U.S. Department of Health & Human Services | National Institutes of Health

National Center for Complementary and Integrative Health

Neurocircuitry of Force-Based Manipulations Workshop

September 17–18, 2019

Meeting Report

Workshop Organizers

Merav Sabri, Ph.D., National Center for Complementary and Integrative Health

James Gnadt, Ph.D., National Institute of Neurological Disorders and Stroke

Workshop Cosponsors

National Center for Complementary and Integrative Health

Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Center for Medical Rehabilitation Research

National Institutes of Health Partners

National Institute of Neurological Disorders and Stroke

National Institute on Aging

National Institute of Biomedical Imaging and Bioengineering

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National Institutes of Health
6701 Democracy Boulevard, Room 602, Bethesda, Maryland
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Executive Summary

This 2-day workshop brought together clinicians, neuroscientists, and bioengineers to highlight current knowledge of biological mechanisms of force sensing and biomechanical force-based manipulations. The workshop was convened to encourage subject matter experts to express their individual thoughts on research gaps in the field of force-based manipulations, issues involving the use of terminology by different disciplines, and potential ways to advance cutting-edge research related to force-based manipulations.

1. Panelists articulated key knowledge gaps, including:

    - What types of biomechanical forces do neural and nonneural cells sense, and how are these involved in therapeutic responses?
    - What types of neurons innervate deep tissues, and what are the spinal and supraspinal circuits that process these inputs?
    - How do mechanosensitive receptors, neurons, and circuits change in pathological conditions that are treated with manual therapies?
    - What is the potential impact of manual therapies on tissue mechanics? Many of the phenomena that might be treated, such as “painful knots” in muscles, are poorly understood.

2. To promote collaborations across disciplines, a common lexicon is needed, including:

    - A comprehensive list of force-based manipulations and the use cases for each
    - A highly generic common language for biomechanics and biological responses
    - The questions to be addressed with computational tools and new devices.

3. Tool development needed to overcome major technology barriers includes:

    - Objective, quantitative measures and physiological readouts of therapeutic responses for rigorous clinical trials and mechanistic research
    - Tools to measure strain/stress fields in deep tissue during dynamic stimulation
    - Validated models of diseases and manual therapies in genetically tractable model organisms
    - Robotics and virtual reality approaches to ethically investigate the impact of social factors and individual differences among therapists on patient responses
    - Computational models that include multiphysics and ways to include historical factors, social factors, and mood.

4. Examples of connections that are needed to promote transdisciplinary, collaborative efforts include:

    - Conferences, such as this workshop, to bring together researchers in different fields to spur synergy between relevant areas of investigation
    - Ensuring that all stakeholders, including clinicians, neuroscientists, bioengineers, funding agencies, patients, and payers, can participate.

Workshop Summary

Day 1 – September 17

Opening Remarks

Dr. Helene Langevin, National Center for Complementary and Integrative Health (NCCIH)

Dr. Langevin, Director of the National Center for Complementary and Integrative Health (NCCIH), opened the workshop, explaining that the focus would be on force-based manipulations—that is, passive application of mechanical force to the outside of the body with therapeutic intent, rather than active movement or exercise. Examples of force-based manipulations include light touch, pressure, thrust, and needling. The purpose of these manipulations is to affect the nervous system or other structures.

The range of applied forces in manipulations is diverse. The forces applied in spinal manipulation are about 1,000 times greater than those in acupuncture needling, and the forces in massage are intermediate between these two. Tissues may react to different forces—or more accurately, to the stresses and strains they induce—with different responses.

Workshop Charge

Dr. Ellen Lumpkin, University of California, Berkeley

Dr. Lumpkin, cochair of the workshop, explained that the objective of the workshop is to assess current understanding of biological mechanisms of force sensing and biomechanical force-based manipulations by bridging the fields of neuroscience, bioengineering, and manual therapies. The goals of the workshop are to:

- Identify research gaps and barriers in the field of force-based manipulations
- Discuss new research opportunities in this field

- Promote collaborations among neuroscientists, manual therapists, physiologists, mathematicians, and engineers to advance cutting-edge research related to force-based manipulations
- Develop and encourage the use of common terminology.

The workshop's product will be a publicly available report that summarizes knowledge gaps, highlights research opportunities, and serves as a roadmap for how researchers can work across disciplines based on their common interest in biomechanical force.

Overview of Clinical Relevance

Dr. Gert Bronfort, University of Minnesota

Dr. Bronfort, cochair of the workshop, presented a high-level overview of the clinical relevance of force-based manipulations. The clinical literature focuses primarily on spinal manipulation, with a much smaller amount of research on massage. The principal clinical applications for spinal manipulation are musculoskeletal conditions such as low-back pain, neck pain, and headaches. For both acute and chronic low-back pain, spinal manipulation has some benefit, according to the most recent reviews, but does not stand out as superior to other therapies. Nevertheless, it is among the nonpharmacologic approaches recommended as first-line therapy in the American College of Physicians clinical practice guidelines for treatment of low-back pain.

For neck pain, spinal manipulation and mobilization have benefits similar to or better than those of other evidence-based interventions. For headache, there is evidence of benefit, particularly for cervicogenic headache but also for migraine, where spinal manipulation may be as helpful as prophylactic medication. This benefit may reflect the presence of musculoskeletal problems in a large proportion of people who have migraine. For massage, the evidence is not as strong, but some trials have provided low-level evidence of effects on musculoskeletal conditions.

To better understand the clinical effectiveness evidence and optimize manual therapies, there is a need to look at the biomechanical, neurophysiological, and psychological effects of these interventions. Psychological effects come into play because the interventions involve a therapeutic ritual and physical touch, which may affect the processing of pain in the brain.

Researchers at the University of Minnesota and University of Pittsburgh are developing technology that uses imaging to obtain real-time information about biomechanical function during spinal manipulation. For example, it is now possible to track individual vertebral segmental motion and relate it to the force applied during manipulation.

Session 1: Defining the Biomechanical Force

Moderator—Dr. Lyndon Joseph (NIA)
Rapporteur—Dr. Julie Fritz (University of Utah)

Stress, Strain, and Stiffness: Why Do They Matter and How Are They Measured?

Dr. Helene Langevin, NCCIH

Dr. Langevin explained that what actually occurs inside a material to which a force is applied is stress, not the force itself. *Stress* is defined as force divided by cross-sectional area of the material, and it is more difficult to estimate than force. Multiple types of stress exist, including tension, compression, and shear. Shear stress—caused by forces parallel to each other—is important in many biological tissues.

Material deformation in response to an applied force is determined by the elastic and viscous properties of the material to which the force is applied. An elastic material is like a spring; it does not matter whether a force is applied quickly or slowly. In viscous materials, the amount of deformation is rate dependent. Biological tissues have both viscous and elastic properties.

The term *strain* refers to change in length divided by initial length—that is, the amount of deformation. Stiffer materials show less strain in response to a given force. Stiffness matters in cellular responses. For example, stiffness will affect how much a membrane deforms in response to a force and may therefore determine whether an ion channel opens. Fibrosis, actions of drugs, or changes in lipid composition can affect the stiffness of membranes. If a cell or tissue responds to strain rather than stress, the impact of stiffness is important.

Ultrasound techniques, including static ultrasound elastography and shear wave ultrasound elastography, have been used to assess stress, strain, and stiffness in tissues or cells. Other techniques include magnetic resonance elastography, atomic force microscopy, and magnetic twisting cytometry. These methods can be used to inform experiments where a downstream effect is measured and there is a need to know what the tissue is responding to. The consistency of tissue matters, and hardness or softness determines how forces are distributed through tissue.

Discussion: In response to questions, Dr. Langevin explained that better imaging is needed to learn about changes in the composition of muscle with aging. Fatty infiltration is not the only process that is going on; fibrotic changes can also occur and may be underestimated. The cancer literature is beginning to reflect the understanding of the importance of stiffness; tumors and cellular receptors behave differently depending on the stiffness of the tumor-associated extracellular matrix.

Optical imaging techniques are beginning to be used, and there are techniques that combine optical and ultrasound components. These techniques are noninvasive, so they can be used repeatedly in human subjects. Wearable devices to measure strain or stiffness would be a valuable future development. For example, wearables could be used to measure changes in stiffness as people become tired throughout the day.

Spinal Mechanics and Robotics
Dr. Greg Kawchuk, University of Alberta

Quantifying tissue loads is important to elucidate tissue properties and the mechanisms of injury and to intervene to assist in recovery. External loads are easy to quantify, but measurement of internal loads is difficult. With a combination of kinematics and external loads, you can derive estimates of loads inside the tissues. Another approach is to measure forces more directly in an *ex vivo* system, then remove a part and see what effect its absence has on the resulting forces in the system. However, removing tissues can change the overall movement of the system; loads may change.

To solve this issue, Dr. Kawchuk has used a technique involving serial dissection with parallel robotics, in which the kinematics of the joint under testing remains constant even if parts are added to or removed from the system. To begin, optical techniques are used to track movements of the joint of interest, such as a vertebra, in response to a force, and then the joint is removed and the kinematics reproduced in the robotic system. This technique allows tissue contributions to be determined by serial dissection. The method can be used to answer questions about the effects of spinal manipulation or other force-based interventions on loads at specific sites and tissues. With this approach, it's possible to determine the

most appropriate location to apply force to affect, or avoid, a particular tissue. Also, different types of manipulation can be compared in this way. However, care must be taken with viscoelastic effects and the dissection sequence.

Discussion: Dr. Kawchuk explained that these techniques may make it possible to reverse engineer what force should be applied to influence specific tissue systems, perhaps even allowing for a personalized approach to manipulation. However, research to date has been conducted using cadaveric models, not living systems, so more data are needed.

Muscle Activation Response
Dr. Julie Fritz, University of Utah

Speaking from her clinical perspective as a physical therapist, Dr. Fritz explained that her focus is on understanding mechanisms so this knowledge can be used to improve clinical protocols. Numerous theories relate the therapeutic effects of spinal manipulation to neurophysiologic mechanisms. In the clinical manual therapy literature, a focus is on key spine muscles that are believed to play important roles in stabilizing the spine.

In preliminary work, Dr. Fritz and her colleagues examined the relationships between mechanical and neurophysiologic changes and clinical outcomes in patients receiving spinal manipulation. Deep trunk muscle activation was assessed with ultrasound, and spinal stiffness was assessed with an indenter device. A conceptual model was developed to understand the muscle activation changes in individuals who were clinical responders versus those who were nonresponders to the treatment.

Later studies have focused on optimizing spinal manipulation treatment protocols for back pain by examining multicomponent interventions that combine manipulation with other therapies known to modulate the same signals (spinal stiffness and muscle activation). In a factorial trial that was just completed, spinal manipulation in combination with strengthening exercise provided superior functional outcomes in patients, although no combination produced a greater effect on spinal stiffness or muscle activation. Future research will focus on evaluating effects of dose, timing, and sequencing of spinal manipulation and other interventions; patient-centered outcomes; evaluation phase trials with optimized spinal manipulation regimens; and attempting to overcome the small treatment effects seen in spinal manipulation clinical trials.

Discussion: Dr. Fritz explained that the patients studied were a mixed group with regard to duration of back pain, but most fit the National Institutes of Health (NIH) definition of chronicity. A threshold level of disability was an inclusion criterion. Patient Reported Outcomes Measurement Information System (PROMIS) measures were not used as primary outcomes. Instead, other psychometric measures in which thresholds defining a treatment responder were validated were used. Stiffness was assessed with a device that measures load deformation. In one study, people with an initial higher level of stiffness and initial higher decreases in that parameter had better responses, but this finding was not replicated in a second study.

General Discussion

Points made in the general discussion included the following:

- Studies in cadaveric models from about two decades ago showed that the strains on the joint capsule in physiological motion were similar to those resulting from spinal manipulation to a single

segment, and that differences in force distributions would lead to differences in the pattern of deformation.

- Future technologies, such as wearables, may make it possible to look at all the stages of motion during a spinal manipulation, not just the endpoints. However, developing wearables that can measure changes in a deep tissue such as the spine is challenging.
- One difference between cadaveric models and living tissue is the absence or presence of reflex responses. However, if the application of force is fast enough, reflexes may not be relevant. Some types of studies could be performed in anesthetized models and models with paralyzed muscles or nerve blocks.
- From a bioengineering perspective, spinal manipulation involves the application of force over a specific area.
- Variability makes research on spinal manipulation challenging. Tissues, the sizes and shapes of individual people, and pathology all vary. How practitioners perform manipulation also varies; physical therapists use methods different from those of chiropractors, and the practitioner's physiology and size also have an impact.
- Different fields, e.g., chiropractic vs. tissue mechanics, talk about dosage issues in different ways. Making the language consistent would enhance understanding. Objective biomarkers for musculoskeletal disorders are needed, but the pathology is still not well understood. The NIH Back Pain Research Consortium (BACPAC) initiative, part of the NIH HEAL (Helping to End Addiction Long-term) initiative, will develop data to help phenotype patients with back pain.
- It's unclear whether tissue stiffness is a promising phenotyping variable because there's much heterogeneity and overlap between symptomatic and asymptomatic people.
- Strategies to understand abnormalities are needed even though different abnormalities may be present in different groups of patients with pain at the same body site.
- The question of whether pain phenotypes are actually caused by variables that can be measured biomechanically has been discussed and studied for decades, but research has not been fruitful. Differences in pain phenotypes do not necessarily correspond to physiologic differences.
- A guest who is a classically trained chiropractor suggested that dry needling may succeed in situations where spinal manipulation would not. Treating soft tissue may be more effective than treating spinal tissue.
- It may be fruitful to have experiments driven by mechanisms rather than symptoms.

Session 2: Peripheral Neural and Extraneural Sensing of Biomechanical Force

Moderator—Dr. James Gnadt (NINDS)
Rapporteurs—Dr. Alex Chesler (NCCIH) and Dr. William Reed (University of Alabama at Birmingham)

Mechanisms of Force Sensation by Ion Channels

Dr. Stephen Brohawn, University of California, Berkeley

Dr. Brohawn explained that ion channels are the cell's fastest sensors and transducers of mechanical force. Some mechanosensors electrically excite cells when they open, while others quiet cells.

TRAAK is a mechanosensitive potassium channel gated by membrane tension. It is very rapidly activated by the application of pressure. When TRAAK is in its closed state, a lipid molecule pops into the channel and prevents ions from moving through. In response to tension, the channel stretches and expands, and these shape changes prevent lipid from blocking the channel. The role of the channel in

mechanosensation is evident in knockout mice, which have mechanical allodynia, and in people with mutations that affect TRAAK, who have complex, recognizable neurodevelopmental abnormalities. TRAAK is expressed throughout the nervous system, but only in nodes of Ranvier in myelinated axons. In rats, TRAAK accounts for a substantial portion of the total nodal potassium current. Current research questions of interest include whether TRAAK serves a mechanoprotective role to prevent ectopic spiking, whether it responds to a fundamental mechanical aspect of salutatory conduction to repolarize the neuron, and whether mechanical force applied to nerves suppresses activity by opening mechanosensitive potassium channels in axons.

Dr. Brohawn contrasted TRAAK with Piezo channels, which are extremely important excitatory channels that are much larger than TRAAK and have a sharper tension response curve. Piezos are cup-shaped, and their unusual shape enables the channel to flatten out, increasing the cross-sectional area. This creates a means by which a small mechanical deformation can lead to a sharp opening.

Discussion: TREK1 and TREK2, which are mechanosensitive potassium channels related to TRAAK, are present in dorsal root ganglion (DRG) neurons but do not appear to be present in the nodes. Precision localization tools have not yet been developed for TREK 1 and 2.

TRAAK contributes to background potassium currents because it is open to some extent (~1 percent) all the time. The lipid site is quite loose, so the lipid frequently moves in and out. Sometimes when the lipid is out, the channel can conduct, but it is a short opening and a low-probability event. The reason why you do not see spikes with movements such as bending the arm is not known, but it could involve a mechanoprotective mechanism.

Cellular Substrates of Touch Sensation
Dr. Ellen Lumpkin, University of California, Berkeley

Skin is innervated by a variety of mechanosensory neurons with different anatomical, molecular, and physiological properties. In the mouse, the different classes of neurons are molecularly distinct, with many types present in hair-bearing skin. A surprisingly large fraction are unmyelinated afferents that respond to low forces, i.e., low-threshold mechanoreceptors.

The neurons can be classified based on their diverse physiological response properties, such as conduction velocity, mechanical threshold (low vs. high), firing pattern (adapting rapidly vs. slowly), preferred stimulus (pressure vs. brush), and receptive field size. Different types of somatosensory neurons are specialized to encode distinct sensory qualities, such as brushing vs. vibration vs. itch.

The functions of the different classes of touch receptors are conserved across mammalian species, including between mice and humans, but their locations may not be. For example, some classes that are associated with hair follicles in mice are not associated with hair follicles in humans.

Types of cells in the skin other than nerve cells can encode or modulate mechanosensory signaling. Merkel cells, keratinocytes, and Schwann cells are examples. The roles of these nonneuronal cells have been revealed by cell-type–specific genetic manipulations, optogenetics, and human microneurography. Work in Dr. Lumpkin's lab has shown that epidermal Merkel cells are necessary and sufficient to drive sustained firing in slowly adapting type I (SAI) tactile afferents in mouse models. Work in other laboratories has confirmed similar effects for keratinocytes and Schwann cells.

Discussion: The behavioral responses to different touch modalities in rodents are similar. In general, a withdrawal response is observed. Therefore, behavior cannot be used to distinguish between optogenetic effects on different cell types.

Sensory Input in Pathological States During Manual Therapy
Dr. Geoffrey Bove, University of New England College of Osteopathic Medicine

Much is known about normal receptor function, but little is known about how receptors function in pathological states or how discharges into the system will be interpreted. Different types of receptors are stimulated by different kinds of manual therapies, including light massage, deep massage, joint mobilization, and joint manipulation. Afferent input in subjects with pathological conditions may be different from that of normal individuals. Manual therapies may have different effects in pathological situations than normal ones, but this is not well understood. Light massage acts on many types of normal skin receptors. Deep massage, mobilization, and manipulation act on position sensors and deep nociceptors, with a heightened response if sensors are sensitized. None of these modalities seem to affect cutaneous nociceptors.

In rats with repetitive motion disorder induced by intense operant training, pathological discharges in a wide variety of primary afferent neurons have been observed. If these changes are consistent with previous reports of effects of inflammation on axons, we would expect that only nociceptors innervating deep structures would be affected. In rats that have done the task for 3 weeks, increases in slow-fiber ongoing activity from deep structures are observed, and by 12 weeks, at which time there is much evidence of damage in terms of fibrosis and neural deficits, the increase is much greater, with a preponderance of C fibers exhibiting ongoing activity. In faster-conducting axons, changes in discharges are also observed.

These data may indicate that axons of deep nociceptors can develop expanded receptive fields as they pass through a nerve due to inflammation or reduced axoplasmic flow. This irregular activity could be perceived as pain. The message here is that damage, including inflammation and even chronic compression (stepping on the hose) of a nerve at one body site may lead to pain at the area that the nerve innervates. Another effect of chronic inflammation of nerves is demyelination, which causes a sort of sterile inflammation. This seems likely to be sufficient to cause interactions within the nerve including ephaptic discharge as action potentials pass by exposed membrane. This sort of discharge could be perceived as paresthesia.

In conclusion, Dr. Bove noted that in pathological conditions, afferent input to the nervous system is altered. It seems likely that manual therapies will affect primary afferent discharge differently in pathological circumstances. However, the ways in which manual therapies affect afferent input are unknown, and it is uncertain whether effects on afferent input influence the effects of manual therapies.

Discussion: In response to questions, Dr. Bove explained that the types of recordings shown in his presentation are observational, and the results are variable. Frequency mapping is not possible with these types of data. Dr. Partap Khalsa suggested that the time between spikes might be meaningful information.

Effect of Neurotransmitters Secreted From Sensory Neurons on Peripheral Nonneural Tissues
Dr. Mary Barbe, Temple University

When noxious stimuli activate afferent signals in sensory nerves, neuropeptides are released that can directly drive several functions in nonneural cells, including leukocyte chemotaxis; immune cell activation; vascular dilation, permeability, and adhesion; and dendritic and T-cell priming. This response, termed neurogenic inflammation, occurs after the release of neuronal factors (e.g., substance P) from peripheral afferent terminals as a consequence of direct stimulation, or antidromic activity from afferent collaterals at or proximal to the target end organ, the midaxonal region of unmyelinated or demyelinated axons (e.g., post injury), dorsal root ganglia, or central terminals of sensory afferents.

However, neurogenic inflammation is only one example of peripheral tissue changes induced after the release of neuronal factors from peripheral afferent terminals. Data from animal models show that one such neuropeptide, substance P, can play a role in peripheral tissue fibrosis. This occurs via signaling of substance P through the neurokinin-1 receptor (NK-1R), which is expressed by a variety of cell types. One source of substance P is increased production and release from nociceptor terminals into the periphery in response to mechanical loading. *In vitro* studies indicate that substance P is involved in tendinopathies. Experiments in animal models of overuse injuries showed that substance P is increased in cells in rat tendons that also show proliferation and thickening. Administration of a neurokinin-1 receptor antagonist that counteracts substance P reduced the proliferation and thickening. Substance P has also been implicated in collagen deposition in dermal tissues. Inhibiting substance P can ameliorate fibrosis in several types of tissues, suggesting that it has potential as a therapeutic target.

Nerves are present in bones. Substance P and other peptides produced by nerve cells are found in bones, and receptors for at least 10 of these neurochemicals have been identified in one or more bone cell types, where they influence bone growth and repair. An NIH initiative on bone healing is using recently developed knowledge of neuropeptides in bone to try to modulate bone growth.

Discussion: In response to questions, Dr. Barbe clarified that the substance P antagonist used in her work is not the same as those that have been found ineffective for treating pain. There are several antagonists with different properties. The antagonists may be more useful for treating fibrosis than treating pain.

Periosteal fractures cause a great deal of pain, but in other situations, such as injecting a needle to the periosteum, no pain may be felt.

Impact of Biomechanical Characteristics of Spinal Manipulation on Trunk Muscle Spindle Response
Dr. William R. Reed, University of Alabama at Birmingham

Manual therapy has a long history, but the circuitry and mechanisms of how it affects the nervous system have only been investigated in the last decade or two. The neurophysiologic mechanisms of spinal manipulation and the roles they play in clinical efficacy still remain for the most part unknown. In the 1970s, it was hypothesized that muscle spindles are an important mediator of the clinical effects of spinal manipulation. There are two types of muscle spindle fibers (bag and chain). Together, they send information back to the central nervous system on length changes in the muscle and the velocity of those changes. In Dr. Reed's *in vivo* model using anesthetized animals, muscle spindles show distinctive patterns of change in discharge during simulated spinal manipulative thrust maneuvers.

Manipulative thrusts are typically rapid, clinically delivered in less than 150 milliseconds (ms), resulting in multiple effects on spindle responsiveness. If thrust displacement is controlled, a graded spindle response is seen with decreasing thrust duration, with an inflection (an increase in the amount of discharge) occurring around 100 ms. If force is controlled, there is a nongraded spindle response with significant increases in spindle discharge occurring at 150 ms or less. Therefore, thrust duration impacts spindle response with manipulative thrust durations of less than 150 ms eliciting the greatest neural response.

There are vast differences in how clinicians perform spinal manipulation, and preload is one of the areas of clinical variance. Dr. Reed and his team found that the smaller the preload magnitude and the longer the duration of the preload, the greater the spindle response during the thrust. However, effects of preload differences on passive signaling properties of the muscle spindle immediately after the thrust were modest and may be clinically inconsequential.

In clinical training, the importance of thrust direction in manual therapy is emphasized, but in our preparation no difference in spindle discharge was observed in response to changes in the direction of a manipulative thrust. Exactly where the thrust is delivered on a vertebra also does not seem to be that important. Three contact sites on the same vertebra produced similar spindle response to spinal manipulation. Only when the thrust was delivered to an adjacent vertebra was a significant decrease in spindle discharge observed. Note that these findings are derived from work on animals without musculoskeletal pathology. In a model with increased spinal stiffness, a shorter duration thrust led to increased discharge, but 60 percent to 80 percent of spindle activity could still be generated by a thrust delivered as far as two spinal segments away from the intended target vertebra. This suggests that while thrust specificity may be ideal, precise segmental accuracy may not be an absolute prerequisite for clinical efficacy. In a study using commercially available spinal manipulation devices that deliver thrusts of extremely short duration (2-3 ms), some spindle afferents required more than 30 s after thrust delivery to return to their prethrust level of resting discharge activity. This suggests that subpopulations of muscle spindle afferents respond differently to extremely short-duration manipulative thrusts. This aspect of spindle response is being further investigated.

Discussion: In response to a question about whether a given tissue or the neurons that innervate it are particularly impactful in responding to a thrust, Dr. Reed said the question is complex because a spinal manipulative thrust stimulates all of the tissues—skin, muscle, ligament, tendon, and joint capsule, among others. There are multiple central inputs from a large variety of mechanoreceptors, and their individual neurophysiological effects have not been separated out.

What a Rare Disease Can Teach Us About Mechanosensation
Dr. Alexander Chesler, NCCIH

Dr. Chesler described PIEZO2 deficiency syndrome and also focused on the opportunity for scientists at NIH to transition between animal and human research, enabling them to understand principles and see differences between species.

Patients with PIEZO2 deficiency syndrome have joint contractions and severe progressive scoliosis, along with a severe lack of proprioception. When blindfolded, they have difficulty walking and reaching for objects. They also have profound deficits in the sense of touch, but not all their mechanosensation is missing. Their acute pain thresholds are normal, as are their thermal thresholds for hot and cold. They

can feel slow stroking on hairy skin (e.g., the arm) but have decreased sensation in glabrous skin (e.g., the palm).

PIEZO2 is a stretch-gated ion channel. It is expressed in diverse types of mechanosensory neurons in mice. If this gene is expressed in a cell that normally does not respond, a greater physical push leads to a larger current out of the cell. PIEZO2 is normally expressed in 70 percent of sensory neurons.

Human touch neurons have been generated in cell culture. *In vitro*–derived touch neurons from people with PIEZO2 deficiency are mechanically insensitive. Correction of the patient's point mutation in the cultured cells restores mechanosensation.

Some species-specific differences in Piezo2 function exist between humans and mice. In mice, Piezo2 is required for trigeminal responses to brushing but not pinching. Human responses may be different.

In both mice and humans, gentle touch sometimes becomes painful, a phenomenon called allodynia. In both species, Piezo2 plays an essential role in allodynia.

The skeletal abnormalities seen in patients with PIEZO2 deficiency and in knockout mice may reflect abnormalities in coordinated movements *in utero* that are important for normal skeletal development.

Discussion: In response to questions, Dr. Chesler said that the lack of abnormalities in pain and cooling responses in PIEZO2-deficient patients may reflect redundancy in the molecules required for these responses. More needs to be known about the molecular mechanisms of human mechanical pain before this topic can be probed further. For cooling, interactions may be present in humans that are not present in mice. Pressure and deep tissue sensation are more intact than cutaneous sensations in PIEZO2-deficient patients. However, it is difficult to obtain clear information about these sensations because the patients have multiple medical issues and have had repeated corrective surgeries.

General Discussion

Points raised in the general discussion included the following:

- Muscle spindle discharge lasts longer than had been expected, with a delay in the return to resting discharge of more than 30 seconds. The muscle spindle discharge may set up a cascade of events that can have a prolonged impact. However, there are apparently subpopulations of spindles that respond in different ways, with different delays before the return to resting discharge. The question of how a brief stimulus can have a prolonged effect is an important one in manipulation research.
- Research on channels could provide a unique opportunity to link our understanding of microscale neuronal activity with events at larger scales, including sensory experience. Much needs to be learned about the channels involved in particular types of sensation first, however. In PIEZO2-deficient humans, various types of inputs have been evaluated while the subjects were in a functional magnetic resonance imaging (fMRI) scanner. For example, a brush stimulus to the palm of the hand was shown to produce no brain activation. Brushing on the forearm produced activation in a small region of the posterior insular cortex but not the somatosensory cortex. So, associations were demonstrated between affect and discrimination.
- Responses to deep tissue massage are difficult to assess because the intervention perturbs the systems you would be attempting to record. It's possible to record responses to touch but not to more intense forms of manipulation during which tissues actually move. In addition, muscle spindles

are difficult to record from because their discharges are so strong. However, there is evidence that they do not have any modulating effect on deep innervating nociceptors.

- Dr. Lumpkin's lab has investigated the mechanical state that activates mechanosensitive neurons and found it was related to stress rather than strain. It is possible that integrins are involved in the connection. Dr. Lumpkin suggested that at the molecular level, mechanosensitive ion channels may have protein tethers that help focus the stimulus energy in a way that leads to very specific outputs. Differences in tension on the membrane activate the channel. Proteins may constrain microdomains around mechanosensitive channels. However, little is known about these microdomains.
- It is not yet known whether TRAAK and Piezo respond to stress or strain. This could be tested by treating a membrane preparation with a stiffener. Some research of this type has been performed. In an *in vivo* skin nerve model, stress and strain can be controlled independently. In this particular model system, the neuron was encoding stress rather than strain.
- It is possible to block substance P and others chemically, without cutting the nerve.
- Stress and strain may not be distinguishable at the molecular level. If either one is applied strongly enough, the other will happen. It may be more important to investigate the underlying physical phenomena of both stress and strain.
- The heterogeneity of textures in the body needs to be taken into consideration. The mechanics of responses are grossly different depending on the type of tissue in which a channel is located. In addition, contact interaction is different from interior stress.
- The issue of whether team science would help to bring together all the fields present at this workshop was discussed. Dr. Chesler mentioned that PIEZO2 is a success story in this regard. Collaboration between groups doing different types of research involving PIEZO2 evolved at a grassroots level within NIH, not in a top-down manner. Dr. Lumpkin commented that a lack of a common language is a barrier to collaboration. It takes time and effort to bridge the terminology of different fields and develop a common language.

Session 3: Spinal Cord Transmission of Force Sensation

Moderator—Dr. Theresa Cruz (NICHD/NCMRR)
Rapporteur—Dr. Victoria Abraira (Rutgers School of Art and Science)

The Cellular and Synaptic Architecture of the Mechanosensory Dorsal Horn
Dr. Victoria Abraira, Rutgers School of Art and Science

Dr. Abraira explained that in comparison to the other senses, touch remains underexplored. Skin is a very large, mechanically complex structure innervated by an equally complex set of sensory neurons. A variety of low-threshold mechanoreceptors (LTMRs) innervate hairy skin. Each of the hair follicles is innervated with a unique combination of LTMRs, each of which extracts a particular feature of a tactile stimulus. The role of each LTMR is like that of an instrument in an orchestra; each one is tuned to particular features, and the many components make up the whole.

LTMRs have connections to the skin and the spinal cord, and some have branches that send signals to the somatosensory cortex. The dorsal column–medial lemniscal pathway (direct pathway) has dominated views of touch perception. However, there is also an indirect pathway involving postsynaptic dorsal column pathways that is less understood but significant. All LTMRs have projections to this pathway. At the time when Dr. Abraira began her research, the LTMR input organization to the indirect

pathway, its contributions to tactile perception, and how LTMR information is represented and processed by this pathway were not understood. Mouse models were used to address these questions.

Labeling the LTMR inputs in a cross-section of the dorsal horn shows that each occupies a specific lamina, but they overlap. Of all the excitatory input into this region, about 30 percent actually comes from the LTMRs. A large proportion comes from local excitatory networks, and a small but statistically significant proportion comes from the cortex; the LTMRs mingle with both. The LTMRs innervate the same or adjacent hair follicles. Injecting a tracer into a small patch of skin in a genetically labeled mouse showed that the LTMRs are organized in tight, somatotopically aligned columns, which may be fundamental units of processing of touch information. Spinal cord interneurons receive the information, process it, and communicate it to the brain. The interneurons of the dorsal horn LTMR recipient zone (LTMR-RZ) receive unique combinations of LTMR subtype-specific inputs. They fall into largely nonoverlapping excitatory and inhibitory populations. To understand the role of the dorsal horn in touch perception, tools were developed to functionally manipulate these neuron types. It was found that each interneuron receives convergent cortical and LTMR-specific input, and each input subtype exhibits divergent contacts. Processing within the dorsal horn is important for touch perception and discrimination. Each interneuron receives convergent information from LTMRs and the cortex, and each input subtype exhibits divergent contacts.

In summary, in the skin, LTMRs are organized in a highly structured manner. LTMRs innervate the hair follicles in a predictable pattern. This type of organization may be essential for the LTMRs to extract particular features of a tactile stimulus. However, synaptic analysis showed that the information is highly distributed across many different interneurons in the dorsal horn. Touch information may reach the spinal cord without any inherent value, and value may be added there. This may account for the fact that touch stimulation may feel quite different depending on the individual's state. Dr. Abraira's lab is investigating how internal states such as pain, affective state, and social state influence spinal cord touch representations, and in the longer term, how associations in supraspinal centers are imposed on spinal cord touch circuits and how the supraspinal centers compute value to elicit appropriate behavioral outcomes.

Discussion: In response to a question about how the new information presented here fits into the historical context of the direct and indirect pathways, Dr. Abraira said that synaptic analyses can be thought of as basic roadmaps. They are anatomical landmarks, and it has been shown many times that the interneurons are important gates for the transmission of pain. Descending modulation probably has a large impact on the ascending pathways. The direct pathway may detect the location of an input, but the indirect pathway may add emotion.

Spinal Integration of Peripheral Inputs: What We Know From Models of Painful Injury
Dr. Beth Winkelstein, University of Pennsylvania

Dr. Winkelstein discussed the ligament as a sensory organ. Afferents and the potential for pain are present in many joints, and models of facet joint damage or capsular injury have been developed. The capsule can be used as a model both in animals and *in vitro*. In many models, whether cadaveric models, test dummies, or isolated specimens, changes that align around different magnitudes of strain can be demonstrated. Ironically, if ligaments are ruptured, the only response is transient pain.

In a model involving a well-characterized facet capsular injury, several sensitivity responses can be seen. Within a day, hyperactivity within the spinal cord can be demonstrated using electrophysiological field

measurements. Alterations in some regulatory proteins are seen. Changes in synaptic organization are seen at day 14 in a persistent pain model. Changes are also seen in network organization in the brain.

In a model of the human capsule, tissue engineering was used to enable study of the tissue. The organization of the capsule differs in different parts of the spine, and regional strains are nonuniform. The location of afferents has a very specific effect on response. Neuron–collagen constructs can be built in such a way that they mimic different kinds of organization in the capsule. Different kinds of loads or stretch are then applied, and questions can be asked about how the local environment dictates the nerves' responses. For example, it can be shown that tension across the capsule induces a change in collagen organization at about 11 percent strain, the same strain at which phospho-ERK (extracellular signal-related kinase) is expressed in the afferents. This point may represent the beginning of injury.

An increase in beta-1 integrin in the DRG after these painful injuries *in vivo* has been demonstrated. Blocking alpha-2 beta-1 integrin can prevent the expression of substance P that would normally be seen in response to these painful stretches. RhoA (Ras homolog family member A) activity increased in an *in vitro* model in response to a painful stretch of the capsule at two different early time points. With rho kinase (ROCK) inhibition, the substance P response normally seen after injury was prevented both *in vitro* and *in vivo*. ROCK inhibition also reduced substance P, microglial activation, and neuronal hyperexcitability.

Discussion: In response to a question about apparent differences in RhoA activation within the treated group, Dr. Winkelstein said that the differences may be regionally specific to local hotspots in strain or in substance P. The collagen organization in the experimental system can be controlled; in the experiment shown, the collagen had a homogeneous random orientation. Modulation of stiffness can change the behavior of the cells, even within the same ligament.

Spinal Cord Transmission of Force Sensation
Dr. Carl Saab, Brown University

Current understanding of pain is fragmented because of a patchy understanding of central nervous system and peripheral nervous system circuits. The patches have been studied in isolation for convenience and because of practical challenges, including separate research programs on the spinal cord and the brain, confounding by anesthesia during experiments, and nonspecific behavioral models.

Circuits are defined as anatomically and functionally connected neurons. They are studied at both the micro scale (one or a few neurons) and macro scale (thousands of neurons). Although much can be learned from individual neurons, groups of neurons together have emergent properties that are only detectable at the macro scale. When neurons form networks, they tend to fire together, which biases the activity of single neurons. Thus, the micro and macro scales are interconnected, and one cannot be studied without the other. In the laboratory, fast-scale time-resolved neural connectivity measures must be used (primarily electrophysiology, although calcium imaging is also used), and cellular specificity and a reliable window to the brain state are necessary.

Pain is a multidimensional experience, but the gold standards for assessing pain in animals (nonspecific reflex behavior) and humans (a verbal analog scale) do not fully capture its complexity. Much is known about anatomical connectivity within the spinal cord, but less is known about functional connectivity. Nonspecific behavioral outcomes to associate functional connectomes with the mind are lacking.

Dr. Saab's group tries to achieve the highest level of specificity possible when intervening in a system, changing one variable at a time, targeting specific populations or subtypes of primary afferents, obtaining reliable behavioral reports from the animals, and using *in vivo* electrophysiology to collect data from both anesthetized and awake animals. A transgenic mouse model has been created using a channel rhodopsin (ChR2) in which exposure to light causes a nociceptive reflex. The model has been used to investigate time-resolved nociceptive input to the dorsal horn and has demonstrated the expected early and late nociceptive volleys, confirming that the animal is experiencing pain. A novel lightweight "backpack" device has been developed to enable electrophysiology of the animals in awake, behaving states. An automated video method has been developed to score reflex behavior, and a training method using reward behavior has been devised that enables the animals to report conscious pain sensations. The ultimate goal is to combine these techniques to elucidate the spinal circuits of pain in awake animals. The availability of optogenetic techniques has played a large role in making this possible.

Discussion: A panelist commented that TRPV1, which was used in Dr. Saab's optogenetic model, is widely expressed. Dr. Saab explained that his lab is now transitioning to a more specific model. In response to a question about the difficulty of getting awake spinal cord recordings because of movement, Dr. Saab said that his lab was using clamping combined with a 3D printing technique and malleable electrodes to overcome this problem.

Dr. Khalsa commented on a model of the brain that focuses on error detection (the brain detects differences between what it expects and what actually happens) and asked whether this model changes the way the actions of the spinal cord are viewed. Dr. Saab explained that the brain is not passively waiting to be hit by stimuli; its main function is to make predictions. The purpose of pain is to disrupt the ongoing predictions and tell the brain that a mistake has occurred. The brain then readjusts in response to this signal. Part of the response involves sending signals to the spinal cord, but this process is not fully understood.

General Discussion

Points raised in the general discussion included the following:

- Neuronal subtypes can be defined based on transcription factors or based on effector molecules and receptors. There is potential for confusion because, for example, CCK has four different types. Ideally, subtypes should define a morphological and physiological population. Cell types have also been defined by intracellular dynamics within a circuit.
- A better understanding of mice as a preclinical model would improve the assessment of the various responses and behaviors that are observed.
- There is a danger in assuming that all reflexes are a reflection of pain or sensation. Other factors, such as the presence of the investigator in the room, also play a role. It's important to distinguish between subliminal messages and those that reach awareness. For example, in Dr. Saab's model, 100 percent of the mice show a reflex response to the light, but only 80 percent have the conscious reaction. One factor that might account for at least some of the missing 20 percent is the freezing behavior typical of prey, but it's also possible that pain did not reach consciousness in some of the animals.

- Little is known about the effects of spinal manipulation on mechanosensitive neurons in paraspinal tissues such as muscles, ligaments, and joint capsules. Addressing this topic will require crossing boundaries across disciplines and anatomic approaches.
- In response to a question about what tools are needed to further research in this field, the panel commented that machines are not the only tools. One type of tool that is becoming important for neuroscience is artificial intelligence (AI). Also, good statistical tools are needed to help interpret rich data sets.
- The clinical effects of spinal manipulation have not yet been matched up with changes in the nerves in the spine. However, with new techniques for recording from moving animals, it may be possible to begin to address this topic. Relevant research is also being done in humans. For example, studies are using functional magnetic resonance imaging to look at injury-related changes at micro levels in truck drivers who have back problems linked to prolonged exposure to vibration. Neuronal changes in response to spinal manipulation may be linked to changes such as improvement or might simply be a biomarker.
- Skin is easier to study than many other tissues because of its uniform thickness and the extensive knowledge of the receptors it contains. Other tissues deeper in the body are more complex and less understood. There is little understanding of the input and how it is received and processed in deeper tissues. This is a critical issue because people mostly suffer pain that is described as coming from deep structures, not coming from the skin.

Wrap-up

Dr. Langevin led a wrap-up of the discussion on the first day, noting that the presentations had moved from receptors to nerves and the DRG and then the spinal cord. There was also discussion about how these structures are surrounded by tissues such as skin, connective tissues, ligaments, muscle, and bone. Pathological conditions that affect the tissues include inflammation, fibrosis, injury, and stiffness, all of which may affect how a mechanical force acts on the tissues.

At the spinal cord level, there is much convergent input from multiple sources, including descending input from the brain, as well as input from psychological states and motor activity, all of which can modulate effects at the periphery such as stiffness. Multiple ion channels and receptors were discussed, and the importance of understanding the effects of inflammation, injury, etc. along the course of a nerve, rather than just at its terminals, was emphasized.

The discussion included the anatomical, physiological, and pathological layers, and to a lesser extent, the treatment layer. It is not surprising that little attention was paid to what the forces are actually doing therapeutically because the underlying pathology needs to be elucidated before the effects of treatment can be understood. This group can make an important contribution by bringing together biomechanics and neuroscience.

Another way of looking at the topics discussed is through three areas: all the possible forces, all the tissues they impact, and the neuroscience elements that are involved. With this type of framework, it could be possible to identify meaningful research questions and distinguish them from unnecessary experiments.

Much is known about the forces in spinal manipulation and how they are distributed into tissues, but the effects on receptors are not understood, and less is known about types of manipulations other than spinal manipulation.

Another emphasis in the discussion was the multiscale nature of the phenomena under consideration. For example, stiffness can be studied at multiple scales. A topic that was not addressed was the role of muscle as an endocrine organ. Force-based manipulations might alter endocrine activity, as exercise does. This is a complex topic. Another area that adds complexity is the chemical and electrical consequences of mechanical effects. For example, chemicals play a role in inflammation. Phenomena such as inflammation may be either local or systemic.

Forces are poorly defined. Their context can affect their meaning. For example, therapeutic forces may be thought of as not being painful, but much of physical therapy is painful, and acupuncture can be painful as well, particularly as performed in China, where strong manipulations are part of the treatment.

Day 2 – September 18

Session 4: Central Processing and Modulation of Biomechanical Force

Moderator—Dr. Merav Sabri (NCCIH)
Rapporteurs—Dr. William Reed (University of Alabama at Birmingham) and Dr. Carl Saab (The Warren Alpert Medical School of Brown University)

Pain Relief by Touch: A Behavioral and *In Vivo* Calcium Imaging Study in Mouse
Dr. Fan Wang, Duke University School of Medicine

Pain relief by vibrotactile stimuli is a well-known phenomenon. It has been shown that vibrotactile stimuli reduce experimental pain in animals and humans and pathological pain in patients. The "gate control theory" and previous neurophysiological investigations in animals indicate a class of wide dynamic range (WDR) neurons, which receive both touch and pain inputs in the dorsal horn, as a possible substrate of the analgesia induced by touch. When pain and touch fibers are stimulated together, the touch fibers activate inhibitory interneurons that in turn suppress the firing of WDR neurons, thereby preventing painful information from being transmitted from these dorsal horn neurons. A similar interaction may occur at the cortical level. A 2006 human study from Japan showed that when a painful stimulus (intraepidermal electrical stimulation, IES A-delta) and a touch stimulus (transcutaneous electrical stimulation, TS A-beta) were both administered, the touch stimulus suppressed both pain responses and subjective pain ratings. Suppression was seen even when the touch stimulus was applied shortly after the painful stimulus, suggesting possible cortical involvement.

To study this interaction at the cortical level in an animal model, Dr. Wang and her colleagues focused on mouse whisking (self-generated whisker movement), which generates a vibrotactile input to the facial skin. Experiments were devised to determine whether mice feel less pain when they're whisking. Pain was assessed using a behavioral measure: mice wipe their faces in response to a noxious stimulus, and the occurrence of wipes can be quantified. Two types of noxious stimuli were used: radiant heat, which induces a wiping response with a latency of about 4 seconds or shorter, and the application of a von Frey filament (which is like a pinprick), both applied to the face/whisker pad. The two stimuli differ not only in response latency but also in the persistence of the noxious stimulus. The von Frey stimulus is a single poke, but the heat stimulus persists.

When full whiskers are present and whisking can occur, the amount of wiping in response to noxious heat or mechanical stimuli is significantly reduced, suggesting that self-generated touch makes the animals less responsive to noxious stimuli. If the whiskers are cut, the motor command that causes

whisking to occur is still present and can be observed by the movement of the entire face, but the vibrotactile stimuli derived from the whiskers are not present because the whiskers are trimmed. In this case, mice remain as sensitive or become even more sensitive to heat and von Frey stimuli. Thus, vibrotactile touch input (reafference) but not motor-efference is required for whisking-induced suppression of face nociception.

An *in vivo* miniscope mounted on the brain of the mouse was used to image cortical responses in this system. Data now being analyzed indicate that S1 somatosensory cortex responses are different with or without whisking.

Discussion: In response to a question, Dr. Wang clarified that the whiskers were simply trimmed. The roots of the whiskers were not removed. Dr. Wang also indicated that wide field imaging of the whole cortex was used to show that S1 is activated by heat and von Frey stimuli, justifying the use of miniscope imaging to study individual neuronal responses in S1 in the presence or absence of self-whisking. A panelist noted that looking at S1 might miss the emotional aspect of pain, and Dr. Wang agreed.

Role of Thalamic Nuclei in Low-Back Pain and Complementary/Integrative Approaches
Dr. William Reed, University of Alabama at Birmingham

In his second presentation, Dr. Reed addressed the role of the thalamus as an important structure to examine in connection with manual therapy. The thalamus was traditionally thought of as just a relay station, but it's actually a gate that has multiple interactions with cortical and subcortical structures. All sensory inputs except olfaction are processed through it. The thalamus processes convergent innocuous mechanoreceptor stimuli (dorsal column pathway) and noxious stimuli (spinothalamic tract). Structural brain imaging has shown that the presence of subacute or chronic low-back pain is not related to significant differences in thalamic gray matter volume (after correction for multiple comparisons).

Recent work indicates that spinal manipulation attenuates activity in the prefrontal cortex. Shortly after spinal manipulation, an effect on the cortical drive to muscles can also be detected. Manipulative therapies cause immediate changes in resting state functional connectivity between several regions of the brain involved in the pain matrix. There are both similarities and differences among the effects of different types of manual therapies—spinal manipulation, spinal mobilization, and therapeutic touch. The thalamus acts as a hub for all sensorimotor connectivity. The concept that spinal manipulation may alter the balance between intracortical inhibitory and excitatory output in the brain is important and in need of additional investigation.

Pain pathways are a dynamic bidirectional interactive network. Chronic pain is a network disorder, not a disorder of a single pathway. Exercise as an active intervention increases the efficiency of the network, and the thalamus plays a key role. Whether passive manipulation has a similar effect on central network efficiency is uncertain but possible. The medial thalamus shows high connectivity with brain areas associated with arousal, attention, and sensorimotor function.

Recent work with integrated positron emission tomography and magnetic resonance imaging (PET/MRI) shows increased brain glial activation in chronic low-back pain. Glial activation was observed in the medial thalamus and pre- and postsomatosensory cortices. Manual therapy interventions may affect neural discharge through dampening glial activation. Ankle joint mobilization has been shown to attenuate spinal glial activation following sciatic nerve injury. This raises the question of whether

manual therapy interventions influence microglial and astrocyte release of chemokines and neuropeptides involved with pain.

Preliminary work in Dr. Reed's lab did not demonstrate a significant change in lateral thalamic trunk mechanical thresholds among wide dynamic neurons following a simulated spinal manipulative thrust. However, among nociceptive specific lateral thalamic neurons, there appeared to be an increase in trunk mechanical threshold following a lumbar manipulative thrust of 85 percent rat body weight when compared to control (nonthrust). No changes in trunk mechanical threshold were found among lateral thalamic neurons in relation to manipulative thrust duration, but the sample sizes were small, and data were not collected from neurons in specific thalamic nuclei.

One of the interesting medial nuclei in the thalamus is the submedius nucleus, which responds to predominantly noxious input. During chronic pain, spontaneous discharge in this thalamic nucleus typically increases. When spontaneous activity was recorded 5 minutes before and after a 100-ms manipulative thrust at 85 percent body weight in a rat, a decrease in activity occurred beginning at 3 minutes postmanipulation. If this happens on a global scale across the medial thalamus, it could suggest a dampening down of ascending affective-related pain response going to the cortex.

Future research directions include determining whether spinal manipulation inhibits mechanical/thermal response in all or only some medial/lateral thalamic subnuclei, measuring thalamic nociceptive responses to spinal manipulation in a low-back pain model, and determining the effect of spinal manipulation on thalamic burst activity and cortical network responses.

Discussion: In response to a question regarding the need to perform more work in models of musculoskeletal pain, Dr. Reed explained that future work will be performed in a back-pain animal model, but the model has not yet been fully characterized. A panelist commented that in work done in monkeys, the medial thalamic nuclei were highly malleable, and this was probably related to emotional modulation. The thalamus may be affecting the perception of pain and the cortex's interpretation of the message.

Brain Circuits of Pain
Dr. Carl Saab, Brown University

Dr. Saab noted that although his previous presentation focused on spinal circuits of pain and the current one focuses on brain circuits of pain, there is a spine–brain continuum, and neither part of the central nervous system should be viewed in isolation.

Because there are limitations on interventions that can be performed in the human brain, computational modeling can be a valuable tool. Dr. Saab and his colleagues are using the publicly available human neocortical neurosolver (HNN) model of S1, a biophysically realistic model based on known local network and molecular parameters, to make predictions about microscale activity of thalamic origin during pain and guide electrophysiology experiments. A signal in S1 can be input back into the computational model to obtain a prediction about the thalamic origin of the signal. The model's predictions of evoked responses to touch and pain needed to be adjusted to account for the recordings observed during pain. This illustrates the way in which computational predictions should be used. They can guide research, but information obtained from them should be taken back to the experimental animal model for testing.

In human subjects with chronic severe back pain, EEG data were recorded using specialized wireless EEG equipment that takes only 2 minutes to put on. Previous EEG literature has shown differences between healthy people and pain patients using conventional statistics. In Dr. Saab's measurements, some differences between the two groups were evident, but if the Bonferroni correction was applied, all significance disappeared. Further investigation showed that averages were similar between healthy people and pain patients, but transient oscillations were different at the subsecond level. Altered gamma activity was detected during pain, with more gamma events per epoch in pain patients, and event power and frequency span were increased, but there was no difference in event duration.

Other research groups are looking at macroscale dynamic changes between pain patients and healthy people. Interesting activity has been observed at the macroscale level in the sensory thalamus, but it's important to look carefully at the statistical analyses.

Relating macro- and microscale findings to one another is important. There is evidence for a causal relation between burst firing in the ventral posterolateral nucleus (VPL) of the thalamus and transient disruption of theta rhythms in S1 and sensory behavioral consequences.

EEG recordings can also be helpful in phenotyping pain patients using AI. It's possible to predict pain intensity using EEG and gait features. Although the EEG data are not fully understood, they contain information that allows the AI agent to make the classification. Further research is needed to understand why this occurs.

Discussion: A panelist commented that some differences between pain patients and healthy people may reflect the active state of being in pain rather than the chronic state of being a pain patient. Dr. Saab explained that his group is analyzing data from chronic back pain patients in whom pain was increased. It's important to recognize that people with chronic pain may differ from healthy people even when they are not experiencing a pain episode. For example, brains of migraineurs who are not experiencing a migraine episode differ from those of healthy individuals.

Bottom-Up and Top-Down Effects of Manual Therapies
Dr. M. Catherine Bushnell, NCCIH

Manual therapies include massage therapy, myofascial therapy, and joint manipulation, as well as alternative therapies that involve touch, such as Reiki, Rolfing, and therapeutic touch. Good evidence of efficacy is not currently available for most of these therapies. Massage therapy has the best evidence for effective pain relief, as demonstrated in studies of chronic low-back pain, chronic neck pain, pain associated with osteoarthritis of the knee, and to a lesser extent, labor pain (NCCIH website: https://nccih.nih.gov/health/massage).

Manual therapies have bottom-up components, such as biomechanical effects on peripheral tissue and activation of primary afferent fibers, sensory and emotional brain regions, and neurotransmitter systems. They also have top-down components, including mood changes, attentional focus, and expectations.

Research on the biomechanical effects of manual therapies has shown that manual therapy can induce remodeling of connective tissues, have anti-inflammatory and antifibrotic effects, and prevent the onset of nociceptor activity. In the brain, these therapies activate both large and small fiber afferent pathways. There's evidence that A-beta activation can inhibit afferent nociceptive pathways at the spinal level. Stimulation of A-beta fibers in the same dermatome has been shown to inhibit laser pain.

Further up in the brain, touch activates the somatosensory cortices, and pleasant touch activates the prefrontal regions. The frontal regions activated by pleasant touch are involved in descending pain modulation; information from the cortex can alter afferent input from the spinal cord. Pleasant touch can reduce pain both through distraction and by improving mood state. Touch is sometimes used as a distraction clinically, for example during dental treatment.

Distraction and mood activate different modulatory circuitry in the brain. Distraction affects the intensity of pain, and mood affects its unpleasantness. Distraction (without changes in emotional state) exerts its effect by reducing pain-evoked activity in sensory pathways. A primary area modulated is the somatosensory cortex. This is consistent with the observed changes in the intensity of the pain rating. Manipulation of emotional state without manipulation of attentional state causes modulation of the anterior cingulate cortex, an affect area.

In conclusion, there's evidence that manual therapies can reduce pain. They may engage spinally mediated A-beta inhibition of nociceptive activity, and they may also engage top-down modulatory circuitry involving attention and emotions.

Discussion: In response to questions, Dr. Bushnell explained that distraction effects are short-lived, but emotional components and expectation effects may persist. Hormonal changes, for example in oxytocin and stress hormones, may occur during manual therapy and persist for a substantial length of time, even without a mood effect. Hormonal effects can even occur in an anesthetized animal in response to manipulation of muscle tissue. Odors can be used to quickly modify moods, but they must be customized for individual people.

Role of Opioids in Superficial and Deep Touch
Dr. Laura Case, NCCIH

Participating by Webex, Dr. Case explained that opioids are very much involved in pain perception. Several brain areas involved in pain perception have high opioid binding potential, and higher ratings of sensory or affective pain are linked to more opioid binding. Dr. Case's research has explored whether endogenous opioids are involved in the perception of pleasant touch and whether endogenous release of opioids during manual therapies, which often involve pleasant touch, might contribute to their analgesic effects.

There is evidence from studies in rhesus monkeys that the rewarding nature of social touch involves opioidergic mechanisms. Young monkeys given acute doses of the opioid antagonist naloxone spent more time in contact with their mothers and actively sought contact. Mature female monkeys solicited grooming more often and received more grooming from their companions. These findings suggest that low levels of opioids may motivate animals to seek potentially opioid-releasing activities like social touch.

Dr. Case and her colleagues examined how opioids affect perception of superficial touch (slow or fast brushing) in humans. Blocking endogenous opioids increased the pleasantness of touch in healthy middle-aged women. However, in this experiment, skin brushing was performed by a visible experimenter, so the effects could have reflected social interaction rather than touch perception.

To separate out the effects of endogenous opioids and to examine the effects of opioid blockade on both superficial and deep touch, perception of both types of touch was examined while human subjects were in a magnetic resonance imaging (MRI) scanner, with little experimenter contact. Deep pressure

was included because it can be pleasant and relaxing; it is used in manual touch therapies and is a complementary treatment for certain psychiatric symptoms. Naloxone reduced the perceived intensity of superficial touch but not deep touch. Naloxone did not alter the pleasantness of either type of touch when examined without social context. However, naloxone strengthened the relationship between mood and pleasant sensation.

In conclusion, manual therapies engage pleasant touch pathways and may lead to endogenous release of opioids. Opioid levels may alter the pleasantness of touch. Top-down factors, such as expectation and visual observation of touch, may affect opioid release. Naloxone-induced changes in touch pleasantness may relate to changes in mood.

Discussion: Previous research has shown that naloxone affects mood, but no effect on mood was seen in Dr. Case's MRI study. This may relate to the levels of naloxone administered. Dr. Bushnell added that participants in the experiment were in a scanner for a long time, so their moods tended to go down, which might have contributed to the lack of effect of naloxone. Studies involving varied levels of naloxone are needed to clarify effects on mood, but there is also a need to directly compare situations involving the presence and absence of social interaction.

Altered Brain Circuitry in Chronic Pain Patients and the Modulation Effect of Peripheral Mechanical Stimulation

Dr. Jian Kong, Massachusetts General Hospital, Harvard Medical School

Chronic pain is associated with widespread changes in brain structure and function. Recent work from Dr. Kong's laboratory has identified brain regions associated with the neuropathology of chronic low-back pain. The patterns were sufficiently distinctive to allow patients to be distinguished from healthy controls. Abnormal medial prefrontal cortex functional connectivity was associated with clinical symptoms of low-back pain.

Dr. Kong discussed acupuncture modulation of pain. There is evidence that acupuncture needle stimulation can change connective tissue displacement and strain in the longitudinal and transverse directions, but how it affects the brain is not as well understood. A study in Dr. Kong's lab compared the effects of four interventions—real, sham, and imagined acupuncture and a control for imagined acupuncture—on heat pain and pressure pain in healthy people. Real and imagined acupuncture had similar analgesic effects on pain threshold. Brain imaging showed responses in the insula and rostral anterior cingulate cortex (rACC) associated with the analgesic effects of real and imagined acupuncture, respectively.

In a small pilot study in patients with chronic low-back pain, real and sham acupuncture treatments were compared, as were high- and low-context treatment situations. In the high-context intervention, the practitioner empathized with the patient and showed confidence in the effects of the treatment. All four treatments (real and sham, each with and without high context), given six times over a 1-month period, reduced pain. Small differences between real and sham were observed, but there was no significant difference between high and low context. Multivariate baseline resting-state functional connectivity measured prior to treatment predicted responses to real and sham acupuncture, and the networks that were predictive for real and sham treatment differed.

Other research by Dr. Kong's group and his collaborators has evaluated the effects of two complementary approaches involving exercise, tai chi and baduanjin (a form of mind-body intervention),

with stationary cycling and a health education control in patients with knee osteoarthritis. All three exercise modalities reduced knee pain over a 3-month period and had similar effects on an inflammation marker. They also had similar effects on brain regions involved in descending opioidergic pathways and reward/motivation systems. The findings are encouraging because they indicate that exercise, including mind-body interventions, can modulate descending pain networks and reward networks simultaneously. All three exercise modalities also modulated the functional connectivity of the dorsolateral prefrontal cortex, which plays an important role in both cognitive attention control and emotion modulation, but the effects of the three modalities were not identical. Baseline connectivity could be used to predict 3-month treatment response.

Discussion: In response to questions, Dr. Kong explained that participants in the exercise study were randomly assigned to the treatments. Randomization would control for differences in participants' familiarity with the approaches. Nevertheless, the complementary approaches might have different effects if introduced in a culture in which they are unfamiliar.

For the acupuncture study on healthy subjects mentioned in the talk, Dr. Kong's group recruited only people who were acupuncture naïve, and the study participants were informed that they might receive a placebo treatment. Results might be different if participants had been deceived.

General Discussion

Points raised in the general discussion included the following:

- Sham acupuncture involves the use of a placebo needle that retracts into the handle of a device and does not pierce the skin. The study participant usually cannot distinguish between this treatment and real acupuncture, and the person doing the manipulations also may not know which treatment is being used. In healthy subjects, there is evidence that the placebo effect produced by sham acupuncture is relatively small, thus real acupuncture tends to produce a better effect than sham acupuncture. The placebo effect in patients with chronic pain tends to be larger; nevertheless, a recent meta-analysis suggests that real acupuncture may produce a significant but moderately better effect than sham acupuncture.
- In some animal studies, the use of a local anesthetic abolished any observable effect of acupuncture, thus demonstrating the role of peripheral afferents. However, the mind also plays a role, as illustrated by the effect of imagined acupuncture in Dr. Kong's research. Before the imagined acupuncture sessions, study participants went through a training session in which they experienced real acupuncture. Panelists suggested that this type of study could be conducted for any intervention and that the imagined intervention may have similarities to mindfulness because the subject is asked to focus on it. Needle stimulation does have effects, though, and it is unclear whether imagined sensations are equally effective.
- A panelist commented that participants in a study in which spinal manipulation was given while the participant was briefly anesthetized could not accurately guess whether they had received treatment until they stood up and attempted to perform movements that had caused them pain. However, this was only a small pilot trial.
- In studies involving brain activity, capturing the brain at one moment in time and averaging measurements taken over a period of time are both valid approaches but may yield different results. A third approach is to watch changes in real time. The brain can move in and out of states in milliseconds.

- Dr. Bushnell clarified that mood can be changed by exposing subjects to pictures or odors, with odors causing particularly rapid change. In real life, social interactions are also important for mood state. The mechanistic effects of long-term mood state changes may be different from those of the rapid changes used in imaging studies.
- Touch does not alleviate pain during allodynia because the touch sensations activate nociceptive pathways in this situation. In effect, touch has been transformed into pain.
- Given a large enough sample size, it might be possible to predict pain using AI based on EEG network analysis. This may be a step forward from the use of more expensive and time-consuming fMRI methods.
- Clinicians can accurately determine whether someone is in pain, but distinguishing acute from chronic pain is more difficult. Current clinical standards for acute and chronic pain depend on the duration of the pain. However, the temporal distinction is arbitrary and may not reflect a biophysical difference. EEG could allow for a distinction to be made based on a biological rationale, and studies could be conducted to determine whether using such a rationale as a guide to treatment would improve outcomes.
- Some people's brains may be predisposed to high-impact chronic pain. If this could be assessed clinically, it might be possible to develop treatments that correct the problem at the brain level. If a biomarker for acute vs. chronic pain is discovered, it may be possible to initiate treatment before pain actually develops.
- It is important to recognize that there may be multiple pain states. It is not possible to train AI without knowing what the states are. There may be different subpopulations of chronic pain patients.

Session 5: Technology Advances for Force-Based Manipulations

Moderator—Dr. Grace Peng (NIBIB)
Rapporteur—Dr. Medha Pathak (University of California, Irvine School of Medicine)

Multiscale Mechanics of Soft Tissue
Dr. Victor H. Barocas, University of Minnesota

Dr. Barocas explained that his group studies mechanics at multiple length scales. They hope to address a fundamental challenge in biomechanics: Biological research yields information on microscale phenomena, but humans are macroscale creatures. What is important is what happens to the whole organism. Phenomena such as touch are extremely complex, involving mechanics, chemistry, transport, electricity, and biology, and they happen at multiple scales. The goal of the group's research is to develop better conceptual and computational models of biological and physiological phenomena involving mechanics.

Creating a computer model involves creating a conceptual model first, which is then put into the computer model, which translates the idea into mathematical and programming terms. The computational model is the formal expression of the hypothesis. Creating a workable computational model requires a scientifically sound conceptual model. Researchers who are not engineers would be well advised to consult with engineers when developing conceptual models.

Dr. Barocas's group studies the lumbar facet capsular ligament, in which events occur at the joint, tissue, and neuron levels. They take a multilevel approach to modeling this system. Different types of data can be included in the multiscale model, and predictions from the model can be compared with

experimental data. For example, the strain fields for several types of spinal motion predicted by the multiscale model were generally but not entirely consistent with experimental data.

Modeling was also used to predict the effects of stretching a neuron in a variety of ways. Two models, one involving microtubule stretch and one involving membrane forces, produced similar but not identical results. Another model investigated the sense of touch in a cluster of capsules in the hand. The transfer of a wave through two nearby Pacinian corpuscles was modeled.

In general, the reason to make a model is to see something that cannot be measured in an experiment. If the experiment were possible, there would be no need for the model. If the quantity of interest cannot be measured, testing the model is difficult. This is a fundamental and huge problem in modeling. When considering making a model, it is important to think about what can be tested experimentally and what cannot be. This is a major challenge going forward.

Discussion: The sample size needed to build a model depends on what one is trying to accomplish. The broader the picture, the larger the sample needed.

Computational Models and Stimulus Devices To Capture Naturalistic Skin-Tactile Afferent Response
Dr. Gregory J. Gerling, University of Virginia

Dr. Gerling addressed two topics from a methods perspective: using computational models to address topics where empirical measurements are not available and moving from a classical stimulus–response paradigm toward naturalistic human-to-human interactions.

Dr. Gerling presented an example of a two-receptor-site conceptual model involving Merkel cells and neurites. Responses to stimulation differ in wild-type, knockout, and Piezo2-deficient animals. Merkel cells and neurites make distinct contributions to the response. A computational model was created from the conceptual model, and its predictions did not fully match what happens in real cells. Based on modeling, a new concept involving a three-receptor-site model was hypothesized. This is an example of taking a conceptual model into a computational space, leading to the generation of a new hypothesis.

A second example involves the modeling of the mouse hair cycle (repeated loss and regrowth of hair). Studies were performed where the skin of animals was dissected and data were gathered about the thickness and stiffness of the hair and skin, both of which vary systematically with the hair cycle. This information was taken into a computational modeling space, and six representative skin models were built. Computational experiments were run, and the indentation magnitude, rate, and spatial geometry could be predicted if force was controlled. This example involves taking a concept into a computational space and using a model to generate data that can be experimentally validated.

Dr. Gerling and others have built mechanical stimulation devices in which experimental parameters are controlled very tightly. A device was used to capture three-dimensional spatial and temporal deformation cues from interaction of a fingertip with soft vs. hard objects. Current work involves the stimulus of human-to-human touch, with unconstrained, unscripted interactions between couples, in which one person touches the forearm of the other to express feelings such as happiness, sadness, attention, or calming. The experiments have shown that there are physical quantities that distinguish the different types of social expression. Efforts are being made to differentiate the expressions based on features within the neural responses.

Discussion: In response to a question, Dr. Gerling said that to accurately quantify deep tissues, a more complicated and robust contact model would be needed.

Human Brain Organoid Technology: From Development to Function
Dr. Medha M. Pathak, University of California, Irvine

Dr. Pathak explained that to fully understand the neurocircuitry that transduces force, it is necessary to understand the molecules involved, measure inputs and outputs, and be able to manipulate these in normal and disease conditions. Often, the necessary biophysical measurements cannot be performed in an animal. Therefore, reduced systems are needed.

Principles from development can be used to create a minimal system *in vitro*. As the brain develops, cells proliferate and migrate, tissues change, and mechanical forces affect how biological processes develop in the brain. It was proposed more than 100 years ago that physical forces dictate organismal growth, but tools to study this hypothesis were not available. In the past decade, this barrier has been crumbling, and it is now known that for normal development to take place, mechanical, chemical, and genetic cues must interact. Dr. Pathak's research focuses on the effects of these interactions, with an emphasis on the mechanotransducer Piezo1.

Traditional approaches to measure the activity of the Piezo1 ion channel using patch clamp electrophysiology disrupt the native cellular state, so Dr. Pathak and her colleagues are using a novel approach to measure its activity. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) imaging, which makes it possible to focus specifically on membrane-associated signals at the interface of the cell and substrate, they have demonstrated Ca^{2+} flickers are produced by Piezo1 wild-type cells even in the absence of externally applied mechanical stimulation, and these signals are missing in Piezo1-knockout cells. The Piezo1 Ca^{2+} flickers are generated by mechanical forces produced by the cell itself. Further investigation showed that localized Piezo1 Ca^{2+} flickers occur at regions of high traction forces.

In mesenchymal stem cells, matrix elasticity directs stem cell lineage specification. Changing the substrate produces different types of cells—neuron-like cells in a soft environment, bone cells in a hard environment, and muscle cells in an intermediate environment. Similarly, in neural stem cells, substrate stiffness influences neuronal versus astrocytic specification. Dr. Pathak's work showed that human neural stem cells express the mechanically activated channel Piezo1. Depending on whether Piezo1 is active or inactive, the stem cells preferentially differentiate into neurons or astrocytes. All this information comes from experiments done *in vitro*, and ongoing work is also being done in genetically engineered mice. However, mice don't replicate all aspects of human development, and some types of biophysical measurements cannot be done in a whole animal.

To overcome these limitations, Dr. Pathak's lab is using human brain organoids, which recapitulate three-dimensional human neural development *in vitro*. The lab's goal is to image mechanotransduction during three-dimensional neural development. Work by others in the field has shown that region-specific brain organoids form neural connections with each other, and a recent proof-of-principle study from Dr. Madeline Lancaster's group has found that brain organoids can make connections with spinal cord tissue when cultured together and control the movement of spinal muscles. Brain organoid technology can also be combined with CRISPR engineering technology and used to study the genetic basis of neurodevelopmental diseases. Moreover, organoids have been generated for the skin and other organs, and thus it is conceivable that organoids could be used to learn about the sense of touch if organoids representing different tissues can be put together in ways that enable them to interact. To do

this, more mature organoids (i.e., with mature cells and vasculature), and better reproducibility in organoid formation will be needed. In effect, the need is for "systemoids" that will re-create minimalistic biological systems. The challenge is to put them together, and this effort will require a collaborative effort across multiple interdisciplinary groups.

General Discussion

Points raised in the general discussion included the following:

- In response to a question about the location of Piezo1 channels, Dr. Pathak described results showing that Piezo1 localization is not restricted to integrin-rich focal adhesions but is present all over the cell membrane. However, Ca^{2+} flickers are only observed in the vicinity of traction forces. Membrane tension has been shown to be sufficient to activate the channel, but additional regulation through protein–protein interactions cannot be ruled out at this stage. Membrane tension within cells is heterogeneous; it is not a global parameter that affects the whole cell.
- The signals produced by Piezo1 Ca^{2+} flickers are very small. Normal epifluorescence imaging would not reveal them. The advantage of the TIRFM technique used is the exquisite sensitivity of the measurement, since the signal-to-background ratio is very high.
- Dr. Pathak explained that the reason why the Piezo1 channel closes at the end of a "flicker" isn't fully understood. This can arise from channel inactivation or due to termination of the mechanical stimulus. Specific mutations in the channel affect the inactivation process, as do some changes in lipid composition.
- Dr. Chesler commented that it takes a substantial stimulus to get Piezo channels to open in the laboratory, suggesting that something about how the channels are activated *in vivo* is not yet understood. An intramolecular reporter of Piezo1, which Dr. Pathak's group is currently working on, would be desirable.
- Computational modeling could be used in combination with real-time imaging of ganglion cells to understand what is happening in deep tissues in an animal. However, if direct measurements are good enough, modeling might not be necessary.
- Techniques are needed for measurements below the surface of the skin. Computed tomography is possible at superficial levels. Ultrasound is possible. Near infrared imaging using a tiny tube close to the tissue of interest could be used. If the interest is in the whole organism, modeling may be the best approach if there are enough data to make reasonable estimates. National Institute of Biomedical Imaging and Bioengineering grantees are developing useful technology. It is important to understand what needs to be measured, why, and in what context. Existing technology or the integration of different types of technology may be adequate.
- *In vitro* and *in silico* models are examples of controlled environments that can be used to test parameters and generate hypotheses. Conceptually, they are no different from animal or human models used in science. Mechanistic models allow you to integrate what is known and what is not known about a system. The point in building them is not just to recapitulate what is known; it is to test new hypotheses. It is not necessary to wait until there are "enough" data before modeling. A concept or theory is enough to start the modeling process.
- Model development is an iterative process. Back-and-forth conversations between the original investigators and the bioengineers who will help to develop the model are essential. Engineers are scientists in their own right, not just tool developers. When teams come together, it is necessary to ensure that the engineering part stands in its own right as a scientific endeavor. The team members need to work together on questions valuable to all of them.

Session 6: General Discussion and Concluding Remarks

Discussion focused on knowledge gaps, connections that are missing but needed for a transdisciplinary effort, major technology barriers to be overcome, and terms to be better defined.

- Panelists noted that some of the force-based manipulations need to be better defined. For example, the distinction between massage therapy and manual therapy may not be clear. It could be valuable for everyone who does research in this field to receive or feel the different therapies. A highly generic common language is also needed.
- The link between the nervous system and specific forces is not well articulated. Circuits and forces are both unknown. There is also a lack of a comprehensive list of force-based manipulations and the use cases for each of them. Another area where work is needed is tool development. Reliable measures of force and biometrics for clinical outcomes are needed. In general, it is difficult to objectively measure clinical outcomes for psychological conditions because of a lack of biomarkers.
- A mouse model of force-based manipulation would be valuable, but the animals may simply be too small. For spinal manipulation, the use of rats is pushing the size limit. Stretching is possible in mouse models, but some types of manipulation—for example, on a limb—may not be.
- There is a need for animal models of diseases that can be used to study the effects of interventions. Normal mice are not suited for all types of experiments.
- Except for spinal manipulation, little is known about the forces present during force-based therapies or the impact of differences between providers. Therefore, one of the first challenges should be finding out what providers are trying to treat, characterizing the forces, and seeing if they cause changes in tissues.
- Simulation programs could be used to emulate force-based manipulations, but a greater understanding is needed of what the therapy is intended to accomplish. Many of the phenomena that might be treated, such as “painful knots” in muscles, are poorly understood.
- On the microscopic scale, it is important to determine whether neurocircuitry plays a role in the effects of therapies. Can the manual intervention be connected with cellular and molecular responses that have an impact on the outcome of the intervention? This question could be addressed with an animal model of a disease and a standardized intervention. In such a model, it would be possible to determine whether specific cell types and molecules are important for a response.
- Greater knowledge of mouse behavior/ethology could lead to the development of better mouse model systems. Mice use touch for a variety of purposes. Their behavior is intricate and not completely understood.
- The difficulties in relating findings in mice to the situation in humans reflects the incomplete understanding of mechanisms.
- Although manipulative therapies are applied to skin, the skin is not usually the tissue being treated. A better understanding of the effects on deep structures is needed.
- Robotics and virtual reality could be used to ethically investigate the impact of social factors and individual differences among therapists on patient responses.
- Standardized force-based treatments do not accurately reflect what is done in practice. For example, the types of standardized manipulations performed in mice resemble using a rolling pin for massage on a patient’s back—something a massage therapist would never do. Large animals such as pigs or dogs may be useful experimental models for realistic manipulations.

- Questions about stress and strain could be addressed by applying the same forces or strains to animals with and without fibrotic conditions and comparing the results. The stress/strain difference may not be useful at the molecular level, but it needs to be addressed at larger levels. Measuring strain in tissue while delivering manipulation would be useful, and better tools are needed to accomplish this. In Dr. Barbe's rat model of arm and hand fibrosis, efforts were made to measure forces inside the animals, but the available tools are inadequate.
- Although it is very helpful to look at neuroscience, a framework is needed for this research. Force-based manipulation therapies are usually used for musculoskeletal conditions, and the conditions and therapeutic indications need to be better understood. The impact of contextual effects may be important.
- If you do not understand the problem a therapy is trying to fix, you cannot understand what the therapy is doing. For example, for back pain, it is usually unclear what the diagnosis is. Pain is a symptom, not a diagnosis.
- Tools and technologies needed include animal genetic models other than mice, computational models that include multiphysics, and ways to include historical factors, social factors, and moods in models. Models need to be holistic and to include multiple sciences.

Presentations By Rapporteurs

Session 1: Dr. Fritz explained that session 1 laid much of the groundwork for subsequent sessions. Key points included distinguishing inputs from effects and defining and clarifying stress, strain, and stiffness as key properties. Going from assessing external loads to internal loads is an important gap. Another gap is at the cellular level, where it is uncertain whether cells respond more to stress or strain. Tools to assess strain and stiffness were discussed, with an emphasis on pure moment testing. The final talk focused on the role of muscles and some strategies to try to integrate some of the ideas into clinical practice.

Session 2: Dr. Chesler reported that the session included developing an understanding of how individual molecules work, including how local forces impact a channel and how to build on that knowledge. Knowing what the channel conducts and where it is localized alters how you think about it. Where a molecule is expressed dictates how the information is received. If there is a dysfunction in one of the molecules, how does this impact a person at a systems level? This topic was explored for Piezo2, using both molecular and person-level information. Dr. Reed added that changes in neuropeptides in the repetitive injury model were discussed, as were changes in substance P and calcitonin gene-related peptide (CGRP) in the pain and injury process. One of the mechanoreceptors in the muscle spindle was discussed, including how mechanical forces affect its discharge during and after a thrust.

Session 3: Dr. Abraira reported that session 3 covered spinal cord transmission of force sensation. The various players in the dorsal horn that might be integrating this type of information were discussed, along with modulation by top-down control. The sensory neurons that innervate ligaments and bones were described, along with their potential roles. The final talk emphasized the emerging properties of circuits. The technology Dr. Saab is developing would facilitate an understanding of the activities of neurons in different states at the dorsal horn level. There is agreement on the need to better understand the sensory neurons and how they interpret the tactile information from force-based manipulations. Application of AI will be necessary to accomplish these goals because of the large amounts of data that must be included.

Session 4: Dr. Saab reported that session 4 was about brain circuits. Panelists discussed research on cortical circuits that was conducted using mouse models, human models involving EEG, and computational models. The issue of gating was brought up at the thalamic level and spinal cord level. The contributors to the gate and circuit were discussed in detail. There was also discussion of a link between neural elements and supportive glial elements. The contextual flavor added to neural circuits in terms of touch perception and mood was discussed. Acupuncture as a therapy was discussed in terms of mechanisms and controlled studies.

Session 5: Dr. Pathak reported that Session 5 focused on tools from a modeling perspective—as an approach to identify the minimal information needed to develop and test hypotheses. An advantage of this approach is that it brings clarity. It forces people to think about what is known, what is not known, and what is most important.

Models can be either computational or experimental. Modeling means trying to look at things in a more reductionist, mechanistic way; in an ideal situation, one would then go back to the complete system with an experimentally testable hypothesis, then incorporate new findings into the model through an iterative process. Suitable approaches are available, but we need to know the questions they can be applied to.

A panelist commented that each of the modeling approaches has benefits for some situations. Dr. Pathak said that the scale of models can vary. A model could focus on determining mechanics of deep tissue at the cellular level, for example, or could focus on the therapist—how therapists approach treatments and patients and whether variation exists between therapists. Until you begin to put things together in a framework, you often do not know what is important.

Wrap-up

Dr. Langevin said that the second day built on the previous day's discussion of what happens in various tissues by extending it to the thalamus and the anterior parts of the cortex. Emotional regulation, the circuits that integrate emotions with pain sensation, and the experiences of pain and therapy were brought into the picture. Other important parts of the picture include behavior, social context, and individual experience, including the patient's internalization of what is happening during therapy (see Figure 1).

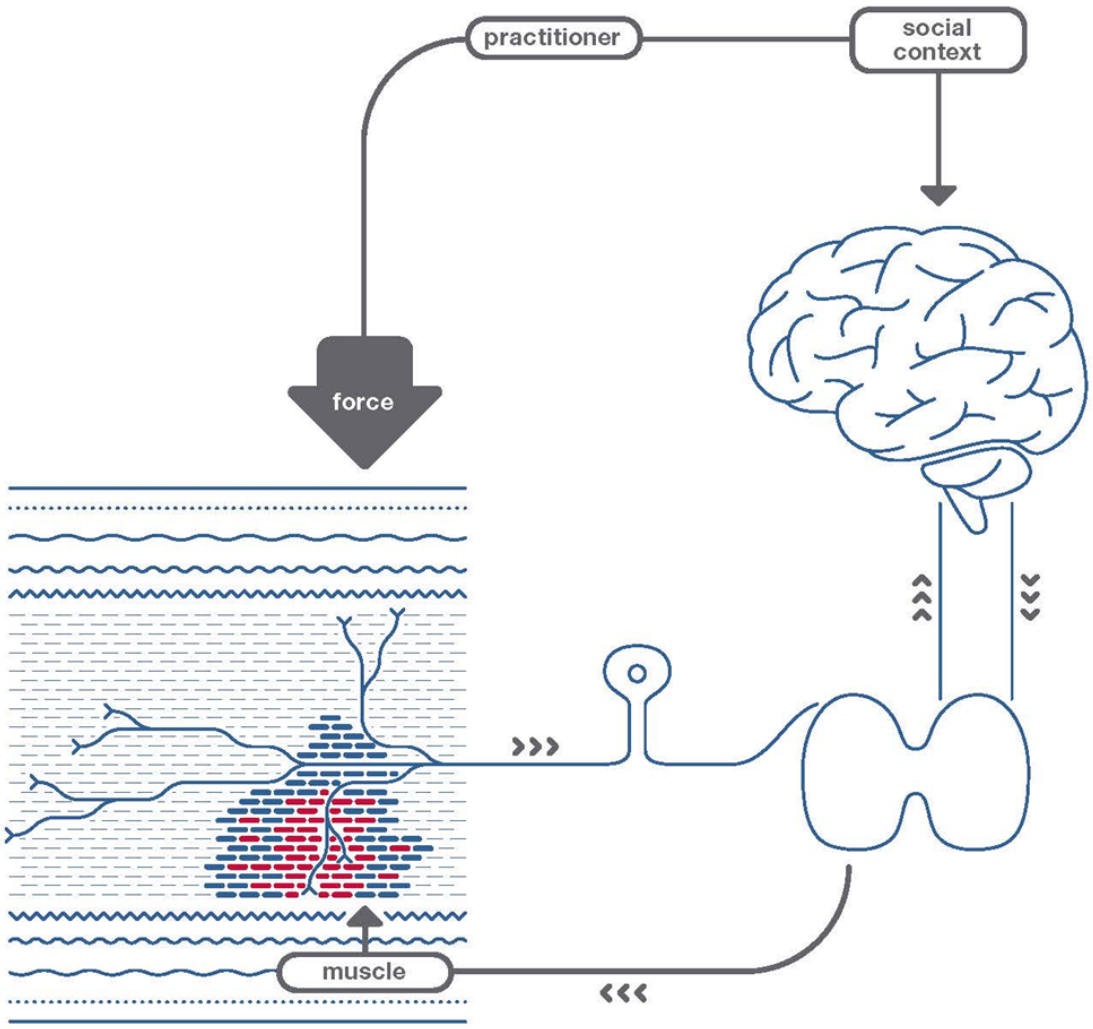

Figure 1. This schematic summarizes the active ingredients essential to a research framework/model as discussed at the workshop.

This framework illustrates an interoceptive component/pathway. The pathway begins with signals in the peripheral tissues (connective tissues, muscle). The signals are detected by the sensory ganglia, which are the dorsal root ganglia, and then transmitted via the spinal cord into the brain in the ascending pathways (afferents) for integration and processing. The signals could also be further transmitted/projected in the descending pathways (efferents) back to the peripheral tissues through the spinal cord. This is the interoceptive circuit involving the muscles and the brain/spinal cord.

The second major component of this framework is how a force-based manipulation can then regulate this circuit. The manipulation is typically delivered by a practitioner, who can influence the circuits from two directions. One is through the delivery of the force to the peripheral muscles and tissues and the other is through interactions with the patient by creating a psychosocial context and expectations. This context can directly influence the patient's brain activity. The social interaction can be projected back through the descending pathways (efferents) to influence peripheral tissue activity.

Some effects of manipulative therapies may last only as long as the manipulation does, but if the person changes behavior, especially motor behavior, as a result of the manipulation, the effects could be prolonged. The sensory experience of the therapy is integrated with other elements, including cognition, emotional regulation, and interaction with the therapist. Contextual effects of manual therapy are not fully understood. In the absence of sham massage, it is not possible to blind the patient as can be done with acupuncture. It is important to be aware that many of the effects of manipulation may be contextual. With acupuncture, research has shown that the effects are not entirely contextual, but it takes very large sample sizes and statistical analysis to detect this. The field of manual therapy is a young one, and not much research has been performed yet. It is important that the pendulum should not swing entirely to the brain and the context; effects at the tissue level should also be considered. The role of the therapist, who provides both the force and the context, must also be taken into consideration.

In conclusion, the roundtable identified gaps and opportunities in the various components of this research framework, specifically the mechanical force and the response to it; how external force and psychosocial context affect the neural circuits of force-based manipulations; the need for computational mechanistic models that include historical factors, social factors, and affect/mood; the technology needs to advance the research; and lastly, the challenges for transdisciplinary efforts. Priorities include the following:

- ***Mechanical force and response***
    - Develop common terminology and metrics to characterize, uniformly define, and quantify the types of mechanical force.
    - Develop and validate objective measures of force, stress, strain, stiffness, and the response to force at multiple levels of analysis (cellular, molecular, extraneural and neural, behavioral, psychological and social).
- ***Circuits.*** Define and quantify external influences such as psychosocial, affect/mood, and expectation effects on various neural circuits of force-based manipulations.
- ***Models.*** Develop computational mechanistic models that include historical factors, social factors, and affect/mood.
- ***Technology needs***
    - Identify/develop methodologies for applying and quantifying forces.
    - Develop technologies for real-time recording and imaging of cells and deep tissue during force-based manipulations
- ***Challenges for transdisciplinary efforts.*** Create new opportunities to connect neuroscientists, engineers, and clinician-scientists.

Appendix A

Neurocircuitry of Force-Based Manipulations Agenda

September 17-18, 2019

6701 Democracy Blvd. (DEM I), Room 602, Bethesda, Maryland

Organizers:
Merav Sabri, Ph.D. (National Center for Complementary and Integrative Health, NCCIH)
James Gnadt, Ph.D. (National Institute of Neurological Disorders and Stroke, NINDS)

National Institutes of Health Partners:
Alison Cernich, Ph.D., A.B.P.P.-Cn. (Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Center for Medical Rehabilitation Research, NICHD/NCMRR)
Theresa Cruz, Ph.D. (Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Center for Medical Rehabilitation Research, NICHD/NCMRR)
Lyndon Joseph, Ph.D. (National Institute on Aging, NIA)
Grace Peng, Ph.D. (National Institute of Biomedical Imaging and Bioengineering, NIBIB)

Workshop Cosponsors:
NCCIH and NICHD/NCMRR

Cochairs:
Ellen Lumpkin, Ph.D. (Helen Wills Neuroscience Institute, University of California, Berkeley), Gert Bronfort, D.C., Ph.D. (University of Minnesota)

Day 1: September 17, 2019

9:00 a.m. **Opening remarks: Helene Langevin, M.D. (NCCIH)**

9:10 a.m. **Workshop charge: Ellen Lumpkin, Ph.D. (Cochair) (Helen Wills Neuroscience Institute, University of California, Berkeley)**

9:20 a.m. **Overview of clinical relevance: Gert Bronfort, D.C., Ph.D. (Cochair) (University of Minnesota)**

9:30 a.m. **Session 1: Defining the biomechanical force**

Moderator- Lyndon Joseph, Ph.D. (NIA)
Rapporteur- Julie Fritz, Ph.D., P.T., F.A.P.T.A. (University of Utah)

- Helene Langevin, M.D. (NCCIH)
    - Stress, strain, and stiffness: why do they matter, and how are they measured?
- Greg Kawchuk, D.C., Ph.D. (Rehabilitation Medicine at the University of Alberta)
    - Spinal biomechanics and robotics
- Julie Fritz, Ph.D., P.T., F.A.P.T.A. (University of Utah)
    - Muscle activation response

10:15 a.m. **DISCUSSION**

10:45 a.m. **BREAK**

11:00 a.m. **Session 2: Peripheral neural and extraneural sensing of biomechanical force**

Moderator- James Gnadt, Ph.D. (NINDS)
Rapporteurs- Alex Chesler, Ph.D. (NCCIH) and William Reed, D.C., Ph.D. (University of Alabama at Birmingham)

- Stephen Brohawn, Ph.D. (University of California, Berkeley)
    - Mechanisms of force sensation by ion channels
- Ellen Lumpkin, Ph.D. (Helen Wills Neuroscience Institute, University of California, Berkeley)
    - Cellular substrates of touch sensation
- Geoffrey Bove, D.C., Ph.D. (University of New England College of Osteopathic Medicine)
    - Sensory input in pathological states during manual therapy, or, what goes up
- Mary F. Barbe, Ph.D., F.A.A.A. (Temple University)
    - Effect of neurotransmitters secreted from sensory neurons on peripheral nonneural tissues
- William R. Reed, D.C., Ph.D. (University of Alabama at Birmingham)
    - Impact of biomechanical characteristics of spinal manipulation on trunk muscle spindle response
- Alexander Chesler, Ph.D. (NCCIH)
    - What a rare disease can teach us about touch

12:30 p.m. **DISCUSSION**

1:00 p.m. **LUNCH**

2:45 p.m. **Session 3: Spinal cord transmission of force sensation**

Moderator- Theresa Cruz, Ph.D. (NICHD/NCMRR)
Rapporteur- Victoria Abraira, Ph.D. (Rutgers School of Art and Science)

- Victoria Abraira, Ph.D. (Rutgers School of Art and Science)
    - The cellular and synaptic architecture of the mechanosensory dorsal horn
- Beth A. Winkelstein, Ph.D. (University of Pennsylvania)
    - Spinal integration of peripheral inputs: what we know from models of painful injury
- Carl Saab, Ph.D. (The Warren Alpert Medical School of Brown University)
    - Spinal circuits of pain

3:30 p.m. **DISCUSSION**

4:00 p.m. **WRAP UP**

4:15 p.m. **ADJOURN**

Day 2: September 18, 2019

8:30 a.m. **Session 4: Central processing and modulation of biomechanical force**

Moderator- Merav Sabri, Ph.D. (NCCIH)

Rapporteurs- William Reed, D.C., Ph.D. (University of Alabama at Birmingham) and Carl Saab, Ph.D. (The Warren Alpert Medical School of Brown University)

- Fan Wang, Ph.D. (Duke University School of Medicine)
    - Pain relief by touch: a behavioral and *in vivo* calcium imaging study in mouse
- William Reed, D.C., Ph.D. (University of Alabama at Birmingham)
    - Role of thalamic nuclei in low-back pain and complementary/integrative approaches
- Carl Saab, Ph.D. (The Warren Alpert Medical School of Brown University)
    - Brain circuits of pain
- M. Catherine Bushnell, Ph.D. (NCCIH)
    - Bottom-up and top-down effects of manual therapies
- Laura Case, Ph.D. (NCCIH)
    - Role of opioids in superficial and deep touch
- Jian Kong, M.D. (Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School)
    - Altered brain circuitry in chronic pain patients and the modulation effect of peripheral mechanical stimulation

10:00 a.m. **DISCUSSION**

10:30 a.m. **BREAK**

10:45 a.m. **Session 5: Technology advances for force-based manipulations**

Moderator- Grace Peng, Ph.D. (NIBIB)

Rapporteur- Medha Pathak, Ph.D. (University of California, Irvine School of Medicine)

- Victor H. Barocas, Ph.D. (University of Minnesota)
    - Multiscale mechanics of soft tissue
- Gregory J. Gerling, Ph.D. (School of Engineering and Applied Science, University of Virginia)
    - Computational models and stimulus devices to capture naturalistic skin – tactile afferent response
- Medha M. Pathak, Ph.D. (University of California, Irvine School of Medicine)
    - Human brain organoid technology: from development to function

11:45 a.m. **DISCUSSION**

12:15 p.m. **LUNCH**

1:30 p.m. **Session 6: General discussion and concluding remarks**

- Rapporteurs report
- Summary- Ellen Lumpkin, Ph.D. (Helen Wills Neuroscience Institute, University of California, Berkeley), Gert Bronfort, D.C., Ph.D. (University of Minnesota)
- General discussion

2:30 p.m. **ADJOURN**

Appendix B

Attendee List

Meeting Organizers

Merav Sabri, Ph.D., NCCIH
James W. Gnadt, Ph.D., NINDS

National Institutes of Health Partners

Theresa Hayes Cruz, Ph.D., director of the ***Eunice Kennedy Shriver*** National Institute of Child Health and Human Development's National Center for Medical Rehabilitation Research (NICHD/NCMRR).

Grace C.Y. Peng, Ph.D., director of Mathematical Modeling, Simulation, and Analysis at the National Institute of Biomedical Imaging and Bioengineering (NIBIB).

Lyndon Joseph, Ph.D., National Institute on Aging (NIA)

Meeting CoChairs

Gert Bronfort, D.C., Ph.D., is a research professor at the Bakken Center for Spirituality & Healing's (CSH) Integrative Health & Wellbeing Research Program at the University of Minnesota.

Ellen A. Lumpkin, Ph.D., is a professor of cell and developmental biology at the University of California, Berkeley in the Department of Molecular & Cellular Biology and the Helen Wills Neuroscience Institute.

Session One

Helene Langevin, M.D., is Director of NCCIH.

Greg Kawchuk, D.C., Ph.D., is a professor in the Faculty of Rehabilitation Medicine at the University of Alberta.

Julie Fritz, Ph.D., P.T., F.A.P.T.A., is a distinguished professor in the Department of Physical Therapy and Athletic Training and the Associate Dean for Research in the College of Health at the University of Utah.

Session Two

Steve Brohawn, Ph.D., is an assistant professor of neurobiology at the University of California, Berkeley

Ellen A. Lumpkin, Ph.D. (see above)

Geoffrey Bove, D.C., Ph.D., is a research professor at the University of New England College of Osteopathic Medicine.

Mary F. Barbe, Ph.D., is a full professor in the Department of Anatomy and Cell Biology at Lewis Katz School of Medicine of Temple University.

William R. Reed, D.C., Ph.D., is an associate professor in the School of Health Professions Department of Physical Therapy at the University of Alabama at Birmingham,

Alexander Chesler, Ph.D., is a Stadtman Investigator in the NCCIH intramural program.

Session Three

Victoria Abraira, Ph.D., is an assistant professor in the Cell Biology and Neuroscience Department at Rutgers University.

Beth A. Winkelstein, Ph.D., is the Eduardo D. Glandt President's Distinguished Professor of Bioengineering and Neurosurgery at the University of Pennsylvania.

Carl Saab, Ph.D., is associate professor, Department of Neuroscience and Department of Neurosurgery, Director of the Center for Pain and Neural Circuits at Rhode Island Hospital, and member of the Carney Institute for Brain Science at Brown University.

Session Four

Fan Wang, Ph.D., is the Morris N. Broad Professor of Neurobiology at Duke University Medical School.

William R. Reed, D.C., Ph.D. (see above)

Carl Saab, Ph.D. (see above)

M. Catherine Bushnell, Ph.D., is Scientific Director of NCCIH.

Laura Case, Ph.D., is a postdoctoral fellow in the Pain and Integrative Neuroscience Branch at NCCIH.

Jian Kong, M.D., is an associate professor at the Department of Psychiatry, Massachusetts General Hospital (MGH), Harvard Medical School.

Session Five

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