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INHIBITORY RIB-RAISING AND MICRONEUROGRAPHIC

MEASUREMENT OF SYMPATHETIC NERVOUS SYSTEM ACTIVITY

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THESIS

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BACKGROUND

The clinical effectiveness of osteopathic manipulative therapy (OMT) techniques that are designed to address the autonomic nervous system (ANS) are untested to current research standards. As the concept of "autonomic imbalance" is frequently ascribed as the etiology of various pathologic conditions, it is paramount to undertake basic research into not only efficacy but also possible mechanistic actions and origins. Osteopathic physicians often utilize treatment regimens and techniques for which the given mechanism of action is simply attributed to "balancing the autonomics". This intuitive concept may finally be at the threshold where enough basic science exists to justify clinical investigations.

Osteopathic manual manipulative techniques have shown effectiveness in the treatment of various musculoskeletal conditions and have been shown to lower perceived pain; supporting the use of manual therapy as an effective treatment modality. A brief review yields the following within just the last four years: Eisenhart showed positive range-of-motion outcomes after ankle sprain in the emergency department[1]. Biondi reviewed the usefulness of cervical manipulation for tension headache[2] and McReynolds demonstrated an equivalent decrease in acute neck with OMT versus intramuscular ketolac in an emergency department setting[3], although the dosing was not maximal. German researchers have shown effectiveness in chronic epicondylopathia humeri radialis[4] and research has led to the demonstration of lowered post-operative pain in hip

or knee arthroplasty[5]. There has also been decreased post-operative pain medication reported in hysterectomy[6] when compared with a control group. OMT has demonstrated a decrease in fibromyalgia symptoms when used with standard care over standard care alone[7].

Low back pain, perhaps the most extensively studied diagnosis in which OMT has been evaluated, has reported numerous positive outcomes including lower levels of narcotic use[8] and decreased pain in both double-blinded and meta-analysis studies[9-11], although there is still considerable debate within this area. There have also been favorable outcomes associated with the management of gait in Parkinson's disease[12] and preliminary work has shown the efficacy in treatment of carpal tunnel syndrome[13, 14].

Most of the aforementioned musculoskeletal conditions are not amenable to traditional therapies and have a high-cost burden on the economy. Traditional treatments generally have a "wait and see" approach combined with analgesics which may not cause harm, but hampers quality of life and income in the interim. The cost effectiveness of OMT is still in the preliminary stages, but there is evidence supporting a superior cost benefit ratio when compared to standard care[15, 16] and since many of these conditions have no other proven treatment modality available patients will often try anything over nothing.

The evaluation of OMT addressing clear autonomic dysfunction is limited. This study closes a small part of that gap by examining the proposed physiologic mechanism of OMT and its' interaction with the ANS. Small studies have documented changes, namely heart rate variability, in autonomic processes in healthy individuals while other,

older studies having found benefit in clinical variables. With few exceptions however, most of these studies lacked a particular technique protocol. Operators were free to use whatever intervention that they chose and most of these studies were not performed under rigorous testing methods with a randomized design.

The technique that was evaluated (inhibitory rib-raising) has a documented history from the origins of osteopathic medicine in the United States, and is currently taught to students in osteopathic medical schools as part of their medical education curriculum. Rib-raising is most often taught to enhance the mechanical motion of the ribs, but other paradigms utilize this technique to either enhance or inhibit sympathetic nervous system (SNS) activity. The evaluation of inhibitory rib-raising or its' proposed mechanism of action has never been rigorously scrutinized to modern scientific standards. The current study was designed to address that gap with both direct and indirect measurement of SNS variable in healthy individuals with the hypothesis that there would be a time-dependent, graded reduction in measured sympathetic nervous system activity (MSNA) in healthy individuals undergoing cold-pressor stimulus.

CHARACTERIZATION OF THE SYMPATHETIC NERVOUS SYSTEM

Galen was the first to anatomically characterize the SNS in the 2nd century A.D. Fifteen centuries later, Thomas Willis (1621-1675) was the first to recognize that the SNS was connected to the spinal cord and ultimately, to the brain. The goal of his work was to describe neuropathology as a result of single physiologic mechanism; the precursor to our understanding of the autonomic nervous system[17].

In the 16th century, François Parfour du Petit cut through the sympathetic ganglion in dogs and described a consequent Horner-like syndrome, thus concluding that sympathetic nerve impulses traveled in a caudal-cranial fashion[18]. Work in sympathetic anatomy and physiology had progressed to the point where by 1852, Brown-Séquard and Bernard established the innervation of vascular smooth muscle and subsequent vasococonstriction[19, 20] upon sympathetic activation.

The current view taught to medical students is the familiar "fight or flight" response. And although this view is a simplistic view of a complex system, it has faithfully guided the thinking of our current generation of physicians. The term "fight or flight" originated with Walter Canon's research in the early 1900's and the Canon model proposed that stimulus applied to any tissue activates the SNS in a graded fashion and that this system provides for a homeostasis of the body[21], a crucial concept of osteopathic medicine.

While most of the peripheral manifestations of the SNS are now well-understood, much work remains in understanding how the brain controls the SNS, and how it can be

regulated. The primary responsibility of the SNS is short and long-term regulation of blood pressure and cardiac drive to meet the demand characteristics of the organism. Short term control of blood pressure appears to be mediated in reflex pathways while longer control is mediated through neuro-humoral pathways, yet some propose a high-degree of integration[22]. The central nervous system does not seem to distinguish between different types of stress and demand and the brain, including the forebrain, has a strong influence in arterial pressure control.

An extensive review of neural control of the SNS is beyond the scope of this project, but some background is necessary. Coote published a thorough, yet brief, review on the subject[23] and current controversies. Research currently proposes that a primary area of sympathetic outflow is the rostral lateral ventral medulla (RVLM). Neurons from this area project directly to vasomotor pre-ganglionic sympathetic neurons in the thoraco-lumbar area and play a major role in the determination of the resting arterial pressure. The RVLM integrates information from both forebrain and limbic areas as well as receiving input from baroreflex pathways and it has been generally accepted that tone is mediated via a GABA/Glutamate pathway[24]. Whether SNA is an intrinsic property (oscillators) of the RVLM or whether it is a result of synaptic drive is currently under debate.

Two other areas are now gaining attention in their contribution to long-term sympathetic regulation. The small neurons of the PVN have been determined to directly innervate the adrenal medulla and several sympathetic organs. This pathway is

independent of the RVLM pathway and is intimately connected to the angiotensin system[25, 26]. Meanwhile, the nucleus tractus solitarius is the afferent terminal of the baroreceptors. These neurons then ascend to the RVLM and may either excite or inhibit sympathetive outflow. The NTS also receives input from the PVN modulating its' actions.

It is apparent that the central command of SNA is complex and integrated. It involves many neurotransmitters, neuromodulators and direct reflex pathways. The key players have only recently been identified and it remains to be seen how the forebrain interacts with these medullary areas.

BASIS OF SYMPATHETICALLY MEDIATED DISEASES

As the physiologic view of disease progresses from that of an individual system dysfunction to global synergistic interactions, the prominence of the sympathetic nervous system in chronic illness becomes better understood and appreciated. The benefits that it provides become toxic when activation is sustained, which is usually idiopathic. The SNA activates as a whole and only under very rare or pathologic states does it manifest in a unimodal fashion (*i.e.* postural orthostatic tachycardic syndrome). The diseases that implicate hypersympathatonia also have multiple developmental mechanisms and sequelae that can be predicted from the varied effects of the sympathetic nervous system. It becomes clear that systemic diseases (such as hypertension, diabetes and heart failure) that implicate hypersympathatonia vary greatly in symptom presentation and this variation is a consequence of a complex interaction between disease course, environment and genetics.

Essential hypertension has been well-characterized as sympathetic nervous system dysfunction, although the root cause still remains elusive. By the 1980's, the suspicion that elevated sympathetic tone was causing essential hypertension in young patients with no or few risk factors was beginning to accumulate evidence. By 1986 well over 80 studies indicated that both norepinephrine release and spillover were increased in essential hypertensives[27-39] under the age of 40. Initially, it was unclear where the excess NE was coming from. Esler used radio-labeled studies to show that adrenal sources could only account for about 30% of the total and concluded that the release must be directly

from the sympathetic nerve terminals[40], as the sympathetic nerves were well-known to directly release NE with stimulation[41-43].

The study which best characterized the hypothesis at the time was conducted by Ferrier *et al*[44]. Normotensive and hypertensive subjects were given trimethaphan to induce ganglion blockade. Cerebrovascular norepinepherine spillover was measured by internal jugular radio-dilution and there was a significant increase (>30%) in hypertensive patients compared with control. Desipramine administration (a centrally acting NE reuptake inhibitor), decreased both whole body spillover NE and cerebrovascular NE levels. The conclusion that essential hypertension was a process of central nervous system activation was further supported by Wallin *et al.* when he was able to demonstrate that either physical increases in SNA in the form of isometric grip, or mentally induced elevations in SNA induced via math problems, correlate with increased norephineprine spillover[45], thereby showing that there can be a strictly neurogenic trigger to sympathetic activation, confirming several decades of cardiologists' observations that high-stress individuals were more prone to myocardial infarction.

Noradrenaline spillover and release measurement is an accurate way to assess sympathetic nervous activity, but its' methodology did not answer the question of whether increased MSNA actually caused essential hypertension and if so, then by what mechanism. Many investigators were simultaneously measuring not only the increases in catecholamines, but they were also using direct microneurography to assess sympathetic nervous activity. These studies showed that there was an increase in resting MSNA in essential hypertensives[46-51] that was more pronounced with the degree of hypertension.

Importantly, microneurographic measurements correlated in a dose dependent fashion with NE release and spillover and confirmed not only the utility of the two methods, but also providing further support for increased MSNA activity in the development of EH[45]. Finally, Grassi has shown that there is not an increase in sympathetic nerve burst activity in secondary hypertensives[52], better delineating that secondary hypertension and essential hypertension are different disease entities. Multiple studies have also shown a correlation between increased microneurographic SNA and alterations in the high-frequency component of heart rate variability in hypertensives; indicating that alterations sympathetic/parasympathetic drive are associated with an increase in sympathetic activity[53-56].

Using heart rate as an indirect measure of sympathetic tone, there is a strong positive correlation between heart rate, which is mediated through β-1 adrenoreceptors, and the development of hypertension as demonstrated by the CARDIA study. CARDIA subjects showed a 1.3mm Hg increase in diastolic blood pressure over a ten-year period for every 10 BPM increase in pulse[57]. In another analysis of the CARDIA study by Dyer, there was a univariate risk of developing Stage 1 hypertension over 10 year follow-up with an elevated HR[58]. The HARVEST study has also found a two-fold increase in the risk of developing Stage 1 hypertension based upon elevated clinical heart after 6 year follow-up[59], thus confirming the risk that an elevated HR may confer. The CARDIA authors speculated that increased sympathetic tone may directly induce smooth muscle proliferation and result in EH due to arteriolar constriction. An increased heart rate may

also cause direct damage due to an increase in myocardial oxygen demand and is also an independent risk factor in cardiovascular mortality[60, 61].

Although essential hypertension has significant negative sequelae in its' own right, it can also lead to the development of heart failure which greatly increases mortality and morbidity. The discovery in 1983 that cultured fetal rat ventricular myocytes hypertrophy in response to NE[62] initiated the elucidation of the sympathetic link to heart failure. The same researchers soon confirmed that direct α_1-receptor stimulation was responsible for this phenomena[63] by preventing hypertrophy with concomitant administration of prazosin and terasozin. Subsequent work using simultaneous verapamil administration to prevent an increase in pressures while undergoing NE infusion also eliminated the possibility that altered hemodynamics were purely responsible for myocyte remodeling[64, 65]. Ultimately it was shown that α_1-receptors induce c-myc expression which induces cell division[66].

In vitro work has also shown that sustained levels of norepinepherine induce vascular remodeling and intimal proliferation[67-69] that correlate with increasing levels of catecholamine exposure. The changes in the muscular wall result in a decrease in vascular compliance due to morphic changes in the smooth muscle cells controlled through the α-1 adrenoreceptor[70]. Animal studies have demonstrated that chronic NE infusion increases vascular hypertrophy and induces atherosclerosis while antagonist infusion attenuates these reponses[71-73]. The synergistic effects of myocyte, vascular and intimal remodeling creates not only a decrease in the ionotropic capabilities of the heart,

but also an increase in myocardial workload that ignites a vicious cycle of fibroblastic proliferation in response to increases stress.

It is not necessary for NE levels to be highly elevated. Laks *et al* was able to produce cardiac hypertrophy at suppressor doses, although at the time it was assumed that this hypertrophy was a result of alterations in flow and wall stresses[74]. Later hemodynamic studied showed that there can be an increase in LV mass without an impairment in cardiovascular dynamics[75] or frank hypertension[76], suggesting that high NE levels alone are not enough to induce frank heart failure, perhaps explaining the gain of function paradox in highly trained athletes[77, 78]. The existence of remodeling in the absence of common clinical signs make diagnosis difficult and may partly explain why there is not a clear cause and effect mechanism of essential hypertension and heart failure.

The degree of abnormal SNA in CHF patients is a strong predictor of mortality as Cohn *et al* showed a positive correlation between plasma norepinepherine levels and mortality in patients with CHF[79, 80]. The SOLVD trial demonstrated that this derangement occurs even in asymptomatic patients whose ejection fractions are less than 35%[81, 82]. This has led to the rationale for beta-blocker therapy despite lower cardiac output[83]. Beta-blocker therapy aimed at reducing SNA tone has recently been validated in decreasing patient morbidity and mortality, with the COPERNICUS[84, 85], MERIT-HF[86], and CIBIS-II[87, 88] trials showing significant beneficial results.

Experimentation with canine models has shown that a key pathologic mechanism in EH is a reduction of baroreceptor output[89], which is under SNS influence. This

abnormality may be an effect of altered Na-K-ATPase activity as administration of digitalis in humans reverses altered vasoconstriction in response to lower body negative pressure[90, 91]. The decreased firing of the baroreceptors, despite adequate volume and filling pressures[92] (baroreceptor resetting), results in an increase in centrally originating autonomic traffic [93] from the rostral ventral lateral medulla (RVLM), which functions as the sympathetic control center of the brain. Some investigators consider baroreceptor resetting to be a consequence of increased SNA, however the pathway to the RVLM can be influenced by afferent discharges and under appropriate circumstances will cause a change in SNA. Research has also demonstrated that baroreceptor reflexes are necessary in the long-term blood pressure control as baroceptor denervation prevents normal sympathoinhibition that normally occurs with the introduction of angiotensin II[94]

The rennin-angiotensin (RAS) regulation pathway thus plays a key role in sympathetic dysfunction. Renin is released by the juxtaglomerular cells in response to direct stimulation by the renal sympathetic nerves. Renin then cleaves angiontensinogen, leaving the product, angiotensin I, to be converted to angiotensin II (AT2) in the lungs. The RAS pathway, and specifically AT2 has been implicated as a major figure in the development of disease. Hypertensive individuals demonstrate an increased baseline of RSNA which is probably of central origin[95] consistent with the existing evidence of alterations in SNA. It has been well-known that renal denervation will relieve hypertension in all mammal species studied and it was recently shown that this is a long-term phenomena[96].

AT2 has a feed forward effect in that AT2 facilitates the outflow of NE in the renal venous system[97, 98]. And administration of an ACE-inhibitor (ACEI) attenuates this response. To better clarify this response it was shown that when rats were given a low-salt diet (to increase RAS) the antinatriuretic response was abolished by an ACEI (captopril) and during a high-salt challenge the antinatriuetic response could be returned to normal by infusion of AT2[99, 100]. Further work has shown that after renal denervation, the ability of AT2 to increase chloride and water re-absorption was reduced by 75%. The conclusion is that AT2 is responsible for only a portion of the renal pressor response and that direct renal sympathetic stimulation may the primary mechanism of this response[101]. Ma *et al.* provided evidence that AT2 directly stimulates the renal nerves in a different fashion than post-ganglionic stimulation and may, in fact, work through AT1 receptors[102] while it was already shown that an intact cathecholinaminergic system is required for proper renal function[103].

AT2 also influences the regulation, and dysregulation, of central sympathetic outflow which has a different (extrarenal) pathway. Strong evidence indicates that AT2 receptors exist in the subfornical organ (SFO) and the area postrema (AP) of the circumventricular organs[95, 104] which alter baroreceptor reflexes. Eshima *et al.* has shown that a certain amount of this dysregulation may be due directly to angiotensin release in the NTS[105].

Although speculative, it can be theorized that certain osteopathic techniques designed to address cranial somatic dysfunction may directly influence the RAS as ablation of the AP prevents the development of hypertension that is created by chronic

AT2 administration in rats. There has also been suggestion that the forebrain RAS may modulate SNA. In this case a rat model was able to demonstrate that ICA injections of captopril modulated both arterial pressure and RSNA. This is distinct from the normal baroreflex mechanisms and may play an enhanced role in pathologic states in which there are altered barorelex mechanics[26]

Modern understanding of sympathetic control has elevated the medulla to the role of the prime mover in the context of long-term (>24 hrs) sympathetic modulation. Primary control areas in the brain have been localized to the rostral ventrolateral medulla (RVLM) and the nucleus tract solitarus (NTS), both of which receive input from the multiple higher and lower neural areas. There are other identifiable areas that influence autonomic traffic including the hypothalamus and the spinal cord; however, their overall contributions are either small or unknown. Unexpected treatment of severe refractory hypertension with patients undergoing microvascular decompression of the RVLM for other pathology appeared occasionally in the literature from the 1980's. An anatomical pathology study performed in 1992[106, 107] concluded that RVLM compression was evident in all post-mortem human subject who had essential hypertension when compared to normo-tensive and renal hypertensive subjects. This study was a powerful piece of evidence for the microvascular compression theory of essential hypertension (Jannett's theory) which had been suggested by Alexander's work in the 1940's. Complete understanding of the interactions of the RVLM and NTS pathways and how compression or inflammation is not yet well-understood and the exact mechanism by which elevated SNA contributes to hypertension is debated[108].

There has been recent evidence showing nitric oxide (NO) release in the NTS is responsible for blood pressure attenuation in spontaneously hypertensive rats[109-112]. Both NO and opiates in the CNS have strong vasodilatory effects. The clear implication in central inhibition of sympathetic activity can not be overlooked[113-117]. And NO release will strongly lower MSNA[118] while NO synthase inhibition will induce hypertension in rats[105, 119].

Much remains to be learned of the details of the SNS and how it integrates with the CNS. And although individual studies address individual components, research does not yet appear ready to postulate a grand scheme of how the various systems interact or are controlled. Nor is the interaction with either genetics or environment understood, this may prove to reveal itself in the years to come.

MANUAL THERAPY AND THE AUTONOMIC NERVOUS SYSTEM

A central tenet to osteopathic medical practitioners is the belief that manual manipulation can influence autonomic tone. A.T. Still, the inventor of the osteopathic medical model, reported in his 1899 work, *Philosophy of Osteopathy*, on the toxic nature of sustained "nervous tone". He stated that nervous inhibition of the superior cervical ganglia can be achieved by sustained manual pressure on the paravertebral muscles. Louisa Burns attempted to quantify this at the beginning of the century[120] with the belief that sympathetic outflow was reduced utilizing inhibitory techniques.

Burns conducted many detailed studies of autonomic techniques on the organic effects in both animals and humans. Using a rat model, she was able to demonstrate an increase in heart rate and blood pressure using electrical stimulation of the sympathetic ganglia, confirming the role of the ganglia in the regulation of the nervous system. She went on to conclude that lesions of these ganglia produced results that were consistent with a decrease in sympathetic tone.

In a second series of studies, she clearly stated that there was a decrease in both heart rate and systolic blood pressure in humans when mechanical pressure via the fingers was applied to the transverse processes of the 8^{th}-10^{th} thoracic vertebrae with the average decrease in systolic BP reported to be ~8mm Hg. She also concluded that these effects were similar to her work in rats, mimicking the artificial lesions in the sympathetic ganglia. Unfortunately there was no published analysis, nor was there any reported control, blinding or randomization procedure. Nor was this work replicated and

its' veracity is a probably a victim of the level of scientific analysis at that time. Her work is, however, the first scientifically designed study to address osteopathic manipulation and inhibition and was incorporated into the osteopathic model of "balancing the autonomics." Northup commented extensively on this in a 1945 speculative article in which he uses an interesting modeling scheme to understand what is essentially now described as hypersympathatonia[121], although its' assumptions and vocabulary are outdated.

Celander, in a series of studies conducted in 1968[122, 123] reported statistically significant reductions in blood pressure and fibrinogen when subjects underwent paravertebral soft-tissue manipulation (not described). Celanders' work used a pre-test/post-test model with a final point fifteen minutes after manipulation and found significance in all measured parameters, with hypertensive subjects showing the greatest response. Numerous studies have been done on the usefulness of manual therapy in the treatment of hypertension[124-127] but most, including Celanders' work, had methodological flaws including the absence of control groups, the overgeneralization of serum results, and limited understanding of the role that the central nervous system plays in autonomic control.

The basic question of this study and many other studies done on manual manipulation is whether or not tactile or somatic stimulus can influence autonomic tone to a sufficient degree that can be measured. The *a priori* requisite of this autonomic response is predicated upon the existence of some pathways or reflex arcs that allow for

local, if not supra-spinal manipulation of basic autonomic processes that are activated by low-threshold or innocuous stimulations.

Furthermore, we propose that these pathways must have some meaningful clinical utility, *i.e.*, they must inhibit sympathetic tone for a meaningful period of time after the cessation of stimulus. There is no question that *noxious* stimuli activate the sympathetic nervous system, as pain will globally increase MSNA through nociceptor activation[128], but it was the discovery of depressor and inhibitory responses that utilize non-nociceptive receptors that may hold some promise as effective treatments.

Sato published a large review in 1997 of the somatosensory influence of the autonomic nervous system that has been done primarily in animals. His review of over 750 published studies showed very conclusive evidence that stimulation of afferent spinal nerves and white-rami in the thoracic and lumbar regions elicited both spinal and supraspinal autonomic reflexes[128]. Examination of different organ systems allowed him to conclude that somato-autonomic reflexes are strongly influenced the characteristic innervation of the target organ, the nociceptor/mechanoreceptor subtype, and the segmental level of stimulation.

Sherrington, in 1906, predicted the existence of a supra-spinal reflex to visceral organs provoked by somatic afferent stimulation and in fact, Alexander[129] was the first to record sympathetically evoked somatic reflexes in anesthetized cats. Stimulation of the sciatic nerve with an amplitude of 8 volts at a 200/s frequency elicited an impulse volley in the sympathetic inferior cardiac nerves with a latency of ~90 ms. This technique involved simple insertion of stimulatory electrodes in the efferent nerve while target

nerve electrode response was measured by oscilloscope. Additional investigation by Alexander in the same published study examined how stimulation of medullary areas resulted in maximal pressor (from rostral stimulation) or depressor responses (from tegmental stimulation) at multiple sites and suggested central integration of these pathways, but it would take several more decades to confirm the location of these areas.

Somato-autonomic reflexes follow response patterns that are characterized by the afferent fibers that are activated. The types of sensory neurons that convert mechanical, chemical, and thermal stimuli into meaningful nervous system traffic have been extensively studied and characterized. It is generally accepted that the receptors that influence the autonomic nervous system are innervated by myelinated Aδ fibers (Type III) and unmyelinated C fibers (Type IV) afferent fibers, yet there exists debate about the influence of muscle spindles and the golgi tendon organs. Approximately 20 percent of these fibers have mechanoreceptors that are activated by low-threshold (innocuous) sensation in muscle, tendon and articular fields. They have been shown to exist in human muscle and tendon in sufficient quantities to lead investigators to believe that they are primarily responsible for the supraspinal and proprioceptive autonomic reflex arcs[130, 131].

Measurement of these reflexes is difficult as there is a high degree of variability between test subjects and the characteristic properties only become apparent after using digital averaging techniques. Nonetheless, three distinct patterns show themselves. The first two, discovered by Schmidt and Sato, were early (25-50 ms latency) and late supra-spinal (80-120ms latency) reflexes that were elicited from the white-rami of the lumbar spine and innervated by the myelinated Aδ fibers[132]. Shortly thereafter, the discovery of a

relatively long latency (200 ms) reflex through the unmyelinated C fibers was discovered. This work also uncovered the existence of propriospinal pathways. The propriospinal pathways integrated with the supra-spinal arcs and thus none of the reflexes could be completely abolished with cervical transaction.

Germane to the present study was the discovery in 1958, of post-excitatory depression. After mass stimulation of afferent somatic nerves, there appears to be a "silent period" of the ANS target nerves (post-excitatory inhibition)[133]. This response is different, depending on which reflex is analyzed (with the early reflex being of a shorter duration than the late reflex) but has been found to exist from about 600ms to 2.2s. Interestingly, post-excitatory inhibitory latency increases as the distance down the spinal column increases. Schaefer's work allowed investigators to infer post-stimulatory effects of somato-sensory stimulus on the ANS, but more importantly suggested that the descending pathways are under some form of supra-spinal integration and subsequent control, which was conclusively shown by Iwamura *et al*[134]. Although Burns proposed that there was a reduction in sympathetic tone by simple mechanical compression, a more complex model emerges in which stimulation of the ascending pathways cause a subsequent inhibition of the descending pathways through interaction of both the spinal and supra-spinal pathways.

The proposed site for sympathetic integration, the rostral lateral ventral medulla (RVLM), seems to mediate this post-excitatory inhibition through GABA projections, as ionophoretically blocking GABA receptor antagonists in the RVLM during hindpaw stimulation greatly abolished vasomotor neuronal traffic[135, 136]. These responses have

been shown to result from nociceptor stimulation of Type II fibers in the dorsal horn of the spinal cord[137]. Inhibition, presumably supra-spinal, was also shown to be present in the adrenal sympathetic nerves[138] and there has been some evidence that the depression occurs as an interaction between arterial baroreceptor afferents and somatic stimulation that is mainly, although not exclusively, integrated in the brain stem[139, 140].

Somato-autonomic reflexes were initially described through sympathetic pathways in animals, however it was already known that the inhalation anesthesia used in these experiments could depress parasympathetic tone. Sato also determined that while some types of stimulus were activating, such as pinching, other types would depress SNA. Sato ultimately concluded that type of response (pressor or depressor) was highly dependent upon the effector organ. Nishijo[141], in his review of the mechanisms of acupuncture has speculated that there is also evidence of parasympathetic reflex arcs. This leads to inevitable confusion in the indirect assessment of autonomic activity, as these two competing pathways may undergo simultaneous activation in both acupuncture and osteopathic manipulative therapy. The literature currently presents a mixed view, indicating both sympathetic and parasympathetic activation and inhibition that seems to be very demand specific[142-145].

Studies using anesthetized rats showed that sustained, but non-noxious, force (0.5kg-3kg) on the thoracic and lumbar vertebrae lead to large decreases in blood pressure, as well as decreases in renal nerve and adrenal nerve activity. When stimulation was examined after cutting the dorsal roots no response was evident. Additionally, stimulation of the *cauda equina* produced no consistent effects. This strongly suggests

that articulatory receptors are required for these responses. The depressor response outlasted the stimulus for a short time and lesions of higher spinal segments obliterated these responses, showing the involvement of ascending pathways[146, 147].

Hudson was able to show autonomic effects through nociception, but additional work has also shown that stretch of skeletal muscle changes differential autonomic tone. Murata[148] used direct neurography in anesthetized cats to show that at the beginning of passive stretch in the hindlimb there was an increase in CSNA burst activity that quickly returned to baseline levels during the stretch. The SNS responses were only partly attenuated by sino-atrial ablation indicating that the autonomic tone was not altered through baroreflex pathways. CSVA followed a different pattern; it decreased to a minimum after 30s of stretch and slowly returned to baseline levels after stretch was discontinued. Only CSVA increased in response to a graded stretch. This establishes not only a different time frames for the SNS when compared to the PSNS but, presumably, different neuroregulatory systems. It also highlights the role of the Type III afferent nerves, which are now known to be activated without nociception in the physiological ranges of motion and rapidly adapt.

The effects of somatic afferent stimulation on the RAS are quite different. It appears that stimulation of the brachial nerve creates pressor response in rats through two mechanisms. Hudson has shown a two-fold increase in plasma renin[100] following 3 Hz brachial nerve stimulation that appears to act at the level of the kidney through AT2. Davis then demonstrated a pressor response and changes in renal function that were greater in decerebrate, carotid-sinus denervated rats[149, 150]. He concluded that the

baroreceptors exert considerable inhibition on reflexes from somatic nerves without central stimulation.

Work has thus shifted the focus from electro-physiologic animal studies to human subjects using mainly indirect assessments of autonomic tone by measuring blood pressure and heart-rate variability (HRV). Many investigators have commented on the utility of using HRV to assess autonomic variability and concluded that there are inherent limitations in the utility of using HRV to attempt to directly assess the influence of sympathetic or parasympathetic activity[151-154]. HRV may have usefulness as a tool for clinical autonomic evaulation[155] but it is not specific enough to determine which effect is accurately being measured. HRV measures are also susceptible to variation in post-synaptic transmission quality, baroreceptor sensitivity and adrenoreceptor sensitivity making is susceptible to phenotypic variation. In the case of the current study, HRV measures limit one to making indirect inference about the origin of increases in the LF domain, limitations that were overcome years ago by correlation of MSNA and noradrenaline measurement[45, 46, 50, 156-159].

There have been few published studies to date using manipulation and direct measurement of MSNA. Prior unpublished work has shown that healthy, resting subjects subjected to the rib-raising technique showed no identifiable changes in MSNA when compared to sham. This fact suggested that a more powerful study design may uncover significant effects and for this reason we decided to use a cold-pressor induced SNS elevation with microneurographic MSNA. The hypothesis of the current design is that there would be a reduction in MSNA in the post-test interval due to descending inhibitory

influence. Three seperate cold-pressor temperatures were chosen to evaluate for a graded response. Although there is a high-degree of intra-subject variability (which occurs with HRV varibilities, as well), this limitation is overcome by using a repeated measures design.

Budgell has recently shown that cervical as well as thoracic manipulation can produce short-term changes in heart-rate variability[160, 161]. There was significant shift in the LF-frequency domain that was indicative of a shift in the ratio of sympathetic to parasympathetic tone which points to a central mechanism, as opposed to a purely local effect. This series of studies utilized a standardized protocol with naïve patients blind to sham or manipulation. Authentic high-velocity low-amplitude manipulation saw a significant increase in absolute (LF) and normalized (LF/total power) HRV spectra. The author concluded, at least in cervical manipulation, the effect of the audible sounds which occur during authentic manipulation may enhance placebo effects. The second consideration in cervical manipulation is the existence of vestibular, sympatho-inhibitory reflexes[162, 163]. There was no report regarding the length of the reported HRV effects.

Delaney[164] also used HRV to address any influence of myofascial trigger point massage therapy (MTPT), analogous to osteopathic myofascial techniques, would have on autonomic tone. The results showed, once again, a significant shift in the LF spectra domain, indicating decreased sympathetic drive to the heart. Unfortunately this study did not compare MTPT to a sham protocol, with the control group simply sitting quietly.

A significant role has been uncovered in neural regulation by baro-sensitive afferent nerve fibers with the discovery that baro-sensitive stretch receptors (lung and arterial) cause a reduction in SNA through baro-sensitive pathways[165] during normal physiologic function. This is in contrast to activation of the Type III and Type IV mechano-receptors which appear to cause a rise is afferent baro-sensitive discharge and an increase in SNA, possibly through interactions between chemo-sensitive receptors and central command[166-168].

Acupuncture studies offer a novel and clinically relevant modality in which to examine somato-autonomic reflexes can be examined for several reasons. First, they are not as susceptible to the sham control problems that inevitably arise anytime a person is touched. Second, they follow relatively consistent protocols due to standardized understanding of the anatomic locations of acupuncture points within the context of Traditional Chinese Medicine (TCM). Third, the degree of therapeutic variability is greatly reduced due a less operator dependent treatment mechanism. There is also considerable overlap in the mechanistic theories of acupuncture and OMT, and some of these theories seem to have been borne out by basic research. These studies not only contribute to overall understanding of physiological phenomena but may give researchers a better perspective in how to approach current work on sympatho-autonomic stimulus.

Modern acupuncture studies use electrically stimulated electrodes inserted at particular points that correspond to their TCM counterparts in animls and humans. Early work focused on the reduction of pain using EA and it is know that low-back and cervical musculoskeletal pain can be reduced by appropriate use of either of EA, usually as an

adjuvant therapy. The Cochrane group has concluded that acupuncture is effective for short-term, immediate relief of certain musculoskeletal pain with moderate supporting evidence for acute, non-complicated neck pain and chronic low-back pain. The effects are small however, with most improvements being of a functional, rather than clinical nature.

Acupuncture has been shown to have some effectiveness as it applies to diseases that involve autonomic dysfunction. Liptak *et al.* was able to show in 1980 that acupuncture reduced ventricular extrasystoles in subjects who had experienced chronic extra-systolic events[169]. Reduction of anginal pain was demonstrated and replicated shortly thereafter[170, 171]. Recent work has shown changes in cardiac function in already healthy, anesthetized, open chest canines[172]. Without EA there was a gradual decrease in most CV variables (MAP, HR, EDV, SV, CO and end-systolic pressure). When compared to EA animals all of these variables increased ~40% over 60min. When EA was stopped these variables decreased to their pre-test levels. This initial SNA activation followed by a depression in MSNA is commonly seen in EA studies and has been speculated in inhibitory OMT techniques.

Chao[173] was able to demonstrate that electro-acupuncture worked through the opiate pathways in a model that reversed the bradykinin induced gallbladder pressor response (BIGPR) by the introduction of naloxone. Direct recordings of RVLM neurons showed decreased sympathetic activity during acupuncture using the BIGPR model[174, 175] in rats. Further work clarified this model by showing that ventral peri-aquaductal grey (vPAG) output was increased during acupuncture, thus lowering RVLM output. The same result was obtained by the injection of the excitatory amino acid, DLH, in the same

region[176]. This was followed by evidence which showed an up-regulation of c-fos(a transcription factor associated with endogenous opiate production) in the RVLM[177] during acupuncture in animal models.

McPartland has shown that OMT therapy can cause an increase in endocannabinoids in humans[178], although this study lacked a protocolized treatment design. AEA (an endocannabinoid) and opiates cause nitric oxide release through cellular signaling pathways in neural human tissue and may also be implicated in attenuating inflammatory mediators. Seagard *et al.* has shown experimental evidence linking AEA microinjection to the activation of CB1 (endocannabinoid) receptors[179] and a concomitant reduction in blood pressure, although it was concluded that this occurred through a GABA-mediated mechanism.

These differing studies align with current evidence of neurogenic hypertension, tending to support the theory of an inflammatory process in the medulla. However, there is currently no accepted mechanism of action for either EA or OMT and these studies are in need of replication. A recent review, in fact, found that physiologic mechanisms should be required in for future funding of acupuncture studies.

These converging lines of evidence point to the priority of the supraspinal effects in somato-sympathetic reflexes. There is currently no literature on whether sympathetic inhibitory techniques actually inhibit nervous system outflow or whether they change nervous system inflow which in turn influences central command of sympathetive output as proposed by Still and Burns. The latter seems more likely, as previous studies have indicated.

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CHAPTER 2: THE CURRENT STUDY

QUESTION

It is currently unknown whether inhibitory rib-raising decreases direct and indirect markers of sympathetic tone, and to what degree. Recent work was not been able to demonstrate effects in healthy, resting individuals. The resting SNA of individuals is low and this study is designed to induce an elevation in SNA in order to magnify the proposed inhibitory process. This is the first study designed to answer to assess whether inhibitory rib-raising changes SNA function when compared to a sham during elevated SNA.

SPECIFIC AIMS

To determine the short term effects (2min interval) of inhibitory rib-raising versus sham (therapeutic touch) on the SNS during a graded sympatho-excitatory stimulus (cold-pressor) by measurement of:

1) MSNA through direct microneurography.,
2) Blood pressure and heart rate through non-invasive monitoring,
3) Pain perception during stimulus using a modified Borg scale.

HYPOTHESIS

It is hypothesized that during cold-pressor stimulus there will be a reduction in muscle sympathetic nervous system activity and pain as compared when undergoing inhibitory rib-raising as compared to sham. This decrease should diminish as temperatures approach a thermo-neutral state.

Sustained inhibitory rib-raising attenuates sympathetic nervous system activity during cold-pressor stimulus

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ABSTRACT

Inhibitory rib-raising (IRR), an Osteopathic Manipulative Treatment (OMT) technique, has been theorized to regulate autonomic imbalance by applying direct pressure to the head of the rib near the sympathetic ganglion, yet no work has been published documenting this effect. An initial study in our lab found no change in SNA in healthy individuals. We hypothesized that rib-raising applied while SNA is elevated will effectively modulate SNA. ***METHODS:*** Twenty healthy subjects by history and physical examination who were naïve to OMT techniques and proposed effects were recruited. IRB approval was obtained. A cold-pressor stimulus was used to produce a pain-mediated elevation of the SNA. MSNA, blood Pressure, and pain were measured during cold-pressor testing. A randomized protocol was used in which IRR and sham touch was performed on all subjects for 2 min in three temperature conditions (2°C, 10°C and 18°C). All data were reduced *post hoc* via a customized digital data acquisition system. ***RESULTS:*** Two-way repeated measures ANOVA showed a significant difference between the sham and treatment groups (p=0.027) in MSNA at the 2°C level. Blood pressure and pain data did not demonstrate significant effects when sham was compared to OMT. *Conclusions:* SNA can be reduced by application of inhibitory rib-raising, but it is likely not mediated by a pain dependent mechanism.

INTRODUCTION

Somato-sensory stimuli and their effects on the sympathetic nervous system (SNS) in humans is poorly understood. Sato and Schmidt[1] established that both inhibitory and excitatory somato-stimulus reflexes are demonstrable in anesthetized animals using electrical stimulation of the somatic nerves, but little is known about the clinical utility of these reflexes. There remain significant questions as to whether or not these pathways can be used in the treatment of disease that are characterized by derangement of the autonomic nervous system.

Somato-autonomic reflexes follow response patterns that are characterized by the afferent fibers that are activated. The types of sensory neurons that convert mechanical, chemical, and thermal stimuli into meaningful nervous system traffic have been extensively studied and characterized[2-5]. It is generally accepted that the receptors that influence the autonomic nervous system are innervated by myelinated A-δ fibers (Type III) and unmyelinated C fibers (Type IV) afferent fibers. Approximately 20 percent of these fibers have mechanoreceptors that are activated by low-threshold (innocuous) sensation in muscle, tendon and articular fields. They have been shown to exist in human muscle and tendon in sufficient quantities to lead investigators to believe that they are primarily responsible for the supraspinal and proprioceptive autonomic reflex arcs[6, 7].

Sato[8] demonstrated that low-grade mechanical load on the spinal column in rats has a sympatholytic response. This response was abolished when the dorsal sensory nerves were transected; leading to the conclusion that this was an afferent mediated reflex arc. After stimulation of afferent somatic nerves, there appears to be a "silent period" of ANS target nerves (post-excitatory inhibition)[9], which has been found to exist from ~600ms to 2.2s. This suggests that the descending sympathetic pathways are under some form of supra-spinal integration and subsequent control, which was conclusively shown by Iwamura[10]. Unfortunately, all studies to date have been done in anesthetized murine or feline models and have not yet been measured in humans.

Inhibitory rib-raising is an Osteopathic Manipulative Treatment (OMT) technique first described by Burns in the early 1900's[11] and is described in current textbooks. The theoretical approach described by Burns is dependent upon the fact that the sympathetic chain ganglia lie anterior to the rib heads. These relay stations modulate peripheral sympathetic traffic in humans. Thus, in theory, pressure on the posterior rib head could activate local reflex arcs and alter sympathetic outflow[12]. However, there has been no published research on this technique in which markers of the SNS have been measured.

Recent work in humans has shown that there is a change in heart-rate variability in humans using thoracic and cervical manipulation[13, 14] indicative of changes in autonomic tone. These studies used a fundamentally different technique from inhibitory rib-raising by utilizing a very short, high velocity (HVLA) thrust to the spinal column.

Nonetheless, Delaney[15] was able to demonstrate a change in HRV utilizing myofascial trigger point massage therapy; a technique which resembles inhibitory rib-raising.

Unpublished wok in our lab found no significant effect of inhibitory rib-raising in resting healthy individuals. However, SNA is generally quite low in health individuals and any sympatholytic effects may be difficult to demonstrate under these conditions. We have postulated that these effects may only occur under sympathoexcitory conditions. Thus, this study was designed to address the hypothesis that inhibitory rib-raising attenuates sympathetic nervous system activity during pain induced sympathoexcitation.

METHODS

Subjects: Twenty volunteers were recruited from the campus of the University of North Texas Health Science Center ranging in age from 22 to 36 years old with 12 Caucasian men and 8 Caucasian women who were naïve to OMT. Subjects were fully informed of experimental protocols and signed human subjects IRB consents approved by the human research committee of the University of North Texas Health Science Center.

All individuals completed a health questionnaire from which it was determined no subjects had significant health issues, including pulmonary, cardiovascular or neurological disease. Individuals were not taking prescribed medications other than oral contraceptives and females were administered a urine pregnancy test to confirm that they were not pregnant. All persons denied being current smokers and were asked to refrain from intense physical activity at least 24 hrs prior to experimentation and refrain from products containing caffeine 12 hrs prior to experimentation.

Experimental Protocol: Subjects were place in the supine position and were outfitted with standard 12-lead EKG, MSNA peroneal nerve probe, and photoplethysmography (FinaPress) of the right middle finger. Subjects were placed in ~5°-10° incline for comfort and remained in this positioning for the course of the experiment (~3 hrs.). Subjects right leg was elevated 10-12 inches off the table at the

knee to isolate the nerve site. Subjects were given a 20 minute rest period prior to experimentation to allow a return to baseline measures.

All subjects underwent a series of cold-pressor stimuli which included a total of six exposures, two each in three different temperature states, 2°C, 10°C and 18°C. Three testing conditions were done with an osteopathic practitioner administering inhibitory rib-raising and three testing conditions were done with sham touch. The order of the testing was determined by a random allocation procedure. A 12 min rest period was given between each stimulus and data recording began 2 min prior to testing to establish a baseline condition. Recording was continued during stimulus and continued for 4 min post-stimulus.

Cold Pressor Stimulus: Cold-Pressor testing has previously been confirmed to cause an increase in sympathetic outflow[16-18]. Cold-Pressor testing was chosen because it is a low-risk, easily reproducible stressor in which minimal movement is required, thus limiting data noise. Subjects' undergoing cold-pressor generally see the following 1) a rise in arterial pressures during the 2 min of CPT, 2) a rise in heart rate (HR) which reaches an apex within the first 30 sec and a return to baseline HR by 2 min, and 3) little rise in MSNA until the last 30 sec.

Subjects' right hand was immersed in a measured cold water bath for 2 min. Bath temperature was attained by a mixture of water and ice and temperature was determined by a standard mercury thermometer and the baths were pre-prepared by a lab assistant. All subjects were able to maintain immersion until the 2 min end-point.

Pain: Pain was measured during cold-pressor stimulus using a modified Borg-scale. The Borg 15-point Rating of Perceived Pain Scale (RPP) was used to rate the subject's perception of pain intensity[19] and has been used to evaluate pain perception during the CPT[20]. Subjects were asked to rate their perceived pain just as their hand is placed in the water and in 15 second intervals using a scale from 6-20 for the full two-minute cold-pressor test.

Sympathetic Nerve Activity: Post-ganglionic MSNA was measured at the peroneal nerve located on the upper and outer aspect of the leg near the fibular head using standard microneurographic techniques[21-24]. Three subjects were excluded due to inability to obtain viable signals, and two subjects were excluded due to excessive noise in the signal. A tungsten electrode was inserted at the nerve and the raw signal was amplified (Nerve Traffic Analyzer, Model 662C-3, University of Iowa Bioengineering, Iowa City, IA) with variable gain that was visually determined in real-time to compensate for signal noise.

Muscle SNA recordings have the following characteristics: 1) pulse-synchronous bursts occurring 1.2-1.4 s after the associated QRS complex, 2) reproducible activation during phase II and III of the Valsalva maneuver, and 3) no activation following a pinch, skin stroking, or startle stimuli. Provocation testing based on these criteria was used to determine appropriate electrode positioning. Data was sampled at a variable rate between 100khz-1000khz and converted to a digital signal via a customized computer set-up utilizing the WinDaq computer program (DATAQ instruments, Akron OH).

Nerve burst activity was assessed by the following criteria: 1) pulse-synchronicity and 2) amplitude of more than 30% of baseline. MSNA was quantified by the total number of bursts during the two minute stimulus interval minus the total number of bursts in the preceding two minute baseline interval.

Hemodynamic measurements: Subjects were fitted with standard limb-lead ECG for determination of heart rate and arterial blood pressure was measured non-invasively by use of a Finapres photoplethysmographic monitor (Finapres blood pressure monitor 2300, Ohmeda, Englewood, CO) placed around the middle finger. This provides a beat-to-beat measure of arterial blood pressure and will be used for estimation of the blood pressure variability and has been shown to be a valid measure of arterial blood pressure[25, 26].

Inhibitory Rib-Raising: Sustained inhibitory rib-raising was performed by bilaterally placing the operator's fingertips on the ipsi-lateral posterior rib heads at the most cephalic portion of the thoracic spine spanning T3-T7 as described in a standard textbook used in the teaching of Osteopathic Medicine[27]. The fingertips were then raised antero-laterally toward the sympathetic chain ganglia for 2 min during cold-pressor stimulus in an attempt to inhibit the thoracic sympathetic chain ganglia. Sham OMT was performed by placing the hands in the same position without the concurrent antero-lateral force. The OMT practitioner was blinded to the stimulus applied and the responses elicited. All attempts were made to maintain consistency of pressure and location during the treatment phase.

Data-Analyses: Statistical evaluation was conducted at a significance level of α=0.05. A repeated measure, two-way ANOVA test was used to assess MSNA, HR, diastolic BP, systolic BP and pain for IRR versus sham treatments at three different treatment conditions of 2°C, 10°C and 18°C. HR, DBP and SBP reflect the mean during the two-minute stimulus phase of testing. Pain was assessed using the Borg scale mean during the two-minute stimulus. The change (response) from baseline during CPS was determined for each physiologic variable under each treatment condition. Pain was assessed using the Borg scale mean during the CPS. If data was determined to be non-parametric, *post-hoc* testing utilized the Holm-Sidak test, a pairwise multiple comparison step-down procedure designed for data that is not normally distributed.

RESULTS

Baseline responses: Baseline measures for physiologic variables were measured prior to each testing condition. There were no significant differences in baseline data for SBP, DBP or MSNA across the pre-intervention baseline periods ($p>0.254$). All baseline variables were normally distributed.

Cold-Pressor Test Responses: Figure 1 is a representative tracing of sympathetic activity and arterial pressure during a 2^{o} cold pressor stimulus. Notable is the large increase in both sympathetic nerve activity (SNA) and arterial pressure during the stimulus and a rapid return toward baseline upon relief of the stimulus. In general, measurement of physiologic variables during cold-pressor testing demonstrated a clear graded stimulus response. Mean MSNA increased as temperature decreased across temperature conditions ($p<0.001$). Mean SBP and DBP showed a similar response, with SBP and DBP increasing as temperature decreased ($p<0.001$). These data were associated with a graded increase in perceived pain as the stimulus intensity was increased, thus, the physiologic responses to cold stimulus correlated strongly with the perceived pain ($r^2>0.6$).

Effects of Treatment Modality: Repeated measures two-way ANOVA testing revealed a statistically significant decrease in MSNA during treatment (OMT) as compared to sham at the 2°C level ($p=0.027$), with a reduction in means of ~7.8 nerve bursts/min during the stimulus (Figure #2). There was no significant effect seen at the 10 °C or 18°C level. MSNA data did satisfy criteria for equal variance, but did not satisfy normality. Pain analysis (Figure #3) showed no interaction or treatment effect in any condition ($p<0.918$), nor was there any identifiable trend. Data did satisfy criteria for equal variance, but did not satisfy normality, as expected. Thus, these data suggest that the IRR treatment did not affect the perception of pain. Systolic blood pressure (Figure #4) showed no significant treatment effect, although there was a modest reduction in SBP during the 2°C condition ($p = 0.32$). Diastolic blood pressure (Figure #5) did not reveal any significant treatment effect in any condition, although there was a modest trend toward a OMT-related reduction in DBP during the 2°C condition ($p = 0.33$).

DISCUSSION

This is the first study to directly examine any osteopathic inhibitory technique and subject it to a sham protocol with direct SNA measurement. This research is also the first in humans to find direct evidence of MSNA attenuation utilizing somato-sensory stimulation, as described by osteopathic medical practitioners. The reduction in MSNA was small, but significant. It is difficult to generalize these results to anyone other than healthy individuals, but we would expect to see a stronger treatment effect in individuals with ANS derangement.

The decrease in MSNA in the IRR group only occurred during high levels of sympathoexcitation. It would also be appropriate to think of IRR as decreasing the *expected* amount of stimulation. The utility of this gestalt is that it may explain why no effects were seen in prior studies with healthy individuals. Although the blood pressure data did not reach a level of significance, it followed the same trend as the MSNA data which allows us to conclude that there seems to be a good degree of internal validation.

The cold-pressor stimulus is a puissant sympathetic stimulus which leads to increases in hemodynamic variables, as well as MSNA[16, 17, 28]. Importantly, Victor *et* al[17] correlated increases in MSNA and BP with increases in MSNA and norepinepherine, suggesting that BP was increased due to catecholamine release. Furthermore Kregel *et al* [20] correlated MSNA with pain sensation, suggesting that MSNA activation is primarily a result of pain. Wirch *et al.*[29] recently has described high-degrees of subject variability in

blood pressure, heart rate variability indices, and baroreceptor sensitivity using 6 min cold-pressor testing. This demonstrated not only strong sympathetic response, but also a significant parasympathetic reduction. Wirch suggested that repeated measures designs were the most appropriate for this type of stimulus.

This study, however, found that pain is not a factor in the reflex arc that accompanies MSNA reduction. We did hypothesize that there would be a reduction in pain, postulating a gating type of mechanism. This was clearly not the case. The contradiction of this argument is that there is mounting evidence that acupuncture, manipulation and other types of manual therapy exert their effects through supraspinal mechanisms.

Animal models have confirmed that some somato-autonomic pathways are independent of supraspinal involvement[30, 31]. Sato[32] has shown that somatic stimulation can decrease adrenal nerve firing in decerebrate cats and has suggested that supraspinal reflexes tend to enhance local spinal reflex effects, although Li *et al*[33] has shown that baroreceptor modulation due to somatic stimulation probably occurs only at the brainstem level.

Burns suggested that the depressor responses in IRR were a local effect of anatomic compression of the sympathetic ganglia[11], although it was not possible during her time to measure these responses. We have speculated that pain and MSNA are related in a causal fashion; that reductions in MSNA are due to decrease in nociception. Studies

have clearly shown that pain is a potent stimulus for SNA, but it may not necessarily follow that a reduction in pain by OMT is the mechanism responsible for reductions in SNA. Recent work by Barkis *et al.*[34] has examined manual treatment in individuals with hypertension but without concomitant complaints of pain. This suggests that the inhibition may be occurring independent of pain pathways.

There is currently no generally theory accepted theory of how OMT would lower SNA. There has been speculation that it is related to NO release in the medulla, and in fact electro-acupuncture studies reveal an increase is c-Fos expression (a marker of NO release) in rat models[35-39]. There has also been recent work suggesting that manual therapy may induce an endogenous release of endocannabinoids[40], although this work was not protocolized to a specific treatment technique. In both cases Seagard *et al.* has demonstrated that endocannabinoid release in the NTS attenuated SNA[41]. Mechanistic action remains speculative at this time.

LIMITATIONS

This study can not address what the mechanism of the reflex arc. It is possible that these reflexes are not related at all to the sympathetic chain ganglia and are remnants of the low-threshold mechano-receptors in either the paravertebral muscles or facet joints of the vertebrae. It has been demonstrated that low-threshold receptors exist in the cervical facet joints[42, 43] and there is no reason to suspect that these would not have the same ANS properties as other receptors.

The random allocation of this study was designed to address the hope that subjects would not immediately understand the difference between the sham treatment and IRR. It is rather simple to determine which one is the actual treatment group as considerable antero-lateral pressure was being applied during IRR. And although subjects were chosen who had no experience with OMT or expectation of treatment, there is still a strong possibility that subjects could reasonably expect which one was IRR by the 3rd trial. Measurement of MSNA has a high-degree of subject variability and a matched control design is fraught with excessively high variability making it difficult to blind placebo and making almost impossible to blind the investigator. This is an ongoing debate in the fields of surgery as well as manual medicine.

Osteopathic medicine has an extensive body-of-work addressing the context of somatic dysfunction and its' role in disease. Some practitioners of OMT have suggested that somatic dysfunction may be caused by primary disease processes and although this is plausible, it has not been conclusively shown. No individual in this study had any

remarkable evidence of somatic dysfunction beyond cursory examination. It would, therefore be purely speculative to extend the clinical utility of this technique without further investigation.

The cold-pressor stimulus is a successful, dynamic modeling of hypersympathatonia. Its main advantage, however, may be its' main drawback. The transient rise of SNA does not necessarily model long-term pathologic states. It is well-known that long-term stresses include alteration in the neuro-hormonal axis that this study was not designed to address. Few studies long-term studies in this area of research are done at a basic science level and the time-frame required for human subjects' research would be a difficult obstacle in study design.

CONCLUSION

OMT addresses disease states. It does not address what happens in the context of a healthy individual when homeostatic mechanisms are functioning correctly. Considerable research exists addressing the fact the there is considerable tonic inhibition exerted on the various systems in the body. There are redundant systems that exist in the forebrain, medulla, somatic nerves, HPA axis and baro-receptors to stabilize and maintain hemodynamic systems and sympathetic outflow.

It was necessary to highly stress individuals to cause measurable deviation in MSNA. This serves to illustrate two concepts, that 1) pathologic ANS dysfunction has overwhelmed the normal homeostatic states and 2) there are corrective reflexes inherent in the body. Both of these concepts align with the tenets of osteopathic medicine and its' approach to the body as a self-correcting mechanism in which disease is an alteration of homeostasis. This study did find that inhibitory rib-raising attenuates MSNA during a stress state. It is the first to quantify this with direct nerve recording and thus, will hopefully serve as a backbone in which to examine the clinical utility of manual therapeutic methods.

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Figure #1: Sample Tracing During Baseline and Cold-Pressor

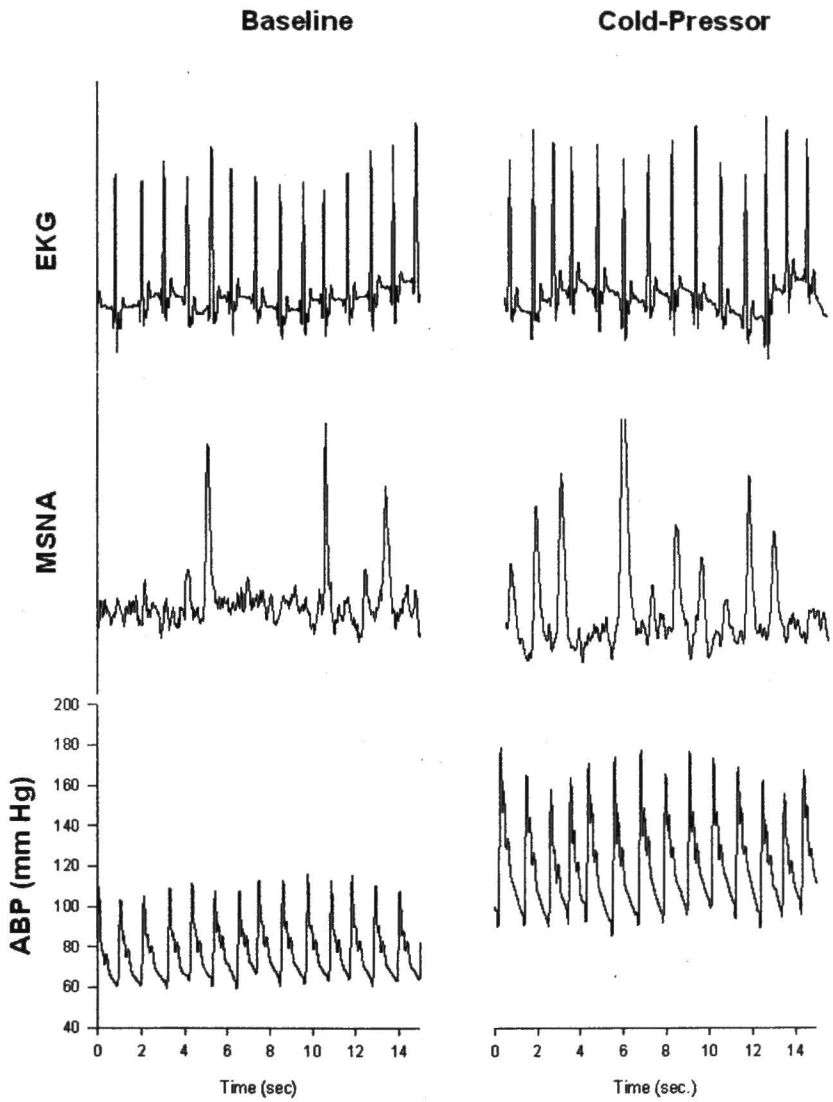
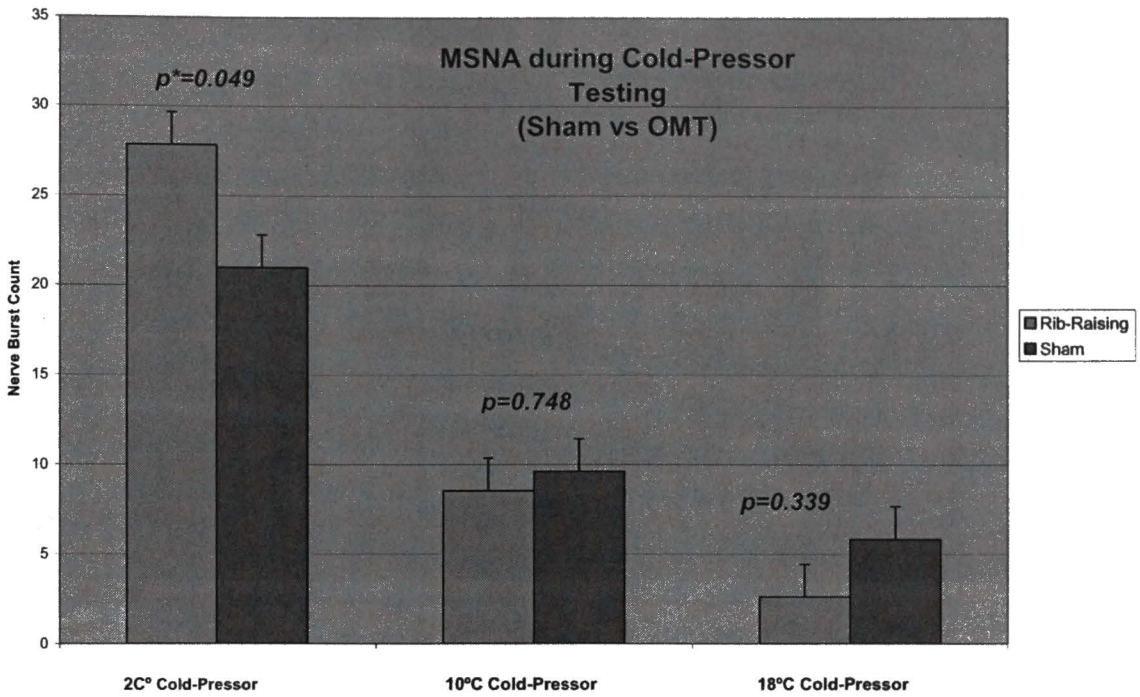


Figure #2: MSNA Change during Cold-Pressor



Figure#3: Pain during Cold-Pressor

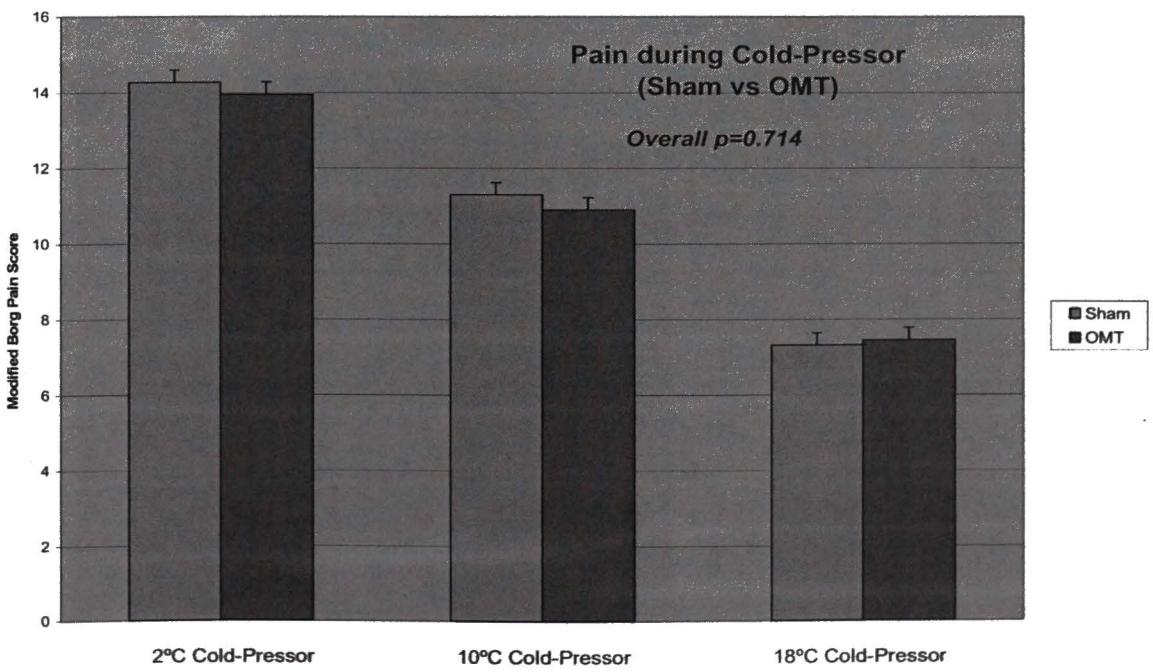


Figure #4: Peak Systolic Blood Pressure during Cold-Pressor

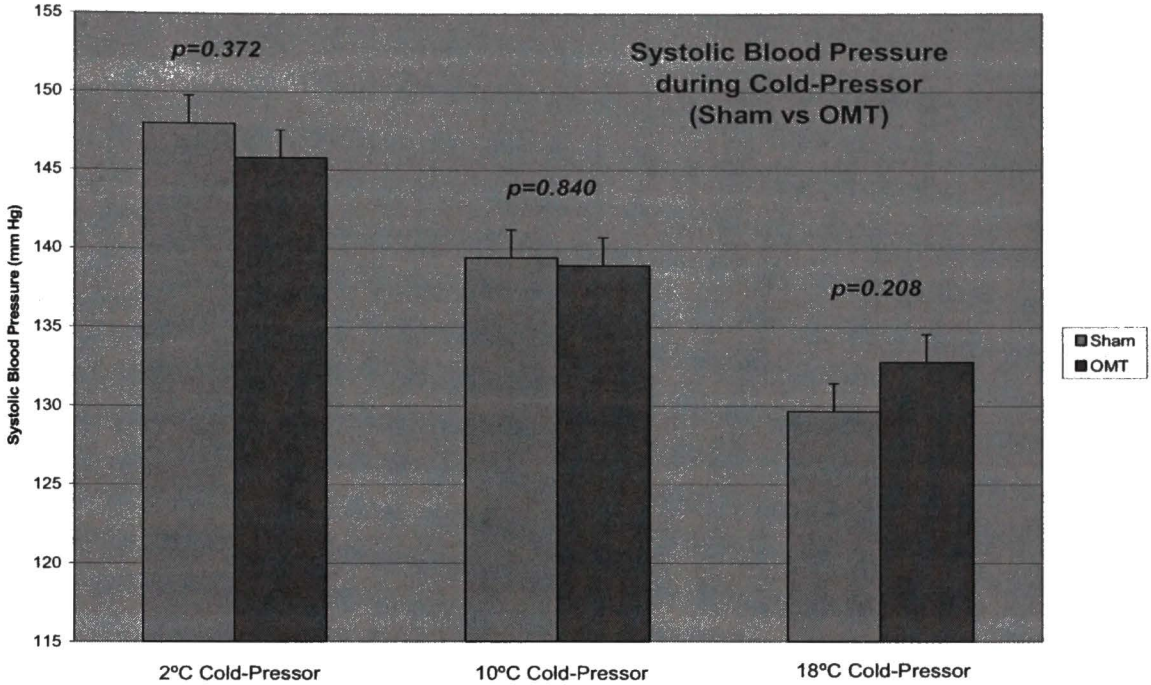


Figure #5: Average Diastolic Blood-Pressure during Cold-Pressor