, unlike in the previous experiments, precooling, cooling, and post-cooling data were all taken consecutively. To analyze the data from these different time ranges, a set of data from ten time points were taken from each level of cooling (precooling, during cooling, and post-cooling), averaged, and then compared. The precooling data was compared to both the cooling and post-cooling data to see if there were any significant differences.

During cooling, the M-wave magnitude profoundly decreased and its latency increased, indicating that with sciatic nerve cooling, the direct muscle response was significantly weaker and slower. Following cooling, the M-wave magnitude did not recover to precooling values, but the latency did. With cooling, the H-wave completely disappeared.

For pre- versus post-cooling values, there was more variability. The M-wave magnitude was consistent across both rats: following cooling, it was lower than its pre-cooling value. The same was found for the H-wave magnitude and the H/M ratio, but the H/M
ratio for R8 was not significant. For the M-wave latency, it did not recover to pre-cooling values in R8, but in R12 the post-cooling average was larger than the pre-cooling average; however, these differences were not significant. Lastly, in R8, the H-wave latency did not recover and in R12 the value following cooling was higher. Taken together, these data indicate that there are significant difference in the M-wave and H-wave between pre-cooling and cooling values, but that the rats did not recover in the same way.
Table 8. Data from R8 and R12 (Sciatic Nerve Cooling), Precooling vs. Cooling. This table shows the differences between precooling and cooling data. Precooling data was taken at 26 °C and cooling data was taken at 0 °C. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8 Average Precooling</td>
<td>2.6625</td>
<td>0.72025</td>
<td>0.270480094</td>
<td>2.479</td>
<td>6.96</td>
</tr>
<tr>
<td>R8 Average Cooling</td>
<td>0.51</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>2.71283E-21</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>R12 Average Precooling</td>
<td>3.1111</td>
<td>0.5876</td>
<td>0.188863045</td>
<td>2.706</td>
<td>7.679</td>
</tr>
<tr>
<td>R12 Average Cooling</td>
<td>1.3468</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>Significance ($\alpha = 0.05$)</td>
<td>2.66691E-25</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
</tbody>
</table>

Figure 10. Data from R8 and R12 (Sciatic Nerve Cooling), Precooling vs. Cooling. This figure illustrates the differences between precooling and cooling data for rats R8 and R12. Titles with an asterisk* have statistically significant differences between the two values. Error bars illustrate standard deviation. (PLEASE NOTE: All data is statistically significant, EXCEPT the H-wave magnitude differences for R12).
Table 9. Data from R8 and R12 (Sciatic Nerve Cooling), Precooling vs. Post-cooling. This table shows the differences between precooling and post-cooling data. Precooling data was taken at 26 °C and post-cooling data was taken after the sciatic nerve had been rewarmed from 0 °C to 26 °C. It also shows significance between the two values, with $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>M-wave Magnitude</th>
<th>H-wave Magnitude</th>
<th>H/M Magnitude Ratio</th>
<th>M-wave Latency</th>
<th>H-wave Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8 Average Precooling</td>
<td>2.6625</td>
<td>0.72025</td>
<td>0.270480094</td>
<td>2.479</td>
<td>6.96</td>
</tr>
<tr>
<td>R8 Average Post-cooling</td>
<td>2.44475</td>
<td>0.65075</td>
<td>0.26610401</td>
<td>2.429</td>
<td>6.853</td>
</tr>
<tr>
<td><strong>Significance ($\alpha= 0.05$)</strong></td>
<td><strong>2.15615E-20</strong></td>
<td><strong>0.038977642</strong></td>
<td><strong>0.720630393</strong></td>
<td><strong>0.422641029</strong></td>
<td><strong>0.000269656</strong></td>
</tr>
<tr>
<td>R12 Average Precooling</td>
<td>3.1111</td>
<td>0.5876</td>
<td>0.188863045</td>
<td>2.706</td>
<td>7.679</td>
</tr>
<tr>
<td>R12 Average Post-cooling</td>
<td>3.0715</td>
<td>0.5528</td>
<td>0.17998795</td>
<td>2.711</td>
<td>7.72</td>
</tr>
<tr>
<td><strong>Significance ($\alpha= 0.05$)</strong></td>
<td><strong>9.99922E-08</strong></td>
<td><strong>0.009889728</strong></td>
<td><strong>0.035357901</strong></td>
<td><strong>0.803076685</strong></td>
<td><strong>0.003380743</strong></td>
</tr>
</tbody>
</table>
DISCUSSION

The purpose of this study was to evaluate the independent effects of thermal deactivation of the primary motor cortex (M1), the spinal cord at the level of the eighth thoracic vertebrae (T8), and the sciatic nerve on spinal cord reflexes. Since there were three arms of this study, there were three separate hypotheses. With M1 deactivated, it was hypothesized that the delayed muscle reflex response, or H-wave, would be less in magnitude and have a delayed latency and that the direct muscle response, or M-wave, would not deviate from baseline levels. For spinal cord cooling, it was hypothesized that that the experimental results would be the same as those for M1 cooling. For sciatic nerve deactivation, it was hypothesized that both the M- and H-wave magnitudes would be retarded and the latencies for both would be delayed. These hypotheses were evaluated based on the H/M magnitude ratio, which reflects spinal excitability (Valero-Cabre, 2004). The effects were believed to be mediated by the basic structure of the peripheral and central nervous systems and the pathways that the reflex responses follow through them. We also wished to see if there were any lasting effects on any of the reflex values due to cooling when compared to baseline, precooling values.

When considering the first two experiments that examined the effects of M1 cooling on spinal cord reflexes, the results were different between the two subjects. In experiments from the first animal, deactivation of M1 had no effect on the excitability of the reflex (via the H/M ratio), but did shorten the latencies of the M response. The results from the second subject indicated that M1 deactivation produced an increase in the excitability of
the reflex, but reduced the latency of the M-wave. However, the basis of this difference is likely to be experimental. In the second subject, the pre- and post-cooling measures were significantly different; as a result, it is unclear whether the cooling had a lasting effect or whether the preparation underwent a shift in the timing and magnitude of the reflex excitability independent of the deactivation. As a result, it is more conservative to suggest that the cooling results from the first subject, which are flanked by unchanging pre- and post-cooling measures, are likely to reflect a real effect of M1 deactivation. Alternatively, the post-cooling deactivation period may have occurred too close to the cooling period and a comparison between the rats may indicate a more complete deactivation of M1 in the second subject.

Regardless, both results are consistent with the purported role of M1 on spinal cord reflexes, and recapitulate the so-called upper motor neuron pattern of damage. This occurs when upper motor neurons, which extend from the cerebral cortex to the end of the spinal cord, are either damaged or, in the case of this study, deactivated (Purves & Williams, 2001). Upper motor neuron signs are characterized by an increase in muscle tone and an increase in spastic movement (so-called spastic paralysis). These data indicate that the motor cortex normally inhibits spinal circuits, and in the absence of this inhibition, the reflex is faster or more excitable, both expectations that fit with the observations from our experiments.
These conclusions from the M1 deactivation are supported by the results in which the spinal cord was deactivated. Deactivation of the spinal cord produced an increase in the H/M ratio, suggesting an increase in reflex excitability. Since the cooling of T8 reduces action potential transmission in the descending motor tracts that relay signals from M1 to the spinal cord, an increase in excitability is consistent with the finding that cooling M1 directly produces an increase in spinal cord excitability. These findings, therefore, reinforce the contention that primary motor cortex provides an inhibitory action on spinal cord reflexes.

It should be noted that the increase in reflex excitability identified in the current study is not consistent with findings from previous studies in which the spinal cord was transected. One study found that with spinal cord transection, the H-wave response increased in magnitude and the H/M ratio significantly decreased, and another didn’t find any differences in the H/M ratio following contusion (Valero-Cabre, 2004)(Thompson, Reier, Lucas, & Parmer, 1992). It is likely, since those studies used damage, that the spinal cord reflexes underwent a decrease due to the effect of spinal shock. Spinal shock reflects decreased spinal cord activity due to a lack of input from the motor cortex (Purves & Williams, 2001). Clinically, the effects of spinal shock include muscle flaccidity, loss of voluntary movement, and reduced tendon reflexes (Boland, Lin, Engel, & Kiernan, 2011). In the present study, we avoided the effects of spinal shock because cooling does not invoke the inflammatory processes that largely underlie the immediate effects of spinal cord injury.
The purpose of deactivating the sciatic nerve was to directly determine whether the recorded waves were mediated through these pathways. The M-wave, which is the electrophysiological manifestation of the nerve-muscle interaction, showed severe attenuation in terms of magnitude and a tremendously delayed and extended time course and the H-wave disappeared completely. The H-wave was replaced with a series of oscillations in the recording that may have been due to inconsistent muscle spindle activity, or residual and delayed movement from the direct activation. While these possibilities are difficult to disentangle, it is clear that the sciatic deactivation profoundly impaired the reflex.

It is interesting to note that there was any signal. Cooling deactivation is thought to completely silence neurons and axons if the right temperature is achieved. In this case, it is likely that not all the axons were completely deactivated; these axons may have been protected from the effect of cooling by being the farthest away from the deactivating isotherm, or it may be that certain large caliber axons were more protected than others.

Limitations of the study.

Three points should be considered as limitations of the study. The first is the limited number of animals – there should be more animals to get a better sense of the effects. The second shortcoming of the current experiments is that the cooling deactivation was not used in this case to provide multiple experiments in the same animal and therefore
reduce the inter-animal variability. Indeed, in many cases, the post-cooling data exhibited changes from baseline, indicating that cooling exerted lasting effects. More time should have been given for the recordings to come back to baseline. Finally, the extent of cortical cooling was insufficiently documented and therefore, difficult to compare between animals.

**Conclusion**
Overall, these data support the idea that motor cortex exerts a net inhibitory effect on spinal cord reflex excitability. These effects ranged from an increase in spinal cord excitability to a decrease in reflex excitability when M1 was deactivated, and was supported by an increase in excitability when the axons relaying signals from motor cortex to the spinal cord were deactivated. Finally, we found that direct cooling of the sciatic nerve eliminated the H-wave and severely attenuated the M-wave.
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CURRICULUM VITAE

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EDUCATION

Boston University School of Medicine, Boston, MA
Master’s in Medical Sciences May 2015

The Ohio State University, Columbus, OH
Bachelor of Arts in History, Bachelor of Arts in Anthropology June 2012
• University Honors Program
• Maximus Scholarship – Awarded on a competitive basis to students in the top three percent of their high school class with competitive standardized test scores ($10,800 total).
• Arts and Sciences Excellence in Scholarship Award Recipient (2011)
• President’s Salute to Undergraduate Academic Achievement (2011)
• Minor in Italian

Università del Salento, Scuola di Italiano per Stranieri, Lecce, Italy
Certificate in Italian language, Summer 2008

Upper Arlington High School, Upper Arlington, Ohio June 2007

COMMUNITY INVOLVEMENT & ACTIVITIES

• Dan’s Courage Crew – Team Captain
  Dan’s Courage Crew is a team I created for the Leukemia and Lymphoma Society’s annual Light the Night Walk. The team has raised nearly $450,000 in the last five years.
• Mortar Board National College Senior Honor Society – Association of Ohio State Class Honoraries Representative, 2011-2012; 2nd Vice President, 2010-2011; Member, 2010-Present
• Wexner Medical Center at The Ohio State University, Volunteer (January 2008-December 2008, September 2011-June 2012)
  o Areas: SICU, Department of Neurology, Pathology Lab, Welcome Ambassador, Ambulatory Surgery Unit
• Phi Alpha Delta Law Fraternity, International - President, 2010-2011; Volunteer Chair, 2009-2010
• Association of Ohio State Class Honoraries – Vice President of Finance and Communications, 2011-2012
• Study Abroad –
  London Honors Program (December 2007)
  Italian Language Program in Lecce, Italy (Summer 2008)
• Kinderitaliano, Indianola Alternative K-8, Columbus, OH (March 2010 – January 2011)
• Slobodna: The New Find, Underwater Archaeology Field School, Key Largo, FL (Summer 2010)

EMPLOYMENT

Clinical Research Assistant, OSU Dept of Anesthesiology, Columbus, OH
April 2012-August 2013
• Clinical research in the Department of Anesthesiology at the Wexner Medical Center at The Ohio State University under Dr. Sergio Bergese. The research team managed about 40 clinical trials, both industry-sponsored and physician-initiated. My work focused mainly on enrolling and managing patients within certain trials, as well as helping develop and write research protocols. Attended and presented at weekly departmental journal club.

Student Assistant, OSU Institute for Materials Research (IMR), Columbus, OH
January 2010 – September 2012
• Administrative work including communication and marketing, event planning and coordination for an annual conference, database research and compilation, as well as office work and driving an hourly shuttle.

Intern, Ohio House of Representatives, Columbus, OH
January 2010 – June 2010
• Intern for State Representative Barbara Sears from Ohio’s 46th District. Work included constituent outreach and communication with district, researching policy issues, drafting memos and letters, as well as assisting with office work.