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Original Research Article

Intense Focused Ultrasound Preferentially Stimulates Subcutaneous and Focal Neuropathic Tissue: Preliminary Results

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Abstract

Objective. Potential peripheral sources of pain from subcutaneous tissue can require invasive evocative tests for their localization and assessment. Here, we describe studies whose ultimate goal is development of a noninvasive evocative test for subcutaneous, painful tissue.

Design. We used a rat model of a focal and subcutaneous neuroma to test the hypothesis that intense focused ultrasound can differentiate focal and subcutaneous neuropathic tissue from control tissue. To do so, we first applied intense focused ultrasound (2 MHz, with individual pulses of 0.1 second in duration) to the rat’s neuroma while the rat was under light anesthesia. We started with low values of intensity, which we increased until intense focused ultrasound stimulation caused the rat to reliably flick its paw. We then applied that same intense focused ultrasound protocol to control tissue away from the neuroma and assayed for the rat’s response to that stimulation.

Results. Intense focused ultrasound of sufficient strength (ISATA of 600 ± 160 W/cm²) applied to the neuroma caused the rat to flick its paw, while the same intense focused ultrasound applied millimeters to a centimeter away failed to induce a paw flick.

Conclusion. Successful stimulation of the neuroma by intense focused ultrasound required colocalization of the neuroma and intense focused ultrasound supporting our hypothesis.

Key Words. Intense Focused Ultrasound; Neuropathic Pain; Localization; Pain Detection; Neuroma

Introduction

Pain is a universally experienced phenomenon with immense personal and societal cost. Unfortunately, current diagnostic tests have limited ability to localize deep sources of pain necessary for diagnosis and associated therapy. For example, imaging studies, physical examination, or provocative diagnostic tests cannot identify sources of pain for 85% of patients with back pain [1] likely due to the mechanical complexity of the spinal column, the depth of the tissue, the fact that anatomical images do not image pain [2], along with changes in nociceptive processing within the spinal chord and brain known as central sensitization [3]. As another example, 50–80% of amputees experience pain that arises through a combination of peripheral sources such as neuromas as well as central sensitization [4–8] with generally ineffective diagnostics and treatments [3,9]. In particular, neuromas readily appear in ultrasound and magnetic resonance imaging (MRI), but determination of their painfulness, as distinct from their presence, requires their stimulation such as via manual palpation, which is generally problematic for most neuromas. As yet another example, cancer metastasized to bone can produce intense pain poorly controlled by drugs including opioids [10]. Ablation of bone metastases via radiosurgery techniques, for example, can reduce this pain [10,11]. Identifying metastases that are painful vs merely present represents an important step in the treatment of these patient’s pain [12].

A more reliable noninvasive evocative diagnostic test is needed to localize and assess candidate deep peripheral source of neuropathic pain with sufficient sensitivity and specificity to improve the treatment of that pain. Intense focused ultrasound (iFU) may form the basis of such an
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**Materials and Methods**

All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Washington under protocol #4084-02, as well as conformed to relevant national guidelines. Adult male Sprague Dawley rats (Taconic) weighing approximately 180 g were housed three per cage under housing conditions of 12 hours light: 12 hours dark (light on at 6:00AM and light off at 6:00PM) at a temperature of 20–22°C with ready access to food and water.

**Animal Model of Focal Neuropathic Pain**

We performed the following surgery on the right rear leg of 15 Sprague Dawley rats (Figure 1), adapting the surgical technique of Dorsi et al. [19]. We surgically exposed the tibial and calcaneal nerves, while keeping them adjacent to one another, sufficiently to free up a 1-cm length of these adjacent nerves starting from just proximal to the ankle. We tied a suture around the distal end of the visible nerve section and then cut the nerve distal to the suture. We then used the suture to guide the now free nerve-end up under the skin proximal to the intact portion of the nerve, securing the transected nerve ending in place with the suture passed all the way through the skin. This nerve ending then formed a subcutaneous neuroma, as in Dorsi et al. [19], here on the medial aspect of the rat’s leg. In this way, we positioned the neuroma to facilitate its stimulation while the animal was in a supine position under light anesthesia.

The original tibial neuroma transposition model as described in Dorsi et al. [19] showed that sham neuroma surgery produced tissue that was no more sensitive than its surrounding tissue in response to mechanical stimulation at 5 days or more after surgery. For this reason, and because here, we seek to assay the ability of iFU to preferentially stimulate a neuroma relative to tissue in the same rat, we decided that it was unnecessary to include a sham surgery group in our study relying upon tissue away from the neuroma for a given rat as its own control (again, Figure 1).

**Behavioral Test—Confirming the Presence of a Sensitive Neuroma**

Dorsi et al. [19] found that the surgery created a sensitive neuroma beginning 5 days after surgery with consistent and lasting sensitivity to mechanical stimulation for the duration of their study (up to 45 days). We therefore performed our tests on each rat starting six days after the surgery ending within 7 weeks of the surgery (Figure 2), during which time each rat underwent two to five tests of evocative test. Several researchers have shown that sufficiently intense iFU can generate sensations in healthy test subjects when applied to tissue both at depth and superficially [13–16], likely through mechanical stimulation, at least for short pulses of iFU [14,17,18]. Therefore, as argued by Gavrilov and colleagues, it is plausible that iFU stimulation applied directly to potentially neuropathic tissue would generate diagnostic sensations that differ in quality or intensity to similar stimulation of surrounding tissue. In this way, iFU, coupled with image guidance, may improve localization, hence identification, of peripheral pain-generating tissue. Making this idea a reality is the long-term goal of our work.

Here, we address a question supportive of our long-term goals, asking: can focal stimulation provided by iFU differentiate focal, subcutaneous neuropathic tissue from nearby tissue?
the ability of iFU to differentially stimulate a neuroma, never more frequently than every other day. Some rats only underwent two tests (compared with five for some) because their neuromas were not reliably responsive to mechanical stimulation—our gold standard, described later—or because some rats would not reliably stay under light anesthesia, which we now describe.

Rats were lightly anesthetized using a mixture of isoflurane (Pitman-Moore, Mundelein, IL, USA) and oxygen via nose cone. Specifically, we induced anesthesia at 4% isoflurane and then adjusted the rats for 5 minutes down to a 1.5% mixture of isoflurane, then to 1.25% for 5 minutes, and similarly down to approximately 1% isoflurane. With the rat acclimated to 1% isoflurane, we used a mechanical stimulator (a thin wooden dowel or von Frey filament) to gently palpate the neuroma. We did this for two reasons: to test for mechanical sensitivity of the neuroma and to localize it in a way independent of the ultrasound. This was necessary because the percutaneous suture holding the neuroma in place was sometimes no longer visible, while other times, the point of sensitivity was few millimeters away from the suture. If we could not elicit a response from the neuroma at 1% isoflurane, we then lowered the isoflurane concentration and tried again. When we found the location where the rat would withdraw its foot when stimulated on that point, but not on an adjacent location within millimeters of the first, we recorded the smallest mass of von Frey that elicited a response and marked that location with permanent ink. Generally, one to two adjustments of anesthetic level were necessary during the von Frey location process. We also marked the leg approximately 1 cm toward the body from the neuroma at a location with an amount of tissue between the skin surface and underlying bone comparable with that found at the location of the neuroma measured using calipers (see Figure 1). We identified this neuroma-free tissue as the control test site.

Ultrasound Device

Figure 3a shows the ultrasound device used for this study: a single-element focused transducer (Sonic Concepts, Bothell, WA, USA) on which a plastic removable cone was mounted, as described in detail in Mesiwala et al. [20]. Mounted permanently on the sides of this cone were two Figure 3 This figure shows (A) a photograph of our ultrasound device, along with the distribution of ultrasound pressure in a direction (B) away from the transducer and (C) parallel to the face of the transducer at the depth of the focus. The dark contour in (B) and (C) marks the region in which the applied pressure is higher than half the value of the maximum pressure, useful for estimating the location of significant biologic stimulus provided by intense focused ultrasound. Note that in (B), the y-axis displays the distance away from the face of the transducer.
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lasers (Digi-Key, Thief River Falls, MN, USA), each pointed to meet at the center of the ultrasound’s focus. With regard to the geometry of iFU (Figure 3b,c), the center of this focus lies 9 mm away from the tip of the cone, with the ultrasound pressure reduced by one-half at the “half-maximum-pressure contour” 6 mm in radius from the focus in the direction away from the transducer face (Figure 3b) and reduced by one-half at a distance of 1 mm in radius in the direction perpendicular to the transducer face (Figure 3c), determined through numerical simulations using Matlab (The MathWorks, Natlick, MA, USA). We confirmed our simulation via measurement of the focus with a needle hydrophone (Onda Corporation, Sunnyvale, CA, USA) in a water-filled tank. The cone itself was filled with degassed water and then capped with a latex membrane to facilitate transmission of the ultrasound from the transducer through the skin surface into the tissue.

We applied iFU with this device using a single 2 MHz ultrasound burst lasting 0.1 second. The signal was created by two function generators (HP33120A, Hewlett-Packard, Palo Alto, CA, USA), and then amplified by a power amplifier (ENI A150, ENI, Rochester, NY, USA) and transmitted to the transducer through a matching network (Sonic Concepts). Together, the function generators were programmed to produce a pulse of the desired length, frequency, and amplitude. The input voltage to the transducer was determined using a Wave Runner LT 322 oscilloscope (LeCroy Corporation, Chestnut Ridge, NY, USA).

We translated that voltage into acoustic intensity, a quantitative measure of the amount of ultrasound available for stimulation emitted by the iFU device. To do so, we used a well-established “force balance” technique [21,22]. Specifically, ultrasound energy emitted by the device beams through degassed water into an ultrasound absorber placed on a scale. The resulting “weight” at a given input voltage, along with calculations of the spatial distribution of ultrasound energy through the half-maximum-pressure contour in the focal plane (Figure 3c) is translated mathematically into a measure of intensity (I_{SATA}). In particular, I_{SATA} is the spatially and temporally averaged intensity over the area enclosed by the half-maximum-pressure contour in the focal plane.

Pilot Study

Prior to collecting the data we present in this article, we performed a pilot study of iFU stimulation on four rats. We first placed the rat under anesthesia and mechanically localized the neuroma as described earlier. We then mounted the iFU device above the approximate location of the neuroma (Figure 4). Through use of a three-dimensional micropositioning stand (Velmex, Bloomingfield, NY, USA), we could move the focus of the iFU transducer laterally and vertically in steps of 100 microns. We started by focusing the lasers attached to the iFU device on the skin above the neuroma (Figure 4b) as determined through mechanical palpation. We then put ultrasound gel (Aquasonic, Parker Laboratories, Inc, Fairfield, NJ, USA) between the transducer and the neuroma to ensure adequate ultrasound coupling. The iFU device was lowered 2 mm below the point where the lasers placed the focus on the skin surface (Figure 4a,c) as determined in our pilot study. Then, beginning at 150 W/cm², we applied 100-millisecond bursts of iFU to the neuroma while increasing the intensity in steps of 10–30% until the rat flicked its paw after application of iFU. When we observed a paw flick, we used the micropositioner to move the transducer’s focus to the control site approximately 1 cm away (Figure 1) and applied the same amount of iFU, observing for the presence or absence of a paw flick. If the rat flicked its paw after application of iFU to the control tissue, we decreased the output of the iFU device and performed the test again first on the neuroma and then again to the control site. (If a rat became restless while under anesthesia, we first increased the concentration of isoflurane then gradually reduced the concentration until the rat did not move, unless the neuroma was mechanically stimulated, or we determined that we could not stabilize that rat for that day. We had to adjust anesthesia in about 50% of tests generally with success. After stabilizing the rat, testing would then proceed as described earlier.) If the rat did not flick its paw after iFU application to the control site, we moved the iFU focus back to the neuroma where we applied the same iFU intensity as before assaying again for a paw flick. We continued in this fashion until we identified the iFU intensity at which we achieved 3/3 responses from the neuroma and 0/3 responses from control tissue in succession. We recorded this intensity as the iFU threshold intensity value for that rat for that day.
Results

iFU and von Frey Threshold Values

We successfully created a neuroma responsive to mechanical testing in eight out of eight rats. Of those eight rats, seven rats responded to both mechanical and iFU testing, while one responded only to mechanical testing. We performed a total of 31 tests on these eight rats (Figure 5a). We found both a mechanical and iFU threshold values for 21 of those tests: 5.7 $\pm$ 2.2 g and 343 $\pm$ 77 W/cm², respectively (Figure 5b). We did not find any correlation between von Frey and iFU threshold, or between isoflurane concentration and either von Frey or iFU.

Of the 10 tests where we did not find both a mechanical and iFU threshold, in six, we found neither mechanical nor iFU thresholds, and in four, we found mechanical but not iFU thresholds. In the four tests where we found mechanical but not iFU thresholds, the rats responded inconsistently to iFU. For example, the rat might have responded to a pulse on the neuroma once but then not again in succession despite our best efforts. Of the six tests in which we did not find either of mechanical and iFU thresholds, in four, we simply could not elicit a mechanical threshold response. During the other two tests, the rats would not stay consistently anesthetized at a low enough level to allow us to perform the studies. Finally, testing the application of iFU at its threshold value for a given rat on a given day away from the neuroma never created a response, nor did testing with von Frey with a force comparable with that necessary to elicit a paw flick when applied to the site of the neuroma.

Discussion

In this study, we tested the ability of iFU to preferentially stimulate subcutaneous neuropathic tissue relative to control tissue. Specifically, our results demonstrated that sufficient iFU applied to focal subcutaneous neuropathic tissue (a neuroma in the leg of a lightly anesthetized rat) caused the rat to flick its paw, while that same amount of iFU applied to nearby or distant tissue did not. We observed this in 21/25 (84%) of tests on animals whose neuroma also responded to mechanical testing—our means of verifying the presence of a painful neuroma. These results support our hypothesis that iFU stimulation of tissue can differentiate focal and subcutaneous neuropathic tissue from normal tissue.
In future studies, however, we intend to map the spatial extent of the sensitive parts of a neuroma using iFU stimulation while the rat is under deep anesthesia, assaying the associated response to iFU stimulation via in vivo electrophysiological measurements at the spinal chord level made on wide dynamic range neurons with a receptor field located at the neuroma level, a technique that has been used in other types of pain models [23]. Eventual human studies could also test iFU's ability to differentiate neuromas from surrounding tissue, which will eliminate the need for anesthesia and move this procedure closer to the clinic. For example, relatively superficial Morton’s neuromas offer one candidate target: physicians regularly localize these neuromas via a combination of manual palpation and imaging [24,25], a useful gold standard.

We note that our specific choices of frequency and pulse duration fit within the existing, larger body of research on the use of iFU for assaying pain. For example, Dalecki and colleagues, and Gavrilov and colleagues [13,14,17] have investigated human sensation using iFU within a range of ultrasound (0.3–5.0 MHz) and duration (5–100 millisecond in single pulses, or multiple pulses per second for 1 to several seconds). Moreover, for the ultrasound protocol we have chosen for this study, this stimulation likely occurs through activation of mechanical receptors by the momentum deposited by the iFU, as studied by Gavrilov and colleagues [14,17,18]. We note that their analysis also highlights the possibility of using longer pulses of ultrasound to simultaneously activate heat-sensitive peripheral nerve receptors as well as mechanical receptors. For this study, we elected to use one frequency and duration, realizing that future work should include exploration of various iFU frequencies and durations to determine ideal values useful for the clinic.

We anticipate that physicians may one day use iFU as a tool for locating and assessing painful neuromas. For example, in an amputee patient with stump pain, physicians could use iFU under image guidance (“ig-iFU”—reviewed in Ter Haar and Coussios [26]) to locate and assay neuromas that may contribute to the patient’s pain. Current techniques for locating potentially painful neuromas in stumps primarily rely on MRI and ultrasound imaging, which are useful for neuromas measuring over 1 cm, but much more difficult for smaller neuromas [24]. Moreover, MRI and ultrasound imaging cannot determine the painfulness of a neuroma, only its existence. The use of ig-iFU may also help localization of painful neuromas that measure less than 1 cm through its ability to focus on a small area as well as for identifying deep painful neuromas regardless of size by facilitating their subcutaneous palpation.

Another study of likely interest would include application of ig-iFU to two cohorts of amputee patients, one with standard amputations and associated neuromas, and the other with targeted muscle reinnervation (TMR). TMR is a technique in which nerves that would normally innervate distal residual limb muscles, and possibly form a neuroma, are transferred to the neuromuscular junction of more proximal muscles so as to control prosthetic devices [27]. Interestingly, though not yet the focus of formal study, we believe difficulties attendant with our use of low levels of anesthesia contributed to those tests (4/25 or 16%) for which we found that mechanical testing could identify the neuroma but iFU could not. We chose to use light anesthesia in this study rather than restraining conscious animals because even while restrained, residual motion would make problematic application of the focus of the iFU device with millimeter precision. However, because of this choice, our use of isoflurane added an element of variability that proved difficult to control at times, owing to the intrinsic variability in rats’ responses to light anesthesia. Each rat therefore required time-consuming, independent exploration of the neuroma by iFU, which was not always possible to quantify on a step-by-step basis owing to the continuous adjustment of isoflurane. For example, sometimes, the rat would shift while lightly anesthetized or even wake up, requiring us to reset the entire experiment. This is why we decided to report merely successful iFU stimulation of the neuroma relative to control tissue in the same rat.

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Figure 5 This figure (A) diagrams the results of our behavioral tests as well as the mean and standard deviation for our measurements, while (B) shows a set of box and whisker plots documenting the distribution of our results when both intense focused ultrasound and von Frey successfully stimulated the neuroma. The line in the box gives the median value, the box encompasses the position of 75% of the values, the whiskers bound 95% of values, and individual points represent individual values that extend beyond the 95% boundary.
patients after the TMR procedure appear to report less pain than standard amputee patients [28]. It would therefore be of interest to use ig-iFU to quantify the sensitivity of neuromas that arise after standard amputation relative to the corresponding nerves at their implantation site of TMR patients.

ig-iFU could also be used to locate and assay other small and subcutaneous peripheral sources of pain. For example, intense pain can be present in patients whose cancer has metastasized to bone, where drugs are not adequately effective [10] in managing the pain. For this application, physicians might use ig-iFU to identify painful metastases vs those merely present [12]. This represents an important step in ablative treatments of bone metastases [10,11].

iFU offers several advantages over existing methods for characterizing peripheral pain. Primarily, it can stimulate subcutaneous structures, where existing methods such as thermodes, lasers, and peltier devices can stimulate only cutaneous structures. Furthermore, iFU is entirely noninvasive. For example, in assessment of severe lower back pain, lumbar provocative discography is often used—a procedure requiring the insertion of needles into spinal discs in order to inject fluids to assay for a diagnostic pain response, with contrast agents to help image the disc as well as check the patency of its capsule [29]. With iFU, one could noninvasively stimulate these subcutaneous discs in a fashion that would assess the sensation threshold for each disc in order to determine which disc, if any, is associated with the pain.

Fibromyalgia represents another malady whose study, and possibly even clinical management, could benefit from a noninvasive and versatile evocative test for deep peripheral sources of pain. One view of fibromyalgia is that subclinical but irritating signals from deep peripheral tissue, especially near joints, chronically stimulate a centrally sensitized nervous system contributing to symptoms of fibromyalgia including chronic pain [2]. Research and some clinical practice utilizes application of heat or pressure pulses to the skin of fibromyalgia patients [4] that produce, on average, more intense sensations or even pain of unusual length when compared with stimulation of health volunteers. Comparable results [4] have been produced by focal application of pressure to the skin after use of a topical anesthetic in a manner shown to activate subcutaneous sensory neurons. However, even with the use of a topic anesthetic, cutaneously applied pressure would activate all subcutaneous structures, while iFU could localize and assay deep potential sources of peripheral irritation for these patients.

Clinicians may one day also use iFU as a means to assay the depth of anesthesia. iFU could be delivered at the site of surgical incision, both cutaneously and subcutaneously, in combination with a brain monitoring method such as the bispectral index [30]. Changes in brain wave activity immediately after iFU stimulation might indicate an insufficiency of anesthetic plane, building on a principle similar to the “toe pinch test” commonly used in animal anesthesia.

In yet another potential application of this technology, physicians may one day use iFU stimulation to track the efficacy of pain management. In a previous publication [31], we demonstrated that the iFU threshold value associated with inflammatory pain (in vivo) had a diurnal variation consistent with the known diurnal variation in inflammatory pain. Motivated by this, we hypothesize that quantitative iFU stimulation (applied blindly as in our human study [16]) may offer a means of tracking changes in pain over longer periods of time during the course of treatment; a reduction in the iFU threshold value for a given patient over time may point to efficacious pain management, for example.

Conclusion

We have tested the hypothesis that iFU can differentiate focal and subcutaneous neuropathic tissue from control tissue using a rat model of a neuroma. These results constitute the first of several steps necessary for achieving the goal of using ig-iFU to localize focal painful tissue pathology within patients. As such, our results suggest that using iFU under image guidance to locate neuropathic damage may one day help guide diagnoses, hence therapy, for patients with chronic back pain, amputees, and cancer patients for tracking the efficacy of pain management (intraoperatively, during clinical exams) among several candidate etiologies.

Acknowledgments and Conflict of Interest

Disclosure/Summary

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The author’s contributions are as follows: experimental design (Dickey, Kliot, Loeser, McClintic, Mourad, Richebe), data collection (Dickey, Gofeld, McClintic, Mourad), data analysis (Dickey, McClintic, Mourad), and write-up (Dickey, Gofeld, Kliot, Loeser, McClintic, Richebe, Mourad). We would like to thank Kate Sweeney for her invaluable help in creating Figures 1 and 4.

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